Free Radical Biology & Medicine, Vol. 7, pp. 435–477, 1989
Printed in the USA.

0891-5849/89 $3.00 + .00

Review Article

REDOX AND ADDITION CHEMISTRY OF QUINOID COMPOUNDS AND ITS BIOLOGICAL IMPLICATIONS

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(*Received* 27 *June* 1988; *Revised and accepted* 9 *September* 1988)

Abstract—The overall biological activity of quinones is a function of the physico-chemical properties of these compounds, which manifest themselves in a critical bimolecular reaction with bioconstituents. Attemps have been made to characterize this bimolecular reaction as a function of the redox properties of quinones in relation to hydrophobic or hydrophilic environments.

The inborn physico-chemical properties of quinones are discussed on the basis of their reduction potential and dissociation constants, as well as the effect of environmental factors on these properties. Emphasis is given on the effect of methyl-, methoxy-, hydroxy-, and glutathionyl substituents on the reduction potential of quinones and the subsequent electron transfer processes.

The redox chemistry of quinoid compounds is surveyed in terms of a) reactions involving only electron transfer, as those accomplished during the enzymic reduction of quinones and the non-enzymic interaction with redox couples generating semiquinones, and b) nucleophilic addition reactions. The addition of nucleophiles, entailing either oxidation or reduction of the quinone, are exemplified in reactions with oxygen- or sulfur nucleophiles, respectively. The former yields quinone epoxides, whereas the latter yields thioether-hydroquinone adducts as primary molecular products. The subsequent chemistry of these products is examined in terms of enzymic reduction, autoxidation, cross-oxidation, disproportionation, and free radical interactions.

The detailed chemical mechanisms by which quinoid compounds exert cytotoxic, mutagenic and carcinogenic effects are considered individually in relation to redox cycling, alterations of thiol balance and Ca^{++} homeostasis, and covalent binding.

Keywords—Quinones, Quinone epoxides, Thioether-quinone conjugates, Hydroxyquinones, Free radicals, Reduction potential, DT-Diaphorase, Superoxide dismutase, Nucleophilic addition, Autoxidation, Excited states, Bioreductive alkylation, Redox cycling, Covalent binding

INTRODUCTION

Quinoid compounds contain the chromatophoric quinoid group (structure **I**; scheme 1); this particular arrangement of double bonds inside and outside the six-membered rings is largely responsible for the chemistry of quinoids. This structure could be considered as a simple cross-conjugated system regardless of the nature of the heterocyclic moiety **X**. Several types of quinoid compounds are found containing oxygen, sulfur, carbon, and nitrogen in the terminal positions. In considering the electronegativity of the heteroatom, it can be predicted that quinoids containing the more electronegative oxygen (quinones; **II**)—or nitrogen (quinone diimines; **III**)—or both (quinone imines; **IV**) heteroatoms are more stable than those containing sulfur (—C=S) or carbon (—C=CH_2).[1]

Quinones are diketones with the structure O=C—(C=C—)$_n$—C=O derived from aromatic compounds (structure **II**); their carbonyl groups are found in the same or separate rings and conjugated with the double bond. Hence, the chemistry of quinones is, in many aspects, similar to that of α–β unsaturated ketones and a broad part of their redox features is based on the electrophilic reactivity determined by the carbonyl groups and the reaction of the polarized double bonds with nucleophiles. Quinones, occurring as benzo-, naphtho-, and anthraquinones, represent the largest class of quinoid compounds and are widely distributed in nature[2]: they are found as components of several functional biological systems, such as electron transport chains and protein carboxylation.[3,4] Metabolically significant quinones possess a terpenoid side chain (ubiquinone or coenzyme Q, plastoquinones, vitamin K, and tocopherylquinone groups) pointing to their location in the lipid phase of membranes. Quinones

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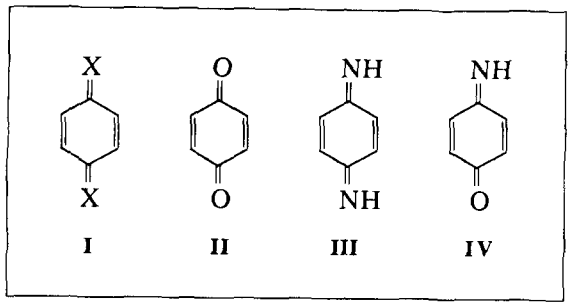


Scheme 1.

are also present as polyaromatic hydrocarbon derivatives in pollutants[5-7] and as active pharmacophores in antineoplastic drugs.[8,9]

Quinone imines possess the group HN=C—(C=C)$_n$—C=NH or HN=C—(C=C)$_n$—C=O (structures **III** and **IV**, respectively); the chemistry of quinone imines is in many aspects similar to quinones including their ability to undergo one- or two-electron reductions to a semiquinone-type free radical or to an diamino-benzene/aminophenol, respectively, as well as addition reactions with nucleophiles.[10] Slight differences between quinones and quinone imines in their ability to enter redox reactions are primarily determined by the electronegativity of the heteroatom, the —C=O function being more negative than the —C=NH function. At variance with quinones, quinone imines occur rarely in nature but are represented among synthetic antitumor agents[11] and as toxic drug metabolites.[12]

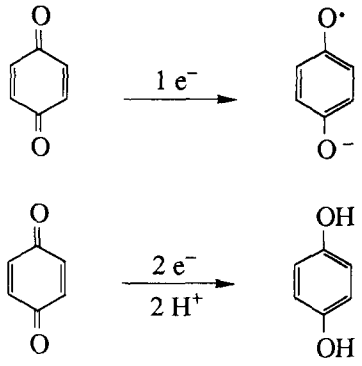
Given the widespread distribution of quinones, the redox features of these compounds seem essential to the understanding of their overall biological activity—encompassing functional, toxicological, mutagenic, and antitumor actions. Different types of redox transitions involving quinones are surveyed here. These include: a) reactions involving only transfer of electrons, such as those comprised in the enzymic reduction of quinones or in the several non-enzymic sources of semiquinones. b) Nucleophilic addition reactions implying either oxidation or reduction of the quinoid ring, the former exemplified in the oxidation of the —C_2=C_3— bond to quinone epoxides by O_2 nucleophiles and the latter in the reductive addition by sulfur nucleophiles to yield thioether-hydroquinone adducts. Further, the electrophilic character of quinones is the basis, in proper instances, for their function as bioreductive alkylating agents with potential antitumor activities. The adduct resulting from the reaction of quinones with nucleophiles is not redox inert but possesses redox properties different from the unconjugated quinone and is capable of undergoing new redox transitions in terms of cross-oxidation, autoxidation, disproportionation, free radical interactions, and enzymic reduction. This subsequent chemistry provides a dynamic aspect to the nucleophilic additions and is likely to contribute in large extent to the overall biological activity of these compounds.

ELECTRON TRANSFER TO QUINONES

Enzymatic reduction of quinoid compounds

Quinones could be one- or two-electron acceptors and their cellular reduction proceeds *via* a one- or two-electron transfer catalyzed by a variety of flavoproteins. NAD(P)H is a two-electron donor and transfer of a hydride equivalent from the reduced pyridine nucleotide to a flavin molecule is a generally accepted mechanism [NADPH + Fp → NADP$^+$ + FpH$^-$].[13] The two-electron reduced flavoenzyme [FPH$^-$ + H$^+$ ⇔ FPH$_2$] can catalyze the reduction of one- or two-electron acceptors. In the former instance, the semiquinone state of the flavin is reported an obligatory intermediate in the step-down reaction from a two-electron donor (FPH$_2$) to a one-electron acceptor [FPH$_2$ + A → FPH· + AH·]. In the latter instance, either a one- or two-electron transfer reaction can take place, though it is likely that the formation of the two-electron reduced acceptor occurs through two one-electron transfer steps [FPH$_2$ + A → FPH· + AH· followed by FPH· + AH· → FP + AH$_2$].

By relating the rate of free radical formation over the rate of enzyme reaction (parameter k), the enzymic reduction of quinones can be classified as a one-electron transfer mechanism (k = 2) (reaction *1*), a two-electron transfer mechanism (k = 0) (reaction *2*), and a mixed-type mechanism (k = 0–2).[14]



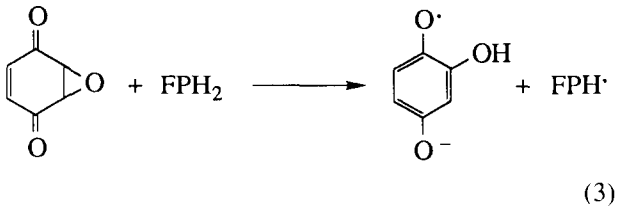
Reaction 1 implies that the intermediate releases only free radicals; the semiquinone free radical species is not immobilized in the active site and is detached from the enzyme without further transfer of an electron. NADH-cytochrome b_5 reductase, NADPH-cytochrome P450 reductase, and chloroplast ferredoxin-NADP$^+$ reductase, all catalyze a typical one-electron reduction of quinones.[13]

Reaction 2 is considered a two-electron transfer

process regardless of the life-time of the potential one-electron transfer intermediate. DT-diaphorase (NAD(P)H:quinone reductase)[15] catalyzes the two-electron reduction of quinones; however, the redox properties of DT-diaphorase have not been studied in detail. A possibility remains that the popular substrate for the enzyme, menadione, is firstly reduced to its free radical form, but may not be easily removed from the active site (probably because of the formation of a rigid complex between the enzyme and menadione)[16] and will be further reduced to its hydroquinone form by a second one-electron transfer step. Another explanation would take into account a more negative reduction potential of the FAD/FAD· couple than that of the FAD·/$FADH_2$ couple; a hydride transfer may be, therefore, a favourable mechanism in the reduction of menadione to menadiol.[17] Among the quinones, those without a side chain are the most active electron acceptors and the activity decreases with increasing length of the side chain. Benzo[a]pyrene quinones are also electron acceptors for DT-diaphorase in the course of their conjugation with glucuronide.[18] Besides quinones, nitro compounds, azodyes, and hexavalent chromium complexes have been shown to serve as electron acceptors for the enzyme.[15]

Quinone epoxides, which can be formed during the HOO^- addition to the 2,3-double bond of quinones[19,20] (see below), are reduced *via* one- and two-electrons by NADPH-cytochrome P450 reductase and DT-diaphorase, respectively.[21] The former (reaction 3) yields primarily an α-hydroxy-semiquinone, whereas the latter (reaction 4) an α-hydroxyhydroquinone. Both mechanisms imply initial reduction of the quinone followed by rearomatization and subsequent epoxide ring opening.

α-Hydroxy-semiquinones, formed during the one-electron reduction of 2,3-epoxy-*p*-benzoquinone by NADPH-cytochrome P450 reductase (reaction 3), disproportionate to 2-hydroxy-*p*-benzoquinone and 2-hydroxy-*p*-benzohydroquinone under anaerobic conditions. The autoxidation of the hydroxy-semiquinone prevails over the disproportionation reaction under aerobiosis. At variance with DT-diaphorase (see below), the product of disproportionation or autoxidation, hydroxy-*p*-benzoquinone, cannot be reduced further by NADPH-cytochrome P450 reductase.[21] This is unlikely prevented by the one-electron reduction potential of hydroxy-*p*-benzoquinone, for it could be expected to be less negative than the potential corresponding to the irreversible one-electron reduction of the quinone epoxide.



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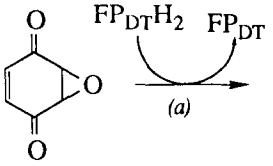
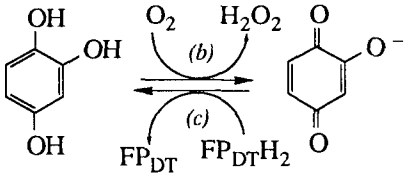




(4)

Hydroxy-*p*-benzohydroquinone, formed upon the two-electron reduction of 2,3-epoxy-*p*-benzoquinone by DT-diaphorase (reaction 4*a*), autoxidizes rapidly (reaction 4*b*) and the autoxidation product, hydroxy-*p*-benzoquinone, is reduced by the enzyme more efficiently than the quinone epoxide (reaction 4*c*) ($k_{4c} >> k_{4a}$). The reduction of the autoxidation product by DT-diaphorase closes an *autoxidation* ⇔ *enzymic reduction* cycle, which accounts for the NADPH consumed in excess over the initial amount of quinone epoxide, as well as O_2 consumption and H_2O_2 formation. Reaction 4*b* is written as a two-electron transfer to O_2, though it is likely to occur in two one-electron transfer steps.

DT-diaphorase also catalyzes the reduction of mono- and dimethyl-substituted *p*-benzoquinone- and 1,4-naphthoquinone epoxides.[20] The very negative reduction potential of quinone epoxides ($E_{1/2}$ values against a Ag/AgCl reference electrode range between −510 and −820 mV for various *p*-benzoquinone- and 1,4-naphthoquinone epoxides[20]) is likely to decrease the rate of their reduction by DT-diaphorase. Because these reduction potentials probably represent a threshold for electron transfer from the flavoprotein, it is difficult to draw a correlation between the reduction potential of the different quinone epoxides and their rate of reduction by DT-diaphorase, as suggested for the one-electron reduction of simple quinones by cytochrome P450- and cytochrome b_5 reductases.[22] Methyl-substituted quinone epoxides are reduced at slower rates than the unsubstituted *p*-benzoquinone epoxide. The decreased rate of enzymic reduction cannot be strictly ascribed to a single physico-chemical property of the quinone epoxides, but it seems to be influenced by both the reduction potential of the quinoid compound (in aqueous solution) and the degree of —CH_3 substitution. The product resulting from the enzymic reduction is in every case an α-hydroxy-hydroquinone, which enters an *autoxidation* ⇔ *enzymic reduction* cycle similar to that in reaction 4*b*,*c*. An

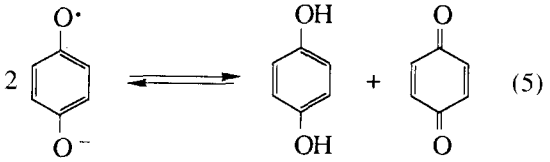
exception is constituted by 2,3-dimethyl-2,3-epoxy-1,4-naphthoquinone, which forms upon enzymic reduction a compound lacking quinoid properties.[20]

Non-quinoid, aliphatic- and aromatic epoxides, as *trans*-stilbene epoxide and 4,5-epoxy-benzo[a]pyrene, respectively, are not substrates for DT-diaphorase, thus suggesting that only quinone epoxides are reduced by the enzyme and that it catalyzes a two-electron transfer to the quinone, thereby regaining aromaticity and leading to epoxide ring opening.[21]

The relative participation of the two key enzymes, DT-diaphorase (two-electron transfer flavoprotein) and NADPH-cytochrome P450 reductase (one-electron transfer flavoprotein), in quinone reduction brings forward an as yet unresolved controversy on their role in the mechanism of action of cytotoxic quinones[23] (see below).

Non-enzymic generation of semiquinones

In addition to the one-electron enzymic reduction of quinones (reaction 1), semiquinones can be generated non-enzymically by: a) *comproportionation reactions* (reaction 5);



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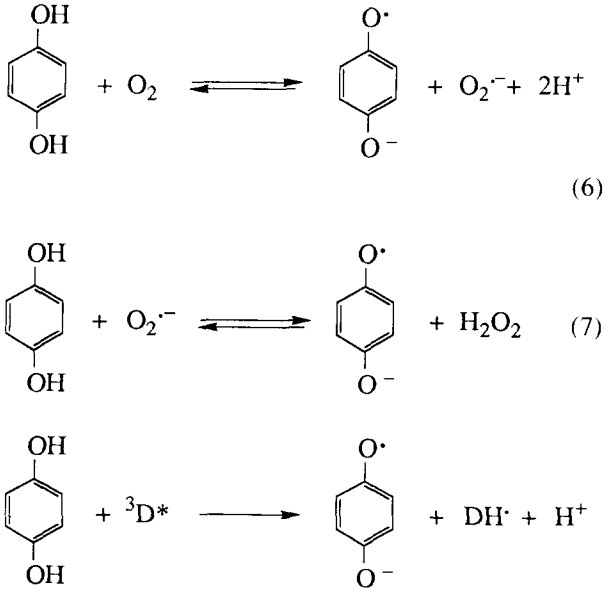
b) *oxidation of hydroquinones* by O_2 (reaction 6), $O_2^{\cdot-}$ (reaction 7), and excited molecules (reaction 8);



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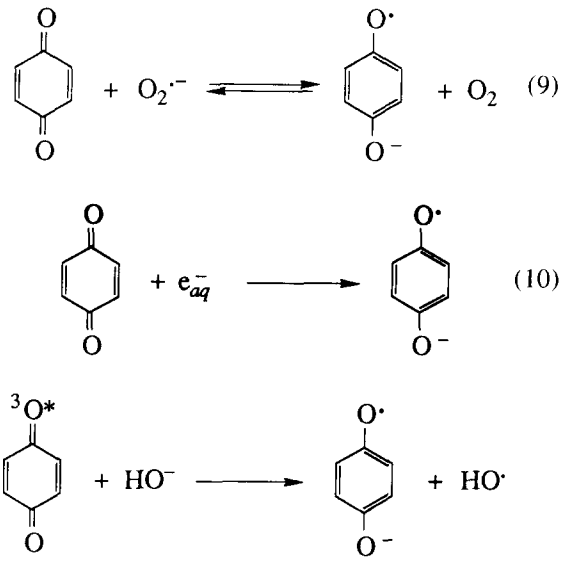
and c) *reduction of quinones* by $O_2^{\cdot-}$ (reaction 9), radiolysis (reaction 10), and reaction of a triplet quinone with suitable electron donors (reaction 11).[24-27]



(9)

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(11)

The general chemical structure of semiquinones shown above is conventionally used, though the radical character and the negative charge in the semiquinones are in reality delocalized to the ring carbon atoms and to the other oxygen atom.

The reaction of $O_2^{\cdot-}$ with *p*-benzohydroquinone (reaction 7) and *p*-benzoquinone (reaction 9) gives the same ESR spectra, suggesting the formation of the same semiquinone species and that $O_2^{\cdot-}$ can act both as an oxidizing and reducing agent depending on the reduction potentials of the substrates.[28] In addition to *p*-benzoquinone, $O_2^{\cdot-}$ reduces duroquinone, vitamin E quinone, 1,4-naphthoquinone, and 2-methyl-1,4-naphthoquinone to yield the corresponding semiquinone radicals. The fact that vitamin E quinone, an irreversible metabolite of vitamin E, is reduced by $O_2^{\cdot-}$ to the semiquinone radical suggests that, like vitamin E, vitamin E quinone may also scavenge $O_2^{\cdot-}$ and protect living cells from the effects of $O_2^{\cdot-}$ in a hydrophobic environment.[28] Although vitamin E was proposed to be oxidized by $O_2^{\cdot-}$ to the corresponding chromanoxyl radicals,[29] other reports indicated that this reaction does not proceed as such.[30,31] However, Trolox radical (α-T·), generated by pulse radiolysis, is efficiently reduced by $O_2^{\cdot-}$ (α-T· + $O_2^{\cdot-}$ + H^+ → α-TH + O_2)[32] with a second order rate constant of $2 \times 10^8\ M^{-1}s^{-1}$, a reaction proceeding at similar rates as the recovery of vitamin E radical by ascorbic acid ($0.4 \times 10^8\ M^{-1}s^{-1}$).[33]

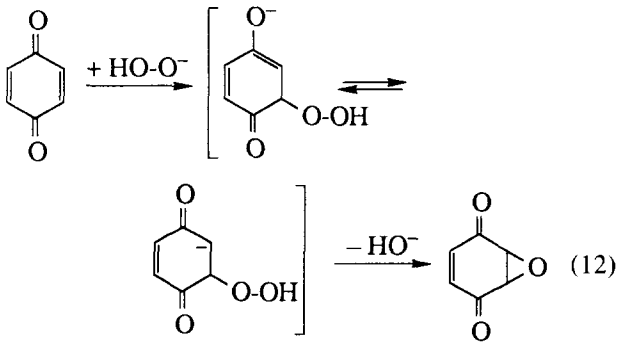
ADDITION OF NUCLEOPHILES TO QUINOID COMPOUNDS

Quinoid compounds provide a structure where the —C=C— bond is conjugated with a group of —M type. This leads to stabilization of the anion formed upon addition and then, the transition state by delocalization of the charge on to the electronegative element. The vast majority of the addition reactions of quinones can be characterized as a 1,4-reductive addition of the Michael type.

Two main types of nucleophilic additions to quinones are covered here, involving either oxidation or reduction of the quinoid ring. The former process is exemplified by the addition of O_2 nucleophiles to the $—C_2=C_3—$ bond of the quinoid ring and formation of a quinone epoxide product. The latter process is illustrated mainly in terms of addition of sulfur nucleophiles with formation of a thioether derivative. The subsequent chemistry of these two primary products is discussed.

Oxygen nucleophiles

H_2O_2-mediated oxidation of quinones: formation of quinone epoxides. The formation of quinone epoxides is attained in the addition of O_2 nucleophiles to *p*-benzoquinones and 1,4-naphthoquinones,[34] as implied in the H_2O_2-dependent oxidation of the 2,3-double bond,[19,20] which is analogue to the reaction of sodium hydroperoxide with naphthoquinones[35] (reaction 12). The reaction is suggested to occur through a nucleophilic addition of HOO^- at the β-carbon of the unsaturated system to give an enolate ion as an intermediate. The carbanionic α-carbon atom on the enolate ions displaces HO^-, breaking the O—O bond in the hydroperoxy group and re-arranging to an epoxide.



(12)

These epoxidations have been shown to take place in neutral- or slightly-alkaline buffered aqueous solutions for both *p*-benzo- and 1,4-naphthoquinones and are examples of the vulnerability of the —C=C— bond conjugated to a carbonyl group. Quinone epoxides are more stable than the parent quinoid compounds. Among quinone epoxides, *p*-benzoquinone epoxide is the least stable, because it undergoes a slow decomposition by H_2O addition reactions leading to epoxide ring opening and formation of α-hydroxyquinones (see below). Quinone epoxides also result from the reaction of $O_2^{\cdot-}$ with several vitamin K analogues.[36]

The epoxidation of *p*-benzo- and 1,4-naphthoquinones is accompanied by changes in their physicochemical properties in terms of changes in electrophilicity, polarity, and reduction potential. The more hydrophilic quinone epoxide is a compound with a weaker electrophilic character than the parent quinone. The half-wave potential ($E_{1/2}$) values for quinone epoxide reduction are about 500 mV more negative than those for the parent quinone compounds lacking the epoxide ring. Because quinone epoxides retain the redox properties of the quinones, they can be analyzed and identified by HPLC with electrochemical detection.[19,20] The reduction of a quinone epoxide is an irreversible event, which precludes any equilibrium with other redox couples and, consequently, the determination of the one-electron reduction potential by pulse radiolysis approaches.

The same mechanism as that illustrated in reaction 12 applies to the *p*-benzo- and 1,4-naphthoquinone compounds with a different degree of $—CH_3$ substitution. The rate of epoxidation is highest for the unsubstituted *p*-benzo- and 1,4-naphthoquinone and it is inversely related to the number of $—CH_3$ substituents.[20] The restraining role of $—CH_3$ against quinone epoxidation may be explained in terms of changes of electron density in the quinone created by the inductive effect of the $—CH_3$ group.[20] This is expressed by the similar second order rate constants for epoxidation of *p*-benzo- and 1,4-naphthoquinone (0.26–0.28 $M^{-1}s^{-1}$), on the one hand, and 2,6-dimethyl-*p*-benzoquinone and 2-methyl-1,4-naphthoquinone (0.07–0.09 $M^{-1}s^{-1}$), on the other. The latter compounds offer a similar degree of 'resistance' to H_2O_2-mediated epoxidation, probably because of the similarities of both molecules in the position of the $—CH_3$ substituents. The disubstituted 2,3-dimethyl-1,4-naphthoquinone undergoes only negligible epoxidation by H_2O_2, thus indicating the restraining role of $—CH_3$ substituents against nucleophilic attack.

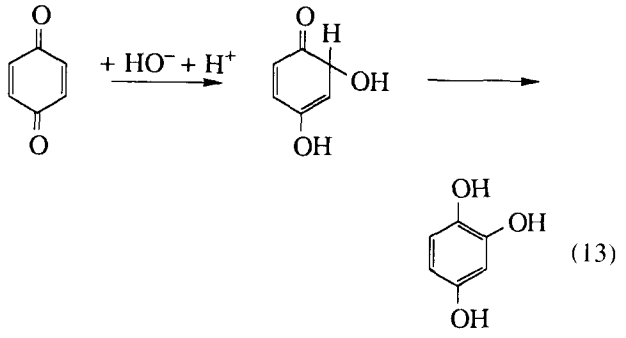
2-Methyl-*p*-benzoquinone shows an intermediate second-order rate constant of epoxidation between the unsubstituted *p*-benzoquinone and the disubstituted *p*-benzoquinone. This may be due to the occurrence of an unsubstituted —C=C— structure in the quinone, where epoxidation could occur unrestricted. Although 2-methyl-5,6-epoxy-*p*-benzoquinone or 2-methyl-2,3-epoxy-*p*-benzoquinone adducts are possible, a prev-

alance of the former is expected according to the electron-donating properties of the —CH_3 substituent (see below).

In summary, the rate of H_2O_2-mediated epoxidation of quinones decreases with the degree of —CH_3 substitution and appears not to correlate strictly with the reduction potential of the quinone,[20] but to be influenced by the inductive effects of the substituents in the electron density of the quinone.

H_2O addition to quinones: formation of α-hydroxy-quinones. *p*-Benzoquinones and 1,2-naphthoquinones are highly unstable towards H_2O, whereas 1,4-naphthoquinones and 9,10-anthraquinones show a lower reactivity towards H_2O, as predicted by perturbed molecular orbital theory studies.[37] H_2O addition reactions to biologically active quinones is expected to be of little significance, for substituents hinder markedly this type of nucleophilic addition.

The H_2O addition to *p*-benzoquinones and 1,2-naphthoquinones results in α-hydroxy-quinone compound formation.[38,39] For the case of *p*-benzoquinones, the mechanism involves a HO^- addition yielding 2-hydroxy-*p*-benzohydroquinone (reaction 13). This product has a very negative reduction potential and participates in cross-oxidation (reaction 14) and autoxidation (reaction 15), the predominance of either reaction being determined by the relative concentration of oxidants, that is, *p*-benzoquinone and O_2. The disproportionation of hydroxy-semiquinone intermediates



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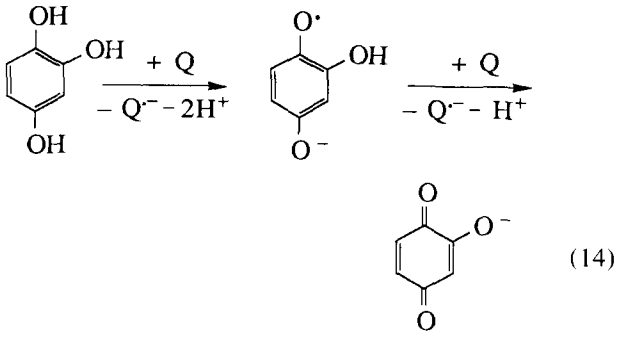

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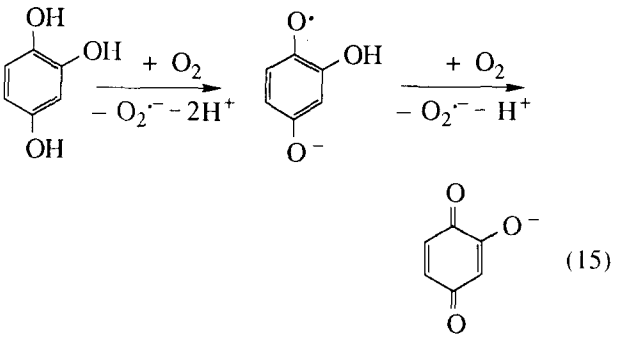

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contributes negligibly to the accumulation of hydroxy-*p*-benzoquinone.

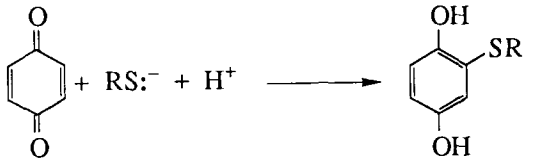
H_2O_2 enhances substantially the spontaneous formation of hydroxy-*p*-benzoquinone in aqueous solutions by mechanisms involving either an oxidation of hydroxy-*p*-benzosemiquinone within the frame of an organic Fenton reaction or a HOO^- addition followed by oxidative elimination of HO^-. Support for an organic Fenton reaction (see *Electronically-Excited Quinoid Compounds* section) could be found in the parallel accumulation of *p*-benzohydroquinone to the half-value of the hydroxy derivative.[19]

α-Hydroxy-quinones have a distinctive chemistry in terms of acid-basic properties (the —OH groups tend to ionize), strong intramolecular hydrogen bonding (with subsequent stabilization of the semiquinone species), and strong influence on reduction potential (the half-wave potential value in aqueous solutions are much more negative than those of the parent quinones). These concepts are discussed below in terms of autoxidation properties of reduced α-hydroxy-quinone species.

α-Hydroxy-*p*-benzoquinone absorbs selectively at λ_{484nm}, probably due to the bathochromic effect of the —OH substituent.[38,39] Methyl substitution of α-hydroxy-quinones exerts minor changes in the UV-absorption band but more specific shifts, generally towards longer wavelengths of the absorption at λ_{484nm}. Hydroxy-semiquinone transient species, obtained by step-wise oxidation of trihydroxybenzene, absorb selectively at λ_{360nm}: pronounced shifts towards longer wavelengths (up to 448 nm) are produced by different degree of —CH_3 substitution.[40] Electrochemical studies of α-hydroxy-*p*-benzoquinones in aqueous solutions indicate that the half-wave potential values of these compounds are considerably more negative (200–300 mV) than those of the parent quinones.[20,41] Methyl substitution of α-hydroxyquinones lowers further the $E_{1/2}$ values. The —OH substituent, with electron-donating properties, determines a reaction center for electron transfer distinct to the carbonyl group, specifically at C_5 in position *para* to the —OH group in C_2 (see below).[41]

Sulfur nucleophiles

The addition of sulfur nucleophiles to quinones has been comprehensively reviewed[34] and it can be characterized as a 1,4-reductive addition of the Michael type (reaction 16). Quinones, containing a polarized double bond, are considered Michael acceptors, whereas the thiol nucleophiles are Michael donors.



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The product orientation in the addition of sulfur nucleophiles to quinones is determined by the substituents and the occurrence of the new sulfur substituent controls in large extent the subsequent chemistry. Among other reactions, the resulting hydroquinone-thioether derivative can undergo autoxidation and cross-oxidation (see below). The ratio [sulfur nucleophile]/[quinone] is important for those quinones which possess a second site for nucleophilic addition and, in general, the main product is the result of two additions, each followed by autoxidation.

p-Benzoquinones and 1,4-*naphthoquinones.* The substituents in *p*-benzo- and 1,4-naphthoquinones determine, on the one hand, the rate of the reaction as well as the product orientation in the thiol addition and, on the other, the following reactions that the hydroquinone-thioether primary product can undergo: cross-oxidation, autoxidation, diconjugation, and free radical reactions.

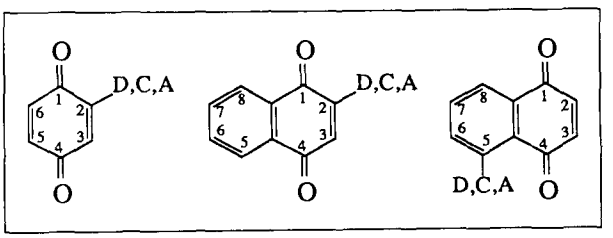
Calculation of the free energy of the lowest unoccupied molecular orbital can be used in the context of frontier molecular orbital theory to explain the orientation of the nucleophilic adduct. Certain generalizations on the site of nucleophilic attack on donor-substituted, acceptor-substituted, and conjugatively-substituted quinone species have been made. The preferred site of attack by nucleophiles is the position having the largest lowest unoccupied molecular orbital coefficient, unless the donor is attached to that position.[42]

p-Benzoquinones. In the *p*-benzoquinone series, the electron-donating properties of substituents such as $-CH_3$, —OH, and $-OCH_3$, selectively diminish the electron-withdrawing ability of one of the quinone carbonyls so that attack of a nucleophile occurs β to the other, more electron-deficient carbonyl group. Electron-withdrawing substituents such as formyl, on the other hand, activate the β-position by resonance. Thus, *p*-benzoquinones substituted with an electron-donating group at C_2 (see Scheme 2)[42] create following order of reactivity towards nucleophiles: $C_5 > C_6 > C_3$. A similar preferential order of attack is expected from conjugatively-substituted *p*-benzoquinones, whereas the order $C_3 >> C_6 > C_5$ is proposed for acceptor-substituted *p*-benzoquinones. For 2-methoxy-*p*-benzoquinone, the preferred site for nucleophilic addition is C_5, whereas for alkyl-substituted *p*-benzoquinone, C_5 and C_6 adducts are expected, though with predominance of the former.

The rate of GSH addition to unsubstituted *p*-benzoquinone ($2 \times 10^6\ M^{-1}s^{-1}$) is decreased by $-CH_3$ substitution: methyl-*p*-benzoquinone ($1.9 \times 10^5\ M^{-1}s^{-1}$) > 2,6-dimethyl-*p*-benzoquinone ($1.7 \times 10^3\ M^{-1}s^{-1}$) ≥ 2,5-dimethyl-*p*-benzoquinone ($6.7 \times 10^2\ M^{-1}s^{-1}$) > 2,3,5-trimethyl-*p*-benzoquinone ($1.6 \times 10^2\ M^{-1}s^{-1}$) > tetramethyl-*p*-benzoquinone (not detectable).[43] The introduction of an electron-withdrawing substituent such as —Br at C_2 increases the rate of GSH addition ($2.4 \times 10^6\ M^{-1}s^{-1}$) as compared to the unsubstituted quinoid compound.[43] The glutathionyl (—SG) substituent following the GSH addition to methyl- and 2,6-dimethyl-*p*-benzoquinone is supposed to be located at C_5, where the lower electron density makes it the most likely site for electron transfer.[41,42] Similarly, the addition of 1-phenyl-5-mercaptotetrazole (HPMT) to 2-methyl-*p*-benzoquinone results in two-fold higher yield of *para*-mercapto adduct than *meta*-mercapto adduct [(C_2 = CH_3; C_5 = PMT) > (C_2 = CH_3; C_6 = PMT)]; no *ortho*-mercapto adduct is formed.[44]

Glutathionyl-*p*-benzohydroquinone shows a specific absorption at $\lambda_{303\,nm}$ (ϵ = 3.6 $mM^{-1}s^{-1}$)[43,45] indicating a 15-nm shift from *p*-benzohydroquinone caused by the —SG substituent; its autoxidation product shows maximal absorption at $\lambda_{367\,nm}$ (ref. 45). Methyl substitution of glutathionyl-*p*-benzoquinone produces only a few nm shift in the absorption wavelength ($\lambda_{298\,nm}$) relative to the parent compound.

1,4-*Naphthoquinones.* The chemistry of GSH addition to 1,4-naphthoquinones is somehow simplified

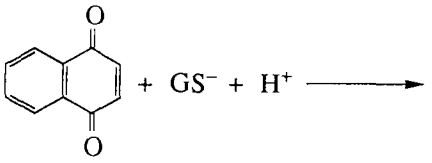


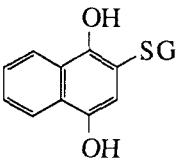
Scheme 2.

over that to *p*-benzoquinones because the adjacent benzene ring in the former limits the nucleophilic addition to one side of the quinoid ring.

In the 1,4-naphthoquinone series, substituents in the quinoid- or the benzenoid ring have different effects on the site of nucleophilic attack. A substituent in the quinoid ring of 1,4-naphthoquinone has a similar effect as that substituent has on the *p*-benzoquinone double bond to which it is directly attached and attack is expected to be limited to the C_3 position. Benzene-ring, donor-substituted 1,4-naphthoquinones at either C_5 or C_6 determine C_3 and C_2 as most preferable sites for nucleophilic attack, respectively, with acceptor-substituted compounds at those positions having opposite effects[42] (Scheme 2).

The addition of sulfhydryl derivatives, among them GSH, to naphthoquinone (reaction 17) and 2-methyl-1,4-naphthoquinone (menadione) proceeds rapidly yielding as primary product the corresponding monothioether adduct.[46-49] Only one orientation of the adduct is possible for the addition of GSH to menadione: 2-methyl-3-glutahionyl-1,4-naphthohydroquinone (k = 28 $M^{-1}s^{-1}$).[49] However, the rate of GSH addition to alkyl-substituted 1,4-naphthoquinones is for benzene ring-substituted naphthoquinones about 100-fold higher than for quinoid ring-substituted naphthoquinones.[49]

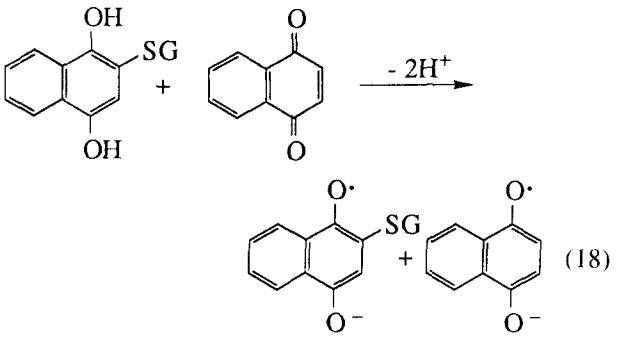




(17)

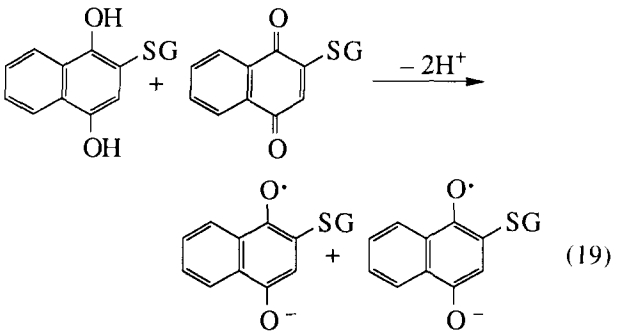
Glutathionyl-1,4-naphthohydroquinone reveals absorption maximum at λ_{343nm} (the —SG substituent shifting 23 nm the absorption of the parent reduced quinone towards longer wavelengths) and its autoxidation product shows a distinct absorption in the visible region (λ_{414nm}).

The GSH addition to 1,4-naphthoquinone and menadione under anaerobic conditions is linked to the formation of a glutathionyl-semiquinone transient species, which can be accounted for in terms of a one-electron oxidation of the glutathionyl-hydroquinone by either cross-oxidation with unreacted quinone (reaction 18)



(18)

or comproportionation (reaction 19).[50]

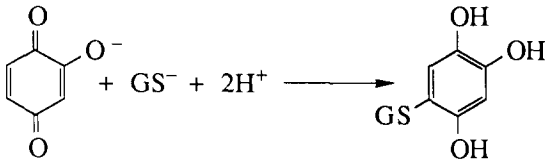


(19)

The supplementation of an aqueous solution of *p*-benzoquinone with N-acetyl-L-cysteine generated a similar ESR signal.[50] The center of the ESR spectrum of the GSH adduct of naphthosemiquinone species is located at a lower magnetic field than that of the semiquinone lacking the —SG substituent by 0.08 G, indicating a larger g value due to spin-orbit coupling of the sulfur atom.[51]

α-Hydroxy-quinones. α-Hydroxyquinones can function as proton donors themselves, and if the —OH substituent is α to the carbonyl group, intramolecular hydrogen-bond formation can stabilize the intermediate semiquinone species.[52,53]

The —OH substituent has different effects on the nucleophilic addition of GSH depending on its location in the quinone (see scheme 2). Because of its electron-donating properties, the —OH group prevents addition on the vicinal carbon. This effect is most evident for 2-hydroxy-1,4-naphthoquinone (lawsone), which does not react with GSH. A —OH substituent in *p*-benzoquinone (reaction 20) or in the adjacent benzene ring of 1,4-naphthoquinone (e.g., 5-hydroxy-1,4-naphthoquinone or juglone) does not prevent nucleophilic addition of GSH. As a matter of fact, the GSH addition to both compounds proceeds at rather high rates,[45,54] and in every case the primary product is a hydroxy-glutathionyl-hydroquinone with particular chemical properties.

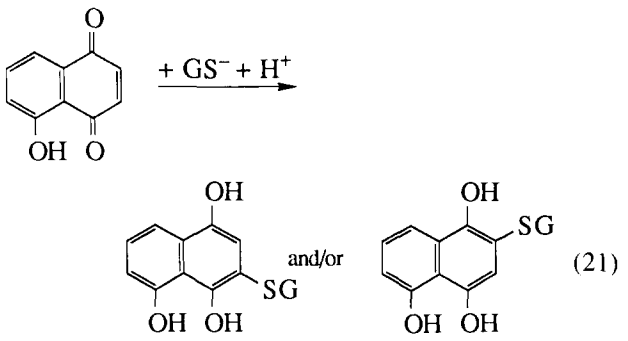


(20)

The —OH substituent affects deeply the resonance structure of the quinoid compound and as a primary effect both carbonyl groups cannot longer be regarded as equivalent reaction centers for electron transfer. Consequently, and for the case of hydroxy-*p*-benzoquinone, the carbon in position *para* to the —OH substituent is endowed with the lowest electron density and turns into a new reaction center for electron transfer.[41] Therefore, the GSH addition to hydroxy-*p*-benzoquinone is expected to yield mainly a 2-hydroxy-5-glutathionyl-*p*-benzohydroquinone primary product (reaction 20). Similar considerations applied to the product orientation in the 1-phenyl-5-mercapto-tetrazole addition to 2-methoxy-*p*-benzoquinone, which yields exclusively a 5-thio-substituted 2-methoxy-*p*-benzohydroquinone.[44] Hydroxy-glutathionyl-*p*-benzohydroquinone absorbs maximally at λ_{300nm}, the —SG substituent producing a 12-nm shift towards longer wavelengths of the absorption maximum of trihydroxybenzene (λ_{288nm}).[45] The autoxidation product of the conjugate absorbs maximally at λ_{343nm} and it also exhibits a resolved absorption at around λ_{520nm} (ref. 45).

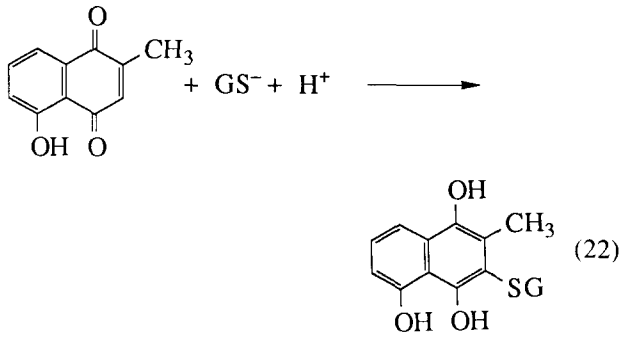
Because of the electron-donating properties of the —OH substituent at C_5 of 5-hydroxy-1,4-naphthoquinone (juglone), it could be expected that the preferred site for nucleophilic attack would be C_2 and most experimental evidence indicate adduct formation at C_2.[42] However, the product orientation in the addition of sulfur nucleophiles to juglone is a controversial topic, inasmuch as it is dependent on the type of nucleophile, which subsequently determines the operativity of either an ionic- or free radical mechanism.[55,56] The product orientation in the sulfur addition to juglone has not been identified and a predominance of a thioether adduct at C_3 has been proposed (reaction 21).[55]

HPLC with oxidative electrochemical detection reveals the formation of a thioether derivative following the addition of GSH in equimolar amounts to 5-hydroxy-1,4-naphthoquinone. Although the —OH substituent in the benzene ring raises the $E_{1/2}$ value of 1,4-naphthoquinone by about 40 mV (−140 and −180 mV against Ag/AgCl reference electrode, respectively), the subsequent —SG substitution lowers the $E_{1/2}$ of 5-hydroxy-1,4-naphthoquinone to −195 mV.[54]



(21)

The adduct resulting from the GSH addition to 2-methyl-5-hydroxy-1,4-naphthoquinone (plumbagin) (reaction 22) possesses a $E_{1/2}$ value of −220 mV, 20 mV more negative than the unconjugated hydroxy-naphthoquinone.[54]



(22)

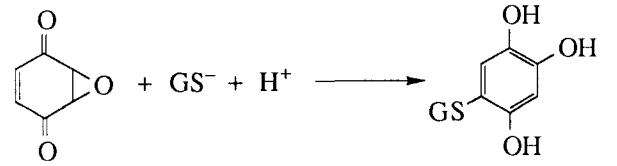
Like the addition of GSH to 1,4-naphthoquinone or menadione, that to 5-hydroxy-1,4-naphthoquinone also yields an ESR signal with a g value of 2,0047 corresponding to the radical anion of glutathionyl-5-hydroxy-1,4-naphthosemiquinone.[50] The spectrum is similar to that observed for the 5-hydroxy-1,4-naphthosemiquinone radical in aqueous solution.[57] The generation of the glutathionyl-5-hydroxy-naphthosemiquinone under anaerobiosis could be accounted for in a manner similar to reactions 18 and 19. It is expected that the hydroxy-semiquinone radical would be a long-lived species in anaerobic conditions due to the stabilization of the radical intermediate by intramolecular hydrogen bonding, which displaces significantly over to the left the equilibrium of the disproportionation reaction[57]: $2\ Q^{\cdot}—OH \Leftrightarrow H_2Q—OH + Q—O^{-}$.

In summary, the electron-donating properties of $—CH_3$ and —OH substituents, respectively, determine the position *para* of benzoquinones as the reaction center for nucleophillic addition. The $—CH_3$ substituents decrease the rate of GSH addition to *p*-benzoquinone, whereas the —Br substituent enhances it slightly. Although the position for nucleophilic addition in 1,4-naphthoquinones is restricted to C_2 and/or C_3 in the quinoid ring, the rate of addition changes dramatically

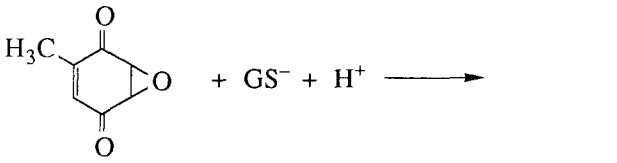
depending on whether the substituent is in the benzene- or the quinoid ring, the former proceeding at faster rates than the latter.[49]

Quinone epoxides

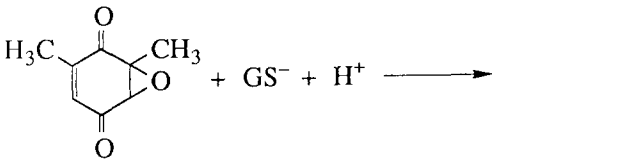
p-Benzoquinone epoxides. The reductive addition of GSH to *p*-benzoquinone epoxides leads to epoxide ring opening, followed by re-aromatization, and primary formation of a hydroxy-glutathionyl-hydroquinone derivative (reaction 23*a–c*).



(23a)

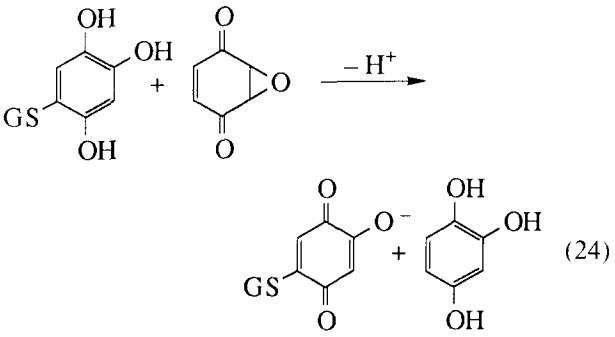


(23b)



(23c)

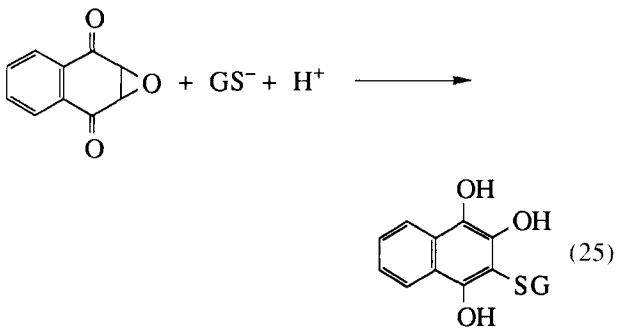
Addition of GSH to unsubstituted *p*-benzoquinone epoxide proceeds at a slower rate than that to *p*-benzoquinones or hydroxy-*p*-benzoquinones. Nucleophilic addition occurs at the site opposite to that bearing the epoxide ring as inferred by the identical absorption spectral properties of this product to that resulting from the nucleophilic addition to hydroxy-*p*-benzoquinone.[45] A common molecular product is expected from the GSH addition to hydroxy-*p*-benzoquinone (reaction 20) and 2,3-epoxy-*p*-benzoquinone (reaction 23a). Autoxidation of the conjugate is accompanied by a shift in absorption towards longer wavelengths (from $\lambda_{300\,nm}$ to λ_{343nm}). The oxidized product, hydroxy-glutathionyl-*p*-benzoquinone, also accumulates under anaerobic conditions, thus indicating the operativity of a cross-oxidation reaction with the unreacted quinone epoxide (reaction 24).[45]



(24)

Methyl substitution of *p*-benzoquinone epoxides decreases by 10–20-fold the rate of GSH addition and shifts the characteristic absorption of the conjugate towards longer wavelengths. The extensive shift observed upon *reductive addition* → *autoxidation* of 2,6-dimethyl-*p*-benzoquinone epoxide (λ_{372nm}) resembles the spectrum obtained with the parent *p*-benzoquinone lacking an epoxide ring. The formation of such a product during the reductive addition of GSH to dimethyl-*p*-benzoquinone epoxide could be understood in terms of an oxidative elimination of the —OH group following reaction 23c.

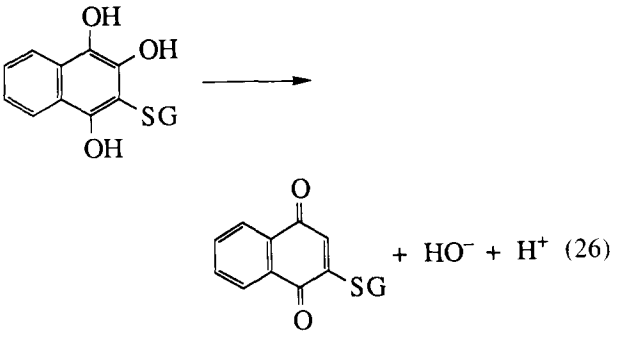
1,4-*Naphthoquinone epoxides.* At variance with *p*-benzoquinone epoxides, the addition of GSH to 1,4-naphthoquinone epoxide is restricted to the side bearing the epoxide group, hence yielding a molecular product with a —SG substituent vicinal to the —OH group, after epoxide ring opening (reaction 25).[58]



(25)

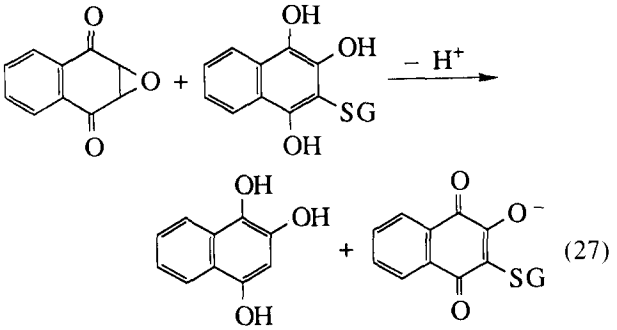
The following reactions are open to the primary product 2-hydroxy-3-glutathionyl-1,4-naphthohydroquinone[58];

a) oxidative elimination (reaction 26) yielding the 3-glutathionyl-1,4-naphthoquinone product;



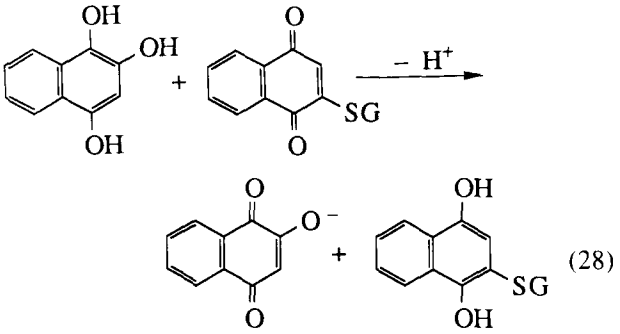
+ HO^- + H^+ (26)

b) cross oxidation with the unreacted quinone epoxide yielding 2-hydroxy-1,4-naphthohydroquinone (reaction 27);



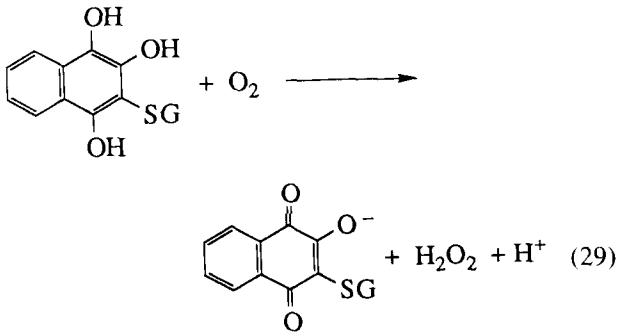
(27)

followed by its subsequent oxidation by 3-glutathionyl-1,4-naphthoquinone to yield lawsone (2-hydroxy-1,4-naphthoquinone) (reaction 28);



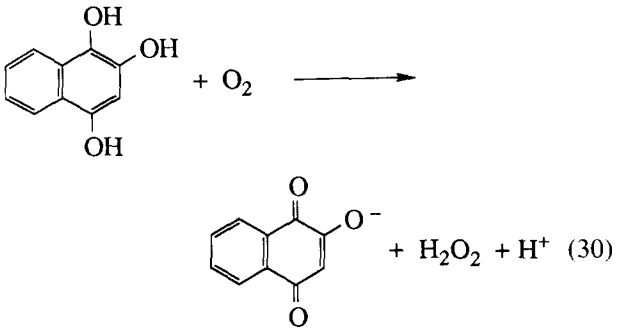
(28)

c) autoxidation of 2-hydroxy-3-glutathionyl-1,4-naphthoquinone (reaction 29), which competes with the cross oxidation reaction illustrated in reaction 27;



+ H_2O_2 + H^+ (29)

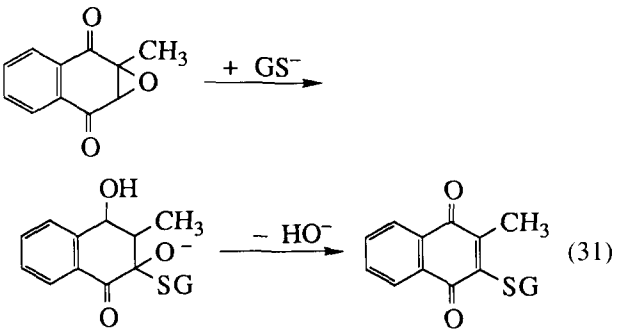
and d) autoxidation of 2-hydroxy-1,4-naphthohydroquinone (reaction 30), which competes with the oxidation reaction shown in reaction 28.



+ H_2O_2 + H^+ (30)

Reactions 26–30 are affected by the pO_2 of the reaction mixture. Thus, under anaerobic conditions, reactions 26–28 are operative, thereby contributing to the particular distribution of molecular products found: 43% 2-hydroxy-3-glutathionyl-1,4-naphthoquinone, 29% 2-hydroxy-1,4-naphthoquinone, and 29% 3-glutathionyl-1,4-naphthohydroquinone. Under aerobiosis, reactions 26–30 are operative to a different extent and affect the distribution of molecular products as follows: 51% 2-hydroxy-3-glutathionyl-1,4-naphthoquinone, 20% 2-hydroxy-1,4-naphthoquinone, and 22% 3-glutathionyl-1,4-naphthoquinone.[58]

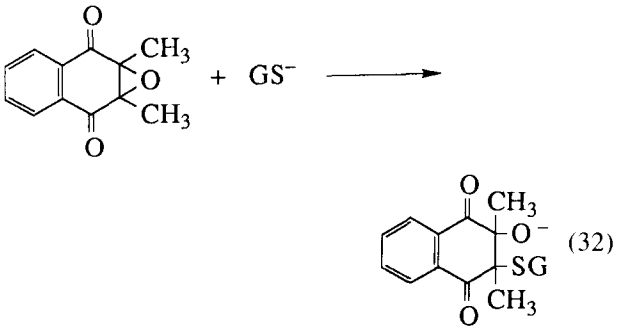
At variance with the GSH addition to the unsubstituted 1,4-naphthoquinone epoxide, that to menadione epoxide yields a single molecular product, 2-methyl-3-glutathionyl-1,4-naphthoquinone, which is formed by oxidative elimination (reaction 31) as suggested by similar product distribution under anaerobic and aerobic conditions. According to these observations, the GSH addition to either menadione or menadione epoxide yields the same end stable molecular product.[58]



(31)

2,3-Dimethyl-2,3-epoxy-1,4-naphthoquinone reacts with GSH at a rate far slower than that of other epoxides in the methyl-naphthoquinone series.[58] The product formed does not possess the redox properties required for its evaluation by HPLC with electro-chemical de-

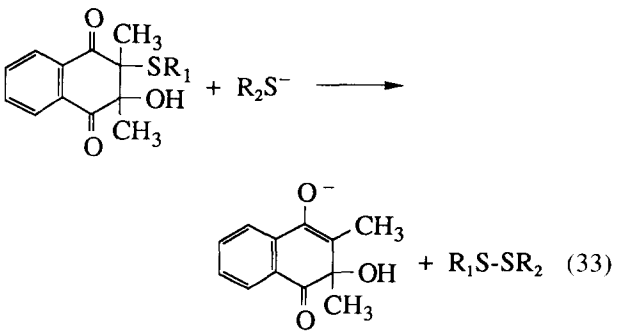
tection, most likely due to loss of its quinoid structure. This can be understood as a consequence of change in the electron configuration in the molecule produced upon GSH conjugation. To regain a quinoid structure two substituents on the quinoid ring would be required to leave, that is, hydroxyl-(HO^-) and glutathionyl anion (GS^-), which would be formally equivalent to a four-electron oxidation. The hypothetical molecule possesses no reducing equivalents and, thus, it will not undergo rearrangement into a quinoid structure. A speculative mechanism is described in reaction 32, where the product should be considered as an intermediate compound which may undergo further rearrangements involving scission between C_2 and C_3.



(32)

The epoxide ring opening following the nucleophilic addition of 1-phenyl-1H-tetrazole-5-thiol to 2,3-epoxy-1,4-naphthoquinones is not facilitated by the carbonyl groups and the presence of substituents in C_2 creates steric hindrance for the reaction with the nucleophile.[59]

By using 2,3-dimethyl-2,3-epoxy-1,4-naphthoquinone as a model for vitamin K_1, the molecular mechanism for vitamin K_1 epoxide reductase was suggested to proceed with thiol addition to open the epoxide ring (analog to reaction 32), yielding preferentially the 2-thio-3-hydroxy-adduct in the case of vitamin K_1 epoxide. Reaction with a second thiolate results in reductive cleavage of this adduct (reaction 33) and elimination of water to yield the quinones (reaction 34).[60,61]



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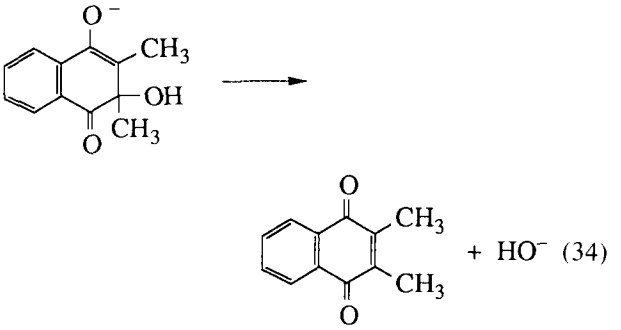
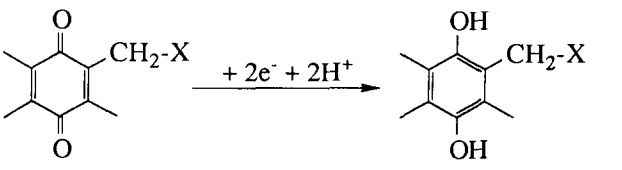


(34)

Bioreductive alkylation

The addition of nucleophiles to quinone methides, formed upon a reductive mechanism followed by oxidative elimination, is termed *bioreductive alkylation*.[62–64] Based on their activation mechanisms, the bioreductive alkylating agents may be divided into three groups: a) The simplest structure is a quinone with a $—CH_2-X$ substituent. The ease of quinone methide formation in this group would be expected to be a function of the electron density of the hydroquinone nucleus. That is, the higher the electron density, the higher the ease of —XH elimination. Thus, quinones in which the substituting groups are electron releasing, are expected to show higher biological activity, though they would be less easily reduced than those with electron-withdrawing substituents. Furthermore, naphthoquinones show higher activity than structurally analogous benzoquinones (which have higher reduction potential values). b) The second group consists of 1,2,4-trihydroxy-9,10-anthraquinones. These quinones undergo a deoxygenation at C_1 under reductive conditions, probably due to a tautomerization. c) The last group of quinones operate via a non-reductive process, where the quinone is in equilibrium with the quinone methide. The feasibility of such a reaction is determined by the occurrence of an acidic hydroxyl group appropriately located to function as an internal acid catalyst.[65]

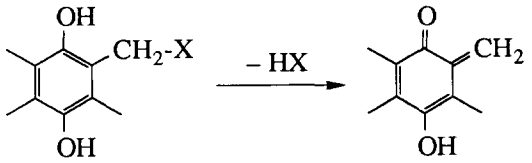
Reduction of quinones with leaving groups. The initial reduction of the quinone containing leaving substituents (reaction 35) is probably an enzymic event, whose efficiency will be determined primarily, but not uniquely, by the reduction potential of the quinoid compound.



(35)

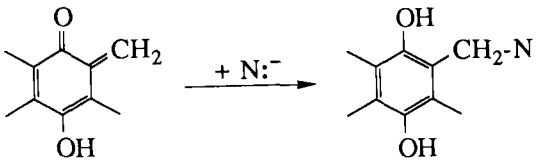
Methyl derivatives of 1,4-naphthoquinone bioreductive alkylating agents involve compounds possessing leaving groups in C_2 or C_6 such as —CH_2Br, —CH_2Cl, or —$CH_2OCONHCH_3$. The $E(Q/Q^{\cdot-})$ (calculated by pulse radiolysis)[66] of these quinones varies from -92 to -150 mV, being slightly more negative when the leaving group is at C_2. The semiquinone form of these derivatives decays by bimolecular collision (disproportionation rate 10^8 $M^{-1}s^{-1}$), without involving intramolecular oxidative elimination.[66] DT-diaphorase has been shown to activate the bioreductive agent 2-methyl-3-bromomethyl-1,4-naphthoquinone and dicoumarol inhibited its mutagenicity to *S. typhimurium* TA 97.[67] A reductive pathway, other than that mediated by enzymes, could involve a thiol-mediated reductive addition to the primary quinoid compound.[49] The reactivity of 2- and 6-methyl derivatives of 1,4-naphthoquinone bioreductive alkylating agents with thiols is substantially decreased when the leaving group is at C_2. The rates are also dependent on the nucleophile used and decrease from 1.6×10^4- to 1.6×10^2 M^{-1} (for the —CH_2Br-substituted quinone at C_6): cysteine > dithioerythritol >cysteamine > GSH.[49]

Oxidative elimination of the leaving group. The intramolecular oxidative elimination (reaction 36) is stereoelectronic controlled in a manner which decreases the activation energy of the reaction.[64] A two-electron reduction of the quinone is required in order to favour oxidative elimination; a pulse radiolysis study on several 1,4-naphthoquinone bioreductive alkylating agents indicated that the elimination observed is the result of the disproportionation of semiquinones, but it does not contribute per se to semiquinone decay.[66] The oxidative elimination of the leaving group proceeds under anaerobic conditions in a manner similar to what described for the HO^- elimination from 2-hydroxy-3-glutathionyl-1,4-naphthohydroquinone (reaction 26), following the reduction of the respective quinone epoxide (reaction 25).[58]



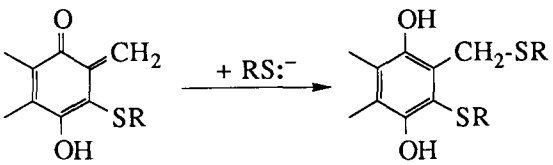
(36)

Reaction of quinone methides with nucleophiles. The quinone methide formed (reaction 35–36) by either mechanism invoked is of a rather modest bimolecular reactivity and, for some of them, the life time was calculated to be about 1 min, its decay taking place (in the absence of suitable nucleophiles) upon protonation.[64] The quinone methide possesses both nucleophilic and electrophilic character and this particular property is perhaps responsible for the low bimolecular reactivity of the quinoid molecule. The reactivity of various quinone methides towards thiols (reaction 37) seems to be higher than towards other types of cellular nucleophiles. Whichever nucleophile might react with quinone methides, their protonation seems to contribute significantly to their decay. If



(37)

reduction of 1,4-naphthoquinone bioreductive alkylating agents proceeded by means of a nucleophilic addition with thiols[49], it could be expected that the thioether of the quinone methide might undergo a second sulfur nucleophilic addition, resulting in diconjugate formation (reaction 38). In this regard, only one nucleophile, *N*-acetyl-cysteine, has been found to have bimolecular reactivity towards daunomycin[64].



(38)

AUTOXIDATION OF QUINOLS: ELECTRON TRANSFER TO OXYGEN

The processes described above, the enzymic reduction of quinones, the non-enzymic generation of semiquinones, as well as the redox component involved in the addition of HO^- and sulfur nucleophiles to quinones, yield hydroquinone-or semiquinone derivatives, which may participate in a) disproportionation-, b) cross-oxidation-, and c) autoxidation reactions. The prevalence of either pathway will be determined by, on the one hand, the inborn chemical features of the quinone, mainly its reduction potential and, on the other, environmental or solvent-related factors, such as availability of H^+, polarity of the medium, effects of solvent cage and solvation energy. It should be born in mind that *every quinone has a chemistry of its own* and, therefore, generalizations regarding their biochemical reactivity should be avoided as a rule.

Reduction potential

The reduction potential of quinoid compounds is, among the intrinsical properties of quinones, the most important factor affecting the rate of electron transfer between donor and acceptor. Moreover, the reduction potential represents the simplest control over free radical reactivity.[68]

Because quinones are one- and two-electron redox species, following reduction potentials at pH 7 can be considered: the one-electron reduction potential [$E(Q/Q^{\cdot-})$], the two-electron reduction potential [$E(Q/Q^{2-})$], and the reduction potential of the intermediate step [$E(Q^{\cdot-}/Q^{2-})$]. The latter, $E(Q^{\cdot-}/Q^{2-})$, is not easily measured in protic media but it can be calculated according to the formula $E(Q^{\cdot-}/Q^{2-}) = 2\,E(Q/Q^{2-}) - E(Q/Q^{\cdot-})$.[24]

Applications of fast, kinetic methods to derive electrochemical potentials, mainly based on pulse radiolysis and flash photolysis, have been recently discussed.[69] The one-electron reduction potential of quinones can be calculated by pulse radiolysis by measuring the equilibrium constant[70] or the approach to equilibrium rate constant[69] for electron transfer between quinone/semiquinone and standard redox couples in aqueous solutions. The electrochemical methods to calculate reduction potentials generally relate to the standard hydrogen electrode (s.h.e.; referred to as the normal or hydrogen electrode potential). The values obtained by using a saturated calomel electrode (s.c.e.) can be converted to those corresponding to s.h.e. by adding 244 mV, whereas those obtained with a Ag/AgCl electrode (some of the data herein) are 222 mV lower than the s.h.e.[71]

The reduction potential of quinones is influenced by the substituents, dissociation constants, $[H^+]$, and solvent effects.[72,73] A brief and rather oversimplified view on these variables and their interrelations is summarized below.

Substituents. The reduction potential of the quinones is affected by the nature, number, and position of the substituent[41]; as a general concept, electron-donating substituents (e.g., —OH, $—NH_2$, $—CH_3$) favour electron-transfer reactions from hydro- and semiquinones, whereas the opposite is expected from electron-withdrawing substituents (e.g., formyl, $—NO_2$, —COOR). Examples on different substitution patterns and their influence on the reduction potentials of quinones are given below.

Dissociation constants. The dissociation constants of hydro- and semiquinones are affected by the substituents in two ways: electron-donating groups raise the pK values, whereas electron-withdrawing groups lower the pK values.[24,74] The pK values of semi-quinones of the *p*-benzoquinone- and 1,4-naphthoquinone series in aqueous solution lie between 4 and 5. The introduction of a electron-donating $—CH_3$ group raises this value in the *p*-benzosemiquinone series with 0.25 units per group ($pK = 4.1 + 0.25$).[24] The formation of intramolecular hydrogen bonding between a suitable substituent (e.g., —OH) with the vicinal carbonyl group ($OH\cdots O—C<$) decreases the dissociation constant (pK_1) of the quinone/semiquinone species,[74] as exemplified by the effect of the —OH substituent in the benzene ring of 1,4-naphthoquinone, which lowers the pK in an additive manner: the first —OH group (at C_5) lowers the pK by 0.45 units, and the second —OH group (at C_8) by about 1.4 units.[75]

H^+ concentration. The redox potential of quinones is dependent on the $[H^+]$ of the medium and the dissociation constants of the species. The $E(Q/Q^{2-})$ values decrease with pH in a fashion involving basically three regions or break points delimited by the pK values of the quinone: [a] the first corresponds to the two-electron/two-proton transfer to the quinone [$QH_2 \Leftrightarrows Q + 2e^- + 2H^+$] and spans to pK_1, [b] the second corresponds to the two-electron/one-proton transfer to give the singly-ionized hydroquinone [$QH^- \Leftrightarrows Q + 2e^- + H^+$] and spans between pK_1 and pK_2, and [c] the third corresponds to the two-electron reduction of the quinone to yield the doubly-ionized hydroquinone [$Q^{2-} \Leftrightarrows Q + 2e^-$] and it is observed at pH values above pK_2. The rate of electron transfer reactions of semiquinones, generally in the order $10^8\ M^{-1}s^{-1}$, is dramatically slowed down upon protonation; similar behaviour is observed for electron transfer from hydroquinones.[76] Thus, deprotonation of hydroquinones and semiquinones, obtained by adjusting to a suitable pH, is often required to observe reversible electron-transfer reactions.[69] At pH 7, semiquinones, having pK values between 4 and 5, will be in their anionic form and participate readily in electron transfer reactions; the so-called stability of hydroquinones, however, will be partly due to the fact that they are protonated at this pH, hence slowing down electron-transfer processes.

Polarity of the medium. The electrochemistry of quinones in aqueous and nonaqueous solutions has been reviewed.[52] The electrochemical reduction of simple quinones in aprotic media differs whether H^+ or H^+ donors are present. In the absence of H^+, quinones are reversibly reduced in stepwise one-electron transfer processes: $Q \Leftrightarrows Q^{\cdot-} \Leftrightarrows Q^{2-}$. The presence of H^+ affects the second one-electron transfer process,

which shifts to more positive potential until it merges with the first-electron transfer reaction and leads to the irreversible two-electron reduction of the quinone. The electrochemical reduction of quinones in aqueous media exhibits a complex behaviour which have the appearance of reversible couples.

The association of a redox agent with a micelle affects the reduction potential in much the same way as the pK value of an acid is changed at the micelle surface. Because the rate of electron transfer is strongly correlated to the difference in reduction potential between donor and acceptor, the association of one or both of these with a micelle will have a substantial effect on the rate of electron transfer.[77]

Electron transfer to O_2

Autoxidation of quinols in oxygenated solutions can theoretically proceed with formation of $O_2^{\cdot-}$ or H_2O_2, as the result of one- or two-electron transfer to O_2. Autoxidation is a non-enzymic process, generally several orders of magnitude slower than the enzyme reactions involving quinones. Quinols may transfer one- or two electrons to O_2 depending on the lifetime of the collisional complex, that is, on the time that the O_2 molecule remains trapped within the solvent cage around the quinol molecule. If this time is short (for the case of relatively water-soluble quinols), one electron will be transferred, because for the transfer of the second electron time is required for spin inversion. For a more rigid solvent cage, time will be allowed for a second electron transfer, and H_2O_2 will be formed. H_2O_2 can also be formed during the oxidation of quinols by $O_2^{\cdot-}$ (reaction 7) via a sequential transfer of a H^+ and a H to give the semiquinone anion radical. The substantially-higher yields of the latter in condensed phase relative to those in the gas phase might be in agreement with the concept that the solvent cage of the activated complex extends its life time to favour a sequential mechanism[78]. These considerations might be useful in explaining the autoxidation of several hydroquinones in terms of either $O_2^{\cdot-}$ or H_2O_2 formation, as well as that of various ubiquinols ($Q_1 > Q_2 > Q_0 > Q_6 \geqslant Q_{10}$) following their reduction by complex I of the respiratory chain[79]. At variance with the oxidation of quinols, the oxidation of semiquinones generates only $O_2^{\cdot-}$, because one-electron transfer is only possible during the bimolecular collision.

The mechanism for transfer of reducing power from a hydroquinone (at neutral pH, formally a two-equivalent H-atom donor) to a one-electron acceptor is open to following considerations[80]: [a] possible reducing couples: ($QH_2/QH_2^{\cdot+}$), ($QH^-/QH\cdot$), or ($Q^{2-}/Q^{\cdot-}$), and [b] possible oxidizing couple: ($O_2/O_2^{\cdot-}$). Alternatively, for H-atom transfer, it requires ($QH^-/Q^{\cdot-}$) or ($QH_2/QH\cdot$) and ($O_2/HO_2\cdot$), respectively.

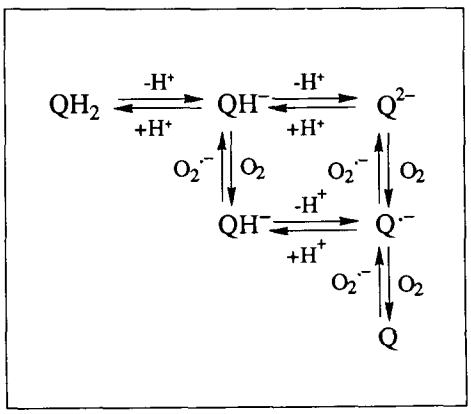
These options were considered in the study of electron transfer between quinols and cytochrome *c*[81], which proceeded by a bimolecular collision including electron transfer from the couple ($QH^-/QH\cdot$) to H^+—cyt. c^{3+}/H^+—cyt. c^{2+}). Thermodynamic considerations indicated a more favourable reaction if the anionic quinol was the electron donor species. The prevalence of electron- to hydrogen transfer is supported by the decrease in reaction rate with increasing ionic strength.[81] The quinol anion and the semiquinone intermediate are involved in protoprotic equilibria ($QH_2 \Leftrightarrows QH^- + H^+$ and $QH\cdot \Leftrightarrows Q^{\cdot-} + H^+$, respectively. Hydroquinone autoxidation is strictly related to pH, the deprotonation reaction preceding the electron transfer to O_2 (Scheme 3).

In summary, the formation of the bimolecular complex between electron donor and O_2 would thus be the rate limiting event and once an appropriate collision has occurred, the electron transfer is rapid. Similar considerations might apply to the production of $O_2^{\cdot-}$ during mitochondrial electron transfer, which could result from the autoxidation of ubisemiquinone as indicated by the occurring one-electron oxidation of ubiquinol by cytochrome c_1 [$UQH_2 + c^{3+} \rightarrow UQH\cdot + c^{2+} + H^+$], followed by autoxidation of the semiquinone [$UQH\cdot + O_2 \rightarrow UQ + O_2^{\cdot-} + H^+$].[79]

EFFECT OF SUBSTITUENTS ON HYDROQUINONE AND SEMIQUINONE AUTOXIDATION

p-Benzoquinone series

Methyl substitution. The introduction of —CH_3 groups into a *p*-benzohydroquinone structure decreases the half-wave potential in an additive manner.[82] Because hydroquinones at pH 7 are protonated, the rate of autox-



Scheme 3.

idation of —CH_3-substituted *p*-benzohydroquinones correlates with the half-wave potential values only after correction of the latter for the change in free energy involved in the dissociation step.[83]

For the *p*-benzosemiquinone series the rate constant for electron transfer between the semiquinone intermediates and O_2 correlates with the reduction potential and no correction for the free energy changes involved in the dissociation step is required because these species are in their anionic form at pH 7. Electron exchange represents here the only electron transfer reaction, for no bonds are broken or formed, thus making the reorientation energy of solvent molecules around the quinone or semiquinone the major contribution to the energy of activation.[84] The introduction of electron-donating —CH_3 groups in *p*-benzosemiquinones decreases the one-electron reduction potential in an additive manner by about 85 mV per —CH_3 group, regardless of their position in the quinone ring [$E(Q/Q^{\cdot-}) = 100 - n85$].[24] The rate of autoxidation of *p*-benzosemiquinones [$Q^{\cdot-} + O_2 \rightarrow Q + O_2^{\cdot-}$] increases with the degree of —CH_3 substitution and, therefore, with decreasing $E(Q/Q^{\cdot-})$ values: *p*-benzoquinone (5×10^4 $M^{-1}s^{-1}$) < methyl-*p*-benzoquinone (1.1×10^6 $M^{-1}s^{-1}$) < 2,6-dimethyl-*p*-benzoquinone (8.8×10^6 $M^{-1}s^{-1}$); this correlates with a decrease in the rate constant of the backward reaction [$Q + O_2^{\cdot-} \rightarrow Q^{\cdot-} + O_2$].[85]

The disproportionation rate of un- and methyl-substituted *p*-benzosemiquinones[85] (reaction 5) is between 10^7–10^8 $M^{-1}s^{-1}$. The stability constant of benzosemiquinones, defined as $K_S = [Q^{\cdot-}]^2/[Q^{2-}][Q]$, reflects the steady-state concentration of semiquinone transient species at a given pH and decreases with increasing number of —CH_3 groups.[24]

Glutathionyl substitution. Glutathionyl substitution is the result of the 1,4-reductive addition of thiols across the double bond of the quinone (reaction 16). The primary product, a glutathionyl-hydroquinone adduct, is prone to undergo one-electron transfers, in terms of autoxidation, disproportionation, and cross-oxidation reactions, and giving rise to the glutathionyl-benzosemiquinone radical (analogue to reactions 18, 19).[50]

The glutathionyl-*p*-benzohydroquinone adduct retains the redox properties of the quinone, thereby making possible its analysis by HPLC with oxidative electrochemical detection ($E_{1/2}$ values calculated against a Ag/AgCl reference electrode for oxidation of glutathionyl-*p*-benzohydroquinone and *p*-benzohydroquinone are +220- and +200 mV, respectively).[19] The half-wave reduction potential ($E_{1/2}$) values of thioether-adducts of *p*-benzoquinone is slightly more negative than the parent compounds.[86] Although no one-electron reduction potential values for the glutathionyl-*p*-benzoquinone conjugate are available, data obtained from diverse glutathionyl-naphthoquinone adducts indicate that $E(Q/Q^{\cdot-})$ values are similar to that of the parent quinone compounds (see below).

Despite the minor changes in $E_{1/2}$ values of the *p*-benzoquinones upon —SG substitution, significant alterations in their oxidation equilibrium, involving both autoxidation and cross-oxidation reactions, are observed.[45,86] Thus, the —SG substituent enhances the rate of autoxidation of the hydroquinone by about 8-fold.[45]

GSH reductive addition to methyl-substituted *p*-benzoquinones is accompanied by a different chemical behaviour of the product, depending on whether the —SG substituent is vicinal to a —CH_3 group. Thus, on the one hand, —SG substitution in 2-methyl-*p*-benzoquinone yields 2-methyl-5-glutathionyl-*p*-benzohydroquinone as primary adduct. The formation of the glutathionyl adduct at C_5, in position *para* to the —CH_3 group at C_2, is expected on the basis of the electron-donating properties of the latter (see scheme 2).[42] Accordingly, the rate of electron transfer to O_2 from 2-methyl-5-glutathionyl-*p*-benzohydroquinone is higher than that from the glutathionyl-conjugated parent compound lacking a —CH_3 group.[45] This may be understood as a consequence of the lowered $E_{1/2}$ value upon —CH_3 substitution of *p*-benzoquinones.

On the other hand, the GSH-reductive addition to 2,6-dimethyl-*p*-benzoquinone, does not produce the additive increase in autoxidation rate as expected if the effect of all substituents were exerted. The nucleophilic addition product contains a —CH_3 group flanking the —SG substituent. This product orientation is not accompanied by a more negative $E_{1/2}$ value, as otherwise caused by —CH_3 and thio-ether groups. This may be explained in terms of steric inhibition, i.e., prevention of the polar effect of one- or both of the substituents from being exercised.[86] With the same quinone and *p*-toluene-thiol as the nucleophile, a more positive $E_{1/2}$ value was found and this consequently hindered cross-oxidation, thereby making the thioether derivative the sole molecular product.[86]

Similarly to what described for *p*-benzoquinones lacking a —SG substituent, superoxide dismutase enhanced the rate of autoxidation of both un- and methyl-substituted glutathionyl-hydroquinones by 6–9-fold,[45] probably by displacing the equilibrium of the reaction $GS—Q^{\cdot-} + O_2 \Leftrightarrow GS—Q + O_2^{\cdot-}$ towards the right.

Hydroxy substitution. Reduced *p*-benzoquinone species with —OH substituents may originate from a) reduction of *p*-benzoquinone epoxides by one- or two-electron transfer flavoproteins[20,21] (reactions 3 and 4); b) reductive nucleophilic sulfur addition to quinone epoxides[58] (reactions 23a–c and 25), and c)

from the monooxygenase-mediated metabolism of benzene.[87]

The occurrence of a —OH group in the quinone ring affects both pK_1 and pK_2 of the corresponding hydroquinone. The explanation of this effect is found in the occurrence of a strong intermolecular bond between the un- and monodissociated —OH groups.[74,88] This in turn affects the reduction potential of the compound, because the dissociation of the hydroquinone is part of the change in free energy during hydroquinone oxidation.[74]

The —OH substituent lowers considerably the $E_{1/2}$ values of *p*-benzoquinones determined in aqueous solutions[20,41,89] and it enhances substantially the rate of autoxidation by 92-fold over that of the parent compound lacking the —OH group.[20,21,45]

Although the considerably lower reduction potential of α-hydroxyquinones in aqueous solutions might be a major variable determining the enhanced rate of autoxidation, other parameters such as the distinctive acid-basic properties and hydrogen bonding capacity of α-hydroxysemiquinones might be equally important.

p-Benzohydroquinones with both —OH and —SG substituents autoxidize at rates 350-fold higher than that of the unsubstituted *p*-benzohydroquinone.[45] It should be noted that the individual effects of —SG and —OH substituents are not additive when present in the same quinoid molecule, but they seemed to potentiate the overall autoxidation.

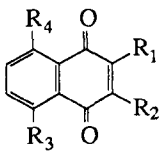
1,4-Naphthoquinone series

A general effect of $—CH_3$, —OH, and —SG substituents on the one-electron reduction potential [$E(Q/Q^{\cdot-})$] and half-wave potential ($E_{1/2}$) of 1,4-naphthoquinones as well as on the rate of electron transfer from the semiquinone species to O_2 [$k(Q^{\cdot-} + O_2)$] are summarized in Table 1.

Methyl substitution. The $E^{\circ\prime}$ values of naphthohydroquinones decrease linearly with the introduction of $—CH_3$ groups in the C_2 and C_3 positions presumably because of an inductive effect.[82] The $E(Q/Q^{\cdot-})$ values of 1,4-naphthosemiquinones decrease with increasing $—CH_3$ substitution [$E(Q/Q^{\cdot-}) = -140$-, -203-, and -240 mV for un-, mono-, and dimethyl-substituted 1,4-naphthoquinone, respectively]. As within the *p*-benzoquinone series, the $k(Q^{\cdot-} + O_2)$ values range between 1.1- and 2.4 $\times 10^8\ M^{-1}s^{-1}$ and are dependent on the $E(Q/Q^{\cdot-})$ values.[24,85,90,91]

Glutathionyl substitution. The —SG substituent is expected to exert only minor changes in the reduction potential of the quinone, in agreement with the relatively-weak electron-withdrawing properties of thioether substituents,[66] which have Hammett constants typically of the order $\sigma_p \approx 0$ and $\sigma_m \approx 0.1$–0.2. Accordingly, the $E(Q/Q^{\cdot-})$ values for menadione ($= -203$ mV) and its glutathionyl adduct ($= -192$ mV) differ by about 11 mV,[66] whereas $E(Q/Q^{\cdot-})$ values for 1,4-naphthoquinone ($= -140$ mV)[24] and its glutathionyl adduct

Table 1. Effect of Substituents in 1,4-Naphthoquinone and 2-Methyl-1,4-Naphthoquinone on the Reduction Potential and Rate of Electron Transfer to Oxygen



R_1	R_2	R_3	R_4	Compound	$E_{1/2}$ (-mV)	$E(Q/Q^{\cdot-})_7$ (-mV)	$k(Q^{\cdot-} + O_2)$ ($MN^{-1}s^{-1}$)	$k(Q + O_2^{\cdot-})$ ($M^{-1}s^{-1}$)
—H	—H	—H	—H	1	180	140	1.1×10^8	2.0×10^8
—OH	—H	—H	—H	2	460	415	—	—
—H	—H	—OH	—H	3	140	93	1.4×10^7	1.5×10^8
—SG	—H	—H	—H	4	225	132	—	—
—SG	—H	—OH	—H	5	195	—	—	—
$—CH_3$	—H	—H	—H	6	225	203	3.3×10^8	5.0×10^7
$—CH_3$	—OH	—H	—H	7	500	—	—	—
$—CH_3$	—H	—OH	—H	8	200	147	8.9×10^7	1.5×10^8
$—CH_3$	—SG	—H	—H	9	265	192	—	—
$—CH_3$	—SG	—OH	—H	10	220	—	—	—

Compounds: 1: 1,4-naphthoquinone; 2: 2-hydroxy-1,4-naphthoquinone or lawsone; 3: 5-hydroxy-1,4-naphthoquinone or juglone; 4: 2-glutathionyl-1,4-naphthoquinone; 5: 2-glutathionyl-5-hydroxy-1,4-naphthoquinone; 6: 2-methyl-1,4-naphthoquinone or menadione; 7: 2-methyl-3-hydroxy-1,4-naphthoquinone or phthiocol; 8: 2-methyl-5-hydroxy-1,4-naphthoquinone or plumbagin; 9: 2-methyl-3-glutathionyl-1,4-naphthoquinone or thiodione; 10: 2-methyl-3-glutathionyl-5-hydroxy-1,4-naphthoquinone.

Data from refs. 19, 24, 54, 58, 66, 75, 92, 93. Half-wave potential ($E_{1/2}$) values in refs. 19, 54 were obtained from hydrodynamic voltamograms by h.p.l.c. with electrochemical detection against a Ag/AgCl reference electrode.

(= −132 mV)[92] differ by about 8 mV. However, the $E_{1/2}$ values for 1,4-naphthoquinone (= −180 mV) and menadione (= −225 mV) are lowered by about 45 and 40 mV upon —SG substitution (−225 mV and −265 mV, respectively).[54]

In spite of the small changes in reduction potential, the autoxidation of the glutathionyl-naphthohydroquinone derivatives—following the reduction of the oxidized counterpart by DT-diaphorase- is 12–16-fold higher than that of the parent naphthohydroquinones.[54]

Hydroxy substitution. For the purpose of this discussion, hydroxy-naphthoquinones can be conveniently classified into two groups: those containing the —OH substituent in the quinoid ring and those containing the —OH substituent in the adjacent benzene ring. To the former group belong 2-hydroxy-1,4-naphthoquinone (lawsone) and 2-methyl-3-hydroxy-1,4-naphthoquinone (phthiocol) originating respectively from the enzymic reduction of 1,4-naphthoquinone- and menadione epoxide as well as from the reductive addition of GSH to 1,4-naphthoquinone epoxide, but not to menadione epoxide.[20,58] To the latter group belong 5-hydroxy-1,4-naphthoquinone (juglone), 5-hydroxymenadione (plumbagin), and 5,8-dihydroxy-1,4-naphthoquinone (naphthazarin), a structure present in certain anthracyclins such as adriamycin.

The position of the —OH substituent in the 1,4-naphthoquinone structure has profound influences on the electrochemical properties of the compound. Naphthoquinones bearing a —OH group in the quinoid ring show a dramatic decrease in the $E_{1/2}$- and one-electron $E(Q/Q^{\cdot-})$ reduction potential values ($\Delta E_{1/2}$ = −280 mV and $\Delta_{E(Q/Q^{\cdot-})}$ = −275 mV for 2-hydroxy-1,4-naphthoquinone).[19,93,94] The decrease in reduction potential can be probably attributed to a strong polar effect exerted by a —OH substituent in the quinoid ring. Further, the occurrence of an intramolecular hydrogen bond[95] might stabilize the corresponding semiquinone species. Moreover, a —OH group in the quinoid ring (e.g., 2-hydroxy-1,4-naphthoquinone) raises the pKa value of the semiquinone ($pKa_{1,4\text{-naphthoquinone}}$ = 4.1; $pKa_{2\text{-hydroxy-1,4-naphthoquinone}}$ = 4.7).[75]

The presence of a —OH substituent in the benzene ring, at C_5 and/or C_8, yields a compound with higher (more positive) reduction potential than the parent 1,4-naphthoquinone.[75,96,97] This effect was reported to be additive and thus 5-hydroxy-1,4-naphthoquinone and 5,8-dihydroxy-1,4-naphthoquinone have $E(Q/Q^{2-})$ values in aprotic medium of 211- and 313 mV more positive than 1,4-naphthoquinone.[98] The increase in the reduction potential caused by the introduction of a —OH substituent are higher in non-polar solutions than in aqueous medium. In the former instances, the $E(Q/Q^{2-})$ value increases by about 200 mV over that of the unsubstituted quinoid compound.[98,99] In the latter instances, the $E(Q/Q^{2-})$ values show a modest increase by about 40 mV.[54] The increase in reduction potential caused by —OH substituents in C_5 and/or C_8 can be attributed to intramolecular hydrogen bonding involving the H of the —OH group and the carbonyl group and additional delocalization of the tautomeric forms.[98] At variance with 2-hydroxy-1,4-naphthoquinone, a —OH substituent in the adjacent benzene ring lowers the pKa value of the semiquinone; the effect of the second —OH group is nearly double that of the first[75]: $pKa_{1,4\text{-naphthoquinone}}$ = 4.1; $pKa_{5\text{-hydroxy-1,4-naphthoquinone}}$ = 3.65; $pKa_{5,8\text{-dihydroxy-1,4-naphthoquinone}}$ = 2.7. A further characteristic of hydroxy-naphthoquinones is a less pronounced shift in λ_{max} from the neutral ($QH^{\cdot}$) to the anionic ($Q^{\cdot-}$) form; for the case of 5,8-dihydroxy-1,4-naphthoquinone, the λ_{max} is in both cases 380 nm.[75]

A shared property of hydroxy-naphthoquinones, independent whether the —OH substituent is in either the quinoid- or the benzene ring is that they show almost no increase in ϵ_{max} in going from the neutral ($QH^{\cdot}$) to the anionic ($Q^{\cdot-}$) form.[75]

The differences in reduction potential between hydroxy-quinones with —OH substitution in either the quinoid or benzene ring can be conveniently summarized in terms of $E_{1/2}$ and $E(Q/Q^{\cdot-})$ in aqueous solutions for the two hydroxy derivatives of 1,4-naphthoquinone: 2-hydroxy-1,4-naphthoquinone and 5-hydroxy-1,4-naphthoquinone. The $E_{1/2}$ values measured against a Ag/AgCl reference electrode are[20,54]: 1,4-naphthoquinone = −180 mV, 5-hydroxy-1,4-naphthoquinone = −140 mV, and 2-hydroxy-1,4-naphthoquinone = −460 mV. The $E(Q/Q^{\cdot-})$ values measured by pulse radiolysis are[75,93,100]: 1,4-naphthoquinone = −140 mV, 5-hydroxy-1,4-naphthoquinone = −93 mV, and 2-hydroxy-1,4-naphthoquinone = −415 mV.

In spite of these differences, both hydroxy-derivatives autoxidize at rates far faster than that of 1,4-naphthoquinone, following their enzymatic reduction by DT-diaphorase[54]: 2-hydroxy-1,4-naphthoquinone (85) > 5-hydroxy-1,4-naphthoquinone (33) > 1,4-naphthoquinone (1); 2-methyl-3-hydroxy-1,4-naphthoquinone (158) > 2-methyl-5-hydroxy-1,4-naphthoquinone (54) > 2-methyl-1,4-naphthoquinone (1). Similarly, in an ESR study comparing various naphthoquinones and anthraquinones, the derived hydroxysemiquinones were extremely reactive towards O_2 (whereas the parent compound lacking a —OH substituent reacted poorly), but in its absence were stable over a limited pH range. The optimal pH for stability

was lowered by the presence of the —OH groups capable of strong intramolecular hydrogen bonding with carbonyl groups, thereby influencing markedly the electron distribution and decreasing the effect of hydrogen bonding of the radicals with solvent molecules.[53,57] Further, anion radicals of certain hydroxyquinones, as naphthazarin, also contain hydrogen bonds with particular splitting constants.[53]

Following the 1,4-reductive addition of GSH to 1,4-naphthoquinone epoxide a hydroxy-glutathionyl-naphthoquinone product is formed (reaction 25), whose $E_{1/2}$ value (−680 mV) seems to represent the sum of the individual values for the glutathionyl- (−220 mV) and hydroxy (−550 mV) derivatives.[58] The autoxidation accompanying the nucleophilic addition to the naphthoquinone epoxide is difficult to attribute to a single reaction (see reactions 23–28), but it is, at any rate, far above that observed with the parent naphthohydroquinone.

In summary, —CH_3 substituents affect the reduction potential of the quinone in an inductive manner, showing an almost linear decrease with increasing number of —CH_3 groups. —OH substituents affect the redox behaviour of quinones in a different manner depending on whether the substituent is present in the quinoid- or adjacent benzenoid ring. Quinones bearing a —OH group in the quinoid ring are mainly influenced by strong electron-donating inductive effects, hence having reduction potentials far below the parent compound. Quinones bearing —OH groups in the neighbouring benzene ring possess reduction potentials slightly more positive than the parent compounds, probably due to an electron-withdrawing mesomeric effect of the —OH substituent (while the inductive effect is not expressed). The reduction potential may be in both cases influenced by the possibility of hydrogen bond formation between the carbonyl and the —OH group, thereby stabilizing the one- and two-electron reduced species. However, in the former case there is also a possibility for resonance stabilization of the oxidized form. —SG substituents exert minor changes in the one-electron reduction potential due to weak inductive/mesomeric effects.

The overall process of electron transfer is a complex event influenced by the interaction of the inborn chemical properties of quinoid compounds with environmental factors, which contemplate homogeneous and heterogeneous systems. However, information on certain properties of quinones such as pK values and reduction potentials is often unavailable, although the latter can be reliably measured by either fast- or conventional electrochemical techniques. Another aspect of no less importance is the solvation of the quinone compounds and the resulting solvation energy. Because only a few phenomenological data are available, it is difficult to establish a correlation between solvation energy, on the one hand, and reduction potential and electron transfer rate, on the other. This concept, which cannot be foreign to the chemical behaviour of quinone compounds in heterogeneous systems, has been rarely considered when trying to relate the chemical reactivity of quinones and the observed overall biological reactivity.

ELECTRONICALLY-EXCITED QUINOID COMPOUNDS

Electronically-excited quinones

Three distinct regions of selective absorption can be recognized in *p*-benzoquinones[101]: a) an intense band with a λ_{max} at about 238 nm; b) a selective absorption showing an inflexion or a peak near 285 nm; and c) a resolved absorption in the visible region (420–500 nm). A detailed study on the electronic transitions of 1,4-naphthoquinone derivatives recognizes four bands—referred to methanol—as a head series[102]: a) an intense band with two peaks at 246–250 nm; b) another intense band which appears as a shoulder of the former at 257 nm; c) a third one, broad and of medium intensity centered at 330 nm; and d) a very low intensity band assumed between 330 and 450 nm. Taking into account the effect of substituents, bands 1 and 3 are attributed to the benzenoid system and labelled benzenoid, whereas bands 2 and 4 are considered due to the quinoid structure and labelled quinoid bands. Furthermore, substitution of the quinoid system causes bathochromic shifts in the quinoid bands and leaves the benzenoid bands almost unchanged.

Semiquinone radicals absorb quite strongly at wavelengths where the parent quinones show little absorption. Protonation and methyl substitution of the radical exert only minor changes on the absorption spectrum.[24]

The most important absorption band of quinones in connection to photoreactivity is that of the longest wavelength in the region 400–500 nm. This band is generally due to a n, π^* singlet–singlet transition ($S_0 \rightarrow S_1$), though in the case of highly substituted quinones it corresponds to a π, π^* transition. The triplet state, formed by intersystem crossing, is the one primarily responsible for chemical reactivity. The energy of the first excited triplet of *p*-benzoquinones lies around 53 kcal.mol^{-1}, whereas that of 1,4-naphthoquinones is around 58 kcal.mol^{-1} (ref. 103).

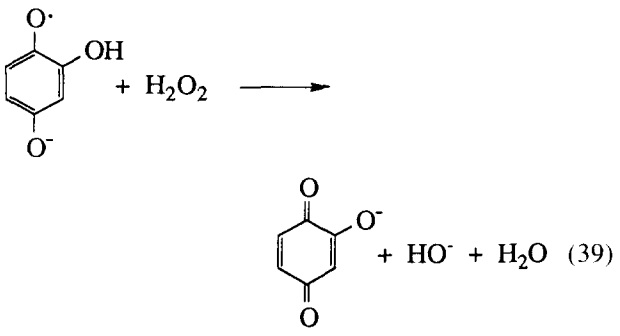
Generation of excited quinones

The behaviour of electronically-excited states of quinones such as those obtained during their photoreduction by laser flash photolysis or other means of

photoexcitation have been widely investigated. The main reactions in the photoreduction of quinones involve light absorption with formation of the triplet excited state ($^1Q_0 + h\nu \rightarrow {}^3Q$), followed by decay to the ground state ($^3Q \rightarrow {}^1Q_0$) or interaction with solvent molecules ($^3Q + SH \rightarrow \cdot QH + S\cdot$). These reactions are of relevance to establish the mechanism of interaction of triplet quinones with bioconstituents as well as the potential use of quinones as cell radiosensitizers.[104]

On the other hand, several cellular redox transitions involving quinones have been shown to be accompanied by generation of electronically-excited states. Examples are given by the chemiluminescence observed during the oxidation of adrenaline, adrenochrome, and related quinones, autoxidation of 6-hydroxydopamine, one-electron activation of menadione, and the free radical- and enzyme-mediated oxidation of semi- and hydroquinones, respectively.[105,106] It is clear that a single molecular mechanism cannot explain the formation of electronically-excited states in all these cases and, moreover, photoemission of biological systems exhibits an intricate spectral distribution, thus revealing that light emission might be more complex than that observed in the relaxation of a sole excited state to the ground state.

Thus, several routes for electronically-excited state formation during quinone redox cycling have been proposed and these include $O_2^{\cdot-}$ disproportionation, peroxyl radical recombination reactions, anion-cation radical annihilation, and oxidation of semiquinones to yield triplet quinones.[107] The latter might be of particular interest and could be understood as involving the primary oxidation of semiquinones by H_2O_2. Thermodynamic calculations indicate that semiquinones having a $E(Q/Q^{\cdot-})$ between -330 and 460 mV can theoretically bring about the one-electron reduction of H_2O_2 (reaction 39).[108] However, competing reactions for reaction 39 will be oxidation of the semiquinone species by O_2 (reaction 9), or $O_2^{\cdot-}$ (reaction 6), or another semiquinone species (reaction 5).



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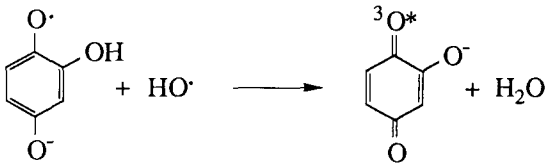
ESR studies of semiquinone radicals generated from naphthoquinones and anthraquinones have shown that these radicals do react stoichiometrically with H_2O_2 (quoted in ref. 108). The reduction of H_2O_2 by the semiquinone species from 2-methyl-1,4-naphthoquinone and several ubiquinones[109–111] and from anthracyclines[112] has been proposed and in some cases shown by ESR with spin trapping techniques. For the latter case, spin-trapping experiments inferred that the radical product is either HO· (from H_2O_2) or an alkoxyl radical (from other organic peroxides), the latter undergoing β scission to give the methyl radical. The rate constant for reaction of radical anion of adriamycin with H_2O_2 is estimated to be 10^4–10^5 $M^{-1}s^{-1}$ (ref. 112). However, it was also shown that the radical form of 9,10-anthraquinone-2-sulfonate formed by flash photolysis could not effect the decomposition of H_2O_2. The formation of HO· in a system containing anthraquinone-sulfonate, a flavoprotein, NADPH, and traces of transition metals was primarily attributed to the decomposition of H_2O_2 by Fe^{2+}, *via* a Fenton reaction.[113] Reduction of EDTA-Fe^{3+} in this system can be accomplished by the radical anion of anthraquinone-sulfonate ($AQS^{\cdot-} + \text{EDTA-}Fe^{3+} \rightarrow AQS + \text{EDTA-}Fe^{2+}$), reaction which, based on the reduction potentials of both couples, is thermodynamically favourable and proceeds with a rate constant $>4.0 \times 10^7$ $M^{-1}s^{-1}$.

The ferricyanide-mediated oxidation of hydroquinones or semiquinones leads to the generation of a triplet semiquinone or a triplet quinone, which upon decay to the ground state, emit at 515- and 568 nm, respectively.[114] A similar reaction is suggested on thermodynamical grounds for the generation of excited triplet hydroxy-*p*-benzoquinone (reaction 40), which upon deactivation to the ground singlet state shows maximal emission at $\lambda_{490-510nm}$ (reaction 41). Photoemission is sensitive to O_2, pointing to the triplet multiplicity of the excited state and proceeds with an efficiency 7.1×10^{-8} photons/hydroxy-*p*-benzoquinone molecule formed.[19]



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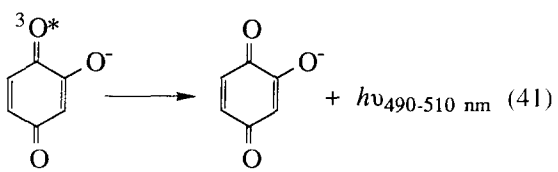

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Generation of electronically-excited states *via* elec-

tron transfer considerably amplifies the potential of photochemistry in the dark[115,116] and is a process involved in the non-enzymatic (potassium ferricyanide-mediated) oxidation of hydroquinone and semiquinone derivatives,[114] the bacterial diaphorase-catalyzed redox transitions involving *p*-benzoquinone,[117] the enzymic oxidation of catechol,[118] and autoxidation of tetrahydroxy-benzohydroquinone.[119] The oxidation of cathecol and tetrahydroxy-benzohydroquinone requires an enediol intermediate which is oxidized either enzymically or spontaneously to a diketo group.[118,119]

Chemical reactivity of excited quinones

Given the chemical reactivity of triplet carbonyls, in several aspects similar to those of alkoxyl radicals,[120] it is expected that their interaction with cellular components, mediated by 1,2-cycloaddition to olefins and/or hydrogen abstraction from suitable donors,[103,107] might partly constitute the basis for their toxicity. Semiquinone species are formed both upon reaction of a triplet quinone with hydrogen donors ($^3Q + RH \rightarrow QH\cdot + R\cdot$) (reaction 11) or another hydroquinone ($^3Q + QH_2 \rightarrow 2QH\cdot$) (analogous to reaction 8) and decay by disproportionation ($2QH\cdot \rightarrow QH_2 + {}^1Q_0$) (reaction 5). The reaction of triplet duroquinone with amines proceeds either *via* chemical reaction (hydrogen or electron abstraction from the amine) or physical quenching; both processes might involve a common intermediate, an exciplex.[26,27] Photoexcitation of menadione in oxygenated solutions is known to lead to photooxidation products of thymine and thymidine, with intermediate formation of pyrimidine radical cation by a charge transfer complex; similar photooxidation products are formed during ionizing radiation on DNA molecules. Menadione was also shown to photosensitize oxidation of nucleic acid and protein constituents.[121]

The cellular milieu is endowed with antioxidants which react readily with triplet carbonyl compounds: for example, triplet ketones are known to react chemically with ascorbic acid, vitamin E, and GSH with rate constants of 1.2-, 6.7-, and $0.67 \times 10^9\ M^{-1}s^{-1}$, respectively.[122] The quenching of triplet benzophenone by GSH can occur *via* a mechanism as that illustrated in reaction 42.[122] All compounds with —SH bonds show similar reactivities towards triplet ketones, whereas the other two aminoacids of GSH, glycine and glutamine, lacking this group, react with the excited state at considerably slower rates. Of note, it might be considered that thioether-hydroquinone adducts, as those formed during the nucleophilic addition of GSH to quinones, might contribute to the quenching of triplet species by electron or H transfer ($GS\text{-}QH_2 + {}^3Q \rightarrow GS\text{-}QH\cdot + QH\cdot$).

$$^3[>C=O]^* + H\text{–}\dot{S}\text{–}R \longrightarrow [>\overset{\delta-}{C}=O \quad \overset{\delta+}{H}\text{–}S\text{–}R] \begin{cases} \nearrow >\dot{C}\text{–}OH + \cdot S\text{–}R \\ \searrow >C=O + H\text{–}\dot{S}\text{–}R \end{cases} \quad (42)$$

CELLULAR ASPECTS OF QUINONE METABOLISM

The cellular aspects of quinone metabolism are diverse and a single mechanism to account for the biological responses to quinones seems at first sight unlikely. However, it is reasonable to assume that, among the diversity of cellular targets, some critical reactions may be unique in causing an initial perturbation of biochemical pathways. Propagation of this effect on interrelated biochemical pathways, comprising a modification of the balance of reactions leading to activation and disposal of the quinoid compounds, may thus result in expression of cytotoxicity.

Only some aspects of quinone metabolism are surveyed here: (I) a listing of the *possible mechanisms for quinone-mediated injury*. (II) The *cellular formation of quinone derivatives and expression of their toxicity*, as principle of primary activation of several xenobiotics. (III) The *cellular detoxication of quinones*, discussed in terms of the possible hydrophobic or hydrophilic metabolic pathways of quinones and the relative participation of one- and two-electron transfer flavoproteins in quinone activation as well as the potential role of superoxide dismutase in quinone toxicity.

Possible mechanisms for quinone-mediated cellular injury

At an experimental level, quinone cytotoxicity is expressed as an initial oxidative damage and subsequent qualitative alterations of related biochemical pathways, which finally leads to irreversible cell impairment and death.[123] In spite of the large body of experimental evidence for quinone cytotoxicity as well as quinones as mutagens and carcinogens, some of these compounds can act as antioxidants as indicated by inhibition of iron-dependent lipid peroxidation of microsomes by *p*-benzoquinone, and 1,2- and 1,4-naphthoquinone, as well as 2-methyl-1,4-naphthoquinone. The mechanism proposed might include a diversion of electrons from NADPH away from the peroxidation process and/or that quinones act directly as antioxidants.[124] The latter possibility seems to be mediated by a mechanism involving trapping by the quinone of lipid peroxyl- and alkoxyl radicals formed during lipid peroxidation.[125]

The toxicity of quinoid compounds seems to be due to the concerted participation of the main processes listed below.[123,126,127] However, it should be noted the general character of these pathways, which are not necessarily shared by every quinone.

Redox cycling. The autoxidation of semi- or hydroquinones, following the enzymic reduction of the oxidized counterparts and within a process of cycling characteristics, yields primarily $O_2^{\cdot-}$. Several reviews on quinone redox cycling are available[128,129] and the reader is referred to them for a detailed discussion.

It is broadly accepted that semiquinone-free radicals arising in the physiological milieu through enzymic activity or redox cycling can lead to cellular damage and exhibit mutagenicity. The production of long-lived populations of semiquinone and ascorbyl free radicals by one-electron redox processes in ascorbate-quinone mixtures exhibits a potent cytotoxic action against Ehrlich ascites tumors in mice.[130] ESR studies have shown that viable cells have an ability to eliminate the free radical populations arising from ascorbate-quinone mixtures and that this ability varies significantly from one cell line to another. The elimination process involves apparently a NAD(P)H-dependent enzyme(s) containing essential —SH groups.[131] The observation of the decay kinetics of free radicals offers a means for studying the enzymic reduction of quinones and semiquinones by suspension of viable cells; the technique is not dependent on the optical properties of the sample and the sensitivity makes it an attractive alternative to other enzymic assay methods. Large differences are observed in the ability of normal *versus* cancerous cells to eliminate free radicals in this assay procedure. This difference may reflect significant variations in the oxido-reductase system of normal and transformed cells.[130]

Alteration of intracellular thiol balance. The alteration of the intracellular thiol balance during quinone biotransformation comprises two main processes, which might be sequentially related: thiol oxidation and thiol arylation, the sum of which is known as thiol loss or thiol depletion.

Thiol oxidation. H_2O_2 formed during quinone redox cycling is reduced by the seleno-protein glutathione peroxidase at expense of GSH and with generation of GSSG; the latter is subsequently re-reduced to the thiol by glutathione reductase at expense of NADPH. When NADPH concentration is not maintained at the normal intracellular level or when glutathione reductase is inhibited, the intracellular GSSG concentration raises. Intracellular accumulation of GSSG is prevented by its extrusion to the extracellular space by a process coupled to a specific ATPase. GSSG can also react directly or enzymically with protein sulfhydryl groups to form glutathione-protein mixed disulfides (Pr—SH + GSSG → Pr—S—SG + GSH). A marked increase in glutathione-protein mixed disulfides is observed during the metabolism of menadione by isolated hepatocytes when DT-diaphorase or glutathione reductase activities are inhibited.[123]

Thiol arylation. Addition reactions with cellular nucleophiles such as GSH, DNA, RNA, and proteins have been pointed as the underlying mechanism for quinone toxicity. Although it is well established that cell killing caused by oxidative stress is preceded by depletion of intracellular GSH, the exact relationship between GSH depletion and cell death has not been clarified yet. It may involve inhibition of vital cell functions critically dependent on GSH or, possibly, nucleophiles other than GSH which become targets for electrophilic attack once GSH has been depleted. Protein sulfhydryl groups can become such a target, as shown by glutathione-protein mixed disulfide formation as a result of increased GSSG formation (a decrease in the [GSH]/[GSSG] ratio) and inhibition of glutathione reductase activity during menadione metabolism in hepatocytes.[132] In summary, the metabolism of menadione by isolated hepatocytes affects the status of both soluble (GSH) and protein thiols by mechanisms involving GSH depletion by oxidation to GSSG, mixed disulfide formation, and conjugation to GSH.[133]

Inhibition of cellular functions. A direct effect of quinones on the *de novo* synthesis of DNA[134] as well as on mitochondrial electron transport[135,136] are well-established examples of inhibition of vital cellular functions during quinone metabolism. Although in the latter case, inhibition is understood as antagonism of the quinones with mitochondrial ubiquinone at a site closely associated with Rieske iron-sulfur cluster,[135] a large part of these effects could be explained by either mechanism outlined above: redox cycling or arylation reactions.

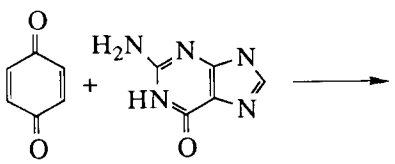
Alteration of Ca^{++} homeostasis. Menadione metabolism is associated with formation of small blebs on the surface of isolated hepatocytes due to a substantial raise in cytosolic Ca^{++}, which alters microfilament organization. Metabolism of menadione in hepatocytes leads to inhibition of the Ca^{++} translocases present in the mitochondria, endoplasmic reticulum, and plasma membrane. Oxidation of pyridine nucleotides and protein thiols leads to an impairment of the Ca^{++}-se-

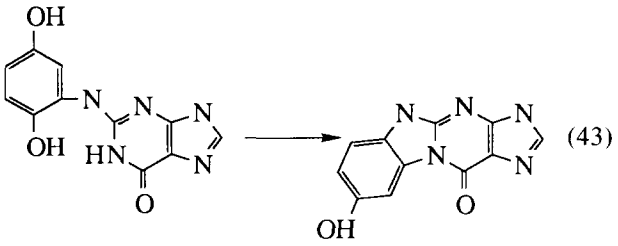
questering capacity of both mitochondria and endoplasmic reticulum, with concomitant increase of cytosolic calcium.[137,138] Selective formation of mixed disulfide with plasma membrane protein thiols upon incubation of hepatocytes with cystamine, is sufficient to cause surface blebbing and cytotoxicity.[123,139,140] Furthermore, proteolytic processes may be involved in the Ca^{++}-dependent hepatocyte injury upon activation of Ca^{++}-dependent proteases.[123]

Covalent binding. Many foreign compounds have been shown to be transformed to chemically reactive metabolites that combine—in some cases irreversibly—with nucleic acids, proteins, and lipids. The relationship of covalent binding to toxicity has been viewed as having a cause-effect character.[141-143]

Metabolic activation usually implies the formation of an "ultimate" or "reactive" intermediate with strong electrophilic character. Its chemical reactivity is displayed by its interaction with bioconstituents and determined by the chemical properties of both the intermediate electrophile and the biological nucleophile. For the case of nucleic acid bases, chemical reactivity can be confined to reactions with either the ring nitrogen atoms or the exocyclic amino or keto groups of the purine and pyrimidine bases. The selectivity of reaction at different sites of nucleic acid bases can be interpreted as the consequence of the random attack of agents on the exocyclic groups through a unimolecular solvolysis (S_N1) mechanism or alkylation of the highly nucleophilic ring nitrogen atoms through a synchronous substitution (S_N2) mechanism. Both mechanisms involved in the alkylation of nucleosides are strongly controlled by the solvent, leaving group, and substituent effects.[144]

The binding of quinones to DNA as a possible cause of DNA damage has been studied to some extent: benzo[a]pyrene quinones bind to DNA in the presence of NADPH and microsomes,[145] menadione has the capacity to bind to hepatocyte DNA,[146] and *p*-benzoquinone can add to deoxyguanosine.[147] The latter finding might be explained by a mechanism as illustrated in reaction 43 involving in a first step the nucleophilic attack of the exocyclic amino group attached to C_2 on the electrophilic C_α of the quinone. This is followed by stabilization due to enolization and further nucleophilic attack by N_1 on the guanine ring at the —OH group in the quinone with loss of H_2O (ref. 147). The biological significance of this model reaction has not been determined yet.





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Benzo[a]pyrene-3,6-quinone has been shown to cause strand breaks in human fibroblasts without oxidative stress and menadione causes strand breaks in both human fibroblasts and rat hepatocytes.[146] The binding of quinones to biomolecules, either covalently or by intercalation, and its significance in mutagenesis is a subject for further study.

The concerted participation of the mechanisms of quinone toxicity listed above might contribute in different extent to mutagenicity and antineoplastic activity.

Mutagenicity of quinones. Several quinones have been shown to be mutagenic in bacterial tester strains, cell cultures, as well as *in vivo*.[148,149] A general mechanism for biotransformation and tumor initiation has not been identified, but the process may involve both oxygen radical formation and alkylation pathways.

Tests of quinone mutagenicity using bacterial tester strains have been performed, both with and without activation of the quinone by liver homogenate plus NADPH (S9 mix). The results varied among the quinones and expression of mutagenicity was dependent on activation by S9 mix. The anticancer quinones adriamycin and daunomycin were shown to be mutagenic to TA98 tester strain in presence of the S9 mix.[150] Hydroxy-naphthoquinones, such as 2-methyl-5-hydroxy-1,4-naphthoquinone, 5,8-dihydroxy-1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone, 5-hydroxy-1,4-naphthoquinone, and 7-methyl-5-hydroxy-1,4-naphthoquinone were all mutagenic to tester strain TA2637 mediated by S9 activation.[151] 2-Methyl-1,4-naphthoquinone, benzo[a]pyrene 1,6-, 3,6-, and 6,12-quinones, pyrene 1,6- and 1,8-quinones, 9,10-phenanthrenequinone, and 1,8-dihydroxyanthraquinone were mutagenic to TA104 with S9 mix supplemented with NADPH, but when the latter was replaced by NADH a drastic decrease in the number of revertants was observed. This indicated that a NADPH-dependent enzyme activity was involved in the expression of mutagenicity. Moreover, mutagenicity of menadione, benzo[a]pyrene-6,12-quinone, 9,10-phenanthrenequinone, and 1,8-dihydroxyanthraquinone correlated with the rate of $O_2^{\cdot-}$ formation and was inhibited by superoxide dismutase, catalase, and a mixture of both.[152] Thus, quinone mutagenicity to bacterial

tester strains can in several cases be explained as a consequence of redox cycling, but exceptions occur such as the mutagenicity of 1,6- and 1,8-pyrenequinone in absence of S9 mix.[153]

Mechanism of action of anticancer drugs. Quinoid compounds have found extensive use as anticancer drugs and are, next to compounds containing the chloroethyl group, the most frequent among clinically approved anticancer drugs in the U.S.A. Anticancer quinoid compounds include the groups: simple quinones, anthracyclines, bis(substituted aminoalkylamino)anthraquinones, mitomycins, aziridinylbenzoquinones, streptonigrins, lapachols, and actinomycin D.[9]

Because of the complex structure of most antitumor quinoid compounds, it is difficult to separate the contributions of chemical reactivity and metabolic pathways to the overall biological activity. Therefore, the basis for the cytotoxic properties of these groups is not clear, though it appears to require the metabolism of the quinoid group and binding of the compound to DNA or other targets in the cell.[9] The contribution of quinone-induced strand breaks to the overall cytotoxicity of an agent may vary considerably and the presence of a quinone group in the structure of several antitumor agents can result in significant cell kill by a mechanism that appears to involve reactive oxygen species. By relating two model compounds, bis(dimethylamino)-benzoquinone, a non-binding quinone agents that serves as a model for the activity of the quinone group, and benzoquinone dimustard, which contains both a quinone group and alkylating groups, it was shown that the cytotoxicity of both was due, at least partly, to the production of reactive oxygen species.[154] Semiquinone radicals are equally important to the contribution of cytotoxicity: the biological conversion of mitomycin C to a reactive species is required to produce intrastrand cross linking of DNA. The one-electron reduction of mitomycin C to its radical anion by NADPH-cytochrome P450 reductase[155] has been shown a critical step sufficient, from a chemical viewpoint, to generate an activated form. The formation of mitomycin C radical anion implies rearomatization of the quinone moiety that favours subsequent loss of methanol between C_9 and C_{9a} upon formation of the aromatic indol moiety.[155] This compound is susceptible to nucleophilic attack as the aziridine ring cleaves and the resulting monofunctional adducts are capable of undergoing secondary enzyme activations. These observations indicate that the key step for initiating this sequence of transformations that leads to metabolite formation is the rearomatization of the quinone ring of mitomycin C, which can be effectively promoted upon the one-electron transfer to the quinone catalyzed by NADPH-cytochrome P450 reductase.[155] The versability of the mitomycin molecule has lead to the observation of other possible reactions involving the subsequent electron transfer from the semiquinone radical anion to O_2 to generate radical oxygen species. This raises the possibility that these species may be involved in the cytotoxicity of this agent.[156]

The metabolism of these quinones, and especially the reduction by flavoenzymes followed by radical formation, is associated with severe side effects such as cardiotoxicity. These complications limit the use of these compounds and this has stimulated the search for derivatives which have less pronounced side effects but still retain the antitumor properties.[64] A number of features of tumor cells may be invoked to explain the specific damage towards tumors produced by anticancer quinones. These may involve, among others, deficiency in protective enzymes such as superoxide dismutase[157] and glutathione peroxidase[158] as well as low concentrations of O_2, the latter being the basis for the activity of bioreductive alkylating agents.[66]

Cellular formation of quinone derivatives and expression of toxicity

The toxicity of several xenobiotics rests on their bioconversion to quinone metabolites by processes involving enzymes and non-enzymic interactions with free radicals. Some examples are given on the activation to quinone derivatives of benzo[a]pyrene, benzene, and 1-naphthol.

Benzo[a]pyrene. Benzo[a]pyrene is co-oxygenated or oxidized to a mixture of 1,6-, 3,6-, and 6,12-quinones; although the precursor of benzo[a]pyrene quinones has not been isolated, it has been suggested to be 6-hydroxy-benzo[a]pyrene. Benzo[a]pyrene quinones are formed by the one-electron oxidation of benzo[a]pyrene during PGH_2 biosynthesis[159] and microsomal mixed function oxidase activity,[160] and during ascorbate-dependent lipid peroxidation.[161,162]

The formation of benzo[a]pyrene quinones by one-electron oxidation occurs in a wide variety of mammalian cells, and this metabolic pathway is predominant under certain conditions. It is almost exclusive when metabolism occurs under peroxidatic conditions as in the case of prostaglandin-endoperoxide synthase or cytochrome P450 and cumene hydroperoxide. The first step involves the transfer of one electron from benzo[a]pyrene to the cytochrome P450-$[FeO]^{3+}$ complex yielding a benzo[a]pyrene radical cation, which reacts with the generated nucleophilic O_2 in the P450-$[FeO]^{2+}$ to form the 6-oxy-benzo[a]pyrene radical. Autoxidation of this intermediate would generate the corresponding benzo[a]pyrene quinones.[160] At variance with the one-electron oxidation of benzo[a]pyrene, its two-

electron oxidation yields 7,8-epoxy-benzo[a]pyrene, which in turn produces benzo[a]pyrene-7,8-dihydrodiol by epoxide hydrase. The difference in stereoselectivity in the metabolism of benzo[a]pyrene to the 7,8-dihydrodiol is the result of different proportions of cytochrome P450 isoenzymes.[160]

Lipid peroxidation-dependent quinone formation seems to contribute to the carcinogenic activation of benzo[a]pyrene by a mechanism implying the direct attack of lipid peroxyl radical on benzo[a]pyrene and subsequent scission of the O—O bond of the peroxyl group.[163]

Although benzo[a]pyrene quinones are confirmed metabolites of benzo[a]pyrene, their contribution to cytotoxicity and mutagenicity is not clear. The question also arises as to whether the quinone pathway results in detoxification.[164]

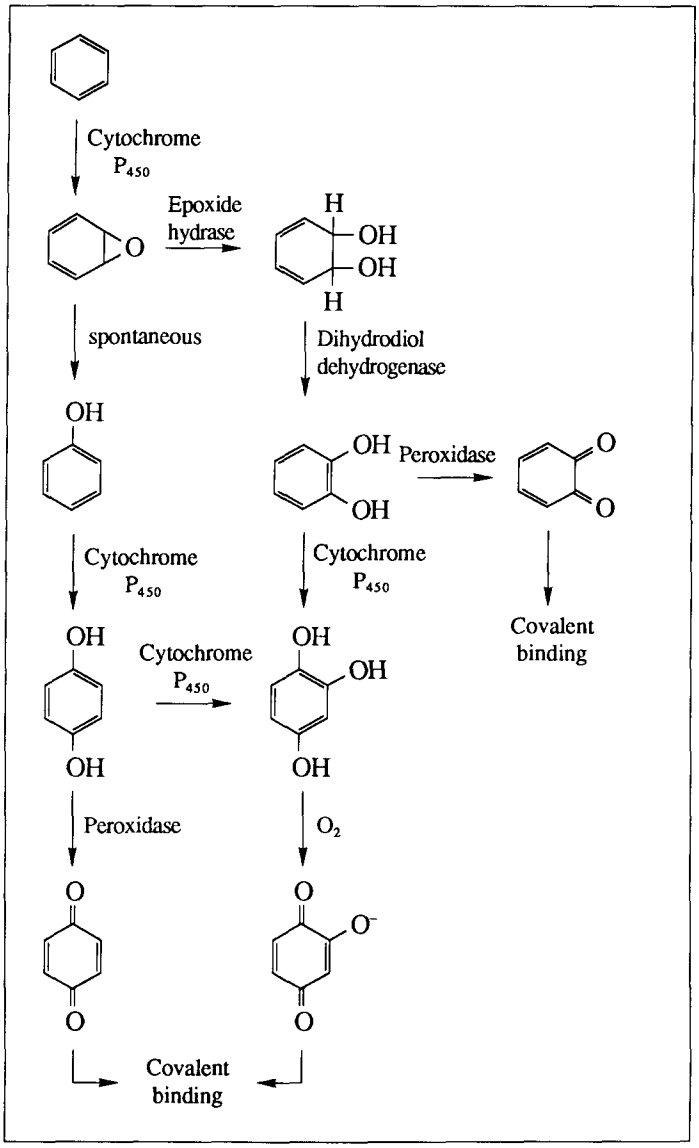
Benzo[a]pyrene quinones are substrates for cytochrome-P450 reductase and DT-diaphorase. The one-electron reduction of benzo[a]pyrene quinones[152,165] has been proposed as the basis of mutagenicity and cell injury, based on the autoxidation of the formed semiquinones[166] within the frame of a conventional redox cycling process. Conversely, the two-electron reduction of benzo[a]pyrene quinones by DT-diaphorase results in the rapid conjugation of the products with UDP-glucuronide catalyzed by microsomal UDP-glucuronyl transferase.[18,167] However, more extensive modification of DNA was observed by either trapping the phenols as glucuronide or conjugating the hydroquinones. An explanation might be the initial inhibition by quinones of the covalent binding of 7,8-diol-9,10-epoxide to DNA, probably by diverting electrons from the reductase to O_2 and away from cytochrome P450. Conjugated hydroquinones would no longer be effective in this process, hence the increased DNA modification.[168] On the other hand, DNA effects benzo[a]pyrene metabolism by altering both the interaction of benzo[a]pyrene quinones with microsomal enzymes and the relative rates of hydration and arrangement of 7,8-dihydrodiol-9,10-epoxide.[168] No definitive experimental evidence is available at present to ascribe the one- or two-electron reduction of benzo[a]pyrene quinones—as well as their conjugation with glucuronide—a definitive role in cytotoxicity and mutagenicity.

Benzo[a]pyrene quinones were toxic, in an O_2-sensitive fashion, to cultured hamster ovarium cells causing both direct cell killing and growth inhibition, the latter probably due to alterations of DNA synthesis.[166] However, benzo[a]pyrene-3-6-quinone was relatively non-toxic to both human fibroblasts and rat hepatocytes.[169] Whether this discrepancy is due to differences in expression of enzyme activity in either tissue is not determined yet.

Benzene. Chronic exposure to concentrations of benzene between 25 and 1000 ppm results in progressive degeneration of the hemopoietic system.[87] Benzene toxicity in mammals requires the primary biotransformation of the molecule to benzene epoxide or phenol, catalyzed by cytochrome P450 monooxygenase system, as well as secondary conversions to cathechol, *p*-benzohydroquinone, and 2-hydroxy-*p*-benzohydroquinone (Scheme 4).[87,170-171] Benzene epoxide was formerly thought to be the ultimate toxic agent, though later studies[172] have shown that it is further converted to phenol (by spontaneous decomposition) or to benzene-dihydrodiol (catalyzed by epoxide hydrolase). The latter compound can be subsequently oxidized to catechol by dihydrodiol dehydrogenase. Phenol and catechol may be hydroxylated a second time forming mainly hydroquinone and hydroxyhydroquinone, respectively. However, phenol is always the main metabolite and it is largely excreted as a conjugate.

The site for metabolic activation of benzene is a subject of debate. Although bone marrow is the main target tissue for the toxic effects of benzene, the main part of its metabolism seems to occur in liver. Benzene metabolites, such as phenol and secondary hydroxylation products, may be subsequently released from the liver and taken up by the bone marrow, where the toxicity is expressed. This hypothesis is only based on the observation that appearance of benzene metabolites in bone marrow correlates with their disappearance in liver.[173] Alternatively, the metabolism of benzene in the bone marrow, whose microsomes are capable of converting benzene to phenol at a rate 4-fold higher than that observed with liver microsomes,[171] may account for the selective toxicity of benzene. Metabolites of benzene other than phenol are not found during the bone marrow microsome-catalyzed activation, which might have been due to binding of the latter to microsomal protein.[171] Unless the pattern of nucleophilic bioconstituents capable of reacting with benzene metabolites in bone marrow microsomes is different from those in liver microsomes, an alternative explanation may depend on the different metabolic pathways present in these tissues.

Benzene metabolites are known to bind to DNA and proteins in vivo and in vitro. This occurs probably through an electrophilic attack on these macromolecules subsequent to the metabolism of benzene to catechol, hydroquinone, or hydroxy-hydroquinone.[147,174-179] None of the latter compounds are reactive electrophiles, and their oxidation products, *o*- and *p*-benzoquinones, are required for chemical reactivity with nucleophiles. The importance of quinone species for microsomal protein binding was further pointed out by the inhibitory effect of both DT-diaphorase,[180] which is known to reduce quinones to hy-



Scheme 4.

droquinones, and GSH, which spontaneously conjugates with quinones at high rates,[181]

The oxidation of catechol, hydroquinone, and hydroxyhydroquinone can be mediated by a) enzyme systems, b) $O_2^{\cdot-}$, and c) O_2.

a) The possibility of enzymic oxidation of hydroquinones was brought forward on the basis of the phenol-stimulated hydroquinone oxidation by horseradish peroxidase. Addition of phenol stimulated both the oxidation of hydroquinone to quinone as well as the binding of [^{14}C]quinone to rat liver protein. The decrease in rat bone marrow cellularity following the co-administration of phenol and hydroquinone was larger than that observed with either compound individually. It was suggested that bone marrow myeloperoxidase could mediate a similar reaction.[182] *o*-Benzoquinone could be formed during the oxidation of cathecol by a peroxidase activity and it binds covalently to the —SH group of proteins. The yield of protein-bound cysteinylcatechols depends on a competition between the intermolecular nucleophilic reaction of —SH groups in the protein and the intramolecular nucleophilic reaction of an —NH_2 group in the side chain.[183]

The hypothesis that $O_2^{\cdot-}$ can stimulate the formation of reactive quinone species is based on the finding that superoxide dismutase can inhibit the binding of benzene- or phenol metabolites in microsomal incubations and that this enzyme causes the accumulation of hydroquinone.[184] This may be dependent on the concentration of phenol used, because at high concentrations of phenol superoxide dismutase did not affect binding and hydroquinone could be detected as a metabolite of

phenol.[170] The fact that superoxide dismutase has been found to accelerate the autoxidation of hydroquinone,[87] makes the above mentioned inhibitory effect of superoxide dismutase more puzzling.

c) The binding of benzene metabolites to cellular macromoles might depend on autoxidation of either hydroquinone or hydroxyhydroquinone but not of catechol, which does not autoxidize.[87] The rate of autoxidation of hydroquinone might be enhanced by superoxide dismutase by displacing, upon removal of $O_2^{\cdot-}$, the equilibrium of the autoxidation reaction ($QH_2 + O_2 \rightarrow Q\cdot + O_2^{\cdot-} + 2H^+$).

1-Naphthol. This compound is selectively toxic to short-term organ cultures of human colorectal tumor tissue,[185,186] probably by a process involving selective accumulation and impaired conjugation of the xenobiotic.

The cytotoxicity of 1-naphthol to tumour tissue would be understood as a direct interference with mitochondrial function or indirectly *via* its metabolism to 1,2- and/or 1,4-naphthoquinone. In hepatocytes 1-naphthol is readily metabolized to its glucuronate- and sulfate conjugates, whereas microsomes catalyze the transformation of 1-naphthol to a series of reactive metabolites which covalently bind to protein.[187]

The possible routes for 1-naphthol biotransformation to 1,2- and 1,4-naphthoquinone metabolites seem to be distinguished by the biocatalyst involved. Thus, following pathways have been described: a) a cytochrome P450-dependent activity, b) a polyphenol oxidase or tyrosinase-dependent conversion, c) peroxidase-dependent activation, d) a $O_2^{\cdot-}$-mediated oxidation, and e) the generation of free radicals during the NADPH- and Fe-dependent lipid peroxidation.

a) 1,4-Naphthoquinone and covalently-bound products are formed during the NADPH-dependent microsomal metabolism of 1-naphthol, whereas 1,2- and 1,4-naphthoquinone as well as covalently-bound products are formed when NADPH is replaced by organic hydroperoxide. These naphthoquinones may arise following hydroxylation of 1-naphthol to either 1,2-dihydroxy-naphthalene or 1,4-dihydroxy-naphthalene and their subsequent oxidation either enzymically or by autoxidation. Neither metabolic pathway is affected by GSH (which reacts with these quinones as illustrated in reaction 17) or ethylenediamide (which reacts rather specifically with 1,2-naphthoquinone), but both compounds inhibit efficiently covalent binding.[188,189] Whereas 1,4-naphthoquinone is the main product at early incubation times, additional products are formed after prolonged incubations: one of them was suggested to be 2-hydroxy-1,4-naphthoquinone (lawsone). The cytochrome P450-dependent metabolism of 1-naphthol (in the presence of NADPH) as well as that of 1,2- and 1,4-naphthoquinone (in the presence of either NADH or NADPH) is related to the formation of $O_2^{\cdot-}$. These observations might be compatible with the suggestion that 1-naphthol may exert its toxicity to isolated hepatocytes and other cellular systems by a mechanism involving the generation of oxygen radicals by redox cycling of the naphthoquinone products.[187] In addition to the naphthoquinones and covalently-bound species, an uncharacterized product is formed during microsomal activation of 1-naphthol, suggested to be a glutathionyl adduct of the naphthoquinones.

b) At variance with cytochrome P450, the polyphenol oxidase, tyrosinase, catalyzes the conversion of 1-naphthol primarily to 1,2-naphthoquinone and small amounts of 1,4-naphthoquinone as well as subsequent covalently-bound products. Inhibition of covalent binding by ethylenediamine, which reacts specifically with 1,2-naphthoquinone but not with 1,4-naphthoquinone, indicates that the *ortho*-quinone is mostly responsible for covalent binding. The metabolic activation of 1-naphthol by tyrosinase to covalently-bound species suggests that 1-naphthol may be of potential therapeutic application in the treatment of cells high in tyrosinase activity such as certain melanomas.[190]

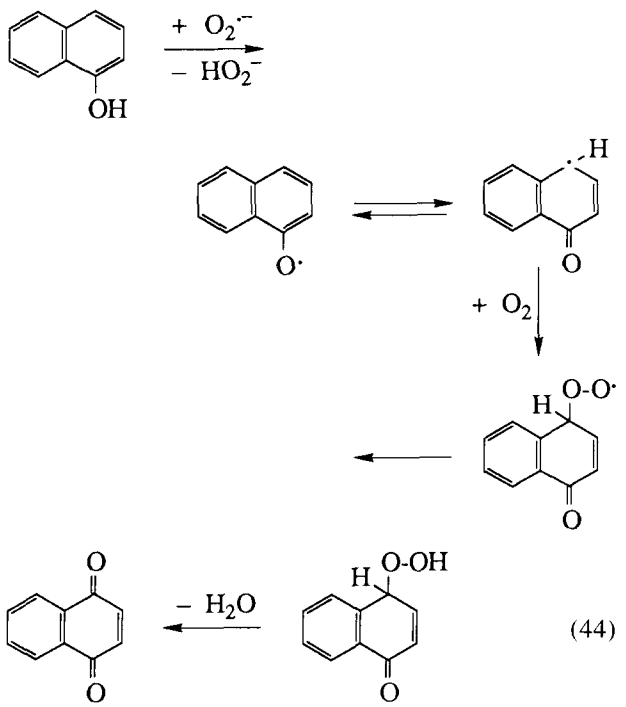
c) 1-Naphthol may also be metabolized by a peroxidase-type mechanism to a naphthoxyl radical in an analogous manner to the formation of phenoxyl radicals from phenols, with subsequent decomposition to catechols and quinones.

Stimulated human neutrophiles can activate 1-naphthol to protein-binding metabolites by both a H_2O_2(myeloperoxidase)-dependent pathway and by a direct $O_2^{\cdot-}$-dependent pathway (see below).[191]

The metabolism of 1-naphthol by horseradish peroxidase/H_2O_2 involves the one-electron oxidation of 1-naphthol to 1-naphthoxyl or a naphthoxyl-derived radical. Formation of covalently-bound products is inhibited by GSH in a dose-dependent manner and by a mechanism which involves the repair of the naphthoxyl radical by GSH to yield GS· and 1-naphthol [Ar-O· + GSH → Ar-OH + GS·]. Accordingly, GSSG formation is stimulated, probably out of the disproportionation of GS· [2 GS· → GSSG].[192] Generation of naphthoxyl radicals by pulse radiolysis in the absence of GSH indicate that these radicals are unstable, decaying by disproportionation with a rate of $1.2 \times 10^9\ M^{-1}s^{-1}$. The upper limit for the reaction rate of naphthoxyl radicals with GSH was set at $3 \times 10^5\ M^{-1}s^{-1}$ at pH 7.4. This rate would correspond to that obtained with high concentrations of GSH, in a way that it could compete effectively with the faster rate of decay of naphthoxyl radicals; further, this rate is not greatly affected by the subsequent fate of GS·, which differs in oxic or anoxic conditions. The one-electron reduc-

tion potential of naphthoxyl radical/naphthol couple at pH 7 was calculated as +560 mV and that of the GS·/GS⁻ couple was assumed to be greater than +865 mV. Because this reaction is thermodynamically unfavourable, in order to ensure recovery of naphthoxyl radicals, the concentration of GSH needs be very high.[192]

d) A $O_2^{\cdot-}$-dependent conversion of 1-naphthol to naphthoquinone derivatives might be of particular relevance, for the naphthoquinone products described above could further potentiate the 1-naphthol conversion upon $O_2^{\cdot-}$ by redox cycling[193]; a plausible mechanism for the $O_2^{\cdot-}$-dependent activation of 1-naphthol is summarized in the sequence of reactions 44.[194] The first step, the $O_2^{\cdot-}$-mediated conversion of 1-naphthol to naphthoxyl radical, is oversimplified and cannot be envisaged as a direct electron transfer from the substrate to $O_2^{\cdot-}$. The actual mechanism contemplates $O_2^{\cdot-}$ acting as a Brønsted base and is further dismutated to HO_2^- and O_2 as well as naphthol anion. The latter is oxidized by O_2 from the dismutation process to the naphthoxyl radical.[194] Only the overall reaction (ROH + $O_2^{\cdot-} \Rightarrow$ RO· + HO_2^-) is illustrated in the first step in the sequences of reactions 44.



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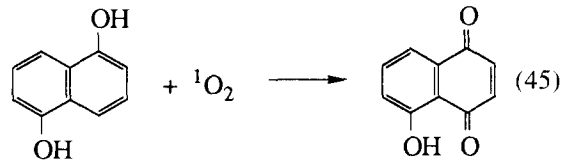
e) Fe-dependent lipid peroxidation of microsomes was found to facilitate the conversion of 1-naphthol to 1,4-naphthoquinone.[195] This pathway was sensitive to desferrioxamine and 6-hydroxy-1,4-dimethyl-carbazol, an inhibitor of lipid peroxidation. Oxygen radicals generated during lipid peroxidation might thus undergo addition reactions with 1-naphthol was subsequent formation of naphthoquinones. Although primarily of photo-chemical and chemical interest, 5-hydroxy-1,4-naphthoquinone or juglone can be generated upon reaction of 1,5-dihydroxynaphthalene with $^1O_2$ (reaction 45), formed upon either thermal splitting of 1,4-dimethylnaphthalene endoperoxide or photosensitization.[196] Thus, $^1O_2$ generated by the recombination of secondary lipid-peroxyl radicals during lipid peroxidation might mediate the conversion of 1-naphthol to 5-hydroxy-1,4-naphthoquinone; the likelihood of such a reaction in biological environments remains, however, to be determined.



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Quinone (di)imines. Quinone (di)imines are nitrogen analogues of quinones where one- (quinone imine) or both (quinone diimine) terminal oxygens are replaced by an imino group (structures **III** and **IV**, respectively, in the Introduction section). At variance with quinones, quinone imines and quinone diimines occur rarely in nature, but they are represented among synthetic antitumour agents[9,11] and as toxic drug metabolites.[12]

Quinone (di)imines as antineoplastic agents. Within the former group, quinone (di)imines displaying in vivo antitumour activity against Sarcoma 180[11] include compounds such as several substituted 1,4-naphthoquinone imines, halogen-substituted 1,4-benzoquinone imines, and *N,N'*-dichloro- or *N,N'*-dibromo-substituted 1,4-benzoquinone diimines with additional substituents (—CH_3, —OCH_3, —NO_2, etc.)[197]; actinomycin D is a quinone imine with activity against human cancer.[198]

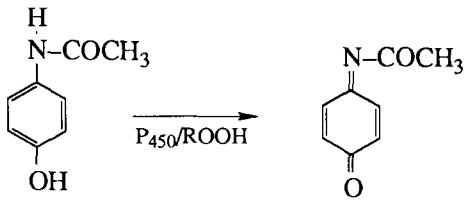
Quinone (di)imines have similar chemical properties as quinones, including their ability to undergo one- and two-electron reduction to a semiquinone free radical and a more stable aminophenol derivative, respectively. In general, quinone imines exhibit a faster rate of one-electron reduction (by NADPH-cytochrome P450 reductase and NADH-cytochrome b_5 reductase) than quinone diimines. The rate of NADPH- or NADH oxidation linked to quinone (di)imine reduction is about 7-fold higher than that observed with simple quinones under similar conditions.[197] Autoxidation is not a common route for semiquinone (di)imines deactivation; for example, 2-amino-1,4-naphthosemiquinone imine and *N*-bromo-1,4-benzosemiquinone imine react rapidly with O_2 with formation of $O_2^{\cdot-}$, whereas *N,N'*-dichloro-2-

chloro-1,4-benzosemiquinone diimine does not. Decay of semiquinone (di)imines through disproportionation or other rapid electron transfer reactions, as well as a high stability of the semiquinone radical, might account for the lack of autoxidation of these species.[197]

Hepatotoxicity and chinese hamster ovary cell toxicity are apparently associated to the rate of reduction (in terms of NADPH oxidation) of the quinone (di)imines but not to $O_2^{\cdot-}$ formation. Overall, there are indications that reactive oxygen species are not a major factor in the growth inhibition by quinone (di)imines. This suggests a wide range of metabolic pathways, which can be ascribed to several quinone (di)imine antineoplastic activities: some of them involve reactive oxygen species formation upon redox cycling, whereas others seem to mediate toxicity exclusively by electrophilic attack.[197] The latter, involving for example reaction with GSH, might be an important mechanism for individual quinone (di)imines such as *N*-acetyl-*p*-benzoquinone imine, but clearly does not apply to every quinone of this type, which might exhibit a distinct chemical reactivity towards other critical cellular necleophiles. Conversely, the ability of quinone (di)imines to cross lipid membranes to reach critical sites within the cell—while still retaining hydrophilicity—is an important feature in determining relative toxicity.[197]

Antitumour quinone imines such as 2-amino-1,4-naphthoquinone imine, *N*,*N*′-dimethyl-indoaniline, and 2-acetamido-*N*,*N*′-dimethylindoaniline are reduced via two-electrons to the less-toxic aminophenol derivatives by partially purified DT-diaphorase. Dicoumarol potentiates the toxic effects of these quinone imines in hepatocytes from phenobarbital-treated rats, but has no effect on hepatocytes from non-induced rats.[199] This led to the conclusion that the effect of DT-diaphorase in protecting cells against quinone imine toxicity was small.

Quinone (di)imines as toxic metabolites. *N*-Acetyl-*p*-benzoquinone imine, formed during the cytochrome P450-mediated oxidation of acetaminophen (reaction 46), has been proposed as the ultimate toxic metabolite in acetaminophen overdosage.[200] The dehydrogenation of acetaminophen to *N*-acetyl-*p*-benzoquinone imine by cytochrome P450 requires cumene hydroperoxide by a mechanism that is clearly different to the cytochrome P450 reactions supported by NADPH. Likely mechanisms contemplate either two-successive one-electron oxidations with formation of intermediate semiquinone species or a concerted two-electron oxidation involving the perferryl form of cytochrome P450.



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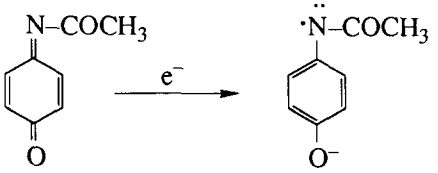
N-Acetyl-*p*-benzoquinone imine can be rapidly reduced back to acetaminophen by various reductants including NADPH, NADH, and GSH. The compound is rapidly reduced *via* two-electrons by partially purified DT-diaphorase; however, dicoumarol potentiates the toxic effect of *N*-acetyl-*p*-benzoquinone imine in hepatocytes from phenobarbital-treated rats, but not in hepatocytes from non-treated rats.[200] The one-electron reduction of the quinone imine by NADPH-cytochrome P450 reductase proceeds at faster rates than the non-enzymic, NADPH-mediated reduction.[201] No ESR signal corresponding to the semiquinone species (reaction 47) formed by the one-electron transfer flavoprotein was found and the free radical, if formed, does not seem to decay upon electron transfer to O_2. Moreover, *N*-acetyl-*p*-benzoquinone imine prevents $O_2^{\cdot-}$ formation during the metabolism of 2,5-dimethyl-*p*-benzoquinone by isolated hepatocytes, probably due to a competitive inhibition of NADPH-cytochrome P450 reductase by the quinone imine.[201] A likely route for semiquinone decay might involve disproportionation to acetaminophen and *N*-acetyl-*p*-benzoquinone imine.



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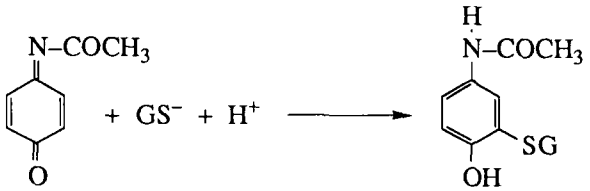
The interaction of *N*-acetyl-*p*-benzoquinone imine with cellular thiols can be summarized in terms of a) electrophilic reaction with GSH yielding a thio-ether adduct (reaction 48); b) oxidation of GSH to GSSG (reaction 49); and c) oxidation and arylation of protein thio groups.[197,202]



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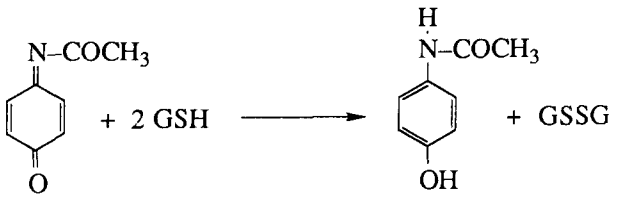


(49)

The pathway in reaction 49 seems to prevail over that in reaction 48, for most of the affected thiol groups during *N*-acetyl-*p*-benzoquinone imine metabolism in hepatocytes can be restored by dithiothreitol.[202] The reaction of *N*-acetyl-*p*-benzoquinone imine with cellular GSH points that part of the intracellular thiol is not readily available for reaction with the quinone imine, probably restricted by compartmentation. The cellular formation of GSSG—by a mechanism as that in reaction 49—is only transient, for it is rapidly reduced back to GSH by glutathione reductase. GSSG accumulation during *N*-acetyl-*p*-benzoquinone imine metabolism by hepatocytes can only be observed upon inhibition of glutathione reductase by 1,3-bis(2-chloroethyl)-1-nitrosourea and, therefore, in cells with a functioning glutathione reductase, only the thioether conjugate of the quinone (reaction 48) is observed.[203] Although *N*-acetyl-*p*-benzoquinone imine can be reduced non-enzymically by NADPH, the oxidation of the pyridine nucleotide during the metabolism of the xenobiotic by isolated hepatocytes can be ascribed to a NADPH-dependent, glutathione reductase-catalyzed reduction of GSSG, inasmuch as this effect is prevented by 1,3-bis(2-chloroethyl)-1-nitrosourea.[202]

Studies with *N*-acetyl-*p*-benzoquinone imine both in a chemical system[204] and with hepatocytes[202] indicate that this compound binds to proteins leading to cross-linking, within a process that could be neither reversed by the addition of dithiothreitol nor increased by the addition of 1,3-bis(2-chloroethyl)-1-nitrosourea, though the latter agent caused a decreased level of protein thiols. *N*-Acetyl-*p*-benzoquinone imine-mediated oxidation of protein thiol groups seems to be linked to alterations of intracellular Ca^{++} homeostasis as a consequence of release of mitochondrial Ca^{++} and inhibition of the high affinity Ca^{++}-ATPase activity of the plasma membrane.[205]

The horseradish peroxidase-catalyzed oxidation of acetaminophen in the presence of GSH leads to the formation of the glutathionyl radical (GS·), which may further dimerize, interact with O_2, or react with GSH. GSSG formation seems not to proceed *via* dimerization reactions, but *via* mechanism(s) dependent on O_2 concentration, such as those involving glutathione disulfide anion radical ($GSSG^{\cdot-}$) or the glutathione peroxysulphenyl radical (GSOO·).[206]

In summary, *N*-acetyl-*p*-benzoquinone imine is subject to redox transitions including solely electron transfer or reductive addition with nucleophiles such as GSH. A four to one partitioning between both processes was found in in vitro systems,[200] though this figure might be altered in vivo, where the concentration of reductants and nucleophiles are continuously in flux and where other enzymes, such as glutathione-S-transferases, may play significant roles in the disposition of *N*-acetyl-*p*-benzoquinone imine.[200]

Cellular detoxication of quinones

In the interest of simplicity, it might be assumed that an early factor determining quinone cytotoxicity would be the partition between a hydrophobic or hydrophilic metabolism of the quinone, primarily controlled by the polarity of the compound.

Hydrophobic metabolism of quinones. Most quinones are relatively hydrophobic compounds and the cellular routes for their detoxication would involve an increase in their hydrophilicity by means of an enzyme-catalyzed conjugation of the corresponding hydroquinones with glucuronate or sulfate. Because the formation of these conjugates is dependent on the presence of a hydroxy group, its chemical requirements would be best encountered by the two-electron reduction of the quinone as that catalyzed by DT-diaphorase or carbonyl transferase. The latter enzyme has been shown as the main quinone reductase in human liver opposed to DT-diaphorase as the main quinone reductase in rat liver.[207] Once the hydroquinone is generated, its participation in subsequent reactions will be influenced by the physico-chemical properties of the hydroquinone and environmental factors. Thus quinones that are good substrates for cellular reductases and have low rate of autoxidation would be easily converted into non-toxic conjugates (upper part of scheme IV).

Hydrophilic metabolism of quinones. GSH reacts with quinones with formation of glutathionyl-hydroquinone conjugates.[43] It is not clear whether this is a detoxication route, because these conjugates autoxidize at higher rates than the corresponding hydroquinones.[45] However, it may be argued that GSH conjugation decreases the number of electrophilic sites in the quinone and, by increasing the hydrophilicity, facilitates the excretion. They are, furthermore, substrates for reductases like DT-diaphorase[54] and may thus close a redox-cycle that leads to the formation of oxygen radicals. It has also been shown that menadione autoxidizes at slower rates when it is bound to membrane[208] and glutathione-conjugation, which makes the qui-

nones more hydrophilic, may thus further enhance the rate of autoxidation (lower part of scheme 5).

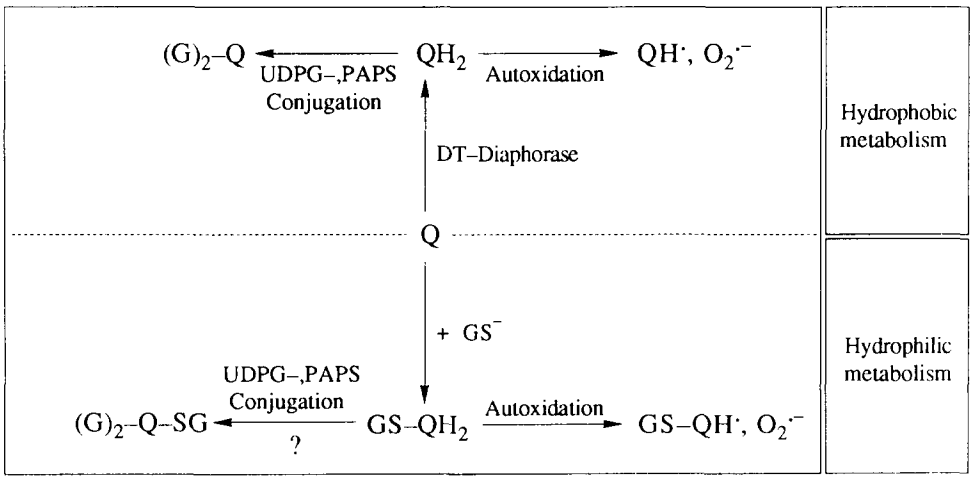
Two-electron- versus one-electron reduction of quinones and their subsequent conjugation. Over the past years NAD(P)H:quinone reductase or DT-diaphorase has been extensively studied in terms of its structure and reaction mechanisms, its biosynthesis and induction, its role as a protective device against the cytotoxicity and mutagenicity of quinone-derived oxygen radicals, and its involvement in vitamin K-dependent protein carboxylation.[15]

It is worth noting that DT-diaphorase occurs in multiple forms in several cellular compartments and that there is strong evidence for a number of DT-diaphorase isoenzymes: for example, two DT-diaphorases, with a "hydrophilic" and a "hydrophobic" character have been separated from mouse-liver cytosol[209]; also multiple species of DT-diaphorase have been demonstrated by means of chromatofocusing of the enzyme purified from the liver cytosol of 3-methyl-cholanthrene-treated rats, work which also revealed that the isoenzyme pattern of DT-diaphorase differed between controls and 3-methyl-cholanthrene-treated rats.[210] The enzyme is induced by a large number of xenobiotics, by a process not involving net increase of newly-synthesized DT-diaphorase[211] and which is controlled by an interaction of two genes.[212] Inducers of DT-diaphorase have been classified in two types: a) flat planar aromatics, such as polycyclic aromatic hydrocarbons, azo-dyes, and β-naphthoflavone, which induce both cytochrome P_1-450 and DT-diaphorase genes and bind to the receptor specified by the Aryl hydrocarbon locus, and b) phenolic antioxidants, such as *tert*-butylhydroquinone, which induce DT-diaphorase but not cytochrome $P_1$450 reductase, the inductive signal depending on the redox lability of the compound.[211] Of note, induction of DT-diaphorase is not always accompanied by increased protection against cytotoxicity.[213]

Based on the complex enzymology and molecular biology of DT-diaphorase, it is not surprising that this enzyme has been ascertained more than one role in cellular metabolism. Thus, a protective role in quinone cytotoxicity has been based primarily on the unique character of the flavoenzyme as a two-electron reductant of quinones, thereby facilitating their terminal conjugation with glucuronide and cell disposition as water soluble products. However, DT-diaphorase may play some role in the metabolism of inorganic compounds and may be involved not only in the detoxification but also in the metabolic activation of carcinogens such as 4-nitroquinoline-*N*-oxide[214] as well as in the reduction of nitro compounds into potent carcinogens.[215,216] Moreover, the two-electron reducing feature of the enzyme would fill the apparent requirements for the activation of bioalkylating agents, thus facilitating the following oxidative elimination and conjugation with cellular nucleophiles (see session on *Reductive Bioalkylation*).[67]

The antioxidant character of DT-diaphorase is based on the conceivable sequence of *two-electron reduction* ⇒ *conjugation* ⇒ *excretion* rationalized for prevention of quinone toxicity and a large body of experimental evidence ranking from in vitro systems, i.e., microsomes, intact cells, and perfused organs, to in vivo systems.

In vitro systems, mainly microsomes from 3-methyl-cholanthrene-treated rats, showed that DT-diaphorase is required for the conjugation of benzo[a]pyrene quinones with glucuronate[18,167] and that dicoumarol potentiates the one-electron reduction of menadione and thereby autoxidation.[208] In a study with rat liver mi-



Scheme 5.

crosomes it was shown that the rate of reduction of benzo[a]pyrene-3,6-quinone was not rate limiting for the formation of glucuronyl conjugates and that more than one enzyme could perform the reduction.[18] In a subsequent study an inhibitory effect on glucuronidation was observed for the DT-diaphorase inhibitor dicoumarol, but this originates from inhibition of a specific UPD-glucuronosyltransferase.[217] Dicoumarol also potentiates menadione cytotoxicity in a) isolated rat hepatocytes, terminally evidenced as an extensive blebbing of the cell surface as a consequence of alterations of intracellular thiol balance and Ca^{++} homeostasis[218,219]; b) embryonic mouse-heart myoblasts[220]; and c) perfused rat liver, evidenced by an increase of low-level chemiluminescence.[221] However, the effect of dicoumarol may involve processes other than DT-diaphorase inhibition, such as mitochondrial uncoupling, thereby potentiating its effects.[219] The one-electron transfer metabolism of the quinones was revealed as mutagenic in the *Salmonella* test, whereas the two-electron reduction by DT-diaphorase was not.[152] Encapsulated DT-diaphorase was shown to protect red blood cells against menadione toxicity.[222]

In vivo, a protective effect of DT-diaphorase against quinone toxicity was shown by using both untreated mice and mice treated with Sudan III, an azo-dye that induces DT-diaphorase, hence increasing their tolerance to streptonigrin.[223,224] Furthermore, preneoplastic hepatocyte nodules, induced in rats by chemical carcinogenesis, contain elevated levels of DT-diaphorase and mRNA,[225–227] which might represent a primary cellular event in the development of resistance to the cytotoxic effects of carcinogens.[228]

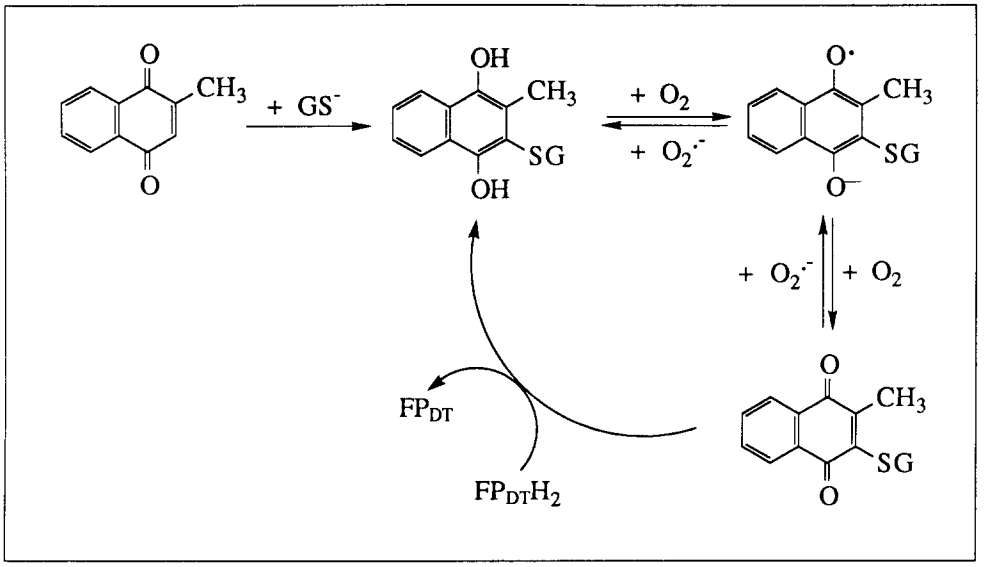
The protective effect of DT-diaphorase seems to extend to those compounds which are metabolized with formation of quinones (see above), as phenol and benzene,[177] benzo[a]pyrene quinones,[229] cathecholamine quinones, and 1-naphthol.[189,191] Phenol-UPD-glucuronosyl-transferase prefers planar phenols as substrates and is inducible by 3-methyl-cholantrene-type inducers.[230]

The role of flavoproteins in the reductive disposition of quinone derivatives has centered the problem of detoxification of various quinoid compounds as a two-electron reduction process *versus* a one-electron reductive process. The former is thought to be facilitated by DT-diaphorase as opposed to NADPH-cytochrome P450 reductase. However, significant amount of hydroquinone species of benzo[a]pyrene-3,6-quinone are formed by NADPH-cytochrome P450 reductase, probably involving disproportionation of the semiquinone species formed. An important aspect of the assessment of these flavoproteins in the disposition of quinone compounds will be the determination of the relative participation of the two reductive mechanisms as well as of the substrate specificity/turnover for particular quinones. Recently, some quinones derived from butylated hydroxytoluene[231] and 3-*tert*-butyl-4-hydroxyanisole[232] have been shown to serve as alkylating agents, thus indicating that mechanisms other than redox cycling might contribute to quinone toxicity. The relative participation of DT-diaphorase and NADPH-cytochrome P450 reductase in the reduction of quinones of polycyclic aromatic hydrocarbons, *tert*-butyl-substituted quinones, and quinone imines indicate differing substrate specificities and V_{max} values for the various quinones and the two flavoprotein-quinone reductases, respectively. The mechanisms of quinone toxicity remain unclear and continued effort is necessary to establish the substrate specificity and K_{cat} of the flavoproteins implicated in quinone reduction.[23]

Formation of glutathionyl-hydroquinone adducts and their possible metabolic routes: antioxidant or prooxidant process? The cellular consequences of the conversion of quinones to glutathione conjugates are determined by the concentration of GSH in a given cell and the dose of the quinoid compound. Hence, depending on the character of this interaction, it could be expected either no significant alterations or a marked depletion of intracellular GSH.

In this context, the toxicity of several methyl-substituted benzoquinones toward isolated rat hepatocytes is directly related to their capacity to react with thiol nucleophiles and consistent with the hypothesis that benzoquinone toxicity is primarily dependent on addition reactions while redox cycling only leads to cell death when antioxidant defenses are impaired.[43] It may be argued that the products of GSH reductive addition to quinones autoxidize rapidly and may participate in redox cycling, but an increase in CN^--resistant respiration was not found in these experiments.

Further, the reaction of quinones with GSH leads to a change in the polarity of the quinone and the glutathionyl conjugates would preferentially distribute in the aqueous cytosolic fraction where ~90% of the DT-diaphorase is located.[15] The glutathionyl conjugates are able to participate in DT-diaphorase-catalyzed reduction and autoxidation reactions as summarized in Scheme 6.[54] Following their reduction by DT-diaphorase, glutathionyl conjugates of 1,4-naphthoquinone, 2-methyl-1,4-naphthoquinone, 5-hydroxy-1,4-naphthoquinone, and 2-methyl-5-hydroxy-1,4-naphthoquinone autoxidize at rates 6-10-fold higher than the parent compounds.[54] However, it remains to be investigated whether glutathionyl conjugation affects the ability of the hydroquinone conjugates to participate in the enzymic processes involving further con-



Scheme 6.

jugation with glucuronide or sulfate (see Scheme 5), thus facilitating their excretion as water soluble products, as found with certain quinones.[167]

In addition to the one-electron reduction of 2-methyl-1,4-naphthoquinone or menadione as a major determinant for its cytotoxicity, other factors such as the rates at which it undergoes either two-electron reduction by DT-diaphorase or 1,4-reductive addition with GSH, can be taken into account. The conjugation of 2-methyl-1,4-naphthoquinone with GSH in vitro[44] was half-maximal at about 1–2 min when the ratio [GSH]/[quinone] was ≅12.5. Thus, there would be two competing reaction pathways that govern the metabolic fate of the quinoid compound: its rapid two-electron reduction by DT-diaphorase (reaction 2) and the 1,4-reductive addition with GSH (reaction 17), the autoxidation product of the latter reaction being a substrate for DT-diaphorase (see scheme 6). The former process would be restrained by both the poor aqueous partitioning of the quinone and lower concentrations of DT-diaphorase associated with intracellular membranes; the latter process would be favoured by the high ratio of thiol to quinone, though restrained by the poor partitioning of the quinone in the aqueous phase.

Glutathione-hydroquinone conjugates, as for example glutathionyl-menadiol, might exert an antioxidant effect by reducing potent oxidizing equivalents such as ferryl-myoglobin. The latter compound is formed during the oxidation of met-myoglobin (Mb^{III} = HX—Fe^{III}) to ferryl-myoglobin (Mb^{IV} = HX—Fe^{IV}—OH) or a ferryl-myoglobin radical (Mb^{IV} = ·X—Fe^{IV}—OH) by hydrogen peroxide.[233] Glutathionyl-menadiol reduces Mb^{IV} to oxymyoglobin ($Mb^{II}O_2$ = HX—Fe^{II}—O_2) or deoxy-myoglobin (Mb^{II} = HX—Fe^{II}) depending whether the assay is carried out under aerobic- or anaerobic conditions, respectively.[234] The two-electron reduction of Mb^{IV} to Mb^{II} or $Mb^{II}O_2$ by glutathionyl-menadiol [HX—Fe^{IV}—OH + GS—Q^{2-} → HX—Fe^{II} + GS—Q + HO^-] requires the quinol moiety as electron donor and it differs from the one-electron reduction of Mb^{IV} effected by either GSH[235] or quinones.[236]

Superoxide dismutase as a superoxide:semiquinone oxidoreductase. Superoxide dismutase is a family of metalloenzymes that play an essential role in protection against oxidative damage by catalyzing the disproportionation of $O_2^{\cdot-}$ ($O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$).[237] At a cellular level, there seems to be a relationship between $O_2^{\cdot-}$-generating quinones and superoxide dismutase activity.[238] The observed inhibition of superoxide dismutase by quinones (i.e., 1,4-naphthoquinone, 1,2-naphthoquinone, and 9,10-phenanthrene quinone, but not by adriamycin and mitomycin C)[239] along with the superoxide dismutase-mediated decrease in O_2 uptake and H_2O_2 production by hepatocytes[48] or microsomes[208] supplemented with menadione, cannot be explained in terms of the classical disproportionation of $O_2^{\cdot-}$ catalyzed by the enzyme.

The autoxidation of hydroquinones, following the two-electron reduction of the quinones by DT-diaphorase, is accompanied by NADPH oxidation in excess over the initial concentration of quinone, O_2 consumption, and H_2O_2 formation. Superoxide dismutase inhibits the autoxidation of hydroquinones in this experimental model by inhibiting these parameters and

altering the distribution and redox status of end molecular products, that is, by enhancing the [hydroquinone]/[quinone] ratio.[240]

This effect is consistent with a superoxide dismutase activity involving the reduction of several semiquinones by $O_2^{\cdot-}$ and which can be termed $O_2^{\cdot-}$-semiquinone oxido-reductase.[240] Its mechanism could be formulated in accordance with current information provided by X-ray[241]

$$\text{SOD-Cu}^{++} + O_2^{\cdot-} \longrightarrow \text{SOD-Cu}^{+} + O_2$$

$$\text{SOD-Cu}^{+} + Q^{\cdot-} + 2H^{+} \longrightarrow \text{SOD-Cu}^{++} + QH_2$$

or quantum chemistry[242,243] studies.

$$\text{SOD-Cu}^{++} + O_2 \longrightarrow \text{SOD-Cu}^{++} \cdots O_2^{\cdot-}$$

$$\text{SOD-Cu}^{++} \cdots O_2^{\cdot-} + Q^{\cdot-} + 2H^{+} \longrightarrow \text{SOD-Cu}^{++} + O_2 + QH_2$$

By either mechanism, a semiquinone can replace the second $O_2^{\cdot-}$. It should be noted that such a mechanism may only apply to those organic compounds which could break down with formation of $O_2^{\cdot-}$. The breakdown of hydroquinones with formation of both $O_2^{\cdot-}$ and semiquinone represents a suitable experimental model for the evaluation of this activity of superoxide dismutase, which does not imply an organic free radical disproportionation, but a mixed function, where $O_2^{\cdot-}$ is still required to react with the copper in the enzyme and subsequently permits the reduction of an alternative organic free radical. However, considering the $E(Cu^{++}/Cu^{+})$ value for superoxide dismutase of +260 mV, the reduction of semiquinones at expense of $O_2^{\cdot-}$ catalyzed by the enzyme would require $E(Q^{\cdot-}/Q^{2-})$ values more positive than that of the Cu^{++}/Cu^{+} couple and in order to compete efficiently with the reduction of $O_2^{\cdot-}$ to H_2O_2 by the enzyme [$E(O_2^{\cdot-}, 2H^{+}/H_2O_2)$ = +940 mV]. For those naphthoquinone derivatives where the $E(Q^{\cdot-}/Q^{2-})$ values are available, such as 1,4-naphthoquinone (+212 mV), 2-methyl-1,4-naphthoquinone (+193 mV), and 2-hydroxy-1,4-naphthoquinone (+107 mV), the reaction seems thermodynamically unlikely, although kinetic factors as the slow and simultaneous formation of $O_2^{\cdot-}$ and semiquinone might alter these relationships.

Independent of the mechanism supporting the inhibition of hydroquinone autoxidation by superoxide dismutase, it might be hypothesized that the joint activities of DT-diaphorase and superoxide dismutase represent a powerful antioxidant mechanism against quinone toxicity: the former enzyme by catalyzing the two-electron reduction of quinones and the latter by preventing the autoxidation of the hydroquinones. Subsequent to their reduction by DT-diaphorase, superoxide dismutase inhibited the autoxidation of several hydroquinones:

a) In the *p-benzoquinone series*, inhibition of autoxidation by superoxide dismutase is observed with hydroxy-*p*-benzoquinones, either un- or methyl-substituted. Superoxide dismutase also inhibits the autoxidation of hydroxy-hydroquinones generated upon either the two-electron enzymatic reduction of 2,3-epoxy-*p*-benzoquinones[240] or nucleophilic addition with GSH,[244] either process followed by re-aromatization and epoxide ring opening.[45] Unlike these hydroxy-*p*-benzohydroquinone derivatives, superoxide dismutase enhances the autoxidation of the unsubstituted *p*-benzohydroquinone as well as that of the mono- and dimethyl-substituted derivatives. This can be rationalized as a displacement by superoxide dismutase of the equilibrium of the autoxidation reactions involving *p*-benzosemiquinones [$Q^{\cdot-} + O_2 \Leftrightarrow Q + O_2^{\cdot-}$] towards the right, thereby making it possible the evaluation of the autoxidation rate of the semiquinone species.

b) In the 1,4-*naphthoquinone series*, superoxide dismutase inhibits the autoxidation of the reduced form of 1,4-naphthoquinone, 2-methyl-1,4-naphthoquinone, 2,3-dimethyl-1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone, 2-methyl-3-hydroxy-1,4-naphthoquinone, and 2,3-dimethoxy-1,4-naphthoquinone. At variance with these naphthoquinones, superoxide dismutase enhances the autoxidation of 1,2-naphthohydroquinone[240] as well as the autoxidation of those hydroxy-naphthoquinones in which the —OH substituent is present in the benzene ring, for example, 5-hydroxy-1,4-naphthohydroquinone (juglone) and 2-methyl-5-hydroxy-1, 4-naphthohydroquinquinone (plumbagin). Although both types of hydroxyquinones, on the one hand, those having —OH substituent in the quinoid ring (e.g., lawsone) and, on the other, those having the —OH substituent in the benzene ring (e.g., juglone), autoxidize at comparable high rates,[54] the effect of superoxide dismutase on their autoxidation is opposite.

Of note, the glutathionyl substitution of *p*-benzo- and 1,4-naphthoquinones does not affect the superoxide dismutase activity towards autoxidation of the parent hydroquinone. Thus, superoxide dismutase inhibits autoxidation of both 1,4-naphthohydroquinone and glutathionyl-1,4-naphthohydroquinone, whereas it stimulates autoxidation of 5-hydroxy-1,4-naphthoquinone and 3-glutathionyl-5-hydroxy-1,4-naphthoquinone.[244]

It is not expected that all semiquinones would be potential substrates for SOD-Cu^{+}. Steric hindrance

factors as well as the reduction potential of the $Q^{\cdot-}/Q^{2-}$ couple will determine the likelihood of this reaction. The geometrical and electronic structure of the copper site is strongly coupled to its formal valence state. Reports on the arrangement of electrostatic charges in SOD-Cu^{++} indicate that sequence-conserved residues create an extensive electrostatic field that directs the negatively-charged $O_2^{\cdot-}$ substrate to the highly positive catalytic binding site at the bottom of the active-site well.[241] Subsequently, reduction of the protein affects the copper site almost exclusively, producing changes in the configuration so that it becomes less symmetric.[245] There might be the possibility that the active site of SOD-Cu^{+} becomes more readily accessible to larger molecules than $O_2^{\cdot-}$, as the semiquinone species shown here.

Following the original discovery of a superoxide dismutase role for erythrocuprein,[237] activities other than the disproportionation of $O_2^{\cdot-}$ have been sought. Firstly, the enzyme seemed to scavenge efficiently $^1O_2$ in an experimental model which did not produce $O_2^{\cdot-}$, thereby the name "singlet oxygen decontaminase".[246] Secondly, superoxide dismutase facilitated the removal of peroxyl radicals[247] by mechanisms implying either a direct catalytic interaction with copper in the enzyme [$ROO\cdot + ROO\cdot \rightarrow ROOH + R(—H) + O_2$ or $ROO\cdot + ROO\cdot + H_2O \rightarrow ROOH + ROH + O_2$] or an indirect effect of superoxide dismutase on the equilibrium concentration of $O_2^{\cdot-}$. Thirdly, it was recently shown that superoxide dismutase catalyzed only poorly the disproportionation of certain semiquinones[248] ($Q^{\cdot-} + Q^{\cdot-} \rightarrow Q + Q^{2-}$) with a rate constant of about $10^6\ M^{-1}s^{-1}$. Lastly, as described above, superoxide dismutase might catalyze the reduction of several semiquinones at expense of $O_2^{\cdot-}$; the requirements for a semiquinone-$O_2^{\cdot-}$ oxidoreductase activity are organic substrates which break down with formation of $O_2^{\cdot-}$, that is, hydroquinones.[240] Thus, it could be expected that the sequential activity of DT-diaphorase and superoxide dismutase might protect selectively against quinone-mediated cell injury.

CONCLUSIONS AND COMMENTS

The physico-chemical properties of a quinoid compound are a function of its chemical structure and functional group chemistry, and they should naturally influence the sequence of events required for the expression of the overall biological activity: cellular uptake, biotransformation, and disposal.

A major determinant in the cellular uptake of quinones would be their polarity, reflected by the partition coefficients, which controls the ability of quinones to cross lipid membranes to reach critical cellular targets, while still retaining their hydrophilic character.[197] It could be speculated that very hydrophobic quinones could be retained for longer times in the lipid phase of the membrane and that this event could be associated with its reduction by membrane reductases. Due to the lack of availability of H^+, the resulting reduced species will be in its anionic form. Given the higher solubility of O_2 in the lipid phase and the more negative reduction potential of the quinone in apolar media, a rapid electron transfer to O_2 could be expected to occur. Conversely, the more negative reduction potential of quinones in apolar media might represent a limiting step for its enzymic reduction.

In interest of simplicity, the biotransformation of quinones may be assumed to contribute to the overall expression of biological activity as a function of a single critical bimolecular chemical reaction between the quinoid compound and a bioconstituent. The nature and extent of this reaction is expected to be a function of the individual physico-chemical properties of both the quinoid compound and the biomolecule. Although the diversity of cellular targets in biological systems does narrow the chemical characterization of a unique bimolecular reaction, attempts have been made to define this single reaction in terms of the redox properties of quinoid compounds encompassing both *sole electron transfer reactions* and *reductive nucleophilic additions*. These redox transitions are not necessarily excluding each other, for quinoid compounds can undergo different biotransformations in parallel.

The detoxication of quinones is conventionally understood in terms of their conjugation with UDP-glucuronate and sulfate. The two-electron reduction of the quinone is a chemical requirement in the course of its conjugation to glucuronide, hence preventing further redox transitions by blocking the carbonyl groups by means of an ester linkage. Thus, the DT-diaphorase-catalyzed reduction of quinones would be expected to precede—and be required—for the disposition of quinones as glucuronide conjugates. The operativity of the conjugation reactions will be apparently determined by those reactions which contribute to decrease the electrophilic character of the quinone, mainly by its conversion to a fully-reduced species, and by the cellular compartmentation of the latter activities in relation to that of UDP-glucuronyl transferases. Thus, the one-electron reduction and the two-electron reductive additions with GSH might prevent further disposition of the quinone species as glucuronate. In the latter instances, however, it remains to be determined whether hydroquinone-glutathionyl adducts could be further conjugated with UDP-glucuronate (see Scheme 5). UDP-glucuronyl transferase is a membrane-bound activity and it could be expected that reduction of quinones at

the membrane surface would be more efficiently followed by formation of glucuronyl conjugates.

The most important inborn physico-chemical property of quinones is their reduction potential and it regulates the redox transitions mentioned above as well as related or subsequent free radical reactions. The reduction potential of quinones is largely influenced by the nature and position of the substituents as well as environmental factors. The inductive- and/or mesomeric character of the substituents affects both the reduction potential and the orientation of the product formed upon nucleophilic addition. In general, substituents with electron-donating character favor electron transfer, whereas those with electron-withdrawing character decrease electron transfer.

Electron-transfer reactions and autoxidation

There is no clear correlation between the rate of enzymic reduction of quinones (either by one- or two-electron transfer processes) and the reduction potential of the quinoid compound.[22,54] A threshold value seems to lie on quinones with very negative reduction potential as 2-hydroxy-1,4-naphthoquinone, which is poorly reduced by NADPH-cytochrome P450 reductase and DT-diaphorase. However, in some instances, a striking correlation between biological activity and chemical reactivity—as modulated through substituent effects—have been observed.[142]

The rate of electron transfer from semiquinones to O_2 is mainly affected by the one-electron reduction potential of the quinoid compounds. At variance with pulse radiolysis studies, the rate of autoxidation following the enzymic reduction of quinones bears no relationship with the reduction potential values. Thus, for pulse radiolysis studies, the rate of autoxidation is inversely related to the one-electron reduction potential, as for example in the series: 5-hydroxy-1,4-naphthoquinone[75] < 1,4-naphthoquinone[24,248] < 2-hydroxy-1,4-naphthoquinone.[93] However, subsequent to the reduction of the above quinones by DT-diaphorase, the following order of autoxidation is found[54]: 2-hydroxy-1,4-naphthoquinone(86) > 5-hydroxy-1,4-naphthoquinone(33) > 1,4-naphthoquinone(1). These observations are further confirmed by the higher toxicity towards isolated hepatocytes of 5-hydroxy-1,4-naphthoquinone than that of 1,4-naphthoquinone and 2-hydroxy-1,4-naphthoquinone.[93] Given the more positive reduction potential of 5-hydroxy-1,4-naphthoquinone and the similar reactivity as 1,4-naphthoquinone towards nucleophiles as GSH, it could be expected that both compounds would exert similar cytotoxic effects. However, the extent of redox cycling with concomitant GSH oxidation is far larger for 5-hydroxy-1,4-naphthoquinone than for the unsubstituted parent compound and 10-fold higher than for 2-hydroxy-1,4-naphthoquinone. In the latter instances, the very negative reduction potential of the quinone might restrain severely its enzymic reduction.

1,4-Nucleophilic addition with GSH and autoxidation

The formation of glutathionyl-substituted hydroquinones upon reaction of GSH with suitable quinones is bound to a loss of the electrophilic character of the quinone and it changes only slightly the reduction potential of the quinoid compound. However,the slight modification created by the —SG substituent may result in a metabolite with an increased chemical reactivity on several accounts: a) the glutathionyl-hydroquinone conjugate autoxidizes at rates far above the parent hydroquinones; b) the autoxidized conjugate can be reduced enzymatically by DT-diaphorase; and c) the —SG substituent, by increasing the hydrophilicity of the quinone, affects its subsequent disposition within the cell. This clearly involves a qualitative change of mechanism and is exemplified in the autoxidation of glutathionyl-1,4-naphthoquinone, following its reduction by DT-diaphorase, which is 16-fold higher than that of the unsubstituted naphthoquinone.[54] Likewise, the nuclephilic addition of GSH to *p*-benzoquinone and 1,4-naphthoquinone, though accompanied only by slight changes in the reduction potential, exerts an increase in the rate of autoxidation of about 8-10-fold.[45,54]

Again, a discrepancy with pulse radiolysis studies is obvious, for the slight changes in reduction potential of 1,4-naphthoquinone upon —SG substitution are accompanied by very small changes in the rate of electron transfer to O_2: 1,4-naphthoquinone[24,248] [$E(Q/Q^{\cdot-}) = -140$ mV; $k(Q^{\cdot-} + O_2) = 1.1 \times 10^8$ $M^{-1}s^{-1}$)] ≅ glutathionyl-1,4-naphthoquinone[92] [$E(Q/Q^{\cdot-}) = -132$ mV; $k(Q^{\cdot-} + O_2) = 1.1 \times 10^8 M^{-1}s^{-1}$)]. This discrepancy might be only apparent, because a) the effect of the cellular milieu on the physico-chemical properties of the quinoid compound and the reactive nucleophile remains vaguely defined and b) the sequence of events between the initial perturbation of a cellular biochemical pathway by this single bimolecular reaction and the observation of a toxic effect might contain several intermediate pathways which are not characterized yet.

The detailed chemical mechanisms by which quinoid compounds exert cytotoxic, mutagenic, and carcinogenic effects have been considered individually and explained in terms of redox cycling, alterations of thiol balance and Ca^{++} homeostasis, and covalent binding. Although not well defined, the cellular com-

partimentalization of quinoid compounds should be a determining factor for their subsequent metabolism. Thus, relatively hydrophilic quinones are assumed to undergo a cellular activation and display different aspects of their chemistry opposite to the more hydrophobic compounds. The overall biological activity observed upon quinone metabolism is expected to correlate partly with the physico-chemical properties of these compounds, mainly their the redox properties, and with a critical bimolecular reaction with cellular target(s).

Acknowledgements—Supported by grants 2703-B89-01XA from the Swedish Cancer Foundation and 4481 from the Swedish Medical Research Council.

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