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BIOREDUCTIVE ACTIVATION OF QUINONES: REDOX PROPERTIES AND THIOL REACTIVITY

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Redox properties and thiol reactivity are central to the therapeutic and toxicological properties of quinones. The use of other physicochemical parameters to establish predictive relationships for redox properties of quinones is discussed, and attention drawn to situations where such relationships may be unreliable. The rates of reaction of semiquinone radicals with oxygen, including those of chemotherapeutic agents such as mitomycin and the anthracyclines, can be predicted with reasonable confidence from the redox properties. The reactions of quinones with thiols such as glutathione produces reduced quinones and radicals, but the reactions are complex and all the features are not well understood.

KEY WORDS: Quinones, semiquinone radicals, thiols, glutathione, superoxide radicals.

INTRODUCTION

Quinones were some of the earliest compounds discovered to form free-radical intermediates upon reduction ('semiquinones') and they play critical roles in electron transport processes in mammals and plants.[1,2] Some chemotherapeutic agents, such as mitomycin-*c*, mitoxantrone and the anthracyclines, are quinones; the radical intermediates of at least some of these are thought to be involved either in their toxicology or their therapeutic action or both.[3] Michael addition of nucleophiles such as thiolate anions to α,β-unsaturated ketones might be expected to be particularly important in the activated case of 1,4-quinones or -quinoneimines not protected by substitution. One of the most biologically-important examples of a quinone-reactive nucleophile is glutathione (GSH), both because of its relatively high concentration (a few millimolar) in many mammalian tissues and the specific catalysis of many glutathione conjugation reactions by ubiquitous glutathione transferases (GSTases).

It is now recognised that reactions of thiols (RSH) with many quinones (Q) are reductive in nature, producing modified quinones (Q-SR) and hydroquinones (QH_2) (see below):

$$2Q + RSH + H^+ \rightarrow Q\text{-}SR + QH_2 \quad (1)$$

Quinones and hydroquinones will always co-exist in equilibrium with semiquinone radicals:

$$Q^{\cdot} + QH_2 \rightleftharpoons Q'H^{\cdot} + QH^{\cdot} \quad (2)$$

and hence thiol reactivity towards quinones may lead to the generation of radicals. One important consequence may be the redox cycling of quinones to generate superoxide ion, and hence potentially damaging hydroxyl radicals *via* the iron-

catalysed Haber–Weiss reaction:

$$QH^{\cdot} \rightleftharpoons Q^{\cdot-} + H^{+} \quad (3)$$

$$Q^{\cdot-} + O_2 \rightleftharpoons Q + O_2^{\cdot-} \quad (4)$$

$$O_2^{\cdot-} + H_2O_2 \xrightarrow{Fe(II)} {}^{\cdot}OH + OH^{-} + O_2 \quad (5)$$

Many of the reactions involved in the interaction of quinones with thiols and of semiquinones with oxygen are fast and require specialised techniques for study, such as stopped-flow spectrophotometry, pulse radiolysis and electron spin resonance (esr) spectroscopy. This paper outlines some of the main features of these reactions, emphasising the use of established concepts in physical chemistry to help predict the magnitude of biologically-important parameters in the interaction of quinones with thiols and oxygen. The reduction potentials of quinone/semiquinone couples, $E(Q/Q^{\cdot-})$ and of semiquinone/hydroquinone couples, $E(Q^{\cdot-}/Q^{2-})$ are of obvious importance in controlling the rate of production and subsequent fate of radicals, either reductively from quinones or oxidatively from hydroquinones. These important properties, too have been best characterised by pulse radiolysis.

REDOX PROPERTIES OF QUINONES

A critical step in the bioreductive activation of quinones is the transfer of a single electron to form the semiquinone, $Q^{\cdot-}$. The rates of such single electron transfer reactions are often dependent upon the energetics of the quinone/semiquinone couple at physiological pH. For many quinones $pK_3 \ll 7$, and in the absence of other prototropic functions with higher pK_a such as ionizable hydroxyl substituents, the mid-point potential will be the same as the standard potential $E^0(Q/Q^{\cdot-})$. Following the application of pulse radiolysis to initiate, and measure the position of, equilibria such as (4) it has been possible to apply this technique to measure the reduction potentials of hundreds of one-electron couples involving unstable free radicals as either reductant or oxidant.[4]

The comproportion equilibrium analogous to (2) between ionized hydroquinone and quinone can have equilibrium constants of the order of unity for simple quinones:[5,6]

$$Q + Q^{2-} \rightleftharpoons 2Q^{\cdot-} \quad (6)$$

Thus at very high pH values, many semiquinones can be considered thermodynamically relatively stable. However, at physiological pH most hydroquinones are dissociated to Q^{2-} to a negligible extent, and $Q^{\cdot-}$ has a short lifetime. There are some notable exceptions where effective semiquinone formation constants are significant even at pH 7,[7,8] and it may not be coincidence that some therapeutically important anthracyclines share the same molecular feature of internal hydrogen bonding with hydroxyl groups.[9] A corollary of this behaviour is that semiquinones are more stable in aprotic solvents such as acetonitrile or dimethylformamide (DMF) than in water, and there are many electrochemical measurements of half-wave reduction potentials of quinones in such solvents.[2]

Whilst such measurements cannot equate to $E^0(Q/Q^{\cdot-})$ in water, their usefulness should not be overlooked. Figure 1 correlates the standard potentials (vs. NHE) in water for methyl-substituted 1,4-benzoquinones with the cyclic voltametric measure-

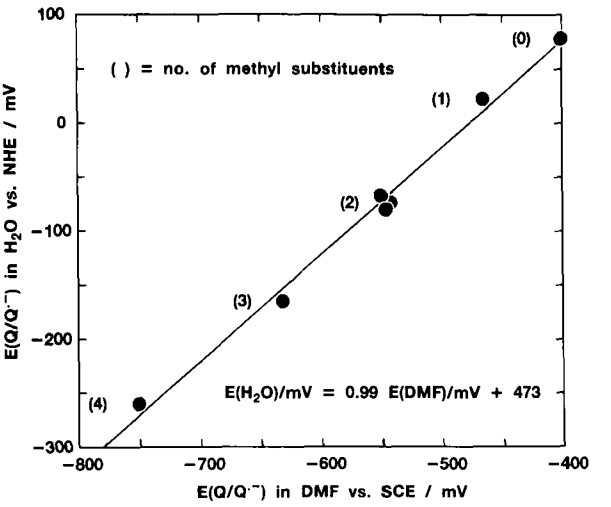

FIGURE 1 Correlation between reduction potentials of methyl-substituted 1,4-benzoquinones in water at pH 7 and in dimethylformamide (DMF).

ments of half-reduction potentials (vs. SCE) in DMF.[4,10] Whilst the numerical values differ (note the SCE standard is 244 mV lower than NHE), the important point is that the correlation line has unit slope (0.99 ± 0.04). About half the intercept or 'off-set' can be accounted for by the differing reference potentials, and the rest reflects unknown liquid junction potentials but especially the solvation energies of the semiquinone anions in the polar solvent, which apparently do not differ in this series sufficiently to cause the slope to deviate from unity.

The corresponding data in Table I for a few representative *p*-quinones suggest that the equation of the straight line in Figure 1 might also be a more general guide to predicting reduction potentials in water, $E^0(Q/Q^{\cdot-})$ for other, more complex quinones *providing* prototropic properties do not interfere. Thus the 'offset' between aqueous and organic potentials in these *p*-quinones is relatively constant at about 0.46 V, and there is no reason to believe that the effects of substituents in other series would be greatly dissimilar to those illustrated in Figure 1 (see below).[11]

Pitfalls in the use of the widely-available electrochemical data to help predict reduction potentials of unknown quinone one-electron couples in water are predict-

TABLE I
Reduction potentials (V) of *p*-quinones in water and DMF $(Q/Q^{\cdot-})$[4,10]

Quinone	H_2O *vs.* NHE.	DMF *vs.* SCE	H_2O-DMF[a]
1,4-Benzoquinone	0.08	−0.40	0.48
1,4-Naphthoquinone	−0.14	−0.58	0.44
9,10-Anthraquinone[b]	−0.39	−0.83	0.44
Juglone	−0.09[c]	−0.39	0.30
Naphthazarin	−0.11[c]	−0.28	0.17

[a]Difference between value in two solvents without correcting for differences in reference potentials (0.24 V).
[b]2—sulphonate salt.
[c]At pH 7.

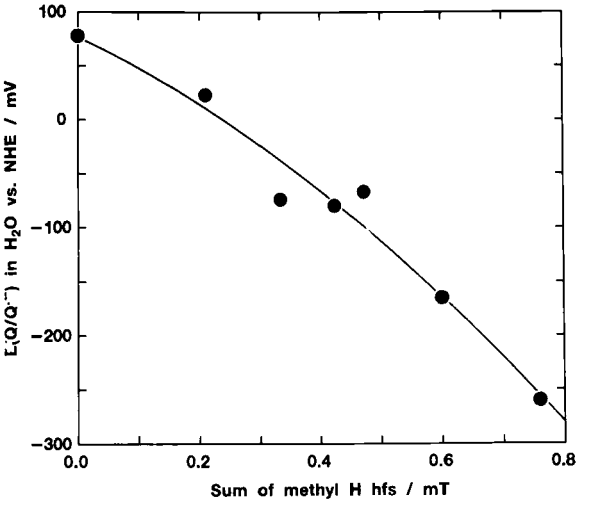

FIGURE 2 Trend between decreasing reduction potentials of methyl-substituted 1,4-benzoquinones with increased spin density on the hydrogen atoms of the methyl substituents, as reflected in the esr hyperfine splitting.

able. The importance of prototropic functions is again illustrated by the comparisons in Table 1 of the potentials for juglone and naphthazarin (5-hydroxy- and 5,8-dihydroxy-1,4-naphthoquinone respectively). Whilst in DMF, the reduction potentials are increased by *ca.* 0.2 and 0.3 V respectively upon substitution of the benzenoid ring in 1,4-naphthoquinone by one (5-) or two (5,8-) hydroxy groups, in water the mid-point potentials of the quinone/semiquinone couples are markedly pH-dependent, and the corresponding substitution only increases the reduction potential by 0.05 or 0.03 V respectively at pH 7.[8] These effects of internal hydrogen bonding have been discussed.[7-10]

Other physicochemical parameters can be used to predict reduction potentials, or more usually difference in analogues resulting from substitution of electron-donating or -withdrawing groups. As with the correlation between reduction potential and the nitrogen hyperfine splitting (hfs) in the electron spin resonance (esr) spectrum of nitroaryl radical-anions (higher N hfs, greater spin density on $-NO_2$, lower reduction potential),[12] so spin density distributions in semiquinones are reflected in differences in reduction potentials. Depew and Wan (see ref.[2]) have discussed how spin densities in semiquinones can be calculated from ^1H or less commonly ^{13}C hfs (^{17}O hfs are rarely obtainable). There are extensive hfs data available for semiquinones.[13] Such calculations or correlations reflecting spin density distributions can range from rigorous and complex to simplistic and trivial. Even the latter may, apparently, be useful for our purposes: such an approach is illustrated in Figure 2, where the reduction potentials of methyl-substituted 1,4-benzoquinones are correlated with the sum of the methyl H hfs (e.g. for duroquinone, $4 \times a^{H}_{CH_3}$). The esr *g*-factor also reflects spin distribution; higher *g*-factors are found for semiquinone radicals than hydrocarbon radicals because of the spin density on the oxygen. This is mirrored in the anomalous chemical behaviour as an oxidant of the 1,3-benzosemiquinone,[14] having a lower *g*-factor than typical semiquinones.

Trends such as those depicted in Figure 2 imply the additivity principle in the Hammett equation:

$$\Delta E \simeq \varrho \Sigma \sigma_p \quad (7)$$

where σ_p is the Hammett *para* substituent constant and ϱ a constant in a single set of analogues. This has been shown by Zuman[11] and others[10] to be useful in correlating shifts in reduction potentials of quinones in aprotic solvents induced by substituents. Prince *et al.*[10] reported $\varrho \approx 0.52$ or 0.36 V for 1,4-benzoquinones or 1,4-naphthoquinones respectively with 2- or 2,3-substituents in DMF. Literature data for 1,4-benzoquinones in acetonitrile also yielded $\varrho \approx 0.53$ V.[10] The unit slope of Figure 1 implies ϱ in water should be similar, and indeed the data in Figure 1 for water at pH 7, if analysed according to eqn. (7) yields a ϱ value of 0.61, comparable to that noted above (0.52) in DMF. Unpublished pulse radiolysis measurements by Dr. I. Wilson (with Dr. G.M. Cohen and the author) of reduction potentials of several 2- or 2,3-substituted 1,4-naphthoquinones in water at pH 7, together with published data,[4] yield a ϱ value of ~ 0.30 V, about that expected from the data obtained using aprotic solvents. The additivity principle breaks down when neighbouring group interactions (e.g. steric inhibition of resonance) is a factor. Thus in water at pH 7, $E(Q/Q^{\cdot -})$ for 2,3-dimethoxy-1,4-naphthoquinone was found by Wilson *et al.* (unpublished work) to be *ca.* 0.06 V *higher* than that for the 2-methoxy analogue (a *lower* value is expected since σ_p (OCH_3) $= -0.28$). A similar positive shift (0.05 V) was found upon substitution of an additional, neighbouring 3-methoxy group in 2-methoxy-1,4-benzoquinone in DMF.[10] Similar effects, and anomalous esr hyperfine interactions, have been reported for methoxy-substituted 1,4-benzoquinones in water.[15] Much greater deviations from Hammett predictions or reduction potential shifts are found on substitution of hydroxy groups capable of internal hydrogen bonding, as expected. Of course, the additivity principle predicts that adding sequential methyl substituents to, e.g. 1,4-benzoquinone should reduce the reduction potential progressively, and that the 3 positional isomers of trimethyl-1,4-benzoquinone should have similar reduction potentials. Both these expectations are confirmed by Figure 1. Similar, 'stepwise' effects of successive methyl substitution has been noted by Swallow (see ref.[1]) upon the effective semiquinone formation constants at pH 7 of 1,4-benzoquinones (log K_2', when concentrations include all prototropic forms). Such effects also reflect the stepwise decrease in the energy gap at pH 7 between the reduction potentials for adding the first and second electron to a quinone, since:

$$E_m(Q/Q^{\cdot -}) - E_m(Q^{\cdot -}/Q^{2-}) = (\mathbf{R}T/\mathbf{F}) \ln K_2' \quad (8)$$

Swallow has discussed these effects in more detail (see ref.[1]).

Some quinoneimines or azaquinones are much more electron-affinic than simpler quinones. Thus 5-aminophthalazine-1,4-dione has $E(Q/Q^{\cdot -}) = 0.24$ V at pH ~ 11.[16] The redox chemistry of some important antitumour benzoquinones and anthracyclines is discussed by Butler and Swallow elsewhere in these proceedings.

REACTION OF SEMIQUINONES WITH OXYGEN

Aside from reduction potentials, arguably the most important property of semiquinones in biology and medicine is the equilibrium (4) with oxygen involving superoxide:

$$Q^{\cdot -} + O_2 \rightleftharpoons Q + O_2^{\cdot -} \quad (4)$$

The position of equilibrium (4) is of course defined by $E(Q/Q^{\cdot -})$ and the relative concentrations of Q and O_2:

$$E(O/O_2^{\cdot -}) - E(Q/Q^{\cdot -}) = (\mathbf{R}T/\mathbf{F}) \ln K_4 \quad (9)$$

Hence if $E(Q/Q^{\cdot -})$ is expressed in volts and given the symbol E the relative *equilibrium* concentrations of semiquinone and superoxide is easily shown to be approximated by the formula:

$$[Q^{\cdot -}]/[O_2^{\cdot -}] \approx ([Q]/[O_2])10^{(17E+2.6)} \quad (10)$$

Of course, although expressions (9) and (10) define $[Q^{\cdot -}]/[O_2^{\cdot -}]$ at equilibrium, the relative availability of semiquinone or superoxide radicals for further reaction depends on the rates of production and removal of the radicals *via* other pathways. As has been discussed elsewhere,[17-20] perturbation of equilibrium (4), for example by removal of $O_2^{\cdot -}$ by superoxide dismutase (SOD) can pull equilibrium (4) to the right even if $K_4 \ll 1$, *i.e.* $E(Q/Q^{\cdot -}) \gg E(O_2/O_2^{\cdot -})$. Thus the production of reduced quinones, QH_2 *via* disproportionation of semiquinones:

$$2Q^{\cdot -} + 2H^+ \rightarrow QH_2 + Q \quad (11)$$

can be efficiently inhibited by oxygen even if the quinone has a higher reduction potential than oxygen, providing other factors are favourable such as superoxide dismutase activity or a low steady-state concentration of semiquinone. Incidentally, most semiquinones can react with SOD, as might be expected from the energetics, but using pulse radiolysis it is easy to show that the reaction is non-catalytic.[21,22]

Quantitative kinetic assessment of such considerations are greatly simplified if it can be assumed that the relaxation time of equilibrium (4) is much faster than the timescale of other reactions involving semiquinone or superoxide. Since in almost all conceivable situations in biology, $[Q^{\cdot -}]$, $[O_2^{\cdot -}] \ll [Q]$, $[O_2]$, the relaxation time τ_4 is given by:

$$\tau_4 \approx (k_{4f}[O_2] + k_{4r}[Q])^{-1} \quad (12)$$

where k_{4f} and k_{4r} are the forward and reverse rate constants. These rate constants are expected, and have been shown,[23] to follow a parabolic dependence of rate constant upon $E(Q/Q^{\cdot -})$, satisfactorily described by the Marcus theory of electron-transfer reactions. Figure 3 shows literature data,[23,24] for electron exchange between semiquinones and oxygen on a Marcus plot. The open symbols refer to simple benzo-, naphtho- and anthra-quinones, whilst the closed symbols refer to more recent data[18,25,26] for more complex, medically-important quinones such as the anthracyclines and mitomycin-*c*. The reactivity of the semiquinone of AZQ with oxygen is close to that expected from the simple Marcus fit to the behaviour of simpler quinones, although the rate constants for reaction of mitoxantrone and other quinones of lower potentials are rather less than the Marcus fit predicts. One might question whether reaction (4) is simple outer-sphere electron-transfer. Regardless of the theoretical predictions, clearly the data in Figure 3 can be used to predict the reactivity of semiquinones with O_2 if $E(Q/Q^{\cdot -})$ is known. For many compounds of interest, with oxygen tensions as low as those which would lead to some radioresistance in radiotherapy (a few micromolar), τ_4 is less than 1 ms even if [Q] is no more than micromolar. However, with (say) 10–20 μmol dm^{-3} SOD present, the half-life for superoxide

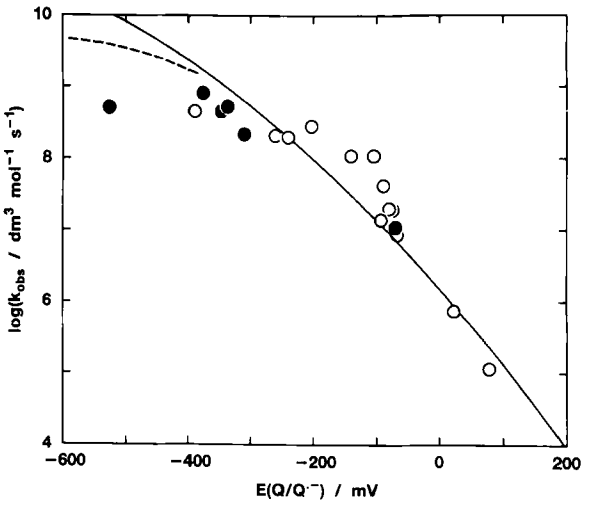

FIGURE 3 Rate constants for reaction of semiquinones with oxygen correlated with reduction potential. Open symbols: simple quinones; closed symbols: chemotherapeutic agents. The solid line is the fit of the Marcus function[23] to all the data, yielding the reorganisation parameter, $\lambda = 77\,\text{kJ mol}^{-1}$, with the dashed line the correction for the rates becoming limited by diffusion rather than by activation.[23]

removal *via* dismutation could be $\sim 20\,\mu s$ and so equilibrium (4) can be pulled to the right even with low O_2 tensions and high $E(Q/Q^{\cdot -})$.

Reduction potentials will obviously be changed from the thermodynamic values in the cellular matrix. Some redox equilibria can be reversed by modest alterations in dielectric constant.[27] However, returning to the comparison earlier of potentials in water and DMF, the potential of the O_2(1 mol dm^{-3})/$O_2^{\cdot -}$ couple is -0.84 V *vs.* SCE in DMF,[28] a value 0.68 V more positive than that in water *vs.* NHE. This shift is higher than that already discussed for 1,4-benzo- and -naphthoquinones, and it thus seems likely that K_4 is changed somewhat by a change in dielectric constant (note the greater solubility of oxygen in lipid than water).

STABILITY OF SEMIQUINONES WITH LEAVING GROUPS

The stabilities of the semiquinones of various potential neoplastic agents which were 1,4-naphthoquinones bearing various leaving groups have been investigated;[29] substituents included halomethyl and acetoxymethyl. Whilst no evidence for unimolecular dissociation of the semiquinones were found on a timescale of tens of milliseconds, radiolytic reduction did release halide from 2-chloromethyl- and 2-bromomethyl-1,4-naphthoquinone. Hence the hydroquinones were potential alkylating agents.

REACTIONS OF QUINONES WITH THIOLS

The Introduction described the reductive nature of the reaction of quinones with thiols, reduction to hydroquinones inevitably leading to semiquinone radical forma-

tion. In a recent study[30] we noted that although the main features of the reactions of thiols with quinones were established many years ago, the kinetics of the reactions had received little attention. Whilst we were able to rationalise the main observations (see below), other studies have not only provided conclusive evidence for free radical formation,[31-33] they have also demonstrated the limitations of the simple reaction scheme[30] by measurement of products other than quinones, hydroquinones and radicals.[34-36]

The reactions of simple 1,4-benzo- or naphtho-quinones with thiols can be summarized as follows.[30] (*i*) If positions 2 or 3 are unsubstituted, nucleophilic attack of thiol upon quinone is likely. (*ii*) The overall stoichiometry of the reaction is (at least with naphthoquinones) two quinone:one thiol. (*iii*) The kinetics of the reaction are first order with respect to both thiol and quinone concentration. (*iv*) The thiolate anion (RS^-) is orders of magnitude more reactive than that of the undissociated thiol, so that around physiological pH, the rate typically increases with pH by about an order of magnitude per pH unit. (*v*) The rate constants typically increases in the order GSH < cysteamine < cysteine and are such that many reactions will occur in seconds at physiological thiol concentrations. (*vi*) The reactions are catalysed by GSTases. (*vii*) Oxidized glutathione (GSSG) can be produced.[33,35,36] (*viii*) Chemiluminescence, possibly from singlet oxygen or quinone excited states, can be observed in oxygenated solutions.[34,35]

Some of these generalisations can be accounted for by a simple scheme. Denoting the thiol conjugate of the oxidized quinone as Q-SR,

$$2Q + RSH + H^+ \rightarrow Q\text{-}SR + QH_2 \qquad (1)$$

we can break down the reaction to two steps, of which the first is slower and rate-limiting:

$$Q + RS^- + 2H^+ \rightarrow (Q\text{-}SR)H_2 \qquad (13)$$

$$(Q\text{-}SR)H_2 + Q \rightleftharpoons Q\text{-}SR + QH_2 \qquad (14)$$

Quinone/hydroquinone exchange (14) is an equilibrium but, at least in the case of menadione, it is over to the right.

Ross[36] discussed the inhibitory effect of catalase on GSSG formation in the menadione/thiol reaction. Certainly superoxide (and hence hydrogen peroxide) can easily result by radical formation *via* (2′):

$$Q\text{-}SR + QH_2 \rightleftharpoons (Q\text{-}SR)^{\cdot} + QH^{\cdot} \qquad (2')$$

and semiquinone reducing oxygen *via* (4). However, the observation of GSSG formation in the reaction of N-acetyl-1,4-benzoquinoneimine (acetaminophen/paracetamol metabolite) with GSH, suggests another possibility.[37]

An alternative route to generating semiquinones not involving disproportionation (2′) would be equilibrium (15):

$$Q + RS^- \rightleftharpoons Q^{\cdot -} + RS^{\cdot} \qquad (15)$$

involving the thiyl radical. The thermodynamics of such electron-transfer equilibria have been discussed in another context.[38] For typical thiols at pH ~ 7, the midpoint potential $E(RS^{\cdot}, H^+/RSH)$ is about 1.0 V,[4] so even in a relatively favourable case such as 1,4-benzoquinone, $\Delta E_{15} \sim E(Q/Q^{\cdot -}) - E(RS^{\cdot}, H^+/RSH) \sim 0.9$ V. Hence K_{15} is $\sim 10^{-15}$ and 5 orders of magnitude lower for menadione. Equilibrium (15) *seems* an

implausible mechanism for generation of semiquinones or GSH oxidation by quinones. It is more likely when $E(Q/Q^{\cdot -})$ is very high, e.g. with tetrachloro-1,4-benzoquinone (chloranil). Radicals were reported many years ago to be produced on mixing chloranil with cysteine.[39] Using (7) we predict $E(Q/Q^{\cdot -}) \sim 0.64$ V so $K_{15} \sim 10^{-6}$ for chloranil. The removal of radicals from equilibrium can easily drive a thermodynamically-unfavourable reaction, as we explained in the aminopyrine/glutathione system.[40]

$$GS^{\cdot} + GS^{-} \rightleftharpoons GSSG^{\cdot -} \quad (16)$$

$$GSSG^{\cdot -} + Q(O_2) \rightarrow GSSG + Q^{\cdot -}(O_2^{\cdot -}) \quad (17)$$

An analogous, unfavourable reaction apparently driven by kinetics was also reported in the interaction of ascorbate and glutathione with a nitrofuran,[41] although reaction as a nucleophile rather than as an electron donor was an alternative possibility.

CONCLUSIONS

It is now possible to build on the wealth of data available, particularly that obtained using pulse radiolysis, to predict some of the properties of quinones central to their role as bioreductive drugs and the oxidative stress which is a characteristic feature of quinone toxicology. The interaction of quinones with thiols, superficially simple, needs further investigation to understand all the features. The distinction between truly hypoxic or anoxic conditions and oxygen tensions such as exist, e.g. in the bulk of tumours or in the liver, is of obvious relevance since an additional complication, not noted above, is the removal of thiyl radicals from equilibria such as (15) by thiyl peroxyl radical formation (18):

$$GS^{\cdot} + O_2 \rightleftharpoons GSOO^{\cdot} \quad (18)$$

in competition with (16), (17).[38]

Other aspects of quinone radical properties and thiol reactivity are discussed in more detail by the distinguished experts at this Symposium: it is hoped this brief overview will provide a stimulus for further discussion.

Acknowledgements

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