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ELECTRON TRANSFER REACTIONS BETWEEN QUINOLS AND QUINONES IN AQUEOUS AND APROTIC MEDIA

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The pathways of redox equilibration of quinols and quinones have been investigated. The rate-limiting reaction involves the couple $QH^-/QH^\cdot$ of the reducing quinol and the couple $Q^{\cdot-}/Q$ of the oxidising quinone. Three general mechanistic points may be surmised: (i) protonation/deprotonation reactions are not rate-limiting; (ii) all transfers occur in one-equivalent steps; (iii) electron transfers, but not hydrogen atom transfers, are the dominant features. In aprotic media, no rapid route of equilibration is available since the ionic species which are necessary for thermodynamically feasible routes of electron transfer cannot exist to any great extent. The relation of these results to models of biological quinone systems is discussed.

Introduction

Quinone redox systems are present in a wide variety of electron transport chains and are used as electron donors, acceptors or redox mediators in many bioenergetic techniques. A knowledge of their physical chemistry would aid experimental design and would provide a means of understanding their biological reactivity and function. Investigations of quinol reduction of cytochrome *c* [1–4] and of ferric iron [5] in solution have shown that the anionic quinol and the anionic semiquinone are the active reductants and that the process is electron transfer and not hydrogen atom transfer. Such a mechanism has been thermodynamically rationalised [3,4]. This report describes a similar approach used to determine the mechanism of equivalent transfer from quinols to quinones in both aqueous and aprotic media. Some conclusions of this study have already been summarised in preliminary form [6].

Materials and Methods

Quinols and quinones. Plastoquinone-1 was prepared by the method of Wood and Bendall [7]. Plastoquinone-9 and ubiquinone-1 were the kind gift of Hofmann-LaRoche Ltd. Other quinones and several quinols were available commercially and were recrystallised when necessary. Other quinols were prepared from the corresponding quinone by a general method: 0.25 g quinone were dissolved in 50 ml diethyl ether (in some cases the quinone concentration was lowered because of the limited solubility of the quinol product in this solvent). A solution of 1 g sodium dithionite in 50 ml water was added and the whole was shaken together in a separating funnel. The quinone could be seen to initially increase in colour due to semiquinone formation and eventually become colourless as full reduction to quinol occurred. The ethereal layer was shaken with a second 50 ml solution of sodium dithionite to ensure completion of the reaction. After removal of the dithionite solution the ethereal layer was washed twice with saturated sodium chloride solution. The ethereal layer containing quinol was then passed through 30 g anhydrous sodium sulphate in a sintered glass funnel, in order to remove water, and the ethereal solution was then dried down by vaccuum dessication at 25°C. Most quinols formed white powders which could be stored

at room temperature in darkness for many months. Quinol solutions were made in acidic ethanol (96% ethanol which contained 10 mM HCl). Most were stable for several weeks when stored at −20°C in this form.

Electron transfer measurements. The initial rates of electron transfer from plastoquinol-1 to ubiquinone-1 in solution were measured spectrophotometrically. Extinction coefficients used were: ubiquinone, 14 $mM^{-1} \cdot cm^{-1}$ at 275 nm; ubiquinol, 3.94 $mM^{-1} \cdot cm^{-1}$ at 290 nm; plastoquinone, 15.2 $mM^{-1} \cdot cm^{-1}$ at 255 nm; plastoquinol, 3.54 $mM^{-1} \cdot cm^{-1}$ at 290 nm. Initial rates of electron transfer from quinols to 2,5-dichloro-*p*-benzoquinone were measured by monitoring formation of the quinol. In general, this was most conveniently measured at 310 nm (with $\epsilon_{mM^{-1} \cdot cm^{-1}}$ of 2.0) where interference from the other quinone system was minimal. For several quinol systems, a correction factor to the extinction coefficient was added. Buffers were as in the figure legends.

n-Hexane was dried over anhydrous calcium sulphate and stock solutions of quinols and quinones were made in diethyl ether for these hydrophobic media experiments.

Nomenclature and calculations. Quinone species nomenclature, thermodynamic calculations and rate constant nomenclature were kept consistent with their usage in Refs. 3 and 4. Rate constant nomenclature included:

$$Q_A^{2-} + Q_B \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} Q_A^{\cdot-} + Q_B^{\cdot-} \quad (1)$$

$$Q_AH^- + Q_B \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} Q_AH^{\cdot} + Q_B^{\cdot-} \quad (2)$$

$$Q^{\cdot-} + O_2 \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} Q + O_2^{\cdot-} \quad (3)$$

Where necessary the subscript 's' was added to the rate constant to indicate a self-exchange reaction, i.e. an electron transfer from one form to another of the same quinone system, e.g.

$$Q_AH^- + Q_A \underset{k_{-5s}}{\overset{k_{5s}}{\rightleftharpoons}} Q_AH^{\cdot} + Q_A^{\cdot-} \quad (4)$$

The following approximated equations, in addition to those of Ref. 4, were also useful:

$$E_0(QH_2/QH_2^{\cdot+}) = E_0(QH_2/Q) + \frac{2.303RT}{2nF}$$

$$\times (pK_B + pK_A - 2pK_s - \log_{10}K_d + 2pK(QH_2^{\cdot+}/QH^{\cdot}))$$

$$E_0(QH^{\cdot}/Q) = E_0(QH_2/Q) - \frac{2.303RT}{2nF}$$

$$\times (pK_B + pK_A - \log_{10}K_d - 2pK_s)$$

$$E_0(QH^{\cdot}/QH^{+}) = E_0(QH_2/Q) - \frac{2.303RT}{2nF}$$

$$\times (pK_B + pK_A - \log_{10}K_d - 2pK_s - 2pK(QH^{+}/Q))$$

$n = 1$ in these formulae and $\frac{2.303RT}{2nF}$ is taken to be 30 mV.

Results and Discussion

The quinone systems chosen for the majority of this study were those of plastoquinone and ubiquinone. The reasons for this were several-fold. Firstly, the species were sufficiently different spectrally so that reaction kinetics could be followed by conventional techniques. Secondly, both reasonably hydrophilic and very hydrophobic derivatives of these systems were available. Thirdly, the relevance of the studies to biological reactions is more emphasised, although it should be noted that these quinones are not behaving in an unusual manner when compared to other quinone systems.

Plastoquinol-1/ubiquinone-1 equilibration in aqueous media

In order to confirm that the reaction observed was simply a second-order reaction between quinol and quinone species, the concentration dependencies of the reaction were determined, and are illustrated in Fig. 1. It may be seen that, up to the concentration limits used, the initial rates of reaction were proportional to both the quinol and the quinone concentrations, i.e. second-order kinetics are exhibited.

The ionic strength dependency of the initial rate of reduction of ubiquinone-1 by plastoquinol-1 is illustrated in Fig. 2. It may be seen that only a small increase in rate occurs on increasing the ionic strength of the reaction medium. This increase is considered to be too small to approximate to an effect on the inter-

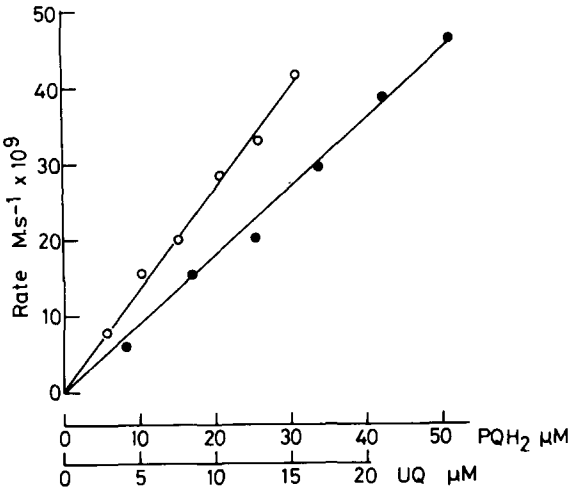

Fig. 1. Concentration dependencies of plastoquinol-1 reduction of ubiquinone-1 in aqueous solution. Reaction medium was 100 mM sodium phosphate with 2 mM EDTA at a pH of 7.54 and at 23°C. Initial rates of reaction were measured as tangents to the decay curves. (○) 17.5 μM plastoquinol-1 and variable ubiquinone-1; (●) 5.2 μM ubiquinone-1 and variable plastoquinol-1.

action of similarly charged species (the initial slope of the plot is less than +0.3). It is therefore concluded that the rate-limiting reaction of quinol reduction of quinone involves at least one uncharged species and

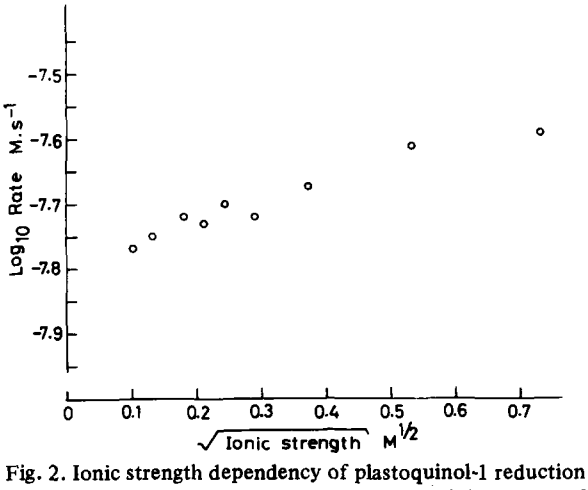

Fig. 2. Ionic strength dependency of plastoquinol-1 reduction of ubiquinone-1 in aqueous solution. The initial rate of reduction of 7 μM ubiquinone-1 by 24 μM plastoquinol-1 was measured as a tangent to the curve. Buffers were 1 mM EDTA with 2.5–10 mM sodium phosphate and appropriate amounts of sodium chloride added to give the desired ionic strength. The pH was adjusted to 7.5 ± 0.05 in each case and temperature was 23°C.

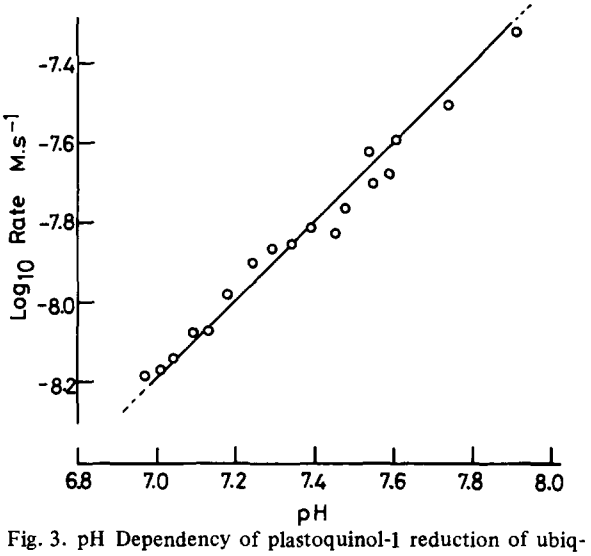

Fig. 3. pH Dependency of plastoquinol-1 reduction of ubiquinone-1 in aqueous solution. Reaction medium was 50 mM sodium phosphate with 2 mM EDTA at 23°C and the pH was varied between 7 and 8. The initial rate of reduction of 7 μM ubiquinone-1 on addition of 24 μM plastoquinol-1 was estimated from a tangent to the decay curve.

that this small ionic strength dependency which is observed is due to a secondary factor.

In order to determine the protonation states of the species involved in the rate-limiting reaction between plastoquinol-1 and ubiquinone-1, a plot of $\log_{10}$ rate vs. pH was constructed in the pH range of 7–8. It may be seen that such a plot (Fig. 3) gives a slope of 1.0 and hence demonstrates that the rate of the reaction is proportional to $[H^+]^{-1}$.

The above results are most easily rationalised in terms of the anionic form of the quinol being the active reductant (cf. quinol reduction of cytochrome *c* [4]). The overall rate of reaction is then governed by the rate constant k_5 (Eqn. 1):

$$\text{Rate} = k_5 \cdot [PQH_2] \cdot [UQ] \cdot K_A \cdot [H^+]^{-1} \quad (5)$$

since when $pH \ll pK_A$,

$$QH^- = \frac{QH_2 \cdot K_A}{[H^+]}$$

(cf. Eqns. 11 and 11a of Ref. 4).

From the data contained in Figs. 1–3, a value for k_5 may easily be calculated for anionic plastoquinol-1 reduction of ubiquinone-1. This value (assuming that

$k_5 = \frac{1}{2}k_{observed}$; see later section) is around $1.3 \cdot 10^5$ $M^{-1} \cdot s^{-1}$ at 23°C. However, inspection of the data listed in Table I of Ref. 4 indicates that the ΔG_0 of this rate-limiting reaction is around +480 mV (given by $E_0(PQH^-/PQH^\cdot)$ minus $E_0(UQ^\cdot/UQ)$) which would give a k_5 value of around 10^{13} $M^{-1} \cdot s^{-1}$. Such a high value is clearly erroneous, since it is greater than the collision rate. A possible explanation for this is that the physical constants for the plastoquinone and ubiquinone systems which are listed in Ref. 4 are significantly in error. However, it is thought unlikely that the ΔG_0 estimate is inaccurate by more than 60 mV and so this cannot be the whole explanation. Instead, it is possible that hydrophobically stabilised complex formation between quinol and quinone occurs in this case such that the observed rate constant is actually a product of several component rate constants.

The ΔG_0 dependency of the rate-limiting step rate constant

The rate-limiting step involves reduction of a $Q^\cdot/Q$ couple by the $QH^-/QH^\cdot$ couple of the donor system at the pH values of these experiments. In order to determine whether a relation existed between rate constant and ΔG_0 of this step, a series of experiments were performed to measure the initial rate of reduction of 2,5-dichloro-*p*-benzoquinone by a variety of *p*-benzoquinol derivatives. The experiments were performed at pH 3.35 so that the reactions were slowed down such that they could be easily measured on a seconds time scale. To go much below this value would require caution, since in many cases contributions to rate from more unusually protonated species began to appear in overall rate. However, at the experimental pH, a knowledge of the quinones physical parameters, obtained from Table 1 of Ref. 4, then allowed the construction of the plot illustrated in Fig. 4. In this figure, the solid line drawn at positive ΔG_0 values has a slope of −60 mV and the experimental points lie close to this line. At negative ΔG_0 values, however, they seem to plateau to zero slope at rate constant values of around $5 \cdot 10^{10}$ $M^{-1} \cdot s^{-1}$. This limit presumably reflects the diffusion-limited maximum value of the rate constant and, since the inflexion is at ΔG_0 values of around 0 mV, it may be deduced that the reaction is always diffusion-limited in the exergonic direction.

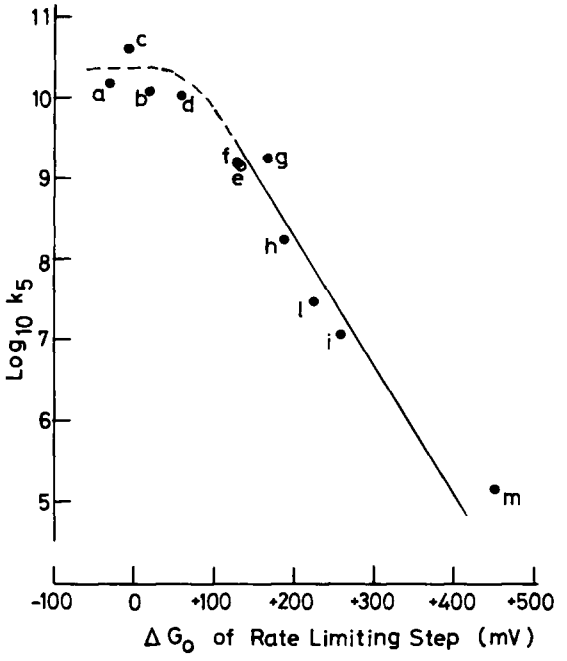

Fig. 4. Relation between ΔG_0 and rate constant of the rate-limiting step of reduction of quinone by quinol. A series of *p*-benzoquinols were used as donors to 2,5-dichloro-*p*-benzoquinone. The reaction medium was 100 mM sodium citrate with 2 mM EDTA at pH 3.35 and 20°C. 100 μM 2,5-dichloro-*p*-benzoquinone was added as acceptor. The rate constant, k_5, was calculated from the initial rate of reduction of quinone on addition of quinol, and assuming that electron flux through rate-limiting step equals half the observed rate of quinone reduction, i.e., $k_5 = \frac{1}{2}k_{observed}$ (cf. Ref. 4). ΔG_0 was given by the formula: $\Delta G_0 = E_0(QH^-/QH^\cdot)_{donor} - E_0(Q^\cdot/Q)_{acceptor}$. Physical data and formulae for these calculations were taken from Ref. 4. Values for phenyl-*p*-benzoquinol were taken as intermediate between hydroquinone and methylhydroquinone values and data for trichloro-*p*-benzoquinol were taken as intermediate between those of 2,5-dichloro- and tetrachloro-*p*-benzoquinol. *p*-Benzoquinol derivatives used were: a, ubiquinol-1; b, plastoquinol-1; c, tetramethyl-; d, trimethyl-; e, thymo-; f, 2,6-dimethyl-; g, 2,3-dimethyl-; h, methyl-; i, unsubstituted; l, phenyl-; m, trichloro-.

The route of the semiquinone electron

The immediate products of the rate-limiting reaction are the protonated semiquinone of the reducing quinol and the anionic semiquinone of the oxidising quinone. These will rapidly equilibrate with the ambient pH to form mixtures of protonated and deprotonated semiquinones according to their pK_s

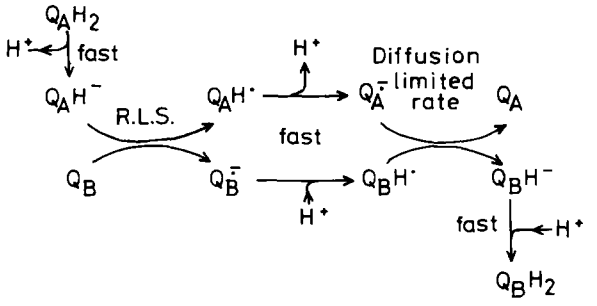

Fig. 5. A major route of equilibration of quinols and quinones in aqueous media. The pathway represents one major route of equilibration on addition of quinol to quinone in aqueous solution at the pH values used in this study. R.L.S., rate-limiting step.

values. Many routes of electron transfer then become possible and the reactions will be complex. Some major routes will include: cross dismutation in a k_{-4} or a k_{-5} type of reaction; self dismutation in a k_{-4s} or a k_{-5s} reaction; reduction of quinone species – rate constants for reduction of quinone by anionic semiquinones have already been measured by an independent technique [8]; reduction of molecular oxygen to superoxide anions, particularly in a k_6 type reaction.

For the plastoquinone system $E_0(Q^\tau/Q) = -165$ mV and for the ubiquinone system $E_0(QH^-/QH^\cdot) = +191$ mV [4]. Since reduction of $UQH^\cdot$ by PQ^τ is a k_{-5} type reaction which is diffusion-limited in the exergonic direction (Fig. 4), it follows that the reaction will be diffusion-limited and will be a major route of completion of the reduction of ubiquinone-1 by plastoquinol-1, at least at pH values close to the semiquinone pK_s. Such a route has been included in Fig. 5, which is a summary of a major route of quinol and quinone equilibration in aqueous solution. Clearly, other possible reactions may occur, the relative magnitudes of which being dependent on pH, ionic strength, concentrations and the physical properties of each individual quinone system.

Plastoquinol-9/ubiquinone-10 equilibration in n-hexane

In order to determine whether the reaction route could be significantly different in aprotic media, the equilibration between the highly hydrophobic plastoquinol-9 and ubiquinone-10 in dry *n*-hexane was studied and compared to the aqueous system mechanism. When 135 μM plastoquinol-9 was added to 85 μM ubiquinone-10, however, no significant rate of reaction between quinol and quinone could be measured and hence the rate constant was too small to be determined. Saturation of the *n*-hexane with water made no detectable difference to this result. To ensure that the lack of reaction was not caused merely by a change in the equilibrium constant of the system by selective alteration of the physical properties of one of the reactants, there reverse reaction of addition of 90 μM ubiquinol-10 to 95 μM plastoquinone-9 was also examined. Again, no detectable reaction occurred and hence the experiment confirmed that the 'stability' of the system was kinetic rather than thermodynamic. A similar experiment was performed with duroquinol as the reductant and 2,5-dichloro-*p*-benzoquinone as the oxidant. In this case, the equilibration rate was measurable and was equivalent to a rate which would have been observed in an aqueous system at a pH of around 2.5.

The results may be rationalised with reference to the aqueous system mechanism- in order to reach a thermodynamically viable route, deprotonation to form the anionic quinol must occur. In hydrophobic media, such ionic species are rare and so equilibration becomes slow. The slowness of the observed rates of equilibration indicate that hydrogen atom transfer routes are still not a fast equilibration mechanism.

Thermodynamic and mechanistic considerations

As has already been demonstrated for the reaction between quinols and cytochrome *c* in solution, the anionic quinol, QH^-, is a much better reductant than the uncharged quinol, QH_2. This has been rationalised thermodynamically in terms of the calculated potentials of the appropriate electron transfer couples. For example, one may calculate for the plastoquinone system that $E_0(QH_2/QH_2^+) > +870$ mV (using the physical data given in Ref. 4 and assuming that $pK(QH^\cdot/QH_2^+) < 0$), see also Ref. 7, whereas $E_0(QH^-/QH^\cdot) \simeq +240$ mV [4]. Similarly, for the plastoquinone system, whereas $E_0(QH^\cdot/QH^+) > +160$ mV (from Ref. 4 and assuming that $pK(Q/QH^+) < 0$), $E_0(Q^\tau/Q) \simeq -165$ mV. Although the deprotonated species may be only a small fraction of the total at pH values below pK, the rate constant is very high and the protonation/deprotonation equilibrium is

rapid. The routes of equilibration within the quinol/quinone system may be rationalised in an analogous way.

Several essential features of the mechanism outlined in Fig. 5 should be emphasised: (i) the overall two equivalent transfer occurs in one equivalent steps. Such a phenomenon was originally recognised by Michaelis [9] and was later elaborated upon by Clark [10]; (ii) the protonation/deprotonation reactions are never rate-limiting. A useful disucussion of this point has been given by Brocklehurst and Dixon [11]; (iii) all transfers are electron transfers rather than hydrogen atom transfers. With such a postulate, the behaviour of quinone systems in aqueous and hydrophobic media is explained. It reflects the very large activation energy which must be involved in the making and breaking of the hydrogen atom covalent bond.

The generality of such one equivalent electron transfer reactions in overall two equivalent hydrogen atom transfers is of interest. In NADH dehydrogenase reactions, for example, hydride or hydrogen atom transfers are implicated [12], although in electrodic reactions of NADH/NAD^+, one-equivalent electron transfer steps have recently been considered [13]. The balance between electron, hydrogen atom and hydride transfers is a subject which will have many mechanistic ramifications.

Implications for biological systems

The results imply that quinone systems are only reactive if they are in a situation where ionic species are feasible. Such a restriction suggests that the biological membrane in which the quinones occur may initially be considered as a two phase system – that of the membrane interfaces where ionic species and hence electron transfers may occur and that of the aprotic membrane interior where the quinols are essentially inactive in electron transfer. Such a notion implies that, in order for transmembrane proton movements to occur during electron transport, the quinones and quinols must per se physically move across the membrane. The alternative notion of hydrogen atom movement in a 'bucket brigade' manner [14] would require a hydrophobic quinol-quinone transhydrogenase activity or at least internally hydrated sites of some sort within the membrane.

Finally, it may be noted that a system which has reactive species at the membrane surfaces and inactive species in the membrane interior forms an ideal tightly coupled hydrogen atom carrying arm of a 'protonmotive loop' as originally envisaged by Mitchell [15].

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