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THE KINETICS AND THERMODYNAMICS OF THE REDUCTION OF CYTOCHROME *c* BY SUBSTITUTED *p*-BENZOQUINOLS IN SOLUTION

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Summary

1. The mechanisms by which *p*-benzoquinol and its derivatives reduce cytochrome *c* in solution have been investigated.

2. The two major reductants are the species QH^- (anionic quinol) and $Q^{\cdot -}$ (anionic semiquinone). A minor route of electron transfer from the fully protonated QH_2 species can also occur.

3. The relative contributions of these routes to the overall reduction rate are governed by pH, ionic strength and relative reactant concentrations.

4. For a series of substituted *p*-benzoquinols, the forward rate constant, k_1, of the anionic quinol-mediated reaction is related to the midpoint potential of the $QH^-/QH^\cdot$ couple involved in the rate-limiting step, as predicted by the theory of Marcus for outer-sphere electron transfer reactions in a bimolecular collision process.

5. A mechanism for the biological quinol oxidation reactions in mitochondria and chloroplasts is proposed based upon the findings with these reactions in solution.

Introduction

In mitochondria and chloroplasts, the oxidation of quinols by their physiological acceptors are reactions of some interest. The potentially proton motive

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Abbreviations: The term hydroquinone is used synonymously with *p*-benzoquinol throughout this work; menaquinol (menadiol) is a common name for 2-methyl-1,4-naphthoquinol; EDTA, ethylenediamine tetraacetate.

function of the quinols has already been noted [1] and regulation of the rate of plastoquinol oxidation as a means of photosynthetic control has been suggested [2–4].

The mechanism of electron transfer from quinol to cytochrome is, however, unclear. For example, it has been pointed out that the potential of the quinol/semiquinone couple is very high, which would make a significant reaction rate with acceptor difficult. In recognising this problem, a number of workers have envisaged a 'quinone binding' site or protein [5–7] such that this potential may be lowered, although it should be noted that any such model must account for the electron-redistributing capacity of the quinone pool [8,9].

We have recently proposed an alternative model for quinol oxidation [10] based on studies of reduction of cytochrome *c* by menaquinol (menadiol) in solution. The biological mechanism must clearly be different from the semiquinone-mediated reduction of cytochrome *c* by *p*-benzoquinol (hydroquinone) [11] or the trimolecular complex-mediated reduction of cytochrome *c* by catechol [12] and we have instead proposed that reduction occurs via the deprotonated quinol, QH^-, in a bimolecular collision reaction. The work reported here is intended to extend these ideas.

Materials and Methods

Quinol substrates

Hydroquinone, 2-methylhydroquinone, trimethylhydroquinone and tetrachlorohydroquinone were obtained commercially and recrystallised when necessary. Other quinols were prepared from the commercially available quinones by a previously described method [13]. Ubiquinone-1 was the kind gift of Dr. C.I. Ragan, Southampton University and of Hofmann-LaRoche Ltd. and plastoquinone-1 was prepared by the method described by Wood and Bendall [14]. Stock solutions of quinols were made up in acidic ethanol (HCl) to minimise autoxidation and were stored in the dark at −20°C. The majority of quinol solutions were stable for several weeks under these conditions.

Reaction kinetics

The reduction of cytochrome *c* (Sigma Type III, from horse heart) was monitored with a Cary 219 spectrophotometer at 550 nm and using an extinction coefficient of 19 $mM^{-1} \cdot cm^{-1}$ [15]. The initial rates of cytochrome *c* reduction on addition of quinol were measured as a tangent to the curve. Unless otherwise stated, reaction buffer was 10 mM 2-(*N*-morpholino)ethanesulfonate, 90 mM NaCl and 1 mM EDTA at a pH of 6 and 20°C and which had been made anaerobic with a flow of nitrogen gas. Initial ferric cytochrome *c* concentration was between $5 \cdot 10^{-6}$ and $20 \cdot 10^{-6}$ M.

Nomenclature, abbreviations and calculation of midpoint potentials of couples

Symbols used are: E_0, standard potential at pH 0; $E_{m,x}$, standard potential at pH x. Quinol and cytochrome species nomenclature is as in Ref. 10.

For calculation of the E_0 values of various couples the following relations, which may be derived from standard formulae, have been employed:

$$E_0(\mathrm{QH_2/QH^{\cdot}}) = E_0(\mathrm{QH_2/Q}) + \frac{2.303RT}{2nF}\,(\mathrm{p}K_\mathrm{B} + \mathrm{p}K_\mathrm{A} - 2\mathrm{p}K_\mathrm{S} - \log_{10}K_\mathrm{d})$$

$$E_0(\mathrm{QH^-/QH^{\cdot}}) = E_0(\mathrm{QH_2/Q}) + \frac{2.303RT}{2nF}\,(\mathrm{p}K_\mathrm{B} - \mathrm{p}K_\mathrm{A} - 2\mathrm{p}K_\mathrm{S} - \log_{10}K_\mathrm{d})$$

$$E_0(\mathrm{Q^{\cdot-}/Q}) = E_0(\mathrm{QH_2/Q}) - \frac{2.303RT}{2nF}\,(\mathrm{p}K_\mathrm{B} + \mathrm{p}K_\mathrm{A} - \log_{10}K_\mathrm{d})$$

where symbols represent:

$$\mathrm{QH_2} \overset{\mathrm{p}K_\mathrm{A}}{\rightleftharpoons} \mathrm{QH^-} + \mathrm{H^+} \tag{1}$$

$$\mathrm{QH^-} \overset{\mathrm{p}K_\mathrm{B}}{\rightleftharpoons} \mathrm{Q^{2-}} + \mathrm{H^+} \tag{2}$$

$$\mathrm{QH^{\cdot}} \overset{\mathrm{p}K_\mathrm{S}}{\rightleftharpoons} \mathrm{Q^{\cdot-}} + \mathrm{H^+} \tag{3}$$

$$\mathrm{Q^{2-}} + \mathrm{Q} \overset{K_\mathrm{d}}{\rightleftharpoons} \mathrm{Q^{\cdot-}} + \mathrm{Q^{\cdot-}} \tag{4}$$

$n = 1$ in these formulae.

Rate constants used for the electron transport reactions are as follows:

$$\mathrm{QH^-} + \mathrm{H^+cyt}c^{3+} \overset{k_1}{\rightleftharpoons} \mathrm{QH^{\cdot}} + \mathrm{H^+cyt}c^{2+} \tag{5}$$

$$\mathrm{Q^{\cdot-}} + \mathrm{H^+cyt}c^{3+} \overset{k_2}{\rightleftharpoons} \mathrm{Q} + \mathrm{H^+cyt}c^{2+} \tag{6}$$

$$\mathrm{QH_2} + \mathrm{H^+cyt}c^{3+} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{QH_2^{\cdot+}} + \mathrm{H^+cyt}c^{2+} \tag{7}$$

and for dismutation:

$$\mathrm{Q^{2-}} + \mathrm{Q} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \mathrm{Q^{\cdot-}} + \mathrm{Q^{\cdot-}} \tag{8}$$

where $K_\mathrm{d} = k_4/k_{-4}$.

$\dfrac{2.303RT}{2nF}$ is taken to be 30 mV in potential calculations.

Results

The autocatalytic and non-autocatalytic reduction of cytochrome c by hydroquinone

As has already been demonstrated by Yamazaki and Ohnishi [11], the reduction of cytochrome *c* by hydroquinone at pH 7 is an autocatalytic process and is predominantly mediated by the semiquinone anion, $Q^{\cdot -}$. The autocatalysed reaction rate depends upon $[H^+]^{-2}$ and is stimulated by the presence of small amounts of quinone in the reaction mixture (Fig. 1). If, however, the reaction is performed at lower pH, conditions can be obtained where no autocatalysis is observed, the reaction depends on $[H^+]^{-1}$, and is not stimulated by the presence of small amounts of quinone. Such a situation is illustrated in Fig. 2.

This difference in behaviour may be understood when one considers the possible routes of cytochrome reduction by the quinol system. At pH 7, the dominant process rapidly becomes reduction via the semiquinone anion (Eqn. 6).

If the reduction were rate limiting, then observed rate would be:

$$\text{Rate} = k_2[Q^{\cdot -}][H^+\text{cyt}c^{3+}] \tag{9}$$

$$= k_2[H^+\text{cyt}c^{3+}]\sqrt{\frac{[QH_2][Q]\cdot K_d}{1+[H^+]/K_B+[H^+]^2/K_AK_B}} \tag{9a}$$

In practice, however, the rate appears to be limited by the rate of dismutation i.e.

$$\text{Observed rate} = k_4[Q^{2-}][Q] \tag{10}$$

$$= \frac{k_4[QH_2][Q]}{1+[H^+]/K_B+[H^+]^2/K_AK_B} \tag{10a}$$

where $[QH_2]$ is the total quinol added. Hence, the rate depends on $[H^+]^{-2}$ at around neutral pH values and is proportional to quinone concentration. We calculate from the gradient of Fig. 2B that $k_4 = 6.3 \cdot 10^8\ M^{-1} \cdot s^{-1}$ under our conditions, which is close to the value of $2.6 \cdot 10^8\ M^{-1} \cdot s^{-1}$ reported by Yamazaki and Ohnishi (termed k_d in their nomenclature) under slightly different conditions [11].

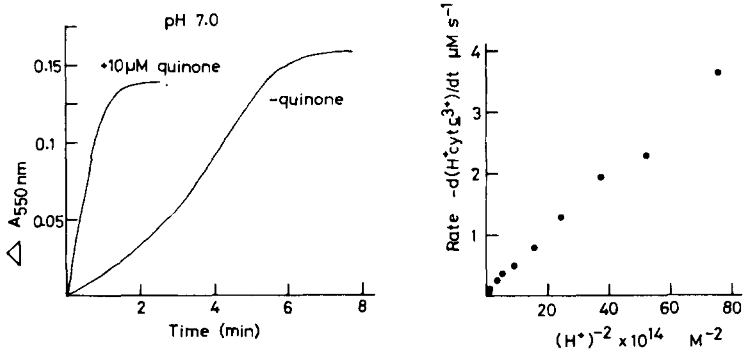

Fig. 1. The autocatalytic reduction of cytochrome *c* by hydroquinone. The reaction mixture consisted of anaerobic 10 mM sodium phosphate, 90 mM NaCl and 1 mM EDTA at a pH of 7.0 and 20°C and with oxidised cytochrome *c* at a concentration of $16.2 \cdot 10^{-6}$ M. The reaction was initiated by addition of $400 \cdot 10^{-6}$ M hydroquinone. In Fig. 1B, the initial rate of reduction in the presence of $10 \cdot 10^{-6}$ M benzoquinone, on addition of $400 \cdot 10^{-6}$ M hydroquinone was determined as a function of pH (range 6.8—7.94). Other conditions were as for Fig. 1A. The plot of rate vs. $[H^+]^{-2}$ is linear over the pH range used.

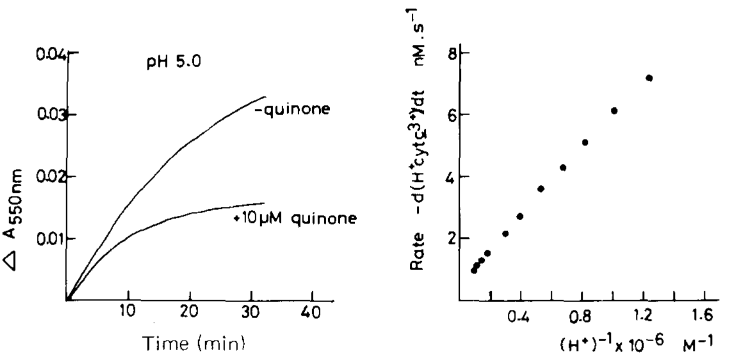

Fig. 2. The non-autocatalytic reduction of cytochrome *c* by hydroquinone. The reaction mixture consisted of anaerobic 10 mM 2-(*N*-morpholino)ethanesulfonate, 90 mM NaCl and 1 mM EDTA at a pH of 5.0 and 20°C and with $16.2 \cdot 10^{-6}$ M oxidised cytochrome *c* added. The reaction was initiated by addition of $3.85 \cdot 10^{-3}$ M hydroquinone in the presence or absence of $10 \cdot 10^{-6}$ M benzoquinone. In Fig. 2B, the initial rate of reduction of cytochrome *c* on addition of $2 \cdot 10^{-3}$ M hydroquinone (in the absence of benzoquinone) is plotted as a function of $[H^+]^{-1}$ (pH range 6.09—4.95). Other conditions were as in Fig. 2A. The plot of rate vs. $[H^+]^{-1}$ is linear over the pH range tested.

At lower pH values, the dominant process can be reduction via the anionic quinol, QH^- (Eqn. 5).

$$\text{Initial rate} = k_1[QH^-][H^+cytc^{3+}] \qquad (11)$$

which approximates, at pH values below neutrality, to

$$\text{Initial rate} = k_1[QH_2][H^+]^{-1} \cdot K_A \cdot [H^+cytc^{3+}] \qquad (11a)$$

As the pH of the reaction is lowered, the rate of the semiquinone-mediated process decreases by a factor of 10^2 for each pH unit, whereas the anionic quinol-mediated process decreases by only a factor of 10 for each pH unit decrease. Hence, a pH will be reached, dependent on experimental conditions and relative rate constants, where the process loses its dominantly autocatalytic character. Under our conditions and concentrations, this change occurs between pH 5 and 7 in the hydroquinone-cytochrome *c* system.

It may be noted in Figs. 1 and 2 that the plots of rate vs. $[H^+]^{-1}$ and $[H^+]^{-2}$ should pass through the origin, given that no pH-independent reactions are occurring. In the case of the plot of the non-autocatalytic rate vs. $[H^+]^{-1}$ this is clearly not the case and a small rate remains after extrapolation to $[H^+]^{-1} = 0$. This is presumably caused by the pH-independent contribution of the reaction of the fully protonated quinol (Eqn. 7).

From the intercept of the plot of Fig. 2B we calculate that $k_3 = 0.013\ M^{-1} \cdot s^{-1}$ and from the gradient that k_1 (observed) $= 1.8 \cdot 10^3\ M^{-1} \cdot s^{-1}$. The plot of rate vs. $[H^+]^{-2}$ in Fig. 1B essentially passes through the origin since the pH-independent k_3 reaction becomes an insignificant fraction of the total rate at these pH values.

As we have already discussed in relation to the menadiol system [10], the anionic quinol-mediated process is of direct relevance to the biological events, since this is the possible biological reaction route. The subsequent experiments have been performed under conditions where the process is non-autocatalytic and where the dominant reaction is via the anionic quinol.

Ionic strength effects on the measured k_1 value of reduction of cytochrome c by anionic benzoquinol, BQH^-

As has been previously noted [16,17,10], the preceding experiment does not distinguish the electron transfer process described by Eqn. 5 from an H-atom transfer process:

$$QH_2 + cytc^{3+} \rightleftharpoons QH^{\cdot} + H^{+}cytc^{2+} \qquad (12)$$

In order to distinguish these possibilities we looked at the effect of ionic strength on the rate of reaction under conditions where the anionic quinol-mediated process was dominant. The result is illustrated in Fig. 3A. It may be seen that an increase in ionic strength caused a marked decrease in reaction rate, a result which indicated that the collisions were between oppositely charged species i.e. the reductant is the charged BQH^- and not the neutral BQH_2, and hence the reaction is an electron transfer rather than an H-atom transfer process.

After subtraction of the pH-independent, QH_2-mediated rate from this process (to calculate this, we used a k_3 value of $0.013\ M^{-1} \cdot s^{-1}$, a value which was found to be ionic strength-independent), we were able to plot $\log_{10}$ rate vs. $\sqrt{I}$ for the QH^--mediated rate (Fig. 3B). At least for the low ionic strength part of this plot, the slope of this line is given by the Debye-Hückel formulation of $-1.018 \cdot z_{\text{donor}} \cdot z_{\text{acceptor}}$ [18]. In the plot in Fig. 3B the low ionic strength part of the plot was a straight line and gave a value of around +1 for $z_{H^+cytc^{3+}}$, i.e. only a single positive charge on the cytochrome is involved in the complex formation, given that $z_{QH^-} = -1$.

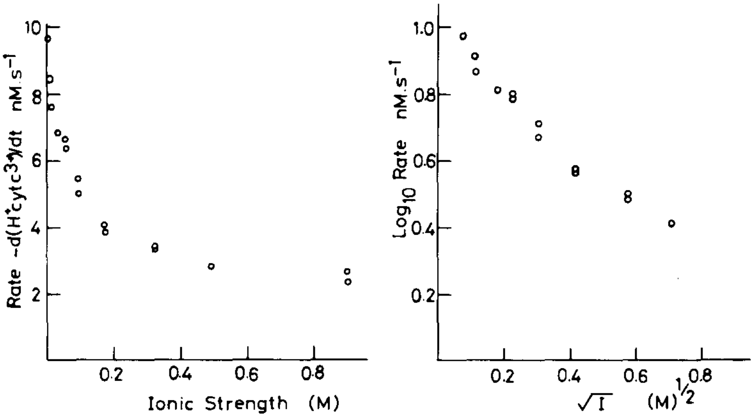

Fig. 3. The ionic strength dependency of the rate of reduction of cytochrome c by anionic quinol. The reaction mixture consisted of anaerobic 5 mM 2-(N-morpholino)ethanesulfonate and 1 mM EDTA at a pH of 6.07 and 21°C, together with $17.76 \cdot 10^{-6}$ M cytochrome c and enough NaCl to make up the desired ionic strength. The initial rate of reduction of cytochrome c was measured as a tangent to the curve on addition of 10^{-3} M hydroquinone.

Measurement of k_1 values for a series of substituted p-benzoquinols

A series of methylated *p*-benzoquinols was used for these studies together with several other derivatives with significantly altered thermodynamic properties. The initial rate of cytochrome c^{3+} reduction on addition of quinol at pH 6 was measured as a tangent to the curve, and since no back reactions were occurring at this initial point, the observed k_1 could be calculated from Eqn. 11 and the fact that $[QH^-] \simeq [QH_2] \cdot 10^{pH-pK_A}$ at the pH of these experiments.

For several of the lower potential quinols a very small but rapid initial rate of cytochrome *c* reduction could be observed on addition of quinol, before the reaction settled into the usual roughly first-order decay reduction process. This rapid phase could be removed by addition of superoxide dismutase and hence is presumably an artifact caused by small residual amounts of oxygen left in the system. This phase was not used for calculation of k_1 values.

The above method was not applicable to the halogenated quinols since at pH 6 the reaction was still significantly autocatalytic and at lower pH values the overall equilibrium constant was such that the reaction did not proceed to a significant extent. Hence, in these cases an alternative technique was used. The reaction medium at pH 3—4 contained initially cytochrome *c* and $200 \cdot 10^{-6}$ M tetramethyl-*p*-benzoquinol (duroquinol). The rate of reaction of this quinol with the cytochrome was very small in this pH range. Halogenated quinol was then added. The reaction proceeded rapidly since, although the rate constant of the reaction was lower than with tetramethyl-*p*-benzoquinol, the pK_A values of these derivatives were much lower and hence the concentrations of QH^- were much higher. The tetramethyl-*p*-benzoquinol, however, kept the chlorinated quinol in the fully reduced state such that a linear reaction rate proceeded for a measurable time and from this rate an observed k_1 value could be obtained. Fig. 4 illustrates this catalytic capacity of the halogenated quinols.

As will be demonstrated in the next section, observed k_1 values obtained in these experiments are actually double the true k_1 values. The accumulated

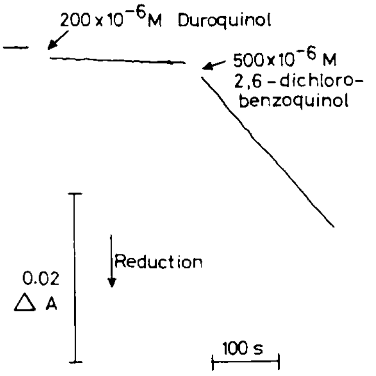

Fig. 4. The catalytic effect of 2,6-dichlorohydroquinone on the reduction of cytochrome *c* by tetramethylhydroquinone. The reaction mixture consisted of anaerobic 100 mM sodium citrate and 1 mM EDTA at 20°C and pH 4.6 together with $19.5 \cdot 10^{-6}$ M cytochrome c. Additions of quinol were made as indicated.

TABLE I

PHYSICAL DATA ON SUBSTITUTED *p*-BENZOQUINOL REDOX SYSTEMS

The data were obtained as described in the footnotes. Some of the values are necessarily approximate estimates but these are useful until more accurate data are available. For measurement of k_1, the reaction medium was 90 mM NaCl and 1 mM EDTA containing either 10 mM 2-(*N*-morpholino)ethane sulphonate at pH 6 (for low-potential quinols) or 10 mM sodium citrate at pH 3—4 (for halogenated quinols). The initial concentration of ferric cytochrome was between 5 and 20 μM and that of quinol between 0.2 and 2 mM.

Quinol	pK_A^a	pK_B^a	pK_S^a	K_d^a	$E_0(QH_2/Q)$ [c] (mV)	$E_0(QH^-/QH^\cdot)$ [d] (mV)	$E_0(Q^{\cdot-}/Q)$ [d] (mV)	$\log_{10}$ (true k_1) ($M^{-1} \cdot s^{-1}$)
Hydroquinone	9.85	11.4	4.1	4.2	+700	+482	+81	2.95
2-Methylhydroquinone	10.05	11.5	4.45	2.0	+644	+412	+7	3.97
2,3-Dimethylhydroquinone	10.43	12.6	4.65	3.6	+588	+390	—86	4.29
2,6-Dimethylhydroquinone	10.35	12.4	4.75	3.0	+588 [e]	+350	—80	4.38
Trimethylhydroquinone	10.8	12.9	4.95	3.3	+529	+279	—166	4.93
Tetramethylhydroquinone	11.25	13.2	5.0	1.3	+466	+221	—264	5.81
2-Methyl-5-isopropylhydroquinone	10.4 [f]	12.5 [f]	4.6 [f]	6.0 [f]	+589	+353	—75	4.36
2,5-Dichlorohydroquinone	7.9	10.0	2.1 [g]	17.3	+723	+623	+223	2.28
2,6-Dichlorohydroquinone	7.9 [h]	10.0 [h]	2.1 [h]	17.3 [h]	+721	+621	+221	2.67
Tetrachlorohydroquinone	5.6 [i]	8.2 [i]	0.0 [g]	50 [j]	+699	+726	+336	2.28
Ubiquinol-1	11.25 [k]	13.2 [k]	5.9 [b]	1.3 [k]	+490 [l]	+191	—240	5.68
Plastoquinol-1	10.8 [m]	12.9 [m]	5.9 [n]	3.3 [m]	+530 [o]	+239	—165	5.98
2,5-Dibromo-6-methyl-3-isopropylhydroquinone	8.4 [p]	10.5 [p]	2.6 [g]	17.3 [h]	+600 [q]	+470	+70	3.93

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[a] From Bishop and Tong [37].
[b] From Patel and Willson [38].
[c] From Clark [39].
[d] Calculated from equations in Methods.
[e] Assumed same as 2,3-dimethylhydroquinone.
[f] Assumed same as 2,5-dimethylhydroquinone.
[g] Calculated assuming pK_A—pK_S = 5.8 for the hydroquinone series.
[h] Assumed same as 2,5-dichlorohydroquinone.
[i] Measured by spectrophotometric pH titration (λ_{max} (QH^-) = 325 nm; λ_{max} (Q^{2-}) = 346 nm).
[j] A first approximation of approx. times 3 K_d of dichloro-derivatives.
[k] Assumed same as tetramethylhydroquinone.
[l] From Erecinska and Wilson [40].
[m] Assumed same as trimethylhydroquinone.
[n] Assumed same as ubiquinol.
[o] From Carrier [41].
[p] Assumed to be value for dichloro-derivative plus 0.5.
[q] By potentiometric titration.

results of the experimental determinations of k_1 are summarized in Table I as true k_1 values.

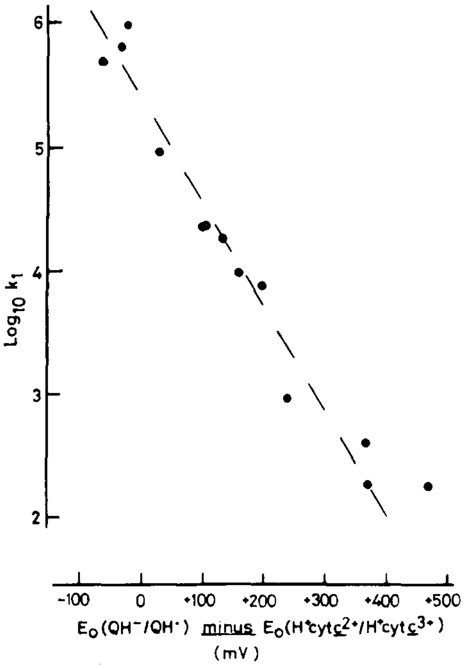
The rate-limiting step in these reactions is the electron transfer during a bimolecular collision between anionic quinol and protonated cytochrome. The pH-independent E_0 values of the $QH^-/QH^\cdot$ couples have been calculated from the data listed in Table I by using the equations of the Methods section. From these values, the ΔG^0 of the reactions could be calculated from

$\Delta G^0 = E_0(QH^-/QH^\cdot) - E_0(H^+cytc^{2+}/H^+cytc^{3+})$ and a value of 254 mV for $(E_0(H^+cytc^{2+}/H^+cytc^{3+})$ [22].

Fig. 5 shows that when $\log_{10} k_1$ is plotted against ΔG^0 for these reactions the points closely follow a straight line of slope —1/118 mV as predicted by the theory of Marcus (Ref. 19, see also Refs. 20 and 21 and Discussion).

The fate of the semiquinone product, $QH^\cdot$

An immediate product of the rate-limiting reaction is the semiquinone, $QH^\cdot$. The pK_S values for these are listed in Table I. It can be seen that the equilib-



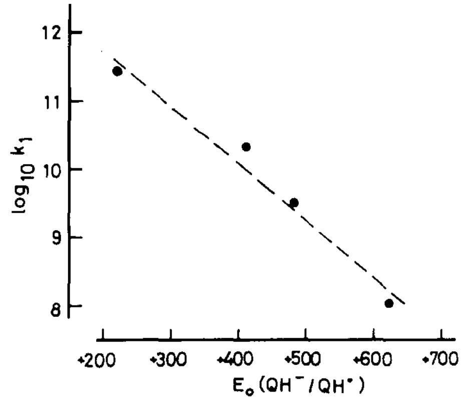


Fig. 5. Marcus plot of the relation between k_1 and $E_0(QH^-/QH^\cdot)$ for a series of substituted benzoquinols reducing cytochrome c. Values of the true rate constant k_1 were determined as described in the text. $E_0(QH^-/QH^\cdot)$ values were calculated from the physical parameters of the quinols which are listed in Table I. The straight line drawn has a slope of —1/118 mV and is the gradient predicted by the theory of Marcus [19—21].

Fig. 6. The relation between k_1 and $E_0(QH^-/QH^\cdot)$ for ferric iron reduction by substituted benzoquinols. Observed k_1 values were calculated as described in Table II. The slope of the line drawn is —1/118 mV, the gradient predicted by the theory of Marcus [19—21].

rium of these will be towards the deprotonated species, $Q^{\cdot -}$, at the experimental pH-values used. Yamazaki and Ohnishi [11] have already measured the rate constant, k_2, (Eqn. 6) for the benzoquinol system and found it to be rapid ($1.8 \cdot 10^6$ $M^{-1} \cdot s^{-1}$ under their experimental conditions). Hence a major fate of the $Q^{\cdot -}$ species, assuming protonation/deprotonation to be more rapid, is reduction of a second cytochrome *c* species.

Dismutation (Eqn. 8) may also contribute to removal of semiquinone, although this will generally be a slow process (Yamazaki and Ohnishi give k_{-4}, k_d in their terminology, as $(7—8) \cdot 10^7$ $M^{-1} \cdot s^{-1}$. Overall rate will be slow since both donor and acceptor are in such low amounts).

Under aerobic conditions it is likely that superoxide anion generation would also occur [23,24]:

$$Q^{\cdot -} + O_2 \rightleftharpoons Q + O_2^{\cdot -}$$

In fact, we found that the initial rate of reduction of cytochrome by quinol in the presence of oxygen plus superoxide dismutase was exactly half of the initial rate when the experiment was performed anaerobically in the normal manner. This observation supports the notion that rate of $Q^{\cdot -}$ removal by superoxide formation > rate of removal by cytochrome reduction > rate of removal by dismutation.

This reduction of a second cytochrome *c* species by semiquinone products leads to the observed k_1 values being twice the true k_1 values which are listed in Table I.

The reduction of Fe^{3+} by p-benzoquinols

Baxendale and Hardy [16] reported evidence for a mechanism of electron transfer from deprotonated quinol to ferric iron but found that no relation existed between the forward rate constant of the reaction and the midpoint potential of the QH_2/Q couple employed. However, the work reported here and in our previous communication [10] suggests that they were not considering

TABLE II

THE RELATION BETWEEN RATE CONSTANT AND $E_0(QH^-/QH^\cdot)$ FOR THE REDUCTION OF FERRIC IRON BY SUBSTITUTED *p*-BENZOQUINOLS

Quinol	k_1^a ($M^{-1} \cdot min^{-1}$)	Observed k_1 ^{b} (for QH^- reaction) ($M^{-1} \cdot s^{-1}$)	Log_{10} (Obs. k_1) ($M^{-1} \cdot s^{-1}$)	$E_0(QH^-/QH^\cdot)$ ^{c} (mV)
Hydroquinone	28	$3.31 \cdot 10^9$	9.52	+482
2-Methylhydroquinone (toluquinol)	126	$2.14 \cdot 10^{10}$	10.33	+412
Tetramethylhydroquinone (duroquinol)	89	$2.63 \cdot 10^{11}$	11.42	+221
2,6-Dichlorohydroquinone	67	$1.12 \cdot 10^8$	8.05	+621

[a] From Baxendale and Hardy [16].

[b] Calculated from observed $k_1 = k_1$ (Baxendale and Hardy) × $10^{pK_A}/60$.

[c] From Table I.

the appropriate parameters. We have recalculated the data of Baxendale and Hardy using our parameters of observed k_1 and $E_0(QH^-/QH^{\cdot})$. The results are summarised in Table II and plotted in Fig. 6. It can be seen that the points lie close to the theoretical line predicted by Marcus of —1/118 mV.

Discussion

For most quinols under normal pH and ionic strength conditions the two major routes of cytochrome reduction involve donation of an electron either from the semiquinone (cf. Refs. 11, 17, 25) or from the anionic quinol (cf. Refs. 16, 17, 26), and these pathways are summarized in Fig. 7. The couples $QH_2/QH_2^{\cdot +}$ and $Q^{2-}/Q^{\cdot -}$ usually play an insignificant part.

This work has concentrated on the measurement of the forward rate constants for cytochrome *c* reduction by a series of anionic quinols. Marcus (Ref. 19, see also Refs. 20, 21) has formulated a theory of outer-sphere electron transfer reactions which predicts a relation between ΔG^{+}, the activation energy, and ΔG^0, the overall free energy change of the rate-limiting step, for a series of like donors to a given acceptor molecule. The general equation may be formulated as

$$\Delta G^{\ddagger} = \tfrac{1}{2}\Delta G^0 + \lambda/4 + w$$

λ is a reorganisation parameter for reactants and products and w is a work term which takes into account the energy needed to bring together reactants and products. If the donors to a fixed acceptor are sufficiently similar, then the

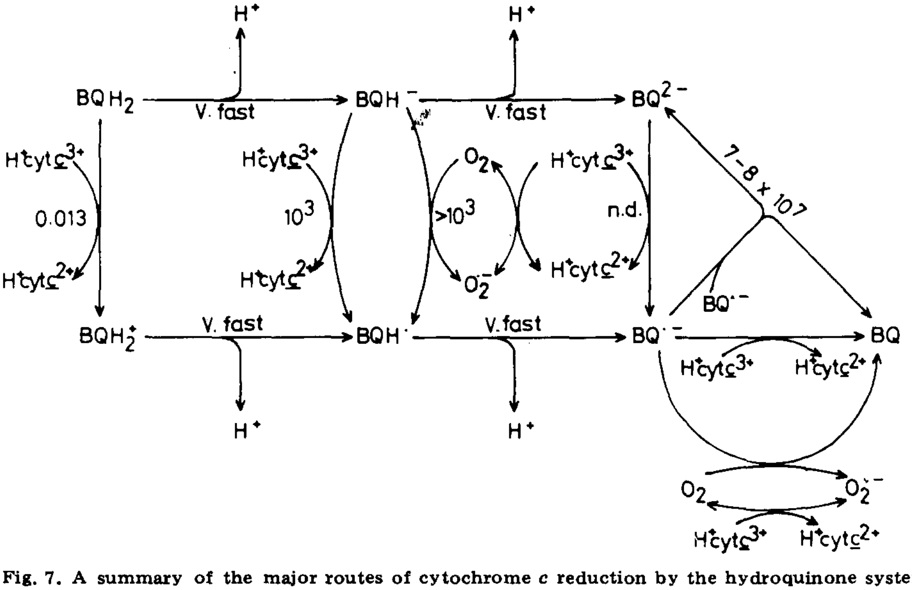


Fig. 7. A summary of the major routes of cytochrome *c* reduction by the hydroquinone system in the presence of oxygen. The reactions have been written in the forward direction of cytochrome reduction and quinol oxidation, although all reactions are reversible. Rate constants are from this work or from Yamazaki and Ohnishi [11]. Rate constants for the reverse reactions may be calculated from the equilibrium constants of the individual reactions which in turn may be calculated from the data in Table I.

reorganisation and work terms will cancel such that the activation energy varies predictably with ΔG^0 and an order of magnitude increase in rate constant for each 118 mV decrease in donor couple midpoint potential is observed. This principle has been reported to hold in some chemical systems [27—29] and is also followed closely with the quinols used in this study (Figs. 5 and 6).

Yamazaki and Ohnishi [11,25] have measured the forward rate constants, k_2, (termed k_r in their work) for the semiquinone-mediated reduction of cytochrome *c* by both benzosemiquinone, $BQ^{\cdot -}$, and menasemiquinone, $MQ^{\cdot -}$. They obtained values of $2.5 \cdot 10^6$ and $2 \cdot 10^8\ M^{-1} \cdot s^{-1}$, respectively. Using the data listed in Ref. 10 and the equations in this paper, one may calculate that E_0-$(MQ^{\cdot -}/MQ) = -160$ mV and $E_0(BQ^{\cdot -}/BQ) = +81$ mV. It is probable therefore that the semiquinone series of reductants also follows the Marcus prediction for the variation of rate constant with ΔG^0.

Of particular interest is the application of these notions to two reactions of biological importance which occur in hydrophobic membrane environments, that of the oxidation of ubiquinol in mitochondria and of plastoquinol in chloroplasts. It is clear that these reactions use the fully reduced quinol and not the semiquinone as initial reductant, since oxidation rate is proportional to quinol concentration and the rate is not inhibited when the quinone pools become highly reduced either in mitochondria [8,9] or in chloroplasts [30,31]. The calculated $E_0(QH^-/QH^{\cdot})$ values from the figures of Table I are +191 and +239 mV for short chain analogues of the ubiquinone and plastoquinone systems, respectively. These values are significantly below the E_m values of their acceptors, presumably cytochrome c_1 ($E_{m,7} \simeq +223$ mV [23]) and cytochrome *f* ($E_{m,7} \simeq +370$ mV [33]), and rapid reaction rates between deprotonated quinol and cytochrome are quite feasible by a bimolecular collision mechanism. A complication for direct analogy of these reactions with our model system is that the biological reactions occur in a hydrophobic membrane in which molecular movements will be somewhat more restrictive than in aqueous solution and molecular collisions will be somewhat less random. A general outcome of this will be that the effective concentrations of the interacting species may be much increased if they both preferentially occupy similar environments and the measured rate constant for the reaction could be substantially increased over the value obtained in aqueous solution. Such a phenomenon might explain the low rates of electron transport between several biomolecules in solution but their rapid redox equilibration in lipid-containing complexes, e.g. ubiquinol → c_1 (or perhaps ubiquinol-Rieske centre [34]) in bc_1 complex [35] and plastoquinol → *f* in *bf* particles [14]. Although at present it may be difficult to quantitate the magnitude of such hydrophobic membrane 'directive' effects, we can at least test whether such a mechanism is operative, for example by studying rate dependence on pH, ionic strength, donor and acceptor concentrations and quinol hydrophobicity in aqueous and hydrophobic membrane reaction milieu.

A product of the rate-limiting anionic quinol reaction will be the semiquinone, $QH^{\cdot}$. This will rapidly dissociate to the $Q^{\cdot -}$ form at around neutral pH. Calculation of the $E_0(Q^{\cdot -}/Q)$ values for the ubiquinone and plastoquinone systems gives values of —240 and —165 mV, respectively. Hence, sufficient energy is easily available for a rapid cytochrome *b* reduction, as would be required in a ‘protonmotive Q-cycle’ type of mechanism as envisaged by Mitchell [1]. Whether this occurs will depend upon the relative rate constants of cytochrome c_1 (*f*) reduction and reoxidation and of cytochrome *b*-566 (or *b*-563, *b*-559$_{\mathrm{LP}}$ in chloroplasts [36]) reduction and reoxidation. Such a situation might give different ratios of protons translocated/electrons transferred with different experimental conditions since overall oxidation and reduction rates will not vary in the same manner as conditions are changed.

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