Hypothesis

A GENERALISED MODEL FOR THE EQUILIBRATION OF QUINONE POOLS WITH THEIR BIOLOGICAL DONORS AND ACCEPTORS IN MEMBRANE-BOUND ELECTRON TRANSFER CHAINS

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1. Introduction

It is well established that quinones form essential components of many biological electron transfer chains and it has been noted that they form ideal hydrogen atom transfer components of Mitchellian protonmotive loops [1]. What is less clear is whether the quinones, which are well in excess of electron transfer chains, are freely mobile species to form a homogeneous pool or whether there are several protein-bound forms which may be considered distinct species and which may structurally comprise a 'solid-state' electron transport chain. Experimentally, results have provided evidence for both points of view. For example, the first order behaviour and kinetic competence of a significant part of the quinone complement [2–4], sigmoidal inhibition with tightly bound inhibitors [4–6], double inhibitior experiments [7], measurement of pool size [8,9], some extraction/reconstitution studies [10–12], substrate additivity measurements [13], and work with reconstituted systems [14,15] all provide evidence of mobility and much of this work points to the quinone as the mobile species. In contrast, potentiometric/kinetic measurements [17–21], stoichiometry estimates [22], some extraction/reconstitution studies [23], detection of stabilised semiquinones [21,24–29], identification of Q-binding proteins [30–32] and studies on the recovery of the chloroplast slow electrogenic phase b [33–35], all point to specific protein-bound quinones and suggest the possibility of a 'solid-state' system.

Abbreviations: Quinone species nomenclature is as in [40]; DBMIB, 2,5-dibromo-6-methyl-3-isopropyl-*p*-benzoquinone; R, the gas constant = 8.31432 J . K^{-1}; T, the absolute temperature; all potentials are relative to the standard hydrogen electrode

2. Thermodynamic considerations

In 1932, Michaelis [36] pointed out the two step nature of many two equivalent redox processes and this was further developed and amplified by Clark [37]. More recently Wikström [38] and Mitchell [39] invoked such a notion in relation to the ordering of electronic reactions in chloroplasts and mitochondria. From this developed the 'Q-cycle' model [39] which now forms a major working hypothesis of many laboratories. In this model, it was suggested that stabilisation of semiquinone species might occur such that rapid enough reaction might be promoted with the appropriate one electron donors and acceptors. For example, as discussed by Mitchell [39], the E_m (UQH_2/UQH^-) is around +365 mV in lipid solution. Binding and semiquinone stabilisation could lower this value significantly so that reaction with its acceptor, at around +250 mV, might then occur at reasonable rates.

I would like to develop these notions further in relation to the quinone redox system:

(1) It may be noted that stabilisation such that the one equivalent E_m is at a value below the E_m of the acceptor species is not a necessity in most cases, since the magnitude of the 'uphill' E_m will merely fix an upper theoretical limit on the forward rate constant – this upper limit will be an order of magnitude less than the diffusion limited rate constant for each 60 mV of 'uphill' E_m difference. Such a consideration is widely used to determine the maximum rate constant of a thermodynamically unfavourable reaction.

(2) More importantly, the couples to be considered should be electron transfer and not hydrogen atom transfer couples. The reasons for postulating this are 2-fold:

(i) In the absence of appropriate molecular polarisation, the activation energy involved in moving a hydrogen atom by the making and breaking of covalent bonds will be large compared to that of moving an electron and therefore a deprotonation/electron transfer sequence offers a thermodynamically feasible route even when pK-values are high;

(ii) In model systems of quinol/cytochrome and quinol/quinone equilibration, it has been demonstrated that electron transfer, but not hydrogen atom transfer processes, are the dominant features [40–42].

The data in [56] may be used to calculate that, for the reaction between duroquinol and 2,5-dichlorobenzoquinone, the activation energy for electron transfer from anionic quinol to quinone is close to RT, whereas the activation energy for uncharged quinol reduction of quinone by hydrogen atom transfer is in excess of 20 RT. As shown in [40–42], it becomes clear that the couples we must consider for the rate limiting step of electron transfer from quinol to acceptor are the ($QH^-/QH^{\cdot}$) couples of the quinone system and appropriately protonated forms for the acceptor species.

When considered in this way, it may be noted that models of, for example, the two electron gate in photosynthesis [21,43–45] do not solve the problem of entry of reducing equivalents into the quinone pool and neither does the notion of the bound quinone 'Z' or 'U' [17–21,33–35] solve the problem of removal of reducing equivalents from the pool, since in both cases we shift to a different step the inevitable problem of reduction or oxidation of the two equivalent Q pool system by one electron transfer steps.

3. An alternative model

One solution to this problem would be that the quinone pool is not directly involved in the electron transfer sequence and that electron transfers proceed only via fixed species. Such a proposal, however, has several serious disadvantages: it does not explain the large body of data, particularly from steady state and approach to steady state experiments, which indicates quinone pool involvement; it does not account for electron transfers between separated domains of components which have been observed in chloroplast systems under certain conditions [46,47]; and it substitutes a 'black box' for a molecular model of proton movements across the hydrophobic membrane.

Instead, I would suggest an alternative model, based on the known physical chemistry and thermodynamics of quinone systems and on the physical chemical interpretation of enzymological reactions. In this model, it is suggested that the observed bound quinone species, which have been termed 'B', 'R', 'Q_s' and 'Q_{II}' on the side of donation to the Q pool [21,26,43,44] and termed 'Z', 'Q_z', 'Q_c', or 'U' [17–21,26,31,33–35] on the side of withdrawal from the quinone pool, may really be intermediates in the one equivalent reduction steps of what may be considered classical enzymological reactions. Reducing equivalents then enter and leave the bulk quinone pool by association/disassociation reactions of the fully oxidised or reduced quinone species from their transient binding sites. Figure 1 illustrates this proposed scheme.

The use of such a scheme has several advantages:

(i) It provides a means of rationalising a large part of the apparently dissonant data which has been summarised in the introduction.

(ii) It removes the problem of redox equilibration of bound and mobile quinone in a way which is in accord with known physical chemistry and thermodynamics of these systems.

(iii) It suggests a novel mechanism for inhibition of the biological reactions by quinone analogues such as DBMIB [48] and provides a number of experimentally testable predictions in the form of expected kinetic behaviour in single and multiple turnover reactions, and in the dependence of observed kinetics on the thermodynamic and physical parameters of the quinone system involved.

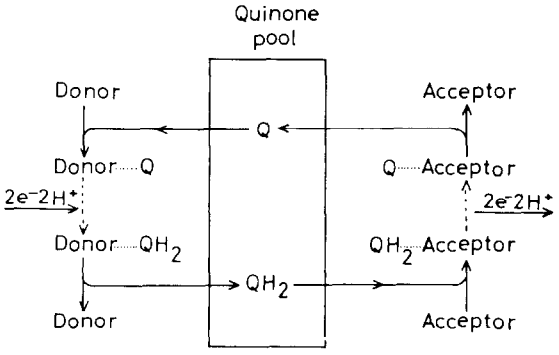

Fig.1. A model of the relation of bound and free forms of quinones in their catalytic redox cycle.

4. The nature of the catalysis

It is proposed that a major catalytic feature of the biological reactions is the presence of a positive charge at the site of quinone binding such that anionic species become stabilised. The positive charge may be in the form of an appropriately protonated amino acid residue or a metal ion, such as Fe^{2+}. Hence, at the site of reduction the midpoint potentials of the operative couples ($Q/Q^{\cdot-}$) and ($Q^{\cdot-}/Q^{2-}$) will be raised and pK-values will be lowered and at the oxidation site the midpoint potentials of the operative couples ($QH^-/QH^{\cdot}$) and ($Q^{\cdot-}/Q$) will be raised and the pK-values will be lowered. Presumably, hydrophobicity plays a part in the binding process and in the holding of molecules in useful orientations, but it is envisaged that there is little steric fitting in the complex in terms of recognition of specific p-benzoquinone or p-benzoquinol derivatives and that rate is determined predominantly by thermodynamics and by the presence of the positive site of interaction close to the redox centre and in a position accessible to the head group of the quinone molecule as it moves in the membrane environment.

The catalytic steps of this model are summarised in fig.2. In the donor side sequence, quinone firstly binds to the catalytic site by a hydrophobic interaction. An electron then arrives, for example, from photosystem II in chloroplasts, and the electrostatically stabilised $D^+Q^{\cdot-}$ complex is formed. Stability of such a complex is presumably reflected in the observations of the rapidly-relaxing semiquinone signal of mitochondria [24,25] and of the biphasic oscillations at the acceptor sides of photosystem II and reaction centres [21,26,43,44]. The $D^+Q^{\cdot-}$ complex will have sufficient kinetic stability such that a second electron may arrive to produce the D^+Q^{2-} complex. The pK-values of this are sufficiently high that it may protonate and the netural QH_2 may leave the positively charged binding site and enter the bulk quinone phase. At the acceptor side, the formation of a complex with the deprotonated QH^- species is promoted by the positive charge at the binding site. In fig.2, the Rieske centre has been illustrated as bearing this positive charge largely on the basis of the studies of Trumpower and coworkers [49] which have demonstrated the role of the Rieske centre in electron transfer between quinol and bc_1 complex and also on the basis of studies which have shown an interaction between the Rieske centre and added quinones [50, 51]. Whether the quinol binds first and then deprotonates or whether it binds after deprotonation will depend on relative rate constants, but will lead to the same overall result of formation of the FeS^+QH^- complex. The positive charge is envisaged as a dissociable amino acid residue on the surface of the binding protein. Electron transfer to the acceptor may then occur, for example, to the Rieske redox centre or perhaps to a cytochrome haem group. It may be noted that the Rieske redox centre does not necessarily undergo redox changes itself during this process. After this transfer, deprotonation to form the electrostatically stabilised $FeS^+Q^{\cdot-}$ complex will rapidly occur. A second electron may then be removed from this complex to produce FeS^+Q. Whether the second electron follows the same route as the first or whether it follows a different path is of interest since this would provide the difference between a linear scheme and, for example, a Q-cycle scheme of electron transfer. The latter would probably require a molecular rearrangement of some sort after the first electron has been transferred. After these electron transfers, the quinone is able to leave the binding site since the complex is no longer electrostatically stabilised, and thus it re-enters the quinone pool.

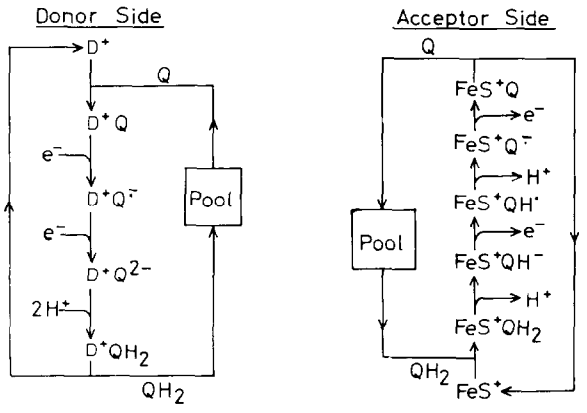

Fig.2. Specific redox and protonation steps during reduction and oxidation of quinones in biological electron transfer chains. See text for details of individual reactions.

It may be noted that the model envisaged suggests the possibility of a variable electron transfer route and hence a variable proton/electron stoichiometry under variable physical conditions. For example, in a Q-cycle scheme, if reoxidation of the species which accepts an electron from QH^- becomes fast compared to the rate of electron donation form $Q^{\cdot-}$ to a b-haem,

then the possibility increases that the $Q^{\cdot-}$ electron will follow the same path as that from QH^-. Such a change would reduce the proton/electron stoichiometry from 2 to 1 at this site. In the chloroplast system, some evidence for such behaviour has been gained by using a series of substituted *p*-benzoquinol derivatives as donors to photo-oxidised *bf* complex [52].

Because the overall redox reactions require rapid protonation/deprotonation steps and because hydrogen atom transfers are considered to be too slow, it becomes apparent that the redox events must occur at sites which can equilibrate with the aqueous phases in which the membrane is located. In the aprotic membrane interior, the overall reactions would not be feasible and quinone/quinol redox equilibrations would not occur [42,56]. Hence, for hydrogen atom transfer across the membrane, the quinones and quinols per se would have to be the mobile species.

5. Kinetic consequences of the model

A consideration of the kinetic behaviour of the above model is informative. To illustrate this, the greatly simplified kinetic model (fig.3) of quinol donation to the *bc* complex is discussed. In this model, quinol binds to its site on *bc* complex to form ultimately a $QH^-\ldots bc_{ox}$ complex, in which electron transfer may take place.

In single turnover situations, assuming an appropriate overall equilibrium constant for binding, most *bc* complexes will have their sites occupied by quinols, if the quinone pool is sufficiently reduced. On flash oxidation of the *bc* complex, observed electron transfer rate will approximately be given by:

$$\text{Rate} = k_e\,[QH^-\ldots bc_{ox}]$$

$$= k_e\,[QH_2\ldots bc_{ox}]\,.\,K_b\,.\,[H^+]^{-1}$$

at pH-values significantly below pK_b

The single turnover experiment will then provide information on the rate constant, k_e, for electron transfer within the quinol–*bc* complex. The thermodynamic behaviour of the quinol when bound is determined by the appropriate on/off rate constants and by the differences in physical constants of the quinone system in bound and free states.

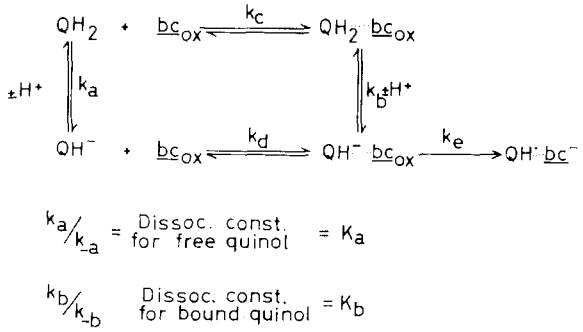

Fig.3. A simplified kinetic scheme to describe kinetic behaviour of quinol reduction of *bc* complex in single turnover and steady state electron transfer conditions.

In the steady state turning over situation, it is postulated that it is the formation of the appropriate electron transfer complex which is the rate limiting step in the overall process. Thus, assuming protonation/deprotonation to be very rapid, observed rate would approximately be given by:

$$\text{Rate} = k_c\,[QH_2]\,.\,[bc_{ox}]\,.\,K_b\,.\,[H^+]^{-1}$$

$$\text{or} = k_d\,[QH_2]\,.\,[bc_{ox}]\,.\,K_a\,.\,[H^+]^{-1}$$

depending on which of the two possible routes indicated in fig.3 is actually the dominant process. In both of these possible situations a pH-dependent rate may be observed, and the observed rate would be proportional to both $[QH_2]$ and to $[bc_{ox}]$, as observed experimentally [2–5,53]. In this situation, since the rate of removal is much greater than the rate of formation, the electron transfer complex will not be observable in the steady state.

It is therefore envisaged that it is the diffusion of quinol (presumably transmembrane diffusion) which is rate limiting in steady state electron transfer, and that in single turnover experiments information of different rate constants may be gained, since the rate limiting step is changed.

6. A mechanism for inhibition by quinone analogues

With the above model, one may define possible mechanisms for inhibition of electron transfers to and from quinone pools by analogues of the naturally occurring quinones, such as DBMIB [48]. Such inhib-

itory quinones have several features in common; they are hydrophobic, they have low pK-values, and their overall midpoint potentials are generally rather high. Any model for their inhibitory action must take into account the odd observation that model system studies predict that they might stimulate, rather than inhibit, electron transfer rate [44].

The behaviour of such quinones in the scheme illustrated in fig.2 may be considered. In the donor side scheme, the inhibitory quinone, Q_I, would compete with the natural one for binding site, hence the required hydrophobicity. Electron transfer could then occur to the $D^+Q_I^{2-}$ stage. However, because of the low pK-values of the bound quinol and the necessity for full protonation to Q_IH_2 to occur before it is able to leave the positively charged binding site, the off reaction of quinol entry into pool will be very low and hence steady state electron transfer will be inhibited. Recently, competition of DBMIB with 'B' in chloroplasts has been suggested by Bowes and Crofts [54] and this proposal would be consistent with the scheme outlined here.

Consideration may also be given to the behaviour of inhibitory quinol, Q_IH_2, (or of quinone, Q_I) at the site of quinol oxidation. With Q_IH_2, initial competitive (with endogenous quinol) binding would occur so that the complex $FeS^+Q_IH^-$ would be formed. Electron transfer could then proceed to the $FeS^+Q_I^-$ or FeS^+Q_I stage. The electron transfer rate constants would be low, however, compared to those of the natural quinone [41,42]. The FeS^+Q_I may also have a rather low off rate constant if the inhibitory molecule is polarised to some extent, or if a quinone–hydroxy adduct is a dominant species. However, from the data of Bishop and Tong [55] the pK of the hydroxy adduct of 2,5-dichloro-*p*-benzoquinone is around 10.8 in aqueous solution and so this latter possibility, even for the bound quinone, seems rather unlikely unless the electrostatic stabilisation effect is very large.

In any case, from the above discussion it may be seen that a rationale for inhibition by quinone analogues may be developed by consideration of individual steps which is not inconsistent with their opposite stimulatory effect on bimolecular collision reactions of quinols with acceptors in solution [41].

7. Discussion

A general model of quinone pool operation in electron transfer chains has been described. It is based on an interpretation of several types of experimental data and is an attempt to produce a model which is consistent both with observed responses of bulk quinone pools and with data which has suggested the possibility of fixed, distinct quinone–protein moieties. The overall outcome is a return to the original concept of the quinones as mobile hydrophobic redox components and to an electron-redistributing function of the quinone in steady state electron transfer. It should, however, be noted that such a concept represents an oversimplification of the true situation since reactants are treated as species interacting in homogeneous membrane solution. That biological systems have a more complex organisation than this becomes increasingly clear. The effects of heterogeneous component organisation and of the restricted diffusion distances of quinones in high protein-content membranes may explain deviation of behaviour (e.g. [13,14]) from that predicted in the simple systems and offers a promising future area of study.

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