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Regulation of Electron Transfer by the Quinone Pool

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Abstract

Strong evidence for a random collisional mechanism for ubiquinone-mediated electron transfer is provided by the characteristic kinetic properties of respiratory chains originally explored by Kröger, A., and Klingenberg, M. (1973), *Eur. J. Biochem.* **34**, 313–323. A kinetic model which leads to this so-called "simple Q-pool behavior" has been described and we use this in reviewing evidence that electron transfer is diffusion-controlled as well as diffusion-coupled. We also consider mechanisms by which the kinetics of electron transfer might deviate from simple Q-pool behavior and how these might be implicated in the regulation of electron transport.

Key Words: Ubiquinone; quinone; electron transfer; electron transport; membrane; lateral diffusion.

Introduction

It is widely, though not universally, accepted that the functional linking of quinone reductases (e.g., dehydrogenases and photosynthetic reaction centers) with quinol oxidases (e.g., the cytochrome *bc* complexes of mitochondria, chloroplasts, and bacteria) is achieved through a pool of quinone and quinol molecules in the membrane which are only transiently associated with their enzymes (e.g., Gutman, 1980, 1985; Rich, 1984; Ragan and Cottingham, 1985). The quinone pool is therefore the lipid phase counterpart of the pyridine nucleotide pools in the aqueous phases of cells, and the various bound forms of quinone, detected by their stabilized semiquinone states, are to be regarded as intermediates in the conversion of enzyme–substrate to enzyme–product complexes in most instances. We should acknowledge, though, that this view is not shared by King (e.g., King and Suzuki, 1984), Yu and Yu (e.g., Yu and Yu, 1980), and possibly Ozawa (e.g., Suzuki and

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Ozawa, 1984) who promote the idea that quinones (in the functionally active state) are associated with apoquinoproteins and therefore that all quinone-mediated reactions occur through protein-bound redox reactions.

The concept of the free, mobile quinone pool can be experimentally tested by two principal approaches. The first involves measurement of the diffusion rates of quinone and quinone-reactive enzymes in biological membranes and can therefore provide evidence for or against the kinetic competence of a random collision model as proposed by Hackenbrock *et al.* (1986). Depending on the values obtained, and here there is some disagreement (e.g., Lenaz, G., 1986), this approach may also indicate whether electron transfer is likely to be diffusion-controlled. On the other hand, if the collisional rates calculated from the diffusion data fall short of the experimental electron transfer rates, then free diffusion is not adequate and a more structured organization of enzymes in the membrane is required as in the dynamic-aggregate model of Ferguson-Miller *et al.* (1986).

The second approach involves measurement of electron fluxes through the quinone pool with a variety of input and output activites, or measurement of quinone oxidoreduction kinetics and steady-state redox behavior, and comparing the results with predictions based on free diffusion or other models. Indeed, such investigations by Kröger and Klingenberg (1973a, b) provided the first real evidence for free diffusion of ubiquinone. It is this kind of approach which will be described in this article. We take as our starting point the observations of Kröger and Klingenberg and the implications that these have for a diffusional model for electron transfer via ubiquinone. We then explore the mechanistic basis of the Kröger and Klingenberg kinetics, and finally describe deviations from such behavior which may be of regulatory significance.

Simple Q-Pool Behavior

In a system in which quinone acts as a freely and rapidly diffusing entity linking reductases with oxidases, which are themselves randomly diffusing in the membrane, it follows that electrons donated into the quinone pool by any one quinone reductase molecule can be withdrawn with equal probability by any quinol oxidase molecule. The quinone redox state is therefore a function of the total activity of the quinone reductases and the total activity of the quinol oxidases regardless of the numbers of molecules actually present. This will be true even if different types of reductase and oxidase are simultaneously operating in a branched respiratory system. The quinone pool therefore serves not only as an intermediary in electron transport but also as a clearing house. Because of this, electron fluxes from different sources communicate

via the pool and are dependent on each other to extents which are a function of their maximum potential turnovers. This dependence is exerted via the quinone redox state.

The manner in which the quinone redox state varies with the relative reducing and oxidizing activities will depend on the detailed kinetics of quinone oxidoreduction by each enzyme. Kröger and Klingenberg (1973a) found that in bovine heart submitochondrial particles, the kinetics were pseudo-first order, i.e., the rate of electron transport through a dehydrogenase to ubiquinone, v_r, was given by $v_r = V_r \cdot Q_0/Q_t$, where Q_0 is the concentration of ubiquinone, Q_t is the concentration of total redox-active ubiquinone, and V_r is the maximum flux obtained when $Q_0 = Q_t$. The quantity V_r is therefore a measure of the total potential activity of the dehydrogenase molecules. A similar expression for oxidation of quinol, $v_0 = V_0 \cdot Q_r/Q_t$, could be combined with the first to give the following expression for the steady-state rate when $v_r = v_0 = v$, namely,

$$v = \frac{V_r V_0}{V_r + V_0} \tag{1}$$

This expression, as well as the first-order behavior, was found to hold with good accuracy for the oxidation of either NADH or succinate and to provide an explanation for the sigmoidicity of inhibitor titration curves (Kröger and Klingenberg, 1973a, b). Adherence to Eq. (1) has been used by a number of investigators as evidence for the participation of a freely mobile ubiquinone pool in electron transfer processes (e.g., Zhu *et al.*, 1982; Heron *et al.*, 1978). However, it should be pointed out that Eq. (1) depends not only on the existence of a random pool but also on strict first-order oxidoreduction of quinone and since there is no *a priori* reason why this should occur, deviations from Eq. (1) or expressions derived from it, particularly minor ones, need not imply any collapse of the homogeneous pool concept. In fact, it is not at all obvious why first-order behavior should be found, particularly over the wide range of conditions employed by Kröger and Klingenberg (1973a, b). Nevertheless, such behavior has been found in a number of systems, and work from our own laboratory with reconstituted membranes has verified directly that overall electron flux is controlled by the total reducing and oxidizing activities and not by the relative numbers of molecules (Heron *et al.*, 1978, 1979; Cottingham and Ragan, 1980).

A Kinetic Model for Simple Q-Pool Behavior

The maximum fluxes, V_0 and V_r, are defined for the condition when all the quinone present is reduced or oxidized respectively, without specifying

the actual concentration. Simple models of Q-pool behavior such as those of Gutman (1980) or Rich (1981) assume that the rate-determining step is the interaction between the quinone and its enzyme so that the velocity is pseudo-first order in quinone. It also follows therefore that V_r, V_0, and, from Eq. (1), v, should be proportional to Q_t. This is simply not the case, the relationship between these parameters being hyperbolic (e.g., Kröger and Klingenberg, 1973; Zhu *et al.*, 1982; Reed and Ragan, unpublished). Indeed, the mitochondrial membrane is nearly saturated with ubiquinone and yet Q-pool behavior is still observed. Moreover, Q-pool behavior is certainly still found when the rate-determining step is elsewhere, as shown by experiments in which the electron flux was lowered by limiting the concentration of the other substrate (e.g., cytochrome *c* in Kröger and Klingenberg, 1973a; NADH in Cottingham and Moore, 1983).

To solve this problem, we have considered a more detailed kinetic model which gives rise to simple Q-pool behavior over a wide range of conditions and takes account of saturation of quinone-reactive enzymes by quinone and quinol (Ragan and Cottingham, 1985). For a dehydrogenase reducing quinone, we consider the following steps:

$$dh_r + Q_0 \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} dh_r \cdot Q_0 \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} dh_0 \cdot Q_r$$

$$dh_r + Q_r \rightleftharpoons dh_r \cdot Q_r$$

$$dh_0 + Q_0 \rightleftharpoons dh_0 \cdot Q_0 \xrightarrow{k_{sub}} dh_r \cdot Q_0$$

$$dh_0 + Q_r \rightleftharpoons dh_0 \cdot Q_r \xrightarrow{k_{sub}} dh_r \cdot Q_r$$

$$dh_0 \xrightarrow{k_{sub}} dh_r$$

In the top line, quinone binds reversibly to the reduced dehydrogenase in a step governed by the rate constants k_{+1} and k_{-1}. The binding reaction may well be diffusion-controlled (e.g., Crofts, 1985), and it can be rate-determining for the overall process although this is not essential. Electron transfer to the bound quinone takes place in a reversible reaction as suggested by Kröger and Klingenberg (1973a) with rate constants k_{+2} and k_{-2}. We also introduce reversible binding of quinol to the reduced or oxidized dehydrogenase and binding of quinone to the oxidized dehydrogenase to give rise to three unproductive complexes. These steps are considered to be governed by rate constants of the same magnitude as those for formation of $dh_r \cdot Q_0$. Introduction of electrons by the dehydrogenase substrate can occur whether quinone or quinol is bound or not and the pseudo-first order rate constant,

Table I. Solutions to the Kinetic Model[a]

Conditions[b]	v	V_r
(a) k_{sub} large $Q_0, Q_r > [dh]$	$\dfrac{\dfrac{k_{-1} \cdot k_{+2}}{k_{-1} + k_{+2}}[dh] \cdot Q_0}{k_{-1}/k_{+1} + Q_t}$	$\dfrac{\dfrac{k_{-1} \cdot k_{+2}}{k_{-1} + k_{+2}}[dh] \cdot Q_t}{k_{-1}/k_{+1} + Q_t}$
(b) As (a) but $k_{-1} > k_{+2}$	$\dfrac{k_{+2} \cdot [dh] \cdot Q_0}{K_s + Q_t}$	$\dfrac{k_{+2} \cdot [dh] \cdot Q_t}{K_s + Q_t}$
(c) $Q_0, Q_r > [dh]$ $k_{-1} > k_{+2}$ $k_{+2} = k_{-2} = k_2$	$\dfrac{\dfrac{k_2 \cdot k_{sub}}{k_2 + k_{sub}}[dh] \cdot Q_0}{\dfrac{K_s \cdot k_{sub}}{k_2 + k_{sub}} + Q_t}$	$\dfrac{\dfrac{k_2 \cdot k_{sub}}{k_2 + k_{sub}}[dh] \cdot Q_t}{\dfrac{K_s \cdot k_{sub}}{k_2 + k_{sub}} + Q_t}$
(d) As (c) but k_{sub} small	$\dfrac{k_{sub} \cdot [dh] \cdot Q_0}{Q_t}$	$k_{sub} \cdot [dh]$
(e) $Q_0, Q_r \not> [dh]$ $k_{-1} > k_{+2}$ k_{sub} large	$\dfrac{k_{+2} \cdot [dh] \cdot Q_0}{\text{const.} + Q_t}$	$\dfrac{k_{+2} \cdot [dh] \cdot Q_t}{\text{const.} + Q_t}$

[a] The derivation of these expressions is given in Ragan and Cottingham (1985). Conditions (a)–(e) are described more fully in the text. [dh] is the total concentration of the dehydrogenase.
[b] The expression for v (and correspondingly, V_r) in condition (a) differs from that given by Ragan and Cottingham (1985) as their equation (14). We are grateful to Dr. J. Siedow for pointing out the error in the original derivations.

k_{sub}, is a composite which in general could be varied by lowering and raising the substrate concentration or by using inhibitors which do not act at the quinone reductase site. Thus, the overall velocity could also be limited either by electron transfer (k_{+2}) or by the substrate input rate (k_{sub}).

A general algebraic solution is not feasible, so we have examined the kinetic consequences of the model under a variety of different constraints which simplify the mathematics. These are summarized in Table I. Case (a) assumes that k_{sub} is very large as is frequently true, for example, when saturating substrate concentration are used. We also assume that Q_0 and Q_r are appreciably larger than the concentration of the dehydrogenase as is true in mitochondrial membranes except at extremes of the redox state. Under these circumstances and irrespective of the relative magnitudes of the other rate constants, the velocity, v, is proportional to Q_0, i.e., simple Q-pool behavior is found. The equation also predicts, though, that V_r will vary hyperbolically with Q_t as required. In case (b) we simplify the expression by replacing k_{-1}/k_{+1} by a dissociation constant, K_s, and by further assuming that the off-rate for quinone from the dehydrogenase is faster than the electron transfer rate, k_{+2}. For chromatophores, there is evidence for this

(Crofts, 1985) and additional support for this argument was discussed by Ragan and Cottingham (1985). However, we stress that this simplifying assumption may not be generally justified or indeed a requirement for simple Q-pool behavior. Case (c) examines the behavior of the model when k_{sub} is not necessarily larger than k_{+2}. In general, simple Q-pool behavior is only found when k_{+2} and k_{-2} are equal, a point which is dealt with in a later section. When k_{sub} is very small (d), the velocity, v, is still proportional to Q_0, but V_r is no longer a function of Q_t as might be expected. Finally, since Zhu *et al.* (1982) had found simple Q-pool behavior even when a large proportion of the pool had been extracted, we examined the behavior when the concentrations of Q_0 and Q_r were not large compared with the concentration of dehydrogenase (or any other quinone-reactive enzymes present). As a close approximation, the same kind of expression for v was found except that K_s was replaced by a constant containing all the concentrations of the quinone- reactive enzymes and their respective dissociation constants for quinone.

To summarize, simple Q-pool behavior arises naturally from a random collision model in which quinone and quinol are bound with equal affinity to the quinone reductase and quinol oxidase. There is no requirement for the binding reaction between enzyme and quinone (which may be diffusion-controlled) to be rate-limiting for the overall process. The results of Gupta *et al.* (1984) indicate that the electron transfer rate in mitochondria is exceeded by the theoretical diffusion-controlled collision rate by factors of only 5 to 45 depending on the reaction and the ionic strength. The collision rate must of necessity exceed the electron transfer rate because of the reversibility of the binding process and the possible occurrence of nonproductive collisions. The latter seems likely since the efficiency of the interaction between quinone and protein will not be promoted by charge effects (cf. cytochrome *c*; Koppenol and Margoliash, 1982). Since we cannot quantify the extents of these processes with existing information, it is not strictly possible to conclude that the random collision rate is in fact adequate for the measured electron flux. However, the existence of simple Q-pool behavior provides good evidence for a free diffusion model, and if this is accepted we can examine the suggestion that electron transport is diffusion-controlled rather than just diffusion-coupled. Strictly, a reaction is diffusion-controlled if the collisional frequency is equal to the reaction rate. In enzyme-catalyzed reactions, we can ask if the second-order rate constant for the interaction between enzyme and substrate is rate-determining for the overall process. This rate constant is frequently within an order of magnitude of the collisional rate and will be dependent on it. From this point of view, the observation of Gupta *et al.* (1984) that the collisional rate exceeds the reaction rate by only an order of magnitude could be taken as strong support for a

diffusion-controlled model. There is a difficulty though if the efficiency (reaction events per collision) is too high. Suppose that the efficiency was one and that every collision led to a reaction. A quinol leaving the dehydrogenase would then be oxidized by the first quinol oxidase molecule that it encountered. One can calculate from the data of Gupta *et al.* (1984) that the collisional frequency between say, Complex I and Complex III, is only one to two times that of electron transfer, so that the relative lateral motion of the proteins is quite slow compared with the reaction rate. There is a possibility then that for several electron transfer events, electrons from a particular dehydrogenase would be accepted by the same quinol oxidase molecule. This is certainly incompatible with simple Q-pool kinetics. In normal membranes, rotational diffusion of the proteins is in fact considerably faster than electron transfer (Poore *et al.*, 1982) which may ensure a random distribution of quinols to their oxidases. Any restriction of quinone and protein diffusion or a nonrandom distribution of proteins in the membrane, though, could lead to deviations from simple Q-pool kinetics as described later.

The ubiquinone diffusion rate used by Gupta *et al.* (1984) of 3×10^{-9} cm^2/sec is a little larger than that used by Crofts (2×10^{-10} to 10^{-9} cm^2/sec) and similar to the 10^{-8} cm^2/sec used by Rich (1984) in his analysis. These authors therefore have reached broadly similar conclusions concerning the diffusional control of quinone-protein binding. However, the fluorescence quenching method of Lenaz and co-workers (Fato *et al.*, 1985) gives a diffusion coefficient which is two to three orders of magnitude greater (approximately 10^{-6} cm^2/sec). If this value is correct, then the collision frequency will be very much greater than the electron transport rate and, strictly, the reaction will not be diffusion-controlled. But even if the second order-rate constant, k_{+1}, is close to the diffusion-controlled limit, electron flux can still show a dependence on k_{+1} and the quinone concentration. Inspection of Table I shows that V_r, V_0, and hence v [Eq. (1)] will vary with Q_t as long as K_s is not negligible, and under these circumstances, v will depend on the magnitude of K_s. This can be arranged regardless of the magnitude of k_{+1} as long as k_{-1} is large enough, i.e., the binding is not too strong. Thus a decrease in electron transfer rate following dilution of the quinone pool (Schneider *et al.*, 1980, 1982) or extraction of quinone (e.g., Ernster *et al.*, 1969) does not constitute definitive evidence for a diffusion-controlled mechanism.

In summary, therefore, we conclude that free diffusion and random collision of ubiquinone with quinone-reactive enzymes is the most probable mechanism of electron transfer. However, neither the existence of simple Q-pool kinetics, nor the decrease in electron flux following depletion of the quinone pool, provide evidence that electron flux is diffusion-controlled rather than diffusion-coupled. This will require agreement on the diffusion coefficient for ubiquinone.

Deviations from Simple Q-Pool Behavior

In principle, we can envisage four situations in which deviations from simple Q-pool kinetics would be produced. The first retains the free and rapid diffusion of quinone but the kinetics of quinone oxidoreduction are no longer first order, i.e., equations of the type given in Table I no longer apply. The mechanism of electron transfer is therefore unchanged, but its kinetic consequences are altered. The second situation is one in which the fluidity of the membrane is greatly reduced. Under these conditions quinol produced by a reductase may be preferentially oxidized by a single adjacent quinol oxidase. Quinol oxidases further from the reductase may not be accessible and the relative numbers of reductases and oxidases now becomes important in determining electron flux. In the extreme this can give rise to what Rich (1984) has termed "solid-state" behavior. The third situation is one in which the reductase and oxidase are physically associated in a stoichiometric supercomplex. Kinetically this "stoichiometric behavior" (Ragan and Heron, 1978) may be indistinguishable from "solid-state behavior" although the underlying causes are quite different. Fourthly one can envisage deviations from Q-pool kinetics if the reductases and oxidases are not randomly distributed in the membrane. This possibility is of particular relevance to branched respiratory chains and the regulation of the distribution of electron flux between the branches. Of course, these various modifications to simple behavior are not mutually exclusive and indeed the effect of nonrandom distribution would be much greater if the quinone diffusion rate were restricted by decreasing the membrane fluidity.

These various situations and their consequences for control of electron transfer are described in more detail below.

Loss of First-Order Kinetics

In Table I, case (c) shows the first-order behavior is encountered when k_{sub} is small only if k_{+2} and k_{-2} are equal. This means that if electron transfer is limited by the availability of electrons from the substrate to the quinone reductase or to the substrate from the quinol oxidase (e.g., NADH or cytochrome *c*), simple Q-pool behavior will not generally be observed. In the chromatophore *bc* complex of *Rh. sphaeroides* the reaction

$$Q_r + (\text{Fe-S})_0 + b^{3+}_{566} \rightleftharpoons Q_0 + (\text{Fe-S})_r + b^{2+}_{566}$$

has an equilibrium constant of 2 (Crofts and Wraight, 1983; Crofts, 1985) which means that k_{+2} and k_{-2} are very similar. However, in bovine heart mitochondria, Rich (1984) has calculated that the equivalent reaction has an equilibrium constant of 70, i.e., $k_{-2}/k_{+2} = 1/70$. This large deviation from

the requirement for simple Q-pool behavior prompted us to look at the theoretical consequences and to try and produce these experimentally. When k_{+2} and k_{-2} are different, the expression corresponding to case (c) of Table I for oxidation of quinol by the bc_1 complex is

$$v = \frac{k_{+2}k_{sub} \cdot [bc_1] \cdot Q_r}{k_{sub}(K_s + Q_t) + k_{-2}Q_0 + k_{+2}Q_r} \tag{2}$$

where k_{+2} is the rate constant for electron transfer from bound quinol to oxidized bc_1 complex and k_{-2} the rate constant for the reverse process. To simplify matters, we assume that the total quinone concentration is saturating ($Q_t > K_s$) and obtain from Eq. (2),

$$v = \frac{\alpha k_{+2}[bc_1] \cdot Q_r}{(\alpha + \beta)Q_t + (1 - \beta)Q_r} \tag{3}$$

where $\alpha = k_{sub}/k_{+2}$ and $\beta = k_{-2}/k_{+2}$. The quantity V_0 is obtained by putting $Q_r = Q_t$ and, combining this expression with Eq. (3),

$$v = \frac{V_0 \cdot Q_r(\alpha + 1)}{(\alpha + \beta)Q_t + (1 - \beta)Q_r} \tag{4}$$

The corresponding equation for simple Q-pool behavior is $V = V_0 \cdot Q_r/Q_t$ and this can be obtained from Eq. (4) either by putting $k_{-2} = k_{+2}(\beta = 1)$ or by increasing $k_{sub}(\alpha)$ as expected. In Fig. 1, v/V_0 is plotted against Q_r/Q_t for various values of α and β. As can be seen, nonlinear dependence on Q_r/Q_t becomes very marked at low values of α as β is decreased. Experimentally, this prediction was tested by measuring the rate of electron flux from NADH to oxygen in a reconstituted bovine heart Complex I–III system as a function of quinone redox state. Both the flux and redox state were varied by titration with rotenone (Fig. 2). Under conditions of excess substrate (NADH and cytochrome *c*) a linear dependence was found (Fig. 2a) in agreement with the finding of Kroger and Klingenberg (1973a) for submitochondrial particles. The slope of this line is V_0 and a straight line of smaller slope was produced when electron flow through the bc_1 complex was partially inhibited by myxathiazol (Fig. 2a). Since myxathiazol acts at the quinol oxidase site, we assume that it was not affecting k_{sub} and no deviation from Q-pool behavior was expected. On the other hand, when electron flow was limited by lowering the cytochrome *c* concentration, the dependence of v/V_0 on Q_r/Q_t was no longer linear (Fig. 2b). The fit to the theoretical curve, assuming a value for β of 0.0143 (or 1/70), is very good. Under these circumstances, the rate of electron flux is very unresponsive to the quinone redox state except when Q_r/Q_t is low, and operates close to its maximum rate, V_0. These experiments therefore not only provide experimental support for the kinetic model of Q-pool behavior

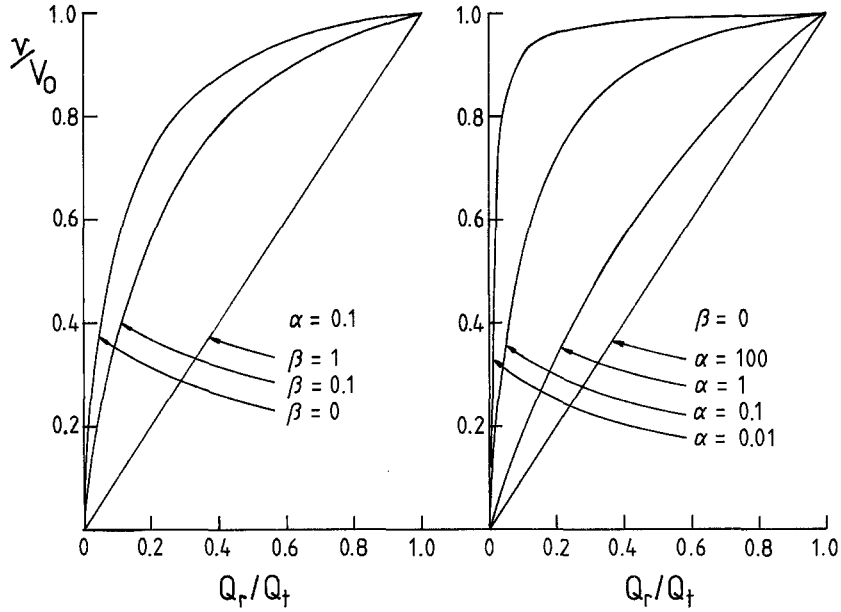

Fig. 1. The dependence of v/V_0 on Q_r/Q_t. The curves show solutions to Eq. (4) with the indicated values of α ($= k_{sub}/k_{+2}$) and β ($= k_{-2}/k_{+2}$).

outlined earlier but also provide one means whereby deviations from simple behavior could arise. A quinone dehydrogenase or quinol oxidase in which the electron transfer reaction is largely unidirectional and whose activity is controlled by substrate availability will operate at a near constant rate over a wide range of quinone redox states. In a branched respiratory chain, this means that its rate will be largely unaffected by the simultaneous operation of other reductases and oxidases. This might be of some regulatory importance, particularly when the various branches have different ATP yields. Deviations from simple Q-pool behavior in branched systems are common, particularly in plant mitochondria. One form which these deviations take is a failure to observe the expected competition between the substrates when these are oxidized simultaneously. The deviation examined above may provide an explanation for this behavior.

Restricted Diffusion of Ubiquinone and Protein

We suggested above that if the number of collisions per electron transfer event was low (in the limit, one), one could envisage a situation where each dehydrogenase transferred its electron via ubiquinone to only very few (and again in the limit, one) quinol oxidase molecules. This effect would depend

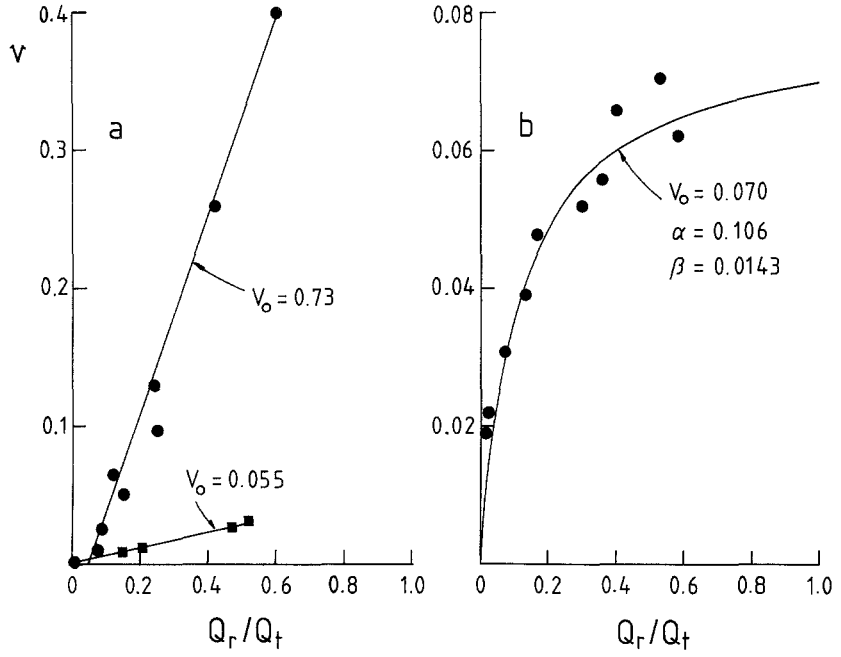

Fig. 2. Deviation from simple Q-pool behavior in reconstituted NADH-cytochrome *c* reductase. Complex I and Complex III mixtures were reconstituted with phospholipid and ubiquinone-10 (Heron *et al.*, 1978). NADH-cytochrome *c* reductase (v, in μmol of cytochrome *c*/min per mg of Complex I protein) and steady-state reduction of ubiquinone (Q_r/Q_t) were measured in the presence of varying concentrations of rotenone. In (a), assay was in the presence of excess cytochrome *c* (●) or additionally in the presence of myxathiazol (■). In (b), velocity was limited by the cytochrome *c* concentration. Note the different scales for v in (a) and (b). To calculate α, the V_0 value in the absence of substrate limitation (0.73) was determined from the slope of the line in (a) and was assumed to be approximately equal to $k_{+2} \cdot [bc]$, since ubiquinone was in excess. The value of V_0 in (b) was estimated to be 0.070 by extrapolation and was assumed to be equal to $k_{+2} \cdot k_{sub} \cdot [bc]/(k_{+2} + k_{sub})$. From this information, k_{sub}/k_{+2} or α was calculated to be 0.106. The curve in (b) was then calculated from Eq. (4) with $V_0 = 0.070$, $\alpha = 0.106$, and $\beta = 0.0143$ (i.e., 1/70).

not only on the number of collisions per event but also on the inability of the dehydrogenase to diffuse laterally or rotationally to the neighborhood of another quinol oxidase on the time scale of several electron transfer events. This kind of behavior has been explored by Rich (1984) using turnovers and diffusion coefficients similar to those of Gupta *et al.* (1984). Under normal circumstances, and using the lower range of ubiquinone diffusion coefficients, either the number of collisions per event or the rotational mobility of the enzymes seems sufficient to ensure a random distribution of quinols to their oxidase molecules. This is required, of course, for the observation of simple Q-pool behavior. However, the margin may not be too great, and any

restriction to diffusion may give rise to deviations. In the limit, "solid-state" behavior ensues. The kinetic consequences of this have been observed in reconstituted electron transfer systems where such behavior was called "stoichiometric."

Stoichiometric Association of Quinone Reductases with Quinol Oxidases

When isolated respiratory complexes (e.g., Complex I and Complex III) in detergent solution are mixed, removal of the detergent results in membranes which catalyze electron transfer from one Complex to the other (e.g., NADH-cytochrome *c* reductase) (Hatefi *et al.*, 1962; Fowler and Richardson, 1963). At a lipid-to-protein ratio typical of the mitochondrial membrane, the kinetics follow simple Q-pool behavior (Heron *et al.*, 1978). However, at a low lipid-to-protein ratio (Ragan and Heron, 1978) or when the lipid is in the gel phase (Heron *et al.*, 1979; Poore and Ragan, 1982a, b), Q-pool kinetics are lost and "stoichiometric" behavior is found. In this, rapid electron transport takes place from a molecule of dehydrogenase to only one molecule of Complex III. Molecules of either protein in stoichiometric excess do not contribute significantly to the electron transport rate. This can most easily be explained by the formation of a stoichiometric "binary" complex in which direct electron transfer via bound quinone takes place. There is circumstantial evidence for the existence or formation of such "binary" complexes in the purified state (e.g., NADH-cytochrome *c* reductase; Hatefi *et al.*, 1961; succinate-cytochrome *c* reductase; Yu and Yu, 1980) but no evidence for their existence in the natural membrane. Moreover, endogenous ubiquinone reduction by NADH in reconstituted Complex I–III occurs to the same extent for "stoichiometric" as for Q-pool behavior (Reed and Ragan, unpublished observations). A more likely explanation for stoichiometric behavior is therefore to be found in the close packing of the proteins at low lipid-to-protein ratio or in gel phase lipid (Poore *et al.*, 1982), their restricted rotational and lateral diffusion (Poore *et al.*, 1982), and the lower diffusion rate of ubiquinone in the less fluid lipid phase (Poore and Ragan, 1982a, b). All of these factors will contribute to the inability of quinone and quinol to be randomly distributed between Complex I and Complex III molecules. It is obvious that "stoichiometric" and simple Q-pool behavior represent opposite extremes, Rich (1984) refers to the possibility of a continuous scale of operation from the solid to the liquid state. Indeed, in reconstituted glycerol-phosphate-cytochrome *c* reductase, an intermediate condition has been observed (Cottingham and Ragan, 1980).

Does the "solid-state" or "stoichiometric" condition have any physiological relevance? It is unlikely that natural membranes would ever be of sufficiently low fluidity to produce this state exactly and it should perhaps be

regarded as an experimental curiosity. However, the general principle that the number of quinol oxidases sampled via the quinone pool by a quinone reductase may be quite small and subject to variation is very important for explaining deviations from Q-pool behavior of the last class, where nonrandom distribution of proteins is the likely underlying cause.

Nonrandom Distribution of Proteins in the Membrane

When the number of collisions between quinone and quinone-reactive enzyme per electron transfer is small, a further deviation from simple Q-pool behavior may become apparent when we consider the distribution of electron flux in branched respiratory chains. Lateral heterogeneity of membrane protein distribution is well established in the appressed and nonappressed regions of the chloroplast thylakoid membrane (e.g., Allred and Staehelin, 1986) and the packing of mitochondrial cristae membranes may produce similar effects although there is as yet no evidence for this. Let us imagine that in such a membrane, two types of dehydrogenase (for example, NADH and succinate dehydrogenase) and two types of quinol oxidase (e.g., the bc_1 complex and an alternative oxidase) are present but not randomly distributed. This offers the possibility for electrons from one substrate to be selectively channelled through one type of oxidase, a type of behavior which is very common in plant mitochondria. For example, exogenous NADH is frequently oxidized preferentially via the cytochrome bc_1 pathway to oxygen while malate is oxidized preferentially by the cyanide-insensitive oxidase (e.g., Gardestrom and Edwards, 1983). The nonrandom distribution of enzymes coupled with a limited diffusion path for quinone implies that the quinone pool no longer behaves homogeneously since quinol produced by one substrate cannot mix with that part of the quinone pool associated with the other dehydrogenase before it is oxidized by the local oxidase. This concept of quinone "domains" has been proposed by Gutman (1980, 1985) to explain some deviations from Q-pool behavior. It should be noted that heterogeneity of the quinone pool does not imply any physical compartmentalization and its description as "domains" is preferable to the use of the term "multiple pools."

The presence of domains prevents the oxidation of any one substrate from being accurately accounted for by simple Q-pool behavior. However, whether one would notice this depends on the kind of experiment performed. For example, if we consider a situation where only a fraction of V_0, α, is accessible to a particular substrate, the velocity v is given by

$$v = \frac{V_r \cdot \alpha V_0}{V_r + \alpha V_0}$$

from Eq. (1). This appears to be the normal equation but with a new lower value of the maximum oxidative flux, αV_0. If independent measurement of V_0 were not undertaken, the existence of domains would not be suspected. On the other hand, the behavior of the ubiquinone is more complicated. One domain is nonreducible (at least in the steady state) while the other is reduced in accordance with the simple Q-pool expression. Thus the overall quinone redox state is a function not only of V_r, V_0, and α but also of the fraction of the total quinone pool in each domain. The latter need not be related to α. In those systems reported not to obey simple Q-pool behavior and in which multiple pools or domains have been proposed, measurements of quinone redox state have not yet been made. This would be desirable since it might lend support to the notion that nonrandom distribution of proteins can occur.

Again we can ask whether this kind of deviation could have any regulatory significance. Certainly, unexpected distribution of electron flux in branched electron transport systems is common and if nonrandom distribution proves to be the cause then it seems very likely that this is by design and intended to modify the normal competition between quinone reductases and oxidases exerted by the quinone pool. Electrons from particular substrates may be directed specifically to certain oxidases thereby enabling them to be oxidized independently of other substrates or independently of the demand of the cell for ATP, for example. Through conformational changes of the membrane in response to the metabolic state of the cell, the protein distribution could vary, thereby providing a regulatory mechanism akin to that found in chloroplasts for the light-harvesting chlorophyll protein (Barber, 1982).

Conclusions

The available evidence strongly favors a collisional mechanism for quinone-mediated electron transfer. At one extreme, purely random collision gives rise to what we have called Q-pool behavior whose kinetic predictions have been experimentally verified in many systems. At the other extreme, quinone reductases and quinol oxidases are functionally paired by the limited diffusion of quinone and protein in the membrane. This extreme has only been observed in reconstituted membranes in which the lipid composition or concentration has been altered. However, we can use the concept in less extreme form, together with nonrandom distribution of membrane proteins, to explain results obtained with natural membranes which are inadequately accounted for by Q-pool behavior, and to propose a mechanism for regulating the direction of electron flux in branched respiratory chains. Direct

experimental evidence for regulation of electron transport by lateral heterogeneity of the membrane is not yet available but measurement of the response of the quinone redox state could provide valuable support for this proposal.

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