Biochem. J. (1987) 247, 657–662 (Printed in Great Britain)

The effect of rate limitation by cytochrome *c* on the redox state of the ubiquinone pool in reconstituted NADH:cytochrome *c* reductase

Jacky S. REED and C. Ian RAGAN*
Department of Biochemistry, University of Southampton, Southampton SO1 3TU, U.K.

The kinetic model of Ragan & Cottingham [(1985) Biochim. Biophys. Acta **811**, 13–31] for electron transport through a mobile pool of quinone predicts that, under certain conditions, the normal linear dependence of electron flow on the degree of reduction (or oxidation) of the quinone should no longer be found. These conditions can be met by reconstituted NADH:cytochrome *c* reductase (Complex I–III from bovine heart) when electron flow is rate-limited by a low concentration of cytochrome *c*. We show that, in such a system, the dependence of activity (varied by inhibition with rotenone) on the steady-state level of quinone reduction is indeed non-linear and very closely accounted for by the theory.

INTRODUCTION

The redox behaviour of the ubiquinone pool in bovine heart submitochondrial particles can be quantitatively accounted for by the observation that reduction of ubiquinone by dehydrogenases and oxidation of ubiquinol by the cytochrome bc_1 complex are both first-order processes in the substrate [1,2]. This model has been found to hold in several other systems, although deviations are also quite common [3–5]. The underlying causes of these deviations from first-order oxidoreduction of a homogeneous quinone pool are not known with any certainty, although compartmentalization of the quinone pool is the most popular explanation [6]. Less attention has been focused on whether or not ubiquinone oxidoreduction is in general a simple first-order process. To explain first-order behaviour it has been proposed that ubiquinone and ubiquinol must be present at low, non-saturating, concentrations (see, e.g., [5]) and that the quinone reductase and quinol oxidase enzymes must be kept fully reduced and fully oxidized respectively (see, e.g., [6]). Since these conditions do not apply in natural membranes, which nevertheless do show apparent first-order oxidoreduction of ubiquinone, Ragan & Cottingham [4] devised a kinetic model that takes account of reversible binding of quinone and quinol to their enzymes. This model could give rise to first-order kinetics of oxidoreduction of ubiquinone even if the ubiquinone concentration were saturating. However, first-order oxidoreduction was not necessarily predicted if the dehydrogenase and cytochrome bc_1 complexes were not maintained in the reduced and oxidized states by substrate and cytochrome *c* respectively. Consider, for example, the reduction of the bc_1 complex by quinol:

$$QH_2 + b\text{-}566^{3+} + (Fe\text{–}S)_o \rightarrow Q + b\text{–}566^{2+} + (Fe\text{–}S)_r$$

where the subscripts o and r refer to oxidized and reduced forms respectively, Q is ubiquinone and *b*-566 is cytochrome *b*-566. The equilibrium constant for this process varies from approx. 2 in chromatophores [7] to 70 in bovine heart mitochondria [5]. The latter high value implies that, at equilibrium, the bc_1 complex is maintained highly reduced, even when the ubiquinone pool is quite oxidized. If the equilibrium condition is maintained by slow reoxidation of the complex by a low, limiting, concentration of cytochrome *c*, the rate of overall electron transport will be largely insensitive to changes in ubiquinone redox state, i.e. first-order behaviour will be lost. The extent to which this occurs will depend on the degree of limitation of the rate by cytochrome *c* and the magnitude of the equilibrium constant.

In the present paper we have used NADH:cytochrome *c* oxidoreductase reconstituted from bovine heart NADH:ubiquinone oxidoreductase (Complex I) and ubiquinol:cytochrome *c* oxidoreductase (Complex III) to demonstrate this effect of cytochrome *c* limitation. We show that the kinetic model of Ragan & Cottingham [4] can quantitatively account for the magnitude of the effect, thereby providing support for the assumptions inherent in the analysis. Lastly we describe the implications of this type of behaviour for the control of electron flux in mitochondrial membranes.

EXPERIMENTAL

Preparations

Complexes I and III were prepared from bovine heart mitochondria by the methods of Hatefi & Rieske [8] and Rieske [9] respectively and stored at −70 °C. A phospholipid fraction was obtained from crude soya-bean lipids as described by Ragan & Racker [10].

Assays

Molar concentrations of Complex I and III solutions were determined from measurement of flavin [11] and cytochrome c_1 [12] respectively. Phospholipid phos-

Abbreviations used: Q, ubiquinone; Q_{10}, ubiquinone-10.

* Present address and address for correspondence and reprint requests: Neuroscience Research Centre, Merck Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, U.K.

phorus was assayed by the method of Bartlett [13]. NADH:cytochrome *c* reductase was assayed by the decrease at 340 nm in a 1 ml final volume containing 20 μmol of potassium phosphate, pH 8.0, 0.1 μmol of NADH, 0.1 nmol of Complex I protein (previously reconstituted with Complex III) and either 20 nmol or 0.08 nmol of cytochrome *c* at 30 °C. The lower concentration was used for rate limitation by cytochrome *c*. Initially, purified bovine heart cytochrome oxidase was added to act as a regenerating system for oxidized cytochrome *c*. However, at the very low rates of electron flux encountered when using 80 nM-cytochrome *c*, trace contamination of Complex I by oxidase was found to be sufficient to maintain the rate. When present, myxothiazol was incubated with the enzyme in the cuvette for 2 min at 30 °C before addition of cytochrome *c*. After the establishment of a steady-state rate, the sample was quenched by rapid mixing with 5 ml of methanol/hexane (3:2, v/v). After centrifugation in a bench centrifuge, the upper hexane layer was removed. The aqueous layer was extracted three times with 1 ml of hexane, and the organic extracts were pooled and evaporated to dryness under vacuum. The residue was dissolved in 150 μl of ethanol, and 100 μl portions were analysed by h.p.l.c. Samples were applied to a Waters Resolve 5μ spherical C_{18} column as described by Takada *et al.* [14], except that the solvent used was ethanol/methanol (3:2, v/v). The $HClO_4$ and $NaClO_4$ used by the abovementioned authors for electrochemical detection of quinone caused degradation of the column packing and was not needed for effective separation of Q_{10} and $Q_{10}H_2$, as shown in Fig. 1. At a flow rate of 1.2 ml/min, retention times for $Q_{10}H_2$ and Q_{10} were 4.4 min and 8.3 min respectively. Detection was at 280 nm. At this wavelength, the absorption coefficients for $Q_{10}H_2$ and Q_{10} are 12000 $M^{-1} \cdot cm^{-1}$ and 3300 $M^{-1} \cdot cm^{-1}$ respectively [15]. Spectrophotometrically standardized Q_{10} in ethanol was used to provide a calibration curve that showed a linear relationship between peak area and amount of Q_{10} up to 800 pmol. Chemically reduced samples of Q_{10} were used to establish the linearity of the response to $Q_{10}H_2$ and the validity of the abovementioned absorption coefficients.

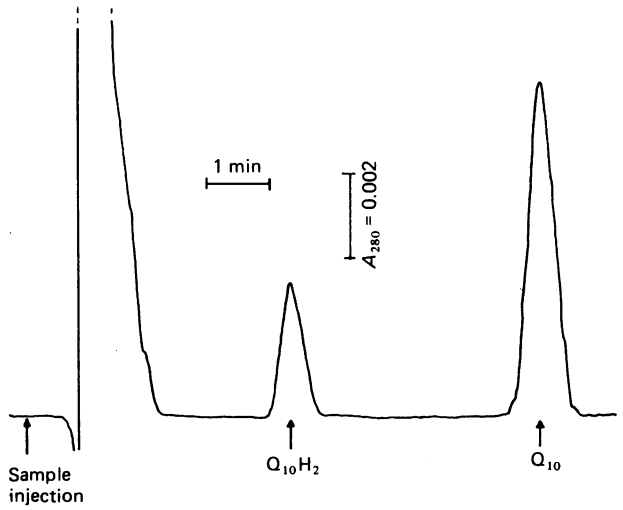

Fig. 1. Analysis of Q_{10} and $Q_{10}H_2$ by h.p.l.c.

A mixture containing 600 pmol of Q_{10} and 735 pmol of $Q_{10}H_2$ was analysed as described in the Experimental section.

Hereafter Q_t refers to the total concentration of redox-active quinone. Particularly in reconstituted systems supplemented with exogenous quinone, a large proportion of the quinone is not reducible by substrate (cf. [1]). Q_t was therefore determined by the amount of quinone reducible by NADH in the absence of cytochrome *c*. The amount reduced in the steady state by NADH in the presence of cytochrome *c* is Q_r, and Q_o is the difference between Q_r and Q_t.

Reconstitution of NADH:cytochrome *c* reductase

Complex I (125 μl of a 30.7 μM solution) was mixed with Complex III (218 μl of a 137 μM solution). Because of contamination of the Complex I by Complex III [8], this gave a final molar ratio of complex III to complex I of 0.2. To the mixture, 125 μl of 5 mM soya-bean phospholipid (prepared by ultrasonic dispersion in water) and 5 μl of 2.98 mM-Q_{10} was added, thereby increasing the endogenous Q_{10} content by approx. 4 mol per mol of Complex I. Portions containing 0.1 nmol of Complex I were diluted to 1 ml for assay of NADH:cytochrome *c* reductase.

Rotenone titrations

After dilution of the sample in the assay cuvette, rotenone (from stock solutions in ethanol) was added to give final concentrations ranging from 0 to 5 mol/mol of Complex I. The volume of ethanolic solution added was less than 10 μl. After 2 min at 30 °C, the assay was started by addition of NADH and cytochrome *c*.

RESULTS

In the original experiments of Kröger & Klingenberg [1], submitochondrial particles were titrated with rotenone to provide progressive inhibition of NADH oxidase activity. Measurement of the steady-state level of reduction of quinone by NADH showed a progressive oxidation as rotenone was increased, and there was a linear relationship between the rate of NADH oxidation and the proportion of quinone in the reduced state, plotted as Q_r/Q_t. The advantages of using a reconstituted system were, firstly, that the ratio of Complex III to Complex I could be manipulated so as to give an initial high level of quinone reduction and, secondly, that the cytochrome *c* concentration could be more readily controlled. In several previous papers [16–19] we have described the properties of the reconstituted NADH:cytochrome *c* reductase. In the presence of ubiquinone and low concentrations of exogenous phospholipid, the interaction between Complex I and Complex III behaves exactly as expected for first-order kinetics of quinone oxidoreduction, as shown, for example, by the dependence of velocity on the molar ratio of the two Complexes [16]. The reconstituted membranes do not present a permeability barrier to either NADH or cytochrome *c*, and the kinetic properties of the system are consistent with all molecules of the complexes having access to a single quinone pool. The reconstituted system consists apparently of vesicular membranes (despite the absence of a permeability barrier) in which the protein molecules are randomly distributed and in rapid motion [19].

In the experiment of Fig. 2, reconstitution was

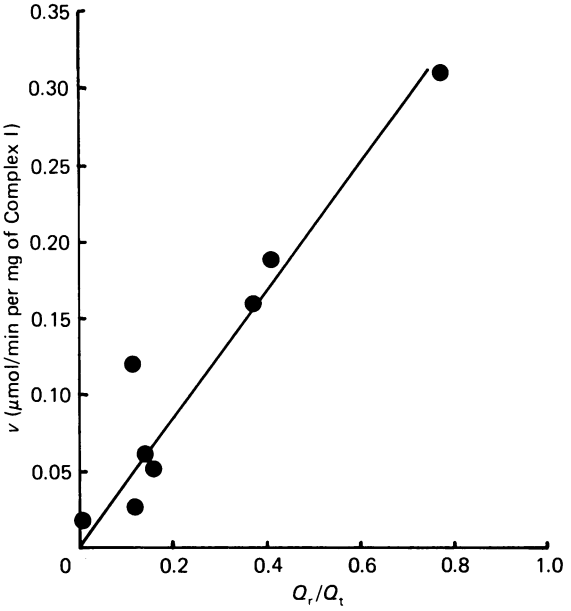

Fig. 2. Dependence of NADH:cytochrome *c* reductase on quinone redox state in the presence of excess cytochrome *c*

Assays were performed as described in the Experimental section on reconstituted Complex I–III pretreated with between 0 and 5 mol of rotenone/mol of Complex I to reduce both v and Q_r/Q_t. V_0' was determined from the slope to be 0.42 μmol/min per mg of Complex I.

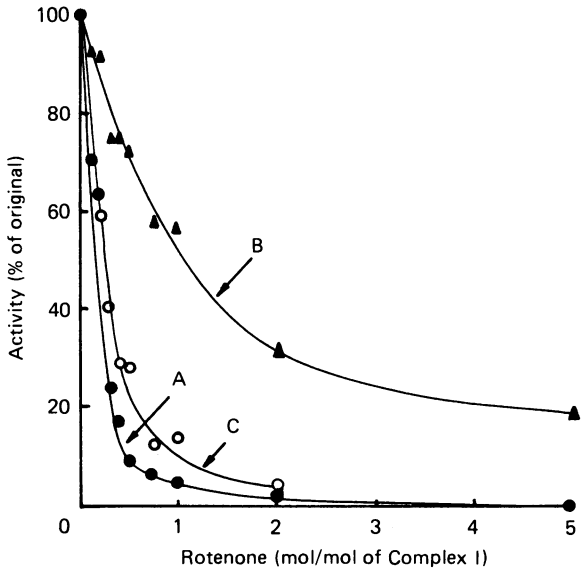

Fig. 3. Rotenone inhibition of NADH:cytochrome *c* reductase

Activity was measured with 20 μM- (A), with 80 nM- (B) or with 20 μM- (C) cytochrome *c* in the presence of 0.55 mol of myxothiazol/mol of Complex III. Maximum rates were, respectively, 0.40, 0.058 and 0.072 μmol/min per mg of Complex I protein.

performed with a low molar ratio of Complex III to Complex I to ensure substantial reduction of the quinone pool. Activity was then decreased by addition of various amounts of rotenone, which also caused oxidation of quinone. When measurement of rate was made with an excess of cytochrome *c* as acceptor, the dependence of velocity on Q_r/Q_t was linear, in agreement with the results of Kröger & Klingenberg [1] and in accord with expectations derived from previous work with this system [16,17].

The same experiment was then repeated either in the presence of myxothiazol or using much lower concentrations of cytochrome *c*. In either instance, the maximum rate was reduced by approximately one order of magnitude. The effect on rotenone titration curves is shown in Fig. 3. As anticipated, more rotenone is required to achieve a given degree of inhibition when activity through Complex III is severely curtailed either by myxothiazol or by cytochrome *c* limitation.

Curve A of Fig. 4 shows the dependence of rate on the redox state of the quinone in the presence of myxothiazol or limitation by cytochrome *c*. The effect of the former treatment is to decrease the slope of the line without greatly affecting its shape. This was expected, since myxothiazol decreases the maximum velocity of the cytochrome bc_1 complex (k_{+2} as defined in the Appendix) without affecting the rate constant for interaction with cytochrome *c* (k_{sub} as defined in the Appendix). On the other hand, when velocity was reduced by cytochrome *c* limitation to a similar extent (Fig. 4, B), the dependence of rate on Q_r/Q_t became distinctly non-linear. The more extreme the limitation the more marked the non-linearity became. Indeed, in the experiment of Fig. 4(C), reduction of the quinone pool could not be detected until the rate of electron flux was an appreciable proportion of its maximum.

The continuous curved lines of Fig. 4 were obtained by fitting to a theoretical equation derived in the Appendix [eqn. (7)]:

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

In this equation, v is the velocity, α is a measure of the degree of limitation by substrate (a low value depicting more extreme limitation), V_0' is the maximum rate of electron flux when $Q_r = Q_t$ and α is very high (determined from the slope of the line in experiments such as Fig. 2), and β is the reciprocal of the equilibrium constant for the reaction of quinol with the cytochrome bc_1 complex and is taken as 1/70 or 0.0143 in the present work.

Thus with V_0' determined from a parallel experiment with an excess of cytochrome *c*, only α is unknown. The line of Fig. 4(A) is the result of a non-linear least-squares fit giving $\alpha = 0.109$ (±0.010, S.E.M.). The data could be fitted equally well if β was decreased to zero, but the fit deteriorated at values of β greater than 0.05, i.e. the results are consistent with value of the equilibrium constant in excess of 20, but cannot distinguish between higher values (see the Appendix). The data of Fig. 4(C) were fitted with $\alpha = 0.058$ (±0.004, S.E.M.), i.e. a more severe degree of limitation. The results in Figs. 2 and 4(A) are much better fitted as straight lines. When α is high, as in these experiments, the above equation reduces to:

$$v = V_0' \cdot Q_r/Q_t$$

i.e. v is linearly related to Q_r/Q_t (see the Appendix).

DISCUSSION

The observation that rate limitation by cytochrome *c* makes ubiquinol oxidation no longer a first-order process

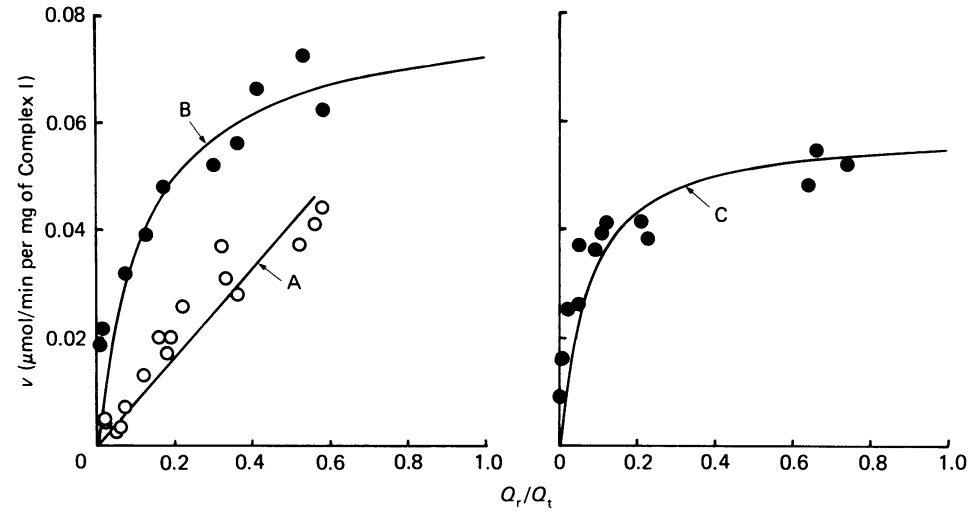

Fig. 4. Dependence of NADH:cytochrome *c* reductase on quinone redox state in the presence of myxothiazol or limiting cytochrome *c*

A, Assays were performed as described in the Experimental section with 20 μM-cytochrome *c* as acceptor and in the additional presence of 0.55 mol of myxothiazol/mol of Complex III. Rotenone was present to decrease v and Q_r/Q_t at 0–5 mol/mol of Complex I. B, Assays were performed with 80 nM-cytochrome *c* as acceptor. Rotenone was present as in A. For this experiment, V_0' was determined as in Fig. 2 to be 0.73 μmol/min per mg of Complex I protein. C, as B, but using different preparations of enzymes. V_0' was 1.0 μmol/min per mg of Complex I protein (results not shown).

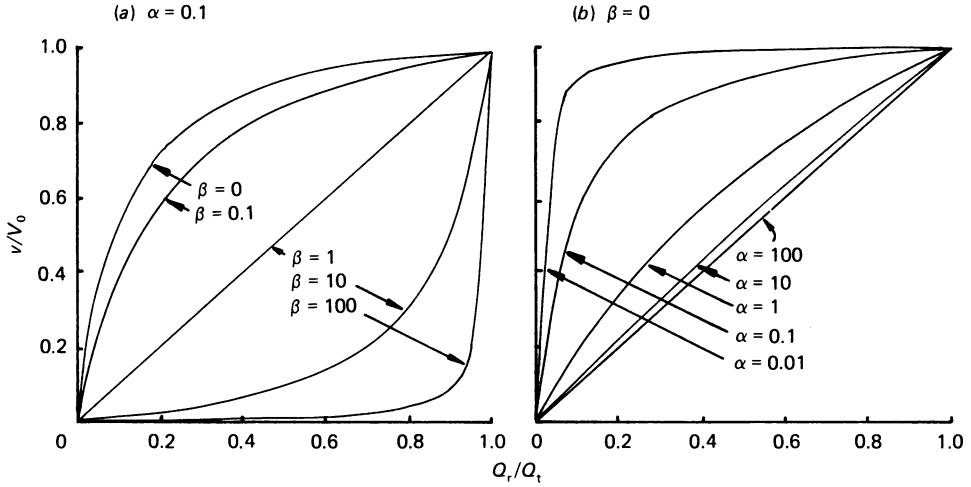

Fig. 5. Theoretical dependence of v/V_0 on Q_r/Q_t as a function of α and β

(*a*) Behaviour of eqn. (4) of the Appendix with $\alpha = 0.1$ and varying β. (*b*) Behaviour of eqn. (4) with $\beta = 0$ and varying α.

is not particularly surprising, although the dependence of the effect on the equilibrium constant for electron transfer between quinol and enzyme is less obvious. Nevertheless, in previous studies of the function of quinones, the possibility that limitation of rate by the availability of the dehydrogenase substrate or oxidized cytochrome *c* could cause a deviation from the simple kinetics of Kroger & Klingenberg [1] has been completely ignored. Certainly, in experiments with intact mitochondria, limitation of flux by the rate of substrate permeability is a very strong possibility. Even if this occurs, however, the extent of deviation from simple first-order behaviour will depend on the equilibrium constant for oxidation of quinol by its oxidase enzyme, and this is not necessarily as large as the value found for bovine heart mitochondria.

Another reason why the effect of substrate limitation has not been previously reported is that measurements of quinone redox state as a function of electron flux are rarely undertaken, and most workers rely on the shape of inhibitor titrations (see, e.g., [20]) or on the extent of competition between substrates (see, e.g., [21,22]) to assess the workings of the quinone pool. The problem with these approaches is that first-order quinone oxido-reduction is invariably assumed, and minor deviations from expected behaviour may then be interpreted as indicating heterogeneity of the quinone pool. For example, consider a branched respiratory chain containing several types of quinone reductases linked to a single quinol oxidase. Suppose that one of these quinone reductases has a terminal redox group of relatively low

midpoint potential so that reduction of ubiquinone is essentially irreversible. Fig. 4 shows that, when the flux through the enzyme is controlled by its substrate, electron transfer becomes largely insensitive to ubiquinone redox state. Thus oxidation of the substrate would not be affected by simultaneous oxidation of other substrates, the competition between branches of the system would not be apparent, and it would be concluded that there are multiple quinone pools present in the membrane. This example illustrates that marked deviations from expected behaviour can arise without any change in the underlying mechanism or in the existence of a homogeneous quinone pool.

Lastly, the quinone pool model of Ragan & Cottingham [4] was designed to provide a mechanism in which first-order oxidoreduction of quinone would be anticipated over the wide range of conditions employed experimentally. The fact that the same model can quantitatively account for deviations from first-order oxidoreduction provides strong support for the basic correctness of the model and the assumptions made in the kinetic analysis.

This work was supported by research grants from the Science and Engineering Research Council and the SmithKline Foundation.

REFERENCES

1. Kröger, A. & Klingenberg, M. (1973) Eur. J. Biochem. **34**, 358–368
2. Kröger, A. & Klingenberg, M. (1973) Eur. J. Biochem. **39**, 313–323
3. Gardestrom, P. & Edwards, G. E. (1983) Plant Physiol. **71**, 24–29
4. Ragan, C. I. & Cottingham, I. R. (1985) Biochim. Biophys. Acta **811**, 13–31
5. Rich, P. R. (1984) Biochim. Biophys. Acta **768**, 53–79
6. Gutman, M. (1980) Biochim. Biophys. Acta **594**, 53–84
7. Crofts, A. R. (1985) in The Enzymes of Biological Membranes, 2nd edn. (Martonosi, A. N., ed.), pp. 347–382, Plenum Publishing Corp., New York
8. Hatefi, Y. & Rieske, J. S. (1967) Methods Enzymol. **10**, 235–239
9. Rieske, J. S. (1967) Methods Enzymol. **10**, 239–245
10. Ragan, C. I. & Racker, E. (1973) J. Biol. Chem. **248**, 2563–2569
11. Ragan, C. I., Galante, Y. M. & Hatefi, Y. (1982) Biochemistry **21**, 2518–2524
12. Rieske, J. S. (1967) Methods Enzymol. **10**, 488–493
13. Bartlett, G. R. (1969) Methods Enzymol. **14**, 485–487
14. Takada, M., Ikenoya, S., Yuzuriha, T. & Katayama, K. (1982) Biochim. Biophys. Acta **679**, 308–314
15. Morrison, L. E., Schelhorn, J. E., Cotton, T. M., Bering, C. L. & Loach, P. A. (1982) in Function of Quinones in Energy Conserving Systems (Trumpower, B. L., ed.), pp. 35–38, Academic Press, New York
16. Heron, C., Ragan, C. I. & Trumpower, B. L. (1978) Biochem. J. **174**, 791–800
17. Heron, C., Gore, M. G. & Ragan, C. I. (1979) Biochem. J. **178**, 415–426
18. Poore, V. M. & Ragan, C. I. (1982) Biochim. Biophys. Acta **693**, 105–112
19. Poore, V. M., Fitzsimmons, J. T. R. & Ragan, C. I. (1982) Biochim. Biophys. Acta **693**, 113–124
20. Zhu, Q. S., Berden, J. A., DeVries, S. & Slater, E. C. (1982) Biochim. Biophys. Acta **680**, 69–79
21. Cottingham, I. R. & Moore, A. L. (1983) Biochim. Biophys. Acta **724**, 191–200
22. De Troostemburgh, J.-G. & Nyns, E.-J. (1979) Eur. J. Biochem. **85**, 423–432

APPENDIX

Theoretical analysis

For reduction of the cytochrome *bc* complex (*bc*) by quinol, we consider the following steps:

$$bc_o + Q_r \overset{k_{+1}}{\rightleftharpoons} bc_o \cdot Q_r \overset{k_{+2}}{\rightleftharpoons} bc_r \cdot Q_o$$

$$bc_o + Q_o \overset{k_{-1}}{\rightleftharpoons} bc_o \cdot Q_o \qquad k_{-2}$$

$$bc_r + Q_r \rightleftharpoons bc_r \cdot Q_r \overset{k_{sub}}{\rightarrow} bc_o \cdot Q_r$$

$$bc_r + Q_o \rightleftharpoons bc_r \cdot Q_o \overset{k_{sub}}{\rightarrow} bc_o \cdot Q_o$$

$$bc_r \overset{k_{sub}}{\rightarrow} bc_o$$

where bc_o and bc_r are the oxidized and reduced forms of the enzyme, Q_o and Q_r the oxidized and reduced forms of quinone, k_{+1} and k_{-1} are rate constants governing the reversible formation of enzyme–quinone complexes and assumed to apply to all four binding reactions, k_{+2} and k_{-2} are rate constants governing the reversible electron transfer from Q_r to the enzyme, and k_{sub} is a pseudo-first-order rate constant covering binding of cytochrome *c* and its reduction by the reduced enzyme. The most obvious simplification inherent in this scheme is the consideration of events occurring only at the quinol oxidase site. However, it seems that the quinone reductase site of the *bc* complex is always effectively saturated with Q_o, even at low Q_o concentrations, and therefore electron flux is modulated by changes in the pool redox state only through the quinol oxidase site [1].

If we assume that Q_o and Q_r are greater than the concentration of *bc* complex and k_{-1} is greater than k_{+2}, we can derive the following equation for the dependence of velocity (v) on Q_r and Q_o:

$$v = \frac{k_2 \cdot k_{sub} \cdot [bc] \cdot Q_r}{k_{sub}(K_s + Q_t) + k_{-2}Q_o + k_{+2}Q_r} \qquad (1)$$

where $K_s = k_{-1}/k_{+1}$ and $Q_t = Q_o + Q_r$. To simplify further analysis, we assume that quinone is saturating ($K_s \ll Q_t$) and obtain:

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

when $\alpha = k_{sub}/k_{+2}$ and $\beta = k_{-2}/k_{+2}$. The maximum velocity, V_0, is obtained when all quinone is reduced ($Q_r = Q_t$), giving:

$$V_0 = \frac{\alpha k_{+2}[bc]}{(\alpha+1)} \quad (3)$$

Combining eqns. (2) and (3), we obtain:

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

The corresponding expression expected for simple first-order dependence of v on Q_r is:

$$v = \frac{V_0 \cdot Q_r}{Q_t} \quad (5)$$

as originally derived by Kröger & Klingenberg [2]. Eqn. (4) collapses to eqn. (5) if $\beta = 1$ or if α is very large. The first of these conditions requires that $k_{-2} = k_{+2}$, which appears not to be the case for bovine heart mitochondria, where β is 1/70 or 0.0143 [3]. The second condition requires that electron transfer should not be limited by cytochrome *c*, a state which is commonly established in work on quinone pools.

The behaviour of eqn. (4) is illustrated in Fig. 5 of the main paper, where deviation from a linear dependence of v/V_0 on Q_r/Q_t becomes more marked as β is increased or decreased from 1 (Fig. 5*a*, main paper) or if α is decreased (Fig. 2, main paper). Note that even if β is zero, i.e. electron transfer is irreversible, values of α in excess of one produce an almost linear dependence of v/V_0 on Q_r/Q_t.

As it stands, eqn. (4) contains two unknown quantities, α and V_0, the velocity when $Q_r = Q_t$. However, these two are not independent variables, since when α is very large, V_0 becomes constant [k_{+2} [*bc*] from eqn. (3)] and can be determined from the slope of the linear dependence of v on Q_r/Q_t in the presence of excess cytochrome *c* (e.g. as in Fig. 2 of the main paper). If we denote this quantity as V_0', eqn. (3) gives:

$$V_0 = \frac{\alpha \cdot V_0'}{1+\alpha} \quad (6)$$

and substituting eqn. (6) into eqn. (4) we obtain:

$$v = \frac{\alpha \cdot V_0' \cdot Q_r/Q_t}{(\alpha+\beta) + (1-\beta)Q_r/Q_t} \quad (7)$$

If we take $\beta = 0.0143$, then there is only one unknown parameter, α, to be fitted. Alternatively, the fit can be made with various values of β to test the robustness of the dependence of the relationship between v and Q_r/Q_t on β.

REFERENCES

1. Venturoli, G., Fernandez-Velasco, J. G., Crofts, A. R. & Melandri, B. A. (1986) Biochim. Biophys. Acta **851**, 340–352
2. Kröoger, A. & Klingenberg, M. (1973) Eur. J. Biochem. **39**, 313–323
3. Rich, P. R. (1984) Biochim. Biophys. Acta **768**, 53–79

Received 27 February 1987/23 June 1987; accepted 20 July 1987