Hypothesis

# PROTONMOTIVE REDOX MECHANISM OF THE CYTOCHROME $b-c_1$ COMPLEX IN THE RESPIRATORY CHAIN: PROTONMOTIVE UBIQUINONE CYCLE

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## 1. Introduction

There are two major interrelated difficulties that have hampered progress in our understanding and rational experimental exploration of the protonmotive and redox functions of the cytochrome $b-c_1$ region of the respiratory chain, using orthodox biochemical theory together with the conceptual facilities of the classical proton-translocating redox loop [1] and some variants of this [1–3] that have so far been explicitly at our disposal. One difficulty is, as pointed out by Slater [4], the apparent lack of a hydrogen carrier for Loop 3. The other difficulty is the peculiar kinetic and thermodynamic behaviour of the *b* cytochromes, which has received much attention [5–11], but not yet a completely satisfactory explanation [12–21].

In this paper I describe the protonmotive Q* cycle that provides a basis, both for explaining the proton-translocating function of the cytochrome $b-c_1$ segment of the respiratory chain, and for rationalising much of the biochemical information about the behaviour and functions of the *b* cytochromes and ubiquinone, which previously appeared to be difficult to interpret simply.

* *Abbreviations:* Q, ubiquinone; $QH^{\cdot}$, ubiquinone semiquinone; deH, dehydrogenase; C side and M side, opposite sides of membrane or of respiratory chain complex corresponding to cytochrome *c* side and matrix side respectively; $\rightarrow H^+/O$, number of hydrogen ions translocated per oxygen atom reduced; State 4, state of steady mitochondrial respiration in the presence of substrate and inorganic phosphate but in the absence of phosphate acceptor.

## 2. The protonmotive Q cycle

The general principle of the protonmotive Q cycle of the cytochrome $b-c_1$-dehydrogenase complexes in the coupling membrane of mitochondria and bacteria is illustrated by the flow diagram in fig.1. The ubiquinone-reactive centres marked deH, $b_T$, $c_1$ and $b_K$ should be taken to represent the reactive groups, associated with the dehydrogenase and cytochrome proteins respectively, which may involve or include the non-haem iron centre(s) thought to participate in this system [22–28]. According to the reversible arrows, the passage of each reducing equivalent through the $b-c_1$ complex, from deH on the M side to $c_1$ (and thence to the natural oxidant cytochrome *c*) on the C side, would be mediated by the diffusion of Q once round its closed orbit, involving two successive one-equivalent reductions and two successive one-equivalent oxidations, via the semiquinone $QH^{\cdot}$ (or its depronated form $Q^{-}$).

As the $b-c_1$ complex is normally plugged through the coupling membrane, it must include a region, between the centres on the C side and the centres on the M side, that normally functions as an osmotic barrier. Presumably the translocation of $QH_2$ and of Q (but not of $QH^{\cdot}$ or $Q^{-}$) depends on appropriate thermally-activated conformational mobility of this condensed osmotic barrier region of the complex, in which phospholipid plays an essential part [21–23]. On the other hand, the diffusion of $QH^{\cdot}$ between $b_T$ and $c_1$, and between $b_K$ and deH, would not involve translocation through this condensed barrier, and it is even possible that little motion of $QH^{\cdot}$ might be required, because the pairs of centres ($b_T$ with $c_1$,

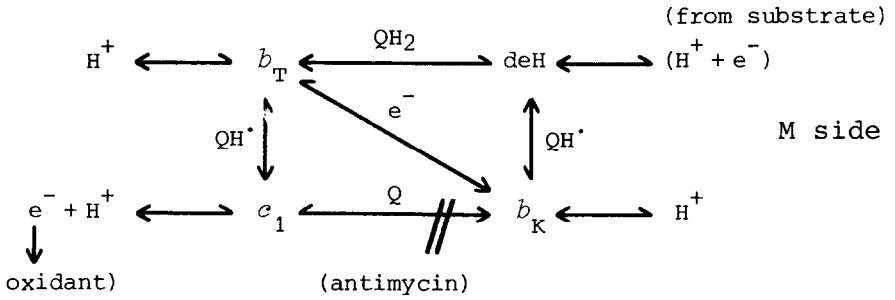


Fig.1. Flow diagram of Q cycle in cytochrome $b$–$c_1$-dehydrogenase complexes. The symbols deH, $b_T$, $c_1$ and $b_K$ represent ubiquinone-reactive centres associated with the dehydrogenase and cytochromes respectively in the complex. For each reducing equivalent passing through the system from deH to $c_1$, the redox functional group Q of ubiquinone passes once round its closed orbit (counter-clockwise in this diagram). The complex includes an osmotic barrier region through which Q and $QH_2$ (but not QH· or $Q^-$) must equilibrate between the C side and the M side. The disconnection of the QH·/Q couple from $b_K$ by antimycin is represented as an interruption of the flow of Q for symbolic simplicity.

and $b_K$ with deH) might be in close apposition. Accordingly, the time constant for equilibration of QH· laterally between the centres on the same side of the complex is expected to be small compared with that for equilibration of $QH_2$ and of Q between the centres on the opposite sides of the complex.

The accepted view that antimycin blocks the respiratory chain on the oxygen side of the $b$ cytochromes [9] implies that it would prevent interaction between $b_K$ and the QH·/Q couple in the Q cycle. For symbolic simplicity, this is represented in fig.1 as an interruption in the flow of Q.

The topological arrangement of the $b$ cytochromes across the osmotic barrier region of the complex corresponds to that proposed in my earlier schemes [1,29,30] and as adopted by Wikström [11]. The new scheme resembles that proposed by Cox, Gibson and co-workers [see 13] for the respiratory chain of *Escherichia coli* in placing ubiquinone on both sides of $b$ cytochromes, although it differs in the proposed topology and chemistry. It also resembles the scheme of Wikström and Berden [19], and is related to the earlier scheme of Baum and co-workers [12], in postulating that a cytochrome $b$ is reduced by the $QH_2$/QH· couple and that the QH·/Q couple is oxidised by a cytochrome $c$: It differs topologically, however, and in the crucial respect that QH· is both oxidant and reductant for $b$ cytochromes in a closed Q cycle.

Much of the research on the cytochrome $b$–$c_1$ complex has been done on preparations that lack significant osmotic properties, and we shall therefore consider the purely biochemical properties of the Q cycle before proceeding to discuss the chemiosmotic properties of the cyclic Loop 2–3 system, in which it is suggested that the protonmotive Q cycle plays a major part.

## 3. Biochemical properties of the Q cycle

It can readily be deduced from the flow diagram of the Q cycle (fig.1) that, if equilibration occurred at uniform total protonic potential only according to the reversible arrows, when the system was poised by substrate in the absence of oxidant for $c_1$ (and in the absence of antimycin), $b_T$ and $b_K$ would equilibrate to the same redox potential $E_h$ ($b$), so that

$$E_h(b_T) = E_h(b_K) = E_h(b) \quad (1)$$

Likewise, deH and $c_1$ would equilibrate, so that

$$E_h(\text{deH}) = E_h(c_1) \quad (2)$$

However, as long as QH· did not equilibrate between the C and M sides of the complex deH and $c_1$ would not equilibrate to the same redox potential as $b_T$ and $b_K$, but these pairs of centres would come to different redox potentials related by the ratio of the activities of QH· on the opposite sides of the complex, or

$$E_h\,(b) = E_h\,(\text{deH or } c_1) - Z \log_{10}\left(\frac{[QH^{\cdot}]_M}{[QH^{\cdot}]_C}\right) \quad (3)$$

where the square brackets denote activity and $Z$ is the conventional factor, 2.303 $RT/F$, having a value of about 60 mV at 25°C.

We accept that, in practice, $QH^{\cdot}$ would equilibrate slowly between the C and M sides of the complex, so that, in the absence of oxidant for $c_1$, $E_h$ (b) would settle towards $E_h$ (deH). We also accept that effective redox equilibration between the $b$ cytochromes and the dehydrogenase would depend on kinetically significant concentrations of all the reactants round the cycle (fig.1); and it would therefore be expected that highly reducing substrate would not be kinetically very effective as reductant for the $b$ cytochromes under these conditions.

Similar reasoning indicates that during steady-state redox activity at uniform total protonic potential in the presence of oxidant for $c_1$ and reducing substrate for deH (when $c_1$ would be at a more oxidising redox potential than deH), $E_h$ ($b$) would settle towards an intermediate potential between that of $E_h$ (deH) and $E_h$ ($c_1$). But, if the rate of withdrawal of electrons from $c_1$ were suddenly increased by oxidant, there would, according to fig.1, be a corresponding increase in the rate of conversion of $QH^{\cdot}$ to Q on the C side and a transient fall in $[QH^{\cdot}]_C$, which could cause a transient reduction of $b_T$ and thence of $b_K$. Only transient reduction of the $b$ cytochromes would be possible under these conditions because the Q produced by $QH^{\cdot}$ oxidation on the C side would soon diffuse across to the M side, where it would begin to reoxidise $b_K$ and thence $b_T$, and $E_h$ ($b$) would settle between $E_h$ (deH) and $E_h(c_1)$, as before.

Antimycin would be expected to stabilise the reduction of the $b$ cytochromes, in the presence of reducing substrate and oxidant, by disconnecting $b_K$ from (oxidation by) the $QH^{\cdot}/Q$ couple while permitting withdrawal of $QH^{\cdot}$ (the oxidant of $b_T$) on the C side. The reducing action of substrate via the dehydrogenase would presumably depend on the supply of the specific oxidant $QH^{\cdot}$ of the deH on the M side, and this, in turn, would depend on the presence of oxidant for $c_1$, because the $QH^{\cdot}$ needed to 'activate' the dehydrogenase would presumably have to be generated by residual antimycin-insensitive redox activity. Thus, in the presence of antimycin, as in its absence, the reduction of the $b$ cytochromes by reducing substrates would be expected to be inhibited when there was no oxidant for cytochrome $c_1$.

This description of the behaviour of the hypothetical Q cycle conforms remarkably closely to the experimental behaviour of cytochrome $b$–$c_1$ preparations and their dehydrogenase complexes, aspects of which have long been recognised as puzzling [5–28]. In particular, the Q cycle appears to explain the fact established by Eisenbach and Gutman [16], by Glazek and co-workers [17] and by DeKok and Slater [14] (but already foreshadowed in the remarkable paper by Baum and co-workers [12]) that the rate of oxidation of the dehydrogenases through the cytochrome $b$–$c_1$ complex is under the control of, or is 'activated by', a substance produced in response to an oxidant reacting with the respiratory chain on the oxygen side of the antimycin-sensitive site. According to the Q cycle concept, this substance is $QH^{\cdot}$, the specific oxidant of the dehydrogenases that reconstitute with the cytochrome $b$–$c_1$ complex.

## 4. Chemiosmotic properties of the cyclic Loop 2–3 system

As illustrated in fig.2, in the classical respiratory chain system, oxidation of the cytochrome $b$–$c_1$ complex would occur via cytochrome $c$ and via cytochrome oxidase which, it is generally agreed, is plugged through the coupling membrane so that it conducts electrons from the C to the M side, where oxygen is reduced and protonated to form water [1,29,31–35]. Inspection of fig.2 shows that the cyclic Loop 2–3 system would exhibit the same $\rightarrow H^+/O$ quotient of 4 for the oxidation of 2H through the deH centre as the classical Loop 2 and Loop 3 scheme [1], and this corresponds to the observed $\rightarrow H^+/O$ quotient of 4 for succinate oxidation, and of 4 + 2 for NADH oxidation (which includes Loop 1) [30]. In the case of ubiquinol oxidation, however, the classical Loop 2 and Loop 3 scheme [1] would be expected to give an $\rightarrow H^+/O$ quotient of 2, whereas the cyclic Loop 2–3 scheme of fig.2 should give a value of 4. The latter is compatible with the observation of Lawford and Garland [36], who found an $\rightarrow H^+/O$ quotient of 4 for ubiquinol oxidation in ox heart mitochondria and pointed out that this cast doubt on the classical Loop 2 and Loop 3 scheme.

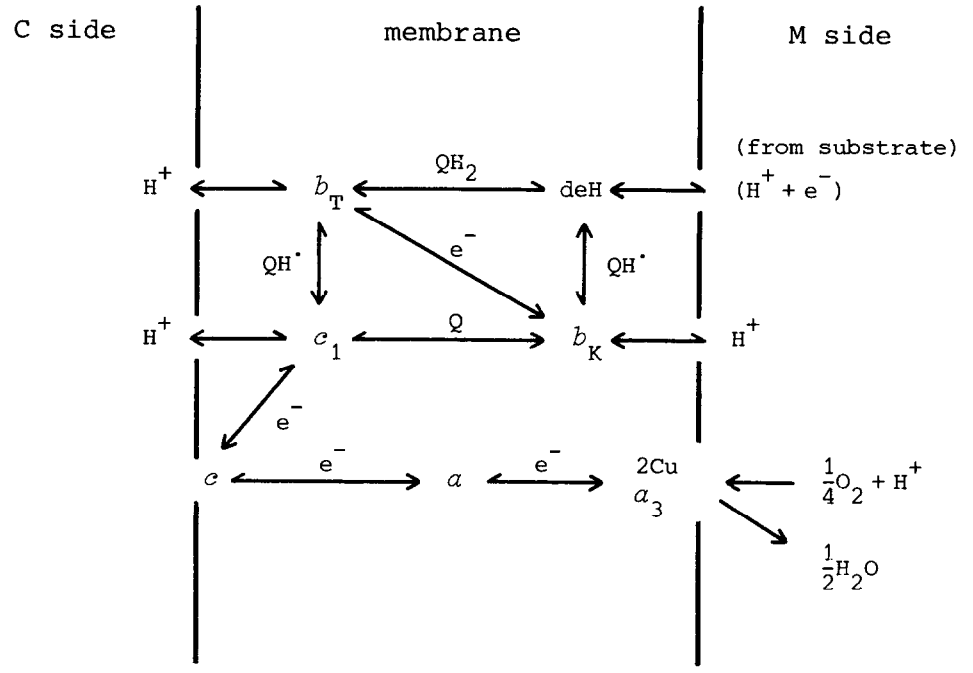


Fig.2. Diagram of proton-translocating cyclic Loop 2–3 system, consisting of the Q cycle system with cytochrome *c* and cytochrome oxidase (*a*, 2Cu, $a_3$) as oxidant for cytochrome $c_1$. When the dehydrogenase centre deH is that of NADH dehydrogenase, the translocation of one $H^+$ per $e^-$ also occurs through Loop 1 (not shown).

To obtain a general appreciation of the effects of electric potential and pH differences on the behaviour of the cytochrome $b$–$c_1$ complex, see [2], let us assume that the total protonic potential of the centres $c_1$ and $b_T$ is higher than that of $b_K$ by an amount $\Delta_p$, and that this protonic potential difference is made up of an electric potential difference $\Delta'\psi$ plus a thermodynamic potential difference $-Z\Delta'$pH, using the conventions that $\Delta'$ or $\Delta$ means the value of the variable on the C side minus that on the M side, and that $\Delta'$ and $\Delta$ refer to the potential differences between the reaction centres and between the aqueous phases on the opposite sides of the membrane respectively, and bearing in mind that $\Delta' p = \Delta p$.

As shown previously [2,29,30] the redox equilibrium between $b_T$ and $b_K$ would be affected by the electric potential difference $\Delta'\psi$, so that

$$E_h(b_T) = E_h(b_K) - \Delta'\psi \qquad (4)$$

Also, the redox equilibria between $c_1$ and $b_K$ would be influenced by $\Delta'$pH [2], so that

$$E_h(b_K) = E_h(c_1) - Z \log_{10}\left(\frac{[QH^\cdot]_M}{[QH^\cdot]_C}\right) + Z\Delta'\text{pH} \qquad (5)$$

Hence,

$$E_h(b_T) = E_h(c_1) - Z \log_{10}\left(\frac{[QH^\cdot]_M}{[QH^\cdot]_C}\right) - \Delta p \qquad (6)$$

In mitochondria respiring in State 4, redox potentiometric and spectrophotometric measurements [11,20,37] indicate that $E_h(b_T)$ is some 150 mV more negative than $E_h(b_K)$, or, according to equation 4, the electric potential difference $\Delta'\psi$ between the $b_T$ and $b_K$ centres is about 150 mV – about three quarters of the electric membrane potential $\Delta\psi$, estimated to be some 200 mV in State 4 [38,39]. Assuming a total protonic potential difference $\Delta p$ of some 300 mV in State 4, $-Z\Delta'$pH would be some

150 mV, and according to equation 5, $E_h$ ($b_K$) would be about 150 mV more negative than $E_h$ ($c_1$) if the activity ratio $[QH^{\cdot}]_M/[QH^{\cdot}]_C$ were unity. The estimates at present available [37] of the redox potentials of the *b* cytochromes relative to cytochrome *c* (or $c_1$) in State 4 indicate that $E_h$ ($c_1$) is near +300 mV and that $E_h$ ($b_K$) and $E_h$ ($b_T$) are near +50 mV and −100 mV respectively. These estimates of $E_h$ ($b_K$) and $E_h$ ($b_T$) are about 100 mV more negative than would be accounted for by the simple cyclic Loop 2–3 system, as presented here, with a $[QH^{\cdot}]_M/[QH^{\cdot}]_C$ ratio of unity.

The general pattern of chemiosmotic behaviour of the theoretical cyclic Loop 2–3 system corresponds well to that of the actual cytochrome system; and it may be premature to expect precise quantitative agreement between predicted and estimated $E_h$ values, especially while there is some doubt about the reliability of the assumptions involved in the experimental estimation of the redox potentials of the *b* cytochromes. Bearing these reservations in mind, however, it is interesting to note that, according to equations 5 and 6, the above experimental values of $E_h$ ($b_K$) and $E_h$ ($b_T$) in State 4 could be accounted for quantitatively if the $[QH^{\cdot}]_M/[QH^{\cdot}]_C$ ratio settled at a value of about 50 in State 4. Presumably, $[QH^{\cdot}]_M$ might settle at a higher level than $[QH^{\cdot}]_C$ either because of net synthesis of $QH^{\cdot}$ on the M side (which could be caused by a redox bypass, conducting reducing equivalents from deH to ($b_K$) or by net transport (which could be protonmotivated) of $QH^{\cdot}$ from the C to the M side in State 4.

## 5. Research prospect

The concepts of the protonmotive Q cycle and of the cyclic Loop 2–3 system, briefly outlined here, provide a simple basis for explaining many biochemical and chemiosmotic properties of the cytochrome $b$–$c_1$ complex of the classical respiratory chain, and also of the whole cytochrome system, that were previously difficult to rationalise.

The idea, implicit in the Q cycle, that $QH^{\cdot}$ is the specific oxidant of the dehydrogenases that reconstitute with the cytochrome $b$–$c_1$ complex, is , I suggest, the natural conclusion towards which much biochemical research has been leading over the last twenty years, since Singer and Kearney [40] discovered that the semiquinone-forming phenazine methosulphate acts as oxidant for soluble succinate dehydrogenase, and Keilin and King [41] first reincorporated a reconstitutively-active soluble succinate dehydrogenase into a particulate respiratory chain system, where the lipophilic oxidant $QH^{\cdot}$ of the dehydrogenase is appropriately generated. However, regardless of the obvious attractiveness of the concept of the Q cycle for rationalising the results of past research, its value as a research tool will depend on its usefulness for the acquisition of more detailed information about the molecular mechanics of respiratory chain systems.

It is hoped that this paper will stimulate more intensive research on the cytochrome $b$–$c_1$ complex and on the cytochrome $b$–$c_1$-dehydrogenase complexes; and, in particular, that it may promote investigations of the participation of $QH^{\cdot}$ in these systems [42–45], with a view to rejecting or developing the Q cycle concept.

Although the Q cycle has been discussed here in the context of the classical mitochondrial cytochrome system, a similar type of Q cycle may operate in other cytochrome systems, such as in *Escherichia coli,* or in phosphosynthetic bacteria, or even in chloroplasts. Perhaps the present brief paper will help to encourage appropriate theoretical and experimental exploration.

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