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Thermodynamic and Kinetic Considerations of Q-Cycle Mechanisms and the Oxidant-Induced Reduction of Cytochromes *b*

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Abstract

In coenzyme Q-cycles, it is proposed that one electron from the quinol reduces the Rieske iron sulfur center ($E_m \sim 280$ mV) and the remaining electron on the semiquinone reduces cytochrome b_T ($E_m \sim -60$ mV). The E_m for the two-electron oxidation of the quinol is ~60 mV and therefore the reduction of cytochrome b_T by quinol is not favorable. As the stability constant for the dismutation of the semiquinone decreases, the calculated E_m for the Q/QH$^\cdot$ couple is lowered to values below the E_m of cytochrome b_T. Contemporary coenzyme Q-cycles are based on the belief that the lower value for the E_m of the Q/QH$^\cdot$ couple compared to the E_m for cytochrome b_T means that the semiquinone is a spontaneous reducing agent for the *b*-cytochrome. The analysis in the paper shows that this is not necessarily so and that neither binding sites nor ionization of the semiquinone *per se* alters this situation. For a Q-cycle mechanism to function, *ad hoc* provisions must be made to drive the otherwise unfavorable reduction of cytochrome b_T by the semiquinone or for the simultaneous transfer of both electrons to cytochrome b_T and cytochrome c_1 (or the iron sulfur protein). Q-cycle mechanisms with these additional provisions can explain the observation thus far accumulated. A linear path which is functionally altered by conformational changes may also explain the data.

Key Words: Ubisemiquinone; complex III; Rieske iron sulfur center; quinone redox couples.

Introduction

There is today more uncertainty about the structure and function of the mammalian electron-transport chain than at any time since the major

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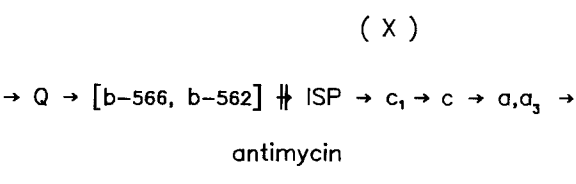


Fig. 1. Simple linear scheme for electron flow through Complexes III and IV. Electrons are inserted via specific dehydrogenases to coenzyme Q (Q) and then sequentially through cytochromes b_{566} (b_T) and b_{562} (b_K) and then through an antimycin-sensitive site to the Rieske iron sulfur protein (ISP) and cytochrome c_1 (c_1). The final stages of the transfer to oxygen use cytochromes *c* and *a*, a_3. "X" signifies a suspected (unidentified) component whose redox state can influence the redox potential of cytochrome(s) *b*. (See Rieske, 1971, and Eisenbach and Gutman, 1975).

components have been known and their relative sequence fixed by a combination of potentiometric and crossover studies. This basic chain as we understood it in 1975 is shown in Fig. 1. The reason for questioning this arrangement was that, according to Mitchell's concept, proton translocation is effected by hydrogen-transporting components of the electron-transport chain arranged alternately with electron-transporting components. There are no known hydrogen-transporting components at energy-transducing site III. Therefore, the energy liberated at site III must be used to drive protons at some other location which Mitchell proposed to be site II. This extra transport of protons at site II employs coenzyme Q functioning in a cycle such that for each electron inserted by a dehydrogenase from the mitochondrial matrix, the cycling electron from coenzyme Q adds an additional proton-carrying vehicle (Mitchell, 1976). In subsequent years, a number of publications from many laboratories gave evidence that protons are transported during the passage of electrons through site III either when inhibitors were present to block site II or when site II was absent as is the case with site III embedded in liposomal membranes (Wikström *et al.*, 1981). Therefore, the original considerations which gave rise to the Q-cycle modification of the electron-transport chain are no longer as compelling as they were. Nonetheless, the Q-cycle concept has continued to grow and attract more adherents. The reason for this is that quite apart from problems concerning site III, the Q-cycle can explain many experimental observations that are difficult or impossible to explain with the simple linear scheme shown in Fig. 1 (Trumpower, 1981a, b). Among these, the main examples are:

1. When the respiratory chain in the presence of reducing substrate and selected inhibitors such as antimycin is poised such that cytochromes *c* and c_1 are reduced and cytochromes *b* are mostly oxidized, the addition of an oxidant

which can selectively oxidize cytochromes *c* and c_1 causes a rapid, almost complete, reduction of cytochromes *b*. This phenomenon is known as the oxidant-induced reduction of cytochrome *b*.

2. When cytochrome c_1 is reduced, the passage of electrons from succinate to cytochrome *b* is greatly diminished. In a system using purified cytochrome *c* reductase, reduction of cytochrome c_1 in the presence of antimycin will totally block the reduction of cytochrome *b* by ubiquinol (Rieske, 1971).

3. Removal of the iron sulfur protein prevents the rapid passage of electrons from succinate to cytochromes *b* and the oxidant-induced reduction of cytochromes *b* in antimycin-treated succinate-cytochrome *c* reductase. Adding back the iron sulfur protein restores these activities.

4. With an intact respiratory chain, oxidizing substrate in the presence of oxygen, antimycin causes the oxidation of cytochromes c_1, *c*, *a*, and a_3 and the reduction of the *b* cytochromes, indicating a blockage of electron transfer between cytochromes *b* and c_1. However, if electron transfer from cytochrome *c* to cytochromes *a*, a_3 is blocked by cyanide or by using a purified system lacking cytochrome oxidase, then electrons are able to go from succinate to cytochrome c_1. This is called a single turnover as opposed to the dynamic situation employing a terminal electron acceptor.

5. EPR measurements have detected the formation of two species of bound ubisemiquinone associated with the respiring ubiquinol–cytochrome *c* reductase complex, one species dischargeable by antimycin, the second species dischargeable by BAL plus oxygen, a treatment that destroys the iron sulfur center of the complex (De Vries *et al.*, 1981; Slater and De Vries, 1980). The antimycin-sensitive Q radical could fit the properties of the $Q^{\cdot-}_{in}$ and the BAL-sensitive Q radical that of the $Q^{\cdot-}_{out}$ of a Q-cycle.

Three forms of the basic coenzyme Q-cycle are shown in Fig. 2. The Trumpower form, which is based on elegant studies using purified succinate cytochrome *c* reductase and the removal and adding back of reconstitutively active iron sulfur protein, is shown in the bottom panel. One of the most appealing features of this scheme is the explanation it offers for the oxidant-induced reduction of cytochromes *b*. The reductant for cytochrome b_T is ubisemiquinone which is formed by the oxidation of ubiquinol. When the system is poised with cytochrome c_1 reduced and the *b* cytochromes oxidized, electrons cannot go from QH_2 to c_1 and the semiquinone reductant of *b* cannot be formed. When an oxidant is added, the cytochrome *b* reductant will be formed. The reduction of cytochrome b_T, however, poses a problem because its midpoint potential has been taken to be about -60 mV and its reductant should have an E_m below this value. On the other hand, the reductant for the iron sulfur protein ($E_m \sim 280$ mV) is QH_2 which should have an E_m of near or

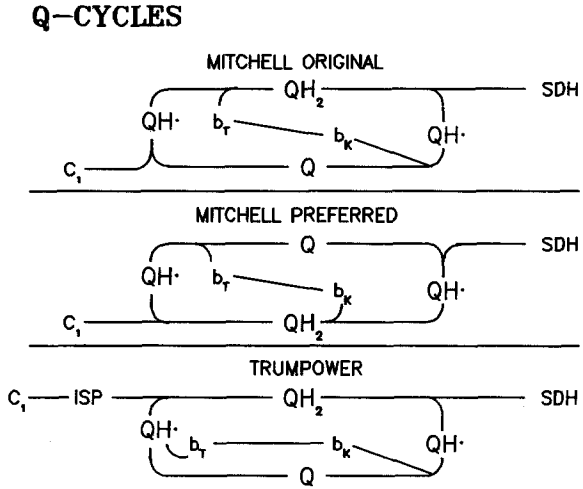


Fig. 2. The Q-cycles are examined here only in terms of electron transport. The cycles normally operate with an electron introduced from a dehydrogenase on the inner surface, represented as SDH (succinate dehydrogenase) on the right side. Electrons are removed on the outer surface by a member of the electron-transport chain shown as c_1 (cytochrome c_1) or ISP (Rieske iron sulfur protein) on the left side. The entering electron either reduces ubisemiquinone ($QH^{\cdot}$) to ubiquinol (QH_2) or reduces the quinone (Q) to the semiquinone. In the original Mitchell scheme, one electron from the quinone is cycled back via the cytochromes *b* to regenerate ubisemiquinone from ubiquinone (Q), whereas the other electron is passed on to cytochrome c_1. In this scheme, the reductant for the *b*-cytochromes is QH_2 and the oxidant is Q. Because of the idea that $QH^{\cdot}$ is a more powerful reductant than QH_2, the scheme was modified to the form shown in the middle panel. In this scheme, an electron is first passed from the quinol to cytochrome c_1 to generate ubisemiquinone, the presumed reductant for the low-potential cytochrome b_T, and the other electron is passed back through the *b*-cytochromes to form ubiquinol from ubisemiquinone. In this scheme, the reducing couple for the *b*-cytochromes is $Q/QH^{\cdot}$ and the oxidizing couple is $QH^{\cdot}/QH_2$. In the Trumpower scheme, one electron from ubiquinol is also passed to cytochrome c_1, via the iron sulfur protein, but the other is passed through the *b*-cytochromes to form ubisemiquinone from the quinone. In this scheme, the same couple $Q/QH^{\cdot}$ is both the reductant and oxidant for the *b*-cytochromes. Antimycin blocks the oxidation of the *b* cytochromes in all of these schemes.

just below that of the iron sulfur protein. As explained by Trumpower (1981a), a practical separation of 350 mV might exist between the E_m's for the two one-electron donor systems $Q/QH^{\cdot}$ and $QH^{\cdot}/QH_2$. The key point of the scheme is that an electron must be removed from the high E_m couple $QH^{\cdot}/QH_2$ in order to form the reduced member of the low E_m couple $Q/QH^{\cdot}$.

The thermodynamic basis for such a relationship between the two semiquinone couples is as follows:

for $QH_2 \rightleftharpoons QH^{\cdot} + e^- + H^+$

$$E_h = E_{m_1} + 60 \log \frac{[QH^{\cdot}][H^+]}{[QH_2]}$$

and for $QH^{\cdot} \rightleftharpoons Q + e + H^+$

$$E_h = E_{m_2} + 60 \log \frac{[Q][H^+]}{[QH^{\cdot}]}$$

at equilibrium

$$E_{m_1} + 60 \log \frac{[QH^{\cdot}][H^+]}{[QH_2]} = E_{m_2} + 60 \log \frac{[Q][H^+]}{[QH^{\cdot}]}$$

$$E_{m_2} - E_{m_1} = 60 \log \frac{[QH^{\cdot}]^2}{[QH_2][Q]} \qquad (1)$$

The expression involving concentrations in Eq. (1) can be recognized as the equilibrium constant for the dismutation

$$Q + QH_2 \rightleftharpoons 2QH^{\cdot}$$

and is referred to as the stability constant for $QH^{\cdot}$

Equation (1) states that the spread between the midpoint potentials for the two couples is determined by 60 log K. For K's above 1, the E_{m_2} for $Q/QH^{\cdot}$ is greater than the E_{m_1} for $QH^{\cdot}/QH_2$, whereas for K's below 1, the relative order is reversed. For the spread described by Trumpower where E_{m_2} is 350 mV below that of E_{m_1}, a K of 1.5×10^{-6} is indicated. According to Mitchell, a K of 10^{-10} would be expected for ubisemiquinone in a hydrophobic environment so that a stabilization of four orders of magnitude is actually called for (Trumpower, 1981a).

Thermodynamic Problems with the Current Q-Cycle Formulation

At the root of this scheme is the concept that when E_{m_2} of the $Q/QH^{\cdot}$ couple is lower than the E_m values of the Q/QH_2, $QH^{\cdot}/QH_2$, and cytochrome b_T couples, $QH^{\cdot}$ formed by oxidation of QH_2 will be an independent and spontaneous reductant for cytochrome b_T. This concept is not justified. As a background for explaining the thermodynamic flaw in this argument, the potentiometric relationships of the three members of the Q-system are shown in Fig. 3. The influence of the stability constant, K, on the E_m values of this system can be seen. When K is greater than 1, appreciable amounts of $QH^{\cdot}$

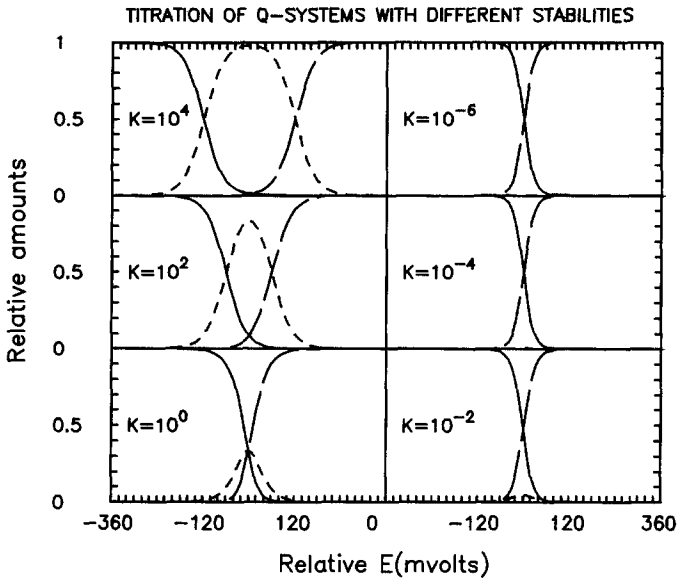


Fig. 3. The relative amounts of QH_2, $QH^{\cdot}$, and Q present at individual voltages are shown by solid, short dashed, and long dashed lines, respectively. E_{m_1} is located where the QH_2 and $QH^{\cdot}$ curves cross. E_{m_2} is at the intersection of the $QH^{\cdot}$ and Q curves. The voltages are shown relative to the midpoint for the two-electron reaction placed at 0 mV. Stability constants, K, for each titration are shown in the panels. The levels of $QH^{\cdot}$ in the titrations with $K < 10^{-4}$ are too low to be seen.

exist and the E_{m_1} for the $QH^{\cdot}/QH_2$ couple is lower than the E_{m_2} for the $Q/QH^{\cdot}$ couple. At $K = 1$, $E_{m_1} = E_{m_2} = E_m$ for the two-electron Q/QH_2 couple and $[QH^{\cdot}] = [QH_2] = [Q] = 0.33$. At values of $K < 1$, E_{m_2} is less than E_{m_1} and the relative concentration of $QH^{\cdot}$ dramatically drops. When $K = 10^{-2}$, at $E = E_{m_2}$, $[QH^{\cdot}]$ represents 1% of the total Q-system and when $K = 10^{-4}$, the semiquinone represents 0.01% of the total. When the value of $K = 10^{-6}$, considered appropriate in the Q-cycle scheme, $[QH^{\cdot}]$ represents 0.0001% of the system or one in a million molecules.

The E_m values of independent couples denote voltages at which 50% of the system is a potential oxidant and 50% a potential reductant. When comparing two such couples, the E_m values indicate relative reducing power. A disproportionating redox system such as the quinone system presents an entirely different situation. This system consists of two one-electron couples and one two-electron couple. Under the conditions evoked for operation of the Q-cycle, at $E_h = E_m$ for each one-electron couple, the two one-electron couples each account for 0.0001% of the system. At $E_h = E_{m_2}$ for the $Q/QH^{\cdot}$ couple,

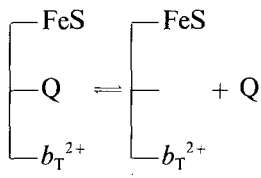
99.9998% of the system is in the form of QH_2 which is not part of the couple. At $E_h = E_{m_1}$ for the $QH^{\cdot}/QH_2$ couple, 99.9998% is in the form of Q which is not part of the couple.

The physical significance of the relationship between a low stability constant and the E_m values of the two one-electron couples of the Q system is that when $QH^{\cdot}$ is formed and present above its low equilibrium concentration, stabilization will require either the loss or acquisition of an electron to form Q or QH_2. This increased tendency to gain or lose an electron effects both one-electron couples equally so that $QH^{\cdot}$ is at the same time both a strong oxidant and a strong reductant. The net effect of these two influences is to make $QH^{\cdot}$ more reactive, but because it is part of two couples in the same medium ($Q/QH^{\cdot}$ and $QH^{\cdot}/QH_2$), it operates with an effective E_m equal to the average of the two, which is the same as the parent two-electron couple, Q/QH_2. The underlying assumption in current forms of the Q-cycle which employ the semiquinone as a reductant for the low E_m cytochrome b_T couple is that the simple act of generating $QH^{\cdot}$ from QH_2 is sufficient for the spontaneous reduction of the b_T-cytochrome, because Q will be formed and the $Q/QH^{\cdot}$ couple has a low E_m. This does not follow from the thermodynamic considerations and, therefore, additional statements about the cycle are required. For example, if a situation arose where $QH^{\cdot}$ was generated in an isolated environment where its only permitted reaction was with oxidized cytochrome b_T, then the low E_m for the $Q/QH^{\cdot}$ couple would be realized and the reduction of cytochrome b_T would occur. The following reactions represent this situation:

$$\begin{matrix} & & \text{FeS}^{+} & & \text{FeS}^{+} \\ \text{QH}_2 + & & \rightleftharpoons & \text{QH}_2 \\ & b_T^{+3} & & b_T^{3+} \end{matrix} \qquad \text{(A)}$$

$$\begin{matrix} \text{FeS}^{+} & & \text{FeS} \\ \text{QH}_2 & \rightleftharpoons & \text{QH} + \text{H}^{+} \\ b_T^{3+} & & b_T^{3+} \end{matrix} \qquad \text{(B)}$$

$$\begin{matrix} \text{FeS} & & \text{FeS} \\ \text{QH} & \rightleftharpoons & \text{Q} + \text{H}^{+} \\ b_T^{3+} & & b_T^{2+} \end{matrix} \qquad \text{(C)}$$



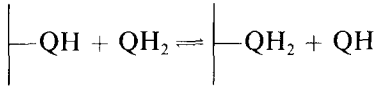
(D)

The operation of this scheme depends on two conditions: (1) that the state represented by the reactant in (C) be highly probable; (2) that the dismutation of the bound semiquinone be highly improbable. Although the oxidant for the quinol in this and other schemes is shown as the iron sulfur protein, kinetic studies reveal that the iron sulfur protein and cytochrome c_1 are essentially at equilibrium (Crofts, 1984; Rich, 1983; T'sai *et al.*, 1983), so that an electron removed from QH_2 is shared by the two acceptors according to their relative E_m values, which are probably not far apart. In effect, therefore, FeS and c_1 comprise a two-electron sink. Because of the low stability of $QH^{\cdot}$, the one-electron oxidation of QH_2 is energetically much less favorable than the two-electron oxidation.

$$QH_2 + (FeS/c_1)^{4+} \rightleftharpoons QH^{\cdot} + (FeS/c_1)^{3+} + H^+ \qquad \Delta G^\circ = -0.5 \text{ kcal}$$

$$QH_2 + (FeS/c_1)^{4+} \rightleftharpoons Q + (FeS/c_1)^{2+} \qquad \Delta G^\circ = -9.2 \text{ kcal}$$

A formulation of the type shown above must consider how the much less favorable one-electron oxidation is selected over the two-electron oxidation. The next problem is that of the effective E_m of the bound semiquinone, which is the same question as the isolation of this species from the free pool where the E_m of the couples involving the semiquinone operate at the E_m of the two-electron couple. The bulk of a variety of kinetic and inhibitor studies indicate the direct participation of the free pool in the operation of the Q-cycle and the oxidant-induced reduction of cytochrome b_T (reviewed in Crofts, 1984, and Hauska *et al.*, 1983). In fact, turnover studies require that the binding of QH_2 to the site, and the unbinding of Q, occur on a submillisecond time scale, and that the binding of the two species is not strong (Crofts, 1984). It is postulated that $QH^{\cdot}$ is held more tightly than either the quinol or the quinone. In the absence of any other assumptions, such rapid equilibration of the site with the QH_2 and Q of the pool also would be expected to allow the following redox reactions:



This, in effect, is a dismutation even though two semiquinones do not directly interact. The bound semiquinone will equilibrate with the pool and therefore have an E_m well above that of cytochrome b_T. This confusion about the relationship between the stability constant of the semiquinone and the relative redox poise between the cytochrome b_T couple and the Q/QH$^\cdot$ couple is illustrated in a very recent review (Hauska *et al.*, 1983) which argues (pp. 117–118) that because the oxidation of quinol forms a strongly reducing semiquinone, previous explanations of the oxidant-induced reduction of cytochrome *b* involving conformational changes and a transient increase in the E_m of cytochrome *b* are unnecessary.

What about the role of pH in Q-systems? If the pK for the dissociation

$$QH^\cdot \longrightarrow Q^- + H^+$$

is near 6 (Trumpower, 1981a), then the semiquinone will be present in two forms and variation of the pH between 6 and 8 will have profound effects on the potentiometric tritration curves. As the pH approaches and exceeds the pK, the charged semiquinone species increases as the protonated form

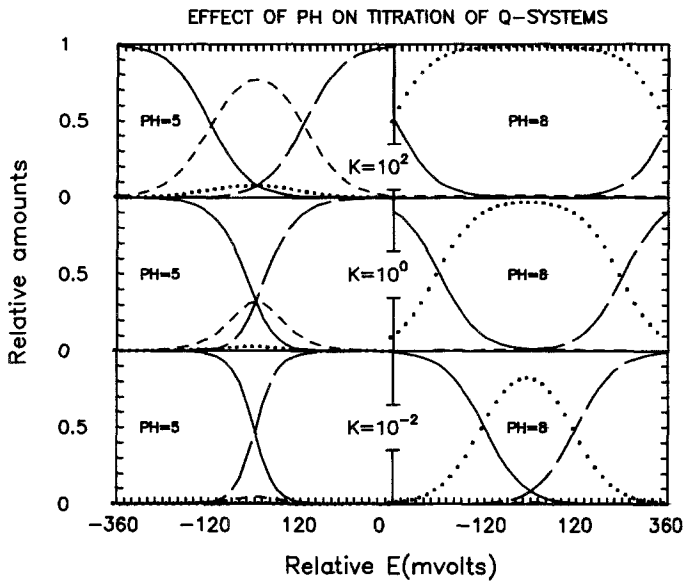


Fig. 4. The relative amounts of QH_2, QH$^\cdot$, Q, and Q$^-$ present at individual voltages are shown by solid, short dashed, long dashed, and dotted lines, respectively. All of the titration curves are shifted −60 mV/pH unit. In the figure, the center has been normalized to 0 mV so that only the effect of pH on the relative positions of the E_m values and shapes of the titration curves are emphasized. The pK_a for the ionization of QH$^\cdot$ is taken as 6.0. The stability constant, K, and the pH relevant to each titration are shown in the panels. E_m values are as described in Fig. 3.

decreases. Then there are four couples with E_m values, namely $QH^{\cdot}/QH_2$, $Q^{\cdot-}/QH_2$, $Q/QH^{\cdot}$, and $Q/Q^{\cdot-}$. Shown in Fig. 4 are titrations for systems with stability constants of 10^2, 10^0, and 10^{-2} at pH's below and above the pK. The relative amounts of $QH^{\cdot}$ are shown with short dashed lines and that of $Q^{\cdot-}$ with dotted lines. At a pH of 1 unit below the pK, 10% as much $Q^{\cdot-}$ is present as $QH^{\cdot}$. When the stability constant is 10^2, the E_m values for the two couples involving the ionized species coincide with the E_m for the Q/QH_2 couple whereas the individual couples involving QH/QH_2 and Q/QH remain 120 mV apart. At a stability constant of 10^0, the QH/QH_2, Q/QH, and Q/QH_2 couples all coincide whereas the small amount of $Q^{\cdot-}$ present crosses the Q curve at a lower voltage than that at which it crosses the QH_2 curve. This situation is analogous to the curves involving $QH^{\cdot}$ when $K < 1$, as shown in Fig. 3. A further lowering of the E_m for the $Q/Q^{\cdot}$ couple below that of the $Q^{\cdot}/QH_2$ at pH 5 occurs when the stability constant is 10^{-2} but the concentration of the ionized semiquinone in this case is minute (e.g., the dotted $Q^{\cdot-}$ is plotted but cannot be distinguished from the base line). Raising the pH above the pK increases the amount of $Q^{\cdot-}$ present and causes a further separation of the E_m values for the two couples involving the charged semiquinone species. This effect corresponds to the effect of an increase in stability constant in the couples involving $QH^{\cdot}$. The newly prominent couples at high pH involving the ionized semiquinone species are potentially effective redox couples insofar as 50% of each couple involves the partially reduced ion. It should be noted, however, that the E_m of the $Q^{\cdot-}/QH_2$ couple remains lower than that of the $Q/Q^{\cdot-}$ couple and so no advantage in terms of current formulations of the Q-cycle is realized.

Other Considerations

The computations on which Fig. 3 is based show that the mole fraction of semiquinone present at the higher E_{m_1} voltage for the $QH^{\cdot}/QH_2$ couple approaches the value of K, as K decreases. Therefore, for a stability constant of 10^{-6}, the mole fraction of semiquinone present at the voltage of E_{m_1} will be 10^{-6}. If equilibrium with the pool is maintained, then only one molecule in 10^6 will be present as semiquinone. The rate of electron transfer to cytochrome b_T would be dependent on the statistics of a contact between the semiquinone and the cytochrome. If the QH_2 which will produce the desired $QH^{\cdot}$ is already *in situ* in a cytochrome b, c_1 assembly matrix, then only one in 10^6 assemblies will be able to react or one assembly would be competent every $1/10^6$ of the time. Considering that isolated Complex III contains only one or less moles of ubiquinone per mole of cytochrome c_1 and the isolated succinate–cytochrome c reductase complex only about five (Ohnishi and Trumpower, 1980), these considerations pose further problems for the Q-cycle models based on this principle.

Items 2 and 3 listed above in favor of a Q-cycle describe restricted or forbidden pathways for electron passage under certain conditions. The Q-cycle provides explanations for these observations. When cytochrome c_1 is reduced, the oxidation of QH_2 is impaired and the proposed reductant for cytochrome b_T, $QH^{\cdot}$, is not made, thus accounting for cytochrome c_1's influence on the reduction of cytochrome *b*. Similarly, if the iron sulfur protein is removed, the path for reduction of cytochrome *b* via $QH^{\cdot}$ is removed. The only other path for electrons from succinate to cytochrome *b* is through the antimycin-sensitive path to cytochrome b_K. The possibility that electrons from succinate have access to the *b*-cytochromes from opposite directions, however, requires some additional assumptions so that this path is not used under normal conditions. Explanations for these observations are not unique to a Q-cycle. A conformational change in the respiratory enzyme complex induced by cytochrome c_1 (or 'Y') (Eisenbach and Gutman, 1975) reduction could slow down the flow of electrons from succinate to cytochrome *b*. This is simply feedback inhibition whereby a later step in a multienzyme process controls the rate of an earlier step. The iron sulfur protein could be involved in these conformational transformations so that in its absence, the binding of antimycin to cytochrome(s) *b* would prevent the passage of electrons from succinate to cytochrome *b*. The concept of conformational changes in the assembly of components comprising complex III is supported by many published observations.

For example, changes in the redox state of complex III are accompanied by changes in the following attributes of structural integrity:

(a) Stability against cleavage by chaotropic reagents with or without antimycin pretreatment (Rieske *et al.*, 1967)
(b) Inactivation by trypsin proteolysis (Baum *et al.*, 1967)
(c) The arrangement of liposomal paracrystalline arrays (Wakabayashi *et al.*, 1972)
(d) Circular dichroic spectra at 220 nm (Berden and Slater, 1972) and at the cytochrome Soret wavelengths (Reed *et al.*, 1978)
(e) Antimycin fluorescence in the antimycin-treated complex (Berden *et al.*, 1975)
(f) The average stretching frequency of buried –SH groups (Rieske *et al.*, 1975)
(g) ESR of a maleimide-linked nitroxide group on the surface (Das Gupta *et al.*, 1979)
(h) The labeling pattern of the subunits of the complex treated with [^{3}H]succinic anhydride in the native and antimycin-treated complex (Ho, 1979)

In addition, although only a single antimycin molecule is bound per two molecules of cytochrome *b* and one molecule of c_1 (Rieske, 1976), the rates of

oxidation and reduction of cytochrome b_{562} and b_{566} are affected differently in antimycin-treated submitochondrial particles (Hatefi and Yagi, 1982).

Item 4 poses the dilemma that antimycin acting on a linear chain would block electron transfer from cytochromes *b* to c_1, yet on a single turnover (i.e., no reoxidation of reduced cytochrome c_1), electrons from succinate appear to pass to cytochrome c_1 in the presence of antimycin (Bowyer and Trumpower, 1981). Trumpower's Q-cycle explains this because the reduction of cytochromes *b* and c_1 is a coordinated event proximal to the antimycin block. Although cytochrome c_1 can be reoxidized, cytochrome *b* will remain reduced. However, recent evidence obtained by stopped-flow kinetic measurements indicates that antimycin does strongly inhibit the reduction of cytochrome c_1 by electrons from Q_1H_2 in the Q_1H_2–cytochrome reductase reaction during the first turnover (Esposti and Lenaz, 1982). The same study found that the reduction of cytochrome *b* preceded that of cytochrome c_1. In a separate study employing stopped-flow techniques (Jin *et al.*, 1981) the initial phase of reduction of cytochrome *b* was faster than the reduction of cytochrome c_1. As the reduction of cytochrome c_1 proceeded, cytochrome *b* went through an oxidation phase. Upon completion of reduction of cytochrome c_1, the cytochrome *b* again became reduced, but at a lower rate than the initial phase of reduction. These studies suggest a linear electron transfer sequence from QH_2 though cytochrome *b* to cytochrome c_1 rather than the branched pathway postulated in the Q-cycle.

Summary

The reduction of cytochrome c_1 and cytochrome b_T by QH_2 is an exergonic reaction with a $\Delta G°$ approximately -1.84 kcal. The alternate reduction of cytochrome c_1 and the Rieske iron sulfur protein, however, with a $\Delta G°$ approximately -9.2 kcal, seems more likely, especially since these two redox centers appear to be in kinetic equilibrium. If the reaction proceeds in two steps where the quinone reduces cytochrome c_1 and forms a semiquinone, the reduction of cytochrome b_T by the semiquinone is a reaction with an unfavorable $\Delta G°$ of approximately 2.8 kcal. The fact that the computed E_m for the $Q/QH^{\cdot}$ couple is low when the semiquinone is unstable does not mean that $QH^{\cdot}$ formed upon oxidation of QH_2 is a spontaneous reductant of the *b* cytochrome. The bound species of semiquinone is probably exposed to the free quinone pool since the site is in rapid equilibrium with both QH_2 and Q.

The unfavorable $\Delta G°$ for the reduction of the cytochrome b_T by the semiquinone does not invalidate the whole cycle which as noted has a favorable $\Delta G°$ of ~ 1.84 kcal. However, previous discussions of the Q-cycle have not dealt specifically with this possibly serious obstacle and, in fact, have

been based on the belief that no problem exists. In a recent summary by Crofts (1984) in which a two-electron gated form of a Q-cycle is discussed, these problems have been noted, but essentially dismissed. It is stated that the dismutation must be prevented, but, at the same time, the rapid equilibration of both bound QH_2 and Q with the pool is documented. As to the problem of the reduction of the low-potential cytochrome b_T by the semiquinone, two explanations are offered. One is that the bound semiquinone becomes isolated from the pool and also oriented so that it can only react with oxidized cytochrome b_T. This situation would endow the semiquinone with the low E_m calculated on the basis of the low stability constant. The other is that mass action would result from the favorable formation of semiquinone during the reduction of the iron sulfur center by the quinol so that the accumulated semiquinone would drive the reduction of oxidized cytochrome b_T. The assumption for this explanation is exactly the same as for the first in that it is required that the bound semiquinone, when formed, be restricted to only a single reaction with oxidized cytochrome b_T. The other problem as to preventing the thermodynamically more favorable two-electron reduction of the iron sulfur, cytochrome c_1 centers, so that only one electron follows this path leaving the other for cytochrome b_T, is dismissed with the assumption that somehow the cell must manage to accomplish this. The general acceptance of the Q-cycle has partly been based on its simplicity and the absence of *ad hoc* assumptions in order to explain such phenomenon as the oxidant-induced reduction of cytochrome *b*, which in terms of a linear chain did require *ad hoc* provisions. The main purpose of this paper is to point out that the simplicity is illusory. Even in the case of the recent review by Crofts where the problems have been discussed, the solutions offered are entirely *ad hoc*.

We have noted that substantial circumstantial evidence, consistent with a Q-cycle mechanism, has accrued. Our intention is to point out a serious weakness in some of its theoretical foundations and not to prove that it cannot or does not operate.

There are two ways a simple Q-cycle mechanism may work. If the transfer of an electron from a bound species of semiquinone to cytochrome b_T was much faster than the reaction of the bound semiquinone with either pool QH_2 or pool Q, then the process would become essentially a two-electron transfer and the problems concerning the thermodynamics and kinetics of semiquinone, as well as this species itself, would be nonexistent. This, of course, leaves unexplained the EPR studies of De Vries *et al.* (1981) which indicate a form of semiquinone with the expected properties of the unstable species. The other alternative is to postulate a machine which uses the energy liberated in the transfer of an electron from QH_2 to the FeS/c_1 center to drive the reduction of cytochrome b_T by the otherwise unsuitable electron donor, the semiquinone. This machine can use binding energies, conformational changes,

ligands, local changes in pH or ψ, etc. to alter the relative redox poise of the semiquinone and cytochrome b_T.

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