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Experimental Observations on the Structure and Function of Mitochondrial Complex III That Are Unresolved by the Protonmotive Ubiquinone-Cycle Hypothesis

John S. Rieske[1]

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Abstract

The current model of the protonmotive ubiquinone cycle as applied to mitochondrial ubiquinol-cytochrome *c* reductase complex (Complex III) is able to explain a number of previously puzzling observations concerning electron-transfer and proton translocating functions of the complex. However, a number of pertinent experimental observations concerning the structure and function of this complex cannot as yet be incorporated into the present version of the ubiquinone cycle. The yet unresolved problems of electron transfer uncovered by these observations include some kinetic and thermodynamic problems, uncertainties in the binding site(s) and mode of binding of ubiquinol and inhibitors, the observed multiple spectroscopic, electrochemical, and kinetic forms of cytochromes *b*, iron-sulfur protein, and cytochrome c_1, the multiple and overlapping effects of inhibitors, and the functional role of conformational changes in the complex. It is concluded that although the Q cycle is a valuable base for the design of future experiments, its mechanism must be reconciled with the above uncertainties as well as with the accumulated evidence that Complex III can exist in two or more interchangeable forms, exhibiting different properties with respect to electron-transfer pathways, inhibitor binding, and spectral and electrochemical properties of the electron-carrier subunits.

Key Words: Ubiquinol-cytochrome *c* reductase; Complex III, protonmotive ubiquinone cycle; structure; function commentary.

Introduction

In this presentation, this author has been placed in the unenviable position of raising questions concerning certain aspects of the protonmotive Q-cycle[2]

[1]Department of Physiological Chemistry, College of Medicine, The Ohio State University, Columbus, Ohio 43210.

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schemes generally accepted currently by most laboratories investigating mitochondrial and photosynthetic electron transport. Not only was the Q-cycle hypothesis first formulated in 1975 by a universally recognized authority and Nobel Laureate in this area, Peter Mitchell (Mitchell, 1975) but, later, slightly modified versions have offered satisfactory explanations for many initially confusing observations on the redox properties and the action of inhibitors on the quinol oxidase complexes found in diverse organisms. However, the success of the Q-cycle model should not blind us to discrepancies, apparent or real, that may appear, since the evolution of a hypothesis to a theory will depend upon modifications made mandatory by these perceived discrepancies that may appear as more experimental data are generated.

Uncertainty in this task is made more severe by the number and complexity of the experimental systems and Q-cycle models and the resulting lack of agreement among different laboratories on much of the accumulated data. This complexity can also be used to justify *ad hoc* assumptions that can be used to nullify many apparent experimental or theoretical discrepancies in the Q-cycle hypothesis. Finally, several variations of the Q cycle have been proposed to fit specific requirements of electron-transfer systems analogous to the mitochondrial ubiquinol-cytochrome *c* reductase that are present in a variety of organisms. Therefore, it is with this feeling of vulnerability that this contribution on the Q-cycle hypothesis has been prepared. To make this contribution manageable only controversies concerning the basic electron-transfer features of the Q-cycle hypothesis as applied to the mitochondrial system will be addressed, since other contributions in this journal will discuss other systems and models in detail.

General Features of the Protonmotive Q Cycle Hypothesis

The protonmotive Q cycle is currently pictured for the mitochondrial QH_2-cytochrome *c* reductase complex (Complex III or bc_1 complex) is diagrammed in Fig. 1. The general features of this scheme can be summarized as follows: (1) Ubiquinol (QH_2) from the ubiquinone (Q) pool in the lipid

[2] Abbreviations used: Q, ubiquinone; QH_2, ubiquinol or reduced ubiquinone; $Q^{\cdot-}$, ubisemiquinone anion; QH; neutral ubisemiquinone; Q-1, Q-2, and Q-3, lower homologs of ubiquinone containing 1, 2, and 3 isoprenoid side chains, respectively; DBH_2, reduced decyl analog of Q-2; antimycin, antimycin A; HQNO, 2-*N*-heptyl-4-hydroxyquinoline-*N*-oxide; UHDBT, 5-*N*-undecyl-6-hydroxy-4,7-dioxobenzothiazol; HMHQQ, 7(*n*-heptadecyl)mercapto-6-hydroxy-5,7-quinolinequinone; DBM1B, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; UHNQ, 2-hydroxy-3-undecyl-1,4-naphthoquinone; BAL, British antilewisite (2,3-dimercaptopropanol); TMPD, *N*,*N*,*N*′,*N*′-tetramethylphenylenediamine.

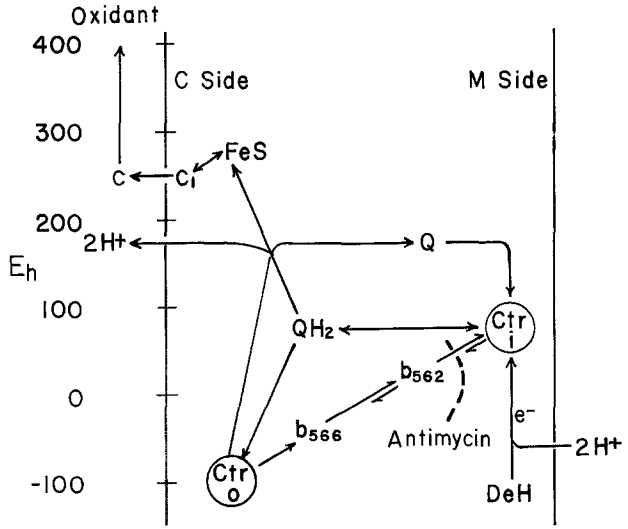


Fig. 1. A protonmotive Q cycle scheme showing the electron-transfer pathways for mitochondrial QH_2-cytochrome *c* reductase complex and the redox potential (mV) relationships among the redox components. Centers i and o are enclosed to indicate that the nature of these centers with respect to proposed ubisemiquinone anions and binding proteins are as yet undecided. DeH and FeS designate a dehydrogenase complex and the iron-sulfur center, respectively.

milieu of the inner mitochondrial membrane is bound to "center o." This bound QH_2 donates one electron to the iron-sulfur center of the iron-sulfur protein ($E_{m7} \sim 280\,mV$), leaving a bound ubisemiquinone anion ($Q^{\cdot-}$). (2) The $Q^{\cdot-}$ then donates the second electron to cytochrome b_{566} heme ($E_{m7} \sim -60\,mV$), which is in rapid electronic equilibrium with the cytochrome b_{562} heme ($E_{m7} \sim +60\,mV$). Two protons are released to the external medium during the oxidation of QH_2 at center o. (3) Reduced cytochrome b_{562} heme donates an electron to center i which together with an electron and two protons generated from NADH or succinate by the dehydrogenases reduce pool Q to regenerate QH_2 and complete the cycle. (4) Centers o and i are purported to be located at the cytosolic (C) and matrix (M) sides of the inner mitochondrial membrane, respectively; thus two protons are taken up at the M side and two protons are released at the C side of the membrane during the transfer of one electron from QH_2 to cytochrome *c*. (5) Antimycin blocks regeneration of QH_2 from Q at center i and myxothiazol blocks electron transfers at center o. The quinone-type inhibitors represented by UHDBT blocks electron transfer between the iron-sulfur center and cytochrome c_1.

Several major experimental observations are explained well by the Q-cycle model. The most puzzling and important of these observations is

what is generally referred to as the oxidant-induced extrareduction (or reduction) of cytochrome *b*. This phenomenon is demonstrated when electron transfer through Complex III is inhibited by antimycin, low temperatures, or a glycerol–water medium, and the complex, partially reduced by electron-donor substrates, is treated with an oxidant that selectively oxidizes cytochrome c_1. This oxidation of cytochrome c_1 induces a rapid reduction of the cytochrome *b* heme groups by electron-donor substrates (Baum and Rieske, 1966; Erecínska and Wilson, 1972; Wilson *et al.*, 1972). Closely related to this phenomenon is the observation that selective reduction of cytochrome c_1 in the antimycin-treated complex inhibits reduction of cytochromes *b* by electron-donating substrates (Rieske, 1971; Wikström and Berden, 1972; Davis *et al.*, 1973).

These phenomena are explained readily by the current Q-cycle model. Oxidation of the high-potential cytochrome c_1 ($E_{m7} \sim 230$ mV) and the iron-sulfur protein ($E_{m7} \sim 280$ mV) allows an electron to be accepted from QH_2 by the iron-sulfur center with a concerted transfer of the second electron to the low-potential catalytic center o and subsequently to cytochromes b_{566} and b_{562}. Reduction of cytochrome c_1 and the iron-sulfur center by ascorbate or ascorbate plus TMPD does not allow the concerted electron transfers from QH_2 to either the high- or low-potential components.

Other major evidences supporting the Q cycle can be listed as follows:

1. Selective removal of the iron-sulfur protein or destruction of the iron-sulfur center by BAL plus oxygen destroys the oxidant-induced reduction of cytochromes *b* (Trumpower, 1981a; Engel *et al.*, 1983; Slater and De Vries, 1980).

2. EPR measurements have detected two species of ubisemiquinones associated with respiring QH_2-cytochrome *c* reductase complex, one species dischargeable by antimycin, the second species dischargeable by BAL plus oxygen. These quinone radicals correspond to semiquinone intermediates associated with catalytic centers i and o, respectively, as formulated in the Q cycle (De Vries *et al.*, 1981; Slater and De Vries, 1980). These data are perhaps the most direct evidence supporting the Q-cycle mechanism.

3. The Q-cycle mechanism predicts that for every electron transferred from QH_2 to cytochrome *c*, two protons will be transported from the inside to the outside surfaces of the inner mitochondrial membrane. This stoichiometry has been observed in mitochondria (Mitchell and Moyle, 1967) and liposomes embedded with purified Complex III (Leung and Hinkle, 1975; Guerrieri and Nelson, 1975).

4. Antimycin, which causes a spectral shift of the reduced cytochrome b_{562}, inhibits the oxidation of the reduced cytochrome by pool Q (Von Jagow

and Böhrer, 1975), whereas myxothiazol, which causes a spectral shift of reduced cytochrome b_{566}, inhibits the oxidant-induced reduction of cytochromes *b* in the antimycin-treated enzyme (Von Jagow *et al.*, 1984).

The above observations by themselves and in the absence of alternative schemes that fit these observations equally well offer a convincing case for the validity of the Q-cycle hypothesis. However, other observations are not as readily incorporated into the Q-cycle scheme; therefore, if not rejection at least some revisions of the currently proposed schemes are called for. Two alternative cyclic schemes, the cytochrome *b* cycle (Wikström and Krab, 1980) and the double Q cycle (De Vries *et al.*, 1982a), have already been proposed and are discussed in other contributions to this discussion series. Because applications of the Q-cycle hypothesis to various electron-transfer systems are also discussed in separate contributions, only data and concepts immediately applicable to the controversial aspects of the Q-cycle hypothesis will be considered in this contribution. For clarity, this discussion will be organized according to the electron pathways of the QH_2-oxidizing and Q-reducing systems, respectively, as postulated in the currently accepted model of the Q cycle.

The Ubiquinol Oxidizing System

The Role of a Ubisemiquinone

As pictured in the Q-cycle scheme (Fig. 1) the QH_2 oxidizing system consists of the high-potential iron-sulfur center ($E_{m7} \sim 285$ mV) and cytochrome c_1 ($E_{m7} \sim 230$ mV) linked through catalytic center o to the low-potential cytochromes b_{566} ($E_{m7} \sim -60$ mV) and b_{562} ($E_{m7} \sim +60$ mV) sequence. Initially, it was postulated that QH_2 reacts directly with the iron-sulfur center, reducing this center with the first electron and leaving an unstable $Q^{\cdot-}$, which then donates the second electron to cytochrome b_{566} (Trumpower, 1976, 1981a). Even though the E_{m7} (~ 70 mV) of the QH_2/Q redox couple in the Q pool is at least 130 mV greater than that of cytochrome b_{566}, and thus very unfavorable for the degree of reduction observed in the oxidant-induced reduction of the cytochrome, the proposed redox equilibration of the $Q^{\cdot-}/QH_2$ couple with the iron-sulfur center ($E_{m7} \sim 280$ mV) would produce a low enough potential ($E_{m7} \sim -140$ mV) for the $Q/Q^{\cdot-}$ couple to reduce cytochrome b_{566}. Although this scheme is attractive in its simplicity, it is beset with some difficulties when examined closely.

The unstable $Q^{\cdot-}$ if accessible to the Q pool would quickly be lost either directly or indirectly through dismutation (Hendler *et al.*, 1985). However, even if electron transfer from the $Q^{\cdot-}$ of center o to cytochrome b_{566} competes

favorably with dismutation (Berry and Trumpower, 1985), a low E_m for the $Q/Q^{\cdot-}$ couple would not automatically ensure the observed degree of oxidant-induced reduction of the cytochrome if the bound $Q^{\cdot-}$ is electronically or molecularly accessible to the Q pool. As pointed out by Hendler *et al.* (1985), at the stability constant of 10^{-6} for the dismutation of $QH^{\cdot}$ corresponding to a difference of 360 mV between the E_m's of the $QH^{\cdot}/Q$ and $Q/QH^{\cdot}$ couples, the potential E_h of the $Q/Q^{\cdot-}$ couple still would be determined by the potential of the pool Q/QH_2 couple. This would in effect amount to an indirect dismutation as shown, where subscripts B and P refer to bound and pool Q, respectively.

$$Q_B^{\cdot-} + Q_P \rightleftharpoons Q_B + Q_P^{\cdot-} \quad (1)$$

$$2Q_P^{\cdot-} + 2H^+ \longrightarrow Q_P + (QH_2)_P \quad (2)$$

Even if the second reaction is very slow because of the low steady-state concentration of $Q_P^{\cdot-}$, this electron exchange could short-circuit the Q cycle by allowing in effect the second electron from substrate QH_2 to reenter the Q pool and subsequently be shunted through the iron sulfur center–cytochrome c_1 pathway or be lost by interaction of $Q_P^{\cdot-}$ with O_2 to form superoxide anion.

Avoidance of this debacle would require that the bound $Q^{\cdot-}$ at center o be shielded from any interaction with the Q pool, yet close enough to the heme center of cytochrome b_{566} to ensure a rapid electron transfer. To accomplish this would require additional assumptions such as significant conformational changes in the polypeptides in the vicinity of center o upon reduction of the iron-sulfur center.

The Oxidant-Induced Reduction of Cytochrome b

The oxidant-induced reduction of cytochromes *b* in antimycin-inhibited cytochrome *c* reductase is the most prominent feature of the Q oxidizing center (center o) of the Q-cycle model. Early models to explain this phenomenon involved a midpoint potential change of the *b* cytochromes modulated by the redox state of either an uncharacterized redox component designated X (Rieske, 1971) or by cytochrome c_1 (Erecínska and Wilson, 1972). Wikström and Berden (1972) instead proposed a mechanism in which QH_2 transfers one electron to cytochrome b_{566} simultaneously with transfer of the second electron to oxygen. Antimycin was postulated to inhibit the reoxidation of cytochromes *b*. Support for this hypothesis was given by an apparent $n = 2$ for the reduction of cytochrome b_{566} when antimycin and cyanide-treated submitochondrial particles were equilibrated with mixtures of succinate and fumarate. This concerted electron-transfer mechanism as proposed by Wikström and Berden (1972) and the requirement that two

protons be translocated across the mitochondrial membrane for every electron transferred from QH_2 to cytochrome *c* across the cytochromes bc_1 segment were foundation cornerstones for the construction of the Q-cycle hypothesis (Mitchell, 1976).

The data of Wikström and Berden (1972) can be incorporated readily in the presently accepted Q-cycle scheme. In the absence of antimycin the *b*-cytochromes can be reduced by QH_2 via both centers o and i. Therefore because both electrons from the pool QH_2 would likely be transferred to the *b* cytochromes, the slope of the redox-titration curve would indicate an *n* value of 2.0 for the reducing substrate, in this case succinate, and an *n* value of 1.0 for the *b* cytochromes as observed. However, in the antimycin-inhibited system only a one-electron pathway from QH_2 to cytochromes *b* via center o would be operative; therefore the slope of the redox titration curve would indicate an $n = 1.0$ for the reducing substrate.

In contrast with the observations of Wikström and Berden (1972), titration of the oxidant-induced reduction of cytochrome b_{566} in antimycin-treated Complex III as well as in deoxycholate-solubilized mitochondria utilizing the Q-1/QH_2-1 couple as the titrant and ferricyanide or oxygen, respectively, as oxidants yielded a slope of 2.0 in both instances with E_m values near 130 mV (Fig. 2) (Rieske *et al.*, 1975; Rieske, 1976). This observed slope of 2.0 is consistent with an *n* value of 2.0 for the Q-1 reducing couple and an *n* value of 1.0 for the cytochrome b_{566} as would be expected for a two-electron equilibration between one molecule of QH_2-1 and two molecules of cytochrome b_{566}. The reason for this discrepancy is at present obscure. One possible explanation would be the presence of two cytochromes b_{566} of differing apparent E_m's in the antimycin-treated enzyme. In this case the slope of the titration curve may be in a transitional potential range between the titration curve of the low- and high-potential species of the cytochrome. This interpretation would imply the presence of a dimeric Complex III with interacting monomeric complexes as proposed by De Vries *et al.* (1982a).

A second possible explanation is that the lower homologs of QH_2 at high concentrations can reduce cytochrome b_{566} by a pathway different from that utilized by QH_2. Indeed, Degli Esposti and Lenaz (personal communication) reported that the kinetics of QH_2-cytochrome *c* reductase activity did not obey Michaelis–Menten kinetics, yielding biphasic double-reciprocal or Eadie–Hofstee plots with respect to varying quinol concentrations. These effects appeared to be highly dependent on the QH_2 homolog or analog used. For instance, the Eadie–Hofstee plot obtained with DBH_2 as reducing substrate was linear, whereas lower homologs of QH_2, e.g., QH_2-3, gave non-linear plots. Unfortunately, the relationships of these effects to properties of the enzyme attributable to the Q-cycle mechanism have not yet been examined.

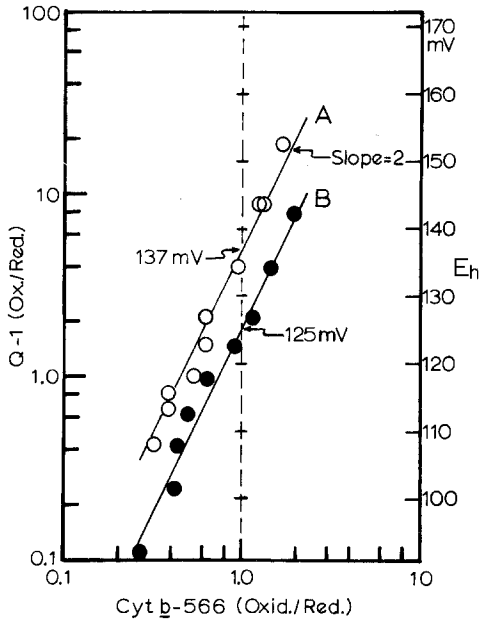


Fig. 2. Potentiometric relationship between the Q-1 and cytochrome *b*-566 redox couples for oxidant-induced reduction of cytochrome *b*. (A) Antimycin-treated (4 nmol/mg protein) bovine-heart mitochondria (2.5 mg protein/ml) solubilized in a mixture containing 0.1 M phosphate (pH 7), 0.1% deoxycholate, 0.2% Na taurocholate and 25 μM ferricyanide. (B) Antimycin-treated (10 nmol/mg protein) Complex III (1.0 mg protein/ml) in a mixture containing 0.1 M phosphate (pH 7), 0.4% Na taurocholate, 10 mM EDTA, and 100 μM ferricyanide. Cytochrome *b*-566 spectra (sample minus oxidized reference) were recorded with an Aminco DW-2 spectrophotometer immediately after addition of 10 μl of appropriate Q-1, QH_2-1 mixtures in ethanol (Q-1 + QH_2-1 = 73 nmol) to 0.7 ml reaction mixture. In experiment (A) the presence of cytochrome oxidase and oxygen maintained cytochromes $c + c_1$ completely oxidized during the measurement of spectra. In experiment (B) the spectra of reduced cytochrome *b* were extrapolated to "zero" time to correct for reoxidation of cytochrome *b*-566 concomitant to nonenzymic reduction of the added ferricyanide by the QH_2-1. Reduced cytochrome *b*-566 spectra were resolved from reduced cytochrome *b*-562 spectra by the procedure of Wilson and Erecínska (1975). Cytochrome *b*-562 remained completely reduced within the potential range examined. (Previously published: Rieske *et al.*, 1975 and Rieske, 1976).

Another factor should be examined in conjunction with this redox titration under conditions of an oxidant-induced reduction of the *b* cytochromes. The postulated concerted reduction of the iron-sulfur center and cytochrome b_{566} by QH_2 would require a highly unstable semiquinone at center o. Berry and Trumpower (1985) have estimated a stability constant of 10^{-11} for the bound $Q^{\cdot-}$ to allow it to be kinetically competent in the reduction of cytochrome b_{566}. With this magnitude of instability one would expect the transfer of the second electron from QH_2 to cytochrome b_{566} to be operationally irreversible, yet the titration of cytochrome b_{566} with the QH_2-1/

Q-1 couple under conditions giving the oxidant-induced reduction of the cytochrome appeared to be essentially at equilibrium and reversible (Rieske, 1971). Obviously, these discrepancies, apparent or real, call for further examination of redox titrations involving the oxidant-induced reduction of cytochrome b_{566} and its role in the Q-cycle hypothesis.

Ubiquinol Binding Sites

Membrane "Sidedness"

The uncomplicated Q-cycle model proposes two binding sites for QH_2, one with the iron-sulfur protein at center o and another at the Q-reducing site associated with center i. To accommodate the protonmotive feature of the Q cycle, these sites are visualized as being located near the cytosolic and matrix surfaces of the inner mitochondrial membrane, respectively. Moreover, cytochrome c_1 is generally accepted to be located near the cytosolic surface of the membrane where it is readily accessible to interaction with cytochrome *c*. This orientation of cytochrome c_1 has also been verified generally by several studies in which its accessibility to various probes in intact mitochondria and the inside-out membrane of submitochondrial particles was compared (D'Souza and Wilson, 1982; Bell *et al.*, 1979; Mendel-Hartvig and Nelson, 1978; Case and Leigh, 1976; Ohnishi and Salerno, 1982; Schneider and Racker, 1971; Beattie *et al.*, 1981). Although the membrane "sidedness" of the iron-sulfur protein has not been established with as much certainty as cytochrome c_1 by measurements of accessibility to protein-modifying probes (D'Souza and Wilson, 1982; Bell *et al.*, 1979), EPR studies employing dysprosium complexes as paramagnetic probes have placed the iron-sulfur cluster near the center of the apoprotein with the surface of the protein calculated to be near the cytosolic surface of the membrane (Ohnishi *et al.*, 1982). Moreover, the generally established close redox linkage between cytochrome c_1 and the iron-sulfur center would favor a cytosolic "sidedness" for the iron-sulfur protein.

With the above information in mind, it is puzzling, therefore, that oxidation of exogenous ubiquinols appears to occur most readily with submitochondrial particles rather than with intact mitochondria. This indicates that the site of QH_2 interaction with Complex III is mostly exposed on the M side of the mitochondrial inner membrane (Degli Esposti and Lenaz, 1982a; Degli Esposti *et al.*, 1982; Lenaz *et al.*, 1982; Lenaz *et al.*, 1981; Lenaz *et al.*, 1978; Papa *et al.*, 1978). This would be consistent with the Q-cycle scheme if exogenous QH_2 could transfer electrons and protons only to a bound endogenous Q at center i or if the iron-sulfur center at or near center o is accessible only to QH_2 that has been transported vectorially across the

membrane from center i. Both cases would require in effect a barrier to the entrance of exogenous QH_2 at the C side of the membrane. However, the existence of elaborate mechanisms of these sorts is not supported by observations that the aerobic oxidation of lower homologs of QH_2 added exogenously to intact mitochondrial membranes does not require endogenous Q (Lenaz *et al.*, 1985).

Other disturbing inconsistencies are raised by the membrane sidedness of the interaction of Complex III with exogenous ubiquinols. In model membrane systems consisting of phospholipid liposomes containing trapped ferricyanide the rate of reduction of the ferricyanide by short-chain homologs of QH_2 in the suspending medium appeared to be limited by the direct interaction of the QH_2 with the ferricyanide rather than by the rate of diffusion of the QH_2 across the membrane (Hauska and Hurt, 1982; Landi *et al.*, 1982). The above data together with the "sidedness" specificity of exogenous QH_2 suggests that exogenous Q binds directly with a binding site of Complex III protruding from the lipid bilayer and not via incorporation first into the lipid bilayer. However, it is difficult to reconcile this suggested mechanism with other evidence that endogenous Q confined within the membrane and through lateral diffusion readily transfers reducing equivalents from the Q reductase complexes to Complex III (Schneider *et al.*, 1985; Zhu *et al.*, 1982).

The Iron-Sulfur Protein as a Binding Site for Ubiquinol

Another facet of the Q oxidizing site open to question is the general assumption that pool QH_2 binds directly with the iron-sulfur protein to subsequently reduce the iron-sulfur center and cytochrome b_{566}. The evidence that this is the case is indirect, involving changes in the EPR spectrum of the reduced iron-sulfur center by the removal or reduction of Q (Siedow *et al.*, 1978; Orme-Johnson *et al.*, 1974; De Vries *et al.*, 1979; Trumpower, 1981a) and the replacement of Q with certain quinone-type respiratory or photosynthesis inhibitors such as HMHQQ (De Vries *et al.*, 1979, 1982b), UHNQ and UHDBT (Bowyer *et al.*, 1980, 1982; Matsuura *et al.*, 1983), and DBMIB (Chain and Malkin, 1979; Malkin, 1981, 1982). Also, the midpoint potential of the iron-sulfur protein is increased by UHDBT and UHNQ (Bowyer *et al.*, 1980, 1982; Matsuura *et al.*, 1983). Yet Yu and Yu (1982) observed no photoaffinity labeling of the iron-sulfur protein by arylazido derivatives of Q. Moreover, none of the spectral and electrochemical perturbations of the iron-sulfur protein observed in intact Complex III have been reported for the purified protein. Also, Gutweniger *et al.* (1981) observed no photoaffinity labeling of the iron-sulfur protein by arylazido derivatives of phospholipids. It could be reasoned that if the iron-sulfur protein is not accessible to added phospholipids it would likewise not be accessible to the highly lipophilic Q.

The photoaffinity labeling data of Yu and Yu (1982) could fit the Q-cycle concept with the labeled cytochrome *b* corresponding to the center i and the labeled 15-kDa polypeptide corresponding to center o interaction sites for Q, with the latter polypeptide being in contact with both the iron-sulfur protein and cytochrome b_{566}. However, this rescue attempt still suffers from some difficulties. In agreement with the Q-cycle mechanism, antimycin is observed to cause a bathochromic shift of the absorption spectral bands of reduced cytochrome b_{562} and to quench the semiquinone EPR signal attributed to protein-bound $Q^{\cdot-}$ at center i and designated as Q_c or Q_i (Yu *et al.*, 1978, 1980; De Vries *et al.*, 1980; Yu and Yu, 1981; Ohnishi and Trumpower, 1980; Bowyer and Ohnishi, 1985). Yet, the labeling of cytochrome *b* by arylazido derivatives of Q was found to be insensitive to antimycin (Yu and Yu, 1982). Superficially, this suggests that either the effect of antimycin on center i is indirect or that the Q-binding polypeptide of 15 kDa is distinct from a purported Q binding site at center i. Another possibility would place the 15-kDa polypeptide between cytochrome b_{566} and the iron-sulfur protein as the Q-binding protein at center o. However, if both the iron-sulfur protein and the cytochrome b_{566} heme group are near the cytosolic surface of the membrane, then the membrane sidedness problem with respect to QH_2 binding remains unresolved.

In support of a direct interaction between QH_2 and the iron-sulfur protein, Shimomura *et al.* (1985) demonstrated an antimycin-insensitive reduction of cytochrome *c* by QH_2 in the presence of a complex reconstituted from purified iron-sulfur protein and cytochrome c_1. The specific activity of the cytochrome *c* reduction was of the magnitude of electron transfer in antimycin-inhibited Complex III and was partially inhibited by superoxide dismutase as was the case with antimycin-insensitive oxidation of QH_2 as catalyzed by Complex III. However, even if this reaction represents a reconstituted antimycin-insensitive pathway of electron transfer in Complex III, it has not yet been determined what relationship this pathway has to the much more active antimycin-sensitive pathway proposed to be utilized by the Q cycle.

Does QH_2 React First with the Iron-Sulfur Center?

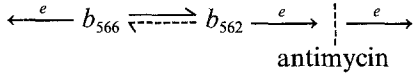
The Q-cycle scheme defines the first reaction of QH_2 with Complex III to be with the iron-sulfur center. As already discussed, this assumption is based primarily on the requirement of the iron-sulfur protein for the oxidant-induced reduction of cytochrome *b* in conjunction with the original Q-cycle formulation of Mitchell (1976). A critical requirement for this model that can be tested experimentally is that the iron-sulfur cluster be reduced by substrate either before or concomitant with reduction of the cytochrome *b* redox

centers. Several rapid kinetic studies have addressed this problem. Jin *et al.* (1981), employing stopped-flow spectrophotometry, observed an initial reduction of cytochrome *b* faster than the reduction of cytochrome c_1 followed by an oxidation of cytochrome *b* as the reduction of cytochrome c_1 continued. Upon completion of reduction of cytochrome c_1, cytochrome *b* again underwent a slower reduction. In a similar stopped-flow measurement, Degli Esposti and Lenaz (1982b) found that cytochrome *b* preceded cytochrome c_1 in being reduced by QH_2-1. Although these studies did not measure the rate of reduction of the iron-sulfur center, the rapid redox equilibration between the iron-sulfur center and cytochrome c_1 (Tsai *et al.*, 1983) would favor a similar relationship between rates of reduction of cytochrome *b* and the iron-sulfur center. A more definitive study was reported by De Vries *et al.* (1983) who measured the rapid kinetics of reduction of duroquinol of cytochrome b_{562}, cytochrome c_1, and the iron-sulfur center of Complex III under a variety of conditions. Generally, the cytochrome *b* was reduced before the iron-sulfur center. On the basis of these kinetic data and the purported existence of two spectroscopic species of the iron-sulfur center and four potentiometric species of cytochrome *b* heme groups, a previously proposed double Q-cycle involving a dimeric Complex III, with each monomer exhibiting a different electron-transfer pathway (De Vries *et al.*, 1982a), was supported. The validity of this model will depend ultimately on a demonstration that a fully functional QH_2-cytochrome *c* reductase can exist only as a dimer, as will be discussed.

The Function of Cytochromes *b*

According to the Q-cycle scheme the cytochrome b_{566}-cytochrome b_{562} sequence serves only to conserve the second electron released during the oxidation of QH_2 by returning it across the intramembrane space to the Q pool at center i. Here it is paired with a proton and a hydrogen atom generated by the Q reductase complexes to reduce the Q to QH_2 and thus complete the cycle. This role of cytochrome *b* appears rather oversimplified considering the chameleon-like properties displayed by this cytochrome. These properties are demonstrated by the multiplicity of spectral, thermodynamic, and kinetic perturbations displayed by the two cytochrome *b* heme groups present in the QH_2 reductase complex (Wikström, 1973; Von Jagow and Sebald, 1980; Reddy *et al.*, 1983). Wikström *et al.* (1980) have also expressed reservations concerning this simplified role of this cytochrome. Considerable evidence has been reported to support a proton-pumping function of the cytochrome *b* chain to supplement or replace vectorial proton translocation by QH_2 as proposed in the Q-cycle hypothesis.

In the Q cycle the two cytochrome *b* hemes are pictured as being able to equilibrate redoxwise very rapidly in a linear sequence. This assumption would preclude kinetic independence of cytochromes b_{562} and b_{566} during oxidation or reduction, especially in an antimycin-inhibited system where electron transfer is greatly restricted. This was supported by Eisenbach and Gutman (1975) who observed that cytochromes b_{562} and b_{566} were reduced at equal rates by substrates in submitochondrial particles treated with antimycin. However, Hatefi and Yagi (1982) showed that the reduction rates of the two cytochrome *b* hemes varied with respect to each other according to the overall flux of electron transfer through the inhibited complex. This effect was found to be caused by a 10-fold difference in rates of oxidation of the reduced b_{562} and b_{566} cytochrome hemes, respectively, through or around the antimycin block. It was suggested that cytochromes b_{566} and b_{562} could be oxidized in opposite pathways with the oxidation of b_{566} heme being at a rate greater than the oxidation of the b_{562} heme as follows:



or that the two *b* cytochromes are not in kinetic sequence. Either of these schemes would be difficult to fit into the Q-cycle mechanism. In the case of the scheme as illustrated above and according to the Q-cycle mechanism the cytochrome *b*-566 would be oxidized by reversal of the electron-transfer pathway from center o to cytochrome b_{566}, whereas cytochrome b_{562} would be oxidized by leakage of electrons through antimycin-inhibited center i. Electron leakage through antimycin-inhibited center i would not be in conflict with the Q-cycle scheme; however, an oxidation of cytochrome b_{566} via center o would be unlikely owing to the energy barrier to the formation of the unstable, bound $Q^{\cdot-}$ from the Q pool.

Center i, the Proposed Site of Reduction of Ubiquinone

Interfacing of Two-Electron to One-Electron Transfer Pathways

Direct evidence for the existence of a bound ubisemiquinone at catalytic center i of the Q cycle is provided by the detection by EPR spectroscopy of a stabilized ubisemiquinone the signal of which is discharged by antimycin, a designated center i inhibitor, but not by myxothiazol, a designated center o inhibitor, or by BAL + oxygen which destroys the iron-sulfur center (Konstantinov and Ruuge, 1977; Yu *et al.*, 1978, 1980; DeVries *et al.*, 1980, 1982b; Ohnishi and Trumpower, 1980). Despite this evidence of a bound ubisemiquinone that fits the Q-cycle model at center i, a great deal of

uncertainty exists in the operational details of this proposed center. Of principal concern has been the interfacing of Q as a single-electron acceptor from the cytochrome *b* chain of the Q cycle with pool Q that has been reduced by the dehydrogenase complexes or by added exogenous QH_2.

The first proposed model of the Q cycle involves an electron transfer from the cytochrome *b* chain to $Q^{\cdot-} + 2H^+$ to form QH_2 and complete the Q cycle (Mitchell, 1976). With the later Q-cycle model of Trumpower (1981a, b) the electron from the cytochrome *b* chain reduces Q to $Q^{\cdot-}$ which is then further reduced to QH_2 by an electron and two protons produced by the dehydrogenase enzymes. One of the presumably attractive features of the Q cycle is its offering of a way to transpose a two-electron transfer system mediated by flavins and quinones to a single-electron transfer mediated by cytochromes and the iron-sulfur center. However, this feature appears to be more apparent than real since the problem still exists at the juncture of the QH_2 pool produced by the dehydrogenases and the Q cycle at center i. One proposed solution is a reverse dismutation of pool Q and QH_2 to yield two molecules of $Q^{\cdot-}$ and two protons which can enter the cycle at center i (Slater *et al.*, 1981). This model suffers from the requirement of two stabilizing $Q^{\cdot-}$ binding sites at center i and a pool of oxidized Q to maintain the reverse dismutation. Indeed, Lenaz's laboratory has shown that endogenous Q is not required for a full initial rate of oxidation of lower homologs of QH_2 in Q-depleted or genetically Q-deficient mitochondria (Cabrini *et al.*, 1981; Degli Esposti and Lenaz, 1982a; Pasquali *et al.*, 1981). Moreover, Rich and Wikström (1986) found that the cytochromes *b* of mammalian Complex III were rapidly oxidized via cytochrome c_1 by a pulse of ferricyanide under conditions where the *b* cytochromes, and presumably endogenous Q, were completely reduced. According to the Q-cycle scheme no oxidized Q could be produced unless an electron were transferred from $Q^{\cdot-}$ at center o to oxidized cytochrome b_{566}. With the *b* cytochromes completely reduced, this reaction could not occur and oxidized Q would not be produced to complete the redox cycle at center i, no matter which mechanism is utilized at this center to facilitate a changeover of a two-electron to a one-electron pathway. Rich and Wikström (1986) concluded that in this case the Q-cycle scheme would have to be modified to allow $Q^{\cdot-}$ at center o to become accessible for interaction at center i, in effect a mechanism similar to that pictured in the *b* cycle as proposed by Wikström and Krab (1980).

Mitchell and Moyle (1985) have proposed a Q-cycle model involving a dimeric complex of cytochrome *c* reductase in which the $Q^{\cdot-}$ produced in each monomer by the single-electron reduction of Q at center i can dismutate to yield Q and QH_2 in the Q pool. The reaction sequence can be represented as follows, with subscripts A and B representing the two Complex III units of the dimer:

$$QH_2 + (c^+)_A + (b^+)_A \xrightarrow{\text{center o}} Q + (c)_A + (b)_A + 2H^+(\text{outside})$$

$$QH_2 + (c^+)_B + (b^+)_B \xrightarrow{\text{center o}} Q + (c)_B + (b)_B + 2H^+(\text{outside})$$

$$(b)_A + Q \xrightarrow{\text{center i}} (b^+)_A + (Q^-)_A$$

$$(b)_B + Q \xrightarrow{\text{center i}} (b^+)_B + (Q^-)_B$$

$$(Q^-)_A + (Q^-)_B + 2H^+(\text{inside}) \xrightarrow{\text{dismutation}} Q + QH_2$$

$$\text{Overall: } QH_2 + 2c^+ + 2H^+(\text{inside}) \longrightarrow Q + 2c^+ + 4H^+(\text{outside})$$

Of course this model would restrict the QH_2-cytochrome *c* reductase activity to dimers of Complex III. A definitive test of this requirement is probably yet to be performed. However, Nalecz and Azzi (1985) reported the isolation and testing of preparations of monomeric and dimeric Complex III, both preparations being catalytically active and displaying identical (inhibitor)/(cytochrome c_1) : enzymic activity stoichiometry. The dimeric preparation differed from the monomeric preparation in displaying biphasic kinetics with respect to the quinol (DBH_2) concentration and a higher activity at saturating quinol concentrations, although these differences were attributed to differences in accessibility of the complexes to the quinol rather than to inherent differences in electron-transfer pathways.

Elimination from consideration of split electron pathways from QH_2 to cytochrome c_1, an example involving a dimeric Complex III, would leave as alternatives cyclic or oscillating electron pathways involving two turnovers. Applied to the Q-cycle, the reaction sequence would be represented as follows:

$$QH_2 + c^+ + b^+ \xrightarrow{\text{center o}} Q + c + b + 2H^+(\text{outside})$$

$$Q + b \xrightarrow{\text{center i}} (Q^-)_1 + b^+(\text{first turnover})$$

$$QH_2 + c^+ + b^+ \xrightarrow{\text{center o}} Q + c + b + 2H^+(\text{outside})$$

$$Q + b \xrightarrow{\text{center i}} (Q^-)_2 + b^+(\text{second turnover})$$

$$(Q^-)_1 + (Q^-)_2 + 2H^+(\text{inside}) \xrightarrow{\text{dismutation}} Q + QH_2$$

$$\text{Overall: } QH_2 + 2c^+ + 2H^+(\text{inside}) \longrightarrow Q + 2c^+ + 4H^+(\text{outside})$$

In this case the Q^- produced at center i at the first turnover would have to be stored in a stabilized, bound form to avoid dissipating side reactions (e.g., reduction of oxygen to superoxide) until the second Q^- is produced at center i during the second cycle.

The two-cycle model as outlined, because of a stored Q^- half of the time, should display a steady-state concentration of Q^- half the concentration

of active Complex III (or cytochrome c_1). Indeed, measurements of the concentration of the antimycin-sensitive ubisemiquinone, designated Q_C, by EPR have shown maximal values of 0.4 per cytochrome c_1 (De Vries *et al.*, 1980) and 0.6 per cytochrome c_1 (Yu *et al.*, 1980); however, these signals were observed only at relatively high pH's (> 8.0) and under nonoxidizing conditions. Under conditions of steady-state electron flux at physiological pH the concentration of $Q^{\cdot-}$ as measured by EPR would be too small to be compatible with the two-cycle model of the Q cycle unless the $Q^{\cdot-}$ is somehow spin coupled to a tightly bound quinone or other moiety that quenches the EPR signal.

Effect of Antimycin on Q-Cycle Electron Transfer

Because antimycin is shown as blocking electron-transfer reactions at center i, the critical junction between the end of one electron-transfer cycle and the beginning of a new cycle of the Q-cycle, its inhibitory effect offers a valuable test of the correctness of the Q-cycle scheme. According to the current model of the Q cycle involving monomeric Complex III, antimycin would block regeneration of QH_2 from Q and reducing equivalents generated by the dehydrogenase complexes, but should not affect the initial reduction by QH_2 of the iron-sulfur center–cytochrome c_1 sequence. Indeed, early applications of this test appeared to confirm this prediction. Measurements of the rate of reduction of cytochrome c_1 by succinate in succinate-cytochrome *c* reductase complex showed no inhibition by antimycin within a time of approximately 1 sec after addition of succinate (Bowyer and Trumpower, 1981). However, by use of rapid stop-flow kinetic measurements with a similar system as well as with QH_2-1 as reductant, Degli Esposti and Lenaz (1982b) reported an inhibition of cytochrome c_1 reduction by antimycin within a time period of a single turnover. Also, they were able to repeat the results of Bowyer and Trumpower by expanding the time frame from milliseconds to seconds. The discrepancy in results was attributed to the normal antimycin-insensitive electron transfer that would appear to be rapid with the concentrated enzyme in the seconds time frame. These results would disallow the monomeric Q-cycle scheme as illustrated by Fig. 1. However, the double Q-cycle scheme as formulated by De Vries *et al.* (1982) would display an antimycin-sensitive reduction of half of the cytochrome c_1 during the first cycle.

Inhibitors and Conformational Changes in Complex III

Inhibitors as Modulators of Conformational Changes

The relatively large number and diverse inhibitory properties of inhibitors of Complex III have proved invaluable in the mapping of electron-transfer

pathways within the complex. As with other studies, however, due to the complexity of the complex, interpretations of the mechanism of action of these inhibitors are not without controversy. The large number of inhibitors catalogued has been classified into three distinct categories according to chemical structure and effects on the kinetic, spectral, and electrochemical properties of Complex III. These are antimycin-like inhibitors, including antimycin, HQNO, and funiculosin; MOA-type inhibitors, characterized by a reactive methoxyacrylate moiety in the molecular structure, including myxothiazol, mucidin, and stigmatellin (Thierbach *et al.*, 1984); and inhibitors containing quinone and naphthoquinone groups, the most potent being UHDBT.

In terms of the Q-cycle hypothesis, the antimycin-like inhibitors are termed center i inhibitors because they inhibit the oxidation of the *b* cytochromes and stimulate the oxidant-induced reduction of these cytochromes. Because antimycin causes a shift in the absorbance spectrum (Berden and Opperdoes, 1972; Dutton *et al.*, 1972) and the EPR spectrum (Dervartanian *et al.*, 1973), it is believed by some investigators that antimycin binds to cytochrome *b*-562 and competes with Q for its binding at center i (Nelson *et al.*, 1972; Roberts *et al.*, 1980). The MOA inhibitors are designated as center o inhibitors because they block the reduction of cytochromes *b* by QH_2 in antimycin-treated Complex III (Becker *et al.*, 1981; Von Jagow *et al.*, 1984). Two of these inhibitors, myxothiazol and stigmatellin, have been shown to perturb the absorption spectrum of reduced cytochrome *b*-566 (Thierbach and Reichenbach, 1981; Becker *et al.*, 1981), and also in antimycin and myxothiazol-resistant mutants of yeast the mutations are found in the mitochondrial gene for cytochrome *b* (Pajot *et al.*, 1976; Colson *et al.*, 1979; Roberts *et al.*, 1980; Thierbach and Michaelis, 1982). On these bases cytochrome *b* generally has been assigned as the binding site for these inhibitors. The quinoid inhibitors are believed to bind at the QH_2 binding site on or near the iron-sulfur protein because they perturb the EPR spectrum and redox properties of the iron-sulfur center as well as showing a competitive effect of Q on their inhibitory activities (Berry and Trumpower, 1985).

Superficially, the inhibitory effects of the inhibitors of Complex III are perceived to fit neatly into the Q-cycle scheme, involving discrete binding sites and electron-transfer pathways. However, more complete data indicate a complex interaction of the inhibitors with the complex. Experiments in which the direct binding of antimycin to resolved subunits of Complex III (Van Keulen and Berden, 1985) and the photolabeling of subunits with azido derivative of antimycin (Ho and Rieske, 1985a) both indicated that the primary binding of the antimycin was not with cytochrome *b* but with a smaller subunit (m = 12–13 kDa). This subunit normally fractionated together with the cytochrome c_1 subsequent to dissociation of the subunits

of Complex III. A similar protein associated with chromatophores of *R. sphaeroides* was found to be specifically labeled with a photoactive analog of antimycin (Wilson *et al.*, 1985). Also, on the basis of studies with antimycin-resistant mutants of yeast, Chevellotte-Brivet *et al.* (1983) concluded that antimycin binds to some other component than cytochrome *b* which is allosterically perturbed by genetic alterations in the structure of cytochrome *b*. Moreover, a more recent examination of the effects of myxothiazol on Complex III has shown a complex range of effects (Von Jagow *et al.*, 1984) including an increase in the midpoint potentials of both cytochromes *b*-562 and *b*-566, effects on reduction of cytochromes *b* and c_1 by succinate or QH_2 similar to those caused by removal of the iron-sulfur protein, a perturbation of the EPR spectrum of the iron-sulfur center, and a removal of midpoint potential increases of the iron-sulfur center caused by hydroxyquinone inhibitors.

Comparative studies involving the respiratory chain, and isolated segments containing Complex III, from various organism and mutants of yeast have also revealed the complexity of inhibitor interactions with Complex III. Yeast mutants that were resistant to antimycin varied in their cross resistance to HQNO and funiculosin, inhibitors that by other criteria bind at the same site as antimycin (Burger *et al.*, 1977). Also, the effects of HQNO and funiculosin on such factors as QH_2-cytochrome *c* reductase activity, spectral redshift of cytochrome *b*-562, oxidant-induced reduction of cytochromes *b*, guanidine-induced dissociation of Complex III subunits, and QH_2-plastocyanin reductase activity of Complex III showed a lack of correlation not only with respect to species source of the Complex III system, but also with respect to the particular property of Complex III perturbed by the inhibitors (Rieske, 1980; Rieske *et al.*, 1984; Tripathy and Rieske, 1985). In a similar vein, Tsai *et al.* (1985) observed that antimycin, funiculosin, myxothiazol, and UHDBT demonstrated effects on the properties of the redox components of yeast Complex III considerably different than the effects of these inhibitors on analogous components of bovine-heart Complex III.

These studies indicated that the inhibitors on the basis of overall effects on Complex III cannot be categorized as discretely with respect to mechanism of action as the Q-cycle scheme indicates. With placement of antimycin and myxothiazol at the extremes of center i and center o inhibitors, the other less potent inhibitors can be arranged as follows with respect to their overall effect on various properties of Complex III as compared to antimycin and myxothiazol: antimycin > HQNO > funiculosin > UHDBT > myxothiazol (Rieske *et al.*, 1984). Of particular interest was the observation that not only antimycin but also HQNO, funiculosin, UHDBT, mucidin, and myxothiazol inhibited the guanidine-induced dissociation of the subunits of Complex III although to a considerably lesser extent than antimycin. The

MOA antibiotics mucidin and myxothiazol were much more effective at pH 8.0 than at pH 7.0, whereas HQNO and funiculosin were much more effective at the lower pH. Because of these accumulated data, it was suggested that all of these inhibitors affect Complex III by blocking specific as well as general conformational changes associated with normal function of the enzyme. This concept would explain the specific effects of antimycin and myxothiazol on the properties of cytochromes *b* and the iron-sulfur center when direct binding of the inhibitors to these subunits of the complex apparently do not occur. Van Jagow *et al.* (1984) have suggested this possibility with respect to the effects of myxothiazol on the iron-sulfur center. It has also been suggested that the overlapping effects of the inhibitors could be explained by multivalent binding of the inhibitors within a cavity or channel within Complex III or between Complex III monomers of a dimeric complex. In this model each inhibitor is in contact with possibly several subunits and thus able to exert several seemingly independent effects (Rieske, 1980).

Functional Conformational Changes

In addition to the evidence supplied by the effects of inhibitors, other evidence has accumulated that support a major role of conformational changes in the electron-transfer function of Complex III. These include redox-linked changes in (a) stabilization of quaternary structure of Complex III (Rieske *et al.*, 1976), (b) arrangement of liposomal paracrystalline arrays of Complex III (Wakabayashi *et al.*, 1972), (c) circular dichroic spectra at 220 nm (Berden and Slater, 1972) and at the cytochrome Soret wavelengths (Reed *et al.*, 1978), (d) antimycin fluorescence in antimycin-treated Complex III (Berden and Slater, 1972), (e) the infrared spectra of buried –SH groups in Complex III (Rieske *et al.*, 1975), (f) EPR spectrum of spin-labeling of Complex III by a maleimide-linked nitroxide group (Das Gupta *et al.*, 1979), and (g) the differential-labeling patterns of subunits of Complex III dual-labeled with ^{3}H- and ^{14}C-labeled succinic anhydride (Ho and Rieske, 1985b). In addition, in a recent study Degli Esposti and Lenaz (personal communication) observed profound effects of the initial redox state of the high-potential components, the iron-sulfur protein and cytochrome c_1, on the substrate-dependent kinetics of QH_2-cytochrome *c* reductase activity as well as on the electronic equilibration between these two components. The data could not be fitted by kinetic effects alone; therefore, it was proposed that only conformational changes in Complex III controlled by the redox state of probably the iron-sulfur center could explain the measured phenomena.

Although there appears to be no doubt that the function of Complex III is intimately linked to profound changes in conformation, the question of

how compatible these changes are to the Q-cycle mechanism must be asked and answered. With our present state of knowledge and understanding concerning these conformational changes, we cannot disprove the Q-cycle mechanism on their account; however, they do advertise the probability that the enzyme functions in a multiplicity of states. This suggestion is substantiated by the detection of multiple spectroscopic and electrochemical species of the electron carriers of Complex III (Wikström, 1973; Rieske, 1976; De Vries *et al.*, 1979; Reddy and Hendler, 1983, 1986). Whether these states represent different conformational forms of components in a single complex or interacting components in a dimeric system is yet to be determined. Nevertheless, the existence of multiple conformational forms of the complex provides a considerable number of possible options to the Q-cycle mechanism that can be reconciled with the described and the yet to be discovered properties of Complex III.

Conclusions

The Q-cycle hypothesis is at this time the most successful model in explaining the electron-transfer pathway in Complex III. Its broad ramifications appear unassailable; however, a number of discrepancies become apparent when the details are examined. Fitting these apparent discrepancies into the broad features of the Q-cycle model will require further modifications as have been attempted with the double Q-cycle and the cytochrome *b*-cycle schemes. A single major discrepancy between experimental data and the Q-cycle model that cannot be reconciled by a moderate modification of the model is sufficient reason to return to the work bench or drawing board. However, although a number of apparent discrepancies and problems are discussed in this review, the experimental difficulties inherent with Complex III with the resulting uncertainty of much of the data compels a wait and see attitude before any definite diagnosis of the condition of the Q-cycle hypothesis can be made. However, in the meantime, additional and more refined data must be obtained to reconcile all of the available experimental information with either the Q-cycle model, a modified version of the Q cycle, or if necessary a completely different model of electron-transfer within Complex III.

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