Journal of Bioenergetics and Biomembranes, Vol. 13, Nos. 1/2, 1981

SHORT REVIEW

New Concepts on the Role of Ubiquinone in the Mitochondrial Respiratory Chain

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Received March 13, 1980; revised June 20, 1980

Abstract

Ubiquinone participates in the oxidation–reduction reactions of the mitochondrial respiratory chain. In addition, this molecule possesses the necessary properties to function as a hydrogen carrier, thereby stoichiometrically coupling proton translocation to respiration by a direct chemiosmotic mechanism. This review discusses recent experimental evidence and new concepts relating to ubiquinone function in the mitochondrial respiratory chain. Emphasis is placed on possible protonmotive mechanisms of ubiquinone function, recent evidence implicating stable forms of ubisemiquinone in the respiratory chain, and properties of the ubiquinone molecule which may relate to its biological function.

Key Words: Ubiquinone; mitochondria; chemiosmotic mechanism.

Introduction

Ubiquinone is the only component of the mitochondrial respiratory chain which is soluble in hydrocarbon solvents and which is oxidized and reduced through reactions which unequivocally involve net release and uptake of protons. Thus this apparently simple, yet deceptively complex, molecule possesses the prerequisite intrinsic properties to function as a transmembrane hydrogen carrier by a direct chemiosmotic mechanism as first suggested by Mitchell (1966). Although there is extensive evidence that ubiquinone is a redox component of the respiratory chain, less is known about whether, and if so how, ubiquinone functions as a redox-linked hydrogen carrier.

This review summarizes recent experimental findings and conceptual

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0145-479X/81/0400-0001$03.00/0

advances bearing on the function of ubiquinone in the mitochondrial respiratory chain with particular emphasis on possible protonmotive mechanisms of ubiquinone function. Earlier research on ubiquinone function in the respiratory chain has been previously reviewed (Crane, 1977; Trumpower and Katki, 1979), and Wraight (1979) has recently reviewed aspects of quinone function in photosynthetic electron transfer systems.

Properties of Ubiquinone Relevant to Its Function in the Respiratory Chain

The structural feature which is most obviously related to ubiquinone function is the 1,4 benzoquinone group which can undergo oxidation–reduction. However, it seems very likely that the ring substituents may confer upon ubiquinone additional properties, which when appropriately amplified by protein, lipoprotein, or metalloprotein ligands may be equally important to its biological function and which may also confer on ubiquinone functional diversity not unlike the more commonly recognized prosthetic groups such as flavin.

It has frequently been pointed out (DePierre and Ernster, 1977; Hauska, 1977a; Crane, 1977; Spisni *et al.*, 1978; and Trumpower and Katki, 1979) that ubiquinone is comparable in length to the transverse dimension of a phospholipid bilayer, as illustrated in Fig. 1. The extended dimension of ubiquinone-10 is 56 Å, including 50 Å due to the 50 carbon isoprenoid sidechain; ubiquinone-6, which is the smallest of the naturally occurring homologues (Crane, 1956) is approximately 36 Å long. Taking into account measurements which place the fluorescence-quenching aromatic nucleus an average of 14 Å from the aqueous interface of the mitochondrial membrane (Chance, 1972), it is obvious that all of the naturally occurring ubiquinones are of sufficient length that their extended conformation would penetrate both halves of a simple phospholipid bilayer. Moreover, it appears that ubiquinone-10 is *too long* to be accommodated within the hydrophobic domain of a simple phospholipid bilayer unless the isoprenoid sidechain is folded.

The trans double bonds introduce a periodic rigidity into the isoprenoid sidechain, and examination of space-filling models indicates that the vicinal methyl groups further constrain intramolecular rotation around single bonds by steric hindrance. The net effect of the polyisoprenoid structure thus appears to be one which restrains the conformational dynamics of the molecule. To the extent that structure is selected for function, it seems probable that the sidechain of ubiquinone has been selected for some function other than flexibility or mobility.

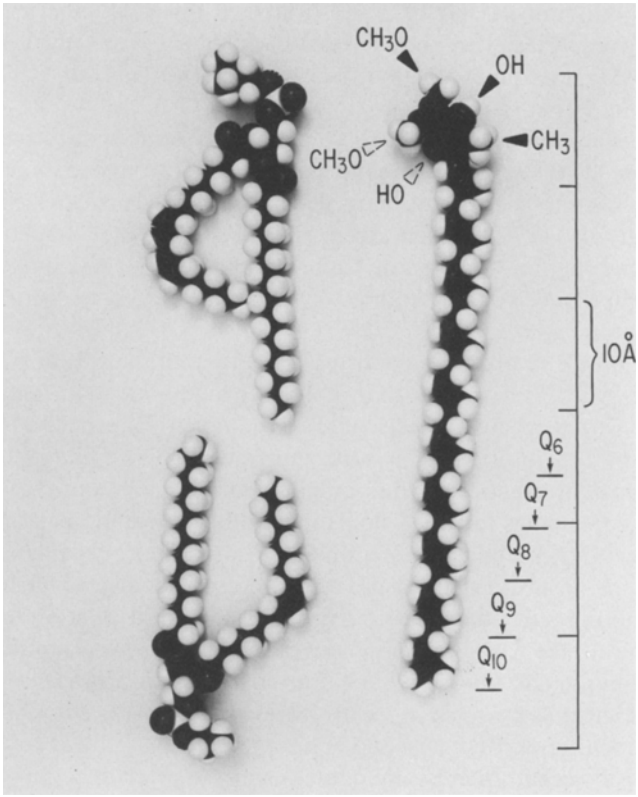

Fig. 1. Space filling models illustrating the extended dimensions of ubiquinone-10 and the naturally occurring ubiquinones relative to a phospholipid bilayer. The lengths of the isoprenoid sidechains of Q_6 through Q_{10} are indicated by the arrows. The vicinal methyl groups are oriented to the right at each isoprenoid double bond. The substituents on the quinone ring are arranged geometrically to conform to the most stable configuration according to molecular orbital calculations (see Fig. 2). The phospholipid to the upper left is 18:0, 18:2 phosphatidyl choline, while that to the lower left is 16:0, 18:1 phosphatidyl ethanolamine. The CPK models were obtained from Ealing Corporation.

Although it is not yet possible to relate the extensive literature of benzoquinone chemistry to ubiquinone function, it should not go unmentioned that p-benzoquinones readily form both π complexes and charge transfer complexes with various metals, including iron (Foster and Foreman, 1974). The latter have been extensively studied in the instance of metal complexes formed with 8-hydroxyquinoline, which is structurally related to the respira-

tory chain inhibitor HOQNO.[2] Substituted 1,4-benzoquinones also form crystalline complexes with themselves in which the π orbital systems are stacked or staggered in a coplanar geometry (Bernstein *et al.*, 1974; Foster and Foreman, 1974).

The electronic interactions in these simpler systems may be relevant to stabilization of the ubisemiquinone pair recently found associated with iron–sulfur cluster S-3 of succinate dehydrogenase (Ruzicka *et al.*, 1975; Ingledew *et al.*, 1976). Likewise, it is conceivable that stacking of the benzoquinone ring may contribute to formation of stable quinone clusters in the model liposome systems studied by Hauska and co-workers (Hauska, 1977a,b; Futami *et al.*, 1979).

The methoxy and alkyl ring substituents lower the midpoint potential of ubiquinone by ~100 mV from that of 1,4-benzoquinone (Morton, 1965), in agreement with expected effects of electron-donating groups (Fieser and Fieser, 1961). Substitution of the benzoquinone ring in all four positions also prevents covalent reaction of this quinone with protein sulfhydryl groups. Sulfhydryl compounds form 1,4-thiol ether adducts with quinones (Snell and Weissberger, 1939), and there is ample precedent that analogues of ubiquinone with an unsubstituted position adjacent to an electron-attracting or weakly donating substituent react irreversibly to form thiol adducts (Redfearn and Whittaker, 1962; Trumpower *et al.*, 1974). This protection may be critical to ubiquinone function in a sulfur-rich domain, and experimentalists might heed nature's example in reconstitution experiments employing unsubstituted quinones (see Redfearn and Whittaker, 1962).

In view of recent interest in one-electron transfer reactions involving ubiquinone and the attendant formation of functionally significant ubisemiquinone (Mitchell, 1976), it is useful to consider mechanisms by which the ring substituents may alter the thermodynamic stability of ubisemiquinone. The crystal structure of ubiquinone is not known. However, molecular orbital calculations (Breen, 1975) indicate that the most stable conformation of ubiquinone is one in which the isoprenoid sidechain rotates into a chromenol conformation and the methoxy groups rotate 60° from a coplanar configuration to assume a trans orientation with respect to each other, as shown in Fig. 2. Depending on the geometry of the methoxy groups, the conformational stability of ubiquinone may be increased 15–75 kcal by a chromenol configuration of the sidechain. Although the conformation implied by these calculations remains to be confirmed by structural studies, the

[2]The abbreviations used are: HOQNO, 2-heptyl-4-hydroxyquinoline-N-oxide; UHDBT, 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole; HMHQQ, 7-*n*-hexadecylmercapto-6-hydroxy-4,8-quinolinequinone; Q, ubiquinone; $QH^{\cdot}$, ubisemiquinone; $Q^{\cdot-}$, ubisemiquinone anion; QH_2, ubiquinol; TTFA, 2-thenoyltrifluoroacetone.

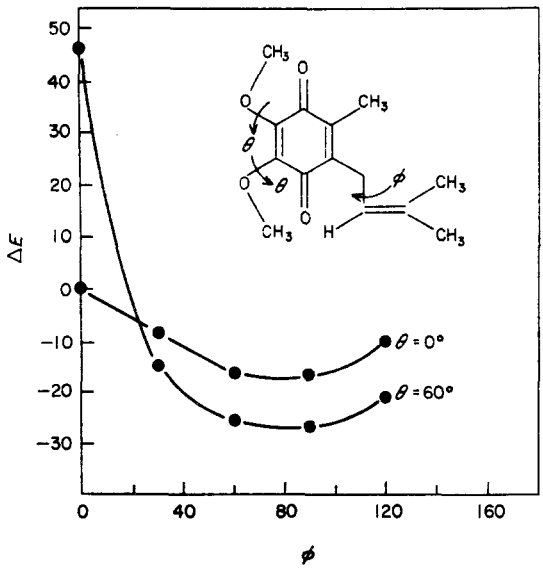

Fig. 2. Rotational energy curves for geometric conformations of ubiquinone as indicated by molecular orbital calculations. The energies (ΔE) are in kilocalories. The reference geometry is the all planar form. θ is the angle of twist around the ring carbon–oxygen bonds of the methoxy group and ϕ is the angle of rotation of the isoprene unit toward the ring. Reproduced from Breen, 1975 with permission.

theoretically predicted structure is consistent with the facile conversion of ubiquinone and ubiquinol to ubichromenol and ubichromanol, respectively (Langemann and Isler, 1965).

The most important factor contributing to semiquinone stability is resonance delocalization of the unpaired spin density (Fieser and Fieser, 1961). The chromenol conformation of ubiquinone places the first double bond of the isoprenoid sidechain sufficiently close to the proximal quinoid oxygen to lower the electron density on the latter and, at the same time, the ring methyl group acquires methine character (Breen, 1975). It is very likely that if this conformation were enhanced by suitable protein ligands it would contribute to ubisemiquinone stability by allowing extended delocalization of the unpaired electron. Further stabilization might be realized by constraining the methoxy groups to a planar geometry, still representing a low-energy form, thus permitting further distribution of the spin density as previously noted with alkoxy-substituted semibenzoquinones (Berger and Rieker, 1974).

Pool Function Behavior, Lateral and Transverse Mobility of Ubiquinone

As evidence evolved that the respiratory chain is segmentally divided into four structurally and functionally discrete lipoprotein electron transfer complexes and that mitochondria have a molar excess of ubiquinone relative to cytochrome b–c_1 complex (for a review, see Hatefi, 1976), it was proposed by Green (1962) that electron transfer between the dehydrogenase complexes and the cytochrome b–c_1 complex was mediated by ubiquinone which "can move in all directions through the lipid" of the inner membrane and is thus "capable of shuttling electrons between pairs of fixed oxidation–reduction components." The mobile carrier hypothesis implies that physical association is not required for electron transfer between complexes and that each electron acceptor and donor complex acts as an independent component of a homogeneous population, serviced by a common pool of ubiquinone.

Kroger and Klingenberg (1973a) tested the mobile carrier hypothesis by measuring the oxidation–reduction kinetics and the steady-state redox poise of ubiquinone in uncoupled submitochondrial particles and correlating these to the overall rate of electron transfer through the respiratory chain. It was found that 80–90% of the total ubiquinone responded to changes in respiratory activity, and the total amount of redox-active ubiquinone was kinetically homogeneous. In addition, with either succinate or NADH as substrate the redox poise of ubiquinone and the respiratory activity were found to be a function of the separately measured activities of electron donor and acceptor, and this "pool function" kinetic relationship extended to measurements where the donor and acceptor activities were partially inhibited by rotenone or antimycin, respectively (Kroger and Klingenberg, 1973b).

The pool-function kinetics were attributed to a homogeneous pool of ubiquinone having sufficiently rapid lateral mobility so that the rates of reduction and oxidation of ubiquinone are determined by the turnover numbers of the dehydrogenase and b–c_1 complex respectively. It was also observed that oxidation of ubiquinol is first order with respect to ubiquinol, and it was noted that these kinetics are somewhat unexpected in that oxidation of a rapidly mobile molar excess of ubiquinol would be expected to exhibit a zero-order transient prior to first-order kinetics (Kroger and Klingenberg, 1973a). Mitchell (1976) has pointed out that the first-order kinetics may reflect restricted mobility of ubiquinol, resulting in a diffusion-limited rate, and the subtle kinetic differences resulting from a heterogeneous population including intermediate redox forms may not be revealed by the macroscopic kinetics analysis.

Ragan and co-workers (Ragan and Heron, 1978; Heron *et al.*, 1978) tested whether isolated electron transfer complexes can manifest pool function interaction by reconstituting NADH–cytochrome c reductase activity

from isolated complex I plus complex III. If the isolated complexes interact as separate and homogeneous populations, the rate of electron transfer from NADH to cytochrome *c* would depend on the complete activities of the ubiquinone reducing and oxidizing systems, and the NADH–cytochrome *c* reductase activity (V_c) would relate to the constituent NADH–ubiquinone reductase (V_{red}) and ubiquinol–cytochrome *c* reductase (V_{ox}) activities according to the equation.

$$V_c = \frac{(V_{ox})\,(V_{red})}{V_{ox} + V_{red}} \tag{1}$$

By varying the ratio of the two respiratory chain complexes it was possible to vary V_{ox} and V_{red} and to test whether the reconstituted NADH–cytochrome *c* reductase activity was equal to the expected pool function rate, based on the activities of the isolated complexes. This approach assumes that the intrinsic activity of complex I is independent of association with complex III, and vice versa. When complexes I and III were mixed in the absence of extra phospholipid they combined with a fixed 1:1 stoichiometry, and the resulting NADH–cytochrome *c* reductase activity reflected the number of stoichiometrically associated complex I–III units (Ragan and Heron, 1978). Thus under these conditions the interacting complexes did not manifest pool function behavior, in agreement with previous findings that isolated respiratory chain complexes reassociate with a fixed ratio (Hatefi, 1966; King, 1966).

However, if the isolated complexes were recombined in the presence of extra phospholipid, approximating the ratio of phospholipid to respiratory chain complexes *in situ,* the resulting NADH–cytochrome *c* reductase activity closely matched expected pool function rates when the complexes were titrated against each other and when the constituent activities were partially inhibited (Heron *et al.,* 1978). In addition, reduction of cytochrome *b* proceeded monotonically, as opposed to the biphasic *b* reduction observed when the complexes interacted with a fixed stoichiometry.

It was proposed that in the natural membrane and in NADH–cytochrome *c* reductase reconstituted with appropriate amounts of phospholipid, electron transfer from NADH to cytochrome *c* proceeds only through complex I–III units which form and dissociate at rates greater than the rate of electron transfer from complex I to complex III (Heron *et al.,* 1978). Lack of pool function behavior was attributed to loss of mobility of the complexes resulting from lowering of the phospholipid-to-protein ratio during isolation of the complexes, thus immobilizing stoichiometrically fixed units. Rapid lateral mobility of the respiratory chain complexes had previously been proposed by Hackenbrock and Hochli (1977), who demonstrated thermotropic lipid-phase transitions, lateral lipid-protein separations, and lateral

translational diffusion of integral membrane proteins in mitochondrial membranes. To further evaluate whether pool function behavior is attributable to lateral mobility of the respiratory chain complexes themselves, rather than to mobility of ubiquinone, measurements must be made of the diffusion coefficients of the respiratory chain complexes and of the lateral displacement required for their interaction *in situ*.

Hauska and co-workers tested whether ubiquinone can transfer hydrogen through a phospholipid bilayer by inserting ubiquinones with various sidechains into liposomes and measuring rates of reduction of internally trapped ferricyanide by external dithionite (Hauska, 1977a,b). Ubiquinones with sidechains containing 3–10 isoprenoid units and a saturated sidechain analog of ubiquinone-9 catalyzed the transmembranous reaction with pseudo-first-order rate constants of 1.2–1.8 sec^{-1}. With ubiquinones-1 and -2 the rate was 20–65 fold slower and not impressively greater than the dithionite leakage rate (Futami *et al.*, 1979). Control measurements established that the transmembrane reduction was not due to a passive leak induced by the isoprenoid sidechain and that the reaction required a redox-active aromatic nucleus.

The reactions catalyzed by long sidechain ubiquinones had activation energies of ~15 kcal/mol, the reactions were higher than first order, and the rates increased as the pH decreased. In contrast, the slow reactions catalyzed by short-chain quinones had activation energies of ~4 kcal/mol, the reactions were first order, and the rate constants increased as the pH increased.

The slow reaction catalyzed by ubiquinones-1 and -2 is probably not relevant to the biological function of the naturally occurring ubiquinones, since the rate is not compatible with the turnover times of the respiratory chain. In addition, these quinones inhibit NADH-linked respiration and are unable to restore such respiration in ubiquinone-depleted mitochondria (Lenaz *et al.*, 1975; 1977; 1978).

The reaction catalyzed by ubiquinones having a sidechain of 15 carbons or longer obviously proceeds by a different mechanism than that of the short-chain homologues. Although there may be subtle differences within the homologous series of ubiquinones-3 to -10 in their interaction with phospholipid bilayers (Spisni *et al.*, 1978), it seems feasible that these ubiquinones "cluster" in some manner as suggested (Futami *et al.*, 1979), forming a relatively immobile transmembrane array by which intermolecular transfer of reducing equivalents mediates reduction of ferricyanide through the liposomal membrane. Transmembranous stacking of the quinoid ring system through a hydrophobic domain may be facilitated by π orbital overlap (Bernstein *et al.*, 1974; Foster and Foreman, 1974). Although the conformational dynamics of the isoprenoid sidechain appear to be limited as discussed above, rotation around single bonds on a relatively slow time scale would

permit approximately 90° folding of variable-length segments of the side-chains and their insertion into the fluid median of a bilayer. The feasibility of π orbital stacking arrangements might be tested by determining whether ubiquinone loaded into liposomes is hypochromic, and equally informative experiments might be designed to test how many ubiquinone molecules per vesicle are required to catalyze transmembranous reduction in small vesicles of the type formed in these studies (Futami *et al.*, 1979).

Whether ubiquinone-loaded liposomes are relevant to the mechanism of ubiquinone function in the respiratory chain is an open question. Such an arrangement does not account for the formation of thermodynamically stable ubisemiquinones (Ohnishi and Trumpower, 1980), and it seems unlikely that such clusters would exist in the phospholipid continuum of the mitochondrial membrane, except to the extent that they might contribute to antimycin-insensitive electron transfer from the dehydrogenases to cytochrome *c*, bypassing the b–c_1 complex.

The Protonmotive Q Cycle

A central premise of the chemiosmotic mechanism of oxidative phosphorylation is that respiration establishes an electrochemical gradient of protons across the inner mitochondrial membrane by a direct ligand conduction mechanism in which transmembranous reduction and oxidation of hydrogen carriers results in stoichiometric and covalent coupling of proton translocation to electron transfer (Mitchell, 1966). In order to reconcile a stoichiometry of $H^+/2e^- = 2$ for each of the three coupling sites with the lack of a recognizable hydrogen carrier in the cytochrome *c* oxidase segment of the respiratory chain, Mitchell (1975a,b; 1976) formulated the protonmotive Q cycle. A description of the *Q* cycle operating under steady-state conditions is shown in Fig. 3. This configuration is identical to that previously proposed (Trumpower, 1976), except that OxF is now identified as the Rieske iron–sulfur protein of the b–c_1 segment (Trumpower and Edwards, 1979a).

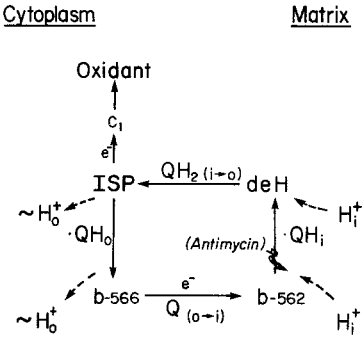

Fig. 3. A protonmotive Q cycle mechanism for electron transfer through the cytochrome b–c_1 complex. ISP designates the iron–sulfur protein of the b–c_1 segment and deH refers to an iron–sulfur cluster of succinate or NADH dehydrogenase.

The Q cycle hypothesis would require that electron transfer through the b–c_1 segment begin with formation of ubisemiquinone ($QH_i^{\cdot}$), or its anion, on the matrix side of the membrane, which then is maintained under steady-state conditions by the Q cycle. The $QH_i^{\cdot}$ is reduced to QH_2 by an iron–sulfur center of the dehydrogenase, resulting in uptake of a proton from the matrix. The QH_2 formed on the matrix side is then oxidized at the cytoplasmic side of the membrane by a reaction in which the iron–sulfur protein of the b–c_1 segment transfers one electron from QH_2 to cytochrome c_1 and a second electron from the $QH_0^{\cdot}$ thus formed to cytochrome b-566, forming Q and releasing 2 H^+ into the cytoplasmic domain. The electron from reduced b-566 is then transferred inward through the membrane to b-562, which in turn reduces Q to $QH_i^{\cdot}$ via an antimycin-sensitive reaction with uptake of a second proton from the matrix domain.

Although this account is simplified in that it ignores the effects of the pK_a of ubisemiquinone on the proton exchange reactions and the mechanistic complications associated with transitions from the steady state and with operation of the Q cycle in a dehydrogenase-free b–c_1 complex, it does illustrate how the transfer of two electrons from the dehydrogenase to cytochrome c covalently translocates 4 H^+ outward across the mitochondrial membrane. If translocation of 4 H^+ is accompanied by liberation of 2 H^+ in the matrix due to succinate oxidation, one would expect the succinate–cytochrome c reductase reaction to generate 4 H^+ in the cytoplasmic domain while only two compensating positive charges (e.g., K^+) would be taken up in the presence of an ionophore. This stoichiometry, which was first measured for the succinate–ferricyanide reductase reaction by Mitchell and Moyle (1967), has been confirmed by Alexandre and Lehninger (1979). If the protonmotive succinate–cytochrome c reductase reaction, for example, is coupled to the electronmotive cytochrome c oxidase reaction so that 2 H^+ released from oxidation of succinate are consumed by synthesis of H_2O in the matrix, the overall succinate oxidase reaction would proceed with $H^+/2e^- = 4$, yielding *net* translocation of 2 H^+ per coupling site. This stoichiometry has been confirmed and enlargements thereon have been analyzed by Mitchell (1979a, b), and recent experiments using dissociation of the oxyhemoglobin complex to accurately measure the initial rate of O_2 consumption provide convincing evidence that the H^+/O quotient for succinate oxidation is 4 (Papa *et al.*, 1980).

The Q cycle requires that there be topographically segregated sites at the cytoplasmic and matrix domains of the membrane, referred to as center o and center i, respectively, for localized redox reactions of ubiquinone (Mitchell, 1976). Center o and center i must each have acceptor/donor activities for both the $Q/QH^{\cdot}$ and $QH^{\cdot}/QH_2$ couples, and the semiquinone intermediates must be insulated from transmembrane reactions, otherwise the

prescribed $H^+/2e^-$ stoichiometry would be lost (for a possible exception, see Mitchell, 1980).

In addition, the $QH^\cdot$ reactivities at center *o* and center *i* must be mechanistically and thermodynamically unique in several respects. Consequently, Mitchell (1976) has noted the important relationship between the midpoint potentials of the ubisemiquinone couples, the midpoint potential of the Q/QH_2 couple, and the equilibration constant for formation of $QH^\cdot$ from Q and QH_2.

The stability constant of $QH^\cdot$ is defined as the equilibrium constant for the reaction:

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

$$K_s = \frac{[QH^\cdot]^2}{[Q][QH_2]} \tag{3}$$

From Eq. (3) and the Nernst equations for the ubisemiquinone couples and the ubiquinone/ubiquinol couple, the following are derived:

$$\epsilon_m\,(Q/QH^\cdot) + \epsilon_m\,(QH^\cdot/QH_2) = 2\epsilon_m\,(Q/QH_2) \tag{4}$$

$$\epsilon_m\,(Q/QH^\cdot) - \epsilon_m\,(QH^\cdot/QH_2) = \frac{RT}{nF}\ln K_s \simeq 60 \log K_s \tag{5}$$[3]

Equations (4) and (5) prescribe that the midpoint potentials of the ubisemiquinone couples are always equidistant above and below, or equal to, the midpoint potential of the Q/QH_2 couple. In addition, as $QH^\cdot$ becomes less stable the potential of the $Q/QH^\cdot$ couple becomes relatively more negative, and the absolute difference $|\,\epsilon_m\,(Q/QH^\cdot) - \epsilon_m\,(QH^\cdot/QH_2)\,|$ becomes larger, as illustrated in Fig. 4. Only when the equilibrium concentration of $QH^\cdot$ becomes greater than one-third of the total quinone content ($Q + QH_2 + QH^\cdot$) does $\epsilon_m(Q/QH^\cdot)$ become more positive than $\epsilon_m\,(Q/QH_2)$.

Ubisemiquinone is a weak acid which dissociates with $pK_a = 6.0$ (Bridge and Porter, 1958), and recent findings indicate that the pK_a's of stable ubisemiquinones detected in isolated succinate–cytochrome *c* reductase complex are comparable to this (Ohnishi and Trumpower, 1980). It is useful therefore to note that at $pH > pK_a$, and at constant $Q^\cdot$ stability, $\epsilon_m\,(Q^\cdot/QH_2)$ manifests a 120 mV/pH unit dependence, while $\epsilon_m\,(Q/Q^\cdot)$ is pH independent. The possible importance of environmentally determined pH and pK_a in stabilization of ubisemiquinone has been pointed out by Crofts (1979).

At a given $\epsilon_m\,(Q/QH_2)$ the fractional concentration of ubisemiquinone

[3] At 25°C and where RT/nF has the conventional units in millivolts.

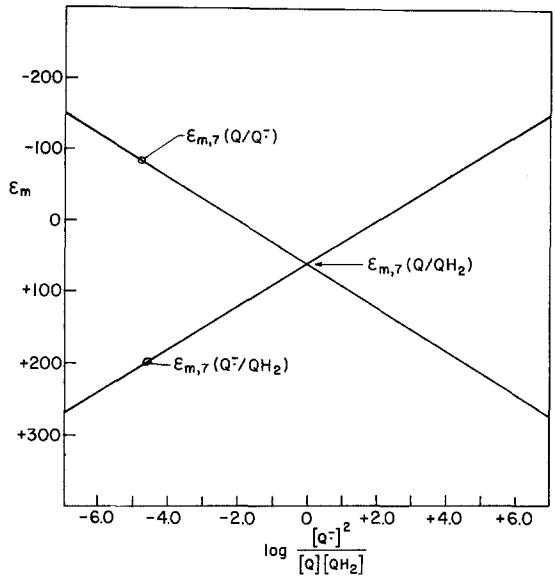

Fig. 4. Relationship between the midpoint potentials of the ubisemiquinone anion couples (Q/Q^- and Q^-/QH_2), the ubiquinone–ubiquinol couple (Q/QH_2), and the equilibrium constant for formation of ubisemiquinone. Calculations were made from Eqs. (4) and (5) in the text, assuming $\epsilon_{m,7}(Q/QH_2) = 60$ mV and $pK_a = 6.0$ for ubisemiquinone.

$[Q^-]$ is related to the apparent potential (ϵ_h) and to the difference between the midpoint potentials of the ubisemiquinone couples $[\epsilon_m\,(Q/Q^-) - \epsilon_m\,(Q^-/QH_2)]$ by equation (6),

$$[Q^-] = \frac{10^{[\epsilon_h - \epsilon_2]/60}}{1 + 10^{[\epsilon_h - \epsilon_2]/60} + 10^{[2\epsilon_h - \epsilon_1 - \epsilon_2]/60}} \tag{6}$$

where $\epsilon_m(Q/Q^-) = \epsilon_1$ and $\epsilon_m(Q^-/QH_2) = \epsilon_2$.

Thus, as illustrated in Fig. 5, if the extent of ubisemiquinone stabilization ($\epsilon_1 - \epsilon_2$) remains constant, $[Q^-]$ varies as a Gaussian function of potential and at a given potential $[Q^-]$ declines exponentially as ϵ_1 (Q/Q^-) and ϵ_2 (Q^-/QH_2) become simultaneously more negative and positive, respectively. It should be appreciated, however, that if the electron transfer complexes contain ligands which stabilize ubisemiquinone, these interactions may vary in response to the membrane potential or pH gradient, and it is noted that the b–c_1 complex undergoes a pronounced structural change which is dependent on the reduction status of the complex (Rieske, 1976).

Although the crossover of redox poise between the b and c cytochromes

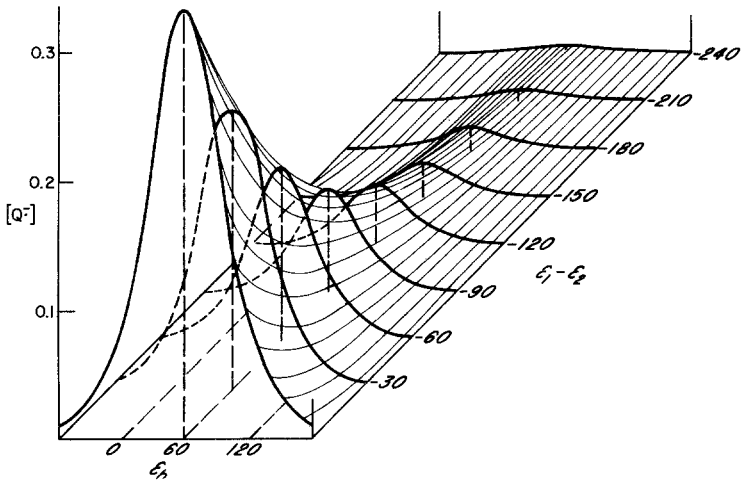

Fig. 5. Relationship between the concentration of ubisemiquinone [$Q^{\cdot-}$], apparent potential (ϵ_h), and increment between the midpoint potentials of the ubisemiquinone couples [$\epsilon_1 - \epsilon_2 = \epsilon_m\,(Q/Q^{\cdot-}) - \epsilon_m\,(Q^{\cdot-}/QH_2)$]. Calculations were made from Eq. (6) in the text, assuming $\epsilon_m\,(Q/QH_2) = 60$ mV and incorporating a constant pH into the midpoint potentials of the semiquinone couples.

which results when antimycin is added to mitochondria in the aerobic steady state (Chance and Williams, 1956) is equally well accounted for by linear or Q cycle mechanisms, other observations are more satisfactorily explained by the Q cycle. Wikstrom and Berden (1972) made the important finding that when the *b* cytochromes were titrated by the fumarate/succinate couple in the presence of antimycin, but not in its absence, two electrons were required for each equivalent of *b* reduced. They proposed that in the presence of antimycin reduction of *b* was obligatorily linked to reduction of c_1 via successive electron transfer reactions involving the two ubisemiquinone couples. Thus the enigmatic oxidant-induced reduction of cytochrome *b* (Chance, 1952; Baum *et al.*, 1967; Erecinska *et al.*, 1972; Lee and Slater, 1972; Chance, 1974) is accounted for by a Q cycle configuration in which electron transfer from QH_2 to c_1 via the iron–sulfur protein generates the prerequisite $QH^{\cdot}$ for reduction of *b*. The Q cycle also accounts for the finding that, in the presence of antimycin, reduction of c_1 and a second, previously unidentified, component prevents reduction of both *b*-566 and *b*-562 by succinate (Trumpower and Katki, 1975, 1979; Eisenbach and Gutman, 1975) and likewise that an oxidant-induced reduction can be demonstrated for both *b* cytochromes (Rieske, 1971; Wikstrom and Berden, 1972; Wikstrom, 1973). As suggested by Fig. 3, the unidentified ascorbate-reducible component previously implicated in the controlled reduction of *b* (Trumpower and Katki, 1979) is most likely the iron–sulfur protein of the *b*–c_1 segment.

The site of antimycin inhibition suggested by Mitchell (1975a) is consistent with evidence that antimycin binds proximal to and induces a shift in the spectrum of *b*-562 (Slater, 1973) and that antimycin inhibits electron transfer from reduced cytochrome *b* to ubiquinone (Von Jagow and Bohrer, 1975). This configuration places c_1 on the O_2 side of the antimycin block (Chance and Williams, 1956), and accounts for how antimycin enhances, but is not required for (Erecinska, 1972; Wilson *et al.*, 1972), the oxidant-induced reduction of cytochrome *b*.

The iron–sulfur protein of the b–c_1 segment has recently been purified in reconstitutively active form (Trumpower and Edwards, 1979b), and this iron–sulfur cluster, which is characterized by an EPR resonance absorbance at $g_y = 1.90$, has been shown unequivocally to be required for electron transfer from succinate and ubiquinol to cytochrome *c* (Trumpower *et al.*, 1980). In addition, experiments with this reconstitutively active protein before it was identified as the iron–sulfur protein of the b–c_1 segment (Trumpower and Edwards, 1979a) showed that it is required for reduction of cytochrome c_1 by succinate and, in the presence of antimycin, it is required for reduction of both cytochromes *b* and c_1 (Trumpower, 1976).

It was therefore suggested that the iron–sulfur protein of the b–c_1 segment functions in a Q cycle mechanism as a ubiquinol/cytochrome c_1–ubisemiquinone/cytochrome *b* oxidoreductase as shown in Fig. 3 (Trumpower, 1976). This function is also consistent with previous evidence (Rieske *et al.*, 1964; Lee and Slater, 1974) placing this iron–sulfur cluster on the O_2 side of the antimycin block. If the configuration shown in Fig. 3 is correct, it requires that the iron–sulfur protein of the b–c_1 segment, possibly in conjunction with other protein ligands, must alter the thermodynamic stability and determine the acceptor/donor specificity of ubisemiquinone at center *o*.

At center *o* the $QH_2/QH_0^{\cdot}$ and $QH_0^{\cdot}/Q$ couples must attain appropriate potentials to reduce cytochromes c_1 ($\epsilon_h = 300$ mV) and *b*-566 ($\epsilon_h \simeq -50$ mV), respectively, suggesting that ϵ_1 ($Q/QH_0^{\cdot}$) $-$ ϵ_2 ($QH_0^{\cdot}/QH_2$) is approximately -350 mV, corresponding to a semiquinone stability constant of 1.5×10^{-6}. Comparing this to an estimated stability constant of 10^{-10} for ubisemiquinone in a hydrophobic environment (Mitchell, 1976), it can be seen that the iron–sulfur protein, or some closely associated polypeptide, must stabilize ubisemiquinone by four orders of magnitude. Although the operating potentials at center *o* are subject to some uncertainty due to the pH dependence of ϵ_m (*b*-566), a smaller increment in potentials would require even greater stabilization of $QH_0^{\cdot}$.

The reactions of $Q/QH^{\cdot}$ and $QH^{\cdot}/QH_2$ at center *o* must be coupled so that the exergonic transfer of an electron to cytochrome c_1 provides the energy for reduction of the low potential *b*-566. This redox "see-saw" relationship (Mitchell, 1976) is implicit in Fig. 4. Mechanistically, this

thermodynamic coupling requires that the reactivities of $Q/QH_0^{\cdot}$ and $QH_0^{\cdot}/QH_2$ must be specifically segregated and dismutations of the common $QH^{\cdot}$ intermediate must be prevented. The function proposed for the iron–sulfur protein (Fig. 3) would therefore require that it be closely associated with both cytochrome c_1 and *b*-566 to form a structurally compact unit. In this regard it may be significant that during purification of the iron–sulfur protein it remained tightly associated with c_1 and could only be dissociated therefrom when the c_1 was denatured by reaction with a mercurial resin (Trumpower and Edwards, 1979b). It is interesting to note that perturbations to the iron–sulfur protein might result in a novel form of "uncoupling," in which dismutation of $QH_0^{\cdot}$ would transfer both electrons from QH_2 to c_1. The resulting electron transfer reaction would manifest a stoichiometry of $H^+/2e^- = 2$ and would be antimycin insensitive.

At center *i* the operating potentials of the dehydrogenase and *b*-562 are sufficiently close (Wilson and Erecinska, 1975) that ϵ_h $(Q/QH_i^{\cdot}) \simeq \epsilon_h$ $(QH_i^{\cdot}/QH_2)$. The stability constant of $QH_i^{\cdot}$ must therefore be close to one, and the equilibrium concentration of $QH_i^{\cdot}$ would be expected to be approximately two orders of magnitude greater than that of $QH_0^{\cdot}$ (see Fig. 5). As mentioned above, the steady-state formulation of the Q cycle shown in Fig. 3 is an oversimplification of the redox reactions at center *i*, in that reduction of the two semiquinone couples is divided between *b*-562 and an iron–sulfur cluster of the dehydrogenase. In order to account for the antimycin-sensitive ubiquinol–cytochrome *c* reductase activity of complex III, center *i* must be capable of reducing Q to $QH^{\cdot}$ and $QH^{\cdot}$ to QH_2 without involvement of the dehydrogenase. In similar fashion, to account for succinate–ubiquinone reductase activity of complex II, the dehydrogenase in these preparations must be capable of reducing both ubisemiquinone couples by an antimycin-insensitive reaction and apparently without involvement of cytochrome *b* (Baginsky and Hatefi, 1969).

These considerations require that both the dehydrogenase and *b*-562 must function as self-contained dismutases (Mitchell, 1976). Comparison of the mechanistic and thermodynamic properties of the semiquinone reactions at center *i* with those of center *o* indicates that whereas the less stable $QH_0^{\cdot}$ must be prevented from dismutating, the more stable $QH_i^{\cdot}$ must undergo dismutation or the equivalent thereof. This difference is an interesting contrast to the more familiar reactions of semiquinones in solution, in which dismutation is commonly equated with instability.

There are two general mechanisms by which the formal equivalent of dismutation might occur. The first is that two molecules of ubiquinone might be bound to the redox center so that the donor would twice reduce Q to $QH^{\cdot}$, whereupon the stably bound semiquinones would dismutate to form $Q + QH_2$. This type of mechanism may be relevant to the succinate–ubiquinone

reductase reaction and may account for the relatively stable ubisemiquinone pair which is formed in proximity to iron–sulfur cluster S-3 (Ruzicka *et al.*, 1975; Ingledew *et al.*, 1976).

The second possibility is that only one molecule of ubiquinone is bound at the redox center and the donor is capable of reducing both Q and $QH^{\cdot}$. This type of mechanism has the interesting feature that the polypeptide ligands at the redox donor center must have sufficient flexibility to accommodate the electronically dissimilar Q and $QH^{\cdot}$ and yet must bind the latter in a relatively stable conformation.

An observation which is not accounted for by a simple Q cycle mechanism is that when the *b* and *c* cytochromes are fully reduced in anaerobic mitochondria, an O_2 pulse causes rapid oxidation of cytochrome *b* along with the *c* cytochromes (see Mitchell, 1980). In the mechanism shown in Fig. 3, oxidation of the *c* cytochromes would generate a *reductant* ($QH_0^{\cdot}$), rather than an oxidant, for cytochrome *b*. A similar situation ensues if reduction of cytochrome *b* by the dehydrogenases precedes reduction of cytochrome c_1, as suggested by the kinetics of the photo-induced reduction of the cytochromes in photosynthetic bacteria (Crofts *et al.*, 1975). Oxidation of the fully reduced *b* cytochromes is accounted for by a linear scheme as suggested by Crofts and co-workers (1975), but the oxidant-induced reduction of cytochrome *b* and the controlled reduction of cytochrome *b* are not. To accommodate these apparently disparate observations, Mitchell (1980) has explored hybrid mechanisms in which aspects of the linear scheme are incorporated into the Q cycle. The problem which must be addressed by further experimentation is how does oxidation of cytochrome c_1 bring about *reduction* of cytochrome *b* which is previously partially reduced, and *oxidation* of cytochrome *b* which is previously fully reduced?

Ubisemiquinone and Ubiquinone Binding Proteins

If there are thermodynamically stable forms of ubisemiquinone which participate with acceptor/donor specificity in the respiratory chain, there almost certainly would be respiratory chain proteins which bind the various redox forms of ubiquinone and thereby alter their physical and chemical properties. There is now evidence for at least two thermodynamically stable forms of ubisemiquinone in the succinate–cytochrome *c* reductase segment of the respiratory chain (Ohnishi and Trumpower, 1980), and it would not be surprising if stable forms of ubisemiquinone were found in complex I. Since large amounts, but not necessarily all, of the ubiquinone can be extracted from mitochondria and functionally reincorporated, ubiquinone might be viewed as a dissociable prosthetic group which normally exchanges between a

bulk immobile pool in the phospholipid continuum and apo-Q-proteins in the respiratory chain complexes. In this regard it should be obvious that previously recognized redox components such as the iron–sulfur proteins or the cytochromes may function as ubiquinone binding proteins, either alone or in conjunction with structurally associated polypeptides. Identifying such hypothetical ubiquinone binding proteins and establishing their topographical and functional relationship to other redox components of the respiratory chain complexes is of central importance to understanding the electron transfer and possible protonmotive functions of ubiquinone.

One approach to identifying proteins which participate in ubiquinone function is to obtain specific inhibitors which disrupt these processes. The structures of several naturally occurring and synthetic analogs which appear to inhibit aspects of ubiquinone function are shown in Fig. 6. Rotenone and piericidin A inhibit NADH–ubiquinone reductase activity of complex I. Both appear to act on the O_2 side of the iron–sulfur centers, and on the basis of binding studies it has been suggested that they bind to identical sites (for a review see Singer and Gutman, 1971). Piericidin A is one of the most potent inhibitors of respiration, and there appear to be two binding sites with equally high affinity for piericidin A, but which contribute unequally to its inhibitory action (Gutman and Singer, 1970). In contrast to piericidin A, there is no obvious structural similarity between the less potent rotenone and ubiquinone. However, rotenone does resemble ubichromenol in certain respects (Fig. 6), lending credence to the possibility that a chromenol conformation may be involved in ubiquinone function in complex I.

Antimycin, which is perhaps the most extensively studied inhibitor of respiration (for a review, see Slater, 1973), blocks electron transfer through the b–c_1 segment and appears to bind proximal to cytochrome b. Evidence that the rate of antimycin binding is increased in ubiquinone-depleted mitochondria and is decreased when ubiquinone is reincorporated (Nelson *et al.*, 1972), and that antimycin differentially eliminates and enhances the EPR signals of two ubisemiquinones in isolated succinate–cytochrome c reductase complex (Ohnishi and Trumpower, 1980), lends support to suggestions that this inhibitor blocks electron transfer between b-562 and the $Q/QH^{\cdot}$ couple (Mitchell, 1975a), possibly by lowering the stabilization of ubisemiquinone by b-562 (Trumpower and Katki, 1979) or a closely associated peptide (Das Gupta and Rieske, 1973).

HOQNO, which is more closely related to the structure of ubiquinone than is antimycin (Fig. 6), causes a crossover between the b and c cytochromes during steady-state respiration and inhibits energy-linked functions in much the same way as does antimycin (Brandon *et al.*, 1972). There is one specific binding site in the b–c_1 complex to which HOQNO binds and from which it is displaced by antimycin (Van Ark and Berden, 1977).

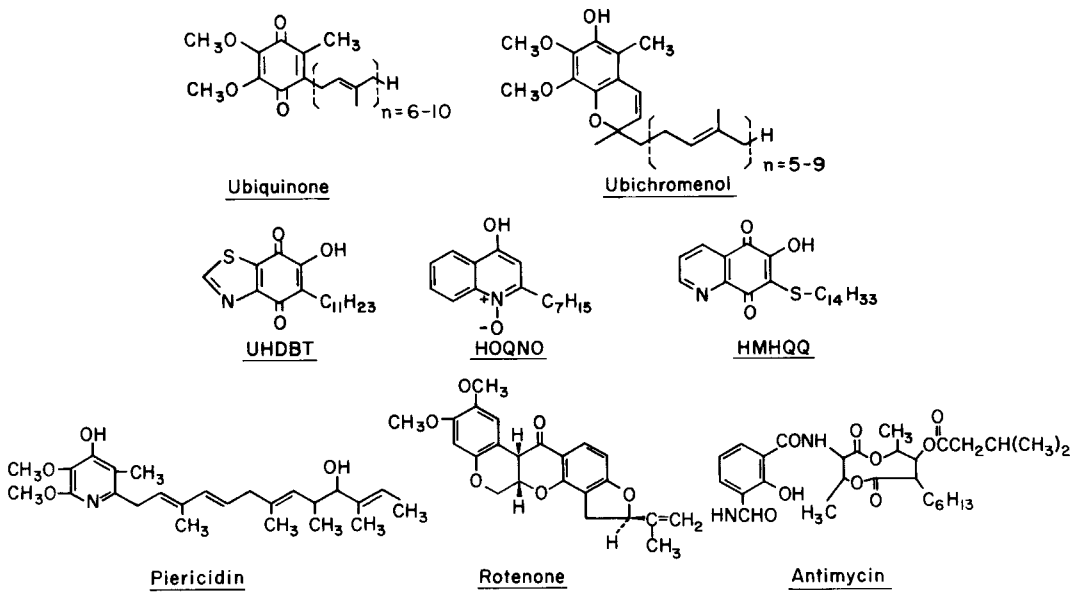

Fig. 6. Structures of ubiquinone, ubichromenol, and mitochondrial respiratory chain inhibitors which appear to disrupt some aspect of ubiquinone function.

Although these results might suggest that antimycin and HOQNO bind at the same site, HOQNO inhibits the oxidant-induced reduction of cytochrome *b* (Eisenbach and Gutman, 1975; Izzo *et al.*, 1978), which is enhanced by antimycin, and it therefore seems likely that these inhibitors act at different sites. These results are consistent with the site of HOQNO inhibition being near to the iron–sulfur protein at center *o* in a Q cycle mechanism. The diminished binding affinity of HOQNO caused by antimycin (Van Ark and Berden, 1977) may reflect the structural dislocation of the iron–sulfur protein in the b–c_1 complex which results from antimycin binding (Rieske, 1976; Trumpower and Edwards, 1979b).

Folkers and co-workers have synthesized more than 30 benzoquinone and naphthoquinone derivatives and tested their efficacy of inhibition of respiration in yeast mitochondria (Roberts *et al.*, 1978). A hydroxy benzoxythiazole analog of ubiquinone, UHDBT, and a hydroxy quinolinequinone, HMHQQ, inhibited both succinate and NADH-linked oxidase activities, and preliminary analysis of their effects on the redox poise of the cytochromes indicated a site of inhibition in the b–c_1 segment. In photosynthetic bacteria, UHDBT inhibits rereduction of photooxidized cytochrome c_2 (Bowyer and Crofts, 1978) and simultaneously prevents oxidation of the Rieske iron–sulfur cluster (Bowyer *et al.*, 1980).

In more recent experiments, UHDBT has been found to be an extremely potent ($K_i < 1 \times 10^{-9}$ M) and specific inhibitor of electron transfer in the b–c_1 segment of mammalian mitochondria (Trumpower and Haggerty, 1980). UHDBT inhibits the oxidant-induced reduction of cytochrome *b* (Bowyer and Trumpower, 1980) and reduction of cytochrome c_1 by succinate in isolated succinate–cytochrome *c* reductase complex (Bowyer and Trumpower, submitted for publication). These effects of UHDBT can be explained by the mechanism in Fig. 3 if the inhibitor binds to the iron–sulfur protein at a site otherwise occupied by ubiquinol or ubisemiquinone and, as a consequence, prevents electron transfer from the iron–sulfur cluster to cytochrome c_1. Whether UHDBT prevents reduction of the iron–sulfur cluster by succinate or ubiquinol remains to be tested.

Yu and co-workers (1977a, b) have isolated and partially purified a protein tentatively identified as a ubiquinone-binding protein. This apo-Q-protein can be recombined with succinate dehydrogenase to form a complex which has TTFA-sensitive succinate–ubiquinone reductase activity (Yu *et al.*, 1977b). The importance of this finding is that purified succinate dehydrogenase, which is otherwise reconstitutively active, is not capable of catalyzing reduction of ubiquinone by succinate (Baginsky and Hatefi, 1969). The apo-Q-protein, which appears to be identical to one of the two low-molecular-weight peptides associated with succinate dehydrogenase in complex II (Capaldi *et al.*, 1977) and which contains 20-50% phospholipid by

weight (Yu *et al.*, 1977b; Yu and Yu, 1979), must stabilize ubisemiquinone sufficiently to bring the Q/QH$^{\cdot}$ couple within range of the fumarate/succinate couple (see Fig. 4) or must confer this stabilizing property on the dehydrogenase.

It is not yet reported whether the succinate dehydrogenase associated protein(s) will stabilize potentiometrically generated ubisemiquinone in the absence of succinate dehydrogenase. However, when this protein and succinate dehydrogenase were added in catalytic amounts to purified b–c_1 complex, a stable ubisemiquinone radical (K_s = 0.25–0.75) was formed when succinate was added as substrate (Yu *et al.*, 1978). The ubisemiquinone radical was abolished by addition of antimycin or TTFA. Since the amount of semiquinone radical formed in these experiments was far in excess of the amounts of apo-Q-protein and dehydrogenase added, these results are evidence for ubisemiquinone formation at a site in the b–c_1 complex by a catalytic process which is blocked by the inhibitors.

Formation of ubisemiquinone by respiring mitochondria was first demonstrated 10 years ago (Backstrom *et al.*, 1970). However, quantitative measurements and thermodynamic analysis of ubisemiquinones in the mitochondrial respiratory chain have only recently been undertaken. Ruzicka *et al.* (1975) discovered an EPR signal at <20°K which was attributable to ubisemiquinone interacting with a second paramagnetic component. The interaction between ubisemiquinone and the second component appeared to be of a magnetic dipole–dipole type, and on this basis it was calculated that the distance between the interacting paramagnetic centers was ≤7.7Å. Ohnishi and co-workers (Ohnishi *et al.*, 1977; Salerno *et al.*, 1977) identified the second paramagnetic component as another ubisemiquinone and showed that the semiquinone pair is oriented perpendicular to the plane of the mitochondrial membrane.

The EPR signal of the spin-coupled ubisemiquinone pair observable at low temperature and the signal of iron–sulfur cluster S-3 of succinate dehydrogenase exhibit identical power saturation behavior, as if this iron–sulfur cluster magnetically relaxes the ubisemiquinone pair at low temperatures (Ingledew *et al.*, 1976). This would account for the rapid relaxation of the g = 2.00 EPR signal from this ubisemiquinone population which is measured at higher temperatures (Konstantinov and Ruuge, 1977).

Two populations of thermodynamically stable ubisemiquinone have recently been demonstrated in isolated succinate–cytochrome c reductase complex (Ohnishi and Trumpower, 1980). The two species of ubisemiquinone can be differentiated on the basis of their power saturation behavior and their response to antimycin, as shown in Fig. 7. At high microwave power (100 mW) the g = 2.00 EPR signal is attributable to the stable ubisemiquinone pair which is proximal to iron–sulfur cluster S-3. The spin intensity of the

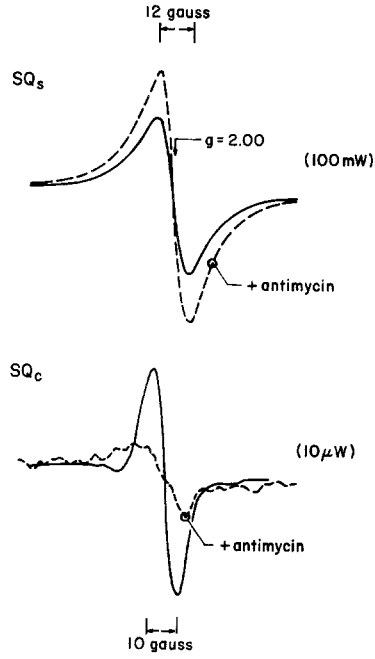

Fig. 7. EPR spectra of the $g = 2.00$ signals arising from stable forms of ubisemiquinone in isolated succinate–cytochrome *c* reductase complex. The upper spectra are attributed to a stable ubisemiquinone (SQ_s) located in proximity to iron–sulfur cluster S-3 of succinate dehydrogenase, which was selectively monitored at high microwave power. The lower spectra are attributed to a stable ubisemiquinone (SQ_c) located in the b–c_1 segment, which was monitored at low microwave power. The dashed lines indicate the spectra in the presence of antimycin. Reproduced with minor modification from Ohnishi and Trumpower, 1980 with permission.

EPR signal from this species, referred to as SQ_s, is enhanced approximately 50% upon addition of antimycin. The EPR signal from the stable ubisemiquinone pair observed at 12°K also increased after addition of antimycin (Ohnishi and Trumpower, 1980). These results suggest that antimycin stabilizes the ubisemiquinone associated with iron–sulfur cluster S-3, which is consistent with previous suggestions that antimycin increases the steady-state concentration of this ubisemiquinone population and thereby decreases the efficacy of inhibition by TTFA (Trumpower, 1978; Trumpower and Simmons, 1979).

The second stable species of ubisemiquinone, referred to as SQ_c, is selectively monitored at low microwave power (10 μW). The $g = 2.00$ EPR signal from SQ_c is almost completely abolished by antimycin (Fig. 7), indicating that the inhibitor destabilizes this ubisemiquinone either by binding directly to a protein involved in semiquinone stabilization or by an indirect effect on ubisemiquinone stability due to a structural rearrangement in the b–c_1 complex (Rieske, 1976). The pH dependence of SQ_c indicates this semiquinone has a $pK_a = 6.5$ in the isolated reductase complex, and estimates of the stability constant gave values between 4.9×10^{-2} and 1.7×10^{-3}, corresponding to $\epsilon_m\ (Q/Q^{\cdot}) - \epsilon_m\ (Q^{\cdot}/QH_2) = -78$ to -166 mV. The existence of more than one form of stable ubisemiquinone in an isolated segment of the respiratory chain is consistent with previous findings that the

power-saturation curve of the ubisemiquinone signal in submitochondrial particles is biphasic (Konstantinov and Ruuge, 1977) and is strongly supportive of the view that bound ubisemiquinones participate as redox components in the mitochondrial respiratory chain.

Acknowledgments

I wish to thank Dr. John Bowyer for providing the derivation and computer program for the calculations used in Fig. 5 and for stimulating discussions during the preparation of this review. I am also grateful to Dr. Donald Schneider for his helpful criticisms and for pointing out the hypochromic effect which would result from clustering of ubiquinone in liposomes. Research from this laboratory is supported by an NIH Research Grant (GM 20379) and by an Established Investigatorship of the American Heart Association.

References

Alexandre, A., and Lehninger, A. L. (1979). *J. Biol. Chem.* **254,** 11555–11560.
Backstrom, D., Norling, B., Ehrenberg, A., and Ernster, L. (1970). *Biochim. Biophys. Acta* **197,** 108–111.
Baginsky, M. L., and Hatefi, Y. (1969). *J. Biol. Chem.* **244,** 5313–5319.
Baum, H., Rieske, J. S., Silman, H. I., and Lipton, S. H. (1967). *Proc. Natl. Acad. Sci. U.S.A.* **57,** 798–805.
Berger, S., and Rieker, A. (1974). In *The Chemistry of the Quinoid Compounds* (Patai, S., ed.), Vol. 1, Wiley, London, pp. 163–229.
Bernstein, J., Cohen, M. D., and Leiserowitz, L. (1974). In *The Chemistry of the Quinoid Compounds* (Patai, S., ed.), Vol. 1, Wiley, London, pp. 37–110.
Bowyer, J. R., and Crofts, A. R. (1978). In *Frontiers of Biological Energetics* (Dutton, P. L., Leigh, J., and Scarpa, A., eds.), Vol. 1, Academic Press, New York, pp. 326–333.
Bowyer, J. R., Dutton, P. L., Prince, R., and Crofts, A. R. (1980). *Biochim. Biophys. Acta,* **592,** 445–460.
Bowyer, J. R., and Trumpower, B. L. (1980). *FEBS Lett.*, **115,** 171–174.
Brandon, J. R., Brocklehurst, J. R., and Lee, C. P. (1972). *Biochemistry* **11,** 1150–1154.
Breen, D. L. (1975). *J. Theor. Biol.* **53,** 101–113.
Bridge, N. K., and Porter, G. (1958). *Proc. Royal Soc. Ser. A* **244,** 276–288.
Capaldi, R. A., Sweetland, J., and Merli, A. (1977). *Biochemistry* **16,** 5707–5710.
Chance, B. (1952). Abstr. 2nd Int. Congr. Biochem. (Paris), p. 32.
Chance, B. (1972). In *Biochemistry and Biophysics of Mitochondrial Membranes* (Azzone, G. F., Carafoli, E., Lehninger, A. L., Quagliariello, E., and Siliprandi, N., eds.) Academic Press, New York, pp. 85–99.
Chance, B. (1974). In *Dynamics of Energy-Transducing Membranes* (Ernster, L., Estabrook, R. W., and Slater, E. C., eds.), Elsevier, Amsterdam, pp. 553–578.
Chance, B., and Williams, G. R. (1956). *Adv. Enzymol.* **17,** 65–134.
Crane, F. L. (1956). In *Biochemistry of Quinones* (Morton, R. A., ed.), Academic Press, New York, pp. 183–206.
Crane, F. L. (1977). *Annu. Rev. Biochem.* **46,** 439–469.
Crofts, A. R. (1979). In *Light-Induced Charge Separation in Biology and Chemistry* (Gerischer, H., and Katz, J., eds.), Dahlem Konferenzen, Berlin, pp. 389–407.

Crofts, A. R., Crowther, D., and Tierney, G. V. (1975). In *Electron Transfer Chains and Oxidative Phosphorylation* (Quagliariello, E., Papa, S., Palmieri, F., Slater, E. C., and Siliprandi, N., eds.), North Holland, Amsterdam, pp. 233–241.
Das Gupta, U., and Rieske, J. (1973). *Biochem. Biophys. Res. Commun.* **54,** 1247–1254.
De Pierre, J. W., and Ernster, L. (1977). *Annu. Rev. Biochem.* **46,** 201–262.
Eisenbach, M., and Gutman, M. (1975). *Eur. J. Biochem.* **59,** 223–230.
Erecinska, M. (1972). *Fed. Proc. Abstr.* **31,** 415.
Erecinska, M., Chance, B., Wilson, D. F., and Dutton, P. L. (1972). *Proc. Natl. Acad. Sci. U.S.A.* **69,** 50–54.
Fieser, L. F., and Fieser, M. (1961). *Advanced Organic Chemistry,* Rheinhold, New York.
Foster, R., and Foreman, M. I. (1974). In *The Chemistry of the Quinoid Compounds* (Patai, S., ed.), Vol. 1, Wiley, London, pp. 257–333.
Futami, A., Hurt, E., and Hauska, G. (1979). *Biochim. Biophys. Acta* **547,** 583–596.
Green, D. E. (1962). *Comp. Biochem. Physiol.* **4,** 81–122.
Gutman, M., and Singer, T. P. (1970). *J. Biol. Chem.* **245,** 1992–1997.
Hackenbrock, C. R., and Hochli, M. (1977). *FEBS Symp.* **42,** 10–36.
Hatefi, Y. (1966). In *Comprehensive Biochemistry* (Florkin, M., and Stotz, E., eds.), Vol. 14, Elsevier, New York, pp. 199–231.
Hatefi, Y. (1976). In *The Enzymes of Biological Membranes* (Martonosi, A., ed.), Vol. 4, Plenum, New York, pp. 3–41.
Hauska, G. (1977a). *FEBS Lett.* **79,** 345–347.
Hauska, G. (1977b). In *Bioenergetics of Membranes* (Packer, L., Papageorgiou, G. C., and Trebst, A., eds.), Elsevier, New York, pp. 177–187.
Heron, C., Ragan, C. I., and Trumpower, B. L. (1978). *Biochem. J.* **174,** 791–800.
Ingledew, W. J., Salerno, J. C., and Ohnishi, T. (1976). *Arch. Biochem. Biophys.* **177,** 176–184.
Izzo, G., Guerrieri, F., and Papa, S. (1978). *FEBS Lett.* **93,** 320–322.
King, T. E. (1966). *Adv. Enzymol.* **28,** 155–236.
Konstantinov, A. A., and Ruuge, E. K. (1977). *FEBS Lett.* **81,** 137–141.
Kroger, A., and Klingenberg, M. (1973a). *Eur. J. Biochem.* **34,** 358–368.
Kroger, A., and Klingenberg, M. (1973b). *Eur. J. Biochem.* **39,** 313–323.
Langemann, A., and Isler, O. (1965). In *Biochemistry of Quinones* (Morton, R. A., ed.), Academic Press, New York, pp. 89–147.
Lee, I. Y., and Slater, E. C. (1972). *Biochim. Biophys. Acta* **283,** 395–402.
Lee, I. Y., and Slater, E. C. (1974). *Biochim. Biophys. Acta* **347,** 14–21.
Lenaz, G., Pasquali, P., Bertoli, E., Parenti-Castelli, G., and Folkers, K. (1975). *Arch. Biochem. Biophys.* **169,** 217–226.
Lenaz, G., Mascarello, S., Landi, L., Cabrini, L., Pasquali, P., Parenti-Castelli, G., Sechi, A. M., and Bertoli, E. (1977). In *Bioenergetics of Membranes* (Packer, L., Papageorgiou, G. E., and Trebst, A., eds.), Elsevier, New York, pp. 189–198.
Lenaz, G., Landi, L., Cabrini, L., Pasquali, P., Sechi, A. M., and Ozawa, T. (1978). *Biochem. Biophys. Res. Commun.* **85,** 1047–1053.
Mitchell, P. (1966). *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation,* Glynn Research, Ltd., Bodmin, Cornwall, England.
Mitchell, P. (1975a). *FEBS Lett.* **56,** 1–6.
Mitchell, P. (1975b). *FEBS Lett.* **59,** 137–139.
Mitchell, P. (1976). *J. Theor. Biol.* **62,** 327–367.
Mitchell, P. (1979a). *Eur. J. Biochem.* **95,** 1–20.
Mitchell, P. (1979b). *Science* **206,** 1148–1159.
Mitchell, P. (1980). In *Oxidases and Related Redox Systems* (King, T. E., Mason H. S., and Morrison, M., eds.), Vol. 3, Pergamon, London, in press.
Mitchell, P., and Moyle, J. (1967). In *Biochemistry of Mitochondria* (Slater, E. C., Kaniuga, Z., and Wojtczak, L., eds.), Academic Press, London, pp. 53–74.
Morton, R. A. (1965). In *Biochemistry of Quinones* (Morton, R. A., ed.), Academic Press, New York, pp. 1–21.
Nelson, B. D., Norling, B., Persson, B., and Ernster, L. (1972). *Biochim. Biophys. Acta* **267,** 205–210.

Ohnishi, T., and Trumpower, B. L. (1980). *J. Biol. Chem.* **255,** 3278–3284.
Ohnishi, T., Salerno, J. C., Blum, H., Leigh, J. S., and Ingledew, W. J. (1977). In *Bioenergetics of Membranes* (Packer, L., Papageorgiou, G. C., and Trebst, A., eds.), Elsevier-North Holland, Amsterdam, pp. 209–216.
Papa, S., Capuano, F., Markert, M., and Altamura, N. (1980). *FEBS Lett.* **111,** 243–248.
Ragan, C. I., and Heron, C. (1978). *Biochem. J.* **174,** 783–790.
Redfearn, E. R., and Whittaker, P. A. (1962). *Biochim. Biophys. Acta* **56,** 440–444.
Rieske, J. S. (1971). *Arch. Biochem. Biophys.* **145,** 179–193.
Rieske, J. S. (1976). *Biochim. Biophys. Acta* **456,** 195–247.
Rieske, J. S., Hansen, R. E., and Zaugg, W. S. (1964). *J. Biol. Chem.* **239,** 3017–3022.
Roberts, H., Choo, W. M., Smith, S. C., Marzuki, S., Linnane, A. W., Porter, T. H., and Folkers, K. (1978). *Arch. Biochem. Biophys.* **191,** 306–315.
Ruzicka, F. J., Beinert, H., Schlepter, K. L., Dunhasse, W. K., and Sands, R. H. (1975). *Proc. Natl. Acad. Sci. U.S.A.* **72,** 2886–2890.
Salerno, J. C., Harmon, H. J., Blum, H., Leigh, J. C., and Ohnishi, T. (1977). *FEBS Lett.* **82,** 179–182.
Singer, T. P., and Gutman, M. (1971). *Adv. Enzymol.* **34,** 79–153.
Slater, E. C. (1973). *Biochem. Biophys. Acta* **301,** 129–154.
Snell, J. M., and Weissberger, A. (1939). *J. Am. Chem. Soc.* **61,** 450–453.
Spisni, A., Masotti, L., Lenaz, G., Bertoli, E., Pedulli, G. F., and Zannoni, C. (1978). *Arch. Biochem. Biophys.* **190,** 454–458.
Trumpower, B. L. (1976). *Biochem. Biophys. Res. Commun.* **70,** 73–80.
Trumpower, B. L. (1978). In *Frontiers of Biological Energetics* (Dutton, P. L., Leigh, J., and Scarpa, A., eds.), Vol. 2, Academic Press, New York, pp. 965–973.
Trumpower, B. L., and Edwards, C. A. (1979a). *FEBS Lett.* **100,** 13–16.
Trumpower, B. L., and Edwards, C. A. (1979b). *J. Biol. Chem.* **254,** 8697–8706.
Trumpower, B. L., Edwards, C. A., and Ohnishi, T. (1980). *J. Biol. Chem.,* **255,** 7487–7493.
Trumpower, B. L., and Haggerty, J. G. (1980). *J. Bioenerg. Biomembr.,* **12,** 151–164.
Trumpower, B. L., and Katki, A. G. (1975). *Biochem. Biophys. Res. Commun.* **65,** 16–23.
Trumpower, B. L., and Katki, A. G. (1979). In *Membrane Proteins in Energy Transduction* (Capaldi, R. A., ed.), Marcel Dekker, New York, pp. 89–200.
Trumpower, B. L., and Simmons, Z. (1979). *J. Biol. Chem.* **254,** 4608–4616.
Trumpower, B. L., Opliger, C. E., and Olson, R. E. (1974). *Chem. Phys. Lipids* **13,** 123–132.
Van Ark, G., and Berden, J. A. (1977). *Biochim. Biophys. Acta* **459,** 119–137.
Von Jagow, G., and Bohrer, C. (1975). *Biochim. Biophys. Acta* **387,** 409–424.
Wikstrom, M. K. F. (1973). *Biochim. Biophys. Acta* **301,** 155–193.
Wikstrom, M. K. F., and Berden, J. (1972). *Biochim. Biophys. Acta* **283,** 403–420.
Wilson, D. F., and Erecinska, M. (1975). *Arch. Biochem. Biophys.* **167,** 116–128.
Wilson, D. F., Erecinska, M., Leigh, J. S., and Koppelman, M. (1972). *Arch. Biochem. Biophys.* **151,** 112–121.
Wraight, C. A. (1979). *Photochem. Photobiol.* **30,** 767–776.
Yu, C. A., and Yu, L. (1979). International Workshop on Membrane Bioenergetics, Abstr., p. 10.
Yu, C. A., Yu, L., and King, T. E. (1977a). *Biochem. Biophys. Res. Commun.* **78,** 259–265.
Yu, C. A., Yu, L., and King, T. E. (1977b). *Biochem. Biophys. Res. Commun.* **79,** 939–946.
Yu, C. A., Nagaoka, S., Yu, L., and King, T. E. (1978). *Biochem. Biophys. Res. Commun.* **82,** 1070–1078.