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Biochimica et Biophysica Acta 1364 (1998) 245–257

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

A reductant-induced oxidation mechanism for Complex I

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Received 19 November 1997; revised 30 December 1997; accepted 5 January 1998

Abstract

A model for energy conversion in Complex I is proposed that is a conservative expansion of Mitchell's Q-cycle using a simple mechanistic variation of that already established experimentally for Complex III. The model accommodates the following proposals. (1) The large number of flavin and iron–sulfur redox cofactors integral to Complex I form a simple but long electron transfer chain guiding submillisecond electron transfer from substrate NADH in the matrix to the [4Fe–4S] cluster N2 close to the matrix–membrane interface. (2) The reduced N2 cluster injects a single electron into a ubiquinone (Q) drawn from the membrane pool into a nearby Q_{nz} site, generating an unstable transition state semiquinone (SQ). The generation of a SQ species is the primary step in the energy conversion process in Complex I, as in Complex III. In Complex III, the SQ at the Q_o site near the cytosolic side acts as a strong *reductant* to drive electronic charge across the membrane profile via two hemes B to a Q_i site near the matrix side. We propose that in Complex I, the SQ at the Q_{nz} site near the matrix side acts as a strong *oxidant* to pull electronic charge across the membrane profile via a quinone (Q_{ny} site) from a Q_{nx} site near the cytosolic side. The opposing locations of matrix side Q_{nz} and cytosolic side Q_o, together with the opposite action of Q_{nz} as an oxidant rather than a reductant, renders the Complex I and III processes vectorially and energetically complementary. The redox properties of the Q_{nz} and Q_o site occupants can be identical. (3) The intervening Q_{ny} site of Complex I acts as a proton pumping element (akin to the proton pump of Complex IV), rather than the simple electron guiding hemes B of Complex III. Thus the transmembrane action of Complex I doubles to four (or more) the number of protons and charges translocated per NADH oxidized and Q reduced. The Q_{ny} site does not exchange with the pool and may even be covalently bound. (4) The Q_{nx} site on the cytosol side of Complex I is complementary to the Q_i site on the matrix side of Complex III and can have the same redox properties. The Q_{nx} site draws QH_2 from the membrane pool to be oxidized in two single electron steps. Besides explaining earlier observations and making testable predictions, this Complex I model re-establishes a uniformity in the mechanisms of respiratory energy conversion by using engineering principles common to Complexes III and IV: (1) all the primary energy coupling reactions in the different complexes use oxygen chemistry in the guise of dioxygen or ubiquinone, (2) these reactions are highly localized structurally, utilizing closely placed catalytic redox cofactors, (3) these reactions are also highly localized energetically, since virtually all the free energy defined by substrates is conserved in the form of transition state that initiates the transmembrane action and (4) all complexes possess apparently supernumerary oxidation–reduction cofactors which form classical electron transfer chains

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PII S0005-2728(98)00031-0

that operate with high directional specificity to guide electron at near zero free energies to and from the sites of localized coupling.

Keywords: Respiration; NADH-ubiquinone oxidoreductase (Complex I); Electron-transfer; Energy-coupling; Ubiquinone; Iron–sulfur cluster

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1. Introduction: many redox centers but few H carriers

The delocalized chemiosmotic model of Mitchell [1] predicted the translocation of six protons and six charges as two electrons were guided from NADH to O_2 via a series of three functionally uniform ‘loops’ that were alternating neutral hydrogen (H) translocations (effected by membrane diffusion of hydrogen carriers) and electrogenic electron (e^-) transfers (directed between a series of fixed cofactors across the membrane). In the model shown in Fig. 1A, each loop arm smoothly and continuously converted respiratory redox energy into a delocalized transmembrane pH gradient and electric potential. Although by the 1970s, the delocalized transmembrane product became well-established and more than enough redox centers were discovered to equip the electron transferring arms of the loops, chemical candidates clearly identified with the hydrogen-carrying arms remained problematical. Ubiquinone (Q) as a natural membrane diffusing 2H carrier remained the only one, acting in the second loop, until in 1975, Mitchell [2], prompted by an allied idea of Wikstrom and Berden [3] presented his Q-cycle model. This showed how the third loop hydrogen carrier could also be ubiquinone, a proposal that has been well-supported experimentally. However, the identity of the 2H and the electron-carrying arms of the first loop remains a mystery. Despite a steady flow of hypothetical schemes for the Complex I [4–7], none have the elegance of the Q-cycle scheme nor have any garnered support.

The sheer enormity of Complex I with its many elaborations of subunits and cofactors has inhibited the testing and maturing of working models (Ohnishi, this issue, pp. 186–206). The escalation in the number of protons reported translocated by Complex I [8–13] coincides with the realization that the flavin and all but one of the seven or eight iron–sulfur clusters are not positioned in the membrane profile, but rather in a long structure that extends well into the aqueous phase of the matrix [14–17]. This simplifies matters considerably when formulating possible models of energy conversion. Flavin and the majority of the iron–sulfur clusters must be removed from the membrane and the associated proton pumping machinery, eliminating the majority of speculative working models [14,18–22] which have recently been discussed in some detail [7]. Surprisingly few candidate players remain for the proton pumping mechanism of Complex I.

Here, we outline a testable hypothetical Complex I model that naturally integrates and extends Mitchell’s now well-established Q-cycle concept. In the original Mitchell formulation (Fig. 1A), the membrane ubiquinone/hydroquinone pool serves as a 2H carrier in the second loop between Complex I (or II) and Complex III. With the Q-cycle formulation, Mitchell introduced a 2H carrier in a cycle around Complex III itself. Here, we suggest a very similar cycle operates around Complex I as well (Fig. 1B). Thus we propose that the membrane pool Q/QH_2 can be the 2H carrier for all three loops of the original Mitchell formulation. Mechanistically, the Complex III Q-cycle is based on the ‘oxidant-induced reduction’ reaction [23]. This overall effects the translocation of two proton charges for every QH_2 oxidized and two ferricytochromes *c* reduced. We suggest that a simple variation of this mechanism can accommodate the position of the Complex I substrate NADH on the matrix side of the membrane and the fact that Q is a substrate oxidant rather than a reductant. The main mechanistic variation compared to Complex III is that the heart of the Complex I energy conversion involves a ‘reductant-induced oxidation’, providing an attractive way of translocating two proton charges from matrix to cytosol per NADH oxidized (Fig. 1C). However, Complex I is driven by a substrate free energy more than double that of Complex III and translocates more than $2H^+$ charges. Thus, we propose that this additional free energy is used to couple the reductant-induced oxidation reaction to an active H^+ pump (Fig. 1D).

2. The electron transfer chain in Complex I

The multiplicity of iron–sulfur clusters [24–26] and the flavin associated with Complex I appear to be located exclusively in the long, ~ 80 Å [27] promon-

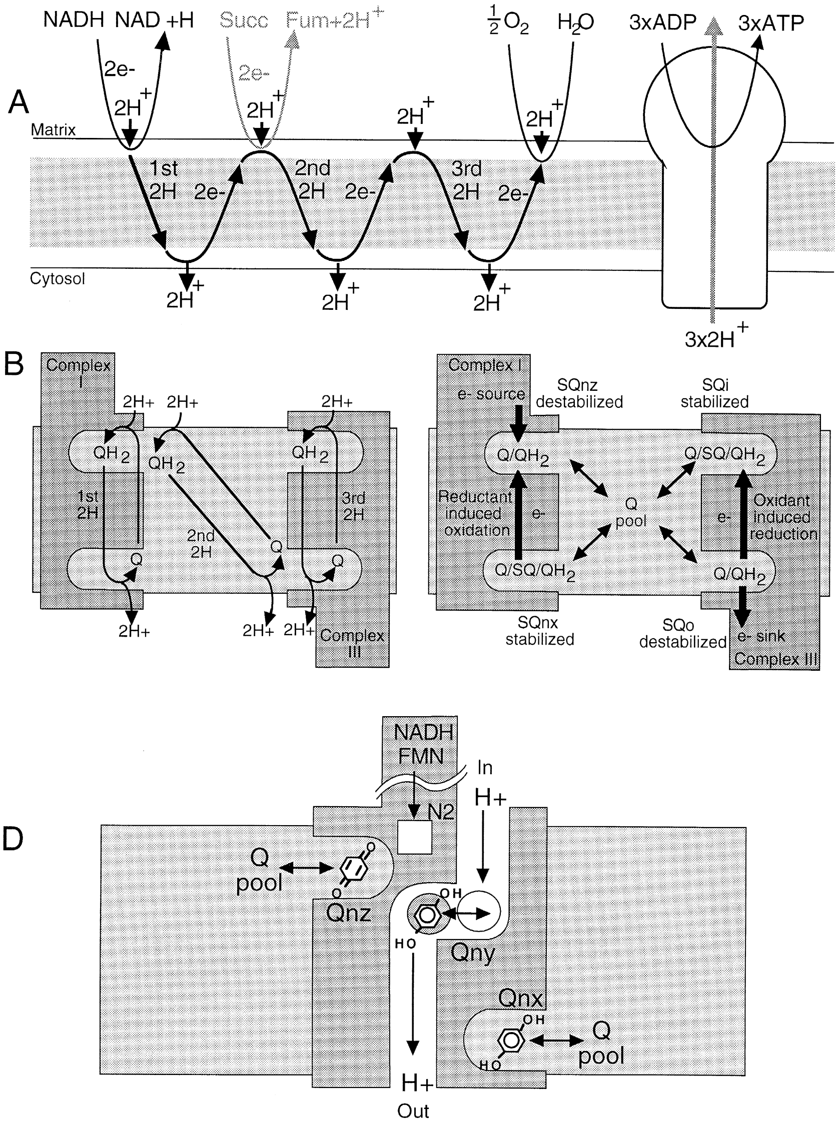

Fig. 1. A: The original formulation of Mitchell's chemiosmotic hypothesis. B: The proposed complementary quinone reactions of Complexes I and III. Both complexes engage in two turnovers of the quinone sites per reaction cycle. The net generation of QH_2 by Complex I complements the net consumption of QH_2 by Complex III. The 2H-carrying arms of the original Mitchell chemiosmotic hypothesis are more obvious from this perspective. C: Another view of the proposed symmetry of the complexes. Both complexes have an exchangeable quinone binding site in which the semiquinone species is unstable (Q_{nz} and Q_o) and an exchangeable quinone binding site in which the semiquinone species is relatively stable (Q_{nx} and Q_i). The proposed reductant-induced oxidation at the Q_{nz} site results in proton binding from the matrix, while oxidant-induced reduction at the Q_o site results in proton release to the cytosol. Diffusable quinone pool connects all these sites. D: The symmetry of complexes I and III are broken by the operation of Q_{ny} in Complex I, which serves to couple electron and proton transfer to transform the greater driving force of Complex I into additional proton pumping.

tory extending into the mitochondrial matrix or microbial cytoplasm [4–7,16,28,29]. These could easily represent the links of an efficient electron transfer chain from NADH to the membrane and a terminal acceptor such as the [4Fe–4S] cluster N2. The flavin is likely to be reduced by the NADH by atom transfer (H^-, i.e., two electron reduction) and then, because of a relatively stable single electron reduced state of the flavin [30], delivers single electrons into the chain. The site of NADH oxidation in the promontory has not yet been located, but if NADH is oxidized at the extreme distant point, an extended chain of the flavin and five to seven iron–sulfur clusters would be separated edge-to-edge by 8–10 Å. This distance is consistent with the ∼10 Å distance estimated from paramagnetic spin–spin interaction between semiflavin and the nearest Fe of cluster N3 [24,30]. Based on principles of intraprotein electron tunneling, [31,32] even with the modest overall free energy drop of 0.07 eV along the chain and assuming typical reorganization energies, the entire 80-Å distance of the promontory can be readily traversed in either direction in microseconds, [33] consistent with experiment [34,35]. Although the low E_{m7} values of cluster N1a has often been the basis of excluding this cluster, we note that thermodynamically unfavorable electron transfer in the middle of an electron transfer chain may be easily overcome by thermal activation, provided that the chain components are sufficiently close that electron tunneling is rapid and not rate limiting. Unfavorable steps are common in analogous redox chains. For instance, in the hydrogenase structure [36], the middle of the chain of three iron–sulfur clusters has a [3Fe–4S] cluster some 0.20 V higher than its flanking [4Fe–4S] cluster partners; yet calculations show submicrosecond electron transfer from one end of the chain to the other [37].

3. Electron transfer chains lead to localized energy coupling sites

In the original Mitchell formulation of the chemiosmotic hypothesis (Fig. 1A), each step of electron and H transfer was given an exothermic free energy which could be more or less evenly harnessed to the overall creation of a transmembrane proton motive force. In contrast, the picture we describe is a series of electron transfers along chains of cofactors with little net expenditure of free energy, returning to the ‘isopotential’ groups of Wilson et al. [38]. These serve to bring electrons and protons to localized coupling sites that exploit oxygen chemistry, either in the form of diatomic oxygen or quinone. At these sites, nearly all the free energy is consumed by coupling of electron and proton transfer events involving reactive intermediates.

The recognition of ‘isopotential’ redox groups as simple redox chains, plus the idea that reactive intermediates (chemical or local proton mediated [39]) were generated at local sites, were two mainstays of proposed local chemical or protonic coupling models of oxidative- and photo-phosphorylation 30 years ago. While the central tenet of Mitchell’s hypothesis that the first useable product of light and oxygen driven electron transfer is a delocalized electrochemical gradient of protons has been preserved over this time, the actual mechanistic picture has moved away from the continuous coupling model of Fig. 1A to that involving local action. Thus in Mitchell’s own ‘Q-cycle’ model of Complex III, the energy coupling mechanism is localized at the Q_o site, and Wikstrom’s description of Complex IV incorporates a highly localized proton pump [40].

At the Q_o site of Complex III, QH_2 and oxidized cytochrome *c* (via cyt c_1 and the [2Fe–2S]) meet twice and each time use half the free energy between these substrates to generate a reductant, a semiquinone (SQ) with the power to drive an electron across the membrane [41,42]. In effect, the Q_o site operates not only as one terminal of a 2H-carrying arm (Fig. 1B), but also as an ‘electron pump’, analogous to the light reactions localized in photosynthetic reaction centers. In Complex IV, local action takes place at the cyt a_3/Cu_B binuclear center, where O_2 binds and meets four electrons delivered from the cytochrome *c* chain (via Cu_A and cyt *a*) bringing the large majority of the substrate free energy to bear on driving protons and electrons across the membrane (see Ref. [43]). We propose a local quinone chemistry coupling site for Complex I that topologically has a pseudosymmetric relationship with the Complex III (Fig. 1C) and mechanistically adds a Complex IV type proton pumping unit (Fig. 1D) to the electron pump established for Complex III. We present our proposal in parallel to that understood for Complex III.

4. The symmetry of Complexes III and I

Complex III is activated by an oxidized cytochrome *c* on the outer, cytosolic side of the membrane, which, via an analogous but short single electron transfer chain of cytochromes c_1, oxidizes the $[2Fe{-}2S]_{Rieske}$ cluster near the same side. The [2Fe–2S] cluster is a relatively strong oxidant ($E_{m7} = 290$ mV) and is the initiator of the primary energy coupling action of Complex III. The [2Fe–2S] cluster considered to be in van der Waals or H-bonded contact with a QH_2 in the Q_o site, abstracts a single electron (with H^+ release) to generate a highly reactive SQ. It has been made clear that this reaction is unfavorable [42,44] (see also Ref. [45]). However, like those commonly identified with the transition state in enzyme catalysis, it is generated thermally and at any instant is in very small concentration; it is followed by an at least equally favorable reaction to achieve stable products.

For Complex I, we propose that a similar reactive SQ is also generated, but on the matrix side and by reduction of a Q, rather than by oxidation of a QH_2 as is the case for Complex III. The most likely reductant that initiates this primary energy coupling action is [4Fe–4S] cluster N2.

Cluster N2 has a long history as a candidate to play an important part of energy conversion in Complex I [46–49]. It differs in several respects from the other clusters of Complex I. (1) The subunit associated with the N2 cluster requires detergent to remove it from the membrane on the matrix side [15,29], and so it is very likely that the N2 subunit occupies a place at the membrane end of the electron transfer chain and that the N2 cluster itself could be in contact with or even slightly within the membrane itself. (2) The equilibrium redox properties of the N2 cluster are the most oxidizing of the group by at least 100 mV (see Table 1) but display a considerable variability that is not understood. (3) The equilibrium oxidation–reduction of the N2 cluster is coupled to proton release-binding over the physiological range [49]. Indeed, impeded kinetics of the interaction of N2 with protons could be partly responsible for the

Table 1
Measured and suggested redox properties of some respiratory chain components

Complex	1 e^- Redox center	E_{m7} (V)	2 e^- Redox center	E_{m7}Q/SQ (V)	E_{m7}SQ/QH_2 (V)	E_{m7} average (V)	log K_{stab}
I	$[2Fe{-}2S]_{N1a}$	−0.38	FMN	−0.389	−0.293	−0.34	−1.6
I	$[2Fe{-}2S]_{N1b}$	−0.25	Q_{nx}	0.09	0.09	0.09	0
I	$[4Fe{-}4S]_{N2}$	−0.15 to −0.05	Q_{ny}		0.19		
I	$[4Fe{-}4S]_{N3}$	−0.25	Q_{nz}	−0.21	0.39	0.09	−10
I	$[4Fe{-}4S]_{N4}$	−0.25					
I	$[4Fe{-}4S]_{N5}$	−0.25					
III	Cyt b_L	−0.09	Q_i	0.03	0.27	0.15	−4
III	Cyt b_H	0.05	Q_{os}	−0.34	0.52	0.09	−14
III	$[2Fe{-}2S]_{Rieske}$	0.29	Q_{ow}	−0.24	0.40	0.08	−11
III	Cyt c_1	0.23					
Diffuser			NAD	−0.92	0.28	−0.32	−20
Diffuser			Q_{pool}	−0.24	0.42	0.09	−11
Diffuser	Cyt *c*	0.28					

These measured and proposed redox midpoint values at pH 7 (E_{m7}) represent a starting point for examining the energetics of the mitochondrial redox complexes. Midpoint values of redox centers vary from species to species. We find that hypothetical redox values for individual couples can vary over a considerable range without impairing overall electron transfer function; thus midpoint values of any species can differ noticeably from these values but still participate in the mechanisms described in this paper. Experimentally-determined E_{m7} for Complex I iron–sulfur clusters [26,49,50] and flavin [30] components reflect bovine heart values, while Complex III values for the transmembrane electron transfer sequence that includes the *b* cytochromes and the ubiquinone couples of the Q_{ow}, Q_{os} and Q_i sites, reflect the values from the photosynthetic bacterium *Rhodobacter capsulatus* which are defined more clearly [41]. NAD, [51] Q_{pool} [42] and cyt *c* are diffusable redox species that may interact with more than one complex. The values for the cyt *c* are from horse heart [52] and for cyt c_1 [53] and the [2Fe–2S] [54,55] are for the bovine heart. The relative stability of the semiquinone states are controlled by the quinone site environment and indicated by log $K_{stab} = \ln[10](E_m SQ/QH_2 - E_m Q/SQ)/RT$; thus increasingly negative values are more unstable.

variation in measured midpoint potential of N2 and for the presence of a slowly reacting resting state. (4) The cluster N2 interacts with two different ubiquinones bound in the membrane domain of Complex I at sites that stabilize the SQ form; the spin-relaxation property of each SQ is different [56–58]. The distance between the fast relaxing SQ, called Q_{Nf}, and N2 was found to be ∼8–11 Å, while the binding site for the slower relaxing SQ, Q_{Ns}, is estimated to be located further away, towards the cytosolic side of membrane [59]. Based on these observations, we propose that the single electron transfer chain reduces the tetranuclear iron–sulfur cluster N2 and that the reduced N2 initiates the primary energy coupling reactions of Complex I.

In our view, initiation occurs by the [4Fe–4S] N2 cluster delivering a single electron to a Q in a site (referred to in this paper as the Q_{nz} site) to generate a highly reactive SQ near the inner, matrix side of the membrane. As with the SQ generated in the Complex III Q_o site, the SQ generated in the Q_{nz} site is a thermally generated unfavorable state. It is quite possible that the SQs, generated in such opposite ways in the Q_o or Q_{nz} sites of Complexes III and I, are intrinsically identical in redox character. In both sites, the SQ can either act as a very strong single electron reductant to get to a more favored Q state, or it can act as a very strong single electron oxidant to get to a similarly more favored QH_2 state. We believe that the essential functional difference in the determination of whether the SQ state of the Q_o or Q_{nz} sites act as an oxidant or a reductant, simply lies in the redox state of the cofactors that interact with them. Thus, if an adjacent redox cofactor is in an oxidized state when a SQ is generated then the cofactor will be reduced by the SQ which itself becomes Q. Conversely, if an adjacent redox cofactor is in a reduced state when a SQ is generated, then it will be oxidized by the SQ which becomes QH_2. The essential functional difference in the determination of the subsequent vectorial movement of protons and charges across the membrane will depend on the positions of the SQ and its neighbors in the membrane profile, and the availability and source of the protons coupled to these highly cooperative oxidation and reduction reactions.

Fig. 1C shows without detail that in Complex III the SQ generated in the Q_o site near the membrane cytosolic side acts as a strong reductant to ‘push’ single electrons to another ubiquinone site (Q_i site) near the matrix side of the membrane, thereby moving a single electronic charge from the cytosolic to the matrix side of the membrane. Fig. 1 also shows how Complex I, despite the differences with Complex III, can likewise move a single electronic charge from the cytosolic to the matrix side of the membrane. In this case, the SQ proposed generated in the Q_{nz} site on the matrix side acts as a strong oxidant to ‘pull’ single electrons from a Q_i-like site near the matrix side (referred to in this paper as the Q_{nx} site).

5. Q_i and Q_{nx} sites as single electron acceptors / donors with relatively stable SQ

It is well-known that the Q_o and the Q_i sites of Complex III differ dramatically in the stability of their SQ states (Table 1). We propose that the same relationship exist between the putative Q_{nz} and the Q_{nx} sites of Complex I. Measurements on the Q_o site ubiquinones have put the log K_{stab} to be < -7 [60], while recent work suggests values of -10 to -14 will be optimal [41]. These very low log K_{stab} values are, of course, the basis of the functional role of the unstable SQ as catalytic initiator in the Q_o site of Complex III [41] and the proposed Q_{nz} site of Complex I. On the other hand the SQ states of Q_i [61–63] and Q_{nx} are expected to be much more stable, display higher stability constants and to be produced even at detectable levels in Complex III or Complex I. Measurements on the Q_i site ubiquinone have put the log K_{stab} between -1 and -4, sufficient for the SQ state to be detected and quantitated by EPR spectroscopy [56,59,64,65]. EPR also provides evidence for relatively stable SQ in Complex I [56–59]. This property is key to its apparently designed role as a facile single electron acceptor (favorably single electron reduced from Q to SQ and from SQ to QH_2 in the Q_i site of Complex III) or donor (favorably oxidized from QH_2 to SQ and from SQ to Q in the Q_{nx} site of Complex I). As we have already suggested for the Q_o and Q_{nz} sites, it is quite possible that the redox properties of the ubiquinone occupants of the Q_i or Q_{nx} sites of Complexes I and III could be intrinsically identical in redox character and in principle interchangeable. The essential difference in function in the Q_i or Q_{nx} sites that determines the vectorial movement of protons and charges across the membrane could be simply a topological one, depending on which side of the membrane provides proton access to the sites, and on whether their neighbors deeper in the membrane profile serve as their oxidants or reductants.

One important parameter that the Q_o, Q_{nz}, Q_i, and Q_{nx} sites may all share along with the membrane ubiquinone pool (Q_{pool}) is the E_{m7} of the QH_2 to Q transition. For the ubiquinone of the Q_{pool} and the Q_o and Q_{nz} sites, this is given as the midpoint of the QH_2/Q ($n=2$) transition. For the Q_i and Q_{nx} sites, it will be the average value of the two QH_2/SQ and SQ/Q ($n=1$) transitions. Table 1 shows that unlike their SQ stabilities which vary up to 10^{12}-fold, the relative affinities of the different sites for the individual QH_2 and Q of the Q_{pool} do not vary greatly. This means that the occupancies of each site by QH_2 or Q approximately mirror the redox state of the Q_{pool}, and that the standard free energy of exchange of QH_2 and Q from the Q_{pool} and the sites is always near zero.

6. The need for two parts in the turnover of Complexes I and III

It was recognized early by Lawford and Garland [19] and detailed by Crofts and Wraight [66] that Mitchell's initial Q-cycle formulation of Complex III had an accounting problem which is taken care of if the system simply turned over twice to complete the expected reduction of two cytochromes *c* (via the [2Fe–2S] cluster) and net oxidation of one QH_2. There is growing evidence for the breakdown of this reaction into two parts, as has been described in detail [41].

The essential energetic action occurs in the oxidation–reductions at the Q_o and the Q_i sites. Fig. 1C emphasizes the view that Complex III oxidizes two QH_2 at the Q_o site, releasing each time $2H^+$ into the cytosol, drives two electronic charges from the Q_o site to Q_i site where Q is reduced to SQ and then to QH_2 and pick up $2H^+$ from the matrix. If we mentally tag and follow the QH_2 released from the Q_i site, it transports 2H moieties across the membrane to be oxidized at the Q_o site; in every double turnover this accounts for two of the $4H^+$ released. The local cycling of Q and QH_2 between the two sites is recognized as the third 2H-carrying arm of the original chemiosmotic hypothesis (Fig. 1A). A more contemporary view of the overall action Complex III is as a pump of two charged H^+ from the matrix to the cytosol per reduction of two cytochrome *c* and *net* oxidation of one QH_2 to Q. We can formally identify the other two protons released by the Q_o site as energetically associated with the 'substrate' QH_2 oxidation at this cytosolic side of the second 2H-carrying arm, still intact from the original hypothesis, emanating from Complex II or Complex I.

We apply the same principles to the Complex I. Thus Complex I: (a) reduces two Q to QH_2 at the Q_{nz} site, each part binding $2H^+$ for a total of $4H^+$ from the matrix; (b) drives two electronic charges from the Q_{nx} site to the Q_{nz} site; and (c) oxidizes one QH_2 first to SQ (first part) and then to Q (second part), releasing $2H^+$ at the Q_{nz} site on the cytosolic side. In this formulation it is clear that Complex I can function in the same way as Complex III. Mitchell's Q-cycle identified the third 2H-carrying arm as ubiquinone, while this Complex I scheme, in proposing Complex I cycling of Q and QH_2 between the Q_{nx} and Q_{nz} sites (2H), allows ubiquinone to be identified as the formal equivalent of the first 2H-carrying arm of the original formulation (Fig. 1A and B). Similarly, in looking at this action together with the two electronic charges translocated, Complex I can be said to function overall as a pump of two charged H^+ from the matrix to the cytosol per oxidation of NADH and net reduction of one Q to QH_2. This will account for two of the $4H^+$ bound at the Q_{nz} site, while the other two can be identified with the second 2H-carrying arm between Complexes I and III of the original formulation that cycles between the Q_{nz} and the Q_o site.

Fig. 1C summarizes a highly conservative and remarkably simple way in which we propose Nature delivers complementary vectorial and energetic transmembrane effects, using analogous properties and mechanistic devices at the two topologically distinct Q/QH_2 catalytic sites of each complex. It is a surprise that in the late seventies Mitchell himself or others since did not apply his then-hypothetical 'Q-cycle' model of Complex III to a related one for Complex I. Perhaps the view was obscured by the large number of cofactors that then were assigned to occupy protein in the membrane profile.

7. Complexes I and III energetics and proton and charge translocation equipment

In Fig. 1C we identify ubiquinone binding sites in Complexes III and I that are the doubly connected terminals for the concerted translocation of electronic charge ($2\ e^-$) and ubiquinone diffusion (2H) that sum to move, or pump, $2H^+$ from the matrix to the cytosol. For Complex III there is strong evidence that the Q_o site accommodates two closely interacting ubiquinone molecules. One of these (Q_{os}) binds strongly and does not exchange with the Q_{pool} during the catalytic cycle; one binds weakly (Q_{ow}) and exchanges rapidly with the Q_{pool} [41]. Observations suggest that the Q_i site may also have a second ubiquinone (H. Weiss, personal communication) but proof for this has yet to be gathered.

In Complex III, ubiquinone diffuses between the sites in concert with electron transfer along an internal chain comprising two, in-series hemes B, called cytochrome b_L and cytochrome b_H. The driving force provided by two electrons passing down the ΔE_m of Complex III (2 electrons times $-0.19\ V = -0.38$ eV) nicely matches that driving force required to move two protons across the prevailing physiological $\Delta\mu_H^+$ (2 times 0.16 to 0.21 $V = 0.3$ to 0.4 eV), in keeping with the overall reversible nature of Complex III.

The ΔG^o of the substrates of Complex III (-0.38 eV) is dwarfed by that of Complex I (-0.82 eV), a value that is in keeping with measurements of translocation of four or more protons and charges [8–13]. Clearly the machinery between the terminal Q_{nx} and Q_{nz} sites must accommodate more than the above-described simple electron transfer chain energetically suited to Complex III. As is shown in Fig. 1D, we believe that there is physically room to equip the transmembrane span between the proposed Q_{nx} and Q_{nz} sites with a localized proton pump. This will not only provide an electron transfer chain across the membrane but will also provide the mechanism to pump additional protons and charges while maintaining the principles outlined in Fig. 1. We propose that the redox element of this localized pump is a third quinone located between the Q_{nx} and Q_{nz} sites, called the Q_{ny} site. In contrast to the key presence of Q_{pool} exchangeable and diffusing ubiquinones of the Q_{nx}, Q_{nz}, Q_{ow} and Q_i sites, it is equally important that the quinone of the Q_{ny} site does not exchange with the pool during catalytic cycle. This is not an unusual situation in electron transfer systems or certain enzymes. We have already mentioned the Q_{os} domain of the Q_o site, the ubiquinone of which is equally strong binding in the Q and QH_2 forms but does not rapidly exchange with the Q_{pool}. The Q_A site ubi- or mena-quinone of the bacterial photosynthetic reaction centers and the Q_A site plastoquinone of the green plant photosystem II reaction centers and the phylloquinone of the photosystem I reaction centers all fulfill this requirement and normally operate only between the Q and the SQ anionic forms. We propose that the Q_{ny} site quinone may also be restricted in its redox states to accommodate single electron exchange and hence a SQ. We consider that the Q_{ny} quinone species is structurally connected to a proton channel. In addition, the Q_{ny} quinone and its attachment to the backbone or side groups of the Q_{ny} site is viewed to operate as a gating component of the proton pump.

Recently Weiss' group searched for an electron and proton transferring component within the membrane arm of Complex I, utilizing ubiquinone-10 depleted complex purified from *Neurospora crassa*. They have detected a novel quinoprotein-like component which is reducible with NADH, and shows an UV absorbance maximum around 305 nm and a minimum around 430 nm, with a redox midpoint potential of higher than -0.10 V. They have postulated this quinonoid compound (called 'X') functioning in the membrane part of Complex I [67]. If the presence of such a species becomes clear, it may correspond to the bound quinone Q_{ny}.

The driving force for this gating mechanism and pump is derived from the two, $\Delta G^o\ -0.30$ eV single electron transfer reaction sequences from the ubiquinones of the Q_{nx} to the Q_{nz} sites. The E_{m7} value of the putative Q_{ny} site ubiquinone couple will be best if between the E_{m7} values of the Q_{nx} and the Q_{nz} sites, adjusted for its actual location in the membrane dielectric profile with respect to the two sites. It will be expected to lie between the E_{m7} of 0.09 V for both the two stable couples QH_2/SQ and SQ/Q

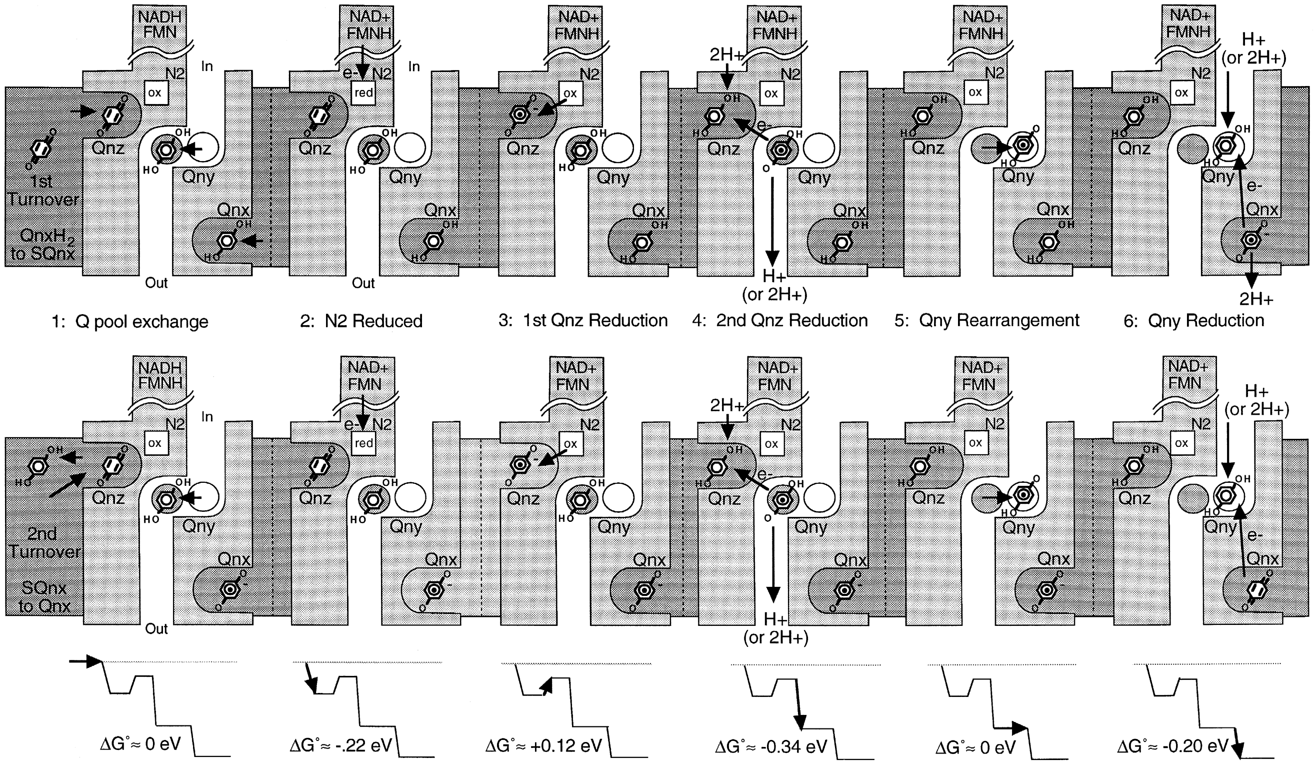

Fig. 2. Hypothetical model for Complex I energy conversion. Complex I is represented as a transmembrane protein with a foreshortened NADH, FMN and iron–sulfur subcomplex which delivers electrons to the [4Fe–4S] cluster N2. There are two quinone binding sites, Q_{nz} and Q_{nx}, that can exchange Q/QH_2 with the membrane pool (Q_{pool}). The Q_{nz} site has access to protons on the matrix side of the membrane and site Q_{nx} has access to protons on the cytosolic side of the membrane. In addition, a non-pool exchangeable quinone Q_{ny} occupies a site that can assume either of two different geometries between the other quinone binding sites. One geometry or conformation (on the right) provides access to the protons on the matrix side of the membrane, presumably through a pore or channel. The other geometry or conformation (on the left) provides access to protons on the cytosolic side of the membrane. The sequence proceeds as follows: 1) NADH arrives at Complex I, Q_{nz} and Q_{nx} sites can exchange with pool. 2) Reduction of N2 occurs by the NADH subcomplex via a chain of FMN and iron–sulfur clusters. 3) N2 reduces a Q drawn from the Q_{pool} into the Q_{nz} site to form an unstable, transition state SQ in an endothermic reaction (approximate relative free energy each step is shown at bottom of figure). 4) The SQ in the Q_{nz} site oxidizes the QH_2 fixed in the Q_{ny} site in a highly exothermic reaction. As SQ of the Q_{nz} site is reduced to QH_2, it binds 2 protons, ultimately drawn from the matrix. As the QH_2 of Q_{ny} site is oxidized to SQ, one (or two) protons are released to the cytosolic channel. 5) While QH_2 in the Q_{ny} site is favored to adopt a geometry with access to cytosolic protons, the newly formed SQ rapidly assumes a geometry with access to protons in the matrix. This need not change the overall free energy substantially. (Steps 3 and 4 represent the reductant-induced-oxidation complementary to the oxidant-induced-reduction reaction in Complex III). 6) In this geometry, the QH_2 in the Q_{nx} site can reduce the SQ in the Q_{ny} site back to the QH_2 and in the process, one or two protons from the channel to matrix are bound. At the same time one or two protons are released from the Q_{nx} site. The figure (top half) shows that as the QH_2 is oxidized in the Q_{nx} site two protons are released into the cytosol leaving a SQ anion (as per the analogous Q_i site in Complex III). This means that no further protons are released from Q_{nx} in the bottom half of the figure; however one proton at each stage could be released. Now reduced, the Q_{ny} site assumes the original geometry, once again with little change in free energy. Q_{nz} exchanges with the pool and the steps 2–6 are repeated. The overall reaction describes two electrons carried by Complex I from the substrates NADH to Q catalyzing the translocation of 4 or 6 proton charges from the mitochondrial matrix to cytosol. Another 2 protons are shown taken up from the matrix for the *net* reduction of substrate Q to QH_2 in the membrane; this initiates the neutral 2H-carrying arm between Complexes I and III. The free energy profile for these reactions is shown at the bottom of the figure. For simplicity we have used energies based on the redox midpoint values; however, under physiological conditions the reduced and oxidized states will not be in equal concentrations and the free energies will vary accordingly. For example, we expect N2 will often be mostly reduced, contributing greater driving force to panels 3–6.

of the Q_{nx} site that serve as alternating electron donors in Parts 1 and 2, respectively, and the 'E_{m7}' of 0.390 V estimated for the (thermally generated) SQ/QH_2 couple of the Q_{nz} site. A reasonable first suggestion for the E_{m7} value of the Q_{ny} site is 0.19 V. Other values can be entertained, but these will impact on the kinetics of the coupling site as they move away from the central value.

Fig. 2 summarizes, step-by-step, the proposed mechanism and energetics of Complex I, using our first suggestions for the E_{m7} values of the quinones of the Q_{nx}, Q_{ny} and Q_{nz} sites.

8. Proton to electron stoichiometries of Q_{ny}

The quinone of the Q_{ny} site could, in principle, take advantage of covalent proton/electron coupling to exchange up to two protons per one electron redox change. However, as the transmembrane electric field builds up, it becomes energetically more difficult to reduce Q_{nz} and oxidize and deprotonate the associated Q_{ny}. From the view point of the 'forward' physiological electron transfers, at high membrane potentials and with enough opposing electric fields, the electrons from NADH would be guided only so far as N2. However, if the Q_{ny} shuttle exchanges a net one proton per one electron redox change, (for example if a local H^+ is borrowed from a nearby residue) the effect of applied field is ameliorated; a greater transmembrane field could build up before this reaction loses its driving force. Eventually, as electrons back up on N2, SQ_{nz} could be trapped upon N2 re-reduction from the low potential NADH–iron–sulfur redox chain. Thus a high transmembrane potential condition could lead to double reduction of Q_{nz} without the action of the Q_{ny} pump or Q_{nx}–Q_{ny} transmembrane electron transfer. This would result in a dramatic loss of efficiency of energy conversion. Indeed, this may occur under some experimental conditions.

Because of the instability important to our hypothesis of Q_{nz}, the experimentally observed SQ states of Q_{Nf} and Q_{Ns} [59] would most likely correspond to Q_{ny} and Q_{nx}, respectively. Rotenone sensitivity of SQ_{Nf} and SQ_{Ns} may correspond to inhibitor occupancy of exchangeable sites Q_{nx} and Q_{nz}; the Q_{Nf} signal would be indirectly rotenone-sensitive since displacement of Q_{nx} by inhibitor leads to the functional inability to form SQ_{ny}. It appears that at least one of the SQ signals is sensitive to the addition of uncouplers that dissipate the transmembrane proton gradient. In our view, the presence of an intact gradient would have relatively little effect on Q_{nx}, while the Q_{ny} directly coupled to the proton channels on either side of the membrane would be the most sensitive.

After the completion of Part 1 and Part 2 and the full catalytic cycle (including the final translocation of 2H from matrix to cytosol from the diffusing Q/QH_2 from the Q_{nx} and Q_{nz} sites), the ΔG^{o} -0.82 eV available from two electrons passing through Complex I will be converted into an equivalent value of $n_p \Delta \mu_{H}^{+}$, where n_p is the number of protons translocated by the complex, consistent (as with Complex III) with the reversible nature of the energy coupled Complex I reactions. If the Q_{ny} site operates between QH_2 and SQH (an exchange of one proton per electron) then there will be four protons and four charges translocated, equivalent to an n_p of $4H^+$. If the Q_{ny} site operates between QH_2 and SQ anion (an exchange of two protons per electron) then there will be, in principle, six protons and six electrons translocated from matrix to cytosol, equivalent to an n_p of $6H^+$. The pK of the Q_{ny} quinone and the local pH at the Q_{ny} site will determine the stoichiometry, which may lead to a transmembrane proton driving force dependent proton to electron stoichiometry. Thus an intermediate stoichiometry around five is also possible. Refer to Table 1 for feasible thermodynamic properties of the three quinones involved in this mechanism.

9. Testable predictions

An obvious prediction of the model of Complex I action presented in this paper is that there should be at least two clear classes of inhibitors [68–70] corresponding to the diffusable quinone binding sites Q_{nx} and Q_{nz}; furthermore, mutational studies [71] that focus on changing sensitivity to the two inhibitor classes will tend to center on residues towards opposite sides of the membrane. This classification of inhibitors would be analogous to the two classes found to act at Q_o and Q_i sites of Complex III, as illustrated by myxothiazol and antimycin. Of course, there are likely to be less specific inhibitors that act at both Complex I sites. There is already some experimental evidence for at least two classes of Complex I inhibitors [68–70].

Also in analogy to Complex III, the proposed mechanism of this paper points to the need for catalytic amounts of oxidized Q and reduced QH_2 in the pool for turnover of Complex I. In principle, a completely oxidized or completely reduced Q_{pool} would introduce a lag in the kinetics of the complex in forward or reverse electron transfer. It should be noted that the requirement for Q or QH_2 at the redox extremes is catalytic and regenerated after each turnover. Thus, one Q or QH_2 could serve many complexes many times. This makes a lag phase difficult to observed experimentally, as with Complex III.

Perhaps most surprisingly, this model predicts that the phenomenon of reductant-induced oxidation should be observable in Complex I. As an antithesis of oxidant-induced reduction in Complex III, a pulse of NADH delivered to Complex I in which Q_{ny} is reduced and oxidized Q pool is available for Q_{nz}, reduction of N2 should lead to oxidation of a Q_{ny} QH_2 to SQ, and if a reduced Q_{pool} is available to Q_{nx}, to oxidation of that QH_2 or SQ. Reductant-induced oxidation should also take place in the presence of selective inhibitors of Q_{nx}; indeed oxidation of reduced Q_{ny} QH_2 to SQ should be more obvious under these conditions.

The postulated redox properties of the functional quinones in this model state that two different SQs should be observable. SQ_{nx} is analogous to SQ_i in Complex III and should be similarly observable in equilibrium redox titrations. The E_m value of the SQ/QH_2 redox couple at the Q_{ny} site should be relatively high, perhaps around 0.19 V, suggesting that this SQ state should be observable in equilibrium redox titrations at potentials well above the Q_{pool} midpoint potential. The oxidizing couple of Q_{ny}/SQ_{ny} would not be observed physiologically, just as the reducing couple SQ_A/QH_2 is observed in the reaction center Q_A site only under extreme conditions. However, during equilibrium redox titrations it may be possible to force the Q_{ny} to become fully oxidized, just as it is possible to force the Q_A site SQ to become fully reduced [72].

The presence of stable SQs should lead to spin interactions that are EPR observable. Thus two different SQs may have different and simultaneous spin interactions with the nearest iron–sulfur cluster N2; the SQ_{ny} should be relatively close, since Q_{nz} is a redox partner of both N2 and Q_{ny}; the SQ_{nx} should be relatively distant, since it participates in transmembrane electron transfer. Observations suggest this to be the case [58,59].

Besides being functionally restricted to a single redox couple, Q_{ny} also acts as a proton pump. Thus its redox properties are expected not only to be pH-sensitive, but also to be sensitive to ΔpH and $\Delta\Psi$, both components of $\Delta\mu_{H}^{+}$. In addition, under conditions of large transmembrane potentials, the effective pH of the Q_{ny} site may approach the pKs of the active QH_2 and SQ states in the Q_{ny} site and lead to a reduction of the H^+/e^- stoichiometry. Alternatively inappropriate electron transfer from QH_2 or SQ in the Q_{nx} site to SQ_{ny} could occur while it is in the proton 'out' (cytosolic side) channel leading to wasteful reabsorption of protons from the outside rather than the inside. Normally this would be prevented by having Q_{ny} rearrangement to the 'in' (matrix side) proton channel upon reduction occur faster than electron transfer from Q_{nx}, which would be about 10 ms for a 12-Å electron transfer. A different source of loss of H^+/e^- may occur during very reducing conditions in which a small amount of transiently reduced SQ_{nz} is reduced again by N2 to form QH_2 at Q_{nz}, because QH_2 at Q_{ny} is unavailable to accept electrons.

Finally, the use of iron–sulfur clusters as elements in a simple electron tunneling chain means that mutagenic changes around their binding sites may be able to modulate the E_m values of the cluster by more than ±0.1 V without destroying Complex I function. In this sense the chain between NADH and cluster N2 is expected to be relatively robust.

10. Comparisons with other Complex I models

The use in our model of reductant-induced oxidation as a mirror image of Complex III and use of a bound SQ/QH_2 proton pump analog of Complex IV are unique. It is these symmetries and analogies that give the model a logical simplicity. This model's ability to deliver to the cytosol four to six protons per oxidation of NADH (depending on the stoichiometry of proton release upon QH_2 oxidation at the Q_{ny} site) is also unusual. In addition, this model suggests that the proton to electron stoichiometry is likely to be dependent upon the transmembrane proton motive force and may be the source of some of the discrepancies in the literature. The stoichiometry is greater than the early models [19,21,56,73,74], and potentially greater than later models [4,7,13,18,20,69,74].

Only quinones are used to manipulate proton motion in our model. Of course, redox active flavins also bind and release protons and can in principle be used as active species in a proton pump. However, with the apparent localization of flavin in the extramembrane domain of Complex I, we are uncomfortable with models that require flavin to play a transmembrane proton moving role [4,13,18–21,74].

The quinone states and their thermodynamics that are used are modeled on quinones described in the better defined Complex III and photosynthetic reaction centers. Indeed, the redox properties of Q_{nz} and Q_{nx} can be identical to Q_o and Q_i in Complex III. In this sense the proton/electron couplings responsible for transmembrane proton motion are local and explicitly defined. The dual proton pumping quinones of the Degli Esposititi model [69] seem obscurely coupled to quinone redox state and seem independent of the principle of charge compensation. Charge compensation concerns also arise at the proposed P_I site in the model of Brandt [7], in which hydroquinone is doubly deprotonated before the first oxidation takes place. Brandt does apply charge compensation later in his model to the N2 site, explicitly recognizing the N2 Bohr proton described by Skulachev [75]. However, we see no need to make a Bohr proton part of an energy coupling event, just as the classic Bohr proton of hemoglobin is not connected with energy coupling. Unlike our model, this model does not include a true proton pump, since every transmembrane proton is carried with an electron and released to the cytosol ultimately by the cytosolic side P_I site.

Our model unifies the mechanisms of the principal respiratory complexes as viewed from the perspective of oxygen redox chemistry. Redox energy is used to move protons across a membrane to create a delocalized electrochemical proton gradient by means of controlling the redox chemistry of oxygen (in the form of O_2 or quinone) at localized sites. By virtue of the relative proximity of single electron redox centers around sites in which SQ is unstable, a kinetic barrier is introduced which prevents both electrons from following the same path. Electrons are carried to and from these sites by tunneling through strings of redox cofactors, while protons are carried by strings of protonatable amino acids.

Acknowledgements

This work was supported by National Institute of Health Grants GM41048 and GM27309 to P.L. Dutton and GM 30736 to T. Ohnishi.

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