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Possible Roles of Two Quinone Molecules in Direct and Indirect Proton Pumps of Bovine Heart NADH-quinone Oxidoreductase (Complex I)

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Abstract

In many energy transducing systems which couple electron and proton transport, for example, bacterial photosynthetic reaction center, cytochrome bc_1-complex (complex III) and *E. coli* quinol-oxidase (cytochrome bo_3 complex), two protein-associated quinone molecules are known to work together. T. Ohnishi and her collaborators reported that two distinct semiquinone species also play important roles in NADH-ubiquinone oxidoreductase (complex I). They were called SQ_{Nf} (fast relaxing semiquinone) and SQ_{Ns} (slow relaxing semiquinone). It was proposed that Q_{Nf} serves as a "direct" proton carrier in the semiquinone-gated proton pump (Ohnishi and Salerno, FEBS Letters 579 (2005) 4555), while Q_{Ns} works as a converter between one-electron and two electron transport processes. This communication presents a revised hypothesis in which Q_{Nf} plays a role in a "direct" redox-driven proton pump, while Q_{Ns} triggers an "indirect" conformation-driven proton pump. Q_{Nf} and Q together serve as ($1e^-/2e^-$) converter, for the transfer of reducing equivalent to the Q-pool.

Keywords

quinone-induced conformation-driven proton pump; quinone-gated proton pump; direct proton pump; indirect proton pump

Introduction

A major role of NADH-quinone oxidoreductase (complex I) is to transfer electrons from NADH to the quinone pool (Q-pool). Accompanying the transfer of two electrons, it is believed that approximately four protons are transported across the membrane to create the proton electrochemical potential ($\Delta\mu_{H+}$). Protein-associated quinone molecules are known to play important roles in energy transducing system. Q_A and Q_B in the bacterial

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photosynthetic reaction center [1-2], Qi and Qo in cytochrome bc_1 complex (complex III) [3-4] and Q_L and Q_H in *E coli* quinol oxidase (bo_3) are good examples [5].

In complex I, the importance of two protein-associated quinone molecules have been emphasized [6-8]. From the measurement of the electron spin relaxation profiles, T. Ohnishi and her collaborators proposed the existence of distinct two semiquinone species, fast relaxing SQ_{Nf} and slow relaxing SQ_{Ns}. The SQ_{Nf} signal is extremely sensitive to the transmembrane $\Delta\mu_H^+$ poise, while that of SQ_{Ns} is $\Delta\mu_H^+$ insensitive. Based on a detailed analysis of the direct spin-spin interaction between reduced cluster N2 and SQ_{Nf}, their mutual distance was calculated as 12 Å. The projection of the N2-SQ_{Nf} vector along the membrane normal is only 5 Å [9]. Therefore, we proposed a directly quinone-coupled, SQ_{Nf}-gated proton-pumping mechanism [10], which differs from the reversed Q-cycle mechanism [11].

Here, we will present a revised proton pump model in which two different mechanisms operate in series. Two protons per electron pair are transported via a quinone-gated “direct” proton pump, and the additional two protons are transported by a quinone-induced conformation-driven “indirect” proton pump. The concept of the “indirect proton transport” has been extensively discussed for complex I [12-16]. This terminology may be somewhat misleading because it gives the impression that the transport takes place independent of the redox energy. Even “indirect” proton pump associated with respiration are still ultimately driven by the exergonic electron transfer reactions between NADH and Q-pool.

A question would be raised: What is the immediate energy source, and how does it communicate to the remote indirect proton pumps?

In this paper, we will present a new hypothesis that the “indirect” pump is triggered by energy related to the site occupancy of Q_{Ns}. The energy is conducted by means of a conformational change of the protein structure to the antiporter homologs to cause the relocation of the channel structure.

Discussion

Direct proton pump mechanism

A scheme of the respiratory chain of complex I is shown in Fig. 1. If we approximate the redox reactions in complex I using the standard redox midpoint potential ($E_{m)}$ instead of the actual redox potential ($E_{h)}$, they cover the range between NADH/NAD^+ ($E_{m,pH7}$= −0.32 eV) and the ubiquinone pool QH_2/Q ($E_{m,pH7}$=+0.09 eV: this was quantitatively measured in photosynthetic reaction center [17]. Thus $\Delta E_{m,7}$ factor for one electron transfer in this redox span is −0.41 eV. The other critical component of the respiratory energy is $\Delta\mu_H^+$ across the inner mitochondrial membrane. Its current optimal values for 2 electron transfer is known in the range of 0.16 to 0.19 eV [17]. The available free energy for proton translocation is $-\Delta G° = n_{e-}\,\Delta E_{m7}$ in eV, where n_{e-} is the number of electrons transferred. Then for 2 electron transfer across the mitochondrial or bacterial cytoplasmic membranes, 5.1 ~ 4.3 protons can be transported with 0.82 eV under standard conditions. From this approximation, complex I has enough redox energy to account for the $4H^+/2e^-$ stoichiometry.

Ohnishi and Salerno published a hypothesis to explain the high ($4H^+/2e^-$) ratio in the coupling of complex I. They used the value of the vertical depth of the electron transport between iron-sulfur cluster N2 and Q_{Nf} of only 5 Å, which was obtained from the analysis of the direct spin-spin coupling [9]. From this, they deduced that the Q_{Nf} is located close to the membrane interface level, and is directly connected to the entrance of the proton well.

However, we recognized that explaining the ($4H^+/2e^-$) ratio by only one Q_{Nf} may be difficult.

In order to solve the problem, we now propose a revised two semiquinone models. As shown on the left side of Fig. 2, Q_{Nf} accepts an electron from iron-sulfur cluster N2 (a) to form SQ_{Nf} which was found to be anionic form below pH 8.5 [9]. Since SQ_{Nf} intensity is increased by the membrane potential poise and it has a higher affinity constant than both Q and QH_2, it binds tightly to the quinone pocket (b). When the semiquinone accepts a second electron from cluster N2, it takes up two protons from the matrix side (N-side). This triggers eversion of the semiquinone-binding protein. When protons bound to the semiquinone are fully reduced to QH_2, 2 protons are released into the proton-well. Two electrons are also transferred one by one to Q_{Ns} (c). This concludes a cycle in which ($2H^+/2e^-$) were vectorially transported by the direct pump.

Indirect proton pump mechanism

The *Klebsiella pneumonia* complex I was reported to act as a Na^+ pump with a stoichiometry of $2Na^+/2e^-$ [18-19]. It was also claimed that *E. coli*, which is a close relative of *K. pneumonia*, acts as a Na^+ pump [20]. However, Stolpe and Friedrich reported that *E. coli* complex I is fundamentally a H^+ pump, but it is capable of performing a Na^+/H^+ antiport [21]. In bovine heart complex I, it has become widely accepted that the Na^+/H^+ antiporter homologs are utilized as the proton channels [21-23].

Although these antiporter homologs are utilized for proton translocation in bovine heart complex I, protons are still pumped across the membrane by the redox energy provided by the electron transport from flavin to quinone, not by an ion gradient as in the antiporters themselves There is no other energy source in complex I. In considering the role of antiporter homologs in the proton pump, several questions may be addressed.

It is interesting that the activity of the antiporter channel is controlled by pH [24]. Effects of pH on the activity of bovine heart complex I was also observed [25]. We do not yet know whether this is related to the mechanism of the antiporters in complex I.

An important point is that the authentic antiporters utilized the electrochemical potential of the Na^+ gradient to pump H^+, while in complex I, the only available driving force is redox energy. An obvious question is how the energy is transferred to the H^+ pump. Investigators in the antiporter field reported that a conformation change causes a reorientation of the channel protein. It also changes the affinity of the key component of the channel to both proton and the monovalent cation [26-28]. Krishnamurthy et al. who studied the Na^+-coupled transporters [29], concluded that the antiporters usually employ proton or sodium transmembrane gradients [30-31]. A hypothetical mechanism for the complex I indirect proton pumps that takes all of these points into consideration is shown in the right side of Fig. 2.

Q_{Ns} is originally in the oxidized form (see (d)). When it accepted an electron, it is reduced to the semiquinone form, which must bind more strongly to the quinone-pocket since it is many order of magnitude more stable than free ubiquinone ($K_{stability}$ = 2.0 at pH 7.8; [8]) (see (e)). Reduction with a second electron causes the uptake of 2 scalar proton from the N-side, producing the quinol ($Q_{Ns}H_2$) which has a much weaker binding constant. When the reduced quinone is released from the quinone-pocket, it causes a large conformational change of the pocket as shown in (f). This change will be conducted through the membrane structure to reach the antiporter homologs to cause the relocation of the channel proteins [27]. We assume that the indirect pump releases two protons to the P-side (intermembrane space).

In this figure, Q_{Nf}-gated direct proton pump (Fig.2(a)-(c)) and Q_{Ns}-induced indirect conformation-driven proton pump (Fig.2(d)-(f)) are shown as suggested by Sazanov et al. [12]. It is suggested that Q_{Nf} binding site is located in the NuoH subunit close to the negative side membrane surface [32-36] and Q_{Ns} -binding site is located on a negative side loop of the NuoN subunit [37].

In summary, Q_{Nf} and Q_{Ns} transport 2 protons each, first by the direct mechanism and then, through an indirect mechanism. Then, both Q_{Nf} and Q_{Ns} jointly perform the role of converter between one electron transfer and two electron transfer processes.

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Abbreviations

Complex I	NADH-quinone oxidoreductase
DBQ	decylubiquinone
SQ_{Nf}	fast relaxing semiquinone
SQ_{Ns}	slow relaxing semiquinone

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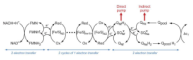

Fig. 1.

A scheme is to show the flows of electrons and protons in bovine heart complex I. Aarrows of direct pump and indirect pump indicate that these two pumps are driven by two kinds of ubiquinone species. At the end of the cycle, bc_1 indicates the coupling to complex III via Qpool.

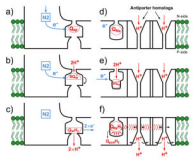

Fig. 2.

Schematic mechanisms of the (a)-(c) Q_{Nf}-gated “direct” proton pump which transports 2 protons, and (d)-(f) Q_{Ns}-induced conformation-driven “indirect” proton pump which also transports 2 protons.