Energy transduction by respiratory complex I – an evaluation of current knowledge

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Abstract
Complex I (NADH:ubiquinone oxidoreductase) is a membrane-bound, multisubunit enzyme. At present, there is no high-resolution structural model available for complex I and its mechanism of energy transduction is unknown. However, the subunit compositions of complex I from both eukaryotic and prokaryotic organisms have been determined, the sequences of their subunits are known and a coherent picture of the redox cofactors present in complex I has been developed. Here, I aim to describe and examine data relating to the mechanism of complex I and to build a framework to facilitate the discussion of possible conclusions and mechanistic models.

Introduction
Complex I (NADH:ubiquinone oxidoreductase) is a complicated, membrane-bound, multisubunit enzyme [1,2]. It catalyses the first step in the respiratory electron-transport chain in mitochondria, namely the reduction of ubiquinone (Q) by NADH, coupled to the translocation of four protons across the inner-mitochondrial membrane (Figure 1) [3].

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\text{NADH} + Q + H^+ + 4H^+_{\text{in}} \leftrightarrow \text{NAD}^+ + QH_2 + 4H^+_{\text{out}} \]

(1)

Therefore, it contributes to the proton-motive force that supports the synthesis of ATP, maintains the \( \text{NAD}^+ / \text{NADH} \) ratio in the mitochondrial matrix and supplies ubiquinol to the cytochrome \( bc_1 \) complex [4,5]. Typically, at steady state, the proton-motive force is close to balancing the potential difference between NADH and ubiquinone, and so the ‘driving force’ for the overall reaction is small. At present, there is no high-resolution structural model for complex I, although the subunit compositions of both eukaryotic and prokaryotic complexes I have already been defined, the amino acid sequences of their subunits are known and a consistent picture of the cofactor composition has been developed [1,2].

The mechanism of energy transduction by complex I is also unknown. This paper summarizes current data relating to the determination of the mechanism of complex I and aims to build a framework to facilitate the discussion of mechanistic proposals.

The location of cofactors and substrate-binding sites
All complexes I contain a common set of 14 subunits, the core subunits, which are considered sufficient for energy transduction [1,2]. Seven of them are predominantly hydrophilic and encoded in the nucleus (the 75, 51, 49, 30 and 24 kDa subunits, TYKY and PSST, according to the nomenclature for bovine complex I) and seven are highly hydrophobic, predicted to be dominated by transmembrane helices and encoded by the mitochondrial genome in eukaryotes (subunits ND1–ND6 and ND4L). Electron microscopy showed that both prokaryotic and eukaryotic complexes I are L-shaped, with one arm in the membrane and the other protruding into the mitochondrial matrix (Figure 1) [7,8]. The resolution of the complexes I from bovine mitochondria [1], Neurospora crassa mitochondria [7] and Escherichia coli [6,9] into subcomplexes suggests strongly that the two arms correspond to the two sets of core subunits: the seven hydrophilic subunits constitute the membrane-extrinsic arm, the seven hydrophobic subunits constitute the membrane-intrinsic arm.

All the known cofactors of complex I, including a non-covalently bound FMN (flavin mononucleotide; the primary oxidant of NADH) and a number of FeS (iron–sulphur) clusters, are bound by the hydrophilic core subunits. There is no conclusive evidence for any cofactor bound by the hydrophobic core subunits (see below for a discussion of quinone binding). Obviously, the idea that all the cofactors are in the membrane-extrinsic domain (Figure 1) is important in the formulation of mechanistic hypotheses for both the redox and proton-pumping reactions. It is probable that complex I contains eight FeS clusters (two [2Fe-2S] clusters and six [4Fe-4S] clusters) [1]. The most consistent model is: the 75 kDa subunit binds two [4Fe-4S] and one [2Fe-2S] [10]; the 51 kDa subunit binds one [4Fe-4S], as well as the FMN and the NADH substrate [11]; the 24 kDa subunit binds one [2Fe-2S] [12]; TYKY binds two [4Fe-4S]; PSST binds a [4Fe-4S] cluster known as cluster N2. However, several points remain controversial and an unambiguous picture is unlikely to be determined before a structural model becomes available. For example, why are the two clusters that must be present in subunit TYKY (since it contains an 8Fe-ferredoxin binding motif) so difficult to observe by EPR spectroscopy?
The redox reaction: oxidation of NADH and reduction of quinone

Figure 2 presents our basis for discussion of the electron-transfer reactions catalysed by complex I (in the absence of a proton-motive force); the redox reaction is thermodynamically favourable and unopposed and so the reverse reaction is negligible.

NADH is oxidized by non-covalently bound FMN

It is probable that the reaction between NADH and FMN is a hydride transfer reaction (Figure 2), to avoid the high-energy NADH radical intermediate. In complex I, the two-electron potential of the FMN is close to that of NADH [11]; so, as demonstrated by protein-film voltammetry experiments, the reaction is thermodynamically reversible [32]. After the oxidation of NADH and reduction of the FMN, FMNH

− reoxidized by the FeS clusters in two one-electron steps, through its thermodynamically accessible semiquinone state (Figure 2). Because the flavin semiquinone radical interacts magnetically with [4Fe-4S] cluster N3, in the 51 kDa subunit, it is probable that cluster N3 is the primary electron acceptor [11].

Electron transfer from FMNH

− to ubiquinone

The active sites for NADH oxidation and quinone reduction are spatially separated in complex I. It is very likely that two electrons are transferred sequentially between the two sites by a ‘chain’ of FeS clusters (Figures 1 and 2), as exemplified by the structures of succinate:quinol oxidoreductase, quinol:nitrate oxidoreductase and formate:quinone oxidoreductase [33–35]. Typically, the intercluster distances are 11–13 Å, so that eight clusters in complex I could span the height of the membrane-extrinsic domain (Figure 1). Furthermore, provided that intercluster distances are short enough, the exact reduction potentials are not critical, and significantly out-of-line reduction potentials, such as the relatively low potential of the [2Fe-25] cluster in the 24 kDa subunit [12],...
Figure 2 | Schematic representation of electron transport through complex I, in the absence of a proton-motive force

Reduction potentials (y-axis) are inverted to represent free-energy changes, and are from studies of complex I from bovine heart mitochondria [11,27–29]; the two one-electron potentials of bound quinone are not known. The schemes on the right-hand side show details of the three stages of the reaction: NADH transfers a hydride to FMN, then FMNH$^-$ is reoxidized in two stages by cluster N3; the electrons are passed (one at a time) along the chain of FeS clusters, given here in the order N3-N1a-N1b-N4-N5-(TYKY clusters)-N2; two electrons are donated, sequentially, to bound quinone. Note that the TYKY potentials have not been defined unambiguously [30], and a wide range of values have been reported for cluster N2 [28,31].

Reduction of ubiquinone

Cluster N2 is proposed to interact magnetically with bound semiquinone in complex I [14,15], suggesting that it is the final cluster in the FeS chain between FMN and bound quinone. The potential of cluster N2 is significantly lower than the (two-electron) potential of ubiquinone (Figure 2), showing that quinone reduction by N2 is thermodynamically favourable. However, the reduction of quinone to quinol requires two electrons and N2 would be required to donate them sequentially, employing the two ‘one-electron’ potentials of bound ubiquinone. These are unknown, as is the molecular mechanism of ubiquinone reduction in complex I. Electron delivery to cluster N2 is expected to be fast, so a semiquinone formed by the addition of one electron will be quickly reduced by a second and thus would be only short-lived. Semiquinone lifetime might be increased (i) by separation of the one-electron quinone potentials, (ii) by alteration of the relative potentials of N2 and quinone or (iii) kinetically. Consequently, it is interesting that SQNf is observed only in the presence of a membrane potential, suggesting a possible coupled proton translocation event [14,15]. Finally, complex I mutants that apparently lack cluster N2 have recently been created in _Y. lipolytica_ and catalyse NADH:ubiquinone oxidoreduction at a reasonable rate [18]. This suggests that cluster N2 is not required for catalysis – it is not consistent with the model presented here and requires further investigation.

Mechanisms of proton pumping

A mechanism for proton translocation by complex I must (i) be reversible, so viable for reverse catalysis, (ii) account for four protons translocated per NADH oxidized, and (iii) be efficient, so that NADH oxidation cannot occur without proton translocation [38]. Currently, there is little direct evidence for any mechanism of energy transduction in complex I, so mechanistic models are inspired largely by other membrane-bound energy-transducing enzymes. (i) Q-cycle mechanisms exploit quinol as a mobile proton/electron (hydrogen) carrier and are based on analogy with the cytochrome _bc_1 complex. (ii) In directly coupled mechanisms, such as that of cytochrome _c_ oxidase, proton transfer across a hydrophobic barrier is controlled directly by a gating reaction, such as a coupled electron-transfer reaction, positioned at (or close to) the barrier. (iii) In indirectly coupled mechanisms, the driving reaction is spatially separated from proton transfer across the hydrophobic barrier, and the two are coupled indirectly by interactions with the protein structure. Indirectly coupled mechanisms are exemplified by the rotary mechanism of F-type ATPases [39], and the conformational changes observed in _Ca_2$^{2+}$-ATPase [40]. They are not associated typically with redox enzymes, in which electron transfer is an obvious ‘in-place’ mechanism for long-range energy transfer. Figure 2 suggests that electron transfer may be (directly or indirectly) coupled with proton...
Two possible mechanisms of proton translocation in complex I

(A) The reverse Q cycle: (1) ubiquinone (Q) accepts one electron from the FeS clusters (from NADH oxidation) and one electron from ubiquinol (QH2) – the ‘reverse bifurcation’ step; (2) the QH2 formed is replaced by a second Q from the membrane; (3) repeat of (1) to generate Q at the cytoplasmic side; and (4) QH2 and Q are replaced, regenerating the starting species. In total, two Qs are reduced, one QH2 is oxidized and two NADH oxidized, and so it cannot be the only energy-trans-

(B) The translocation of two protons is coupled with the reduction and oxidation of cluster N2. A low-potential electron is delivered to N2ox (from NADH), energizing the protein (perhaps by altering the protein configuration around the active site) and forming N2red*. The high-energy state is capable of doing work, and its relaxation to the ‘normal’ state (N2red) is coupled with the translocation of two protons, requiring 0.4 V (2 × proton-motive force). Consequently, the normal state, N2red, has an apparent reduction potential of +0.06 V and is still capable of reducing bound (semi)quinone, completing the redox reaction. Similar schemes could be proposed also for the oxidation and reduction of a semiquinone, or for the binding and dissociation of Q and QH2, for example.

translocation either at cluster N2 or at ubiquinone, since, at these points, the electron loses a significant amount of potential energy.

The Q-cycle mechanism

Dutton et al. [41] proposed that the mechanism of complex I is equivalent to that of the cytochrome bc1 complex operating ‘in reverse’ (Figure 3), with the FMN and the FeS clusters acting only to supply low-potential electrons. However, the Q cycle alone accounts only for two protons pumped per NADH oxidized, and so it cannot be the only energy-trans-

Figure 3

Proton transfer coupled with the reduction and oxidation of cluster N2

Cluster N2 has long been implicated in the proton-pumping mechanism of complex I. It is proposed to be the direct electron donor to bound quinone and so is probably positioned appropriately for controlling proton transfer across a hydrophobic barrier (in, or close to, the membrane domain). The reduction potential of cluster N2 is pH-dependent (indicating that a proton is taken up by the cluster or by a nearby residue when the cluster is reduced) [27,28] and it responds to the membrane potential also [28,42]. However, it is not clear whether the pKox and pKred values associated with the reduction and oxidation of cluster N2 are sufficiently far apart to form an efficient proton pump, and a redox titration (at equilibrium) may not be relevant to (cyclic) catalytic turnover. Finally, recent Fourier-transform infrared studies on complex I from E. coli have suggested that a number of residues change their protonation state upon a change in the oxidation state of cluster N2 and that a redox-coupled conformational change may take place [43,44]. Therefore, although there is considerable circumstantial evidence to support proton pumping powered by cluster N2, at present there is no conclusive evidence to justify the formulation of specific mechanistic hypotheses. Figure 3 presents an example of a cycle that may describe how the proton-motive force is coupled to the reduction potential of cluster N2. However, note that such a scheme does not contain any information about the molecular mechanism and that similar schemes could be constructed for the reduction and oxidation of a semiquinone or for the binding and dissociation of substrate also.

Evidence for an indirectly coupled proton-transfer mechanism

In complex I, the FeS clusters provide an obvious route for long-range energy transfer between the hydrophilic and hydrophobic domains, and so it is not clear why complex I should adopt indirect coupling to couple a redox reaction (or a chemical transformation) with proton transport across the membrane. Furthermore, note that a conformational change does not necessarily imply an indirect coupling mechanism. For example, a conformational change takes place during catalysis by the cytochrome bc1 complex, but forms part of the mechanism for the bifurcation of electron transfer at the Qo site [45]. Conformational changes have been reported in complex I. A ‘horseshoe’ conformation has been reported [46]
(but has been questioned by other researchers [47]), complex I from E. coli was reported to ‘open up’ upon its reaction with NADH (although the structural change is not specific to any particular part of the structure) [48], and changes in the cross-linking patterns and trypsinolysis of the hydrophilic subunits of bovine and E. coli complex I, upon reaction with NADH substrates, have been reported [48,49]. However, these are preliminary observations and it is clear that further data are required before they may form the basis of mechanistic hypotheses for proton pumping by complex I.

References

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Received 18 January 2005