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Energy conversion, redox catalysis and generation of reactive oxygen species by respiratory complex I

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ABSTRACT

Complex I (NADH:ubiquinone oxidoreductase) is critical for respiration in mammalian mitochondria. It oxidizes NADH produced by the Krebs' tricarboxylic acid cycle and β-oxidation of fatty acids, reduces ubiquinone, and transports protons to contribute to the proton-motive force across the inner membrane. Complex I is also a significant contributor to cellular oxidative stress. In complex I, NADH oxidation by a flavin mononucleotide, followed by intramolecular electron transfer along a chain of iron–sulfur clusters, delivers electrons and energy to bound ubiquinone. Either at cluster N2 (the terminal cluster in the chain) or upon the binding/reduction/dissociation of ubiquinone/ubiquinol, energy from the redox process is captured to initiate long-range energy transfer through the complex and drive proton translocation. This review focuses on current knowledge of how the redox reaction and proton transfer are coupled, with particular emphasis on the formation and role of semiquinone intermediates in both energy transduction and reactive oxygen species production. "This article is part of a Special Issue entitled Respiratory complex I, edited by Volker Zickermann and Ulrich Brandt".

1. Introduction to complex I

Complex I (NADH:ubiquinone oxidoreductase) [1] plays a central role in the cellular metabolism of humans and many other organisms. It oxidizes NADH in the mitochondrial matrix to regenerate the NAD^+ pool, sustaining the Krebs' tricarboxylic acid cycle and the β-oxidation of fatty acids. The two electrons from NADH oxidation are transferred through the enzyme and used to reduce ubiquinone to ubiquinol in the inner mitochondrial membrane, supplying the rest of the electron transport chain with electrons for the reduction of oxygen to water. The free energy produced by the redox reaction is captured and used to transport protons across the mitochondrial inner membrane, building the proton-motive force (Δp) that is consumed to support ATP synthesis and the import and export of metabolites and proteins to and from the mitochondrion. In addition, reactive oxygen species production by complex I is an important contributor to mitochondrial and cellular oxidative stress [2]. Complex I dysfunctions are caused by genetic, environmental, and pathological factors, and have been linked to both impaired catalytic ability and enhanced superoxide production [3]. Their molecular, mechanistic, and energetic consequences remain poorly understood, highlighting the need for improved basic knowledge of the enzyme's molecular structure and function.

Complex I from *Bos taurus* heart mitochondria is the most studied mammalian complex I, and has been adopted as a closely-related model for the human enzyme. The forty-five (known) proteins in mammalian complex I [4,5] comprise 14 conserved 'core' subunits that are sufficient to catalyze energy transduction, and 31 'supernumerary' subunits [6,7]. The supernumerary subunits vary between species: they have been accumulated through evolution and surround the core complex. Some supernumerary subunits are known to have specific roles, and as a cohort they have been proposed to have roles in regulation, protection against reactive oxygen species, assembly, and stability [8]. Here, we concentrate on the mechanism of complex I, and thus on the 14 core subunits; we refer to the subunits throughout by their names in *B. taurus* (regardless of the species concerned).

The core subunits form two distinct domains that are reflected in the L-shape of the complex (see Fig. 1). Seven hydrophilic core subunits (encoded by the nuclear genome) constitute the redox domain that extends into the mitochondrial matrix, and seven hydrophobic core subunits (the ND subunits encoded by the mitochondrial genome) are contained in the mitochondrial inner membrane. The structures of the core subunits were determined first in the bacterial enzymes from *Thermus thermophilus* and *Escherichia coli* [9–11], then their conserved structures were modeled using medium-resolution structural data

Abbreviations: CW, continuous wave; ESEEM, electron spin echo envelope modulation; EPR, electron paramagnetic resonance; FeS, iron–sulfur; HYSCORE, hyperfine sub-level correlation; SMP, submitochondrial particle; SQ, semiquinone.

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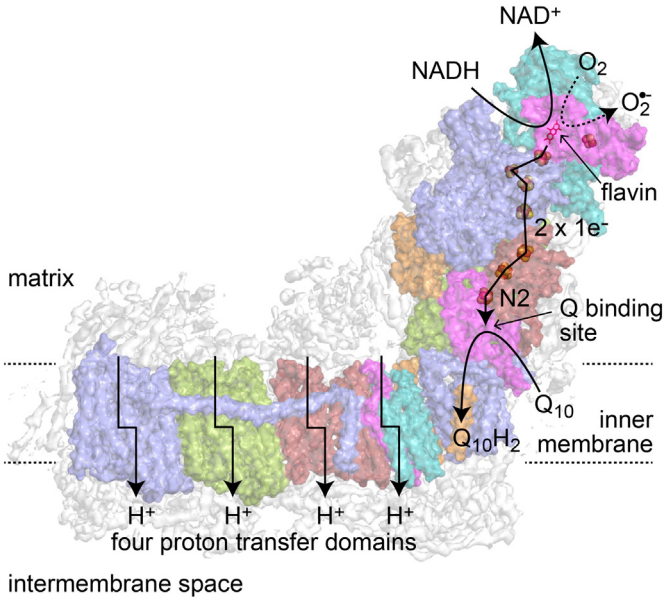

Fig. 1. Overview of the structure and reactions catalyzed by mammalian complex I. The cryoEM density for the complete enzyme is shown in white, the fourteen subunits of the core enzyme are shown in color [5], and the reactions are indicated schematically. NADH is oxidized by a flavin mononucleotide at the top of the hydrophilic domain, then electrons are passed along a chain of iron–sulfur clusters (ending in cluster N2) to reduce bound ubiquinone. Four protons are transferred from the matrix to the intermembrane space for each NADH oxidized. The reduced flavin cofactor also reacts with molecular oxygen to form reactive oxygen species.

from the mammalian enzyme from *B. taurus* [5], and the yeast enzyme from *Yarrowia lipolytica* [12].

NADH is oxidized by a non-covalently bound flavin mononucleotide at the top of the hydrophilic domain, in the 51 kDa subunit. Then a chain of seven iron–sulfur (FeS) clusters (one $[2Fe–2S]^{2+/1+}$ and six $[4Fe–4S]^{2+/1+}$ clusters) transfers electrons from the flavin to the quinone-binding site. An unusually-positioned additional $[2Fe–2S]^{2+/1+}$ cluster is located on the opposite side of the flavin, separate from the main chain of clusters [9]; it is likely to be important for the structure around the flavin site and has no known distinct functional role [13]. The final cluster in the chain (cluster N2), which donates electrons to the bound ubiquinone substrate, is positioned more than 20 Å above the membrane surface and ~12 Å from the likely binding site for the ubiquinone headgroup [11]. A 30 Å long chamber connecting a narrow entry point in the membrane to this binding site has been proposed as the access route for the ubiquinone, and to accommodate its highly hydrophobic isoprenoid tail [11]. In the membrane domain, four antiporter-like structural motifs have been identified that are likely to transport one proton each per catalytic cycle. Three of these proton transfer units are formed by subunits ND2, ND4 and ND5 [10], and one by ND1, ND6 and ND4L [11]. Each of them contains two related half-channels that connect the external aqueous phase to the central membrane plane where a set of structural indicators for gated proton transfer, including charged residues and the loops of broken transmembrane helices, are located [10]. Strikingly, a long transverse α-helix runs along the membrane plane; together with supporting structure on the intermembrane face it may be involved in maintaining the integrity of the membrane domain [10]. As the transporter domains are positioned far away from the FeS cluster chain and the quinone-binding site, using the redox reaction to drive proton transport requires long-range energy transfer through the protein. The mechanism of redox-proton coupling is currently the least understood aspect of the mechanism of this huge and complicated enzyme.

In this review we focus on the redox chemistry of complex I, and particularly on current knowledge pertaining to the mechanism by which the redox energy is captured and used to initiate proton translocation. We begin with a brief overview of the 'upstream reactions' that deliver electrons to the coupling site (NADH oxidation and intramolecular electron transfer) then discuss the evidence for coupled chemistry at cluster N2 (the terminal cluster in the FeS chain), and the mechanism of ubiquinone reduction. In particular, we examine evidence for the involvement of semiquinone intermediates in catalysis. We close by considering how current knowledge of the redox mechanism of complex I contributes to understanding its mechanisms of energy transduction and reactive oxygen species production.

2. Generating the electrons for ubiquinone reduction

2.1. Reversible oxidation of NADH by the flavin

In mitochondrial complex I, NADH oxidation by the flavin mononucleotide is both 'fast' [14] and 'reversible' [15]. It almost certainly occurs by hydride transfer, because the highly unstable nature of the radical intermediate $NAD^{\bullet}$ disfavors stepwise processes and because the structure of the hydrophilic domain of *T. thermophilus* complex I with a nucleotide bound in the flavin site [16] shows the nicotinamide ring juxtaposed on the flavin ring system in an orientation consistent with a direct hydride transfer reaction [17]. The structure also revealed stacking interactions between three phenylalanine residues and the adenine ring of the nucleotide that are critical for nucleotide binding; they may help to capture the nucleotide and orientate entry of the nicotinamide ring into the cavity [18].

NADH oxidation by the flavin occurs much faster than the full catalytic cycle (proton-coupled NADH:ubiquinone oxidoreduction) of complex I can turn. Thus, it is not rate limiting in catalysis, and to study the flavin site reactions NADH oxidation must be coupled to the rapid reduction of an 'artificial electron acceptor' directly by the flavin so it is not controlled by the slow downstream steps of the full cycle. Kinetic studies using *B. taurus* complex I have revealed that the apparent second order rate constant for NADH binding (represented by $k_{cat}^{NADH}/K_M^{NADH}$, where k_{cat}^{NADH} is the (maximum) rate of NADH oxidation observed at saturating NADH concentration and K_M is the Michaelis constant, equivalent to the NADH concentration required for half the maximum rate) is ~7.5×10^7 M^{-1} s^{-1}, approaching the diffusion-controlled limit, and that k_{cat}^{NADH} (which includes both reversible hydride transfer and NAD^+ dissociation) is greater than 15,000 s^{-1} [14,19]. In comparison, the maximum rate of NADH:ubiquinone oxidation that has been observed is ~400 s^{-1} [20,21].

Here, we use a thermodynamic definition of the term 'reversible'. The fact that complex I can catalyze 'reverse electron transfer' (ubiquinol:NAD^+ oxidoreduction, driven by Δp) establishes it only as an enzyme that can catalyze backwards, not as a thermodynamically-reversible catalyst that operates with a substantial rate in either direction under only minimal driving force [22]. The thermodynamic reversibility of mammalian complex I was established by varying the driving force for NADH:ubiquinone oxidoreduction to each side of the point at which it balances Δp [23], and the reversibility of NADH oxidation by the flavin, facilitated by the flavin's low reduction potential [24], was established electrochemically [15]. The rapid and reversible oxidation of NADH by complex I indicates that the oxidized and reduced states of the flavin rapidly come to redox equilibrium with the NAD^+ and NADH in solution — a feature that was instrumental in defining the reduced flavin as the site of reactive oxygen species production (see below) [25].

Although knowledge of the flavin site in complex I is relatively well developed, the identities of the intermediates that dominate during catalysis (and so govern reactive oxygen species production) remain unclear. Scheme 1 shows how NADH oxidation must progress across a network of states, according to the different (as yet unknown) binding constants and nucleotide concentrations present, and identifies those 'poised' states in which it is likely the system waits until turnover is initiated by ubiquinone binding.

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