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Review

A modeling and simulation perspective on the mechanism and function of respiratory complex I☆

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ABSTRACT

Respiratory complex I is a giant redox-driven proton pump, and central to energy production in mitochondria and bacteria. It catalyses the reduction of quinone to quinol, and converts the free energy released into the endergonic proton translocation across the membrane. The proton pumping sets up the proton electrochemical gradient, which propels the synthesis of ATP. Despite the availability of extensive biochemical, biophysical and structural data on complex I, the mechanism of coupling between the electron and proton transfer reactions remain uncertain. In this work, we discuss current state-of-the-art in the field with particular emphasis on the molecular mechanism of respiratory complex I, as deduced from computational modeling and simulation approaches, but in strong alliance with the experimental data. This leads to novel synthesis of mechanistic ideas on a highly complex enzyme of the electron transport chain that has been associated with a number of mitochondrial and neurodegenerative disorders.

1. Introduction

Plants, animals and microorganisms fulfill their energetic requirements in the most optimal ways. They utilize highly complicated, yet efficient, metabolic pathways for their continuous growth and development. Depending on the environmental conditions, they are capable of re-engineering their metabolic pathways for efficient energy production. For instance, the nitrogen fixing bacterium *Bradyrhizobium japonicum* terminates the respiratory chain into two types of cytochrome oxidases; aa_3-type that is expressed under normal aerobic conditions, whereas the other cbb_3-type oxidase is employed only under low oxygen tensions due to its very high affinity for dioxygen ($K_M \sim 7$ nM) [1]. This shows that organisms employ multiple strategies to cater their needs for one of the most fundamental molecules in biology, ATP (adenosine triphosphate). In the power house of eukaryotic cells, mitochondrion, ATP is synthesized predominantly through oxidative phosphorylation. In this process, several membrane-bound respiratory complexes catalyze electron transfer reactions from NADH ($E_{m,7}$ $NAD^+/NADH \sim -320$ mV) to the terminal electron acceptor, dioxygen ($E_{m,7}$ $O_2/H_2O \sim +800$ mV). These electron transfer reactions are tightly coupled to proton pumping across the membrane leading to the formation of electrochemical proton gradient (or proton motive force, *pmf*), which is about 150–200 mV depending upon the respiration state of mitochondria. The *pmf* then drives the ATP synthase to generate ATP from ADP and P_i through a rotary mechanism as proposed by Boyer [2], and confirmed by X-ray studies and biophysical measurements [3,4]. The rotary-catalysis mechanism of ATP synthase is now well supported by a wealth of experimental data [5], and also by a number of modeling and simulation studies [6,7], which provided detailed mechanistic insights not easily obtained from the experiments. One major unresolved question in the field is how the proton transfer in the F_O part [8,9] is coupled to the ATP synthesis in the F_1 part of the enzyme.

In the classical "linear view" of the electron transport chain (ETC), the respiratory enzymes (ETC complexes and ATP synthase) float in the sea of lipids, and the sequential electron transfer from complexes I/II → III → IV is tightly coupled to the proton pumping across the membrane. In this process of long-range electron transfer, mobile electron careers such as the membrane-bound quinone and water-soluble cytochrome *c* play key roles. In the inner mitochondrial membrane, ubiquinone (Q_{10}, quinone with ten isoprene units) dynamically shuttles between complexes I, II and III transferring electrons coupled with the uptake and release of protons. The ubiquinone structure resembles that of the lipids with a polar head group and a long (ca. 30 carbon) hydrophobic tail. Free energy simulations show that the head group of Q prefers certain locations along the bilayer normal [10,11], and that the Q molecules

☆ Note: All amino acid numbering corresponds to complex I from *Thermus thermophilus*, unless otherwise stated.

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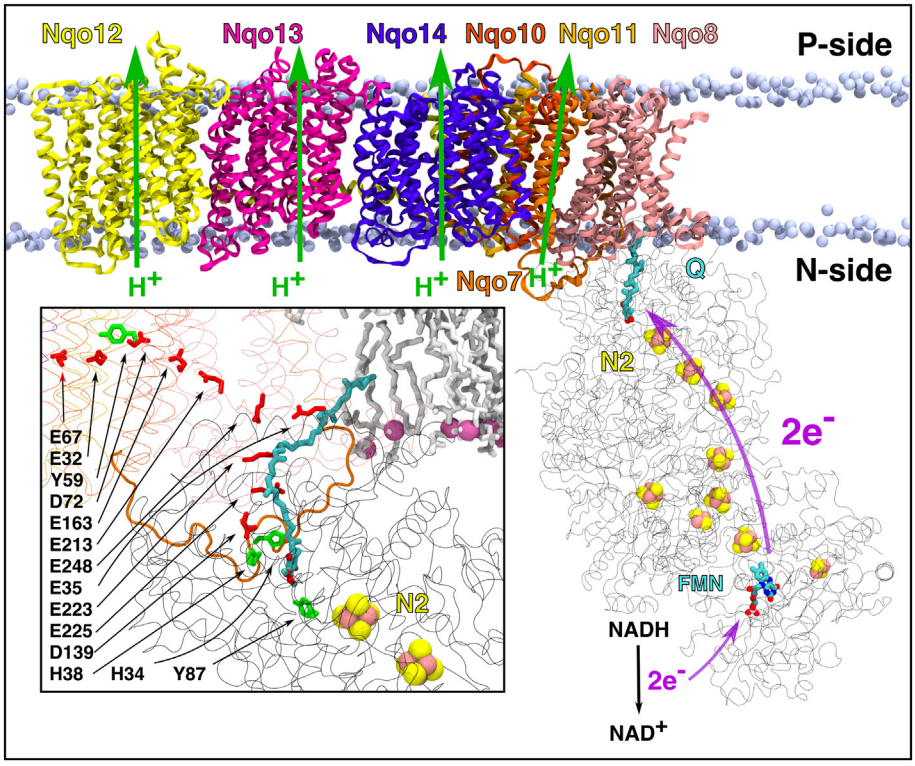

Fig. 1. The 14 core subunits of complex I from *Thermus thermophilus*. The membrane-bound subunits and hydrophilic domain are displayed. Iron-sulphur clusters are shown in yellow and pink spheres, together with FMN and a quinone molecule. Phospholipid phosphorus atoms (light blue spheres) mark the membrane boundary. The electron transfer and proton pumping routes are shown in purple and green arrows, respectively. Inset shows highly conserved charged and polar amino acid residues that provide connectivity to the hydrophilic axis in the middle of the membrane domain, together with the functionally critical Tyr87 from Nqo4 subunit. Bilayer is also shown with white sticks and pink spheres, in which the tail of a Q_{10} molecule extends into. The dynamic loop of Nqo7 is highlighted in a thicker orange representation (see also Fig. 7 below).

undergo rapid *flip-flop* between the two leaflets of the bilayer, which may be important for the optimal turnover of the ETC [11].

A second dominant view in the field is of mitochondrial *supercomplexes*, in which individual respiratory complexes join together to form a single unit, albeit in different stoichiometric amounts [12]. These so called *respirasomes* catalyze redox-driven proton pumping in a much tightly packed environment (see also recently solved structures [13,14]), which may be necessary to prevent ROS formation [15,16]. The advantages of having respirasomes as functional entities over individual complexes are not fully understood, and the topic remains highly debated [17]. Interestingly, respiratory supercomplexes (III + IV) have been studied with coarse-grained molecular dynamics (MD) simulations imitating the crowded inner mitochondrial membrane environment [18]. Results reveal an important role of cardiolipin molecules in gluing the respiratory complexes together in a variety of conformational arrangements [18], in agreement with earlier data [19].

In contrast to limited knowledge on supercomplexes, individual respiratory complexes have been studied for several decades through different types of biochemical, biophysical and structural techniques [20–22]. They have been characterized in both detergents and liposomal conditions through site-directed mutagenesis studies, spectroscopic and as well as state-of-the-art electrometric techniques [20]. Thanks to these immense efforts, the molecular mechanisms of complexes III and IV are relatively well-understood. Some delicate questions remain to be answered, in which computational approaches are playing the most important role. See refs. [23, 24] and refs. [25, 26] for recent computational work on complexes IV and III, respectively. In detailed mechanistic discussions below, this existing mechanistic knowledge on cytochrome oxidase (complex IV), cytochrome bc_1 (complex III) and Photosystem II is utilized to delineate complex I working principles.

Among all the ETC enzymes, complex I remains least understood, especially because of its large size and very high complexity. The respiratory complex I comprises 14 to 45 subunits, with mass ranging from 500 kDa to 1 MDa in bacteria to mitochondria, respectively. Earlier, research on complex I progressed slowly due to the unavailability of any suitable biochemical assay to estimate activity and efficiency of the enzyme. However, currently highly active preparations of complex I are being used in various labs for its mechanistic explorations (see refs. [27, 28]). The crystal or cryo EM structures of complex I [29–35] have provided the most spectacular insights into enzyme architecture, in particular, it is now absolutely clear that all three respiratory enzymes (I, III and IV) utilize completely different strategies to generate *pmf*, even though some microscopic elements and principles may be shared. In the light of these well-understood respiratory and photosynthetic enzymes, we discuss some novel mechanistic ideas on complex I in strong alliance with computational methods and tools.

Computational approaches such as molecular dynamics (MD) simulations are particularly effective when applied to static structural data because they allow spatio-temporal resolutions to be gauged that are currently outside the scope of expensive experimental instruments. Emerging techniques such as super resolution microscopy spans time and length scales of ca. 15 millisecond and 20 nm, respectively. Furthermore, the time-resolved crystallography captures atomic resolution images of processes that occur in femtoseconds to hundreds of picoseconds [36]. However, it is the reactions that occur in hundreds of nanoseconds to hundreds of microseconds for which there are no experimental techniques that would provide high resolution data. Examination of such processes can indeed be performed by modeling and simulation techniques. An additional factor that makes biomolecular computations highly attractive is their ability to simulate catalytic states that are inaccessible to experiments (such as high energy states of low occupancy). In the discussions below on complex I mechanism, such aspects will be explored.

2. Respiratory complex I

2.1. General structure

Respiratory complex I (Fig. 1) is a large membrane protein found in the inner mitochondrial membrane as well as in the plasma membrane of many bacteria. It couples the two-electron exergonic reduction of