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Review

Insights into the mechanism of electron transfer and sodium translocation of the Na^+-pumping NADH:quinone oxidoreductase ☆

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ABSTRACT

Na^+-NQR is a unique energy-transducing complex, widely distributed among marine and pathogenic bacteria. It converts the energy from the oxidation of NADH and the reduction of quinone into an electrochemical Na^+-gradient that can provide energy for the cell. Na^+-NQR is not homologous to any other respiratory protein but is closely related to the RNF complex. In this review we propose that sodium pumping in Na^+-NQR is coupled to the redox reactions by a novel mechanism, which operates at multiple sites, is indirect and mediated by conformational changes of the protein. This article is part of a Special Issue entitled: 17th European Bioenergetics Conference (EBEC 2012).

1. Introduction

It is generally considered that conservation of energy during respiration is a process that involves the transport of protons across biological membranes. In mitochondria, and in most bacteria and archaea, the major enzyme complexes of the respiratory chain contribute to the generation of a proton electrochemical gradient that is used as an energy source [1,2]. However, in certain marine and pathogenic bacteria, Na^+ gradients have an essential parallel role in primary conservation of energy, as well as in ionic homeostasis [3–5]. One of the most important Na^+ transporters in bacteria, is the Na^+-pumping NADH:quinone oxidoreductase (Na^+-NQR), which has the ability to directly couple electron transport to the generation of a sodium gradient.

Na^+-NQR serves as the entry point for electrons into the respiratory chain, transporting electrons from NADH to the quinone pool, and using the free energy of this reaction to pump Na^+ across the cell membrane. The resulting Na^+ gradient provides power for cellular processes that include: ATP synthesis, rotation of the flagellar motor, transport of nutrients, and operation of the multi-drug transporter, which is responsible for resistance to some types of antibiotics [6–12].

Consistent with its function, which combines redox reactions and Na^+ transport, Na^+-NQR has several distinctive structural and functional properties. The evidence indicates that Na^+-NQR is not a variant of the typical respiratory enzymes or Na^+ transporters, but has features in common to both types of enzymes. Na^+-NQR is closely related to a family of enzymes known as RNF, originally described as *Rhodobacter* nitrogen fixation protein. Members of the RNF family are a diverse group of redox linked Na^+ pumps, involved in a variety of physiological roles [13–17]. Due to the similar composition of subunits and cofactors, it is highly likely that RNF and Na^+-NQR operate through the same basic mechanism.

In this review, we summarize advances in mechanistic studies on Na^+-NQR together with some relevant aspects of the RNF family. We discuss new evidence indicating that coupling between the redox reactions and the transport of Na^+ is indirect, and possibly mediated by conformational changes of the protein.

2. Distribution of Na^+-NQR

Na^+-NQR was originally discovered and characterized in the marine bacteria *Vibrio alginolyticus*, by the research groups of Unemoto and Dimroth [18–21]. For many years the enzyme was thought to be largely confined to marine species, possibly representing an adaptation to the high Na^+ content of seawater [22–25]. However, Hayashi et al. also found Na^+-NQR in *Haemophilus influenzae*, which is an airborne human pathogen [26]. Subsequently, with the advent of whole-genome sequencing, Na^+-NQR has been found in the

Abbreviations: Na^+-NQR, Na^+-pumping NADH:quinone oxidoreductase; RNF, *Rhodobacter* nitrogen fixation protein; HQNO, 2-n-heptyl-4-hydroxyquinoline N-oxide; CCCP, carbonyl cyanide m-chlorophenylhydrazone; $\Delta\Psi$, membrane potential

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genomes of other pathogenic bacteria, including *Vibrio cholerae*, *Chlamydia trachomatis*, *Yersinia pestis*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, and *Porphyromonas gingivalis* [27]. To date, the enzyme has been found in more than 100 different bacteria, including numerous marine and pathogenic species, as a well as in other groups of bacteria.

3. Biochemical properties

The net redox reaction catalyzed by Na^+-NQR is the same as that carried out by the H^+-pumping NADH:quinone oxidoreductase (Complex I), found in mitochondria and many bacteria [28]. However, these two respiratory enzymes are not homologous and have a different composition of cofactors and subunits, and likely a different catalytic mechanism [29,30].

The initial evidence for the operation of a respiratory Na^+ pump came from experiments on sub-bacterial particles of *V. alginolyticus* by Tokuda [31]. At high pH and high Na^+ aerobic respiration was able to generate a membrane potential, even in the presence of the protonophore CCCP, indicating that the potential was due to pumping of some ion other than H^+. This led them to the discovery of a previously undescribed enzyme, the Na^+-pumping NADH:quinone oxidoreductase (Na^+-NQR)[32]. Subsequently, the enzyme was isolated and reconstituted into phospholipid vesicles [32–36]. In these preparations the redox reaction was shown to be accompanied by electrogenic transport of Na^+ into the vesicles. Since no other proteins were present, this definitively showed that Na^+-NQR is a redox-driven primary Na^+ pump and that the Na^+ gradient is not the result of a secondary process, such as the activity of an antiporter.

3.1. Polypeptide composition

Na^+-NQR is an integral membrane complex made up of six subunits: NqrA through NqrF with a total molecular weight of aprox. 200 kDa [32,34–38] (Fig. 1). In most bacteria, the genes coding for these subunits are organized into a single operon (nqr) that contains six open reading frames. Of the six subunits, NqrB, D and E are very hydrophobic, consisting largely of transmembrane helices. NqrF and C have one and two helices, respectively that anchor them in the membrane, but also include large cytoplasmic domains. NqrA, the largest subunit in the complex, is hydrophilic and does not contain any transmembrane helices. Topological studies, using reporter-gene fusions have shown that NqrA is located on the cytosolic side of the membrane [39]. NqrB contains nine transmembrane helices and has its N-terminal located in the periplasmic space. It includes the binding site for one of the two FMN cofactors and possibly for Riboflavin, and its hydrophobic domains contain seven conserved, negatively charged residues whose location and character make them good candidates to be involved in Na^+ translocation. NqrC includes two transmembrane helices and has its N-terminal located in the periplasmic space. The binding site for the other FMN cofactor is located in the soluble domain of this subunit. NqrD and NqrE each have six transmembrane helices, but they are situated in opposite orientations in the membrane. In NqrD, the N and C termini are both located on the cytoplasmic side of the membrane, whereas, in NqrE, both are in the periplasm. These two subunits do not bind any cofactor(s), but they include several conserved acid residues that are important for Na^+ transport. NqrF has only one transmembrane helix, which is attached to a large cytoplasmic domain, where the NADH binding site together with the FAD and the 2Fe-2S cofactors are located [35,40–47].

3.1.1. Catalytic activity: ion specificity

Quinone reductase activity is highly dependent on Na^+ concentration. In the absence of Na^+, the reaction proceeds very slowly (turnover number 60 s^{-1}), but this activity is stimulated eight to nine times by Na^+ ($k_{cat} = 460\ s^{-1}$) with a Km^{Na+} of 2.5 mM. Recently, we have shown that in addition to Na^+, Na^+-NQR can also translocate Li^+. Lithium stimulates enzyme turnover three times ($k_{cat} = 180\ s^{-1}$), with a Km^{Li+} of 3.5 mM. Steady state kinetic measurements using mixtures of Na^+ and Li^+ show that Na^+-NQR contains at least two cation-binding sites and that both ions compete for these site(s). Moreover, the cation binding sites have positive cooperativity: binding to the first site increases the affinity of the second site by a factor of four. Equilibrium measurements using $^{22}Na^+$ show that the enzyme can bind up to three sodium ions, and that neither the stoichiometry nor the binding affinities at the three sites differ between the oxidized and reduced forms of the enzyme [48]. As discussed below, this result has important implications for the mechanism of Na^+ translocation.

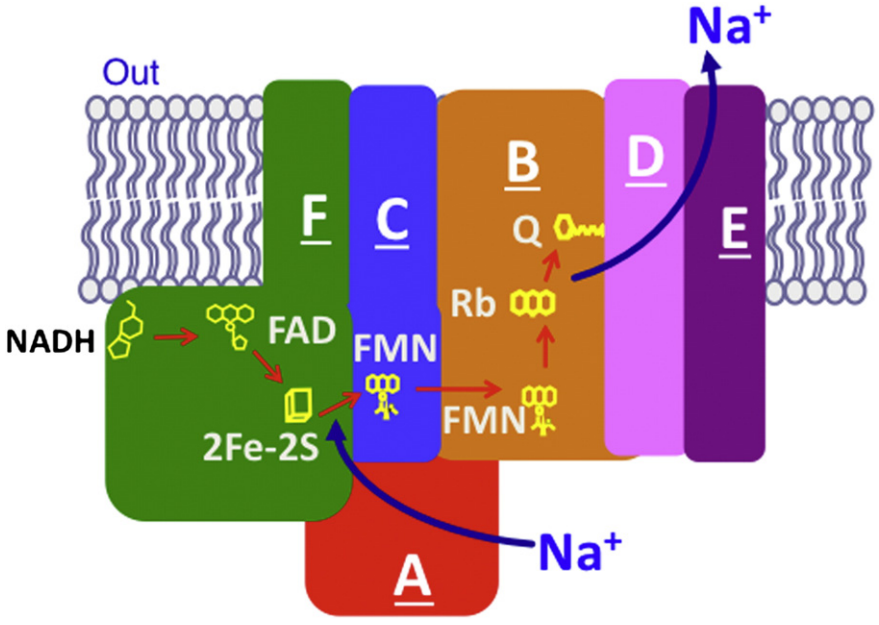

Fig. 1. Topology, cofactor and subunit composition of Na^+-NQR. Electron transfer pathway and Na^+ translocation are shown.