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The Na^+-Translocating NADH:quinone Oxidoreductase (NDH I) from *Klebsiella pneumoniae* and *Escherichia coli*: Implications for the Mechanism of Redox-Driven Cation Translocation by Complex I

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Eukaryotic complex I integrated into the respiratory chain transports at least 4 H^+ per NADH oxidized. Recent results indicate that the cation selectivity is altered to Na^+ in complex I (NDH I) isolated from the enterobacteria *Escherichia coli* and *Klebsiella pneumoniae*. A sequence analysis illustrates the characteristic differences of the enterobacterial, Na^+-translocating NDH I compared to the H^+-translocating complex I from mitochondria. Special attention is given to the membranous NuoL (ND5, Nqo12) subunits that possess striking sequence similarities to secondary Na^+/H^+ antiporters and are proposed to participate in Na^+ transport. A model of redox-linked Na^+ (or H^+) transport by complex I is discussed based on the ion-pair formation of a negatively charged ubisemiquinone anion with a positively charged Na^+ (or H^+).

KEY WORDS: NADH:quinone oxidoreductase; NDH I; Na^+ transport; sodium bioenergetics; *Klebsiella pneumoniae; Escherichia coli*.

INTRODUCTION

The electrogenic NADH:quinone oxidoreductase (complex I) is the primary electron entry site in many eukaryotic and bacterial respiratory chains. Complex I (or NDH I) from bacteria is composed of 14 subunits encoded by the *nuo* operon and has been studied with respect to primary sequence, cofactor content, and regulation in various microorganisms (Friedrich, 1998; Dupuis *et al.*, 1998; Finel, 1998; Yagi *et al.*, 1998; Bongaerts *et al.*, 1995). Since every subunit present in the bacterial complex I has a homolog in the mitochondrial enzyme, the smaller bacterial complex I is regarded as a model for the analysis of the structure and function of its larger counterpart. Complex I from *E. coli* is an L-shaped multisubunit complex with an approximate mass of 530 kDa (Guénebaut *et al.*, 1998). Upon purification, the complex tends to disrupt into a peripheral (subunits NuoE, F, and G) and a connecting fragment (subunits NuoB, the fused subunits NuoC and D, and NuoI) harboring the FMN and Fe–S clusters, and a membranous fragment composed of the subunits NuoA, H, J, K, L, M, and N (Friedrich, 1998). For a detailed description of the redox properties and localizations of the different Fe–S clusters, the reader is referred to van Belzen *et al.* (1997), Ohnishi *et al.* (1999) and Yano *et al.* (2000). The oxidation of NADH is catalyzed by the peripheral NuoF subunit harboring FMN and a [4Fe–4S] cluster. Subsequently, electrons are delivered via additional Fe–S centers to the cluster(s) with the highest midpoint redox potential, termed N2 (or 2). Cluster N2 is thought to be ultimately oxidized by ubiquinone.

Complex I from *E. coli* exhibits some distinct catalytic properties, like the reduction of menaquinone or demethylmenaquinone, in addition to ubiquinone (Tran and Unden, 1998), and the translocation of Na^+ ions (Steuber *et al.*, 2000). This review summarizes the experimental evidence for Na^+ transport by complex I from *E. coli* and a close relative, *Klebsiella pneumoniae* (Krebs *et al.*, 1999). Like *E. coli*, *K. pneumoniae* is an enterobacterium and belongs to the gamma subdivision of

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proteobacteria. In the first part of the review, characteristic differences in the primary sequences of the Nuo subunits of the enterobacterial complex I compared to the H^+-pumping counterparts will be outlined, following the idea that the exchange of conserved amino acid residues, or the presence of additional redox cofactors, might reflect the altered catalytic properties of the enterobacterial complex. Special attention is given to the membranous NuoL (ND5) subunits that possess striking sequence similarity to secondary Na^+/H^+ antiporters. In the second part, the implications of Na^+ transport by the enterobacterial NDH I on the mechanism of cation transport by complex I, in general, will be discussed.

EXPERIMENTAL EVIDENCE FOR NA^+ TRANSPORT BY COMPLEX I FROM *Klebsiella pneumoniae* AND *Escherichia coli*

The conclusion that complex I from *K. pneumoniae* and *E. coli* catalyzes primary Na^+ transport is based on four lines of evidence, namely, measurements of respiratory Na^+ transport by native membrane vesicles, identification of subunits from complex I in Na^+-stimulated NADH:Q oxidoreductase fractions, growth studies and determination of Na^+ transport in a Na^+/H^+ antiporter-deficient *E. coli* strain, and controls performed with an *E. coli* mutant devoid of complex I.

The respiratory Na^+ transport into native membrane vesicles from *K. pneumoniae* or *E. coli* during the oxidation of NADH or the complex I-specific substrate deaminoNADH with oxygen did not collapse in the presence of a protonophore, indicating that the transport was accomplished by a primary Na^+ pump and not by the combined action of a proton pump and the Na^+/H^+ antiporter(s). In the case of *K. pneumoniae*, the rates increased even threefold in the presence of protonophore, resulting in an internal Na^+ concentration that was three times higher than the external Na^+ concentration (Fig. 1). With both *E. coli* and *K. pneumoniae* vesicles, the Na^+ transport was severely inhibited by the complex I-specific inhibitor rotenone (Krebs *et al.*, 1999; Steuber *et al.*, 2000) (Figs. 1 and 2). An enrichment of *K. pneumoniae* complex I was achieved following the Na^+-stimulated Q reductase activity during purification. This increase in enzymic activity of complex I in the presence of its coupling ion is reminiscent of other primary Na^+ pumps. N-terminal sequencing of two polypeptides present in the Na^+-stimulated NADH:Q oxidoreductase confirmed the identity with the corresponding complex I subunits (Krebs *et al.*, 1999). Further support for the hypothesis that complex I from *E. coli* acts as a Na^+ pump came from studies

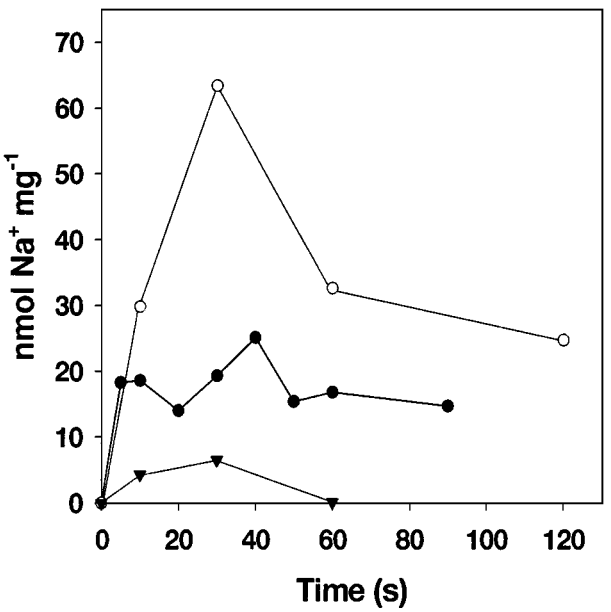

Fig. 1. Respiratory Na^+ transport catalyzed by complex I. The oxidation of deaminoNADH by inverted membrane vesicles of *Klebsiella pneumoniae* with oxygen as electron acceptor drives the transport of Na^+ ions into the vesicles (•). The uptake is stimulated by the protonophore CCCP (○), but inhibited by the complex I-specific inhibitor rotenone (▼).

with mutant strains lacking either secondary Na^+/H^+ antiporters or complex I. The antiporter-deficient strain did not tolerate high concentrations of NaCl in the growth medium under conditions where complex I was repressed, but was not affected by Na^+ ions if grown under complex I-inducing conditions. On the other hand, with membrane vesicles from the *E. coli* mutant devoid of complex I, the rate of respiratory Na^+ transport was severly reduced (Steuber *et al.*, 2000) (Fig. 2). Taken together, these data clearly point to Na^+ transport by complex I from *K. pneumoniae* and *E. coli*.

An important observation is the decrease in Na^+ transport and Q reductase activity of complex I upon aerobic cell rupture of *K. pneumoniae* or *E. coli* cells. Hereby, the two-electron reduction of ubiquinone is followed by dual-wavelength spectrophotometry, since the noncoupled, Q-mediated oxidation of NADH without the formation of the fully reduced substrate ubiquinol is readily observed, even with inactivated complex I preparations. For example, the NADH-driven Na^+ transport by native membrane vesicles from *K. pneumoniae* increased from 2 nmol/min^{-1}/mg^{-1} in aerobically (Dimroth and Thomer, 1989) to 180 nmol/min^{-1}/mg^{-1} in anaerobically prepared vesicles (Krebs *et al.*, 1999), with a concomitant increase in Q reductase activity from 20 nmol/min^{-1}/mg^{-1} to 200 nmol/min^{-1}/mg^{-1}. The

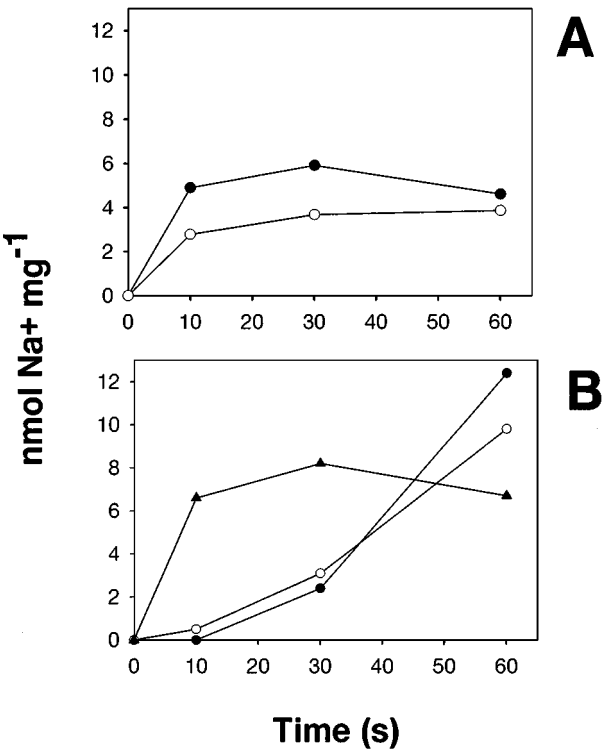

Fig. 2. Comparison of NADH-driven Na^+ transport into membrane vesicles from *E. coli* EP432 or *E. coli* ANN021. The *E. coli* strains EP432 (deficient in the Na^+/H^+ antiporters NhaA and NhaB) and *E. coli* ANN021 (deficient in complex I) were grown on glycerol and fumarate in the presence of 350 mM NaCl. Na^+ uptake into membrane vesicles from *E. coli* EP432 was followed in the presence of 10 μM CCCP with NADH (panel **A**) as electron donor in the absence (•) or presence of 5 μM rotenone (○). Panel **B** shows NADH-driven Na^+ uptake into membrane vesicles of *E. coli* ANN021 in the presence of 5 μM rotenone (▲), 50 μM CCCP (•), or 5 μM rotenone and 50 μM CCCP (○).

inactivation of complex I in the presence of oxygen might be due to an unspecific oxidative damage, but could also indicate the specific requirement for an internal redox cofactor in the reduced state.

THE *nuo* OPERON OF *Klebsiella pneumoniae*

The sequencing of the complex I genes from *E. coli* encoded by the *nuo* operon (Weidner *et al.*, 1993; Blattner *et al.*, 1997) revealed that the *E. coli* NDH I is quite distinct compared to the complexes from mitochondria or α-proteobacteria. The Nuo subunits from *Rhodobacter capsulatus* (Dupuis *et al.*, 1998), *Rickettsia prowazekii* (Andersson *et al.*, 1998) or *Paracoccus denitrificans* (Xu *et al.*, 1993) are more related to the eukaryotic, mitochondrial complex I than to complex I from *E. coli* or other enterobacteria like *K. pneumoniae* and *Salmonella typhimurium* (Wong *et al.*, 1999). For example, the overall sequence similarity of central subunits in the peripheral complex I fragment from *E. coli* with eukaryotic complex I from the yeast *Yarrowia lipolytica* is only 39–52%, compared to 46–77% sequence similarity of the yeast complex with the NDH I from *P. denitrificans* (Djafarzadeh *et al.*, 2000). The genome of *K. pneumoniae* has been sequenced [Wong *et al.*, 1999; (http://genome.wustl.edu/gsc/Projects/bacteria.shtml)], but the data are still erroneous and the annotation of the genes has not yet been completed. Like in *E. coli*, there is only one copy of the *nuo* operon on the genome from *K. pneumoniae* (Fig. 3). Both the *E. coli* and the *K. pneumoniae nuo* genes are arranged consecutively and are preceded by the *lrhA* gene coding for a gene regulator similar to those of the LysR family (Bongaerts *et al.*, 1995). The N-terminal sequences of the NuoF and NuoG subunits that were identified in the Na^+-stimulated NADH:quinone oxidoreductase from *K. pneumoniae* (Krebs *et al.*, 1999) are identical to the sequences of the NuoF and NuoG subunits translated from the DNA sequence. Complex I from *K. pneumoniae* exhibits an overall DNA sequence identity of 84% to the enzyme from *E. coli*. From this high degree of sequence identity, it is reasonable to assume that both complexes have very similar molecular properties. Compared to complex I from eukarya and α-proteobacteria, the enterobacterial NDH I subunits reveal several peculiarities, like C-terminal extensions on the NuoA (ND3, Nqo7), NuoB (PSST, Nqo6), and NuoG (75 kDa, Nqo3) subunits and insertions on the NuoG and NuoI (TYKY, Nqo9) subunits. Note that both the NuoB and NuoI subunits are located in the connecting fragment and are likely candidates for harboring cluster N2. Another important difference between the eukaryotic and the *E. coli* complex I are four additional

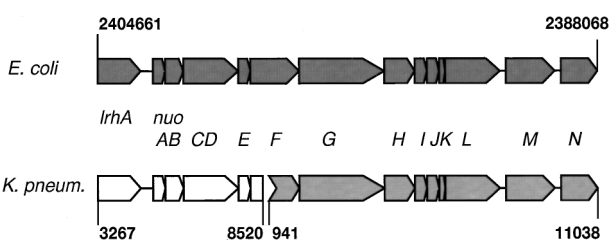

Fig. 3. Comparison of the *nuo* operon from *Escherichia coli* and *Klebsiella pneumoniae*. Numbers indicate the position of the *nuo* genes on the *E. coli* genome (Blattner *et al.*, 1997) or on contig697 (3267–8520) and contig845 (941–11038) from *K. pneumoniae* (Wong *et al.*, 1999). The contigs 697 and 845 overlap in contig405, indicating that like in *E. coli*, the *nuo* genes of *K. pneumoniae* are arranged consecutively in a single operon. In both organisms, an intergenic region upstream of the *nuo* locus is followed by the *lrhA* gene coding for a gene regulator similar to those of the LysR family (Bongaerts *et al.*, 1995).

cysteine residues on the NuoG subunit (Cys_{230}-X_2-Cys_{233}-X_3-Cys_{237}-X_{27}-Cys_{265}, *E. coli* numbering) that might ligate an additional Fe–S cluster tentatively assigned as binuclear cluster N1c from EPR studies (Leif *et al.*, 1995). These additional cysteine residues are also found on the NuoG subunit from the *K. pneumoniae* complex I. The connecting fragment of complex I is made up of subunits NuoB (PSST, Nqo6), NuoI (TYKY, Nqo9), NuoC (30 kDa, Nqo5), and NuoD (49 kDa, Nqo4). In *E. coli*, the *nuoC* and *nuoD* genes are fused, resulting in a NuoCD subunit that is shortened by 30 amino acids (Friedrich, 1998). The amino acid sequence derived from the corresponding DNA fragment suggests that a short-ended, fused NuoCD subunit is also present in the *K. pneumoniae* complex I. Inhibitors acting on the Q site(s) of complex I were used to identify subunits that might participate in Q binding. Biochemical data point to the NuoB subunit (Schuler *et al.*, 1999), whereas mutational analysis indicates the participation of the NuoD subunit (Darrouzet *et al.*, 1998). As outlined above, both subunits belong to the connecting fragment of the Na^+-translocating, enterobacterial complex I and clearly differ from the corresponding subunits of the eukaryotic, H^+-pumping counterpart. These alterations in the putative Q-binding subunits of the enterobacterial complex I could be important for the binding or reduction of naphtoquinones, in addition to ubiquinone.

COMPARISON OF THE NuoL (ND5) SUBUNIT OF COMPLEX I WITH SECONDARY Na^+/H^+ ANTIPORTERS

The observation that Na^+ is transported by complex I from *K. pneumoniae* and *E. coli* raises two fundamental questions: Are Na^+ ions transported instead of (or in addition to) protons, and which subunits of complex I contribute to Na^+ translocation? Without a direct assay for cation transport by complex I, the assignment of transport functions to distinct subunits is speculative and based on sequence comparisons.

It has been early recognized that the membranous NuoL (ND5, Nqo12), NuoM (ND4, Nqo13), and NuoN (ND2, Nqo14) subunits have very likely arisen from a common ancestor (Kikuno and Miyata, 1985) and show sequence similarity to Na^+/H^+ antiporters, which seemed to support their participation in H^+ translocation (Friedrich, 1998). The novel class of multisubunit Na^+/H^+ antiporters related to NuoL was discovered by complementation of an alkali-sensitive mutant of *Bacillus* C-125 and restored secondary, $\Delta\mu H^+$-driven Na^+ efflux from whole cells (Hamamoto *et al.*, 1994). The authors identified a single open reading frame encoding for a hydrophobic protein that exhibited striking sequence similarity to the membranous ND5 subunit (NuoL homolog) of complex I. Especially in the N-terminal region, three well-conserved domains were recognized (Fig. 4). However, the expression of this putative Na^+/H^+ antiporter did not increase the NADH dehydrogenase activity in *Bacillus* membranes (Hamamoto *et al.*, 1994). A corresponding gene, *mnhA*, was identified in *Staphylococcus aureus* and was shown to be part of an operon encoding seven genes (*mnhA* through *G*), with *mnhA* and *mnhD* encoding hydrophobic polypeptides with strong similarities to NuoL and NuoN, respectively (Hiramatsu *et al.*, 1998). From heterologous complementation studies using Na^+/H^+ antiporter-deficient or alkali-sensitive *E. coli* strains, the authors concluded that all seven gene products of the *mnh* operon were required in order to confer Na^+/H^+ antiport activity. Such a large antiporter with an approximate mass of 200 kDa was unexpected compared to the single-subunit, bacterial antiporters described thus far (Padan and Schuldiner, 1993). Genetic studies with the corresponding *mrp* operon in *Bacillus subtilis* showed that MrpA-dependent Na^+/H^+ antiport is dependent on the other *mrp* gene products (Ito *et al.*, 1999), while the *mrpF* gene product (*mnhF* in *S. aureus*) functions in Na^+ or cholate efflux independently of any other *mrp* gene product (Ito *et al.*, 2000). In summary, it seems likely that the *nuoL* homolog (*mnhA* or *mrpA*) encode structural genes for Na^+/H^+ antiporters, but that other gene products of the multisubunit Na^+/H^+ antiporter operons may also be required for Na^+ extrusion (Ito *et al.*, 2000). Based on these studies, it is proposed that the membraneous NuoL subunit of complex I from *E. coli* or *K. pneumoniae* participates in Na^+ binding or transport. Most likely, active Na^+ pumping is only catalyzed by the entire complex, but membranous subcomplexes may have the capacity for passive Na^+ (or H^+) translocation as observed with the F_0 part of the F_1F_0-ATP synthase. Assembly studies performed with complex I showed that distinct parts of the membrane arm are formed that might also represent functional units. In the fungus *Neurospora crassa*, a small intermediate containing the ND5 (NuoL, Nqo12) and ND2 (NuoN, Nqo14) subunits was identified (Schulte *et al.*, 1994). Recent biochemical (Sazanov *et al.*, 2000) and structural (Sazanov and Walker, 2000) data suggest that the large ND5 (NuoL) subunit is situated at the distal end of the membrane domain of bovine complex I and is connected to the neighboring ND4 (NuoM) subunit by a thin stalk.

A switch from a H^+ pumping to a Na^+-transporting primary pump can be achieved by the exchange of only few conserved amino acid residues that act as ligands for Na^+ ions, as exemplified for subunit *c* of the membranous

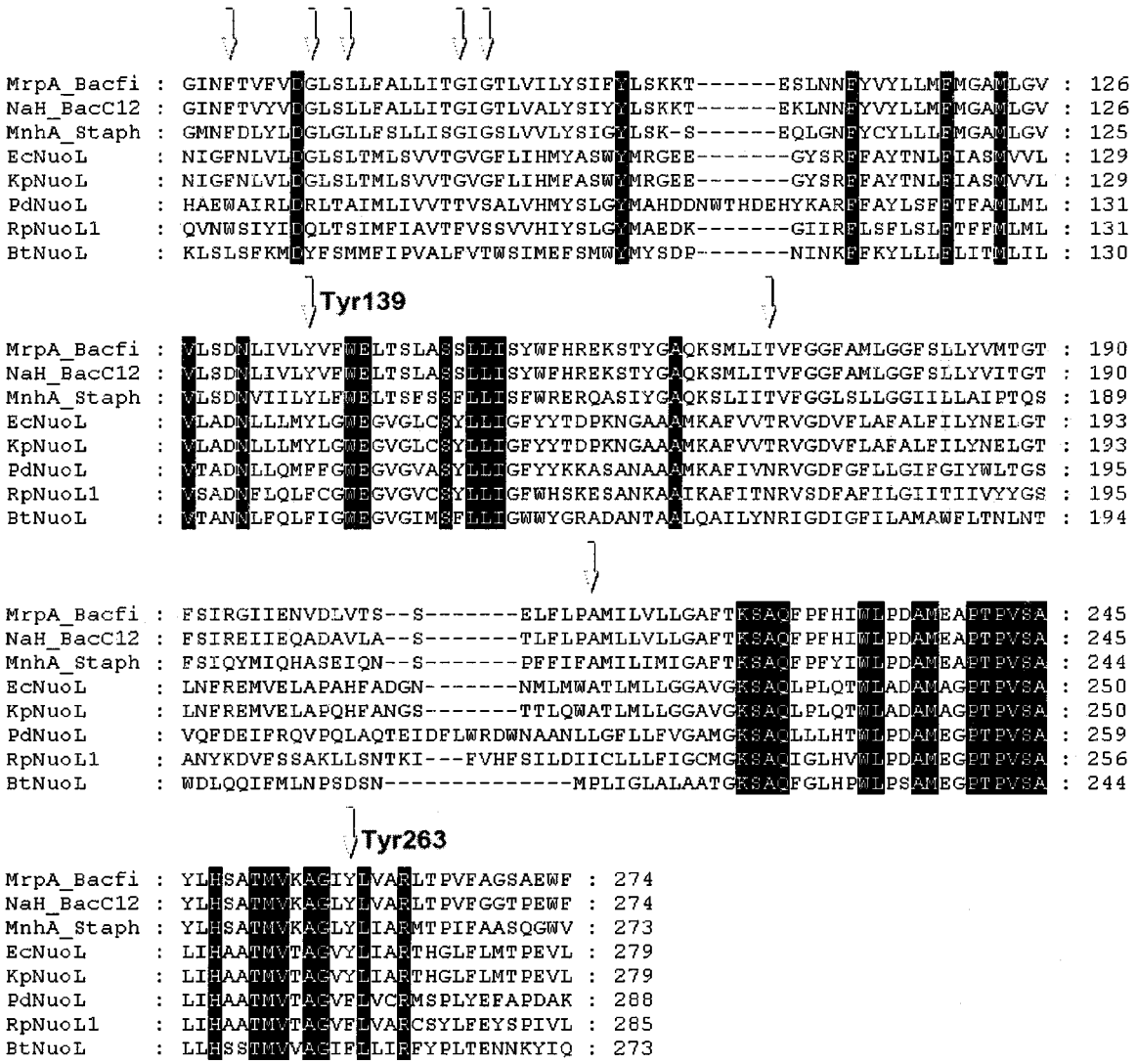

Fig. 4. Conserved domains in secondary Na^+/H^+ antiporters and the NuoL (ND5, Nqo12) subunits of complex I. The N-terminal part of the sequences of Na^+/H^+ antiporters from *Bacillus* strain C-125 (accession number BAA06609) (Hamamoto *et al.*, 1994), *Bacillus subtilis* (YufT, designated as MrpA, accession number Z93937) (Ito *et al.*, 1999), and *Staphylococcus aureus* (MnhA, accession number BAA35095) (Hiramatsu *et al.*, 1998) were aligned with the NuoL (ND5, Nqo12) subunits from *E. coli* (accession number AE000317) (Blattner *et al.*, 1997), *Klebsiella pneumoniae* (Wong *et al.*, 1999), *Paracoccus denitrificans* (accession number L02354) (Xu *et al.*, 1993), *Rhodobacter capsulatus* (accession number AF029365) (Dupuis *et al.*, 1998), *Rickettsia prowazekii* (Andersson *et al.*, 1998), and *Bos taurus* (accession number P03920) (Anderson *et al.*, 1982) using the program ClustalX (Jeanmougin *et al.*, 1998). Conserved residues are indicated by astericks. The arrows indicate residues in the complex I-related domains of Na^+/H^+ antiporters that are conserved in the NuoL subunits of the Na^+-translocating NDH I from *E. coli* or *K. pneumoniae*, but not in the H^+-pumping eukaryotic complex I or in NDH I from α-proteobacteria. Since Tyr139 and Tyr263 are polar residues in predicted transmembrane regions (von Heijne, 1992), they are prime candidates for the liganding of Na^+.

F_0 part from the Na^+-dependent F_1F_0-ATP synthase from *Propionigenium modestum*. Mutagenesis studies showed that the binding of Na^+ is accommodated by three amino acid residues on subunit *c* that act as ligands (Q32, S66, E65), while for H^+ transport, a single Glu (E65) is sufficient (Kaim *et al.*, 1997). In the NuoL subunit, critical residues that determine the cation specificity are probably found in domains that are related to Na^+/H^+ antiporters (Fig. 4). If the principles of Na^+ or H^+ binding established for the F_1F_0-ATP synthase are also valid for other primary Na^+ pumps, one could speculate that residues that are conserved in the enterobacterial NuoL subunits

and the Na^+/H^+ antiporters, but not in the H^+-pumping, eukaryotic complex might act as ligands for Na^+. Hereby, polar residues in hydrophobic, transmembrane domains (von Heijne, 1992) like Tyr139 and Tyr263 are prime candidates for conferring the Na^+ specificity (Fig. 4).

COUPLING OF ELECTRON TRANSFER TO Na^+ TRANSPORT

The finding that the electrogenic NADH:Q oxidoreductases (NDH I) from *K. pneumoniae* and *E. coli* are capable of Na^+ translocation has important implications for the mechanism of cation translocation by complex I, in general. Detailed descriptions of putative mechanisms of H^+ translocation by complex I are given in the reviews of Brandt (1997, 1999) and Dutton and colleagues (Dutton *et al.*, 1998). Since only H^+, but not Na^+ can be transported across the membrane by diffusion of the proton carrier QH_2, a mechanism of cation translocation by complex I from *K. pneumoniae* and *E. coli* that exclusively relies on a Q cycle-type mechanism (Mitchell, 1976) can be excluded. As a consequence, a Na^+-translocating redox pump is likely to operate. As outlined above, the similarity of the NuoL (ND5, Nqo12) subunit from complex I to Na^+/H^+ antiporters suggest a role of the former in cation transport. The contribution of the NuoL subunit is also supported by the evolutionary relationship of some complex I subunits to membrane-bound hydrogenases. These multisubunit complexes catalyze the reversible oxidation of H_2 to $2H^+ + 2e^-$ utilizing specialized H^+ pathways. Together with the NuoB (PSST), NuoC (30 kDa), NuoD (49 kDa), NuoH (ND1), and NuoI (TYKY) subunits, the NuoL (ND5) subunit is, therefore, thought to be part of the catalytic core of complex I (Friedrich and Scheide, 2000).

In the following, it is assumed that cations follow a conserved translocation pathway provided by the NuoL subunit to or from a single site of redox-driven cation translocation, with either Na^+ or H^+ being the preferred substrate. A single translocation pathway for either Na^+ or H^+ is also found in the Na^+-translocating ATPase from *Propionigenium modestum*, where protons are translocated only in the absence of Na^+ (Dimroth *et al.*, 2000). Another possible scenario that is considered less likely is presence of two distinct coupling sites (and transport pathways) in the enterobacterial complex I, one specific for Na^+ and the other for protons.

The electrons derived from NADH oxidation by complex I are ultimately delivered to the Fe–S cluster(s) with the highest midpoint redox potential, termed N2 (or 2). It seems to be generally accepted that cluster N2 donates electrons to an as yet unidentified, enzyme-bound quinone or quinoid cofactor acting as a proton-pumping element. Reoxidation of this cofactor by external Q acting as substrate is thought to be linked to H^+ translocation (Brandt, 1997; Dutton *et al.*, 1998).

As outlined above, a conserved cation transfer path and translocation mechanism for both the Na^+-coupled enterobacterial and the H^+-coupled eukaryotic complex I are likely. However, while transported H^+ can intermediately be trapped under formation of quinol or a neutral semiquinone radical, Na^+ ions cannot be covalently linked to the putative quinoid redox pump during turnover. It is, therefore, proposed that the central pumping unit of the Na^+-translocating complex I is a quinoid group carrying a negative charge that attracts a Na^+ from the negative side of the membrane. During reoxidation of the cofactor, the Na^+ is pushed to the positive side of the membrane, hereby generating an electrochemical potential. This concept is based on the electroneutrality principle that states that a negative charge generated in an environment of low dielectric strength is compensated by the uptake of a positive charge (Rich *et al.*, 1995). The central redox cofactor of the Na^+-translocating complex I is thought to be a bound quinoid group that shuttles between the fully oxidized and the one-electron reduced, anionic state (Fig. 5). The quinoid cofactor is most likely reduced by the high-potential cluster N2 of complex I, generating an enzyme-bound semiquinone that is deprotonated at physiological pH (pK_a $QH^\bullet/Q^{\bullet-} = 4.9$) (Rich, 1984). Upon formation of the bound semiquinone anion, Na^+ is taken up from the negative side of the membrane, forming an ion pair with the negatively charged semiquinone. The one-electron oxidation of the semiquinone by external Q (substrate) is coupled to the extrusion of Na^+ against the electrochemical potential to the positive side of the membrane. A second cycle of Na^+ uptake and extrusion is initiated by the incoming second electron that again generates a semiquinone anion. This time, the bound semiquinone anion is oxidized by the substrate semiquinone remaining bound to the enzyme and the two-electron reduced product quinol is formed (Fig. 5). The formation of quinol from semiquinone is an exergonic reaction ($E^{\circ\prime}$ $QH_2/QH^\bullet + H^+ = +190$ mV) that drives the unfavorable reduction of quinone to semiquinone ($E^{\circ\prime}Q^{\bullet-}/Q = -240$ mV) (Rich, 1984) in the preceding reaction steps. Following this idea for the H^+-translocating complex I, the protonation of the anionic quinoid cofactor could result in the formation of a neutral semiquinone. The subsequent oxidation of the quinoid cofactor could be tightly coupled to proton release. Net transport across the membrane would require a gating mechanism that forces H^+ (or Na^+) to enter the active site from the negative side and to escape to the positive side of the membrane. A fast-relaxing semiquinone

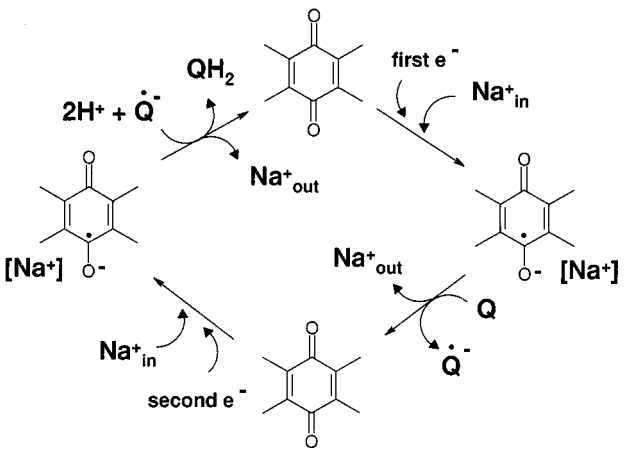

Fig. 5. A putative mechanism of Na^+ translocation by complex I. The central redox cofactor of the pump is a bound quinoid compound (symbolized by the quinoid ring) that shuttles between the oxidized and one-electron reduced, deprotonated state (semiquinone anion). Q, $Q^{\bullet -}$, and QH_2 indicates the oxidized, one or two-electron reduced state of external quinone acting as substrate. Upon reduction by the first electron, the bound quinone is converted into the semiquinone anion and Na^+ is taken up from the negative side of the membrane to form the ion pair ($Q^{\bullet -}$ [Na^+]). Upon reoxidation of the semiquinone anion by external Q, Na^+ escapes to the positive side of the membrane. This one-electron transfer reaction converts the external Q into the semiquinone state, in which it probably remains enzyme-bound until it becomes fully reduced to the quinol in the second cycle. The second cycle of Na^+ uptake and extrusion is initiated by the incoming second electron. Per NADH oxidized, two electrons are transferred to quinone, and two Na^+ are translocated.

species (SQ_{Nf}) has been detected in the complex I segment of the bovine respiratory chain that is affected by the electrochemical proton potential posed across the membrane. The narrow line width of the EPR spectrum of SQ_{Nf} indicates that it is in the anionic form (Yano *et al.*, 2000). If this energy-coupled semiquinone species (SQ_{Nf}) detected in coupled submitochondrial particles is dependent on the electrical component of the proton motive force, it is most probably in the anionic state. A neutral semiquinone would not be affected by the transmembrane voltage, while a positively charged semiquinone is not formed under physiological conditions (Brandt, 1999). A reaction scheme that is based on charge compensation of the semiquinone anion by the coupling cation yields a transport stoichiometry for complex I of $1Na^+$ (or $1H^+$)/e^- (Fig. 5). The transport stoichiometry of complex I has not yet been determined, since neither mitochondrial nor bacterial complex I has been purified in an active state that allows reconstitution into proteoliposomes. From studies with submitochondrial particles or whole *E. coli* cells, the ratio protons translocated to electrons transferred by complex I is considered to be $4H^+/2e^-$ (Brandt, 1997; Galkin *et al.*, 1999; Wikström, 1984) or $3H^+/2e^-$ (Bogachev *et al.*, 1996), respectively. It should be considered that the reduction of quinone by complex I under concomitant uptake of two protons might occur at the negative side of the membrane, while the oxidation of the quinol by another respiratory enzyme could result in the liberation of protons at the positive side of the membrane. By this simple arrangement, the transport stoichiometry measured for the complex I segment in native membrane vesicles could increase to $2Na^+ + 2H^+/2e^-$ or $4H^+/2e^-$, respectively. In *E. coli*, fumarate respiration with NADH depends on the electrogenic complex I (Bongaerts *et al.*, 1995). The high-resolution structure of the *E. coli* fumarate reductase revealed two menaquinones located close to the negative or the positive aspect of the membrane, respectively (Iverson *et al.*, 1999). For the NADH:fumarate segment of the *E. coli* respiratory chain, maximal H^+/e^- ratios of 1.7 (menaquinone) or 2.5 (demethylmenaquinone) have been calculated (Tran and Unden, 1998). This overall transport of approximately four protons/$2e^-$ could be explained by the action of complex I that transports 2 H^+ (or Na^+)/$2e^-$ under reduction of menaquinone at the negative aspect of the membrane and the release of two protons at the positive side of the membrane during menaquinol oxidation by the fumarate reductase.

CONCLUDING REMARKS

The existence of a Na^+-dependent enterobacterial complex I gives strong experimental support for the operation of a primary redox pump in complex I. The altered cation selectivity of the enterobacterial complex I will have several experimental advantages, like the direct determination of the Na^+/e^- ratio, or the identification of amino acid residues that participate in Na^+ binding.

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