ELSEVIER

Biochimica et Biophysica Acta 1364 (1998) 134–146

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

The NADH:ubiquinone oxidoreductase (complex I) from *Escherichia coli*

Thorsten Friedrich *

Institut für Biochemie, Universität Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany

Received 19 November 1997; revised 29 January 1998; accepted 30 January 1998

Keywords: NADH dehydrogenase; Complex I; NADH:ubiquinone oxidoreductase; Electron microscopy; EPR spectroscopy; FeS-cluster; *Escherichia coli*

1. Introduction

The aerobic respiratory chain of *Escherichia coli* contains several primary dehydrogenases, including NADH dehydrogenase, succinate dehydrogenase, and D-lactate dehydrogenase, which oxidize organic substrates and deliver the electrons to membrane bound ubiquinone-8. The ubiquinol is then reoxidized by reductases, and the terminal oxidases. This exergonic process is coupled with the endergonic translocation of protons across the cytoplasmic membrane. The resulting membrane potential is used to drive energy-consuming processes like ATP synthesis, solute transport and flagellar motion [1,2].

The number of NADH dehydrogenases and their role in energy transduction had been under debate for a long time. Today, it is widely accepted that *E. coli* possesses two different NADH dehydrogenases called NDH-I and NDH-II [3,4]. The NDH-II is a single polypeptide enzyme with FAD as the only redox group [5,6]. It is a non proton-pumping NADH:ubiquinone oxidoreductase. The NDH-I is homologous to the mitochondrial complex I and is therefore also called complex I. Like the mitochondrial complex, it is a multi-subunit enzyme and couples the electron transfer from NADH to ubiquinone with a proton translocation according to the overall equation:

$$NADH + Q + 5H_n^+ \rightarrow NAD^+ + QH_2 + 4H_p^+ \qquad (1)$$

where Q refers to ubiquinone, and H_n^+ and H_p^+ to the protons taken up from the negative inner and delivered to the positive outer side of the membrane. The H^+/e^- stoichiometry of the enzyme in the cytoplasmic membrane has been determined to be at least 1.5 [7]. The stoichiometry of the mitochondrial complex I equals 2 [8]. The NDH-I is characterized by its prosthetic groups, namely 1 FMN and up to 9 iron–sulfur (FeS) clusters, by its sensitivity to a number of naturally occurring compounds, e.g., the antibiotic piericidin A or the acarizide annonine (Refs. [9,10], see also article by M. Degli-Esposti, this volume), and by its high number of subunits. The bacterial

Abbreviations: bp, Base pair; EPR, Electron paramagnetic resonance; d-NADH, deamino-NADH; FMN, Flavin mononucleotide; FeS, Iron–sulfur; NDH-I, Proton-pumping NADH:ubiquinone oxidoreductase; NDH-II, Non-proton-pumping NADH:ubiquinone oxidoreductase; Q, Ubiquinone; QH_2, Ubiquinol

* Corresponding author. Fax: +49-211-81-15310; E-mail: thorsten.friedrich@uni-duesseldorf.de

PII S0005-2728(98)00024-3

complex I is made up of 14 different subunits [11]. Two of these are fused in *E. coli* resulting in a 13 subunit enzyme. The mitochondrial complex contains many additional polypeptides [11–13]. Therefore, the bacterial complex I is considered as a minimal structural form of a proton-pumping NADH:ubiquinone oxidoreductase [14].

The initial confusion about the nature of the *E. coli* NADH dehydrogenases arose from mutants that were unable to grow on mannitol as sole carbon source [15]. These mutants were incorrectly believed to contain single-locus mutations. One mutation was mapped to min 22 at the *E. coli* chromosome, with the corresponding gene *ndh* [5]. The encoded protein was purified as a single polypeptide enzyme of 47 kDa, which is now known as NDH-II. This enzyme restored the KCN-sensitive NADH oxidation of an *ndh*$^-$ mutant strain without contributing to a membrane potential. From these experiments, it was concluded that *E. coli* contains only one NADH dehydrogenase not being energy converting [16,17].

However, this was contradictory to the finding that *E. coli* cells respiring on malate show a higher H^+/O ratio than those respiring on succinate, and that *E. coli* is capable to reduce NAD^+ by a reverse electron flow from succinate or glycerol-1-phosphate in an uncoupler-sensitive manner [18,19]. The presence of two different types of NADH dehydrogenases was also concluded from the detection of at least two NADH dehydrogenases by immunological methods [20]. Furthermore, it was demonstrated that one of the two NADH dehydrogenases reacts with both NADH and deamino (d)-NADH as substrates, the d-NADH reductase activity being linked to the generation of a membrane potential in the presence of KCN and ubiquinone-1 [21]. EPR spectroscopic characterization of aerobically grown *E. coli* membranes showed the presence of at least two binuclear and two tetranuclear FeS clusters, which are associated with NADH dehydrogenase [22].

The presence of two different gene loci for NADH dehydrogenases was demonstrated by introducing an insertional mutation in the *ndh* gene. The resulting strain still showed membrane-bound NADH dehydrogenase activity from a second enzyme. The locus of the second enzyme was mapped to min 51 on the *E. coli* chromosome [23]. This locus was cloned and sequenced, and the complex was isolated as a multisubunit complex homologous to the eucaryotic mitochondrial complex I [3,24]. The gene locus of NDH-I was named *nuo* (from *N*ADH:*u*biquinone *o*xidoreductase). The presence of both coupled and non-coupled NADH dehydrogenases is required for optimal growth under several growth conditions [25,26].

2. Physiological role of complex I

The respiratory chains in bacteria do not only generate a proton motive force, but also rapidly remove an excess of reducing equivalents in order to recycle NAD^+. Under certain growth conditions, the recycling is more important than the maximum energy yield of respiration [25,26]. Recently, it has been shown that *E. coli* mutants lacking complex I have a competitive disadvantage during stationary growth phase [27]. This phenomenon might be explained with the lower amount of energy that can be obtained from any carbon source given the poor availability of nutrients in the stationary phase. Furthermore, complex I mutants grow poorly on acetate, or on some amino acids as sole carbon source [28]. In the same study, these mutants were reported to fail to produce the L-aspartate chemotactic band on tryptone swarm plates. The reason for this phenotype is most likely that NADH cannot be sufficiently recycled, and that the resulting large $NADH/NAD^+$ ratio inhibits tricarboxylic acid cycle enzymes and the glyoxylate shunt [28].

In *Salmonella thyphimurium*, it has been observed that complex I mutants are unable to activate ATP-dependent proteolysis under starvation conditions [29]. In *Myxococcus xanthus*, complex I is necessary for protein secretion during vegetative growth, development, and sporulation [30]. From all these findings, it can be concluded that complex I mediates an important metabolic switch in bacteria. Therefore, the expression of this complex has to be regulated very tightly.

3. Genes and regulation

The genes of the *E. coli* complex I are organized in the so-called *nuo*-operon, a 15,020-bp DNA region at min 51 of the chromosome (Fig. 1b) [3]. The locus

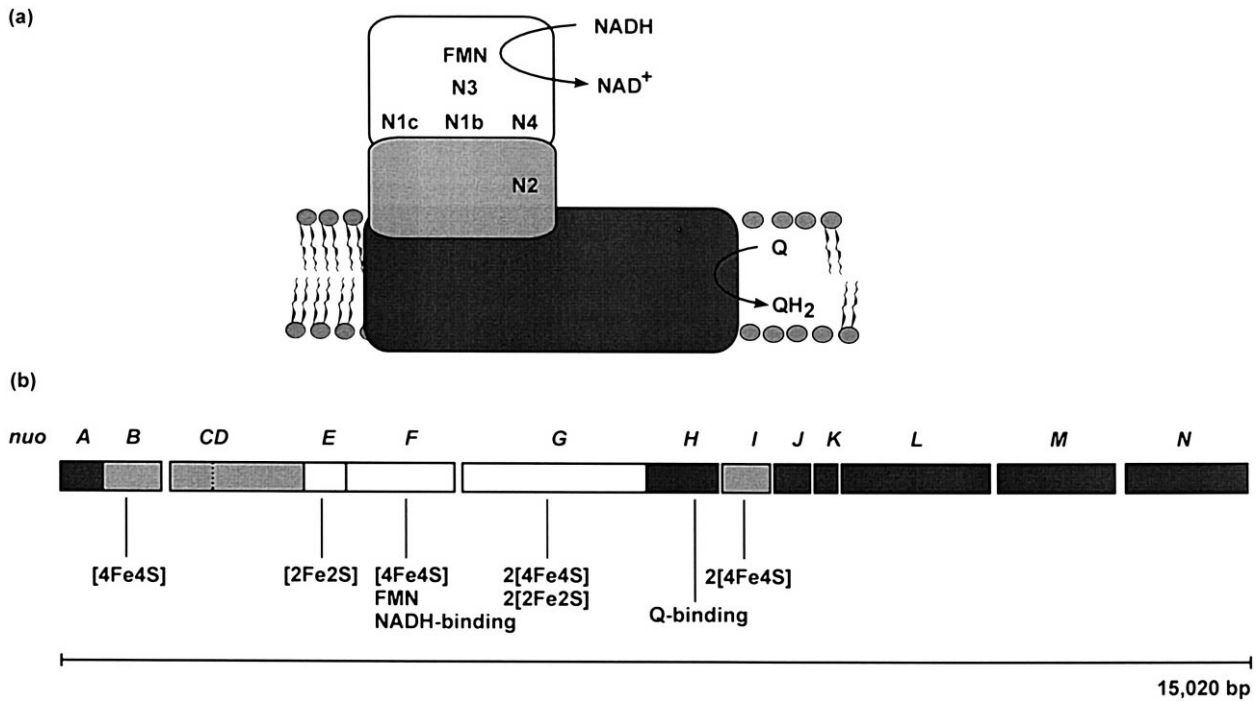


Fig. 1. Topological model of complex I and the *nuo*-operon. (a) Arrangement of the *E. coli* complex I fragments and (b) order of the corresponding genes. A–N refer to the genes of the operon, N1b, N1c, N2, N3 and N4 to the EPR-detected FeS clusters and Q to ubiquinone. The binding sites of the cofactors and substrates as predicted from the sequence are indicated. The NADH dehydrogenase fragment and the genes encoding the subunits of this fragment are drawn in white, the connecting fragment and its genes are shown in light grey, and the membrane fragment and its genes are shown in dark grey.

contains 13 structural genes named *nuoA-N*. Due to a sequencing error, it was initially believed that *nuoC* and *D* constitute separate genes [3], as it is the case in all other bacteria [13], and that the *nuo*-operon coded for 14 polypeptides. A TGA stop-codon in the 3′-region of *nuoC* is present in the sequence of the *nuo*-operon [3], as well as in the sequence of the *E. coli* genome published by Blattner et al. [31]. Due to a mismatch between both sequences upstream of that stop codon, the TGA is in frame in the *nuo*-sequence but not in Blattner's sequence. Since *nuoCD* codes for one protein that is present in a complex I preparation from *E. coli* (see below), the latter is correct [32]. The genes *nuoC* and *D* are also fused in *Buchnera aphidicola* [33]. The homologue of *nuoCD* in the formate hydrogenlyase of *E. coli* is also fused to one gene *hycE*, coding for the large subunit of the hydrogenase [13,34,35]. Due to another sequencing error, *nuoG* is 255 bp larger than published in Ref. [3]. The genes *nuoG* and *H* meet each other, and there is no intergenic region between them as published earlier. The molecular mass of NuoG as deduced from the DNA sequence is in agreement with its apparent mass determined from SDS-PAGE (Ref. [32] and Table 1).

The order of the 14 or 13 genes, respectively, is highly conserved within the domain of bacteria. It is also found in *Paracoccus denitrificans*, *Rhodobacter capsulatus*, *Thermus thermophilus*, and *Synechocystis* [3,11,36–38]. Preparations of the *E. coli* complex can easily be cleaved resulting in a fragmentation pattern that reflects the order of the genes in the *nuo*-locus (see below).

As expected, the *nuo*-genes are co-transcribed, since the insertion of a kanamycin resistance cassette in *nuoB*, *D*, *F*, *H*, or *I* leads to a complete loss of complex I as measured by immunological, EPR spectroscopic and kinetic methods (A. Berger and T. Friedrich, unpublished data). The polar effect of the resistance cassette inhibits the proper transcription of the genes located downstream. This has also been described for the *nuo*-operon of *S. thyphimurium* [39]. A detailed genetic analysis of the non-polar *Δ*(*nuoF-L*) and *Δ*(*nuoG*) *E. coli* mutants revealed the inability to assemble a functional complex I [40]. The *Δ*(*nuoG*) mutant lacks 85 amino acids at the C-terminus of NuoG. This mutant could be complemented with a wild-type *nuoG* allele showing the recessive nature of the mutation. Although all *nuo*-genes seem to be transcribed in the *Δ*(*nuoG*) mutant, there is no assembled complex I in this mutant [40]. Destruction of the last gene of the *E. coli nuo*-operon, *nuoN*, by insertion of the resistance cassette leads to the assembly of the NADH dehydrogenase fragment (see below) of the complex in the cytoplasm (A. Berger and T. Friedrich, unpublished data).

The promotor region of the *nuo*-operon consists of a 560-bp upstream region. Expression of the *nuo*-op-

Table 1
Nomenclature and properties of the complex I subunits of *E. coli*

Designation of the subunit	Molecular mass (kDa)	No. of predicted membrane helices	N-terminal sequence	Predicted function
NuoA	16.3	3		
NuoB	25.1	1/0[a]	MDYTLT	1 × [4Fe–4S]
NuoCD	67.9	0	MVNN/TVLQ[b]	Proton channel
NuoE	18.6	0	MHENQ	1 × [2Fe–2S]
NuoF	49.5	0	MKNIIRTPE	NADH binding; FMN; 1 × [4Fe–4S]
NuoG	100.5	0	MATIHV	2 × [4Fe–4S]; 2 × [2Fe–2S]
NuoH	36.5	8		Ubiquinone binding
NuoI	20.6	1/0[a]	MTLKF	2 × [4Fe–4S]
NuoJ	19.9	5		
NuoK	11.4	3		
NuoL	66.3	13		Proton translocation
NuoM	56.7	12		Proton translocation
NuoN	46.1	10		Proton translocation

[a]The number of predicted membrane spanning helices of these subunits varies depending on the used prediction method.
[b]This is an internal amino acid sequence (No. 233 to 236) obtained after proteolytic digestion of NuoCD.

eron is regulated by O_2 and nitrate via the regulators ArcA, NarL, FNR, the integration host factor, and by other factors including C_4-dicarboxylates [41]. The expression is not stimulated by growth on substrates supplying high amounts of NADH, e.g., glucose or glycerol. The reason might be that maximal energy conversion is only performed under conditions of NADH limitation. Out of the three quinones present in *E. coli*, namely ubiquinone ($E_{m,7} = +110$ mV), demethylmenaquinone ($E_{m,7} = +40$ mV), and menaquinone ($E_{m,7} = -80$ mV) [42], only ubiquinone and possibly demethylmenaquinone are expected to be used by complex I for thermodynamic reasons. Nevertheless, it has been shown by Tran et al. [43] and Unden and Bongaertz [44] that the alternative NADH dehydrogenase II is more important in aerobic respiration than complex I, while the latter is essential for respiration on fumarate and dimethylsulfoxide. Both NADH dehydrogenases participate in nitrate respiration [43]. This implies that complex I is able to use menaquinone as electron acceptor, since menaquinone and demethylmenaquinone are the major quinones at growth on fumarate and dimethylsulfoxide [44]. This has consequences for the mechanism of complex I because difference of the redox potential of NADH and quinone is roughly 190 mV lower when using menaquinone as acceptor compared to ubiquinone (see below).

The expression rate of the *nuo*-operon was increased fourfold by replacing its 5′-promotor region by the T7-RNA-polymerase promotor and transforming the mutants with an inducible plasmid coding for T7-RNA-polymerase [45]. This has been confirmed by measuring d-NADH/ferricyanide activity, and by EPR spectroscopy. Other membrane proteins, however, can be overproduced 30-fold by using this method [46,47]. This indicates that there are most likely *cis*-acting regulatory elements that do not allow a higher expression of the *nuo*-operon than it has been achieved.

4. Protein composition

The *E. coli* complex I has been purified to homogeneity by liquid chromatography in the presence of an alkyl polyglucoside (APG) detergent [24] and in the presence of dodecyl maltoside [45]. Both preparations are made up solely of the 13 subunits encoded by the *nuo*-genes (Fig. 2). Six subunits are peripheral proteins. NuoB, E, F, G and I have been assigned to the corresponding *nuo*-genes by N-terminal sequencing (Ref. [24] and Table 1). Subunit NuoCD was proteolytically cleaved in the preparation in APG so that its N-terminal sequence was found in a 25-kDa protein, while an internal sequence of NuoCD was found in a 65-kDa polypeptide [24]. Using the preparation in dodecylmaltoside, NuoCD has been assigned to the 70 kDa polypeptide as shown in Fig. 2 [45]. The remaining 7 subunits are very hydrophobic proteins predicted to fold into 54 α-helices across the membrane. Their homologues in the eucaryotic com-

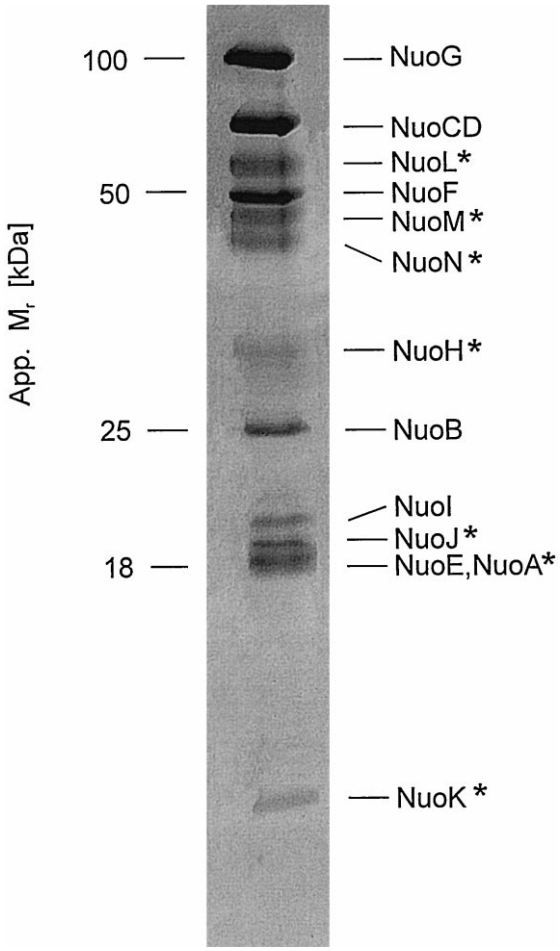


Fig. 2. SDS-PAGE of the *E. coli* complex I. The numbers refer to the apparent molecular masses in kDa. Asterisks indicate the 7 membrane subunits that are mitochondrially encoded in the eucaryotic complex I. These subunits are poorly stained with coomassie blue.

plex are mitochondrially encoded. They have been assigned to the *nuo*-genes by their apparent molecular mass in SDS [24]. One non-covalently bound FMN, two binuclear (N1b and N1c), and three tetranuclear (N2, N3 and N4) EPR detectable FeS clusters have been found in both preparations. The binuclear FeS cluster N1a found in other species was not detected. Since the sequence motif presumed to harbour this cluster is present, we suggest this cluster to be present in the *E. coli* complex while not being reducible with NADH [24,48,49].

After reconstitution into artificial phospholipid membranes, both preparations show NADH/ubiquinone-2 reductase activity, which is sensitive to inhibitors. The affinity to NADH and ubiquinone is not changed compared to the values obtained with membrane preparations [24,45]. The NADH/ubiquinone-2 activity of the enzyme in dodecylmaltoside, as well as its sensitivity to piericidin A and annonine VI is increased compared to the preparation in APG. This might reflect a higher portion of active enzyme in this preparation [10,24].

5. Fragmentation of complex I and relationship of the fragments to other enzymes

By raising the pH and changing the detergent to Triton X-100, the complex I preparation in APG is readily cleaved into three parts, called NADH dehydrogenase fragment, connecting fragment and membrane fragment [24]. The genes of the subunits that make up the individual fragments are clustered together in the operon, except that *nuoA* and *nuoI* have changed places (Fig. 1). Thus, the fragility of the isolated complex helped to recognize its modular structure [11,24].

The water-soluble NADH dehydrogenase fragment is made up of the subunits NuoE, F, and G. It contains the FMN- and NADH-binding site and the EPR-detectable FeS clusters N1b, N1c, N3, and N4. This fragment was obtained as a stable preparation with a molecular mass of approximately 170 000 Da [24]. It is capable of transferring electrons from NADH to ferricyanide with the same K_m values as the entire complex. It therefore represents the electron input part of complex I. The fragment also catalyzes the reduction of water-soluble ubiquinone analogues with a lowered affinity, indicating the presence of non-physiological binding site(s) [24]. The fragment was overproduced and isolated from *E. coli* [32]. Although it is made up of the subunits NuoE, F, and G, overexpression of the corresponding genes leads to overproduction of three apoproteins that are not assembled. With coexpression of *nuoB* and *CD*, the cofactors are incorporated, and the fragment is properly assembled. It is most likely that at least NuoCD acts as a base on which the NADH dehydrogenase fragment is built up [32]. Like the NADH dehydrogenase fragment obtained by splitting of the entire complex I, the overproduced fragment contains the EPR detectable FeS clusters N1b, N1c, N3, and N4 with spectral characteristics identical to those of the corresponding clusters in complex I [32].

The NADH dehydrogenase fragment represents a protein module that switches reversibly from one two-electron to two one-electron transfer steps. It is evolutionarily related to the diaphorase part of a bacterial NAD^+-reducing hydrogenase [12,13,50,51]. The NAD^+-reducing hydrogenase from *Alcaligenes eutrophus* is made up of the α, β, γ, and δ subunits. The β/δ dimer functions as a hydrogenase, the α/γ dimer as a diaphorase [52]. The diaphorase part contains one FMN and several FeS clusters [53–55]. The N-terminus of the α subunit is homologous to NuoE, its C-terminus to NuoF. The γ subunit is homologous to the N-terminus of NuoG. This hydrogenase is not only present in *A. eutrophus* and *Rhodococcus opacus* [56] but also in cyanobacteria like *Anabaena variabilis, Desulfovibrio fructosovorans* and *Synechocystis* sp. PCC6803 [57–59]. Sequence comparisons point to a divergent evolution of the NADH dehydrogenase fragment and the diaphorase part of the hydrogenases after phylogenetic separation [13].

The connecting fragment is made up of NuoB, CD, and I and contains the EPR detectable FeS cluster N2 [24]. This fragment is only soluble in the presence of detergent, although none of the subunits is unambiguously predicted to form membrane spanning helices. The fragment is assumed to connect the NADH dehydrogenase fragment with the membrane fragment. A fragment consisting of the homologues of NuoB, CD, and I was isolated from *Synechocystis* membranes [60]. The connecting fragment might constitute another structural module. In addition, NuoB

and NuoCD show a very weak homology to the small and the large subunits of hydrogenases [3,12,13,61–63]. NuoB and the small subunit of hydrogenases share a sequence motif of three conserved cysteines that most likely bind an FeS cluster (see below). Most of the residues of the large subunit of hydrogenases that are discussed to be part of a proton pathway are conserved in the NuoD portion of NuoCD. The conserved amino acids in the large subunit of hydrogenases that bind the active site Ni atom are not found in NuoCD [3,64].

The membrane fragment is composed of NuoA, H, and J–N which are the homologues of the mitochondrially encoded subunits of the eucaryotic complex [3]. They do not contain any known cofactor like flavin or FeS clusters. Nothing is known about the function of these subunits with the exception of NuoH, which contains the ubiquinone binding site [65]. They are most likely involved in proton translocation [12,13]. *Rhizobium meliloti* contains the so-called *pha* genes (from *pH a*daption; EMBL database, Acc. Nr.: X93358) that code for membrane intrinsic proteins constituting a new type of K^+/H^+ antiporter (P. Putnoky, personal comm.). A gene system related to the *R. meliloti pha*-locus was also found in *Bacillus C-125* coding for an Na^+/H^+ antiporter [66]. PhaA and D of *R. meliloti* are related to NuoL and M of complex I, respectively. The degree of sequence identity among these putative bacterial antiporter proteins exceeds clearly that of any of them with the corresponding complex I subunits. NuoL and PhaA, as well as NuoM and PhaD, are predicted to fold in a similar way through the membrane [12,13].

NuoL, M and N very likely share a common ancestor [67]. NuoL and M are more closely related to each other than either of them to NuoN. This implies that NuoM is either the common ancestor of, or an evolutionary intermediate between NuoN and NuoL. Taking it all together, it is most likely that NuoL, M, and N are involved in proton translocation.

6. Redox components

A detailed discussion of the FeS clusters of complex I is provided by T. Ohnishi in this volume. Five FeS clusters have been detected by EPR spectroscopy in the *E. coli* complex I and its fragments (Table 2). The binding sites for most of the FeS clusters of the NADH dehydrogenase fragment have been proposed. The binuclear cluster N1c has uniquely been found in complex I from *E. coli*. It is presumed to be located on NuoG, which contains a typical cysteine motif for binding this type of cluster. This motif is not found in the homologous subunits of other organisms, with the exception of *S. thyphimurium* and *Th. thermophilus* [29,38], from which the complex has not yet been isolated. In analogy to the results reported for the homologous subunit of *P. denitrificans* and beef heart complex I [68,69], the subunit NuoG should also bind the binuclear cluster N1b and the tetranuclear cluster N4 (Table 2). Furthermore, NuoG contains an atypical but conserved sequence motif for the ligation of an additional tetranuclear FeS cluster [3,70]. The high amount of iron found in complex I

Table 2
EPR spectroscopic and thermodynamic properties of the FeS clusters of the *E. coli* complex I

Cluster	Location	Type	Field position			$E_{m,7}$ (mV)	pH dependence
			g_x	g_y	g_z		
N1a[a]	NuoE	[2Fe2S]	1.93	1.94	2.03	−330	?
N1b	NuoG	[2Fe2S]	1.94	1.94	2.03	−230	−
N1c[b]	NuoG	[2Fe2S]	1.92	1.95	2.00	−250	−
N2	NuoB	[4Fe4S]	1.91	1.91	2.05	−220	+
N3	NuoF	[4Fe4S]	1.88	1.92	2.04	−270	−
N4	NuoG	[4Fe4S]	1.89	1.93	2.09	−270	−

[a]This cluster has not been detected unambiguously by EPR spectroscopy in the *E. coli* complex I.
[b]This cluster has only been detected in *E. coli* by EPR spectroscopy.

preparations would allow the presence of such an additional FeS cluster that has not yet been detected by EPR spectroscopy. NuoF is presumed to contain the FMN binding site and the tetranuclear FeS cluster N3 in analogy to the results obtained with *Neurospora crassa* and beef heart (Table 2) [71,72]. The binuclear FeS cluster N1a found in other species should be present on NuoE, although it was not detected in the NADH reduced preparation [24], while the sequence motif for binding this cluster is present on NuoE [3,49].

The connecting fragment of the *E. coli* complex I contains the FeS cluster N2 [24]. Among the subunits that make up this fragment, only NuoB and I contain binding motifs for the ligation of FeS clusters (Table 1) [3]. The FeS cluster N2 deserves special attention because it is located close to the membrane [48] and has a pH-dependent midpoint potential (Table 2). For this reason, this cluster has been discussed to be involved in proton translocation [48]. There are three conserved cysteines in NuoB that could bind a FeS cluster. The third cysteine of this motif is followed by a proline, suggesting a tetranuclear cluster to be present in this subunit. The fourth ligand could be a conserved glutamate that is located N-terminal from the cysteine motif. NuoI contains two typical ferredoxin motifs for the ligation of two tetranuclear FeS clusters [3].

To determine the location of the FeS cluster N2, we have replaced all cysteines of the putative binding motifs in NuoB and I individually to alanine by unmarked mutations in the chromosome (A. Schlitt, V. Spehr and T. Friedrich; manuscript in preparation). The signal of the FeS clusters of complex I in the mutant membranes strongly overlaps with signals from other enzymes. We isolated the complex I from the site-directed mutants NuoB^{Cys64Ala}, NuoB^{Cys129Ala} and NuoI^{Cys102Ala}. The EPR spectra of these mutants at 40 K do not show any difference to the 40 K spectrum of the wild-type enzyme, indicating that the binuclear FeS clusters have not changed in these mutants. The signal of the cluster N2 is not present in the 13 K spectra of both NuoB mutants, while the 13 K spectrum of the NuoI mutant is essentially the same as the wildtype (Fig. 3 and A. Schlitt, V. Spehr and T. Friedrich, manuscript in preparation). From these data, we conclude that cluster N2 is located on NuoB. It is very unlikely that NuoI does not bind any FeS cluster while containing two typical binding motifs. This would imply that there are two additional FeS clusters in NuoI that have not yet been detected by EPR spectroscopy.

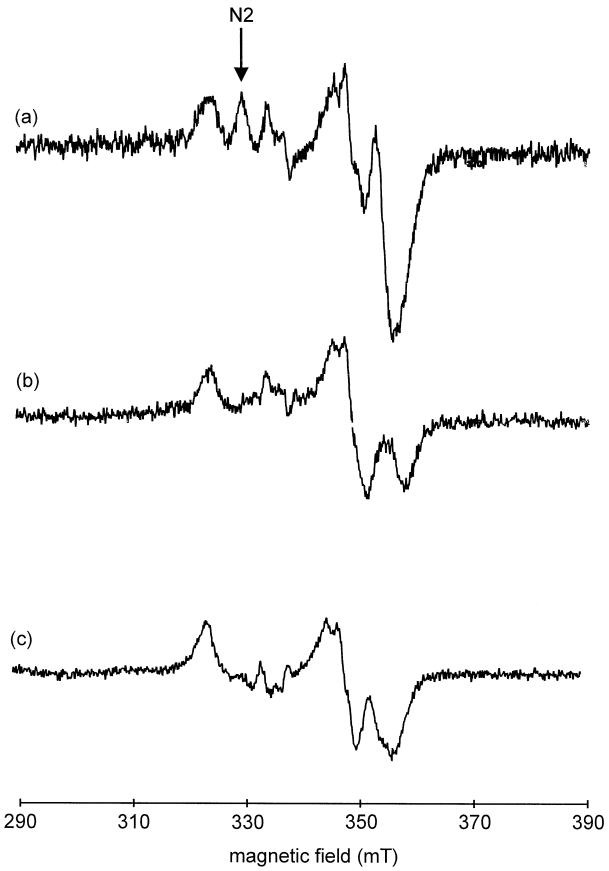


Fig. 3. EPR spectra of isolated complex I from (a) wild-type, (b) the NuoB^{Cys129Ala}, and (c) the NuoB^{Cys64Ala} mutants. The g_z signal of N2 is indicated by an arrow. Spectra were recorded at 13 K and 5 mW microwave power. Other EPR conditions were: microwave frequency, 9.44 GHz; modulation amplitude, 0.6 mT; time constant, 0.064 s; scan rate, 17.9 mT/min. Samples were reduced with a 100-fold molar excess of NADH in the presence of redox mediators.

7. Three-dimensional gross structure

Electron microscopy of two-dimensional membrane crystals of the mitochondrial complex I from *N. crassa* revealed that roughly half of the protein mass is buried in the membrane, while the remainder protrudes into the mitochondrial matrix [73]. By means of a single particle analysis of negatively

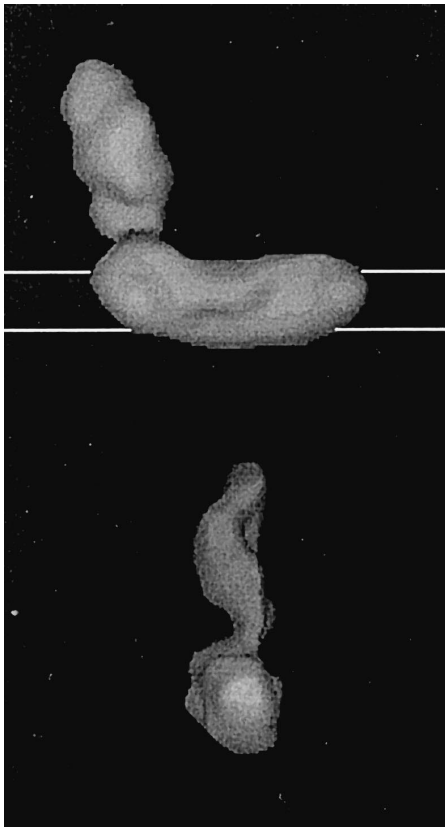


Fig. 4. Three-dimensional model of the *E. coli* complex I. The lower view is rotated by 90° clockwise about the axis of the peripheral arm with respect to the upper view. The membrane arm is shown horizontally. The lipid bilayer is symbolized by two horizontal lines at the top separated by 4 nm.

stained complex I by transmission electron microscopy and random conical tilt reconstruction, it was shown that the mitochondrial complex I has a general 'L-shape' made of a peripheral arm and a membrane arm [74].

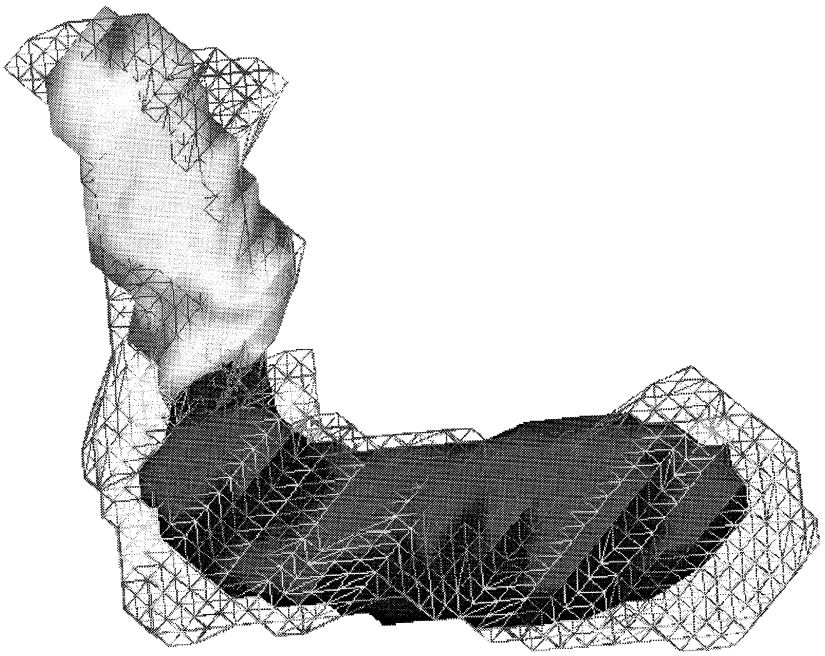
Electron micrographs of the complex I preparation in dodecyl maltoside and the three dimensional reconstruction of the images showed that the *E. coli* complex I has the same L-shaped structure as the mitochondrial complex from *N. crassa* (Fig. 4) [75]. In contrast to the *N. crassa* complex I, the *E. coli* complex shows an intrinsic variability around the junction between the peripheral and the membrane arm. It is unclear whether the same variability of the angle between the two arms exists when the complex is buried in its native membrane environment. The junction between the two arms of the bacterial complex is much smaller than it is in the mitochondrial complex, in which it is stabilized by additional proteins (Figs. 4 and 5). The small contact between the two arms of the bacterial complex may be the reason why isolation of complex I from bacteria is such a difficult task.

The similarity in structure and handedness of the peripheral arms of the bacterial and the mitochondrial complex allowed us to assign the peripheral and membrane arm of the bacterial complex. Furthermore, the two structures could be compared by aligning them (Fig. 5). Most of the mass difference between the two models is located around the membrane arm, but additional protein is also present in the peripheral arm of the mitochondrial complex, particularly at the junction between the two arms. The extra density is evenly distributed around the mitochondrial membrane arm. From this comparison, it becomes clear that, although the *E. coli* complex has only half the mass of the *N. crassa* complex, the lengths of the peripheral arms are similar, while the membrane arm of the *E. coli* complex is only slightly shorter than that of *N. crassa*. Both arms of the *E. coli* complex are much thinner than the arms of the *N. crassa* complex. In other words, the structural framework bearing the complex I machinery is made up of two elongated protein moieties, one protruding from the membrane, one buried in the membrane, and both arranged perpendicular to each other [75].

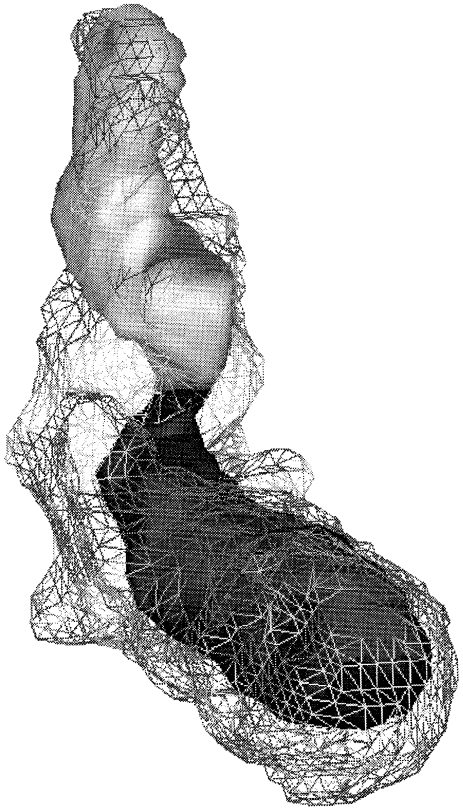
Most of the additional subunits of the mitochondrial complex I may only have a structural role in stabilizing the complex and keeping its redox groups at defined distances. This may prevent electrons from escaping the respiratory complexes and reacting with oxygen to produce highly toxic radicals. The extra

Fig. 5. Alignment of the models for the *E. coli* and *N. crassa* complex I. (a) The *N. crassa* complex I is shown as a grey wire model surrounding the solid *E. coli* complex I model with the peripheral arm colored in light grey and the membrane arm colored in dark grey. (b) A view of the model with the membrane arms pointing towards the reader. (Courtesy of Dr. Vincent Guénebaut, Dept. of Biochemistry and Biophysics, UCSF, San Francisco, CA).

(a)



(b)



proteins would therefore make energy transduction safer for the eucaryotic cell.

8. Mechanism

The mechanism of electron transfer and its coupling to proton translocation is poorly understood. The peripheral arm oxidizes NADH and transfers the electrons to the membrane arm. This is performed by the FMN and the isopotential FeS clusters N1a, N1b, N1c, N3, and N4. These clusters are all located in the NADH dehydrogenase fragment and span a midpoint-potential range from −330 to −230 mV [24]. According to measurements carried out with several eucaryotic complexes and the bacterial complexes from *P. denitrificans* and *R. sphaeroides* [49], the FeS cluster N2, which is located in the connecting fragment, has been designated as the complex I high-potential FeS cluster. In these organisms, the midpoint potential of N2 varies from −150 to −130 mV [49]. However, in *E. coli* the midpoint potential of N2 is −220 mV, in other words, just 10 mV higher than that of the isopotential FeS clusters. The electron transfer from the isopotential FeS clusters to the cluster N2 can therefore not be coupled with proton translocation. Nevertheless, as the cluster N2 most likely injects the electrons into the membrane arm, its redox reaction might possibly be connected with a proton translocation process. It is noteworthy that NuoCD, which is also located in the connecting fragment, most likely contains a proton pathway that might be linked to the redox reaction of cluster N2. This proximal section of the electron pathway covers a midpoint potential range from −320 mV to −220 mV in *E. coli*, i.e., less than one quarter of the redox potential difference between the substrates NADH ($E_{m,7} = -320$ mV) and ubiquinone ($E_{m,7} = +110$ mV) or menaquinone ($E_{m,7} = -80$ mV).

The low midpoint potential of N2 might reflect the ability of the *E. coli* complex I to react with menaquinone. In that way, the loss of the available redox potential energy of 190 mV when using menaquinone instead of ubiquinone is partly compensated. If most of cluster N2 should be in the reduced state during the steady-state reaction, its actual potential might be much lower than its midpoint potential. The so adjusted difference of the redox potential between N2 and ubiquinone or menaquinone might be sufficient for the translocation of four protons across the membrane. This hypothesis has to be tested by careful studies.

In the membrane arm, following a yet unknown mechanism, the redox-driven proton translocation and ubiquinone reduction must take place. To maintain a reversible electron transport through the complex, the membrane arm should contain redox group(s) with an intermediate redox potential. Otherwise, the gap in the potential between N2 and ubiquinone, namely 280 mV, would be too large to allow a reversible electron flow. Furthermore, the H^+/e^- stoichiometry of two [7,8] implies the existence of two coupling sites in the membrane arm. Its elongated shape would provide enough space for a serial arrangement of the two sites. The first spectroscopic evidence for such a novel redox group located in the membrane arm of complex I from *E. coli* and *N. crassa* has been obtained. An unknown component can be detected in the membrane arm by transient UV/VIS spectroscopy following partly reoxidation of the NADH-reduced complex. To reveal the role of this component in the mechanism of complex I, its structure has to be identified [76].

Acknowledgements

I gratefully acknowledge the work of the colleagues, who worked with me on the complex I from *E. coli*: A. Berger, M. Braun, S. Bungert, M. Kerstan, H. Leif, D. Scheide, A. Schlitt, V. Spehr, and U. Weidner. I also thank my ‘external’ collaborators T. Ohnishi, K. Leonard and V. Guénebaut. I am grateful to B. Brors for stimulating discussions and critical reading of the manuscript, and to H. Weiss for his critical and stimulating guidance during the past years. The work from my lab is supported by the Deutsche Forschungsgemeinschaft.

References

[1] Y. Anraku, Annu. Rev. Biochem. 57 (1988) 101–132.
[2] Y. Anraku, R.B. Gennis, Trends Biochem. Sci. 12 (1987) 262–266.
[3] U. Weidner, S. Geier, A. Ptock, T. Friedrich, H. Leif, H. Weiss, J. Mol. Biol. 233 (1993) 109–122.

[4] T. Yagi, Biochim. Biophys. Acta 1141 (1993) 1–17.
[5] J.G. Young, B.L. Rogers, H.D. Campbell, A. Jaworowski, D.C. Shaw, FEBS Lett. 116 (1981) 165–170.
[6] A. Jaworowski, G. Mayo, D.C. Shaw, H.D. Campbell, I.G. Young, Biochemistry 20 (1981) 3621–3628.
[7] A.V. Bogachev, R.A. Murtazina, V.P. Skulachev, J. Bacteriol. 178 (1996) 6233–6237.
[8] M. Wikström, FEBS Lett. 169 (1984) 300–304.
[9] M. Londershausen, W. Leicht, F. Lieb, H. Moeschler, H. Weiss, Pestic. Sci. 33 (1991) 427–438.
[10] T. Friedrich, P. van Heek, H. Leif, T. Ohnishi, E. Forche, B. Kunze, R. Jansen, W. Trowitzsch-Kienast, G. Höfle, H. Reichenbach, H. Weiss, Eur. J. Biochem. 219 (1994) 691–698.
[11] T. Friedrich, K. Steinmüller, H. Weiss, FEBS Lett. 367 (1995) 107–111.
[12] T. Friedrich, H. Weiss, Origin and evolution of biological energy conversion, in: H. Baltscheffsky (Ed.), VCH Publishers, New York, 1996, pp. 205–220.
[13] T. Friedrich, H. Weiss, J. Theor. Biol. 187 (1997) 529–540.
[14] H. Leif, U. Weidner, A. Berger, V. Spehr, M. Braun, P. Van Heek, T. Friedrich, T. Ohnishi, H. Weiss, Biochem. Soc. Trans. 21 (1993) 998–1001.
[15] I.G. Young, B.J. Wallace, Biochim. Biophys. Acta 449 (1976) 376–385.
[16] I.G. Young, A. Jaworowski, M.I. Poulis, Gene 4 (1978) 25–36.
[17] A. Jaworowski, H.D. Campbell, M.I. Poulis, I.G. Young, Biochemistry 20 (1981) 2041–2047.
[18] K. Matsushita, H.R. Kaback, Biochemistry 25 (1986) 2321–2327.
[19] R.K. Pool, B.A. Haddock, Biochem. J. 152 (1975) 537–543.
[20] P. Owen, H.R. Kaback, Biochemistry 18 (1979) 1413–1422.
[21] K. Matsushita, T. Ohnishi, H.R. Kaback, Biochemistry 26 (1987) 7732–7737.
[22] S.W. Meinhardt, K. Matsushita, H.R. Kaback, T. Ohnishi, Biochemistry 28 (1989) 2153–2160.
[23] M.W. Calhoun, R.B. Gennis, J. Bacteriol. 175 (1993) 3013–3019.
[24] H. Leif, V.D. Sled', T. Ohnishi, H. Weiss, T. Friedrich, Eur. J. Biochem. 230 (1995) 538–548.
[25] M.W. Calhoun, K.L. Oden, R.B. Gennis, M.J. Teixeira de Mattos, O.M. Neijssel, J. Bacteriol. 175 (1993) 3020–3025.
[26] O.M. Neijssel, M.J. Teixeira de Mattos, Mol. Microbiol. 13 (1994) 179–182.
[27] M.M. Zambrano, R. Kolter, J. Bacteriol. 175 (1993) 5642–5647.
[28] B.M. Prüß, J.M. Nelms, C. Park, A.J. Wolfe, J. Bacteriol. 176 (1994) 2143–2150.
[29] C.D. Archer, X. Wang, T. Elliot, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 9877–9881.
[30] K. Laval-Favre, B. Letouvet-Pawlak, T. Friedrich, J. Alexandre, J.F. Guespin-Michel, Mol. Microbiol. 23 (1997) 1043–1052.
[31] F.R. Blattner, G. Plunkett III, C.A. Bloch, N.T. Perna, V. Burland, M. Riley, J. Collado-Vides, J.D. Glasner, C.K. Rode, G.F. Mayhew, J. Gregor, N.W. Davis, H.A. Kirkpatrick, M.A. Goeden, D.J. Rose, B. Mau, Y. Shao, Science 277 (1997) 1453–1462.
[32] M. Braun, S. Bungert, T. Friedrich, Biochemistry, 1998, in press.
[33] M.A. Clarke, L. Baumann, P. Baumann, Curr. Microbiol. 35 (1997) 122–123.
[34] A. Videira, J.E. Azevedo, Int. J. Biochem. 26 (1994) 1391–1393.
[35] M. Sauter, R. Böhm, A. Böck, Mol. Microbiol. 6 (1992) 1523–1532.
[36] X. Xu, A. Matsuno-Yagi, T. Yagi, Biochemistry 32 (1993) 311–968.
[37] A. Dupuis, A. Peinnequin, M. Chevallet, J. Lunardi, E. Darrouzet, B. Pierrard, V. Procaccio, J.P. Issartel, Gene 167 (1995) 99–104.
[38] T. Yano, S.S. Chu, V. Sled, T. Ohnishi, T. Yagi, J. Biol. Chem. 272 (1997) 4201–4211.
[39] C.D. Archer, T. Elliot, J. Bacteriol. 177 (1995) 2335–2342.
[40] H.J. Falk-Krezesinski, A.J. Wolfe, J. Bacteriol., 1998, in press.
[41] J. Bongaerts, S. Zoske, U. Weidner, G. Unden, Mol. Microbiol. 16 (1995) 521–534.
[42] U. Wissenbach, D. Ternes, G. Unden, Arch. Microbiol. 158 (1992) 68–75.
[43] Q.H. Tran, J. Bongaertz, D. Vlad, G. Unden, Eur. J. Biochem. 244 (1997) 155–160.
[44] G. Unden, J. Bongaertz, Biochim. Biophys. Acta 1320 (1997) 217–234.
[45] V. Spehr, A. Schlitt, V. Guénebaut, T. Friedrich, submitted.
[46] K. von Meyenburg, B.B. Jörgensen, T. van Deurs, EMBO J. 3 (1984) 1791–1797.
[47] J.H. Weiner, B.D. Lemire, M.L. Elmes, R.D. Bradley, D.G. Scraba, J. Bacteriol. 158 (1984) 590–596.
[48] T. Ohnishi, J. Bioenerg. Biomembr. 25 (1993) 325–330.
[49] V. Sled', T. Friedrich, H. Leif, H. Weiss, S.W. Meinhardt, Y. Fukumori, M.W. Calhoun, R.B. Gennis, T. Ohnishi, J. Bioenerg. Biomembr. 25 (1993) 347–356.
[50] S.J. Pilkington, J.M. Skehel, R.B. Gennis, J.E. Walker, Biochemistry 30 (1991) 2166–2175.
[51] J.E. Walker, Q. Rev. Biophys. 25 (1992) 253–324.
[52] B. Friedrich, E. Schwarz, Annu. Rev. Microbiol. 47 (1993) 351–383.
[53] K. Schneider, R. Cammack, H.G. Schlegel, Eur. J. Biochem. 142 (1984) 75–84.
[54] A. Tran-Betcke, U. Warnecke, C. Böcker, C. Zaborosch, B. Friedrich, J. Bacteriol. 172 (1990) 2920–2929.
[55] A. Erkens, K. Schneider, A. Müller, J. Biol. Inorg. Chem. 1 (1996) 99–110.
[56] B. Bowien, H.G. Schlegel, Annu. Rev. Microbiol. 35 (1981) 405–452.
[57] O. Schmitz, G. Boison, R. Hilscher, B. Hundeshagen, W. Zimmer, F. Lottspeich, H. Bothe, Eur. J. Biochem. 233 (1995) 266–276.
[58] J. Appel, R. Schulz, Biochim. Biophys. Acta 1298 (1996) 141–147.

[59] S. Malki, I. Saimmaime, G. de Luca, M. Rousset, Z. Dermoun, J.P. Belaich, J. Bacteriol. 177 (1995) 2628–2636.
[60] S. Berger, U. Ellersiek, D. Kinzelt, K. Steinmüller, FEBS Lett. 326 (1993) 246–250.
[61] T. Friedrich, U. Weidner, U. Nehls, W. Fecke, R. Schneider, H. Weiss, J. Bioenerg. Biomembr. 25 (1993) 331–337.
[62] S.P.J. Albracht, Biochim. Biophys. Acta 1144 (1993) 221–224.
[63] G. Voordouw, Adv. Inorg. Chem. 48 (1992) 397–422.
[64] A. Volbeda, M.H. Charon, C. Piras, E.C. Hatchikian, M. Frey, J.C. Fontecilla-Camps, Nature 373 (1995) 580–587.
[65] T. Friedrich, M. Strohdeicher, G. Hofhaus, D. Preis, H. Sahm, H. Weiss, FEBS Lett. 265 (1990) 37–40.
[66] T. Hamomoto, M. Hashimoto, M. Hino, M. Kidata, Y. Seto, T. Kudo, K. Horikoshi, Mol. Microbiol. 14 (1994) 939–946.
[67] R. Kikuno, T. Miyata, FEBS Lett. 189 (1985) 85–88.
[68] T. Yano, T. Yagi, V.D. Sled', T. Ohnishi, J. Biol. Chem. 270 (1995) 18264–18270.
[69] W.J. Ingledew, T. Ohnishi, Biochem J. 186 (1980) 111–117.
[70] I.M. Fearnley, J.E. Walker, Biochim. Biophys. Acta 1140 (1992) 105–134.
[71] W. Fecke, V.D. Sled', T. Ohnishi, H. Weiss, Eur. J. Biochem. 220 (1994) 551–558.
[72] S. Chen, R.J. Guillory, J. Biol. Chem. 256 (1984) 8318–8323.
[73] G. Hofhaus, H. Weiss, K.R. Leonard, J. Mol. Biol. 221 (1991) 1027–1043.
[74] V. Guénebaut, R. Vincentelli, D. Mills, H. Weiss, K.R. Leonard, J. Mol. Biol. 265 (1997) 409–418.
[75] V. Guénebaut, A. Schlitt, H. Weiss, K.R. Leonard, T. Friedrich, J. Mol. Biol. 276 (1998) 105–112.
[76] H. Weiss, N. Amling, A. Abelmann, T. Rasmussen, T. Friedrich, U. Schulte, submitted.