Substrate-induced Conformational Change in Bacterial Complex I*

Received for publication, February 11, 2004, and in revised form, March 17, 2004
Published, JBC Papers in Press, March 22, 2004, DOI 10.1074/jbc.M401539200

Aygun A. Mamedova, Peter J. Holt, Joe Carroll, and Leonid A. Sazanov‡

From the Medical Research Council Dunn Human Nutrition Unit, Wellcome Trust/Medical Research Council Building, Cambridge CB2 2XY, United Kingdom

The mechanism coupling electron transfer and proton pumping in respiratory complex I (NADH-ubiquinone oxidoreductase) has not been established, and it has been suggested that it involves conformational changes. Here, the influence of substrates on the conformation of purified complex I from Escherichia coli was studied by cross-linking and electron microscopy. When a zero-length cross-linking reagent was used, the presence of NAD(P)H, in contrast to that of NAD+, prevented the formation of cross-links between the hydrophilic subunits of the complex, including NuoB, NuoI, and NuoCD. Comparisons using different cross-linkers suggested that NuoB, which is likely to coordinate the key iron-sulfur cluster N2, is the most mobile subunit. The presence of NAD(P)H led also to enhanced proteolysis of subunit NuoG. These data indicate that upon NAD(P)H binding, the peripheral arm of the complex adopts a more open conformation, with increased distances between subunits. Single particle analysis showed the nature of this conformational change. The enzyme retains its L-shape in the presence of NADH, but exhibits a significantly more open or expanded structure both in the peripheral arm and, unexpectedly, in the membrane domain.

NADH-ubiquinone oxidoreductase (complex I, EC 1.6.5.3) is the first enzyme of the mitochondrial and bacterial respiratory chains. It catalyzes the transfer of two electrons from NADH to quinone, coupled to the translocation of about four protons across the membrane (for reviews, see Refs. 1–4). Complex I is one of the largest known membrane protein complexes. In terms of protein content, the simplest version is the prokaryotic enzyme, which has 13–14 subunits with a combined molecular mass of about 550 kDa (5). All of the subunits of bacterial NADH-ubiquinone oxidoreductase complex I (also referred to as NDH-1)³ have analogues in the mitochondrial enzyme (4). Therefore, NDH-1 represents a useful “minimal” model for the study of the structure and function of complex I. In contrast to other enzymes of the respiratory chain, the atomic structure of complex I is not known, and the mechanisms of electron transfer and proton pumping are not established.

Electron microscopy has shown that both the mitochondrial and the bacterial enzymes have a characteristic L-shaped structure. One arm is embedded in the membrane and the other, the peripheral arm, protrudes into the mitochondrial matrix or bacterial cytoplasm (6–11). An alternative conformation of complex I from Escherichia coli has been proposed recently (12), but the existence of this conformation was not confirmed in our laboratory (13). The membrane and peripheral arms contain mainly hydrophobic and hydrophilic subunits, respectively. Dissociation of complex I by chaotropes and detergents indicates that all of the redox centers of the enzyme (FMN and up to 8–9 iron-sulfur clusters) are located in the peripheral arm (2, 14–16). With the E. coli enzyme, the resolution of the peripheral arm into a dehydrogenase fragment containing subunits NuoE, NuoF, and NuoG, and a connecting fragment containing subunits NuoCD, NuoB, and NuoI, has been described (17). On the basis of further fragmentation studies, we have proposed recently a more detailed model of subunit arrangement in bacterial complex I, in particular in the membrane domain. In this model, the large hydrophobic subunits NuoL and NuoM are located in a distal part of the membrane arm, spatially separated from the redox centers of the peripheral arm (18).

Two different models for the mechanism coupling electron transfer and proton pumping in complex I are currently being discussed: direct (redox-driven) and indirect (conformation-driven) (1, 4). The first model employs modifications of the Q cycle and assumes that some electron carriers are located in the membrane and are directly involved in proton translocation (19, 20). In this case, quinone-binding sites and proton-translocation machinery need to be located close to the peripheral arm with its redox centers to allow direct interaction. In the second model, the catalytic module with electron carriers is distinct from the proton-pumping module. The two modules can be spatially separated; energy transduction takes place through long range conformational changes (4, 21–23). Sequence comparisons have suggested that subunits NuoL, -M, and -N have evolved from a common ancestor related to K⁺/Na⁺/H⁺ antiporters and, thus, are likely to be involved in proton translocation (1, 24, 25). Therefore, the distal locations for subunits NuoL and -M (18) would be consistent with an indirect coupling mechanism. A combination of direct and indirect mechanisms, to account for a high H⁺/e⁻ ratio in complex I, has also been discussed (1, 22).

Evidence indicating conformational changes in complex I came from studies with the bovine enzyme, where the patterns of cross-linking between subunits (23) and the extent of proteolysis (26) changed significantly in the presence of NAD(P)H, but not NAD+. These data indicate that NAD(P)H binding induced significant conformational change in bovine complex I. Both NADH and NADPH bind to the FMN-containing 51-kDa cytoplasmic domain (26).
NADH, compared with NAD⁺, purified complex I from E. coli adopts a more open conformation with increased distances between hydrophilic subunits. Single particle analysis of electron micrographs of complex I treated with either NAD⁺ or NADH has allowed us to visualize this conformational change for the first time. The enzyme retained its overall L-shape but exhibited significantly expanded structure in the presence of NADH, compared with NAD⁺. This difference was observed for both the peripheral domain, which was suggested by our cross-linking data, and, unexpectedly, for the membrane domain also.

**Experimental Procedures**

**Materials**—Dodecyl-maltoside (DDM) was purchased from Anatrace (Maumee, OH). Complete™ EDTA-free protease inhibitor mixture tablets were obtained from Roche Applied Science. Bio-scake DEAE column came from Bio-Rad, other chromatography columns were from Amersham Biosciences, pipericidin A was obtained from Fluka (Gillingham, UK), and cross-linkers were from Pierce Perbio Science (Tattenhall, UK). All other chemicals were purchased from Sigma.

**Protein Purification**—Complex I was purified from E. coli BL21 cells grown under limited oxygen, as described previously (13). Purified complex I was stored in small aliquots under liquid nitrogen and used as required. Activity assays using freshly obtained pipericidin A stock (Fluka) indicated that the preparation is fully sensitive to this specific inhibitor, confirming the native state of the enzyme. The IC₅₀ for pipericidin A was 10 μM in detergent solution (20 mM MES, pH 6.0, 50 mM NaCl, 5 mM CaCl₂, 0.05% DDM, and 100 μM NADH, and 100 μM decyl-ubiquinone) and 4 μM in the presence of added E. coli lipids (0.25 mg/ml).

**Cross-linking**—To establish conditions for the formation of specific cross-links between complex I subunits, a wide range of cross-linkers with different functional groups and various spacer arm lengths were surveyed. The products of cross-linking reactions were analyzed by SDS-PAGE followed either by staining with Coomassie Blue dye or by immunodetection. The concentrations of complex I and cross-linkers, as well as the times of incubation, were optimized to avoid formation of protein aggregates. The optimal concentrations of cross-linkers were in the range 0.1–0.25 mM, with a 2-h incubation at 4°C. The protein was diluted to 0.5 mg/ml (for immunodetection) or to 1.5 mg/ml (for Coomassie Blue staining) in a buffer containing 50 mM MES and 0.2% DDM. EDTA at 2 mM was also included to avoid NuoG degradation in the presence of NADP/H. Control experiments have shown that patterns of cross-links did not change if, instead of EDTA, reaction mixtures contained 5 mM CaCl₂ (as in buffers for single particle analysis). For reactions with DEDQ, the buffer pH was 6.0; for sulfo-SMCC, sulfo-SMPB, and BS², the pH was 6.5, so that the pH was as close to optimal as possible for each reagent, but within the range needed for complex I to be stable (stable range, pH 5.5–6.5). Control incubations (Fig. 1) were performed in the same buffer as for EEDQ. Stocks of sulfo-SMCC, sulfo-SMPB, and BS² were prepared in 50 mM MES, pH 6.5. Stock of EEDQ was prepared in methanol and diluted 10-fold in 50 mM MES, pH 6.0, immediately prior to use. The final concentration of organic solvent in the reaction mixture was less than 2%. Cross-linkers were added last to the reaction mixtures. After incubation with cross-linkers, the reactions were stopped by adding an equal volume of SDS-PAGE sample buffer, which contained 0.25 μM Tris-HCl, pH 9.15, 40% SDS, and 50 mM dithiothreitol. Solubilized samples were subjected to SDS-PAGE using pre-prepared Novex Tris-glycine polyacrylamide gels containing a 10–20% acrylamide gradient (Invitrogen).

**Immunoblotting**—For SDS-PAGE, biotin-labeled markers (Amersham Biosciences) were used as molecular mass standards. After SDS-PAGE, polypeptides were electroblotted onto polyvinyldene difluoride membranes (Immobilon-P, Millipore) in a buffer containing 10 mM NaHCO₃, 3 mM Na₂CO₃, and 0.01% SDS, in a Bio-Rad Mini-cell at 30 V for 220 min. Polyclonal antibodies against E. coli complex I subunits NuoL and NuoB were kindly provided by Prof. T. Friedrich (Albert-Ludwigs-Universität, Freiburg, Germany) and those against Paracoccus denitrificans complex I subunits NuoA (NuoG analog), NuoO (NuoE analog), and NuoG (an analog of the C part of NuoCD) were kindly provided by Prof. T. Yagi (Scripps Research Institute, La Jolla, CA). Antibodies were used at 1:20,000–1:100,000 dilution. Immunodetection was performed using anti-rabbit horseradish peroxidase, streptavidin-horseradish peroxidase, and the ECL kit according to its instructions (Amersham Biosciences).

**Electron Microscopy and Single Particle Analysis**—Protein was diluted to about 20 μg/ml in a buffer containing 20 mM MES 6.0, 50 mM NaCl, 5 mM CaCl₂, 0.1% DDM, and either 2 mM NAD⁺ or 2 mM NADH. This buffer composition was shown previously to be optimal for protein stability and activity (13). After incubation on ice for 5 min, the samples were applied to carbon-coated copper grids freshly glow-discharged in air. After a 2-min incubation, excess buffer was removed by blotting and the grid was washed twice with the same buffer containing no protein or detergent and then stained with 1% uranyl acetate for 10 s. Images were recorded with a Philips Tecnai 12 microscope operating at 120 kV and a magnification of 42,000×, on Kodak SO163 film. Electron micrographs were checked for astigmatism using an optical diffractometer. Films were digitized on a Zeiss-SCAI scanner at a 7-μm step size (corresponding to 1.67 Å at the specimen level) and demagnified by linear interpolation on the computer to obtain a pixel size corresponding to 5 Å.

Single particle analysis was performed with the IMAGIC 5 software package (30). About 1100 particles were picked for each dataset (NAD⁺ and NADH) and boxed off (480 Å × 480 Å) from the micrographs. The particle images were normalized and band-pass filtered using a low frequency cut-off of (1/500) Å⁻¹ and a high frequency cut-off of (1/15) Å⁻¹ and centered by translational alignment to a rotationally averaged total sum. The centered particle images were grouped into 24 classes using multivariate statistical analysis. All 24 classes were used as references for a multi-reference alignment, and the process was iterated until stable class-sum images were obtained. After the final iteration, aligned particle images were grouped into 24, 12, or 6 classes using multivariate statistical analysis, and class-sum images were calculated and compared.

**Analytical Methods**—Protein concentrations were determined by the Bradford method (40) (Bio-Rad) with bovine serum albumin as standard. The positions of all individual subunits of complex I on our SDS-PAGE system were determined previously by mass-spectrometry (13). For the identification of the 120-kDa cross-linked product by mass-spectrometry, complex I was treated by sulfo-SMPB at 0.5 mM concentration and sulfo-SMCC at 1.0 mM concentration in a 1:1 molar ratio followed by a 220 min incubation. Polyclonal antibodies against E. coli complex I sequences using the ProteinProteins database (Micromass, Altrincham, UK) essentially as described previously (13). Peptide mass fingerprint data were calibrated using trypsin autolysis peptides and a matrix-derived mass (15) or with added peptide standards (renin substrate, angiotensin I, and ACTH clip 18–39, with monoisotopic molecular masses of 1296.665, 1758.933, and 2465.199 Da, respectively) used to obtain sample masses for calibrating non-spiked data. Monoisotopic peak mass lists were screened against protein sequence databases against SwissProt) against “in-house” databases containing only E. coli complex I sequences using the ProteinProteins program (Micromass), as well as against protein sequence databases using MASCOT via a web interface (available on the World Wide Web at www.matrixscience.com).

**RESULTS**

NADP/H-induced Proteolysis of Subunit NuoG—Before proceeding with the study of near-neighbor relationships between subunits of E. coli complex I using cross-linking reagents, control experiments were performed in which the enzyme was incubated at 4°C with different substrates and analyzed by SDS-PAGE. Surprisingly, we found that treatment of complex I with NADH induced limited proteolysis: three major additional protein bands with apparent molecular masses of about 90, 75, and 55 kDa, as well as a minor band at about 60 kDa, appeared on SDS gels after more than 30-min incubation of complex I with NADH (Fig. 1, lane 2). The degree of proteolysis was close to maximal after 2-h incubation with NADH and was...
only slightly greater after 24–48 h. This proteolysis was not prevented by the addition of EDTA-free protease inhibitor mixture (Fig. 1, lane 3). It could be prevented by the addition of EDTA (Fig. 1, lane 4), but was not stimulated by the addition of CaCl₂ (Fig. 1, lane 5). The addition of NAD⁺, adenine nucleotides ADP or ATP, or the SH-reducing agent dithiothreitol, did not lead to proteolysis (Fig. 1, lanes 6-9). Only trace amounts of possibly similar high molecular mass degradation products or impurities could be observed without the addition of NADH (Fig. 1, lanes 1 and 6-9). Addition of complex I inhibitors rotenone and piericidin A, quinone analogue decyl-ubiquinone (all at 100 μM), or the reducing agent sodium dithionite at 10 mM did not lead to proteolysis either (data not shown). However, the addition of NADPH led to effects similar to those of NADH (Fig. 1, lane 10). If NADH was added together with an excess of artificial electron acceptor K₃Fe(CN)₆ sufficient to oxidize all NADH through complex I, proteolysis was not prevented by the addition of EDTA-free protease inhibitor mixture (Fig. 1, lane 4). When NADH was added together with an excess of CaCl₂, the addition of NADH led to effects similar to those of NADH (Fig. 1, lanes 2 and 3 as compared with lanes 1 and 4). Thus, binding of NADPH to subunit NuoF, followed by reduction of complex I and its iron-sulfur clusters, likely led to a more open conformation of subunit NuoG or its surroundings, making it more susceptible to proteolysis than in the presence of NAD⁺ without any added substrates.

Cross-linking of Complex I Subunits in the Presence of Substrates—Two water-soluble hetero-bifunctional reagents, which link amines to sulphydryls, were found to be effective in producing specific cross-links. They are sulfo-SMCC (N-sulfoisocyanimidyl 4-[(N,N-limifomethyl) cyclohexane-1-carboxylate, a 11.6 Å linker) and sulfo-SMPB (N-sulfoisocyanimidyl 4-(p-maleimidophenyl)butyrate, a 14.5 Å linker). The homobifunctional reagent BS₂ (bis(sulfoisocyanimidyl)suberate, a 11.4 Å linker), which links amines together, proved effective also, as did EEDQ (N-ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline, no spacer arm). This “zero-length” reagent links carboxyl groups to nucleophilic side chains of polypeptides that are in direct contact with each other.

The effects of sulfo-SMCC and sulfo-SMPB were broadly similar, with data obtained for sulfo-SMCC shown in Fig. 2A. As can be seen from the Coomassie-stained gel, a major additional protein band of about 120 kDa was observed in the presence of cross-linker, and the pattern did not change significantly upon addition of NAD⁺ or NADPH. Immunoblotting of identical gels with antibodies against NuoI and Nqo5 (NuoCD) indicated that the 120-kDa band contains these subunits (Fig. 2A). This conclusion was confirmed by mass-spectrometry analysis using peptide mass mapping after in-gel tryptic digestion of the 120-kDa band. We obtained 19 peptides with matching masses (44% coverage) for subunit NuoCD and 4 matching peptides for subunit NuoI (21% coverage). When sulfo-SMPB was used, analysis of the similar 120-kDa band showed 28 matching peptides (59% coverage) for subunit NuoCD and the same 4 peptides for NuoI. As the total molecular mass of NuoCD+NuoI is about 89 kDa, it is likely that an additional subunit was cross-linked to NuoCD and NuoI. Immunoblotting of identical gels with antibodies against Nqo1 (NuoF) and Nqo2 (NuoE) did not reveal any cross-linked products (data not shown), whereas the NuoB antibody recognized different cross-linked products (see below). The molecular mass of NuoG is too high for it to be a component of the 120-kDa (NuoCD+NuoI) band. Therefore, it is likely that the additional subunit in this cross-linked product, not detected by mass-spectrometry, is hydrophobic. Hydrophobic Nuo subunits are not readily identified by peptide mass mapping (13). The additional subunit may be NuoH (36 kDa), judging from its molecular mass and likely proximity to the peripheral arm (18). The apparent small shift in molecular mass of NuoI in the presence of cross-linkers might indicate a modification of this subunit, such as an internal cross-link.

The NuoB antibody recognized cross-linked products of a somewhat higher molecular mass than the 120-kDa band, as

![Conformational Change in Complex I](image-url)
was clear from comparisons with biotin-labeled molecular mass markers. The identity of the subunit(s) cross-linked to NuoB in this high molecular mass band remains to be established, but it is likely to be a subunit to which antibodies were not available (namely NuoG and all the hydrophobic subunits). A cross-linked product of NuoB and NuoI was detected weakly at 45 kDa, but this was more obvious with other cross-linkers (see below). Another weak band was observed at about 150 kDa with both NuoB and NuoI antibodies and may correspond to a cross-linked product of NuoB, NuoI, and another unidentified subunit(s).

These data confirmed the spatial proximity of subunits NuoCD, NuoI, and NuoB as components of the connecting domain of the complex. Importantly, of all subunits, only NuoB showed a significant change in the cross-linking patterns in the presence of substrates. The amount of NuoB-containing high molecular mass cross-linked product was much higher in the presence of NADH or NADPH than without substrates or with NAD$^+$ (Fig. 2A). This indicates that in response to substrate binding, NuoB has a higher mobility than the other subunits of the connecting domain.

In the presence of the cross-linker BS$^3$, a major product similar in size to that observed with sulfo-SMCC (120 kDa) was generated, and it also contained subunits NuoCD and NuoI (Fig. 2B, NuoCD and NuoI). Immunoblotting with antibodies against Nqo1 (NuoF) and Nqo2 (NuoE) did not reveal cross-linked products. Again, only the NuoB subunit showed a significant change in cross-linking patterns upon incubation of complex I with NAD(P)H. The amount of the highest molecular mass cross-linked product containing NuoB increased, and a new band appeared just below it in the presence of NAD(P)H (Fig. 2B, NuoB).

When the zero-length cross-linker EEDQ was used, a clear change in cross-linking patterns was observed upon treatment of complex I with NAD(P)H. As can be seen even from Coomassie-stained gel, the number of cross-linked products was much lower in the presence of NAD(P)H (Fig. 3). Immunoblotting showed that subunits NuoB, NuoI, and NuoCD are involved in a large number of cross-links when complex I is incubated with no added substrates or in the presence of NAD$^+$. These products disappear completely in the presence of NAD(P)H (Fig. 3). Immunoblotting with anti-Nqo2 (NuoE) antiserum revealed a similar pattern, although this antibody was less specific than others and reacted with other proteins also (data not shown). Immunoblotting with an antibody against Nqo1 (NuoF) did not reveal any cross-linked products (data not shown). One major cross-linked product was a NuoI$^+$NuoB band at 45 kDa, which disappeared in the presence of NAD(P)H (Fig. 3). In the absence of NAD(P)H subunit NuoB, NuoI, and NuoCD were cross-linked to several other subunits also, because the molecular masses of several cross-linked products did not coincide (Fig. 3, NuoB, NuoI, and NuoCD). Some individual cross-links may be prevented just by relative domain movements within subunits; however, taken together, these data demonstrate that upon NAD(P)H binding, E. coli complex I adopts a more open conformation, with increased distances between many subunits, including those of the connecting domain. The increase in distances is likely to be within 11 Å, as we did not observe such a dramatic NAD(P)H-induced change in cross-linking patterns when sulfo-SMCC or BS$^3$ were used (Fig. 2). Subunit NuoB again appeared to be the most mobile: with this subunit alone, an additional cross-linked product of 125 kDa (likely involving NuoG or hydrophobic subunit(s)) was observed above) was observed in the presence of NAD(P)H (Fig. 3).

We have previously reported that E. coli complex I dimerizes after several days of incubation at 4 °C and can aggregate in very low ionic strength solutions (13). For the studies reported here we used freshly prepared or freshly unfrozen preparations of complex I under moderate ionic strength conditions, which are optimal for enzyme activity and stability and do not lead to the dimerization of the enzyme (13). Size-exclusion chromatography performed with complex I after cross-linking under the conditions used here confirmed the absence of dimers or larger aggregates of the enzyme (data not shown). Thus, the cross-links observed are formed within a monomer of complex I.
between 160–six classes. Each of the two datasets contained about 1100 particles, and each class contained an approximately similar number of particles, datasets were processed identically. The images were grouped carbon on the grid. About 80% of the particles were in the the enzyme, due to the preferred orientation of complex I on the series. The majority of the particles represented side views of, raw images of side views of the complex.

Fig. 4. Single particle analysis. Complex I was incubated with either NAD$^+$ or NADH and negatively stained for electron microscopy. A and B, raw images of side views of the complex. C and D, class-sums of such images obtained after multi-reference alignment and classification into six classes. Each of the two datasets contained about 1100 particles, and each class contained an approximately similar number of particles, between 160–200. Bar, 20 nm.

Single Particle Analysis of Complex I in the Presence of Substrates—To establish the nature of the conformational changes suggested by our cross-linking experiments, we performed electron microscopy and single particle analysis of complex I incubated with either NAD$^+$ or NADH under otherwise identical conditions. Electron micrographs of negatively stained particles revealed mostly monomers of complex I, and the proportion of dimers was negligible due to conditions used, as discussed above. A clear difference in the appearance of the NAD$^+$- and NADH-treated enzyme was obvious even from raw images of side views of the complex, without any further processing. In both cases, complex I retained its L-shape, but in the presence of NADH, it had significantly thicker, wider arms than in the presence of NAD$^+$ (Fig. 4A and 4B). These observations were reproduced with several independent preparations of complex I. Without added substrates, the majority of the particles appeared similar to the enzyme incubated with NAD$^+$ (data not shown). This finding was in agreement with cross-linking and proteolysis data, which showed similar patterns for the enzyme both with no substrates added and with NAD$^+$ (Figs. 1–3).

To proceed with image processing, electron micrographs of complex I incubated with either NAD$^+$ or NADH were taken in the same session on the microscope with the same alignment and defocus (−1.5 μm), to ensure identical magnification in a series. The majority of the particles represented side views of the enzyme, due to the preferred orientation of complex I on the carbon on the grid. About 80% of the particles were in the “flip” orientation, and 20% in the “flop” orientation. This result is presumably due to the asymmetrical nature of the molecule which is reflected in image processing, as the majority of classes show the “flip” orientation. The particles were selected for processing only on the basis of their L-shaped appearance, without any further selection. About 1100 particles were picked for each dataset (with NAD$^+$ and with NADH). Then the two datasets were processed identically. The images were grouped into 24, 12, or 6 classes by multivariate statistical analysis and multi-reference alignment, using the IMAGIC 5 software suite (30). We found that by grouping the images into 6 classes, as shown in Fig. 4C and 4D, all of the major types of particles were represented. By grouping the images into 12 or 24 classes, only sub-populations of these 6 classes were obtained (data not shown).

From a comparison of class-sum images it was clear that all class-sums obtained with complex I in the presence of NAD$^+$ were very similar to each other, either in “flip” (5 classes) or “flop” (1 class on the right) orientations (Fig. 4C). The same was true for NADH, with all 6 classes showing very similar structures (Fig. 4D). However, there was a very clear difference between images obtained with NAD$^+$ as compared with NADH; although the lengths of the peripheral and membrane arms were identical in both cases (20 nm), both arms were significantly thicker in the presence of NADH as compared with NAD$^+$ (Fig. 4C and 4D). The apparent thickness of both arms increased from about 70 Å on average in the presence of NAD$^+$ to about 90 Å on average in the presence of NADH, as could be observed with either individual classes or the whole dataset. The average protein density is likely to be lower in NADH-treated enzyme, but this is not reflected significantly in our reconstructions, which were performed in negative stain and thus show mainly an outline of the molecule. The fact that both arms of the assembly had identical lengths in two models confirmed that the observed differences reflected real conformational changes and that they were not due to artifactual differences in magnification or negative staining. The fact that the lower half of an “L” in Fig. 4C and 4D was featureless and was excluding stain much more strongly than the other half, indicated that this is the membrane domain of the complex, as observed previously (10, 13).

Thus, single particle analysis showed that upon NADH binding, E. coli complex I adopts a significantly more open, ex-
Importantly, our data showed that subunit Nuob was the most mobile connecting domain subunit because only this subunit formed additional cross-links in the presence of NAD(P)H, most likely with Nuog or hydrophobic subunits, as discussed (Figs. 2 and 3). This indicates that in the presence of NAD(P)H, Nuob moves away from Nuof closer to either Nuog or some subunits of the membrane arm. They may include Nuoa, which has been suggested to interact with Nuob (33), or Nuoh, which may contain a ubiquinone-binding site (4). It has been suggested that subunit Nuob coordinates iron-sulfur cluster N2 (34), which has a high pH-dependent midpoint potential and is, therefore, proposed to reduce ubiquinone and play a key role in proton translocation (4). The mobility of subunit Nuob, which we observed, would be consistent with such a role and may reflect movements of this subunit during the catalytic cycle.

It is possible that during catalysis, the enzyme cycles between an “open” conformation (reduced state, with NADH bound) and a “closed” conformation (oxidized state, with NAD+ bound or without any substrates), shown in Fig. 5. Our finding that the overall conformation, not only of the peripheral domain but also of the membrane domain, changes so significantly during the cycle was quite unexpected. One might have predicted rather more subtle changes in the arrangement of transmembrane helices during proton translocation. However, the observed gross change of the structure may reflect a cooperative aspect of proton translocation by complex I, because as many as three similar antiporter-like subunits (Nuol, -M, and -N) are likely to be involved in the process. A significant part of the observed expansion of the membrane domain may be caused by a re-arrangement of the hydrophilic loops, because the hydrophobic surface exposed to lipids should remain approximately similar in any conformation, spanning about 5 nm of the lipid bilayer.

Earlier studies with the bovine enzyme have shown that rotenone binding prevents the formation of many cross-links between hydrophilic subunits (35). Therefore, it is possible that inhibitor binding at or close to the ubiquinone-binding site in the hydrophilic domain of the complex can induce a conformational change similar to that induced by NADH binding to the peripheral domain. Alternatively, it is possible that without any added substrates, the enzyme might fluctuate between the two conformations, and rotenone binding locks it in the “open” conformation. Recent kinetic studies with the bovine enzyme have shown that NADH binding to the complex results in conformational change of the ubiquinone-binding site, greatly increasing its affinity toward hydrophobic substrates and inhibitors (36), which is in agreement with these proposals. We have not observed enhanced proteolysis in the presence of rotenone, piericidin A, and decyl-ubiquinone, indicating that these inhibitors and substrate do not induce conformational change in E. coli complex I, at least in the purified enzyme in solution. The reason for the difference with bovine complex I (35) is likely to be the much lower affinity of E. coli complex I for the inhibitors. It is known that rotenone is a poor inhibitor of the E. coli enzyme; the IC50 with our preparation was only about 100 μM, whereas for the bovine enzyme it was about 2 nm. For piericidin A, the IC50 with our preparation was between 4–10 μM (see “Experimental Procedures”), and the Kd for decyl-ubiquinone was about 10 μM (data not shown). These rather low affinities, combined with limited solubility of these hydrophobic substances, might prevent the observation of a rotenone-like effect with the bacterial enzyme.

Our results show that the catalytic cycle of complex I is not likely to involve large movements of the peripheral arm relative to the membrane arm, as discussed elsewhere (12, 37). The conformational change shown in Fig. 5 is, simultaneously,
more modest and more dramatic than has been suggested before: we observed only an “opening up” of the structure instead of movements. It is, however, a very significant expansion involving the whole of the assembly, including the membrane domain. Such conformational change, where the length of both arms and the angle between them remain constant, is likely to be more energetically effective than large relative movements of arms. Previously, a transition on a similar scale from the open, expanded structure of the hydrophilic part of Ca\(^{2+}\)-ATPase to the closed, compact structure was observed upon Ca\(^{2+}\) release (38) or nucleotide binding (39). This conformational change was brought about by movements of cytoplasmic domains and was coupled to drastic rearrangement of transmembrane helices (38). As proton translocation in complex I is coupled to NADH/NAD\(^+\) binding and release, it is possible that proton-binding sites on hydrophobic subunits involved in translocation are accessible to different sides of the membrane in the two conformations shown in Fig. 5. Binding of NADH to subunit NuoF, which is located close to the tip of the peripheral arm, induced a conformational change as far as the tip of the membrane domain (location of subunit NuoL), separated by the distance of about 300 Å. This shows that long-range conformational changes are indeed a part of the mechanism of complex I, as has been discussed widely in the field.

Acknowledgments—We thank Prof. T. Friedrich (Albert-Ludwigs-Universität, Freiburg, Germany) and Prof. T. Yagi (Scripps Research Institute, La Jolla, CA) for the kind gift of antibodies.

REFERENCES

1. Friedrich, T. (2001) J. Bioenerg. Biomembr. 33, 169–177
2. Walker, J. E. (1992) Q. Rev. Biophys. 25, 253–324
3. Videra, A. (1998) Biochim. Biophys. Acta 1364, 89–100
4. Yagi, T., and Matsuno-Yagi, A. (2003) Biochim. Biophys. Acta 121, 125–133
5. Yagi, T., Yano, T., DiBernardo, S., and Matsuno-Yagi, A. (1998) Biochim. Biophys. Acta 1364, 89–100
6. Leonard, K., Haker, H., and Weiss, H. (1987) J. Mol. Biol. 194, 277–296
7. Hofhaus, G., Weiss, H., and Leonard, K. (1991) J. Mol. Biol. 221, 1027–1043
8. Djafarzadeh, R., Kerscher, S., Zwicker, K., Radermacher, M., Lindahl, M., Schagger, H., and Brandt, U. (2000) Biochim. Biophys. Acta 1459, 230–238
9. Grigorieff, N. (1998) J. Mol. Biol. 277, 1033–1046
10. Guennéhaut, V., Schlitt, A., Weiss, H., Leonard, K., and Friedrich, T. (1998) J. Mol. Biol. 276, 105–112
11. Peng, G., Fritzsche, G., Zickermann, V., Schagger, H., Mentele, R., Lottspeich, F., Bostina, M., Radermacher, M., Huber, R., Stetter, K. O., and Michel, H. (2003) Biochemistry 42, 3032–3038
12. Botcher, B., Scheide, D., Hesterberg, M., Nagel-Steger, L., and Friedrich, T. (2002) J. Biol. Chem. 277, 17970–17977
13. Sazanov, L. A., Carrrell, J., Holt, P., Toime, L., and Fearnley, I. M. (2003) J. Biol. Chem. 278, 19483–19491
14. Friedrich, T. (1998) Biochim. Biophys. Acta 1364, 134–146
15. Finel, M., Skobel, M., Albracht, S. P., Fearnley, I. M., and Walker, J. E. (1992) Biochemistry 31, 11425–11434
16. Sazanov, L. A., Peak-Chew, S. Y., Fearnley, I. M., and Walker, J. E. (2000) Biochemistry 39, 7229–7235
17. Leif, H., Sled, V. D., Ohsishi, T., Weiss, H., and Friedrich, T. (1995) Eur. J. Biochem. 230, 538–548
18. Holt, P. J., Morgan, D. J., and Sazanov, L. A. (2003) J. Biol. Chem. 278, 43114–43120
19. Dutton, P. L., Moser, C. C., Sled, V. D., Daldal, F., and Ohsishi, T. (1998) Biochim. Biophys. Acta 1364, 245–257
20. Brandt, U. (1997) Biochim. Biophys. Acta 1318, 79–91
21. Brandt, U., Kerscher, S., Drose, S., Zwicker, K., and Zickermann, V. (2003) FEBS Lett. 545, 9–17
22. Sazanov, L. A., and Walker, J. E. (2000) J. Mol. Biol. 305, 455–464
23. Belogrudov, G., and Hatefi, Y. (1994) Biochemistry 33, 4571–4576
24. Fearnley, I. M., and Walker, J. E. (1992) Biochim. Biophys. Acta 1140, 105–134
25. Kikuno, R., and Miyata, T. (1985) FEBS Lett. 189, 85–88
26. Yamaguchi, M., Belogrudov, G. I., Matsuno-Yagi, A., and Hatefi, Y. (1997) Biochim. Biophys. Acta 1339, 239–336
27. Hatefi, Y., and Bearden, A. J. (1976) Biochim. Biophys. Res. Commun. 69, 1032–1038
28. Galante, Y. M., and Hatefi, Y. (1978) Methods Enzymol. 53, 15–21
29. van Heel, M., Harauz, G., Orlowa, E. V., Schmidt, R., and Schatz, M. (1996) J. Struct. Biol. 116, 17–24
30. Hammersberg, T., and Radmark, Ö. (1999) Biochemistry 38, 4441–4447
31. Zharova, T. V., and Vinogradov, A. D. (1999) Biochim. Biophys. Acta 1320, 256–264
32. DiBernardo, S., and Yagi, T. (2001) FEBS Lett. 508, 385–388
33. Flemming, D., Schlitt, A., Spehr, V., Bischof, T., and Friedrich, T. (2003) J. Biol. Chem. 278, 47602–47609
34. Gendal, J. A., and Anderson, W. M. (1985) J. Biol. Chem. 260, 12890–12894
35. Hans, N., Nakashima, Y., Shinzawa-Itoh, K., and Yoshikawa, S. (2003) J. Bioenerg. Biomembr. 35, 257–265
36. Holt, P. J., Morgan, D. J., and Sazanov, L. A. (2003) J. Biol. Chem. 278, 28938–28943
37. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
Substrate-induced Conformational Change in Bacterial Complex I
Aygun A. Mamedova, Peter J. Holt, Joe Carroll and Leonid A. Sazanov

J. Biol. Chem. 2004, 279:23830-23836.
doi: 10.1074/jbc.M401539200 originally published online March 22, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M401539200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 7 of which can be accessed free at
http://www.jbc.org/content/279/22/23830.full.html#ref-list-1