Journal of Bioenergetics and Biomembranes, Vol. 25, No. 4, 1993

Minireview

NADH-Quinone Oxidoreductase, the Most Complex Complex

Tomoko Ohnishi[1]

Received March 10, 1993; accepted March 15, 1993

INTRODUCTION

The previous two issues in this series (Vol. 25, Nos. 1 and 2) focused on cytochrome oxidase and cytochrome bc_1 complex. This issue presents a synopsis of recent work on the NADH-ubiquinone oxidoreductase (also known as Complex I). This enzyme has a reputation as the most complicated and least understood among mitochondrial proton-translocating enzymes, which is mostly due to its extreme structural complexity and the difficulty of applying optical spectroscopy to analyze the redox state of intrinsic chromophores within the membrane. However, during the last several years there has been a major breakthrough in the field of Complex I, particularly with respect to its structural aspects. They are highlighted by a recent electron microscopic image of *Neurospora crassa* Complex I and the determination of the primary sequences of many subunits of mitochrondrial Complex I, as well as simpler bacterial counterparts of Complex I. This issue covers timely topics in this field written by leading investigators. For additional comprehensive reviews, see Hatefi, 1985; Ragan, 1987; Weiss *et al.*, 1991; Yagi, 1993; Walker, 1992.

Bovine heart Complex I, which is composed of 41 different subunits with a total molecular weight of 907 kDa (Fearnley and Walker, 1992), is by far the largest proton-translocating oxidoreductase in the mitochondrial respiratory chain. It catalyzes the transfer of electrons from NADH to ubiquinone which is coupled with the vectorial transfer of protons across the mitochrondrial membrane. This leads to the formation of $\Delta\tilde{\mu}_{H^+}$ which drives ATP formation. The stoichiometry of proton transfer has been scrutinized in many laboratories, and the current consensus value is $4H^+/2e^-$, which distinguishes Complex I from the two other aforementioned H^+-translocating complexes of the respiratory chain both of which have a stoichiometry of $2H^+/2e^-$. Complex I contains one FMN, two binuclear clusters N1a and N1b, four tetranuclear iron-sulfur clusters N2 to N5 (Ohnishi, 1979; Ohnishi and Salerno, 1982; Beinert and Albracht, 1982), and two distinct species of tightly bound ubisemiquinones which differ in their spin relaxation and in redox properties (Burbaev *et al.*, 1989; Vinogradov *et al.*, 1991; Vinogradov, this issue). The thermodynamic profiles of individual redox components are determined by low-temperature electron paramagnetic resonance, which is the most powerful technique available for this complex. However, the detailed electron transfer pathway has not yet been established. The difficulty lies in the much faster redox equilibration within the complex in comparison to the reaction of the complex with its substrate, NADH, and also in the lack of specific inhibitors which can dissect the intermediate electron transfer steps. There have been several attempts to resolve Complex I; earlier use of chaotropic reagents resolved Complex I into three distinct fractions, namely, two water-soluble fractions, flavo-iron sulfur protein[2] (FP) and iron-sulfur protein (IP), and a water-insoluble hydrophobic precipitate (HP). This fractionation of huge Complex I provided an excel-

[1] Department of Biochemistry and Biophysics, University of Pennsylvania, Johnson Research Foundation, Philadelphia, Pennsylvania 19104-6089.

[2] FP fraction consists of 51, 24, and 10 kDA subunits; IP fraction contains 75, 49, 30, 18, 15 and 13 kDa as major subunits. IP and HP fractions are neither homogeneous nor monodisperse.

0145-479X/93/0800-0325$07.00/0

lent basis for sequence determination and some useful data on the possible location of redox components (Ohnishi, 1979; Hatefi, 1985). Recent development of a new isolation procedure yielding a more defined bovine heart Complex I by nonionic detergent extraction and ion exchange chromatography and resolution of this Complex I into subfractions using milder detergents promises further useful information (Finel *et al.*, 1992; Finel, this issue).

In addition to mammalian Complex I, Complex I from *Neurospora crassa*, another eukaryote, has been extensively studied. This Complex I is composed of ~ 32 subunits with a total molecular weight of ~ 700 kDa (Weiss *et al.*, 1991). The electron microscopic analysis of isolated particles of *N. crassa* Complex I and the 3D-reconstruction of 2D membrane crystals have revealed a unique low-resolution structural outline with an unusual L-shape which is presented as a montage picture on the cover of this issue (Hofhaus *et al.*, 1991). A large hydrophilic arm is protruding out from the N-side of the membrane, and a longer hydrophobic arm is extending within the membrane. By utilizing their newly devised gene replacement method or by growing cells under specified conditions, Weiss' group has demonstrated that the peripheral and membrane parts of *N. crassa* Complex I are synthesized independently from each other. The former part contains the NADH binding site, FMN, and low-potential iron sulfur clusters, while the latter contains a high-potential cluster, N2 (Weiss *et al.*, 1991; Weidner *et al.*, 1992).

In recent years there have been significant advances in numerous areas of biological research attributable to the application of molecular biological techniques. The field of Complex I is no exception. The amino acid sequence of all nuclear-encoded subunits in bovine heart Complex I (Walker 1992; Fearnley and Walker, 1992) and many of the counterpart subunits of *N. crassa* Complex I (Nehls *et al.*, 1992 and references cited therein) have been derived from the DNA sequence. A structural feature which emerged from the primary sequence data is that fully conserved cysteine-rich sequence motifs, which suggest subunits harboring iron-sulfur clusters, are found in six nuclear-encoded subunits, but not in mitochondrially encoded ones. NADH and FMN binding sites were suggested to be located in the 51 kDa subunit of the hydrophilic FP fraction. Another interesting finding is that none of these hydrophilic subunits seem to contain hydrophobic stretches long enough to form the transmembranous helical structure, whereas all the mitochondrially encoded polypeptides contain numerous transmembranous helices.

P. denitrificans, *Rb. sphaeroides*, and *Rb. capsulatus* belong to the α subgroup of the purple bacteria from which mitochondria may have evolved (Yang *et al.*, 1985). As seen in two preceding issues of this series, cytochrome oxidase (Anraku and Gennis, 1987; Brown *et al.*, 1993) and cytochrome bc_1 (Trumpower, 1990) complexes of these bacteria show considerable spectral and functional similarity to their mitochondrial counterparts. However, the bacterial enzymes consist of far fewer polypeptides; 3–4 instead of 10–13. Both the high sequence homology between corresponding subunits of bacterial and mitochondrial enzymes and the ease of bacterial genetic manipulations have made the bacterial enzymes excellent model systems for the structure–function analysis of eukaryotic respiratory complexes. On the other hand, some complexities are encountered in most bacterial systems because they contain redundant branched respiratory chains. For example, there are two distinct membrane-bound NADH dehydrogenases, which are designated as NDH-1 and NDH-2 (Matsushita *et al.*, 1987; Yagi, 1991). The former consists of multiple subunits containing FMN and iron-sulfur clusters and is coupled to the formation of $\Delta\mu_{H^+}$, while the latter consists of a single subunit containing only FAD and is not energy coupled. Redox centers in NDH-1 have been characterized *in situ* in several bacterial systems, as described in this volume (Sled' *et al.*). Because of the labile nature of bacterial enzymes (Berks and Ferguson, 1991), intact NDH-1 has not been isolated. However, an important step was taken when structural genes of NDH-1 from *P. denitrificans* were cloned and the DNA-derived primary sequences of their polypeptides were obtained (Yagi, 1991, 1993). Its gene cluster consists of 14 structural genes which exhibit a high sequence homology to the bovine heart counterpart subunits, and contains an additional six unidentified reading frames (URFs). This bacterial NDH-1 consists of seven homologues of nuclear-encoded and seven of mitochondrially encoded subunits of bovine heart Complex I. Three hydrophilic subunits, namely 51 (FP), 24 (FP), 75 (IP), and two less hydrophilic subunits, 23 (IP or HP), and 20 (IP or HP) kDa, harbor the cysteine-rich sequence motifs which are fully conserved in bovine heart, plants, and cyanobacterial counterparts so far known (Walker, 1992).

Very recently the entire primary sequence of the *E. coli* NDH-1 has also been determined (Weidner *et al.*, 1993). It was found to contain only 14 structural genes which are homologous to their *Paracoccus* counterparts and which are in the same gene order, but with no URFs (Friedrich *et al.*, this volume). Therefore, this set of *E. coli* structural genes seems to provide a minimal subunit structure essential for the proton-translocating NADH-Q oxidoreductase. This NDH-1 is about the same size as that of the *E. coli* F_1F_0 ATP synthase. Thus, this bacterial Complex I provides a decently sized enzyme to study. Sequence identity of *E. coli* NDH-1 subunits to bovine heart equivalents is lower (average 34%) than that of *P. denitrificans* (57%) (Weidner *et al.*, 1993). However, cysteine-rich iron-sulfur sequence motifs are also fully conserved in the *E. coli* enzyme. The 14-subunit NDH-1 from this *E. coli* (wild-type G strain) has already been isolated (Leif, H., Friedrich, T., and Weiss, H., unpublished data). Preliminary EPR analysis of this enzyme in my laboratory, both *in situ* and *in vitro*, has revealed the presence of several distinct iron-sulfur clusters analogous to their mammalian counterparts (Sled' *et al.*, this issue). Although EPR spectral features of the iron-sulfur clusters seem to be somewhat different from the mammalian type, their general thermodynamic profile (one each of low and high E_m components and several pool clusters at an intermediate E_m range) is clearly discernable. Based on the cysteine-rich sequence motifs, the presence of 7 to 8 iron-sulfur clusters is predictable. Their subunit assignment should be verified by site-directed mutagenesis and EPR analysis, as was successfully conducted with iron-sulfur clusters of the succinate-quinone oxidoreductase (Ackrell *et al.*, 1922; Hederstedt and Ohnishi, 1992). Two independently evolved functional modules in the catalytic domain (proximal and distal parts of the electron transfer pathway) have been recognized, which exhibit a high sequence homology with two independent bacterial enzymes, namely, the hydrophilic NAD^+-reducing hydrogenase of *Alcaligenes eutrophus* and the hydrophobic nonenergy-producing hydrogenase of the *E. coli* formate hydrogenlyase, respectively (Weiss *et al.*, 1991; Friedrich *et al.*, this issue). The most intriguing question at present is the cluster identity and membrane topography of the putative iron-sulfur clusters in 23 and 20 kDa subunits (Dupuis *et al.*, 1991; Arizmendi *et al.*, 1992) in the distal module of the catalytic domain. Two putative [4Fe-4S] clusters in the 23 kDa subunit may be low E_m clusters magnetically and/or thermodynamically coupled as in the case of bacterial eight-iron ferredoxin (Mathews *et al.*, 1974) or FrxA and FrxB clusters in the photosystem I (Golbeck and Bryant, 1991), both of which may be difficult to analyze because of heavily overlapping signals from other low E_m clusters in the $g = 1.94$ region of the magnetic field. EPR signals of some clusters, such as cluster N5, can be substoichiometric to the complex or undetectable due to the dominant $S = 3/2$ ground state of their spins (Park *et al.*, 1991), which requires a much higher spin concentration for their detection. This problem may be overcome by overexpressing individual subunits bearing specific clusters of interest, if successfully obtained in the intact form; this work is currently in progress (Yagi *et al.*, in this volume; Walker, personal communication). An even more interesting topic is the putative iron-sulfur cluster in the 20 kDa subunit which carries only three conserved cysteines. The third cysteine is accompanied by a conserved proline residue, suggesting a [4Fe-4S] structure. The third residue downstream from the first cysteine is a conserved glutamate residue which is a better candidate than cysteine to provide a ligand, as in the case of a [4Fe-4S] cluster in *Pyrococcus furiosus* ferredoxin (Park *et al.*, 1991) or the binuclear high E_m cluster S1 (Ackrell *et al.*, 1992). An alternate ligand candidate is a water molecule in addition to three cysteines as seen in activated aconitase (Robbins and Stout, 1989). These possibilities are consistent with the unique properties of the cluster N2 (Ohnishi, 1979; De Vault, 1976; Gutman *et al.*, 1972) and they are testable utilizing bacterial systems.

The photosynthetic bacteria are also expected to offer attractive advantages for future genetic manipulations and spectroscopic analysis. The EPR and thermodynamic features of iron-sulfur clusters in *Rhodobacter* NDH-1 are remarkably close to the mammalian counterparts, even more than those of *P. denitrificans* (Sled' *et al.*, this issue). NDH-1 of *Thermus thermophilus* HB-8, which contains only low E_m iron-sulfur clusters but is energy coupled at Site I, is suitable toward attempting 3D crystallographic analysis of its Complex I structure because of much higher stability of the enzyme at higher temperatures and with denaturing chemical reagents.

Recent developments of microbial Complex I systems with their molecular genetic knowledge, if successful, will soon open a new avenue for active structure–function analysis of the NADH-Q oxido-

reductase segment of the respiratory chain, as exemplified by successful ligand assignments of bacterial cyt. *bo* (Lemieux *et al.*, 1992) and cyt. aa_3 oxidase (Shapleigh *et al.*, 1992) systems.

Regarding the mechanism of energy coupling Site I, every possible model has been proposed in order to explain the $4H^+/2e^-$ stoichiometry. Most of them are composed of two energy-transducing subsites; the flavin-cycle and the Q-cycle (Ragan, 1987; 1990 and references cited therein). The newest model is an energy-coupled dismutation between specifically bound paired ubisemiquinones yielding a H^+ stoichiometry of $1H^+/2^-$ (Vinogradov, this issue) combined with a *b*-cycle type flavin cycle ($3H^+/2e^-$), both of which are connected conformationally to the H^+ channel within the membrane (Walker, 1992; Pilkington *et al.*, 1993). At the present stage of Site I coupling studies, it is important to determine the topographical location of redox active centers of Complex I, specifically cluster N2, within the mitochondrial or bacterial cytoplasmic membranes.

Another very important research area we could not include in this issue is the major progress in the area of mitochondrial myopathies and neuropathies. These diseases predominantly affect organs with the highest demand for energy such as the muscle tissue and brain which are most frequently associated with Complex I defects. Seven out of a total of 13 mitochondrial genes encode subunits of Complex I. The protein synthesis turnover is much faster in the mitochondrion than in the nucleus, and the possibility of mutation is much greater in the former which increases the likelihood of the occurrence of these diseases. For example, a single amino acid mutation in the mitochondrial genes, such as in ND-4, ND-1, or ND-5, causes Leber hereditary optic neuropathy (Wallace, 1992; Howell *et al.*, 1991). Recently it was found that the ND-4 defect may cause a problem in the interaction between Complex I and NAD-linked dehydrogenase in the mitochondrial matrix (Majander *et al.*, 1991). There are many subunits (at most 34 in bovine heart, 8 in *E. coli*) whose function is unknown. This was the first observation which suggested a functional role for the ND-4 subunit. This field is becoming one of the most important areas studying degenerative human diseases, including idiopathic dystonia, Parkinson's, and Huntington's disease. Extensive review articles are available on the topic (Morgan-Hughes *et al.*, 1990; Singer and Ramsay, 1992).

REFERENCES

Ackrell, B. A. C., Johnson, M. K., Gunsalus, R. P., and Cecchini, G. (1992). In *Chemistry and Biochemistry of flavoenzymes*, Vol. 3 (Mueller, F., ed.), CRC Press, Boca Racon, Florida, pp. 229–297.

Anraku, Y., and Gennis, R. B. (1987). *Trends. Biochem. Soc.* **12**, 262–266.

Arizmendi, J. M., Skehel, J. M., Ruunswick, M. J., Fearnley, I. M., and Walker, J. E. (1992). *FEBS Lett.* **313**, 80–84.

Beinert, H., and Albracht, S. P. J. (1982). *Biochim. Biophys. Acta* **683**, 245–277.

Berks, B. C., and Ferguson, S. J. (1991). *Biochem. Soc. Trans.* **19**, 581–587.

Brown, S., Moody, A. J., Mitchell, R., and Rich, P. T. (1993). *FEBS Lett.* **316**, 216–223.

Burbaev, D. S., Moroz, I. A., Kotlyar, A. B., Sled, V. D., and Vinogradov, A. D. (1989). *FEBS Lett.* **254**, 47–51.

De Vault, D. (1976). *J. Theor. Biol.* **62**, 115–139.

Dupuis, A., Skehel, J. M., and Walker, J. E. (1991). *Biochemistry* **30**, 2954–2960.

Fearnley, I. M., and Walker, J. E. (1992). *Biochim. Biophys. Acta* **1140**, 105–134.

Finel, M., Skehel, J. M., Albracht, S. P. J., Fearnley, I. M. and Walker, J. E. (1992). *Biochemistry* **31**, 11425–11434.

Golbreck, J. H., and Bryant, D. A. (1991). *Curr. Top. Bioenerg.* **16**, 83–177.

Gutman, M., Singer, T. P., and Beinert, H. (1972). Biochemistry **11**, 556–562.

Hatefi, Y. (1985). *Annu. Rev. Biochem.* **54**, 1015–1069.

Hederstedt, L. and Ohnishi, T. (1992) in *Molecular Mechanisms in Bioenergetics* (Ernster, L., ed), Elsevier; Amsterdam, pp. 163–198.

Hofhaus, G., Weiss, H., and Leonard, K. (1991). *J. Mol. Biol.* **221**, 1027–1043.

Howell, N., Kubacka, I., Xu, M., and McCullough, D. A. (1991). *Am. J. Human Genet.*, **48**, 935–942.

Lemieux, L. J., Calhoun, M. W., Thomas, J. W., Ingledew, W. J., and Gennis, R. B. (1992). *J. Biol. Chem.* **267**, 2105–2113.

Majander, A., Huoponen, K., Savontaus, M.-L., Nikoskelainen, E., and Wilkström, (1991). *FEBS Lett.* **292**, 289–292.

Mathews, R., Charlton, S., Sands, R. H., and Palmer, G. (1976). *J. Biol. Chem.* **249**, 4326–4328.

Matsushita, K., Ohnishi, T., and Kaback, H. R. (1987). *Biochemistry* **26**, 7732–7737.

Morgan-Hughes, J. A., Schapira, A. H. V., Cooper, J. M., Holt, I. J., Harding, A. E., and Clark, J. B. (1990). *Biochim. Biophys. Acta* **1018**, 217–222.

Nehls, U., Friedrich, T., Schmiede, A., Ohnishi, T., and Weiss, H. (1992). *J. Mol. Biol.* **227**, 1032–1042.

Ohnishi, T. (1979). In *Membrane Proteins in Energy Transduction* (Capaldi, R. A., ed.), Marcel Dekker, New York, pp. 1–87.

Ohnishi, T., and Salerno, J. C. (1982). In *Iron-Sulfur Proteins* Vol. IV (Spiro, T. G., ed.), Wiley, New York, pp. 285–327.

Ohnishi, T., Robertson, D. E., Harmon, J. H., and Hatefi, Y. (1992). *Biochim. Biophys. Acta, EBEC Short Rep.* **7**, 29.

Park, J. -B., Fan, C., Hoffman, B. M., and Adams, M. W. W. (1991). *J. Biol. Chem.* **266**, 19351–19356.

Pilkington, S. J., Arizmendi, J. M., Fearnley, I. M., Runswick, M. J., Skehel, J. M., and Walker, J. E. (1993). *Biochem. Soc. Trans.* **21**, 26–31.

Ragan, C. I. (1987). *Curr. Top. Bioenerg.* **15**, 1–36.

Ragan, C. I. (1990). *Biochem. Soc. Trans.* **18**, 515–516.

Robbins, A. H., and Stout, C. D. (1989). *Proc. Natl. Acad. Sci. USA* **86**, 36–39.

Shapleigh, J. P., Hosler, J. P., Tecklenburg, M. M. J., Kim, Y., Babcock, G. T., Gennis, R. B. and Ferguson-Miller, S. (1992). *Proc. Natl. Acad. Sci. USA* **89**, 4786–4790.

Singer, T. P., and Ramsay, R. R. (1992). In *Molecular Mechanisms in Bioenergetics* (Ernster, L., ed.), Elsevier, Amsterdam, pp. 145–162.
Trumpower, B. L. (1990). *Microbiol. Rev.* **52**, 101–129.
Vinogradov, A. D., Sled, V. D., Maklashina, E. O., Burbaev, D. S., and Moroz, I. A. (1991). *Biol. Chem. Hoppe-Seyler* **372**, 553.
Walker, J. E. (1992). *Q. Rev. Biophys.* **25**, 253–324.
Wallace, D. C. (1992). *Science* **256**, 628–632.
Weidner, U., Nehls, U., Schneider, R., Fecke, W., Leif, H., Schmiede, A., Friedrich, T., Zensen, R., Schulte, U., Ohnishi, T., and Weiss, H. (1992). *Biochim. Biophys. Acta* **1101**, 177–180.
Weidner, U., Geier, S., Stock, A., Friedrich, T., Leif, W., and Weiss, H. (1993), *J. Mol. Biol.* in press.
Weiss, H., Friedrich, T., Hofhaus, G., and Preis, D. (1991). *Eur. J. Biochem.* **197**, 563–576.
Yagi, T. (1991). *J. Bioenerg. Biomembr.* **23**, 211-225.
Yagi, T. (1993). *Biochim. Biophys. Acta* **1141**, 1–17.
Yang, D., Oyaizu, Y., Oyaizu, H., Olsen, G. J., and Woese, C. R. (1985). *Proc. Natl. Acad. Sci. USA* **82**, 4443–4447.