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The energy-transducing NADH-quinone oxidoreductase (NDH-1) of *Paracoccus denitrificans*

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The NADH-quinone (Q) oxidoreductase (complex I or NDH-1) is one of three energy-transducing enzyme complexes of the respiratory chain in mitochondria and bacteria [1,2]. It is the point of entry for the major fraction of electrons that traverse the respiratory chain eventually resulting in the reduction of oxygen. It may be the most intricate membrane-bound enzyme known to date, being composed of more than 35 unlike polypeptides. Of these, 7 are encoded by mitochondrial DNA (ND-1, -2, -3, -4, -4L, -5 and -6) [3,4].

Although three decades have passed since the first isolation of complex I from bovine heart mitochondria, information on its structure and mechanism of action is still limited [5,6]. This enzyme complex contains a noncovalently-bound FMN molecule and 5 binuclear and 3 tetranuclear iron-sulfur clusters, of which 2 binuclear and 3 tetranuclear clusters are EPR-visible. Bovine heart mitochondrial complex I has been resolved into 3 fractions with the use of chaotropes: a flavoprotein (FP, 3 polypeptides), an iron-sulfur protein (IP, 8 polypeptides) and a hydrophobic protein fraction (HP, more than 24 polypeptides) [1,7]. The only other isolation of complex I from mitochondria was carried out by Weiss and coworkers using *Neurospora crassa* [5]. The isolated *Neurospora* complex I, like the bovine enzyme, contains more than 30 polypeptides. The three-dimensional structure of the *Neurospora* enzyme has been studied by electron microscopy of membrane crystals [5]. The data suggest that complex I may have an L-shaped structure with 2 domains.

Information on the functional role of the individual polypeptides in complex I is even more limited. Chen and Guillory [8], using a tritiated photoaffinity NAD analogue, have shown that the 51-kDa polypeptide of bovine complex I is the NADH-binding subunit. Recently, it was shown by our laboratory that N,N'-dicyclohexylcarbodiimide (DCCD) inhibits the activity of complex I coupled to H^+ translocation, and that the subunit modified by DCCD is the mitochondrial ND-1 gene product [9,10]. Studies of Earley et al. [11] have suggested that the rotenone-binding subunit of complex I is also the ND-1 gene product. There are results suggesting that the 24-kDa (FP), 75-kDa (IP), and 23-kDa (HP) subunits may bear iron-sulfur clusters [12,13]. Furthermore, one polypeptide appears to be an acyl-carrier protein although the homologue of *E. coli* is located in the cytoplasm instead of the membrane [14]. The function of the remaining 30 or more polypeptides remains largely unknown.

Research on the NADH-Q oxidoreductase complex has taken on greater significance since the finding that many mitochondrial diseases involve structural and functional defects at the level of this enzyme complex [15]. Examples include a variety of neuromuscular diseases. Many cases of Leber's hereditary optic neuropathy also appear to be associated with a defect in complex I resulting from a single nucleotide change in the mitochondrial DNA converting the 340th amino acid of the ND-4 gene product from an arginine to a histidine [16]. The compound, 1-methyl-4-phenylpyridinium, an inhibitor of complex I, produces Parkinsonism, suggesting a link between Parkinson's disease and complex I [17]. Furthermore, capsaicin, another inhibitor of the mammalian complex I, is known to produce neuronal insensitivity to several types of physicochemical stimuli [18]. Parker et al. [19,20] have reported that both Parkinson's disease and Huntington's disease may be associated with functional defects in the human complex I.

Paracoccus denitrificans a 'free-living mitochondrion', has an aerobic respiratory chain that appears to be evolutionally related to the mitochondrial one [21]. The *Paracoccus* NDH-1, complex III, and cytochrome

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oxidase all closely resemble the mitochondrial enzymes in terms of electron carriers [22]. However, both complex III and cytochrome oxidase are structurally simpler than their mitochondrial counterparts [23,24]. Therefore, we have made attempts to purify the NDH-1 from *Paracoccus* membranes. The isolated enzyme complex is composed of approx. 10 dissimilar polypeptides [25]. However, as described below, the genetic data suggest that this preparation may lack several subunits. The enzyme complex prepared using dodecylmaltoside appears to be composed of approx. 15 polypeptides (T. Yagi, unpublished data).

One of the advantages of using a bacterial system is the high probability that the structural genes of an enzyme complex may constitute an operon. This has been illustrated for other oxidative phosphorylation enzyme complexes. In eukaryotes, it is difficult to demonstrate that a polypeptide is a component of an enzyme complex rather than just a copurified contaminant unless functional and/or structural justification for the polypeptide is provided. However, a polypeptide encoded by a structural gene in an operon is generally found to be an essential component of the enzyme complex coded by this operon. Another advantage of studying a bacterial enzyme complex is the facility of gene manipulation.

Recently, the identification of the NADH-binding subunit (M_r 50000) of the *Paracoccus* NDH-1 was accomplished in this laboratory by direct photoaffinity-labeling of this complex with [^{32}P]NAD(H) [26]. the *Paracoccus* NADH-binding subunit was isolated by preparative SDS gel electrophoresis and was shown to cross-react with antiserum to its bovine counterpart [26,27]. Elucidation of the primary structure of the subunit will enhance our understanding of the NADH-binding site, the prosthetic group-binding sites and the mechanism of electron transfer from NADH.

In this laboratory, the cloning of the structural gene encoding the *Paracoccus* NADH-binding subunit has been successful and the complete nucleotide sequence of the gene (*NQO1*) has been determined [28]. DNA sequencing surrounding the *NQO1* gene indicated that the structural genes encoding subunits of the *Paracoccus* NDH-1 constitute a gene cluster (possibly an operon). The entire DNA sequence of the *Paracoccus* NDH-1 gene cluster has been determined [29–31]. The gene cluster is composed of 14 structural genes and 6 URFs. Table I shows structural genes encoding subunits of the *Paracoccus* NDH-1 and their bovine counterparts. The order of the 14 structural genes in this gene cluster is *NQO7*, *NQO6*, *NQO5*, *NQO4*, *NQO2*, *NQO1*, *NQO3*, *NQO8*, *NQO9*, *NQO10*, *NQO11*, *NQO12*, *NQO13*, and *NQO14*. Upstream of the *NQO7* gene an open reading frame was detected which encodes a predicted polypeptide homologous to the UV repair enzyme A of *E. coli* and *Micrococcus lysodeikticas*. Downstream of the *NQO14* gene, an open reading frame was located which is homologous to biotin acetyl-CoA-carboxylase ligase of *E. coli*. Provided that the gene cluster contains all the structural genes of the *Paracoccus* NDH-1, it may be concluded that the *Paracoccus* NDH-1 is structurally simpler than its mitochondrial counterparts as we anticipated. A simpler structure is also supported by the results of the isolation and immunoprecipitation of this enzyme complex (T. Yagi, unpublished results).

TABLE I

Paracoccus gene	Bovine homologue
NQO1	FP 51 kDa
NQO2	24 kDa
NQO3	IP 75 kDa
NQO4	49 kDa
NQO5	30 kDa
NQO6	20 kDa [a] (*ndhK*)
NQO7	HP ND3 product
NQO8	ND1 product
NQO9	23 kDa (*ndhI*)
NQO10	ND6 product
NQO11	ND4L product
NQO12	ND5 product
NQO13	ND4 product
NQO14	ND2 product

[a] This polypeptide has been isolated from the bovine IP by Masui et al. [32]. Whether the 20-kDa polypeptide is a component of IP remains to be determined (see 32).

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