Review

The respiratory-chain NADH dehydrogenase (complex I) of mitochondria

Hanns WEISS, Thorsten FRIEDRICH, Götz HOFHAUS and Dagmar PREIS
Institut für Biochemie der Universität Düsseldorf, Federal Republic of Germany

(Received October 2, 1990) — EJB 90 1178

Contents
Introduction
Protein and redox components
Smaller, related forms of NADH dehydrogenase
Assembly and evolution
Three-dimensional gross structure
Sequences and functions of subunits
Electron and proton pathways
Human diseases associated with NADH dehydrogenase deficiency
References

Introduction

In mitochondria, electrons are transferred from NADH to O\textsubscript{2} through a chain of three large enzyme complexes, namely NADH:ubiquinone oxidoreductase (NADH dehydrogenase or complex I), ubiquinol:ferricytochrome c oxidoreductase (cytochrome reductase or complex III), and ferrocytochrome c:O\textsubscript{2} oxidoreductase (cytochrome oxidase or complex IV). The function of these enzyme complexes is to link electron transfer with proton translocation out of the mitochondrion. In doing so, they generate a transmembrane proton motive force which subsequently drives ATP synthesis by the H\textsuperscript{+}-ATPase (complex V, for a review see [1]).

NADH dehydrogenase catalyses the following reaction:

\[
\text{NADH} + \text{ubiquinone} + 5 \text{H}^+_2 = \text{NAD}^+ + \text{ubiquinol} + 4 \text{H}_2
\]

where the subscripts N and P refer to the negative inner and positive outer side of the mitochondrial inner membrane. Well known inhibitors of the reaction are rotenone and picrocin A. The enzyme is by far the largest and most complex among the proton translocating enzymes of mitochondria. It comprises some 30 different subunits of which most are nuclear-encoded and imported from the cytoplasm. Some, however, as in cytochrome reductase, cytochrome oxidase and H\textsuperscript{+}-ATPase, are encoded by genes on the mitochondrial DNA and synthesized within the mitochondrion. One FMN, a still not exactly defined number of iron-sulfur clusters, and probably one form of bound ubiquinone participate in the electron pathway through the enzyme. It is because of this enormous complexity that NADH dehydrogenase has remained the least understood among the proton translocating enzymes of mitochondria (for a review see [2]).

The more recent interest in NADH dehydrogenase has come from several directions. (a) Sequences of a number of subunits of the enzyme have been determined, allowing its relationship to different bacterial enzymes to be traced. The functions of several subunits were determined in this way. (b) Simpler, related forms of the NADH dehydrogenase have been discovered in fungi and bacteria and opened a new way of studying the role of the many protein and redox components of the enzyme. (c) Comparative electron microscopic and biochemical studies have revealed that the enzyme is constructed of two distinct parts. They are assembled, and have possibly emerged in evolution, independently of each other; they contribute to different segments of the electron pathway and are arranged in a different manner with regard to the membrane. (d) These new findings in combination with more direct functional data lead us to propose a mechanistic model for the redox-driven proton pumping of NADH dehydrogenase. (e) A broad spectrum of degenerative mitochondrial diseases has been associated with defects in NADH dehydrogenase. Further classification of these diseases and, hopefully, their better medical treatment, require a deeper understanding of the genetics, structure and the function of the enzyme.

This review will preferentially focus on the above topics, discussing reports only from the more recent literature. Comprehensive reviews on NADH dehydrogenase dealing with the stage of knowledge at that time appeared several years ago [1, 2].

Protein and redox components

Thus far NADH dehydrogenase has been isolated and characterized in detail only from bovine heart and the filamentous fungus Neurospora crassa. The bovine heart enzyme is prepared by fractional solubilization of mitochondrial membranes with bile salts and subsequent ammonium sulfate and ammonium acetate precipitation [1, 2]. The preparation is reported to be highly active but is in polydisperse state. NADH dehydrogenase of N. crassa is isolated by chromatographic steps performed in Triton X-100 solution [3]. The preparation is in monodisperse state and sedimentation equilibrium analysis corrected for bound phospholipid and detergent gave a protein molecular mass of approximately 700 kDa [3]. A similar value resulted from the flavin content and the sum of the
When treated with chaotropic agents such as NaBr, the enzymes 51, 24, and 25 have been discussed in detail previously [1, 2]. The fraction consists of three polypeptides (apparent molecular masses 51, 24, and 25 kDa) and can dehydrogenate NADH and transfer the electrons to a variety of water-soluble electron acceptors [1]. The iron-sulfur protein fraction comprises six polypeptides (apparent molecular masses 75, 49, 30, 18, 15, and 13 kDa) and the hydrophobic fraction contains most of the remaining subunits. Because the resolution of the enzyme caused a considerable alteration of EPR spectroscopic properties of the iron-sulfur clusters, their clear assignment in the three fractions was not possible [10]. When the N. crassa NADH dehydrogenase is treated with NaBr, all those subunits which are found in the bovine flavoprotein and iron-sulfur protein fractions together are stripped off. A subcomplex that, in terms of subunit composition, resembles the hydrophobic fraction of the bovine enzyme, resists the degradation and can be isolated in monodisperse state [9]. All mitochondrially encoded and only a few nuclear-encoded subunits of NADH dehydrogenase are found in the hydrophobic fraction [8] and, hence, all polypeptides of the flavoprotein and iron-sulfur protein fractions are nuclear-encoded [9, 11, 12] (Fig. 1).

While it is not questioned that NADH dehydrogenase contains 1 mol FMN/mol, the number and the nature of iron-sulfur clusters in the enzyme are still under debate. The authors of this review have no expertise in electron paramagnetic resonance (EPR) and magnetic circular dichroism (MCD) spectroscopy of iron-sulfur clusters and, therefore, do not wish to voice an opinion as to the discrepancies amongst the experts in this field. For extensive reviews the readers are referred to [13 -15]. Clearly established in the mammalian [10, 13 -17] and fungal enzyme [18] are the [2Fe-2S] cluster N-1 and the [4Fe-4S] clusters N-2, N-3 and N-4. In the mammalian NADH dehydrogenase, clusters N-1, N-3 and N-4 form an isopotential group with midpoint redox potentials ($E_{m,n}$) around $-250 \, \text{mV}$. Cluster N-2 has a more positive redox potential of approximately $-50 \, \text{mV}$ although values of $-100 \, \text{mV}$ to $-120 \, \text{mV}$ have also been reported [19, 20]. The redox potentials for the clusters N-1, N-2, N-3, N-4 in the N. crassa enzyme are $-330, -150, -230,$ and $-300 \, \text{mV}$, respectively [18]. Most authors believe that the clusters are present at concentrations comparable to FMN, but Albracht and his group [21, 22] consistently report cluster N-1 to be present at half the concentration of the other clusters. The existence in bovine NADH dehydrogenase of a fifth, consider-ably more negative ($-400$ to $-500 \, \text{mV}$) binuclear cluster, called N-1a, has been reported by the group of Ohnishi [10, 14]. In the N. crassa enzyme no experimental evidence for two species of cluster N-1 were found [18]. There is EPR spectroscopic evidence for a protein-bound ubiquemiquinone in NADH dehydrogenase, the signal of which is partially sensitive to rotenone [17, 23].

Substrate channeling of NADH from mitochondrial matrix dehydrogenases to NADH dehydrogenase has been reported to occur by transient enzyme—enzyme binding [24]. Sensitivity of the binding to low NADH concentrations was interpreted as indicating a high specificity for the binding. Identification of the subunit(s) that mediate(s) the binding might throw some light on the role of the many subunits that do not directly participate in electron transfer and proton tranlocation.

Smaller, related forms of NADH dehydrogenase

The complexity of NADH dehydrogenase has hampered progress in the study of the function of the many protein and redox components in the enzyme. Therefore, attempts were made to find related, simpler forms of the enzyme. When in N. crassa the mitochondrial protein synthesis is inhibited with chloramphenicol, a smaller form of the NADH dehydrogenase is made in place of the large enzyme, found normally. This small NADH dehydrogenase has an approximate molecular mass of 350 kDa and consists of some 13, exclusively nuclear-encoded, subunits [9]. All of them appear to be identical to nuclear-encoded subunits of the large enzyme. Our previous conclusion drawn from in situ peptide mapping experiments [9], namely that several subunits of the small form are not identical but are homologous to subunits of the large form, has not yet been confirmed by sequencing of cDNA and genomic DNA [25, 26].

Most remarkably, none of the subunits of the small form are found in the hydrophobic fraction of the large form and vice versa, i.e. with regard to their protein components, the small form and the hydrophobic fraction are complementary parts of the large form (Fig. 1). The small form has the same site for NADH as the large form, contains FMN and the iron-sulfur clusters N-1, N-3, and N-4, but is devoid of cluster N-2 [18].

The two forms of the N. crassa NADH dehydrogenase further differ in their catalytic site for ubiquinone which has a lower affinity and is insensitive to rotenone or priericidin A in the small form [9]. As will be discussed in greater detail below, the electron pathway in the large form is extended at the ubiquinone site relative to the electron pathway in the small form. The fungus appears to adapt the mitochondria to the small NADH dehydrogenase, because the ubiquinone content in the mitochondrial membrane of the chloramphenicol-treated cells is 5–8 times higher than that of the mitochondrial membranes from untreated cells [9]; (T. Friedrich and H. Weiss, unpublished result).

Also, Candida utilis appears to alternate between a large and a related small form of the NADH dehydrogenase. Cells growing in the early exponential phase were reported to have no site I phosphorylation (i.e. proton-translocating NADH dehydrogenase), rotenone sensitivity, and iron-sulfur clusters N-1 and N-2. Towards the late stationary growth phase, they all appear concomitantly [27]. The EPR spectra examined by the authors can, however, be interpreted as indicating a loss of only cluster N-2 under conditions of rotenone insensitivity [18].