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Proteomic approach to characterize mitochondrial complex I from plants

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ABSTRACT

Mitochondrial NADH dehydrogenase complex (complex I) is by far the largest protein complex of the respiratory chain. It is best characterized for bovine mitochondria and known to consist of 45 different subunits in this species. Proteomic analyses recently allowed for the first time to systematically explore complex I from plants. The enzyme is especially large and includes numerous extra subunits. Upon subunit separation by various gel electrophoresis procedures and protein identifications by mass spectrometry, overall 47 distinct types of proteins were found to form part of *Arabidopsis* complex I. An additional subunit, ND4L, is present but could not be detected by the procedures employed due to its extreme biochemical properties. Seven of the 48 subunits occur in pairs of isoforms, six of which were experimentally proven. Fifteen subunits of complex I from *Arabidopsis* are specific for plants. Some of these resemble enzymes of known functions, e.g. carbonic anhydrases and L-galactono-1,4-lactone dehydrogenase (GLDH), which catalyzes the last step of ascorbate biosynthesis. This article aims to review proteomic data on the protein composition of complex I in plants. Furthermore, a proteomic re-evaluation on its protein constituents is presented.

1. Introduction

In mitochondria, ATP formation is coupled to oxygen consumption. Prerequisite for this process is the so-called oxidative phosphorylation (OXPHOS) system, which consists of five multiprotein complexes (termed complexes I–V), cytochrome *c* and the lipid ubiquinone (Hatefi, 1985). The complexes I–IV form part of an electron transfer chain (also called the respiratory chain), which catalyzes NADH oxidation by reduction of oxygen to water. Complex V catalyzes phosphorylation of ADP. This endergonic reaction is driven by a proton gradient across the inner mitochondrial membrane which is generated by the exergonic electron transfer reactions of the respiratory chain.

1.1. Size and function of complex I

With an approximate mass of 1000 kDa, complex I (NADH:ubiquinone oxidoreductase, EC 1.6.5.3) is the largest protein complex of the mitochondrial OXPHOS system. Its major function is the transfer of two electrons from NADH (matrix side) to ubiquinone (inner mitochondrial membrane). Coupled to this reaction four protons are translocated from the mitochondrial matrix into the intermembrane space (reviewed in Brandt, 2006; Friedrich and Böttcher, 2004; Lazarou et al., 2009; Remacle et al., 2008; Vogel et al., 2007; Zickermann et al., 2008, 2009). Complex I was first purified from bovine heart mitochondria about 50 years ago by Hatefi et al. (1962). Since then, it has been studied in different eukaryotes including mammals (*bos taurus* (Carroll et al., 2003; Hirst et al., 2003), *homo sapiens* (Murray et al., 2003)), fungi (*Neurospora crassa* (Marques et al., 2005), *Yarrowia lipolytica* (Abdrakhmanova et al., 2004), and *Pichia Pastoris* (Bridges et al., 2010)), the green algae *Chlamydomonas reinhardtii* (Cardol et al., 2004) and in higher plants (Heazlewood et al., 2003; Klodmann et al., 2010; Meyer et al., 2008; Sunderhaus et al., 2006). In bacteria, a more simple form of complex I is present in the cytoplasmic membrane (Weidner et al., 1993) designated NADH dehydrogenase-1 (NDH-1). It only has half of the size of the eukaryotic complex I (550 kDa) (Yagi et al., 1998) and often is considered to represent a "minimal model" of complex I because it contains all subunits necessary to perform its major functions. The NDH-1 complex has been extensively studied in *Escherichia coli* and *Thermus thermophilus* resulting in the recent elucidation of its structure by X-ray crystallography (Berrisford and Sazanov, 2009; Efremov et al., 2010; Hinchliffe and Sazanov, 2005; Sazanov and Hinchliffe, 2006; Sazanov, 2007). Currently available information on complex I in various species is summarized on "Complex I home page" (http://www.scripps.edu/mem/ci/).

1.2. Shape of complex I

Complex I has an L-like shape with a peripheral arm protruding into the mitochondrial matrix and a membrane arm embedded in

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the inner membrane (Fig. 1). Each arm consists of at least seven "core subunits" also found in the bacterial enzyme. For a long time, crystallization of the complex did not succeed due to its size and hydrophobicity. Instead, analyses by electron microscopy allowed elucidating its overall structure (Böttcher et al., 2002; Clason et al., 2010; Dudkina et al., 2005; Grigorieff 1998; Guénebaut et al., 1997, 1998; Morgan and Sazanov, 2008; Peng et al., 2003; Radermacher et al., 2006). Insights into the internal subunit arrangement within complex I were obtained by controlled destabilization of the purified enzyme using chaotropes or different detergents and the subsequent characterization of the generated subcomplexes. Based on these experiments, first topological models have been presented (Brandt, 2006 and references within, Lazarou et al., 2009; Radermacher et al., 2006; Zickermann et al., 2008, 2009).

1.3. Subunit composition of complex I

Besides the 14 core subunits, eukaryotic complex I contains about 30 extra subunits, several of which are conserved in different groups of species. Overall, 18 of these subunits of the especially well characterized bovine complex (Carroll et al., 2002, 2003, 2006; Hirst et al., 2003) likewise are present in most other eukaryotic model systems like *N. crassa, Y. lipolytica*, *C. reinhardtii*, and *A. thaliana* (Cardol et al., 2004; Gawryluk and Gray, 2010; Morgner et al., 2008). The function of the eukaryotic extra subunits is largely unclear so far. Some of them were suggested to be important for assembly of the mitochondrial enzyme, which has to be build up by proteins encoded by two different genomes localized in the nucleus and the mitochondria (Brandt, 2006). Other subunits exhibit structural similarity to proteins of known function, e.g. the apoptosis factor GRIM19 (bovine complex I subunit B16.6 (Lazarou et al., 2009; Remacle et al., 2008) and subunit NDUFA13 in human complex I (Fearnley et al., 2001; Lu and Cao, 2008; Murray et al., 2003; Zhang et al., 2003)) and the mitochondrial preprotein translocase protein TIM17/22 (bovine complex I subunit B14.7). The remaining 10–15 extra subunits seem to be specific for subdomains of the eukaryotic kingdom. Only for some of them a biological function was proposed: complex I of mammals and fungi includes an acyl carrier protein which indeed was shown to be involved in mitochondrial fatty acid biosynthesis (Carroll et al., 2003; Sackmann et al., 1991; Zensen et al., 1992). Furthermore, an extra subunit in fungi resembles rhodaneses which are involved in FeS cluster biosynthesis (Cipollone et al., 2007; Mueller, 2006). Finally, complex I of plant mitochondria includes additional subunits as discussed below.

Different nomenclatures are used for complex I subunits in different organisms. In this review, we use the bovine names (Carroll et al., 2003, 2006; Hirst et al., 2003). For plant specific subunits, names are taken from the literature or represent accession numbers of the corresponding *Arabidopsis* genes taken from the TAIR database (www.arabidopsis.org).

1.4. The two complex I arms

The structure of the peripheral arm of bacterial complex I has been analyzed by X-ray crystallography. In *E. coli*, it consists of seven subunits which are also present in all eukaryotic complexes analyzed so far, namely the 75, 51, 49, 30, 24 kDa, PSST, and TYKY subunits. In *Thermus thermophilus* an additional frataxin-like subunit occurs (Nqo15) which is specific for this organism and its closest relatives (Hinchliffe et al., 2006). These "core" subunits of the peripheral arm comprise all redox prosthetic groups for electron transfer from NADH to ubiquinone: a flavin mononucleotide (FMN) and eight to nine iron–sulfur (FeS) clusters (Berrisford and Sazanov, 2009; Ohnishi, 1998; Hinchliffe and Sazanov, 2005; Hinchliffe et al., 2006; Sazanov and Hinchliffe, 2006; Sazanov, 2007; Yagi and Matsuno-Yagi, 2003).

Until recently, structural characterization of the hydrophobic membrane arm of complex I was entirely based on electron and immune-electron microscopy analyses (Abdrakhmanova et al., 2004; Baranova et al., 2007a,b; Clason et al., 2007). Like the peripheral arm, the membrane arm of bacterial complex I contains seven subunits termed ND1, ND2, ND3, ND4, ND4L, ND5 and ND6. They are extremely hydrophobic and in eukaryotes are encoded by the mitochondrial genome. Three subunits of the membrane arm (ND2, ND4 and ND5) show homology to a special type of Na^+/H^+ antiporters (Fearnley and Walker, 1992; Mathiesen and Hägerhäll,

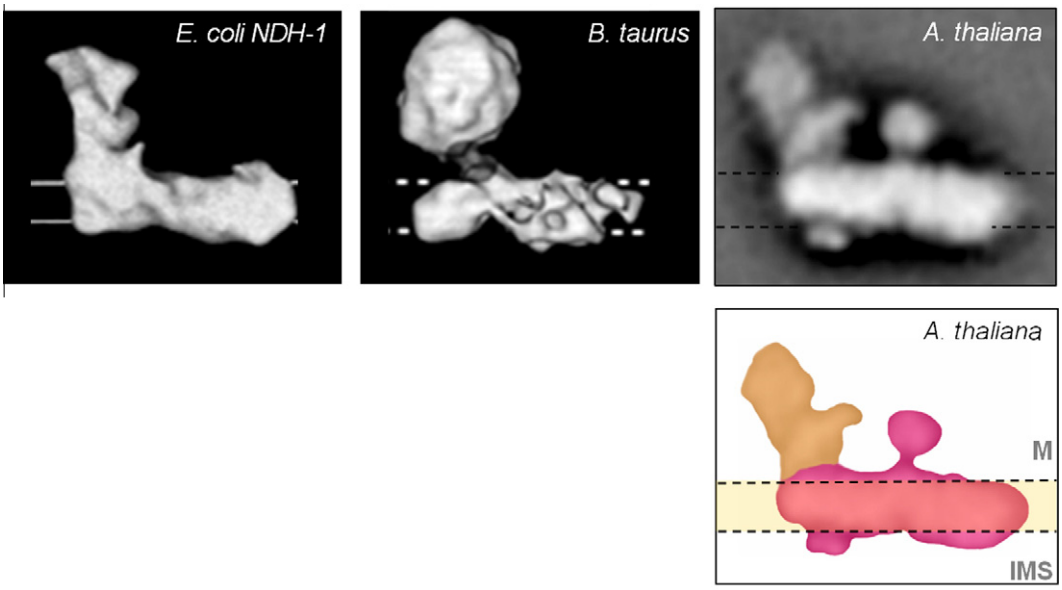

Fig. 1. Structure of complex I from *E. coli*, *B. taurus* and *A. thaliana* as revealed by single particle electron microscopy. A model of plant complex I from *A. thaliana* is given to the right. The position of the inner mitochondrial membrane is indicated in yellow. M: matrix; IMS: inter membrane space. Images were taken from Guénebaut et al. (1998) (*E. coli*), Grigorieff (1998) (*B. taurus*), and Sunderhaus et al. (2006) (*A. thaliana*), with permission.