A novel chaperone-like subunit of complex I from Thermus thermophilus

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NADH-ubiquinone oxidoreductase (complex I) is the first and the largest enzyme in the respiratory chain of mitochondria and most bacteria. Complex I catalyses the transfer of two electrons from NADH to quinone, coupled to the translocation of about four protons across the membrane. The mitochondrial enzyme contains 45 different subunits, while the bacterial enzyme consists of 13–15 different subunits. Analogues of all conserved bacterial subunits are found in the mitochondrial enzyme. We study bacterial complex I as a "minimal" model of the mitochondrial enzyme. It is an L-shaped assembly, with the hydrophobic arm embedded in the membrane and the hydrophilic arm protruding into the bacterial cytoplasm.

Recently, we have crystallised the entire complex I from Thermus thermophilus and determined its architecture by molecular replacement with the structures of the hydrophilic and the hydrophobic domains [1]. We have now identified a novel T. thermophilus protein of about 14.2 kDa, which is not part of the nqo operon. This protein is present in approximately 50% of the total purified complex I, depending on the cell growth and conditions. It does not appear to be necessary for activity, including proton-pumping. However, it is essential for crystallisation of the intact complex — it is involved in crystal contacts, and may also stabilise the junction between the two main domains, as it binds to the hydrophilic arm close to the hydrophobic domain. Although there are many chaperones/assembly factors known for the mitochondrial complex I, this is the first case of a chaperone-like protein described for bacterial complex I.

Reference

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6P5

Studies on protein/substrate interaction of type II NADH: quinone oxidoreductase from Escherichia coli

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Type II or alternative NADH dehydrogenases (NDH-II) are monomeric enzymes with a molecular mass of ≈50 kDa containing FAD as the only prosthetic group. They are membrane associated proteins that catalyse NADH:quinone oxidoreduction but do not directly contribute to the establishment of the membrane potential. NDHs-II are present in the three domains of life. In eukaryotes, their presence has been observed in plants, fungi and protozoa. In several organisms, especially in pathogenic such as Staphylococcus aureus, only NDH-II is expressed, which raises the possibility for rational design of specific drugs interacting at the level of this enzyme, which is not present in animal mitochondria. Furthermore, NDHs-II have been suggested to be used in gene therapy correcting NADH:quinone oxidoreductase activity in pathologies with malfunctioning complex I such as neurodegenerative disorders. Thus, not only for its importance in bioenergetics, but also in other areas such as health a deep knowledge of NDH-II is demanded.

Although being a simple enzyme and having been isolated for the first time more than thirty years ago, fundamental questions regarding its structural/functional relation are still unanswered. Its structure, the substrate binding sites, and its membrane association mode are still unknown. Also its functional mechanism has to be investigated, namely concerning the interaction with the substrates and the way FAD is reduced by NADH and oxidised by the quinone.

In this work conformational changes in NDH-II from Escherichia coli due to interactions with its substrates (NADH and quinone) were monitored. For that, changes in the fluorescence of tryptophan residues in response to ligand binding were followed. In order to identify the structural elements/motifs involved in substrate interaction, surface enhanced infrared absorption (SEIRA) spectroscopy was applied.

It was observed that the protein reacts with NADH and with different quinones promoting the interaction of the ubiquinone analogue, apparently, more pronounce conformational changes. The involvement of Trp and Glu/Asn residues in the binding of NADH and ubiquinone is suggested. The obtained results prompted us to extend the project to the study of NDHs-II from organisms that synthesize different quinones. A comparative study showing the differences between ubiquinone and menaquinone bindings will be showed.

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6P6

Biochemical characterisation of A/D conformational changes of mitochondrial Complex I

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