**S4.P1**

**Isolation and identification of mitochondrial complex I subunits by three-dimensional electrophoresis: Applications**

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Mitochondrial complex I or NADH:ubiquinone oxidoreductase catalyses the oxidation of matrix NADH by membrane ubiquinone and is a major entry point for electrons into the respiratory chain. It is the biggest complex of the mitochondrial respiratory chain, composed of at least 44 subunits with a molecular mass of about 1 MDa [1;2]. 14 subunits found in the bacterial enzyme represent the minimal catalytic core unit providing the catalytic activity of the enzyme while the function of the 30 remaining so-called accessory subunits found in the mammalian enzyme is still under debate. The study of these accessory subunits represents a challenge in the understanding of the mammalian complex I functioning, regulation and assembly. Analysing 44 subunits at the same time is not trivial, therefore we specifically implement three-dimensional electrophoresis to study the organisation of the enzyme. Here, we present a quick and reliable method for complex I subunit separation, and a reference “map” of the distribution of all complex I subunits after separation by BN/dSDS-PAGE without the commonly used electroelution step to extract protein from a BN-PAGE. The obtained subunit distribution from complex I isolated by BN-PAGE matches the distribution of the subunits of purified bovine complex I [3]. This map was further used to gain information on the accessibility of the mammalian complex I subunits using two different approaches. The intact, fully active enzyme was labelled with amino acid specific fluorescent probes or submitted to a limited proteolysis. The most accessible subunits presumably located at the surface of complex I (such as B8, B18 and AQQD) were found. This map can now be used to quickly identify every subunit for numerous applications, including enzyme assembly studies, DIGE-like approach or cross-linking.

**References**


**S4.P2**

**The role of proton and sodium ions on the bioenergetics of Escherichia coli. The involvement of respiratory complex I**

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Respiratory complex I plays a central role in energy transduction. It catalyzes the oxidation of NADH and the reduction of quinone coupled to ion translocation across the membrane, thereby establishing an electrochemical potential. Its crystallographic structure has been resolved [1], but still, the mechanisms of quinone reduction, ion translocation and their coupling are unknown. The nature of the ion(s) translocated by this enzyme is still a highly discussed issue, being H⁺ and Na⁺ possible candidates. It is generally accepted that H⁺ is the coupling ion in complex I [2], however in the case of some bacterial complexes I Na⁺ has been proposed to have that role [3]. It was also shown that some bacterial complexes I are capable of H⁺ and Na⁺ translocation, but in opposite directions, with H⁺ being the coupling ion [4]. In the present work, we aim to address the role of the two different ions, H⁺ and Na⁺, in the overall cellular bioenergetics of the bacterium Escherichia coli. Particularly, we study the influence of pH and sodium ions on the growth of E. coli KNabc (a strain mutated in all known Na⁺/H⁺ antiporters) and on E. coli KNabc complemented with the nhaA gene (a gene that codifies for NhaA). We also investigate the influence of proton and sodium ions on the redox activity of E. coli respiratory complex I, as well as, on the ion transport process. NADH:ubiquinone oxidoreductase activity was determined at different pH values (6.8, 7.5 and 8.2) and at different sodium concentrations (0–250 mM). Transport assays in the same conditions were also performed. Implications on the coupling mechanism of complex I are discussed.

**References**


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**S4.P3**

**Mutagenesis of complex I from Thermus thermophilus**

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NADH:ubiquinone oxidoreductase (complex I) is situated on the inner mitochondrial membrane, or the plasma membrane of most bacteria. Complex I couples the transfer of two electrons from NADH to ubiquinone with the translocation of four protons across a membrane [1] and is the first complex in the electron transport

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chain. The 14 core subunits of the complex are conserved from prokaryotes through to higher eukaryotes. The prokaryotic complex lacks the ~30 subunits that are found in the mitochondrial enzyme, making it an attractive model by which to probe the structure and mechanism. Recently, the crystal structure of complex I from Thermus thermophilus has been determined to a resolution of 3.3 Å. It suggests that the proton translocation is driven by co-ordinated conformational changes [2]. Due to the established crystallisation methods and the genetic toolbox available for T. thermophilus, this species was chosen to be a model in which to probe the mechanism of proton pumping, by site-directed mutagenesis. The systems by which genetic manipulation can be carried out in T. thermophilus have been established and used to previously knock out a variety of genes. When applied to complex I subunits however, no chromosomal gene knock out has been obtained. When screening for mutants using PCR it was consistently found that both the wild type (WT) copy of the gene and the introduced knock out cassette are found within the cell, which displays a WT phenotype. Some controversy in the literature exists as to whether T. thermophilus exists as a monoploid or a polyploid bacterium. Our results appear to be consistent with the view that T. thermophilus maintains one copy of its chromosome and that illegitimate recombination is taking place within the 200 kbp megaplasmid that it harbours. In addition to mechanistic mutations the role of two supernumerary subunits [2] that are unique to T. thermophilus were probed. The role of these is at present unclear; Nqo15 is speculated to be frataxin-like and may play a role in the assembly of iron–sulphur clusters whereas Nqo16 is postulated to be an assembly factor for the complex. An antisense RNA knockdown approach was used to probe the role of these subunits, the results of which are discussed.

References

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S4.P4
Unraveling the coupling mechanism of complex I and the role of antiporter-like subunits
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Respiratory complex I plays a central role in energy production by coupling electron transfer between NADH and quinone to ion translocation across the membrane, thereby establishing an electro-chemical potential. This L-shaped enzyme consists of hydrophilic and membrane domains. The membrane domain includes seven hydrophobic subunits and the three largest subunits, NuoL, M and N (Escherichia coli nomenclature), are homologous to each other and to Na+/H+ antiporter complex (Mrp) subunits [1,2]. Previous studies indicate that complex I from Rhodothermus marinus transduces energy by two different processes: proton pumping and Na+/H+ antiporting [3]. This work aims at evaluating the ability of the isolated complex I to translocate H+ and Na+ across the membrane and to determine the stoichiometry of the process. Moreover, we intend to evaluate the role of the individual membrane antiporter-like subunits. In order to achieve our goal, the enzyme was purified from R. marinus and incorporated into liposomes. The proteoliposomes were characterized by Dynamic Light Scattering (DLS). The existence of NADH:quinone oxidoreductase activity and the formation of membrane potential after addition of the substrates proved that the incorporation was successful. H+ and Na+ translocation were monitored by fluorescence spectroscopy and 23Na NMR, respectively. The antiporter-like subunits of complex I (NuoL, NuoM, NuoN) are composed of two structural inverted repeat structures. Each of these subunits and in the case of NuoL, each individual inverted repeat, were cloned and expressed together with a Maltose binding protein (MBP). The role of the individual subunits, namely in ion transport is investigated.

References

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S4.P5
Role of LdcI for the assembly of the Escherichia coli complex I
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NADH:ubiquinone oxidoreductase (complex I) is the main electron entry point in the respiratory chains of eukaryotes and bacteria. It couples the transfer of electrons from NADH to ubiquinone with the translocation of protons across the membrane. Complex I of Escherichia coli consists of 13 subunits, named NuoA-N, an FMN and 9 iron–sulfur (Fe/S) clusters [1]. Very little is known about the assembly of bacterial complex I, however it is reasonable to assume that the complex is assembled by joining together prefabricated smaller parts. The inducible lysine decarboxylase (LdcI) was identified as a possible assembly factor. Deletion of NuoL resulted in production of two distinct complex I populations, an active and an inactive one. While the active one contained all known cofactors and all subunits except NuoL, the inactive one lacked the most distal Fe/S cluster N2 and was associated with LdcI [2]. To further investigate the interaction between complex I and LdcI in vivo FRET measurements were performed.

References

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