S4.P6

Respiratory supercomplexes from *Ustilago maydis*, do they work as a whole unit? Structural and functional analysis

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The mitochondrial complexes of the electron transport chain generate the electrochemical proton gradient which is used for the ATP synthesis. In the last decade it has been shown that these complexes could be assembled in supercomplexes (solid model for electron flow). This evidence contrasts with the free diffusion model for electron flow through single complexes. Flux control analysis at the respiratory complexes from bovine heart mitochondria and submitochondrial particles shows flux control coefficients (Ci) for complexes I, III2 and IV of 1.06, 0.9, and 0.26, respectively (1). In contrast, in potato mitochondria Ci values for complexes I, III2 and IV were 0.89, 1.11, and 1.15 respectively. However, in the experimental conditions reported, the presence of single complexes could not be excluded. In this work the *Ustilago maydis* respiratory supercomplexes were solubilized with digitonin, a soft-detergent, and isolated by a sucrose gradient. Supercomplexes contained the activities of complexes I and IV, while complex III2 was identified by MS/MS. Stoichiometry of *U. maydis* supercomplexes was I:III2:2–3:IV:2–4 and other element, such as the alternative NADH dehydrogenases and the alternative oxidase were not present. NADH oxidation and oxygen reduction were performed by *U. maydis* supercomplexes; addition of coenzyme Q1 and cytochrome c increased the respirosome activity which was sensitive to rotenone, antimycin A or cyanide. Although substrate channeling inside supercomplexes remains to be demonstrated, in our experimental conditions the *U. maydis* respirosome works as a functional unit.

Reference


This work was supported by Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (IN214914, IN209614) from Universidad Nacional Autónoma de México (UNAM). Meztli Reyes-Galindo is a student (309210120) of the AFINES program of Facultad de Ciencias Biológicas (511021118) of Universidad Nacional Autónoma de México and fellow to CONACYT (254400).

doi:10.1016/j.bbabio.2014.05.054

S4.P7

Molecular basis of Leigh-like syndrome in respiratory complex I

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Reactive oxygen species (ROS) are known to be involved in Parkinson’s and Alzheimer’s diseases as well as other neurodegenerative diseases such as the Leigh-like syndrome, Leigh-like syndrome, also termed subacute necrotizing encephalopathy, is a neurodegenerative disease of childhood with an estimated incidence of 1:40,000 births [1]. It is clinically characterized by muscular hypotonia, respiratory insufficiency and brain stem and basal ganglia dysfunction. The NADH:ubiquinone oxidoreductase (complex I) is one of the major sources of ROS in mitochondria [2]. Tyr204 and Cys206 of subunit NDUFV1, in close vicinity to the NADH binding site, are patho-physiologically relevant as indicated by heterozygous mutations in a patient suffering from Leigh-like syndrome [3]. To gain insights into the function of these amino acids we introduced the orthologous mutations Y178C and C180G in subunit NUoF of the *Escherichia coli* complex I by X-Red mediated mutagenesis. The ROS production by the variants was determined by the Amplex Red assay revealing that the Y178CF variant shows a 5-fold increased ROS production while that of C180GF was not altered. The NADH:decyl-ubiquinone oxidoreductase activity of both variants was halved. ThermoFAD experiments demonstrated that neither the FMN-content nor the FMN-binding was significantly influenced by the mutations in contrast to what has been reported in the Literature [3].

References


doi:10.1016/j.bbabio.2014.05.055

S4.P8

Comparing the rates of ATP production driven by NADH or succinate oxidation to measure the H⁺: 2e stoichiometry of complex I

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NADH:ubiquinone oxidoreductase (complex I) of the mitochondrial electron transport chain (ETC) catalyses the two electron oxidation of NADH and reduction of ubiquinone. The energy from the redox reaction is coupled to the pumping of protons across an energy transducing membrane to build a proton motive force (Δp) that can be used to drive ATP synthesis (1). The question of how many protons are pumped by each oxidation of NADH has long been debated (2). Recent structural (3) and functional (4) data have suggested that the value is 4 H⁺/NADH. However, due to lingering issues with current methods, the development of a relatively simple and elegant technique for measuring the H⁺:2e stoichiometry of complex I (n) is still required for confirmation of this critical value and for analysis of medically relevant mutant complex I. Here, we describe experiments that aim to compare ATP production by coupled submitochondrial particles (SMPs) oxidising succinate or NADH. The H⁺:succinate stoichiometry is known to be six (5,6), due to pumping by complexes III and IV. The H⁺:NADH stoichiometry of complex I is thus (n + 6). In addition we present a new and simple method for measuring the rate of complex II catalysis; created out of necessity for this project, it has been proven a valuable tool in other areas of our kinetic studies. The complex II coupled enzyme assay can be used in SMPs, with the isolated enzyme, and in tissue samples (7).
in many debilitating human disorders. Complex I catalyzes the transfer of electrons between NADH and quinone, coupled to the translocation of protons across the membrane. The structure of bacterial complex I from *Thermus thermophilus*, consisting of 14 conserved “core” and two extra subunits, of 536 kDa in total, was determined recently [1]. Mitochondrial complex I contains up to 30 “accessory” subunits in addition to the 14 “core” subunits and is about 1 MDa in total. The role of “accessory” or “supernumerary” subunits is currently not clear, mostly because the structure of mitochondrial complex I is not known. We use complex I from mitochondria of *Neurospora crassa* fungi as a model enzyme for structural studies. It is a canonical form of eukaryotic complex, which shares the majority of subunits with mammalian enzyme [2,3]. A novel procedure for isolation of complex I from *N. crassa* has been developed, yielding pure and monodisperse sample, suitable for structural studies. Many from about 40 subunits expected to be present in the complex were identified by mass-spectrometry. Preliminary analysis by single particle cryo-EM indicates a familiar L-shape of the complex, which will be compared to EM reconstructions of complex I from other species.

**References**


**S4.P9**

**Structure of the NADH binding site of respiratory complex I**

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The bacterial NADH:ubiquinone oxireductase, respiratory complex I, couples the transfer of electrons from NADH to ubiquinone with the translocation of protons across the membrane. It harbors one FMN and up to 10 Fe/S clusters depending on the species. The X-ray structure of *Thermus thermophilus* complex I revealed the structure of the NADH binding site being composed of a modified Rossmann fold to bind FMN and NADH [1]. We solved the structure of the NADH binding site of complex I from *Aquifex aeolicus* in the oxidized and reduced state with bound substrates at resolutions higher than 2 Å [2]. The redox reaction is accompanied with a structural rearrangement in the active site. The structural flexibility was restricted by site-directed mutagenesis. The effect of the mutations on the structural change in the active site was proven by structural analysis of the variants. The homologous mutations were introduced in the *Escherichia coli* complex I leading to a fully assembled complex that is unable to oxidize NADH. Thus, the redox-dependent conformational change in the active site is essential for NADH oxidation.

**S4.P11**

**Proton transfer pathways in the Respiratory Complex I**

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Respiratory Complex I is a redox-driven proton-pump, which drives proton-pumping across the mitochondrial inner membrane and bacterial cytoplasmic membrane by reduction of quinones. The established electrochemical proton gradient provides the driving force for active transport and synthesis of ATP and is thus crucial for biological energy conversion. Complex I comprises a membrane domain with three antiporter-like subunits, catalyzing the proton-pumping process, and a soluble domain, responsible for reduction of quinones by electron transfer from NADH. Remarkably, site-directed mutagenesis experiments show that mutation of titratable residues in the antiporter-like subunit, –20 Å away from site of quinone reduction, inhibits both proton-pumping as well as quinone reductions. To explain this long-range proton-coupled electron transfer mechanism, both indirect and direct coupling models have been suggested. However, despite the recent elucidation of the complete intact structure of Complex I, the molecular principles of the coupling principles remain elusive. We present here results from large-scale classical and hybrid quantum-classical (QM/MM) molecular dynamics (MD) simulations of Complex I, embedded in

**References**

