Removal of Endogenous Phospholipids of Rhodobacter Sphaeroides Cytochrome C Oxidase affects the Flexibility of the Enzyme

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energized status of the inner membrane caused dramatic structural alterations in the channel region. In an energized membrane, TMS2 formed a continuous α-helix that was inaccessible to the aqueous intermembrane space. Upon depolarization, the helical periodicity of TMS2 was disrupted and the channel became exposed to the IMS. Real time kinetic measurements confirmed that changes in TMS2 conformation coincided with depolarization. This analysis is extended to the soluble receptor domain of Tim23, where we show protonmotive force-coupled structural changes and key protein interactions that are mediated by specific lipids within the inner membrane. These results reveal how the energized state of the membrane drives functionally relevant structural dynamics in membrane proteins that are coupled to processes such as channel gating.

1869-Pos Board B599

Evolutionary Perspective on the Coupling Mechanism of Complex I and Related Enzymes

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Complex I is an energy transducing enzyme present in the three domains of life. This enzyme catalyzes the oxidation of NADH and the reduction of quinone coupled to charge translocation across the membrane. In this way, it contributes to the establishment of the transmembrane difference of electrochemical potential which is used for ATP synthesis, solute transport and motility. The research on this enzyme has gained a new enthusiasm, especially after the resolution of the crystallographic structures of bacterial and mitochondrial complexes. Most attention is now dedicated to the investigation of the energy coupling mechanism.

In this work, we made a thorough investigation of complex I and group 4 [NiFe] hydrogenases and established a third member of this family of proteins: the energy-converting hydrogenase related complex. We observed that four subunits (NuoB, D, H and antiporter-like) are common to the 3 types of complexes and we have denominated these subunits as the universal adaptor. We further explored the properties of the adaptor by investigating the structural characteristics of the antiporter-like subunit. We observed that the adaptor contains an antiporter-like subunit with a long amphipathic α-helix. The long helix is a common denominator that has been conserved through evolution. This should reflect a key role of such helix in the coupling mechanism of this family of enzymes.

We are currently investigating the structural motifs involved in Na^+/H^+ antiporter activity in complex I and related complexes. These findings are a step forward in the investigation of the coupling mechanism of complex I.

1870-Pos Board B600

Water Gated Transitions in Proton Pumping of Respiratory Complex I

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Respiratory Complex I or NADH:ubiquinone oxidoreductase is a redox-driven proton-pump. Powered by quinone reduction, Complex I drives the energetically uphill translocation of protons across the mitochondrial inner membrane and bacterial cytoplasmic membrane. 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 that includes three antiporter-like subunits, involved in 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 mutations of titratable residues in the antiporter-like subunits, ~200 Å away from site of quinone reduction, inhibit both proton pumping and quinone reduction. 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 of large-scale classical and hybrid quantum-classical (QM/MM) molecular dynamics (MD) simulations of Complex I, embedded in biologically realistic environments. Our simulations indicate that water molecules provide important elements in the proton-pumping process. Our findings may form a basis for understanding long-range energy transduction in Complex I, and mechanistic similarities to other redox-driven proton-pumps such as cytochrome c oxidase and bacteriorhodopsin.

1871-Pos Board B601

Dielectric Heterogeneity in the Cytochrome B6F Complex

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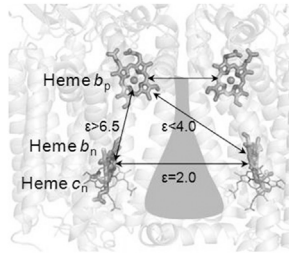
Electron transfer in the dimeric cytochrome b_6f complex, which includes four *b*-type hemes organized as two pairs in symmetric monomers, was studied by simultaneous measurement of the kinetics of heme reduction by dithionite and an associated amplitude increase of Soret band split circular dichroism (CD) spectra diagnostic of heme-heme exciton interactions, for which similar kinetics were determined. Based on inter-heme distances and orientations from crystal structures of the complex, the increase in the split CD signal is dominated by interaction between the two intra-monomer *b*-hemes, located on the electrochemically negative and positive sides of the complex, whose midpoint oxidation-reduction potentials, E_m, determined by titrations of isolated complex, differ by 75-100 mV. Kinetics are fit best by preferential reduction of the intra-monomer heme pair. Equilibration of transferred electrons would, however, predict preferential reduction of the two higher potential hemes, one in each monomer. Heterogeneity of the dielectric constant is implied, a consequence of structure inhomogeneity, and/or dielectric reorganization in response to electron transfer. The largest dielectric constant exists between the intra-monomer *b*-hemes, resulting in a lower energy state of the reduced intra-monomer heme pair relative to any other heme pair.



1872-Pos Board B602

Removal of Endogenous Phospholipids of Rhodobacter Sphaeroides Cytochrome C Oxidase affects the Flexibility of the Enzyme

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The crystal structure of cytochrome *c* oxidase (COX) from *Rhodobacter sphaeroides* shows two phospholipids intercalated inside subunit III and four at the interface between subunits I, III and IV. These phospholipids are necessary for electron transfer, but their exact function in the structure of COX is still unclear.

Phospholipids were removed from COX by incubation with molar stoichiometric amounts of phospholipase A_2 for 3 hours at 4 °C in 20 mM MOPS pH 7.2, 20 mM $CaCl_2$ and 0.2 % dodecyl maltoside. The enzyme was then washed on a cytochrome *c*-affinity column; phosphorous, iron and copper content was determined by inductively coupled plasma-mass spectrometry. Wild-type enzyme contained an average of 5 moles phosphorous per mole enzyme, while the delipidated enzyme contained less than one. Electron transfer activity in the treated enzyme was decreased 30% and it exhibited suicide inactivation. Inhibition of electron transfer activity and suicide inactivation were reversed by the addition of 1 mg/ml asolectin. The time dependence of α-chymotrypsin digestion of the enzyme showed that subunit I was digested at a faster rate in the delipidated COX, suggesting a more open conformation in the lipid-depleted COX. To further assess COX conformational flexibility upon delipidation, both COX forms were labeled in subunit III with a sulfhydryl group-directed fluorophore, N-iodoacetylaminoethyl-1-aminonaphthalene-5-sulfonate (IAEDANS). Fluorescence anisotropy measurements showed a 50% increase in the rotational rate of AEDANS-labeled delipidated COX. This increase in flexibility of subunit III affects the flexibility of the adjacent subunit I as shown by the higher chymotrypsin digestion rate of subunit I in the delipidated enzyme. Taken together, these data provide an explanation of the low turnover rates and suicide inactivation, both of which occur in COX in the absence of phospholipids.

1873-Pos Board B603

Energy Transfer in a Molecular Motor in Kramers' Regime

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We present a theoretical treatment of energy transfer in a molecular motor described in terms of Brownian motion on a multidimensional free-energy landscape. We implement the classical analog of the tight-binding model of quantum mechanics to transform the continuous diffusion equation to a discrete master equation that is analytically tractable [1]. This treatment applies for multidimensional non-separable periodic potentials enabling energy transfer between degrees of freedom to be described explicitly in both the strong and