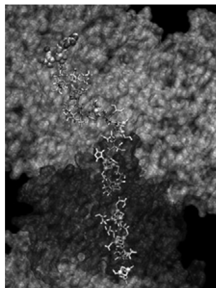
CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector

The nature of the coupling charge of *Rhodothermus marinus* CpI was investigated using inside-out membrane vesicles. We observed that this CpI is able of H^+ and Na^+ transport, although to opposite directions. The H^+ is imported and Na^+ is exported from the vesicles, indicating that the H^+ is the coupling ion of the system [4]. The Na^+ transport is specific of CpI activity, being sensitive to its inhibitor rotenone, and stimulated by the presence of CCCP, a protonophore. We observed that although neither the catalytic reaction nor the establishment of the ΔpH requires the presence of Na^+, its presence increased the H^+-transport. We proposed a model for the coupling mechanism of CpI, suggesting the presence of two different energy coupling-sites, one working as a H^+-pump (Na^+ independent), and the other functioning as a Na^+/H^+-antiporter (Na^+ dependent) [4]. This model was reinforced by studies performed in the presence of the Na^+/H^+-antiporter inhibitor, 5-(*N*-ethyl-*N*-isopropyl)-amiloride (EIPA) [5].
In order to establish whether the antiporter site was exclusive of *R.marinus* CpI we addressed ion translocation by the two most studied bacterial enzymes. We observed that *Ecoli* CpI also presents the antiporter activity, but that from *Pdenitrificans* does not. We proposed a correlation between the type of quinone used as substrate and the presence of the antiporter activity[6].

[1] Efremov etal(2010) *Nature 465, 441*.
[2] Hunte, C.etal(2010) *Science 329, 448*.
[3] Efremov and Sazanov(2011) *Nature 476, 414*.
[4] Batista, etal(2010) *BiochimBiophysActa 1797, 509*.
[5] Batista, etal(2011) *ACS ChemBiol 6, 477*
[6] Batista and Pereira(2011) *BiochimBiophysActa 1807, 286*.

2915-Pos Board B685
Electron Tunneling in Respiratory Complex I
Tomoyuki Hayashi, Alexei A. Stuchebrukhov.
University of California, Davis, Davis, CA, USA.
NADH:ubiquinone oxidoreductase (complex I) plays a central role in the respiratory electron transport chain by coupling the transfer of electrons from NADH to ubiquinone to proton pumping across the membrane. Until now, the atomistic details of electron transfer have remained unknown. In this study, electron tunneling along seven Fe/S clusters in complex I is examined in atomistic resolution by using the tunneling current theory and computer simulations [1]. Distinct electron tunneling pathways between neighboring Fe/S clusters are identified; the pathways primarily consist of two cysteine ligands and one additional key residue. The identified key residues are further characterized by sensitivity of electron transfer rates to their mutations, examined in simulations and their conservation among complex I homologues. Internal water between protein subunits is identified as an essential mediator enhancing drastically the overall electron transfer rate to achieve the physiologically significant value. With the water included, negative slope of the distance dependence of the electron transfer rates becomes close to a typical 1.4 in natural logarithm . The unusual electronic structure properties of Fe/S clusters in complex I explain their remarkable efficiency of electron transfer.



[1] T.Hayashi, A.A.Stuchebrukhov, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 19157

2916-Pos Board B686
The Role of Acid Residues in Na^+ Uptake and Binding in Na^+-NQR from Vibrio Cholerae
Blanca Barquera, Oscar Juarez, Michael E. Shea, Jonathan Cho,
Darcie Cook.
RPI, Troy, NY, USA.
The Na^+-translocating NADH:quinone oxidoreductase (Na^+-NQR) is the gateway for electrons into the respiratory chain of *Vibrio cholerae* and many other pathogenic bacteria. Na^+-NQR is unique among respiratory enzymes in that it pumps sodium rather than protons.
The character of Na^+ binding to the enzyme is crucial to understanding the coupling between Na^+ translocation and the redox reaction. We are using functional studies_steady state and transient kinetics and equilibrium binding_in combination with site-directed mutagenesis to investigate the interaction of Na^+ with Na^+-NQR. Recently, we have focused on conserved acid residues in membrane spanning regions as candidates for Na^+ binding site ligands. Here, we describe results on two of these residues, which are both involved in Na^+ uptake by the enzyme: NqrB-D397 and NqrE-E95.
Replacement of either residue by a neutral amino acid (Ala) results in a large increase in the apparent Km for Na^+. In the case of NqrB-D397, replacement by Glu or Cys, produced smaller changes in Km^{app}, indicating that the size and charge of the residue at this position both modulate Na^+ binding. Stopped-flow kinetic measurements show that mutations at both positions exert their effect specifically at one internal electron transfer step: 2Fe-2S center àFMN$_C$. These results are consistent with the earlier finding that this is the first Na^+ dependent electron transfer step in the enzyme reaction. The results are discussed will be discussed in the context of our current model of Na^+-NQR as a redox driven Na+ pump, that operates on the basis of kinetic rather than thermodynamic coupling.

2917-Pos Board B687
Suicide Inactivation in *Rhodobacter Sphaeroides* Cytochrome *c* Oxidase Lacking Subunit III Coincides with Release of Cu_B and Major Conformational Changes in Subunit I
Lawrence J. Prochaska[1], Robert R. Geyer[1], Jonathan P. Hosler[2],
Audie Thompson[2], Lakshman Varanasi[2], Gerald M. Alter[1], Aimin Liu[2].
[1]Wright State University, Dayton, OH, USA, [2]University of Mississippi Medical Center, Jackson, MS, USA.
Cytochrome *c* oxidase from *Rhodobacter sphaeroides* shares homology with the three subunit core of the mitochondrial form. Subunits I (SI) and II contain the redox centers (Cu_A, heme *a*, and the binuclear center oxygen binding site, heme a_3 and Cu_B) of the enzyme. Subunit III (SIII) functions to inhibit turnover induced suicide inactivation by maintaining proton uptake into the D pathway and by stabilizing the heme a_3-Cu_B active site. Metal analysis of I/II oxidase, as determined by ICP-OES, shows that suicide inactivation leads to the release of one copper; EPR spectroscopy indicates that the missing copper is Cu_B. Limited proteolysis of wild-type and I/II oxidase using α-chymotrypsin showed no differences in the pattern of proteolytic digestion, however, SI of I/II oxidase was digested at a faster rate. MALDI-TOF and protein sequencing showed that the cleavage sites are localized to the N and C-termini of SI. Suicide-inactivated I/II oxidase exhibits a completely different digestion pattern, including the release of a peptide (AA 237-258 in SI; identified by MS-MS) that is located in a region above Cu_B in the enzyme. In an attempt to identify the oxidative reaction which leads to suicide inactivation, the two-electron reduced PM and three-electron reduced F intermediates were generated chemically in I/II oxidase. After incubation, the I/II oxidase was assayed for O_2 reduction activity and no inactivation was observed, suggesting that build up of strongly oxidizing chemical intermediates at the active site does not lead to suicide inactivation. These results suggest that suicide inactivation in I/II oxidase is triggered by a mechanism leading to Cu_B loss with concomitant conformation changes in SI.

2918-Pos Board B688
New Ligands of the Conserved Steroid Binding Site of Cytochrome c Oxidase
Carrie Hiser, Leann Buhrow, Jian Liu, Shelagh Ferguson-Miller.
Michigan State University, East Lansing, MI, USA.
Cytochrome *c* oxidase (*Cc*O), the terminal enzyme in the electron transfer chain of mitochondria and many bacteria, requires the uptake of protons for activity via two pathways, the D-path and the K-path. Among the mutants of *Rhodobacter sphaeroides* (*Rs*) *Cc*O that inhibit proton uptake in the K-path is E101A in subunit II, which removes a key carboxyl at the entrance to the pathway and decreases the activity of the purified enzyme over 20-fold. Micromolar levels of the bile acids cholate or deoxycholate were shown to stimulate E101A activity 10-fold, and crystals of wildtype *RsCc*O grown in the presence of deoxycholate (Qin *et al*., *Biochemistry* 47:9931-9933, 2008) showed a single deoxycholate molecule bound close to E101 in the same location as a cholate molecule observed in bovine *Cc*O crystal structure (PDB 1OCC). Evidence of protective effects of steroids against *Cc*O inhibition in bilirubin neurotoxicity and in Alzheimers (Vaz *et al*., *J. Neurochem.* 112:56-65, 2010; Tillement *et al*., *Steroids* 71:725-735, 2006) suggest that this site could be physiologically relevant to regulation of *Cc*O.
Here we use the *RsCc*O mutant E101A as a sensitive assay to further investigate the nature of this conserved site. Activity assays show that certain other steroids (cholesterol hemisuccinate) and lipidic molecules (phytanic acid, arachidonic acid) and some detergents affect *RsCc*O in a manner that indicates that these ligands also bind at the same site. The studies reveal a high degree of specificity and suggest possible physiological regulators. Crystallographic and computational approaches are underway to identify additional potential ligands and to determine the physiological significance of this conserved binding site. (NIH GM26916)

2919-Pos Board B689
A Kinetic Model of Proton Transport in a Multi-Redox Center Protein: Cytochrome c Oxidase
Johannes Srajer, Andreas Schwaighofer, Asmorom Kibrom,
Christoph Nowak, Renate L.C. Naumann.
AIT Austrian Institut of Technology, Vienna, Austria.
Simulations of electrochemical measurements are presented, making use of a model system comprising cytochrome c oxidase (CcO) immobilized in a strict orientation on an electrode. This allows studying direct electron transfer (ET) to