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doi:10.1016/j.bbabbio.2010.04.294

#### 11P.14 Yeast cytochrome *c* oxidase: A model system for determining the specific role of bound phospholipids

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Cytochrome *c* oxidase (EC 1.9.3.1; CcO) catalyzes the transfer of electrons from ferrocytochrome *c* to oxygen, a reaction coupled to proton translocation across the inner mitochondrial membrane. One feature that most directly impacts upon the structural and functional integrity of CcO is cardiolipin (CL) tightly bound to the enzyme. Hypotheses have been put forward that (i) CL acts as “glue” to stabilize the multi-subunit complexes; and (ii) CL functions as a “proton antenna” to facilitate proton entry into the active site of enzyme. One of the best ways to test these hypotheses is site-directed mutagenesis of *Saccharomyces cerevisiae* CcO. The enzyme is structurally similar to mammalian CcO and is amenable to genetic manipulation of its structure. Towards this goal, CcO was isolated from baker's yeast. The enzyme was extracted from mitochondria using dodecyl maltoside and purified by high performance Q-Sepharose column chromatography. The resulting enzyme has the expected oxidized and reduced visible absorption spectrum, and a molecular activity of about  $120 \text{ s}^{-1}$  when assayed spectrophotometrically using ferrocytochrome *c* as a substrate. The subunit composition of yeast CcO was analyzed by a combination of RP-HPLC and mass spectrometry. Seven major HPLC elution peaks were detected using absorption at 214 nm. The identity of the 8 nuclear encoded subunits that eluted from the RP-HPLC column was determined by electrospray ionization mass spectrometry. Normal phase silicic acid HPLC analysis of the extracted from isolated CcO phospholipids confirmed that a small number of phospholipids including CL, phosphatidylcholine, and phosphatidylethanolamine co-purified with CcO. Treatment of CcO with phospholipase A2 resulted in partial inactivation of the enzyme indicating the important functional role of at least some of these bound phospholipids.

This work was supported by the National Institutes of Health (GMS 24795 to N. C. Robinson).

doi:10.1016/j.bbabbio.2010.04.295

#### 11P.15 Membrane-facilitated proton transfer to the surface of a membrane-spanning proton transporter

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A key step in energy metabolism of a living organism is translocation of protons across a membrane, conducted by membrane-spanning proton transporters of the respiratory chain. The electrochemical gradient maintained by these transporters is utilized,

for example, for synthesis of ATP. In the present study we used Fluorescence Correlation Spectroscopy (FCS) to investigate the interplay between the components of the respiratory chain and the membrane, and the effect of the membrane on the proton transfer in the energy-conservation machinery. The FCS technique was used earlier in our laboratories to study membrane-facilitated proton transfer, by determining the protonation kinetics of a fluorescein molecule anchored to the surfaces of membranes of different composition. The results from these studies showed that the protonation rate of the fluorescein molecule increased upon incorporation of the probe into a membrane. This acceleration in the rate was interpreted in terms of a proton-collecting antenna, composed of the lipid molecules, that acts to facilitate protonation of the surface-bound probe [1]. Here we have used the FCS technique to investigate the interplay between the membrane surface and the protein surface of one of the proton transporters of the respiratory chain. A fluorescein molecule was covalently linked to the surface of cytochrome *c* oxidase from *Rhodobacter sphaeroides*. The protonation kinetics was determined for the fluorescein molecule linked to the detergent-solubilized protein as well as to the protein incorporated into di-oleyl-phosphatidylglycerol vesicles. The results show that the protonation rate increased by a factor of about 400, from about  $7 \times 10^{10} \text{ s}^{-1} \text{ M}^{-1}$  for the detergent-solubilized oxidase to about  $3 \times 10^{13} \text{ s}^{-1} \text{ M}^{-1}$  upon incorporation into vesicles. Collectively, these results indicate that there is proton transfer to the protein surface facilitated by the membrane surface [2].

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doi:10.1016/j.bbabbio.2010.04.296

#### 11P.16 Characterisation and flash photolysis of carbon monoxide adducts of heme-copper binuclear model compounds

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In studies of heme proteins including cytochrome *c* oxidase, carbon monoxide has often been used as a surrogate for the physiological reactant dioxygen. Investigations employing CO flash photolysis have been useful in probing the dynamics and coordination chemistry of the heme-Cu<sub>B</sub> binding pocket after CO photolysis [1, 2]. Therefore, we have carried out systematic studies of CO coordination and photodissociation, on a series of biomimetic models of the binuclear Fe/Cu (heme/trisimidazole) active site of cytochrome *c* oxidase. Based upon a porphyrin core, all these models have a covalently linked pyridine in the proximal site of the porphyrin but they differ strongly by the environment around the copper and their rigidity. In order to explore the influence of copper (I) on the heme-bound CO, we have used cryogenic difference FTIR spectroscopy and a Nd-Yag laser for photodissociation. In the absence of copper bound in the distal pocket, the CO stretching frequency of (L)Fe<sup>II</sup>-CO is observed at  $1978\text{--}1982 \text{ cm}^{-1}$  for all complexes. Interestingly, the more rigid model compound showed two Fe-CO stretching frequencies upon addition of copper (I), one in the same region as the model without copper and one strongly downshifted by about  $33 \text{ cm}^{-1}$ . Upon photolysis, the CO was transferred from the heme to the copper (I) ion. This was not the case for