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Abstracts

S11 Terminal Oxidases

Lectures

11L.1 Ligand binding, electron and proton transfer in NO-reducing heme-copper oxidases

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Heme-copper oxidases (HCuOs) are the terminal components of the respiratory chain in the mitochondrial membrane or the cell membrane in many bacteria. These enzymes reduce oxygen to water and use the free energy from this reaction to maintain a proton-motive force across the membrane in which they are embedded. The HCuOs of the *cbb*$_3$-type are only found in bacteria, often pathogenic ones since they have a high O_2 affinity enabling the bacteria to colonize semi-anoxic environments. *Cbb*$_3$-type (C) oxidases are highly divergent from the mitochondrial-like aa$_3$-type (A) oxidases, and within the HCuO family, *cbb*$_3$ is the closest relative to the most divergent member, the bacterial nitric oxide reductases (NOR). NORs reduce NO to N_2O without coupling the reaction to the generation of any electrochemical proton gradient. The significant structural differences between A-type HCuOs and C-type HCuOs as well as NORs are manifested in the lack in *cbb*$_3$ and NOR of most of the amino acids found to be important for proton pumping in the A-type, as well as in the different binding characteristics of ligands such as CO, O_2 and NO. Despite these differences, and in contrast to NORs, *cbb*$_3$-type oxidases are proton pumps [1, 2]. Investigations of ligand binding and reduction in *cbb*$_3$s and NORs have provided insights into the mechanism of O_2 and NO reduction as well as the proton-pumping mechanism in all HCuOs (see e.g. [3]). In this presentation, results from these studies will be discussed with the focus on the relation between proton transfer and ligand binding/reduction. Furthermore, the influence of the lipid membrane on these reactions will be discussed.

References
[1] E. Arslan, et al., FEBS Lett. 470 (2000) 7–10.
[2] M. Toledo-Cuevas, et al., Biochim. Biophys. Acta 1365 (1998) 421–434.
[3] Y. Huang, et al., Proc. Natl. Acad. Sci. USA 105 (2008) 20257–20262.

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11L.2 Choreography of electron and proton transfer in cytochrome *c* oxidase

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Cytochrome *c* oxidase (CytcO) is a multisubunit membrane-bound enzyme, which catalyzes oxidation of four molecules of cytochrome c^{2+} and reduction of molecular oxygen to water. The free energy of this reaction is conserved in part by transfer of the electrons and protons from opposite sides of the membrane, and in part by linking the O_2-reduction reaction to pumping of 1 H^+/e^- across the membrane. This pumping stoichiometry can be altered by introduction of specific mutations, as far away as about 25 Å from the catalytic site, within one of the proton pathways of the enzyme [1], to obtain essentially any stoichiometry in the range 0–1. In many cases these mutations leave the proton-transfer rate through the pathway unaffected. Understanding the molecular mechanism of such partial or full uncoupling of proton pumping from O_2 reduction is important for understanding the molecular design of the proton-pumping machinery in the wild-type Cyt*c*O [2]. One striking feature of all uncoupled mutants studied to date is that the (apparent) pK_a of an internal proton donor, Glu286, located deeply within a proton pathway, is either increased or decreased (from 9.4 in the wild-type oxidase) (e.g. [3]). Nonetheless, our results suggest that the pK_a itself is not a determinant of the proton-pumping stoichiometry as there are cases where proton pumping is maintained even with a shifted Glu286 pK_a. We speculate that the apparent link between changes in the pumping stoichiometry and the Glu286 pK_a reflects local changes in hydrogen bonding, which determine the proton-transfer rates to the catalytic site and acceptor site of pumped protons. The results establish a link between specific structural changes in the proton pump and, its thermodynamic and kinetic properties.

References
[1] P. Brzezinski, R.B. Gennis, J. Bioenerg. & Biomem. 1 (2008).
[2] K. Faxén, G. Gilderson, P. Ädelroth, P. Brzezinski, Nature 437 (2005) 286.
[3] A. Namslauer, A.S. Pawate, R.B. Gennis, P. Brzezinski, Proc Natl Acad Sci USA 100 (2003) 15543.

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