Dissertation Abstract

Terminal oxidases of the respiratory chain are membrane-bound protein complexes that catalyze the reduction of oxygen to water. The protons needed for water formation are taken up from the inside of the membrane. Furthermore, the enzyme uses some of the free energy released in the oxygen reduction reaction to pump four protons over the membrane. This very complicated reaction proceeds with a fast rate *in vitro*; every second about 1000 water molecules are produced and 2000 protons are taken up from the bulk solution of which 1000 are translocated across the membrane. Although much is known about the mechanism of oxygen reduction, its coupling to proton pumping and the mechanism by which the enzyme is able to pick up protons with a high rate is still to be unraveled.

The main focus of this thesis work is the mechanism of proton transfer in terminal oxidases from the bacterium *Rhodobacter sphaeroides* and the archaea *Acidianus ambivalens*. Cytochrome *c* oxidase from *R. sphaeroides* is a useful bacterial model enzyme for its eukaroytic counterpart because of their remarkable similarity. The quinol oxidas from *A. ambivalens,* on the other hand, is structurally very different from the eukaryotic enzyme. It lacks all the residues of one of the two proton pathways, the D-pathway, including the Glu286 that has been proposed to constitute the proton-pumping element of the mitochondrial-like terminal oxidases.

The reaction between the reduced enzyme and oxygen was studied using time-resolved optical spectroscopy. The different reaction steps during oxygen reduction, including proton uptake from bulk solution, were found to be the same for the *A. ambivalens* enzyme as for the mitochondrial-like enzymes. The *R. sphaeroides* cytochrome *c* oxidase double mutant EA(I-286)/IE(I-112), which was constructed to mimic the *A. ambivalens* enzyme was also shown to reduce oxygen to water *and* to pump protons.

The effect of zinc ions on proton-transfer reactions in the wild-type enzyme from *R. sphaeroides* was investigated. The presence of Zn^{2+} reduces the rate of proton uptake from the bulk solution >>20-fold. This effect is explained by Zn^{2+} binding to the entrance of one of the proton pathways.

The results are discussed in the context of the proton uptake and proton pumping ability of terminal oxidases.

Key words: cytochrome aa_3, electron transfer, proton transfer, proton pumping, oxygen reduction, *Rhodobacter sphaeroides*, *Acidianus ambivalens*

ISBN: 91-628-5282-5