

EXAFS Investigations of Cytochrome-c Oxidase: Substrate and Inhibitor Binding

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The integral membrane protein cytochrome-c oxidase catalyzes the conversion of dioxygen to water as the terminal reaction in aerobic respiration. The redox energy of this reaction step is converted into a proton gradient across the plasmatic or mitochondrial membrane. The gradient is utilized for the biosynthesis of ATP.

In this complex enzyme which contains several metal sites, the homodinuclear Cu_A center acts as the primary electron acceptor whereas the heterodinuclear Fe_{a₃}-Cu_B center binds the substrate. Both of them have been characterized by EXAFS and X-ray diffraction studies in the native (oxidized) form. The EXAFS investigation of the reduced enzyme from *Thermus thermophilus* (ba₃ oxidase) has also been reported. The reduction of the enzyme reduces the oxidation states of all copper atoms to +1 and that of iron within Fe_{a₃} to +2 without effecting the number of ligands.

The coordination sphere of iron within heme a₃ of the ba₃ oxidase of *T. thermophilus* consists of five nitrogen donor functions and an additional ligand. This ligand (substrate or inhibitor) acts as a bridge towards the copper atom of Cu_B which binds three histidine residues.

The present EXAFS study contributes to the question of the mode of substrate or inhibitor binding to the metals of the heterodinuclear site. To this end, the native as well as the inhibited forms of the oxidized and the dithionite-reduced ba₃ oxidase of *T. thermophilus* have been measured by XAS. The combination of the information obtained from the iron and the copper K-edge EXAFS leads to the exact spatial arrangement of the atoms within the binding site. The structure analysis clearly shows that the coordinated units (substrate/inhibitor) are bound to the copper and iron atom simultaneously in *end-on* fashion.