STRUCTURE AND POSSIBLE MECHANISM OF ACTION OF CYTOCHROME C OXIDASE FROM *PARACOCUS DENITRIFICANS*.

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The cytochrome c Oxidase from the soil bacterium *Paracoccus denitrificans* could be crystallized with the help of antibody Fv-fragments (1). The subsequent X-ray structure analysis revealed the structure of the four protein subunits, and the arrangement and mode of binding of the metal centers involved in electron transfer (2). In agreement with results from site-directed mutagenesis studies, two possible pathways for proton transfer, one for those being consumed upon reduction of dioxygen and one for those being pumped across the membrane could be identified. The absence of electron density in the azide inhibited enzyme for a histidine side chain, which was postulated by site-directed mutagenesis and spectroscopy to be a ligand to CuB of the oxygen binding site, was used to propose a histidine shuttle/cycle mechanism for proton pumping. The structure of intermediates of the redox reaction is currently being determined.

References:


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A mechanism of Cytochrome c Oxidase Proposed by the Crystal Structures at Different Oxidation and Ligand Binding States.

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The X-ray crystallographic structure of beef heart cytochrome c oxidase on reduction of the fully oxidized enzyme induces a movement of a segment from Gly49 to Asn55 of subunit I. The conformational change induces a big change in the environment of an aspartate residue, Asp 51, from a buried and hydrogen-bonded state in the interior to a state partially exposed towards the cytosolic side releasing the hydrogen bonds which suggests a significant change in pK. Two possible hydrogen bond networks which are likely to be the proton wires are detectable extending from the Asp51 to the matrix side. Both networks go through points very near the heme a, which suggests the involvement of the heme to the proton pumping. A μ-peroxo bridge between Fea3 and CuB is found at the fully oxidized state and a hydroxide anion on Fe2+a3 at the fully reduced state. These structures suggest that the proton pumping at the system including Asp51 is coupled to the dioxygen reduction at the heme a3-CuB site with the electron transfer from heme a to the dioxygen reduction site.
The Use of Mutants to Investigate the Putative Proton Pumping Channels in Cytochrome c Oxidase

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In a collaboration with the groups of Dr. Alexander Konstantinov (Moscow State University) and Dr. Peter Brzezinski (Göteborg University) we have been examining mutants of the cytochrome c oxidase from Rhodobacter sphaeroides. This is a prokaryotic homologue of the mitochondrial oxidase and, like the eukaryotic enzyme, couples the reduction of dioxygen (to water) to the generation of voltage and pH gradients (i.e., protonmotive force) across the membrane. The recently determined X-ray structures contain apparent channels (D-channel and K-channel) that have been proposed to facilitate the movement of protons within the enzyme, both for the pumped protons and for those that are needed to combine with reduced oxygen species. Mutants that appear to block these channels have been examined to determine the influence on both steady-state and single-turnover kinetics. It is demonstrated that mutants in the two channels each effectively inhibit steady state turnover, but apparently at different steps in the catalytic cycle. The K362M mutation in the K-channel, for example, appears to block the initial reduction of the heme-copper bimetallic center prior to the reaction with dioxygen. Presumably, this is due to the inhibition of uptake of protons which are needed to stabilize the reduced metal centers.
Origin and assembly of bacterial quinol oxidase

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Cytochrome bo-type ubiquinol oxidase from *Escherichia coli* belongs to the heme-copper respiratory oxidase superfamily and acts as a redox-coupled proton pump. It is composed of four subunits and subunits I, II and III show structural homology to the counterparts of cytochrome c oxidase from which quinol oxidase appears to be derived during evolution. To probe the structural features of the quinol oxidation site, we isolated and characterized the quinone analogue-resistant mutants of cytochrome bo and found that a soluble part of subunit II contains two structural domains specific for quinol oxidases. We suggest that a proto-type of quinol oxidase, which was evolved from cytochrome c oxidase through gene duplication, lost the CuA ligands for oxidation of ferrocytochrome c in a cupredoxin-fold and acquired new domains for the oxidation of quinols. Assembly of cytochrome bo is similar to that of bacterial cytochrome c oxidases. We examined the functional role of subunit IV (i.e., a supernumeral subunit of the oxidase complex) by deletion and alanine-scanning mutagenesis. Results suggest that subunit IV functions as a domain-specific molecular chaperone and assists the correct assembly of the heme-copper binuclear center in subunit I where dioxygen reduction and proton pumping take place.