inside surface is located at the D-pathway entrance. The zinc binding affinity for the second site suggests that the zinc site is tightly coupled with the proton-pumping site. Recently, we analyzed Zn/Cd-binding to monomeric CcO which gives crystal packing different from that in the dimeric CcO crystal. The x-ray structural analysis showed Zn-binding to the Zn2, Zn3 and additional sites including the site near the K-pathway entrance. Several Zn-binding sites have been found on the outside surface. However none of them is located on the subunit I surface from which pumping protons exit.

Keywords: cytochrome oxidase, metal-biomolecule interactions, proton transfer

P04.03.186

Acta Cryst. (2008). A**64**, C289

Structural analysis for lipid/protein interactions in bovine heart cytochrome *c* oxidase

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All 13 lipids, including two cardiolipins, one phosphatidylcholine, three phosphatidylethanolamines, four phosphatidylglycerols and three triglycerides were identified in a crystalline bovine heart cytochrome *c* oxidase (CcO) preparation. The chain lengths and unsaturated bond positions of the fatty acid moieties determined by mass spectrometry suggest that each lipid head group identifies its specific binding site within CcO. Binding of dicyclohexylcarbodiimide to the O_2-transfer pathway of CcO causes two palmitate tails of phosphatidylglycerols to block the pathway, suggesting that the palmitates control the O_2 transfer. The phosphatidylglycerol with vaccenate (*cis*-Δ^{11}-octadecenoate) was found in CcO of *Paracoccus denitrificans*, a possible ancestor of mitochondrion. This indicates that the vaccenate is conserved in bovine CcO in spite of the abundance of oleate (*cis*-Δ^{9}-octadecenoate). The X-ray structure indicates that the protein moiety selects *cis*-vaccenate against *trans*-vaccenate for the O_2-transfer pathway. These results suggest that vaccenate plays a critical role in the O_2-transfer mechanism and that the lipid binding specificity is determined by both the head group and the fatty acid tail.

Keywords: cytochrome oxidase, mass spectrometry, protein-lipid interactions

P04.03.187

Acta Cryst. (2008). A**64**, C289

Inter- and intra-molecular complex structures of Cu-containing nitrite reductase with cytochrome *c*

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Copper-containing nitrite reductase (CuNIR) is known as a key enzyme in biological denitrification, catalyzing one-electron-reduction of nitrite to the gaseous nitric oxide. CuNIRs are homotrimers with two distinct Cu sites per ca. 35-kDa monomeric unit. The type 1 Cu site (T1Cu) buried within each monomer relays an electron from the redox partner protein to the type 2 Cu site (T2Cu) where NO_2^- is reduced to NO. Despite much effort by several groups, a crystal structure of the protein-protein complex state between a CuNIR and its redox partner(s) has not been determined. This difficulty is probably a reflection of the low free energy for complex formation and short lifetime that is conducive to rapid electron transfer in such complexes. As a first topic, we report the X-ray crystal structure analysis at a resolution 1.7 Å of a binary protein-protein complex between blue CuNIR and its redox partner protein cytochrome c_{551} from *Alcaligenes xylosoxidans* GIFU1051. The CuNIR-Cyt c_{551} interface is largely hydrophobic, covering ca. 500 Å^2 of surface on each molecule. The closest distance from heme-edge to T1Cu is 10.5 Å. Second is the X-ray crystal structure analysis of the novel CuNIR from the versatile marine Antarctica bacterium *Pseudoalteromonas haloplanktis* TAC125. This enzyme consists of two distinct functional domains, belonging to the new type of CuNIR. The N-terminal domain contains two copper atoms, T1Cu and T2Cu, and is homologous of the well-known CuNIRs. The *c*-type heme attached C-terminal domain is combined with the N-terminal domain by a linker region. Using both high-resolution X-ray data, structural and mechanistic insights into the multiple electron transfer reaction from heme *c* to T1Cu, following to the T2Cu for the reduction of NO_2^- to NO, are given.

Keywords: electron transfer, metalloproteins, protein complex structure

P04.03.188

Acta Cryst. (2008). A**64**, C289–290

Crystal structure of cytochrome P450 105A1 in complex with 1 α ,25-dihydroxyvitamin D3

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The bacterial enzyme cytochrome P450SU-1 (CYP105A1) from Streptomyces griseolus has been known for its ability to convert vitamin D3 into its active form in two steps. We determined the crystal structures of hyperactive mutant (R84A) of P450SU-1 (CYP105A1) in complex with and without the final reaction product 1alpha,25-dihydroxyvitamin D3. The product is bound between B', G, and I helices within the distal pocket at the distance of 11 angstroms from the heme iron. The loop after K helix shows remarkable conformational difference upon product binding, resulting in the different shape of active site pocket. Nonetheless, orientation of 1alpha,25(OH)2D3 is similar to that of VD3 in human CYP2R1, suggesting a common substrate-binding mode for 25-hydroxylation. R84A shows a 32-fold increase in 25-hydroxylation activity compared with the wild type enzyme. A plausible explanation for this effect of the R84A mutant is that the loss of the interaction with the Arg84 side chain the in B' helix opens