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

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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

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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

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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

a direct channel to the active site and increases the adaptability of hydrophobic amino acids. Further Mutational analysis revealed that the 25- and 1alpha-hydroxylations have several important residues in common. Substrate docking studies also indicate that 1alpha(OH)D3 and 25(OH)D3 bind to the common site in two distinct orientations that present opposite ends of the sterol to the heme iron. We propose an underlying mechanism for two-site hydroxylation in the activation of VD3 by CYP105A1 and provide a successful example of structure-guided design to increase the activity.

Keywords: heme enzyme structure and function, monooxygenases, vitamin D

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Cu/Zn superoxide dismutase structure of the heavy-metal-tolerant *Cryptococcus liquefaciens* strain N6

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The deep-sea yeast *Cryptococcus liquefaciens* strain N6 shows high tolerance towards heavy metals and can grow in the presence of 50 mM $CuSO_4$. Enzymatic analysis indicated that copper ions induced the Cu/Zn superoxide dismutase (SOD) activity of strain N6, and its expression increased with increasing $CuSO_4$ concentrations. Although an essential trace element, copper ions can initiate oxidative damage and affect important cellular events. On the other hand, SOD protects against copper toxicity by converting superoxide to hydrogen peroxide and oxygen. The strain N6 Cu/Zn SOD (*Cl*-SOD1) contains a copper and a zinc ion in the active site, and has an activity four-fold higher than the *Saccharomyces cerevisiae* Cu/Zn SOD. The crystal structure of *Cl*-SOD1, at 1.2 Å resolution, reveals several significant residue substitutions in the enzyme compared to other Cu/Zn SODs. In the electrostatic loop, notably, His135 and Pro136 replace two well conserved linear residues while Thr133 substitutes a highly conserved glycine, causing an inward dragging of the turn region of the electrostatic loop. The highly conserved Asn143 side chain, interacting with His135, also has rotated approximately 90°. The electrostatic loop has been shown to play a role in copper uptake, and the copper ion reportedly contributes more than the zinc ion to the kinetic stability of SOD. In *Cl*-SOD1, replacement with Pro136, which has the lowest conformational entropy, introduces rigidity into the loop structure while substitution of the conserved glycine, which has the highest conformational entropy, with Thr133 decreases loop flexibility. These substitutions may confer the electrostatic loop greater stability, which in turn may possibly lead to more efficient copper uptake and a more stabilized copper-bound form.

Keywords: superoxide dismutase, copper tolerance, loop stability

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Crystal structure of TTHA1429 from *Thermus thermophilus* HB8

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TTHA1429 is a metallo β-lactamase superfamily protein from an extremely thermophilic bacteria *Thermus thermophilus* HB8. The metallo β-lactamase superfamily proteins, first identified as class B β-lactamases, include glyoxalase II's, rubredoxin oxygen:oxidoreductases, phosphorylcholine esterases, and tRNA maturases. The superfamily members possess an $\alpha\beta\beta\alpha$-fold and a di-metal binding site, but the substrate binding pocket and the residues involved in metal coordination differs among each other. Although the function of TTHA1429 remains unknown, the fact that its homologues are present in many thermophilic bacteria and archaea implicates that TTHA1429 homologues are important for the adaptation to thermal environment. To analyze the structural and functional properties of TTHA1429, we have determined the 2.1-Å crystal structure of TTHA1429 in a zinc-bound form. TTHA1429 exhibited a unique putative substrate binding pocket with the di-metal binding site at the bottom. The loop regions which the electron densities couldn't be observed were located at the entrance of the putative substrate binding pocket. It suggests that the loop regions work as the lid of the pocket. Also, the residues involved in metal coordination of TTHA1429 were identical with glyoxalase II's though its metal content was different from glyoxalase II's.

Keywords: metallo enzyme X-ray crystallography, thermophilic proteins, beta-lactamases

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Metalloporphyrin binding to the NEAT domain of IsdA

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The cell wall anchored components of the Isd heme transport system all contain at least one near transporter (NEAT) domain. Previously, we characterized by X-ray crystallography heme binding to the IsdA NEAT domain, demonstrating five-coordinate heme iron through a single Tyr166. Interestingly, this structure revealed that His83 is non-coordinating, though it is in close proximity to the heme iron atom. To investigate the mechanism of heme binding in NEAT domains, several point mutations within the binding pocket of the IsdA NEAT domain were generated and characterized by combinations of X-ray crystallography and electronic spectroscopy. Unexpectedly, mutating the heme-iron coordinating Tyr166 to Ala or Phe does not completely abrogate heme binding in vitro. X-ray crystal structures of the Tyr166Ala variant reveal heme-iron coordination is accomplished through His83, suggesting a role in loading or unloading heme. The metal-substituted Co-protoporphyrin IX has been co-crystallized with the wild-type IsdA NEAT domain. This structure reveals similar five-coordinate binding revealed in the Tyr166Ala variant, with the metal coordination through His83. These structures show that the lack of His83 participation in heme binding in the native NEAT domain is not due to steric hinderance. In addition, significant flexibility in the IsdA NEAT domain binding pocket allows heme to be stably bound in multiple orientations which may facilitate rapid binding and release as heme is relayed through the cell wall to the membrane.