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.

Keywords: heme, transport, bacteria

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Structure of protochlorophyllide reductase reveals a mechanism for greening in the dark

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Chlorophyll (Chl) is a tetrapyrrole macrocycle containing Mg and a phytol chain. The Chl biosynthetic pathway consists of the multi-enzymatic reactions. An asymmetric conjugated double bond system of Chl a, which is crucial for efficient light absorption, is formed in the penultimate step of biosynthesis, reducing protochlorophyllide (Pchlide) to form chlorophyllide a. Photosynthetic organisms adopt two different strategies for the reduction of Pchlide; one is the light-dependent Pchlide oxidoreductase that requires light for the catalysis, and the other is dark-operative Pchlide oxidoreductase (DPOR) that operates even in the dark. The greening ability of plant in the dark is attributed to the activity of DPOR. We show a crystal structure of the DPOR catalytic component NB-protein from *Rhodobacter capsulatus* at 2.3 Å resolution. Overall structure with two copies of homologous BchN and BchB subunits is similar to that of nitrogenase MoFe protein. Each catalytic BchN-BchB unit contains one Pchlide held without any axial ligations from amino acid residues and one Fe-S cluster (NB-cluster) coordinated uniquely by one aspartate and three cysteines. Intriguingly, NB-cluster and Pchlide are arranged spatially as almost identical to P-cluster and FeMo-cofactor in MoFe protein, illustrating a common architecture to reduce chemically stable multi-bonds such as porphyrin and dinitrogen.

Keywords: chlorophyll synthesis, nitrogenase-like enzyme, iron-sulfur cluster

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Structure, stability and flexibility of a psychrophilic iron superoxide dismutase

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The Antarctic eubacterium *Pseudoalteromonas haloplanktis* (Ph) produces a cold-active iron superoxide dismutase (SOD). PhSOD is a homodimeric enzyme, that displays a high catalytic activity even at low temperature [1-2]. The structure, stability and dynamics of PhSOD have been determined and compared with those of its mesophilic counterpart from E. coli (EcSOD). PhSOD was found to have structure and stability very similar to Ec-SOD. However, the psychrophilic protein shows an increased flexibility of the active site with respect to its mesophilic homologue. Two PhSOD mutants (C57S and C57R) have been also characterized. The C57R mutation significantly alters the half-denaturation temperature of the protein. The structural and dynamic changes induced by this mutation with respect to the C57S and wild-type structure were correlated with modifications in the thermal stability of the mutant. Altogether these data illustrate how evolution can adjust psychrophilic enzyme sequences to alter the flexibility, without compromising the overall protein structure.

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Keywords: cold adapted enzymes, superoxide dismutase, protein structures

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X-ray structures of redox partner proteins for *Hyphomicrobium* Cu-containing nitrite reductase

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Cu-containing Nitrite Reductase (NIR) is a soluble enzyme involved in bacterial denitrification, catalyzing one-electron-reduction of nitrite into nitric oxide. Crystal structure of novel hexameric NIR (HdNIR) from *Hyphomicrobium denitrificans* has been reported [1]. The overall structure of HdNIR shows a trigonal prism-shaped molecule, in which a monomer is organized into a unique hexamer. Each monomer is composed of an N-terminal region like a blue copper protein and a C-terminal region having homology with well-known trimeric NIRs. In the case of trimeric NIRs the type 1 Cu site buried within each monomer relays an electron from the redox partner protein to the type 2 Cu site where is catalytic site. On the basis of several data for NIRs from various organisms, a hydrophobic patch region of the surface on the type 1 Cu of NIR has been proposed as the binding site for the partner protein. While, in the case of HdNIR, the hydrophobic patch region were covered by its N-terminal region. Recently, we have revealed that two electron transfer proteins, a cytochrome *c* (HdCyt c_{550}) and a blue copper protein, pseudoazurin (HdPaz), act as electron donor for HdNIR in the periplasm [2]. To study the interactions between two proteins and HdNIR, we determined the crystal structures of HdCyt c_{550} and HdPaz at resolutions 1.50 and 1.18 Å, respectively. HdCyt c_{550} exhibits the typical cytochrome *c* folding having five α-helices. While, HdPaz possesses eight β-strands, forming two β-sheets, and two C-terminal α-helices. We discuss the interactions of HdNIR with HdCyt c_{550} and HdPaz by comparing their structures.

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Keywords: electron transfer, blue copper protein, cytochromes

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Structure of vitamin D_3 hydroxylase, a novel cytochrome P450 from *Pseudonocardia autotrophica*

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