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The asymmetric architecture of 2Fe-2S IscU, a scaffold protein for iron-sulfur cluster biosynthesis

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Iron-sulfur (Fe-S) clusters are ubiquitous prosthetic groups that are required to maintain fundamental life processes. The assembly of Fe-S clusters in several bacteria as well as eukaryotic mitochondria is achieved by a multicomponet system, called ISC machinery. In this machinery, IscU serves as a scaffold for assembly of a nascent Fe-S cluster, prior to its delivery to an apo-protein. The Hsp70-type molecular chaperon HscA and the J-type cochaperone HscB comprise a specialized chaperone system that selectively binds IscU and facilitate the cluster transfer process. We have determined the crystal structure of holo-IscU from the hyperthermophilic bacterium *Aquifex aeolicus* at 2.3 Å resolution, using multi-wavelength anomalous diffraction of the [2Fe-2S] cluster. IscU formed an asymmetric homotrimeric structure harboring only one [2Fe-2S] cluster that was coordinated by three cysteines and one histidine (Cys36, Cys63, His106, and Cys107) on the surface of just one of the protomers. The cluster was buried inside the homotrimer by the neighboring protomers. The three protomers were conformationally distinct from each other, and associated around a non-crystallographic pseudo-three-fold axis. This unique trimeric holo-IscU architecture was clearly distinct from other known monomeric apo-IscU/SufU structures, indicating that asymmetric trimer organization would be involved in the scaffolding function of IscU. This oligomeric holo-IscU structure provides mechanistic implications concerning the initial event in the assembly of Fe-S cluster, as well as the event in the transfer to target apo-proteins.

Keywords: iron sulfur clusters, iron sulfur proteins, crystal structure analysis

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Crystallographic study of Cu, Zn superoxide dismutase in extreme pressure conditions

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High-pressure macromolecular crystallography is now a mature and powerful technique (Girard et al., 2007). We have investigated the effect of high pressure perturbation on crystal and molecular structures of oxidized bovine Cu,Zn superoxide dismutase (Cu,Zn SOD). This enzyme belongs to a family of metalloenzymes that catalyze the dismutation of the superoxide anion into dioxygen and hydrogen peroxide. Cu,Zn SOD has a key role in the cellular defence against oxidative damage. X-ray absorption spectroscopy had previously revealed (Ascone et al., 2000) that the metal site local structure is preserved beyond 1 GPa. X-ray crystallography was performed at ID27 beamline (ESRF, Grenoble, France) at room temperature. In the crystalline state, the secondary and tertiary structures are preserved up to at least 1 GPa in spite of the fact that the enzyme is a dimer. The structure of oxidized Cu,Zn SOD at 0.57 GPa has been refined at 2.0 Å resolution, using a highly-complete data set. The effects of pressure on different regions of the molecule, including the active site, have been analyzed with reference to the oxidized and reduced PDB structures at room and liquid nitrogen temperature respectively. Moreover, advantages and limitations in coupling macromolecular crystallography with X-ray absorption spectroscopy for high-pressure studies, in non-crystalline and crystalline states, will be discussed.

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Keywords: superoxide dismutase, dimer, high-pressure

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Insights into iron transport in *Helicobacter pylori* ferritin

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Ferritin, a biomineralizing protein found in organisms from all kingdoms, catalyzes the oxidation of iron into a ferric oxide core within the protein shell. Ferritin-mediated iron storage is required for the colonization and survival of *Helicobacter* pylori in human gastric mucosa, protecting the bacteria against acid-amplified iron toxicity. Here we report the crystal structures of H. pylori ferritin (Hpf) in four distinct forms: apo, low-iron bound, high-iron bound, and acidic pH. Despite high degree of sequence and structural homology with other ferritins, Hpf may use a pair of distinct histidine residues His93 and His96 located in proximity to the ferroxidase center as a novel metal binding site. At the high-iron bound form, a quartet of His149 at the entrance of the 4-fold symmetry channels undergoes marked conformational changes upon binding of Fe ions, which was found to be tightly coupled to Fe translocation. Hpf may have evolved to possess the dual transport mechanism for the control of irons.

Keywords: *Helicobacter pylori*, ferritin crystal structure, iron transport

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Structure of a member of glycoside hydrolase family 61: Are these true glycoside hydrolases?

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The first structure of a protein classified in family 61 of glycoside hydrolases is presented. The crystal structure of Thielavia terrestris GH61 isoform E was determined at a resolution of 1.9 Å by Multiple Isomorphous Replacement. The fold is a β-sandwich consisting of two sheets and is a variation of the fibronectin type III fold. The structure shows significant structural similarity to the chitin-binding protein CBP21 of Serratia marscecens and shows a conserved Zn-binding site which is likely to be functionally relevant. No hydrolytic activity has been reported for this protein, although it potentiates the activity of enzyme cocktails used for the degradation of cellulosic wastes. Together with the information available in the literature, serious doubts can be cast on the nature of family 61 proteins as true glycoside hydrolases, we propose instead that they have an accessory function in cellulose degradation, in analogy to the one shown for chitin binding protein.

Keywords: biotechnology, plant cell wall, chitin binding protein

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Crystal structure and mechanism of cytochrome P450 StaP that constructs the indolocarbazole core

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Staurosporine isolated from Streptomyces sp. TP-A0274 is a member of the family of indolocarbazole alkaloids that exhibit strong antitumor activity. A key step in staurosporine biosynthesis is the formation of the indolocarbazole core by intramolecular C-C bond formation and oxidative decarboxylation of chromopyrrolic acid (CPA) catalyzed by cytochrome P450 StaP (StaP). We have solved x-ray crystal structures of CPA-bound StaP. Hydrogen-bonding interactions of two carboxyl groups and CH-pai interactions with indole rings hold the substrate in the substrate-binding cavity with a conformation perpendicular to the heme plane. Based on the crystal structure of StaP-CPA complex, we propose that C-C bond formation occurs through an indole cation radical intermediate that is equivalent to cytochrome c peroxidase compound I. Theoretical QM/MM calculation of a catalytic intermediate (CPA-Fe(IV)=O pai cation radical) suggests that the indole cation radical can be formed in the catalytic process and that the spin density of the indole cation radical is controlled by the surrounding H-bonding network. Our crystallographic and theoretical studies provide valuable insights into the process of staurosporine biosynthesis, combinatorial biosynthesis of indolocarbazoles, and the diversity of cytochrome P450 chemistry.

Keywords: indolocarbazole, heme, P450

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Bacsu PerR : Metal binding sites and unambiguous highlights of 2-oxo-His in the oxidized protein

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In Bacillus subtilis, the PerR protein is a metal-dependent sensor of hydrogen peroxide that regulates the adaptive response to H_2O_2. PerR is a dimeric zinc protein with a regulatory metal-binding site that coordinates either Fe^{2+} (PerR-Zn-Fe) or Mn^{2+} (PerR-Zn-Mn). Here we present the structural studies of both active forms: the crystal structure of the PerR-Zn-Mn protein and X-ray absorption spectroscopy experiments of PerR-Zn-Fe. While most of the peroxide sensors use redox-active cysteines to detect H_2O_2, it has been shown that reaction of PerR-Zn-Fe with H_2O_2 leads to the oxidation of one histidine (H) residue that binds the Fe^{2+} ion. This metal-catalyzed oxidation of PerR leads to the incorporation of one oxygen atom into H37 or H91. However the exact position of the added oxygen is still unknown. This study presents the crystal structure of the oxidized PerR protein (PerR-Zn-ox) that clearly shows a 2-oxo-histidine residue in position 37.

Keywords: 2-oxo-histidine, metalloproteins, protein-DNA complex

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Crystallization and SAXS of α -Actinin 2

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α-Actinin is involved in cytoskeletal network and it is composed of an N-terminal actin binding domain (ABD) connected by a neck to a central rod domain composed of 4 spectrin-like repeats (SR) and a C-terminal calmodulin like domain (CaM). Its functional unit is an anti-parallel homodimer allowing the protein to crosslink actin filaments. Different isoforms are present in human cells: isoforms 1 and 4 are found in non-muscle cells, while the isoforms 2 and 3 are present in muscle cells. The described work is related to the muscle isoform 2. To try to understand the α-actinin structural mechanisms underlying its regulation, the full length α-actinin 2 was expressed, purified and submitted to crystallization experiments. No crystals grew with the wild type protein in any standard conditions; therefore thermofluor experiments were performed to find a buffer where the protein is more thermally stable. Mutations in the primary structure were then designed to decrease the surface entropy of the protein and lysine methylation assays were performed. Both strategies helped enhancing the propensity of the protein to crystallize and several hits were identified. Crystal optimization is ongoing with success. In an attempt to understand α-actinin molecular architecture a small angle X-ray solution scattering (SAXS) experiment was carried out on the full length and on the half dimer construct (ABD-SR1-SR2// SR3-SR4-CaM). The low-resolution data of the molecular envelope presented the expected protein shape and dimensions, if compared to the solved structures of the individual domains. Reported biochemically information on the interaction points between CaM