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A Tri-nuclear Metal Cluster in Reduced Mouse Ribonucleotide Reductase R2 Subunit

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Ribonucleotide reductase (RNR) is the enzyme that converts ribonucleotides to their corresponding deoxyribonucleotides. The R2 protein reacts with ferrous iron and dioxygen to generate a tyrosyl radical that is essential for enzymatic activity.

Here we present a structure of mouse R2 soaked in ferrous iron, ascorbate, and methanol. In addition to the expected di-nuclear iron cluster, a tri-nuclear metal cluster is observed. The tri-nuclear cluster is located ~10 Å from the di-nuclear cluster and is attached to the protein by a two cysteines and the protein backbone. It is not yet clear whether the observed tri-nuclear cluster is an artefact from the soaking conditions or if it has some biological relevance.

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Keywords: ribonucleotide reductase, trinuclear, diiron cluster

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Structural Analysis of the Interaction between Plant Sulfite Reductase and Ferredoxin

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Plant type ferredoxin (Fd) is reduced via photosystem I or by Fd:NADPH oxidoreductase and donates reducing equivalents to various Fd-dependent enzymes. Sulfite reductase (SiR) is one of such enzymes, catalyzing six-electron reduction of sulfite to sulfide. SiR contains siroheme and [4Fe-4S] cluster as redox centers and our ongoing x-ray crystallographic analysis of maize SiR has revealed its active site structure consisting of these two prosthetic groups. SiR forms an electron transfer complex with Fd and this inter-molecular interaction is stabilized mainly through electrostatic force between acidic residues of Fd and basic residues of SiR. We have also been investigating the interaction by NMR spectroscopy. When ^{15}N-labeled Fd was titrated with SiR, NMR chemical sift changes were observed on ^{1}H-^{15}N HSQC spectra. The data allowed us to map the interaction sites for SiR on the 3D structure of Fd. Site-specific Fd mutants lacking acidic residues with the large chemical sift perturbation showed lowered affinity to SiR both in the kinetical assay and static interaction analysis, confirming the NMR assignment of the interaction sites. We have introduced a series of mutations on the basic amino acids of SiR and selected mutants with a lowered affinity to Fd. These SiR mutants exhibited little activity in the assay of Fd-dependent sulfite reduction. We will present a detailed interaction mapping of SiR and Fd based on the combined results.

Keywords: iron-sulfur proteins, protein-protein interactions, NMR spectroscopic investigations

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Rv0805: Cyclic Nucleotide Phosphodiesterase from *M. tuberculosis*

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Cyclic nucleotides play a crucial role in signaling pathways as second messengers and are generated by nucleotide cyclases. The intracellular level of cyclic nucleotides is controlled by the presence of cyclic nucleotide phosphodiesterases. In our efforts to understand the role of cyclic nucleotides in bacterial systems, our attempt is to systematically characterize the relevant proteins from mycobacteria. Bioinformatic analyses revealed the presence of a protein Rv0805 from *M. tuberculosis* related to a recently characterized phosphodiesterase from *E. coli* (Class III). We developed a large-scale expression and purification procedure for Rv0805, which was shown to be a potent cAMP-phosphodiesterase. Rv0805 appears to be expressed mainly as a dimer in several expression strains of *E. coli*. The protein is partially nicked, which however doesn't seem to disturb the dimer formation. The biological role of the cleavage is not known yet. The position of the proteolytic cleavage was determined by the N-terminal sequencing and mass spectrometry of the nicked protein. Rv0805 is a metallo-enzyme. Highly concentrated solutions of the enzyme (several 10 mg/ml) are colored brown. The presence of iron and manganese in the active site was shown by the Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Crystallization of the phosphodieterase Rv0805 is in progress.

Keywords: cyclic nucleotides, phosphodiesterase, metallo enzymes

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Manganese Superoxide Dismutases and Substrate Mimic Derivatives

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P4

Superoxide dismutases (SODs) are enzymes that catalyse the elimination of the oxygen-derived free radical superoxide, making an aerobic existence more viable. Our research interest is in manganese SODs from *Escherichia coli* [1,2] and *Deinococcus radiodurans,* an extremophile, which can tolerate very high radiation exposure and dessication. Presented here are four new structures: the *E. coli* iron-substituted MnSOD with bound azide (a substrate mimic/inhibitor) to 2.2-Å resolution, the *E. coli* Y174F-MnSOD complexed with azide to 1.5 Å (the first ordered Mn^{II}/Mn^{III} structure), the wild-type form of MnSOD from *D. radiodurans* to 2.0 Å, and *D. radiodurans* MnSOD with bound azide to 2.0 Å.

The binding of azide to wild-type, mutant and wrong-metal MnSODs is associated with a change in coordination of the metal centre. Azide binding also leads to major changes of the water structure of the solvent-access funnel, especially near the conserved Tyr34 (*E. coli* numbering). Azide is observed to bind quite differently to that previously reported for an MnSOD [3], and adopts an orientation very similar to that reported for wild-type FeSODs [3].

[1] Edwards R. A., et al., *J. Biol. Inorg. Chem.*, 1998, **3(2)**, 161-171. [2] Edwards et al., *J. Am. Chem. Soc.*, 1998, **120(37)**, 9684-9685. [3] Lah et al., *Biochemistry*, 1995, **34(5)**, 1646-60.

Keywords: metalloenzymes, superoxide dismutases, azide

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Structure of the 2[4Fe-4S] Ferredoxin from *Pseudomonas aeruginosa* at 1.32 Å Resolution

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The 2[4Fe-4S] ferredoxin from *Allochromatium vinosum* (AlvinFd) has been proved recently to be the prototype of a sub-family of 2[4Fe-4S] Fds, characterized by very negative and widely different reduction potentials of the two $[4Fe\text{-}4S]^{2+/+}$ clusters (–430 to –485 mV and –585 to –675 mV, versus NHE), in contrast to the

clostridial Fds, whose clusters have more positive and essentially the same potential (~ –400 mV). Structural information for AlvinFd is available at 2.1 Å resolution [1]. A recently established member of the AlvinFd sub-family is *Pseudomonas aeruginosa* Fd (PaFd), which also shows the above unusual redox properties. The PaFd structure has been determined to 1.32Å resolution, the highest up to now for the AlvinFd sub-family. The detailed structural information that this structure affords, i.e. the precise geometry of the [4Fe-4S] clusters combined with the conformation of the surrounding residues and the electrostatic properties of the protein around and between the two clusters, the EPR spectroscopy studies, as well as a thorough comparison with existing high resolution structures of [4Fe-4S]-containing proteins, provide structural reasons for the unusual redox properties of the Alvin-like 2[4Fe-4S] Fds.

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Keywords: ferredoxin, metallo proteins, *pseudomonas aeruginosa*

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Dynamic Structures and Reaction Mechanism of Active Fe-type Nitrile Hydratase

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Nitrile hydratase from *Rodococcus* sp. N-771 is the enzyme that catalyzes the hydration of nitriles to the corresponding amides, and contains a mononuclear non-heme iron as the reaction center (Fe-type NHase). The center is photo-reactive, inactivated by nitrosylation and activated by photo-driven NO release. The photo-activated Fe-type NHase loses the activity within 24 hours under aerobic conditions. Previous studies have revealed that the post-translationally modified cystein sulfenate (αCys114-SO^-) of active enzyme is further oxidized under the aerobic conditions to cystein sulfinate (αCys114-SO_2^-).

In order to avoid the further oxidation, a crystallization system was constructed under anaerobic conditions of less than 0.1% (v/v) oxygen concentration. The really active structure of intact Fe-type NHase was studied by X-ray crystallography, including complex structures with butyric acid as an inhibitor/stabilizer and with cyclohexyl-isocyanide (ch-NC) as a substrate analogue. We also crystallized the inactive nitrosylated NHase under the anaerobic conditions in the complex form with ch-NC, and dynamic structure changes were observed after photo-activation at a time-resolution of 30min using the large-angle oscillation technique (LOT) at a RIKEN beamline: BL45XU, SPring-8. Based on the results obtained, we will discuss the role of αCys114-SO^- in the nitrile hydration mechanism of Fe-type NHase.

Keywords: nitrile hydratase, cysteine sulfenate, dynamic structure

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Structural Studies on *Cerebratulus lacteus* Mini-Hb K(E10)W and L(G12)A Mutants

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A very short hemoglobin (CerHb; 109 amino acids) binds O_2 cooperatively in the nerve tissue of the nemertean worm *Cerebratulus lacteus* to sustain neural activity during anoxia. The structure of oxygenated wild-type CerHb displays a substantial editing of the globin fold which makes CerHb unique among the known globin fold evolutionary variants [1].

Here we present the crystal structures of two CerHb mutants: Lys(E10)Trp (at 2.3 Å resolution) and Leu(G12)Ala (at 1.6 Å resolution) and its complex with xenon atoms (at 2.3 Å resolution).

The single mutation Lys(E10)Trp, intended to perturb the protein heme binding, has also a dramatic and unexpected effect on the H-bond network stabilizing the O_2 ligand, and it makes the protein more susceptible to heme-iron oxidation.

The Leu(G12)Ala mutant and its complex with xenon atoms map a wide protein matrix tunnel connecting the distal site to a surface cleft between the E and H helices, thus suggesting a novel ligand access to heme**.**

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Keywords: hemoglobin, mutagenesis, Xe binding

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Structure of the MntC Protein: Mn^{2+} Import in Cyanobacteria is Redox Controlled

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Cyanobacteria have unique Mn requirements due to the essential role of Mn in Photosystem II and the low concentrations of Mn^{2+} in fresh and sea water. We have determined the crystal structure of the MntC solute binding protein (SBP) component of the high-affinity manganese ABC-type transport system from the cyanobacterium *Synechocystis* sp. PCC 6803 to 2.9Å using a combination of MAD phasing and molecular replacement. The trimeric structure was refined to an R/R_{free} of 0.23/0.29, and anomalous difference diffraction maps show the presence of Mn^{2+} in the binding site, the first SBP structure containing bound Mn^{2+}. The Mn^{2+} binding site has a distorted tetrahedral geometry, with E220 and D295 situated closer to the ion than H89 and H154. This geometry may be due to a disulfide bond between C219 and C268. Reduction of the disulfide bond *in vitro* and *in crystal* releases bound Mn^{2+}. Sequence homology comparisons show that only cyanobacterial Mn SBPs contain conserved cysteine residues, and we thus propose that reduction of the disulfide bond by a redox active protein alters the position of E220 thereby modifying the affinity towards the bound metal. To more fully understand the import of Mn, we have cloned both the MntC and MntB permease from the thermophilic cyanobacterium *T. vulcanus*. The *T. vulcanus* MntC contains the conserved cysteines and binds Mn^{2+} *in vitro*. The protein was crystallized and structure determination is in progress. Expression experiments of the transmembrane MntB are under way.

Keywords: ABC transporter system, photosynthesis, redox control

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Crystal Structure of a Eukaryotic FeSODs Suggests Intersubunit Cooperation during Catalysis

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Superoxide dismutases (SODs) are a family of metalloenzymes that catalyze the dismutation of superoxide anion radicals into molecular oxygen and hydrogen peroxide. Iron superoxide dismutases (FeSODs) are only expressed in some prokaryotes and plants. A new and highly active FeSOD with an unusual subcellular localization has recently been isolated from the plant Vigna unguiculata (cowpea). This protein functions as a homodimer and, in contrast to the other members of the SOD family, is localized to the cytosol. The crystal structure of the recombinant enzyme has been solved and the model refined to 1.97 Å resolution. The superoxide anion binding site is located in a cleft close to the dimer interface. The coordination geometry of the Fe site is a distorted trigonal bipyramidal arrangement, whose axial ligands are His43 and a solvent molecule, and whose in-plane ligands are His95, Asp195, and His199. A