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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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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