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.

[1] Moulis J.-M., Sieker L.C., Wilson K.S., Dauter Z., *Prot. Sci.*, 1996, **5**, 1765.

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**.**

[1] Pesce A., Nardini M., Dewilde S., Geuens E., Yamauchi K., Ascenzi P., Riggs A.F., Moens L., Bolognesi, M., *Structure*, 2002, **10**, 725.

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