

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 1.32 Å 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 *Rhodococcus* 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 ($\alpha\text{Cys114-SO}^-$) of active enzyme is further oxidized under the aerobic conditions to cystein sulfinate ($\alpha\text{Cys114-SO}_2^-$).

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 $\alpha\text{Cys114-SO}^-$ 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 O₂ 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 O₂ 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: Mn²⁺ 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 Mn²⁺ 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 2.9 Å 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 Mn²⁺ in the binding site, the first SBP structure containing bound Mn²⁺. The Mn²⁺ 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 Mn²⁺. 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 Mn²⁺ *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

comparison of the structural features of cowpea FeSOD with those of homologous SODs reveals subtle differences in regard to the metal–protein interactions, and confirms the existence of two regions that may control the traffic of substrate and product: one located near the Fe binding site, and another in the dimer interface. The evolutionary conservation of reciprocal interactions of both monomers in neighboring active sites suggests possible subunit cooperation during catalysis.

Keywords: crystal, metalloenzyme, FeSOD

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Towards the Crystal Structure of *Saccharomyces cerevisiae* Frataxin

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Due to the toxicity and insolubility of ferrous iron a conserved mitochondrial protein, frataxin has been suggested to be involved in iron delivery to the biosynthesis of iron-sulfur cluster and heme. Moreover, frataxin shows iron chaperone properties and iron-dependent oligomerisation. Insufficient production of frataxin results in Friedreich ataxia, an autosomal neuro-degenerative disease.

Frataxin from *Saccharomyces cerevisiae* is activated by Fe(II) in the presence of O₂ and assembles stepwise into a 48-subunit multimer that sequesters more than 2000 atoms of iron. In the first reaction monomeric frataxin is assembled into a trimeric form. Ferrochelatase catalyses the last step in the heme biosynthetic pathway, the insertion of ferrous iron into protoporphyrin IX to form heme *b*. Studies of a direct interaction between ferrochelatase and frataxin show interaction between dimeric ferrochelatase and trimeric frataxin.

Crystallisation of the trimeric form of yeast frataxin has given well defined three dimensional crystals in two different crystal forms. X-ray data has been collected at the synchrotron beamline at MAX-lab, Lund, Sweden. The crystal structure of the frataxin trimer will be presented. The structural basis of frataxin oligomerization and metal binding will be discussed.

Keywords: iron storage, heme synthesis, X-ray crystal structure

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The Crystal Structure of NEP and its Complexes with Inhibitors

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Neprylsin (NEP; EC 3.4.24.11) is a mammalian, zinc-dependant type II membrane protein consisting of a short N terminal cytoplasmic domain of 27 amino acids, a transmembrane region of 22 hydrophobic residues, and a large extracellular domain of some 700 residues. NEP has many functions in humans and is principally involved in turning off regulatory peptide signals in the brain as well as in the metabolism of a number of smaller regulatory peptides of the cardiovascular, inflammatory, and immune system. Its substrates include the enkephalins, substance P, atrial natriuretic factor, bradykinin, and endothelins. NEP down-regulated in a number of cancers, especially of the prostate. Lately it has been shown that NEP is involved in metabolism and removal of the neurotic amyloid β -peptide, the deposition of which in the brain is part of the initiation of Alzheimer's disease.

We describe the crystal structure of the soluble extracellular domain of rabbit NEP (residues 55-700) at 2.2 Å resolution. There are two molecules in the asymmetric unit and the structure reveals an extra metal molecule bound to the active site Zn, but not coordinated by NEP as well as several glycosylated residues. We have solved and refined the crystal structures of rabbit NEP complexed with competitive inhibitors, thiorphan and phosphoramidon at 3 Å and 2.8

Å resolution, respectively.

Keywords: macromolecular synchrotron X-ray crystallography, metalloprotein structures, enzyme inhibitors

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Crystal Structures of Cyanobacterial Heme Oxygenases

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Heme oxygenase (HO) catalyzes heme degradation utilizing O₂ and reducing equivalents. In mammals, HO is involved in iron homeostasis, whereas in plants, algae, and cyanobacteria, it is utilized for producing photoreceptor and light-harvesting pigments.

We determined structures of heme bound HOs from cyanobacterium, *Synechocystis* sp. PCC 6803 (Syn HO-1 and Syn HO-2) by molecular replacement using mammalian HO structure. Syn HO-2 crystals were non-merohedral twin and diffraction data were detwinned for refinement. Overall folding and heme environment of each Syn HO-1 and Syn HO-2 is similar to that of mammalian HO, however, two characteristic features are seen in Syn HO-1 and Syn HO-2. One is charge distribution; basic patch of Syn HO-1, where Syn HO-1 interacts with redox partner, is narrower than that of mammalian HO. Different charge distribution between Syn HO-1 and mammalian HO would reflect the different molecular size between their redox partners. The other is oligomeric state; Syn HO-2 forms dimer although other HOs including Syn HO-1 are monomer. Different oligomerization between Syn HO-1 and Syn HO-2 would contribute to the selection of their redox partners four ferredoxin paralogs in this bacterium.

Keywords: heme protein, oxygenase, redox proteins

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Crystal Structure of Nitrile Hydratases: Possible Industrial Usage

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Nitrile hydratase (NHase) is a metalloenzyme that catalyzes hydration of nitriles to corresponding amides. It is of major interest because of its use for synthesising industrial products such as acrylamide and nicotinamide [1]. NHases typically consist of two subunits (α and β) with similar molecular masses (23 and 25 kDa) and either a single non-heme Fe^{III} or non-corrinoid Co^{III} per $\alpha\beta$ dimer [1].

We have purified, crystallised and determined the structure of wild type (WT) and mutant NHases from *Bacillus RAPc8*. The space group was determined to be primitive tetragonal (p41212). The WT structure was solved at 2.1 Å using molecular replacement (MR) with a 65% homologue from *P. thermophila*.

The 2.5-3.0 Å data from isomorphous crystals of F36L, F52L, F55L, Y67A and W76G mutants were solved by MR using the WT structure. An interesting result came from the F55L mutant map, showing apparent flexibility of F52. The flexible F52 might be related to substrate access to the active site. In order to understand this we are doing activity tests for the F55L mutant. The other interesting result came from normal mode analysis about the flexibility of the protein. Our aim is to combine structural data with biochemical results to understand the mechanism of this enzyme better, and so search for possible improvement in activity for industrial biotransformation.

[1] Cowan D.A., Cameron R.A., Tsekoa L.T., *Advances in Applied Microbiology*, 2003, 52, 123.

Keywords: model building, enzyme active site, metalloenzymes