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

P.04.03.19

Acta Cryst. (2005). A**61**, C213

Towards the Crystal Structure of *Saccharomyces cerevisiae* Frataxin

Tobias Karlberg[a], Oleksandr Gakh[b], Isaya Grazia[b], Salam Al-Karadaghi[a], [a]*Department of Molecular Biophysics, Centre for Chemistry and Chemical Engineering, Lund University, Sweden.* [b]*Department of Pediatric and Adolescent Medicine, Mayo Clinic, College of Medicine, Rochester, Minnesota, USA.* E-mail: Tobias.Karlberg@mbfys.lu.se

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 O_2 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

P.04.03.20

Acta Cryst. (2005). A**61**, C213

The Crystal Structure of NEP and its Complexes with Inhibitors

Pawel Grochulski[a], Lucie Gonneville[b], Jurgen Sygusch[b], [a]*Canadian Light Source, University of Saskatchewan.* [b]*Département de biochimie Faculté de Médecine Université de Montréal, Montréal, Canada.* E-mail: pawel.grochulski@lightsource.ca

Neprysilin (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

P.04.03.21

Acta Cryst. (2005). A**61**, C213

Crystal Structures of Cyanobacterial Heme Oxygenases

Masakazu Sugishima[a], Yoshinori Hagiwara[a], Catharina T. Migita[b], Xuhong Zhang[c], Tadashi Yoshida[c], Keiichi Fukuyama[a], [a]*Department of Biology, Graduate School of Science, Osaka University, Osaka, Japan.* [b]*Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi, Japan.* [c]*Department of Biochemistry, Yamagata University School of Medicine, Yamagata, Japan.* E-mail: sugi2@bio.sci.osaka-u.ac.jp

Heme oxygenase (HO) catalyzes heme degradation utilizing O_2 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

P.04.03.22

Acta Cryst. (2005). A**61**, C213

Crystal Structure of Nitrile Hydratases: Possible Industrial Usage

Özlem Taştan Bishop[a], Tsepo Tsekoa[a], Trevor Sewell[b], Rory Cameron[a], Muhammed Sayed[a], Donald Cowan[a], [a]*Dept. Biotechnology, UWC.* [b]*Electron Microscopy Unit, UCT, South Africa.* E-mail: obishop@uwc.ac.za

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 αβ 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 2.1Å using molecular replacement (MR) with a 65% homologue from *P. thermophila*.

The 2.5-3.0Å 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., TsekoaL.T., *Advances in Applied Microbiology*, 2003, **52**, 123.

Keywords: model building, enzyme active site, metalloenzymes