transfer of the γ-phosphate of GTP (or ITP) to form PEP and GDP (IDP) as the first committed step of gluconeogenesis. Recent kinetic, NMR and EPR studies have demonstrated that pH dependent changes occur with respect to the environment of the active site Mn^{2+}[1]. Structures of the Mn-, Mn-PEP- and Mn-PEP-GDP-PEPCK complexes presented here provide evidence for important changes that occur at the catalytic metal site along the catalytic pathway. The structures show an interesting Mn binding site that is composed of deprotonated lysine, histidine and aspartate residues. In addition, the involvement of a previously unrecognized cysteine sulfhydryl in the Mn-PEPCK complex is demonstrated. Upon formation of the PEPCK-Mn-PEP or PEPCK-Mn-GDP binary complexes cysteine 273 coordination is lost as the loop it resides in occupies a different conformation. The involvement of cysteine 273 in the coordination of the Mn^{2+} in the Mn--PEPCK complex provides the structural basis for previous observations that catalytic activity is stimulated by β-mercaptoethanol, and inhibited by Zn^{2+} and modification or ionization of cysteine 273. This suggests that stabilization of the cysteine coordinated metal complex traps the enzyme in a catalytically incompetent metal complex and may represent a mechanism of oxidative regulation. These structures of catalytically relevant complexes in conjunction with the previous kinetic data provide detailed insight into the mechanism of catalysis of this important metabolic enzyme.

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Keywords: enzyme catalysis, biochemical crystallography, metallo enzyme X-ray crystallography

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Crystal Structures of *Bacillus cereus* AdoP Complexed with Substrates

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Purine nucleoside phosphorylases (PNP, E.C. 2.4.2.1) catalyze the reversible phosphorolysis of purine (deoxy)nucleosides, generating the corresponding purine base and (deoxy)ribose 1-phosphate. PNPs purified from a broad range of organisms can be ascribed to two main categories on the basis of substrate specificity, molecular mass, subunit composition and amino acid sequence: low-molecular-mass homotrimers specific for 6-oxopurines, and high-molecular-mass homohexamers, accepting both 6-oxo- and 6-aminopurines [1].

Bacillus cereus adenosine phosphorylase (AdoP) belongs to the high-molecular-mass PNP class on the basis of amino acid sequence homology and molecular mass determination, but it differs from the other members of this subfamily because it exhibits a high preference for adenosine over inosine.

To investigate the structural basis of the unusual substrate specificity shown by *B. cereus* AdoP, we determined the structures of the wild type enzyme and an active site mutant, both complexed with substrates. Comparison of the different structures provides insights to the unique substrate preferences of *B. cereus* AdoP.

[1] Bzowska A. *et al*., *Pharmacol Ther*, 2000, **88**(3), 349-425.

Keywords: purine nucleoside phosphorylase, substrate specificity, structure comparison

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Crystallographic Study of the Archaeal DNA Repair Enzymes: EXOIII and APE

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The apurinic/apyrimidinic (AP) sites occur frequently and spontaneously. They are both cytotoxic and highly promutagenic due to a lack of coding information. All organisms have mechanisms to repair this DNA damage, specifically by the base excision repair (BER).

The AP site endonuclease (APE) catalyzes an important step in BER pathway, in which the enzyme first recognizes the AP site and then cleaves the DNA backbone 5' to the AP site. The exodeoxyribonuclease III (EXOIII) is 3' to 5' directed DNA exonuclease. Although these two enzymes belong to the same family and their primary sequences are similar to each other, they have different nuclease activities.

To reveal structurally the reaction mechanisum and the specific recognistion of DNA, we crystallized EXOIII and APE from *Sulfolobus tokodaii* strain7.

*St*EXOIII and *St*APE crystals are obtained using the vapor diffusion method. *St*EXOIII crystal diffracts at 1.7 Å resolution with R_{merge} of 7.7% using synchrotron radiation at 100K. It belongs to the spacegroup $C222_1$ with unit cell dimensions of a = 48.0, b = 155.0, c = 75.3 Å. There is a monomer in an asymmetric unit.

Keywords: protein crystallization, DNA repair enzymes, archean

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Study of Substrate-Complexed Formylglycinamide Ribonucleotide Amidotransferase

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Formylglycinamide ribonucleotide amidotransferase, also known as PurL, catalyzes the fourth step of the purine biosynthetic pathway. PurL catalyzes the ATP-dependent synthesis of formylglycinamidine ribonucleotide from formylglycinamide ribonucleotide and glutamine [1]. Two types of PurLs have been detected. The first type, found in eukaryotes and Gram-negative bacteria, consists of a single polypeptide chain (140 kDa) and is designated large PurL. The second type, small PurL, (80 kDa) is found in Gram-positive and archea bacteria and requires two additional gene products (PurS and PurQ) for activity.

The proposed reaction mechanism of PurL remains mostly uncharacterized [2]. PurL is also a member of a protein superfamily that contains a novel ATP-binding domain [3]. To characterize the active site of the enzyme, structures of several complexes of small PurL from *Thermotoga maritima* were determined. These complexes show a conformational change in the protein not seen in the native structures [4]. They also provide insight into the positioning of the substrates in the active site and the identification of catalytically important residues, thereby elucidating aspects of the mechanism, as well as the signature sequence of the novel ATP-binding domain.

[1] Buchanan, Hartman, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 1959, **21**, 199-261. [2] Schrimsher *et al.*, *Biochemistry*, 1986, **25**, 4366. [3] Li *et al*., *Structure*, 1999, **7**, 1155. [4] Anand *et al.*, *Biochemistry*, 2004, **43**, 10328.

Keywords: amidotransferase, purine biosynthesis, ATP binding

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Structural Studies of Infestin 4, a Factor XIIa Inhibitor

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Infestin is a protein from *Triatoma infestans*, the main Chagas disease vector in Brazil, composed of seven Kazal-type domains and is further processed to yield a few serine protease inhibitors with different specificities. Infestin 3-4 are the last two domains of the infestin gene and are found *in vivo* in the insects anterior midgut [1].

The last domain, infestin 4, has been cloned, expressed and purified, showing remarkable inhibitory activity towards the human factor XIIa of the coagulation cascade. Crystals of infestin 4 were grown using the sitting-drop vapour-diffusion method with PEG 8000 as precipitant. X-ray diffraction data were collected to a maximum

resolution of 1.4 Å using a synchrotron radiation source. Crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 25.76, b = 45.38 and c = 56.77 Å. Initial phases were calculated by molecular replacement using an edited rhodniin molecule as the search model. Currently the structure is under the final stages of refinement with an R = 0.203 and R_{free} = 0.214. We shall discuss the implications of this structure in the light of its biological inhibitory function against factor XIIa.

[1] Campos I.T.N., et al., *Insect Biochem. Mol. Biol.*, 2002, **32**, 991.
Keywords: infestin, *Triatoma infestans*, serine-protease inhibitor

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Structural Studies on 3-hydroxyanthranilate-3,4-dioxygenase
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3-Hydroxyanthranilate-3,4-dioxygenase (HAD) catalyzes the oxidative ring opening of 3-hydroxyanthranilate in the tryptophan-based quinolinate biosynthetic pathway. The enzyme requires Fe^{2+} as a cofactor and is inactivated by 4-chloro-3-hydroxyanthranilate (ClHAA). The structure of HAD from *Ralstonia metallidurans* was determined at 1.9 Å resolution. The structures of HAD complexed with the inhibitor ClHAA and either molecular oxygen or nitrous oxide were determined at 2.0 Å resolution, and the structure of HAD complexed with the substrate 3-hydroxyanthranilate was determined at 3.2 Å resolution. HAD is a homodimer with a subunit topology that is characteristic of the cupin barrel fold. Each monomer contains two iron binding sites. The catalytic iron is buried deep inside the cupin barrel. The other iron site forms an FeS_4 center close to the solvent surface. The two iron sites are separated by about 24 Å. Based on the crystal structures of HAD, mutagenesis studies were carried out and a new mechanism for the enzyme inactivation by 4-chloro-3-hydroxyanthranilate is proposed.
Keywords: dioxygenase, cupin, enzyme mechanism

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Stucture of *S. typhimurium* Formylglycinamide Ribonucleotide amidotransferase
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Formylglycinamide ribonucleotide amidotransferase (FGAR-AT) catalyzes the ATP-dependent conversion of formylglycinamide ribonucleotide, and glutamine to formylglycinamidine ribonucleotide (FGAM) and glutamate in the purine biosynthetic pathway. In eukaryotes and Gram negative bacteria, FGAR-AT is encoded by the purL gene as a multidomain protein. In Gram positive bacteria and archaebacteria FGAR-AT is a complex of three proteins: PurS, PurL and PurQ. We have determined the structure of FGAR-AT from *Salmonella. typhimurium* at 1.9 Å resolution. The structure reveals four domains: an N-terminal domain structurally homologous to a PurS dimer, a linker region, an FGAM synthetase domain homologous to an aminoimidazole ribonucleotide synthetase dimer, and a triad glutaminase domain. A structural ADP molecule was found bound. A glutamylthioester intermediate was found in the glutaminase domain at C1135. The N-terminal domain is hypothesized to form the channel through which ammonia passes from the glutaminase domain to the FGAM synthetase domain.
Keywords: formylglycinamide ribonucleotide amidotransferase, ATP-grasp motif, protein evolution

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Towards the Structure of the BchI Hexameric Complex of Magnesium Chelatase
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The insertion of Mg^{2+} into protoporphyrin IX is catalyzed by the enzyme magnesium chelatase and represents the first committed step in the chlorophyll and bacteriochlorophyll biosynthetic pathways. Magnesium chelatase, in organisms that synthesize bacterio-chlorophyll, consists of three subunits, known as BchI, BchD and BchH. The structure of the monomeric BchI has been determined by X-Ray crystallography and it belongs to the chaperone-like, ATPase associated with a variety of cellular activities, (AAA) family of ATPases. Examination by electron microscopy of BchI solutions in the presence of ATP demonstrated that BchI, like other AAA proteins, forms oligomeric ring structures, [1]. The ATP hydrolysis is proposed to trigger major conformational changes in the hexamer, as has been shown for other AAA modules, [2]. Thus, it is crucial to fixate the complex in one single conformational state using non-hydrolysable substrates to create a homogenous environment. Needle shaped protein crystals have been obtained in different conditions. Ongoing optimization of crystallization conditions will hopefully give single crystals suitable for X-ray diffraction.

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Keywords: AAA-domain, ATPase, biosynthesis

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Crystal Structure of Glutaredoxin of CxxC1 Type from Poplar
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Glutaredoxins are small ubiquitous oxidoreductases of the thioredoxin family. The major function of glutaredoxin and thioredoxin is to selectively reduce disulfide bridges of target proteins. One of the major differences between glutaredoxin and thioredoxin is that the latter is reduced by various thioredoxin reductases whereas the former requires glutathione. The functions of plant glutaredoxins are not well understood compared to those of *E. coli* and animals. Nineteen poplar glutaredoxins have been annotated based on its genome information and they are classified into three classes [1]. We started crystal structure analysis of poplar glutaredoxin of CxxC1 type belonging to one of the three classes for deepening our understanding of plant glutaredoxins.

The glutaredoxin was overexpressed in *E.coli* cells and crystals were obtained by the hanging drop vapor diffusion method. X-ray diffraction data with three wavelengths were collected at 100K on beam line BL5A of the Photon factory, Tsukuba up to 2.3 Å resolution. The crystals belonged to space group $P6_1$ with unit cell dimensions a=b=97.5 Å and c=91.7 Å. The structure has been determined by the multiple-wavelength anomalous dispersion method. The polypeptide chain was traced in the electron density map and is now being refined. The molecule has a structure typical of the glutaredoxin fold but contains several new features.

[1] Rouhier N., Gelhaye E., Jacquot J.P., *Cell Mol Life Sci*, 2004, **61**, 1.
Keywords: redox enzyme, protein structure determination, MAD

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The Structure of *Yersinia pestis* Dehydroquinase
Abelardo M. Silva, Sandra B. Gabelli, Wei Cao, Elena Afonina, John