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Two thirds of the clinically useful antibiotics are naturally produced in actinomycetes, especially in *Streptomyces* species. These antibiotics vary highly in their chemical structures, some examples being amphenicols (chloramphenicol), polyketides (tetracyclin) or aminoglycosides (streptomycin).

Enzymes involved in the synthesis of aminoglycoside antibiotics (AGAs) are organized in large gene clusters containing 24 or more enzymes. The AGA family can be further divided into several subfamilies; the NEO subfamily takes the common precursor paromamine, which is further modified to AGAs such as neomycin, ribostamycin and lividomycin. [1]

Lividomycin B and neomycin B - members of the NEO subfamily - are produced by enzymes of the LIV/NEO gene cluster. The aminotransferases that catalyze the terminal transamination reaction (LivB and NeoB respectively) utilise the cofactor pyridoxal-5'-phosphate (PLP). LivB catalyzes the transamination reaction of 6'''-oxoparomomycin to the antibiotic paromomycin, which is also a precursor of lividomycin B, whereas NeoB performs the transamination of 6'''-oxoneomycin C to neomycin C. [1]

LivB and NeoB were expressed in *Streptomyces sp*., purified via Ni-affinity chromatography and crystallized. The structure of LivB could be solved using the "magic triangle compound" I3C [2] for SAD phasing, and that of NeoB by molecular replacement using the LivB structure as search model. Soaking of LivB crystals with the cofactor PLP, an amino donor and the end product paromomycin yielded crystal structures of the PLP-bound enzyme and the complex structure of LivB with an aldimine paromomycin-PLP intermediate. The latter represents a molecular snapshot of a key intermediate of the enzymatic reaction - the transamination at the 6''' position of the lividomycin B. These structures provide a basis for analyzing substrate specificities of other carbohydrate-modifying aminotransferases.

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Keywords: aminotransferase, aminoglycoside antibiotics, streptomyces

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Nicotinamide mononucleotide adenylyltransferase displays alternate binding modes for nicotinamide nucleotides

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Methanobacterium thermoautotrophicum nicotinamide mononucleotide adenylyltransferase (NMNAT) catalyzes the synthesis of nicotinamide adenine dinucleotide (NAD+) from nicotinamide mononucleotide and adenosine triphosphate. NAD+ plays a central role in cellular processes as it functions as a coenzyme in reduction-oxidation reactions and as a substrate in DNA ligation and protein ADP ribosylation reactions. The crystal structure of NMNAT complexed with NAD+ and sulfate implicated active site residues in binding and catalysis. Site-directed mutagenesis was used to further characterize the roles played by these residues. Arg11 and Arg136 were mutated to lysine residues. Arg47 was changed to lysine and glutamic acid, the amino acid found at the corresponding position in the Methanococcus jannaschii enzyme. Surprisingly, when expressed in Escherichia coli, all these mutants trapped a molecule of NADP+ in their active sites. This NADP+ was bound in a conformation quite different from the one displayed by NAD+ in the native enzyme complex. When NADP+ was co-crystallized with wild-type NMNAT, the same structural arrangement was observed. Keywords: Enzyme, Nucleotide, Protein.

Keywords: enzyme, nucleotide, protein

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Crystal structure of hypoxanthine guanine xanthine phosphoribosyl transferase from the thermophilic archeon *Sulfolobus solfataricus*

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Hypoxanthine guanine xanthine phosphoribosyltransferase (HGXPRTase) acts in the salvage of nucleotides by catalyzing the formation of 6-oxopurine nucleotide monophosphates IMP, GMP and XMP from D-phosphoribosyl-α-1-pyrophosphate (PRPP) and the respective nucleobases. Several pathogenic parasitic protozoa are dependent on purine salvage making HGXPRTase an attractive drug target [1].

We have determined the crystal structure of the HGXPRTase from the thermophilic archaeon *Sulfolobus solfataricus* to 1.8 Å resolution. *Ss*HGXPRTase together with product XMP (5 mM) was crystallized by vapour diffusion using NH_4SO_4 at pH 5.0 and the structure was solved using molecular replacement ($P3_12_11$, a=b=132.2 c=103.0). *Ss*HGXPRTase is a tetramer in the crystal having XMP and a sulfate ion bound in its active sites.

In addition synchrotron data have been recorded on crystals of *Ss*HGXPRTase obtained in the presence of substrate PRPP diffracting to 2.3 Å resolution ($P4_12_12$, a=b=73.7 c=142.6). These structures may provide valuable insight into the function of the HGXPRTases.

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Keywords: phosphoribosyltransferase, HGPRT, nucleotide salvage

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Crystal structure of enoylpyruvate transferase in Streptococcus pneumoniae

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Enoylpyruvate transferase catalyzes the first step in the peptidoglycan synthesis pathway and it is also a fosfomycin target. We are trying to solve the three-dimensional structures of enoylpyruvate transferase in different *Streptococcus pneumoniae* strains to elucidate their catalytic mechanism. We have solved the crystal structure of enoylpyruvate transferase from *Streptococcus pneumoniae* strain D39. Crystal needles were obtained by a hanging drop technique with

a precipitant solution containing $CaCl_2$, PEG 4000 and a pH 7.5. We have collected X-ray datasets up to 2.1 Å resolution, revealing an orthorhombic crystal system with the unit cell parameters: a= 46.18 Å, b= 80.82 Å, c= 104.5 Å, α=β=γ=90º. Space group is $P2_12_12_1$ and there is one molecule per asymmetric unit. The phasing method used to solve this structure was molecular replacement. The three-dimensional structure shows the general fold for this protein and presents valuable information about its function.

Keywords: macromolecules, mechanism, structure

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The structure of a novel bifunctional dehydratase/isomerase from fungal secondary metabolism

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Several fungi contain a dehydratase/isomerase that converts metabolites from lignin degradation into small compounds that show anti-tumor and antimicrobial effects against both Gram-positive and Gram-negative bacteria. The reaction occurs in two steps, a dehydratase reaction resulting in a relatively stable reaction intermediate followed by a unique isomerase step that leads to the final product. The enzyme is a dimer of 200 kDa, and the enzyme subunit (900 amino acids) does not show significant overall sequence homology to any protein of known structure. The X-ray structure of this enzyme has been solved using SAD to 2.0 Å resolution. The enzyme belongs to the all beta class of proteins and consists of three domains. Anomalous diffraction experiments revealed the presence of three Zn2+ ions and removal of zinc resulted in the loss of catalytic activity. Complexes with substrate and a reaction intermediate allowed the identification of the two active sites and suggest that two of the zinc ions participate in catalysis. The two active sites are about 20 Å apart, which requires that the reaction product of the first step has to diffuse from the enzyme in order to reach the second active site. This observation is consistent with NMR data that show release of this intermediate into the bulk solution. The crystal structures of the enzyme-ligand complexes allow some mechanistic conclusions that can be probed by other biochemical methods.

Keywords: macromolecular crystallography, bifunctional enzyme, dehydratase/isomerase

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Structures of exo-β-D-glucosaminidase, a chitosan degrading enzyme from *Trichoderma reesei*

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The 93-kDa extracellular protein composed of 892 amino acids from *Trichoderma reesei*, named Gls93, is an *exo-β*-D-glucosaminidase (GlcNase), belonging to glycoside hydrolase family 2. Gls93 catalyzes the degradation of chitosan into *β*-D-glucosamine (GlcN) monomers. For crystallographic studies, Gls93 was overexpressed in *Pichia pastoris* cells. The recombinant Gls93 had two molecular forms of approximately 105 kDa (Gls93-F1) and approximately 100 kDa (Gls93-F2), with the difference between them being caused by N-glycosylation pattern.

Initial crystal screenings for Gls93-F1 and Gls93-F2 produced several microcrystals in a week. Trials to improve the crystallization conditions were performed by varying the pH, the buffer system and the precipitant concentration. As a result, rectangular parallelepiped-shaped crystals were obtained from different conditions. Both crystal forms of gls93-F1 and gls93-F2 were suitable for X-ray structure analysis[1]. Initial phase determination for the Gls93-F1 crystal was performed by the molecular-replacement (MR) technique using the coordinate set of a bacterial GHF2 GlcNase, *A. orientalis* CsxA (PDB ID 2VZS; van Bueren *et al*., 2009), which has approximately 37% amino-acid sequence identity to Gls93, as a search model. Initial phase determination for the Gls93-F2 crystal was performed by the MR method with coordinate set of Gls93-F1. We have also successfully determined the crystal structure of Gls93 complexed with glucosamine. The crystal structures of Gls93-F1, Gls93-F2, and Gls93-F1/glucosamine were refined at 1.8, 2.4, and 2.6 Å resolutions, respectively. In addition, affinities of oligo sugars to the Gls93 enzyme were examined by surface plasmon resonance (SPR). The present crystal structure analyses and SPR measurements provide useful information for the substrate recognition mechanism of Gls93.

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One residue substitution in PcyA leads to unexpected changes in tetrapyrrole substrate binding

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Phycocyanobilin:ferredoxin oxidoreductase (PcyA) catalyzes the sequential reduction of the vinyl group of the D-ring and A-ring of biliverdin IXα (BV), using reducing equivalents provided by ferredoxin. This reaction produces phycocyanobilin, a pigment used for light-harvesting and light-sensing in red algae and cyanobacteria. The crystal structure of PcyA-BV reveals that BV is specifically bound in the PcyA active pocket through extensive hydrophobic and hydrophilic interactions. During the course of a mutational study of PcyA, we observed that mutation of the V225 position, apart from the processing sites, conferred an unusual property on PcyA; V225D mutant protein could bind BV and its analog BV13, but these complexes showed a distinct UV-vis absorption spectrum from that of the wild-type PcyA-BV complex (Fig. 1). In order to gain a better understanding of this non-canonical spectrum, we have determined the crystal structures of V225D mutant protein in complex with BV and with BV13.

The crystal structures of BV- and BV13-bound forms of V225D protein revealed that gross structural changes occurred near the substrate-binding pocket, and that the BV/BV13 binding manner in the pocket was dramatically altered (Fig. 2). That is, the orientation of the bound BV in the V225D protein was inverted relative to the PcyA-BV complex along the axis connecting the γ-position and the mid-point of the A- and D-rings. Furthermore, the BV in the V225D protein was translated by approximately 3 Å from the corresponding position in PcyA-BV. Protein folding in V225D-BV/BV13 was more similar to that of substrate-free PcyA than that in PcyA-BV; the "induced-fit" did not occur when BV/BV13 was bound to the V225D protein. The