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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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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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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