at 2.4 Å and 2.25 Å, respectively. This is the first reported structure of a prokaryotic UCK. The overall structure is highly similar to that of human uridine cytidine kinase 2 (UCK2). Structural comparison of the nucleoside-binding site between ttCK and human UCK2 revealed that most amino acid residues around the base moieties are identical between them, except for Tyr59 and Tyr93 in ttCK, corresponding to Phe83 and His117 in human UCK2. These two residues are located near the N4 amino group and the N3 nitrogen atom of cytosine: these atoms differ between cytosine and uracil. Many UCK homologues have a Phe and His residue at the position equivalent to Tyr59 and Tyr93 in ttCK, respectively. As the next step, we prepared ttCK mutants with amino acid substitution at Tyr59 and Tyr93. The substrate specificity of Y59F was the same as that for wild type. In contrast, Y93H had activity for both uridine and cytidine. Whereas the replacement of Tyr93 with Phe or Leu, unaffected the substrate specificity of ttCK, the replacement with Gln, Asn and Glu endowed ttCK with phosphorylation activity toward uridine. These results indicate that a hydrophilic residue at position 93 permits UCK to accept uridine as substrate. A potential hydrogen donor at this position may enable UCK to interact a keto group of uridine specifically. In addition to ttCK, other UCK homologues of 26 species including pathogens have tyrosine at the position equivalent to Tyr93, which predicts that these are cytidine-specific UCKs. This study points to the critical need for experimental studies even of enzymes whose annotation has been accepted.

Keywords: nucleoside, kinase, mutation

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Structure-based catalytic optimization of a type III rubisco

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The Calvin-Benson-Bassham cycle is responsible for carbon dioxide fixation in all plants, algae, and cyanobacteria. The enzyme that catalyzes the carbon dioxide-fixing reaction is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Rubisco from a hyperthermophilic archaeon *Thermococcus kodakarensis* (*Tk*-Rubisco) belongs to the type III group, and it shows high activity at high temperatures. We have previously determined the crystal structure in the apo-form of this enzyme [1]. We have also found that replacement of the entire α-helix 6 of *Tk*-Rubisco with the corresponding region of the spinach enzyme (SP6 mutant) results in an improvement of catalytic performance at mesophilic temperatures, both *in vivo* and *in vitro*, whereas the former and latter half replacements of the α-helix 6 (SP4 and SP5 mutants) do not yield such improvement [2]. We report here the crystal structures of the wild-type *Tk*-Rubisco and the mutants SP4 and SP6, and discuss the relationships between their structures and enzymatic activities [3].

A comparison among these structures shows the movement and the increase of temperature factors of α-helix 6 induced by four essential factors. We thus supposed that an increase in the flexibility of the α-helix 6 and loop 6 regions was important to increase the catalytic activity of *Tk*-Rubisco at ambient temperatures. Based on this structural information, we constructed a new mutant, SP5-V330T, which was designed to have significantly greater flexibility in the above region, and it proved to exhibit the highest activity among all mutants examined to date. The thermostability of the SP5-V330T mutant was lower than that of wild-type *Tk*-Rubisco, providing further support on the relationship between flexibility and activity at ambient temperatures.

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TTP binding in the active conformation of dCTP deaminase: dUTPase

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The bifunctional dCTP deaminase:dUTPase is a homotrimeric enzyme, which is closely related to the monofuncional dCTPases and dUTPases. The enzyme catalyses the deamination and the phosphate hydrolysis of dCTP to dUMP and it is end-product inhibited by dTTP. Until now the structures of the wild type *Mycobacterium tuberculosis* bifunctional enzyme in its apo form (pdb-entry 2qlp) and with TTP (pdb-entry 2qxx) bound have been solved [1] and the *Methanocaldococcus jannaschii* bifunctional enzyme in the apo form (pdb-entries 1ogh, 2hxb, 1pkh, [2,3]) is also known. Furthermore, two variants of the *M. jannaschii* enzyme have been crystallized: the E145Q variant with diphosphate and magnesium (pdb-entry 3GF0) and the E145A variant of the *M. jannaschii* enzyme with α,β-imido dUTP and magnesium (pdb-entry 2hxd) [4]. It has been concluded that the bifunctional dCTP deaminase:dUTPase exists in two mutually exclusive conformations. An active form, which resembles the apo-form and the structure where α,β-imido dUTP binds and an inactive form that binds dTTP. To investigate the inhibition by dTTP we have made several mutant enzymes, one of which is able to bind dTTP in the active conformation. Furthermore, the wild type enzyme and this mutant enzyme have been enzymatically characterized and interestingly, the mutant enzyme, which binds dTTP in the active conformation, is still inhibited by dTTP.

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Keywords: nucleotide metabolism, bifunctional dCTP deaminase: dUTPase, dTTP inhibition

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Structural studies of the aminotransferases LivB and NeoB

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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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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