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.

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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.

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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