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Fatty acid breakdown is an essential process in all organisms, and the complex pathway require several sets of enzymes working in different cellular compartments. β-oxidation cycles are the center of the fatty acid breakdown, in each round the lipid chain shortening by a C_2 unit. The acyl-CoA oxidase family catalyzes the first and rate limiting step in the perixosomal β-oxidation cycle, where acyl-CoA is converted to *trans*-2-enoyl-CoA. *Arabidopsis thaliana* have 4 different acyl-CoA oxidases, each with different chain length specificities. The structure of acyl-CoA oxidase 1 (ACX1), specific for long chain lipids, has previously been determined in our group.

We here present ACX1 in complex with acetoacetyl-CoA; the first structure of an acyl-CoA oxidase in complex with a substrate analogue. The fatty acyl moiety could be modeled between the isoalloxazine ring of FAD and the putative catalytic residue Glu424, forming hydrogen bonds to the backbone nitrogen of Glu424 and to N5 of FAD. Glu424 has moved to better accomodate the inhibitor, and is also more ordered in the complex structure. This confirms Glu424 to be the catalytic residue. The beginning of helix G and the end of helix H has moved slightly, and an interesting rotamer change of His374 and Tyr278 can be observed, bringing both residues closer to the CoA tail of the modeled ligand.

Keywords: acylCoA oxidase, beta oxidation, peroxisome

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Structural Studies on Sulfurtransferase/Phosphatase Enzymes

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Sulfurtransferases are widespread enzymes that *in vitro* catalyse the transfer of a sulfur atom from a donor molecule to cyanide [1]. In order to elucidate the molecular basis of sulfur transfer reaction and identify the structural determinants for enzyme selectivity, crystallographic analyses were carried out on three different sulfurtransferases: *Azotobacter vinelandii* rhodanese (RhdA) and *Escherichia coli* GlpE and *Escherichia coli* SseA, a MST enzyme.

The crystal structure of the RhdA has been determined with the method of Multiple Isomorphous Replacement and refined at 1.8 Å resolution in the sulfur-free and persulfide-containing forms [2]. GlpE crystal structure has been determined at 1.06 Å and displays a three-dimensional fold similar to that of either RhdA domains [3]. Notably, GlpE is also structurally similar to the catalytic domain of the human cell cycle-control Cdc25 phosphatase. The distinct substrate specificity, sulphur for rhodanese enzymes and phosphate for Cdc25 phosphatases, appears to be primarily consequence of the different active site loop length in the two enzymes. These structural findings provide guidelines for the identification of the as yet unknown biological role of this protein. Also the crystal structure of SseA, solved at 2.8 Å resolution by molecular replacement method [4].

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Keywords: sulfurtransferases, phosphatases, rhodanese

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Regulation of *Sulfolobus solfataricus* Uracil Phosphoribosyl-transferase

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UPRTase is a salvage enzyme that catalyzes the formation of UMP from PRPP (5-phosphoribosyl-1-α-diphosphate) and uracil.

CTP and UMP can independently bind to *Ss*UPRTase and simultaneous binding of CTP and UMP strongly inhibits the enzyme. A structure to 2.8 Å resolution of *Ss*UPRTase-CTP-UMP has already been determined [1].

GTP causes a 20-fold increase in the turnover number k_{cat} and raises K_M for PRPP and uracil by 2- and >10-fold, respectively [2]. In order to make an *Ss*UPRTase-GTP-PRPP complex, the enzyme must be depleted for the product UMP. Co-purified UMP binds so strongly to *Ss*UPRTase that an unfolding and refolding procedure was necessary to remove it. UMP-depleted *Ss*UPRTase (5 mg/mL) with 5 mM GTP and 5 mM PRPP was crystallized by vapour diffusion with PEG8000 at pH 6.5. Synchrotron data to 2.8 Å resolution has been recorded ($P6_422$, a=b=122.2 Å, c=62.2 Å) and the structure determined by molecular replacement.

*Ss*UPRTase in solution as well as in crystals is tetrameric with 222 symmetry. The allosteric binding sites for CTP/GTP are situated in the middle of the tetramer ca. 24 Å from the active sites. Transformation from inhibited to activated structure involves structural changes in the quaternary structure along with major active site movements.

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Keywords: nucleotide metabolism, protein regulation, enzyme catalysis

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Crystal Structure of Glutathione-dependent Dehydroascorbate Reductase from Spinach Chloroplast

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Glutathione-dependent dehydroascorbate reductase (GSH-DHAR) catalyzes the reduction of dehydroascorbate (DHA) to ascorbate using reduced glutathione as the electron donor. GSH-DHAR existing in chloroplast plays a pivotal role in the regeneration of ascorbate, which is oxidized to scavenge active oxygen species in the process of photosynthesis. The catalytic mechanism of the GSH-DHAR from spinach chloroplast is intriguing, because the specific constants for DHA and GSH are much higher than those of the other characterized DHARs.

Here, we report the three-dimensional structure of GSH-DHAR from spinach chloroplasts at 1.65 Å resolution, which has been determined by the multiwavelength anomalous dispersion (MAD) phasing method. The crystal structure reveals a monomeric form, which corresponds to the results observed in the analyses of gel filtration and dynamic light scattering. The structure is mainly composed of the similar domain to that of previously solved thioltransferase [1] and an extra alpha-helical domain. The catalytically essential cystein was completely reduced, because it was crystallized in the solution including high concentration of DTT. The model study using the coordinates of glutathione transferases suggested that the putative glutathione binding site was formed by the amino residues corresponding to those of the other glutathione transferases. These observations support that glutathione could be bound near the catalytically essential cystein. We will show the detailed reaction mechanism to describe how it establishes its high specificities.

[1] Katti S. K., *et al*., *Protein Sci*., 1995, **4**, 1998.

Keywords: crystal structure, dehydroascorbate, reductase

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Snapshots Along the PEPCK Catalytic Pathway

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Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the reversible decarboxylation of oxaloacetic acid with the concomitant

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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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