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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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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.

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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