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STRUCTURE OF A NOVEL PECTATE LYASE FROM *AZOSPIRILLUM IRAKENSE*

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Pectate lyases are the major pectinases that play a key role in the development of the soft-rot disease. Besides in phytopathogens, pectin depolymerization has also been reported in non-pathogenic plant associated bacteria such as the N2-fixing endosymbiont Rhizobium and the N2-fixing soil bacterium *Azospirillum irakense*. A gene from *A. irakense* encoding a pectate lyase (termed PelA) was isolated by heterologous expression of the gene in *Escherichia coli*. Analysis of the corresponding amino acid sequence revealed no homology to other bacterial, plant and fungal pectinases leading to the classification of the enzyme in a new pectate lyase family (family 10). The *A. irakense* PelA has been crystallized using the hanging-drop vapor diffusion method at 277K. These crystals are hexagonal with cell dimensions of a = b = 85.55 Å, c = 230.13 Å, γ = 120°, and space group $P6_522$ having one molecule per asymmetric unit2. Diffraction data to a resolution of 1.97 Å were collected at synchrotron facilities, as well as a three-wavelengths MAD data set on a Hg derivate crystal to a resolution of 2.6 Å. The preliminary structural results show that PelA does not have the characteristic parallel β-helix fold of the polysaccharide pectate lyase families.

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REACTION INTERMEDIATES ANALYSIS OF ACCD AND ITS HOMOLOGUE

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In higher plants, acc (1-aminocycropropane-1-carboxylate) is a precursor of hormone ethylene that initiates fruit ripening and regulates various processes in growth and development. Several soil microorganisms have acc deaminase, a pyridoxal 5'-phosphate (plp) dependent enzyme, which catalyzes cyclopropane ring opening; the degradation of acc into 2-oxobutyrate and ammonia. Unlike other plp-dependent enzymes, the substrate of this enzyme has no a-hydrogen atom. Thus, a unique mechanism for the bond cleavage is required. In this study, six types of crystal structures have been determined including mutants and homologue protein.
Several yACCD (from yeast *Hansenula saturnus*) mutants lost ACCD activity and were crystallized in the presence of substrate ACC. One of them, K51T made a main absorption band at around 330 nm, and loss of stereospesificity of the reaction to D- and L-serine. The 420 nm absorption was recovered by adding of ACC and structure determination was succeeded at this condition. The structure of yACCD reaction intermediates, K51T-ACC complex shows that PLP rotated to form Schiff base of ACC-PLP. On the other hand, hyperthermophilic archaebacteria *Pyrococcus horikoshii* OT3 has ACCD-homologue ORF named PH0054 whose amino acid sequence identity is 25% with other ACCD. However, recombinant PH0054 did not show ACCD activity. Crystal structure of PH0054-ACC complex shows similar active site environment with yACCD but a little difference is recognizable. The different conformation around active sites between yACCD and PH0054 complexes reveals that circumstance around PLP strictly controls enzyme activity.

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STRUCTURAL STUDIES OF ENZYMES INVOLVED IN PEROXISOMAL β-OXIDATION

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Peroxisomal β-oxidation is the predominant pathway of fatty acid breakdown in plants. The β-oxidation is facilitated by three enzymatic steps catalyzed by: Acyl-CoA oxidases (ACX), a multifunctional protein (MFE) and a 3-ketoacyl-CoA thiolase (thiolase). The first step of the β-oxidation is catalyzed by ACX, a flavoenzymes responsible for converting acyl-CoA to 2-trans-enoyl-CoA. The existence of ACXs as a family of enzymes that differs in size, subunit composition and substrate specificity (short-, medium-, and long-chain specific) has been demonstrated in several plant species(1). The second step in the β-oxidation is catalyzed by MFE possessing 2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase as well as isomerase and epimerase activity. A thiolase catalyzes the final cleavage of the ketoacyl-CoA to acetyl-CoA and an acyl-CoA shortened by two carbons. Studies have indicated metabolon formation and channeling of β-oxidation metabolites(2). The three enzymes has been cloned from *Brassica napus* (oilseed rape) and by recombinant *E. coli* expression, purification, crystallization and structure determination the enzymatic mechanism of the individual enzymes as well as the interaction between the enzymes are being studied.

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STRUCTURE OF HUMAN PHOSPHOSERINE PHOSPHATASE

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Phosphoserine phosphatase (psp) is the enzyme responsible for the third and last step in the major pathway of L-serine biosynthesis. It catalyzes the Mg^{2+}-dependent hydrolysis of L-phosphoserine. The reaction mechanism of many phophatases or phosphotransferases involves the formation of a catalytic intermediate in which the phosphate detached from the substrate is bound to the side chain of a serine, histidine, cysteine [1] or aspartate residue present in the catalytic site. Psp belongs to a recently identified class of phosphotransferases forming a phosphoaspartate intermediate during catalysis.
Crystals of psp were grown in the $C222_1$ space group with 2 molecules in the asymmetric unit [2]. Diffraction data were collected to 1.53 Å and the structure was solved by the MAD method making use of a selenomethionyl derivative. Refinement of the structure is currently being done. The psp structure comprises two molecules in each asymmetric unit. A monomer consists of two major domains: a core α/β domain and a four-helix-bundle domain. The two domains come together and form a pocket. The active site is within a closed environment between the core α/β domain and the four-helix-bundle domain.

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