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X-ray crystallographic analysis of type I pullulanases from *Klebsiella pneumoniae* and *Bacillus subtilis*

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Pullulanase (EC 3.2.1.41) catalyzes the hydrolysis of α-1,6-glycosidic linkage of α-glucans such as amylopectin and pullulan. The enzyme is classified to family 13 of glycoside hydrolase together with α-amylase and isoamylase. In order to elucidate the enzymatic mechanism of pullulanase, we have determined the crystal structures of the enzyme from *Klebsiella aerogenes* (KPP) and *Bacillus subtilis* (BSP) by M.I.R and MR, resspectively. KPB structures of apo and complexes with glucose (G1), maltose (G2), matotriose (G3), matotetraose (G4) and isomaltose (isoG2) were refined at 1.6-1.9 Å resolution. BSP structures of apo and complexes with G2, and α-cyclodexrin (αCD) were refined at 2.1-2.3 Å resolution. KPP is consist of five domains (N1, N2, N3, A and C), while BSP has four domains lacking N1 domain of KPP, which is a new type of starch-binding domain with one calcium site (CBM41). In catalytic A domain, BSP has shorter loops (res. 446-559, 771-783 and 913-945 in KPP are deleted) and resembles to isoamylase [1] more than KPP except for a few loops characteristics to both enzymes. The subsite mapping of KPP by the binding of the oligosaccharides clearly showed the parallel binding of two oligosaccharides in the active site (subsites +2~-1' and -1~-4). The catalytic acid (Glu706 in KPP and Glu435 in BSP) and nucleophile (Asp677 in KPP and Asp406 in BSP) are situated at the junction of the two sugar binding sites (between +1 and -1). One αCD was found to bind in the edge of the active cleft of BSP around subsite +2 of the oligosaccharide-binding site. The side chain of Phe476, which is in the pullulanase specific loop, occupied the hole of αCD from its hydrophobic side. These findings provide an important structural evidence to account the different enzymatic properties between pullulanase and isoamylase.

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Three-Dimensional Structure of Rat-Liver Acyl-CoA Oxidase in Complex with Fatty Acid

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Fatty acid degradation by b-oxidation in mammals is known to proceed in two different cellular compartments, mitochondria and peroxisomes. The former contains the pathway initiated by acyl-CoA dehydrogenases (ACDs) while the latter fosters the one initiated by acyl-CoA oxidases (ACOs). ACO is a flavooxidase with non-covalently bound FAD and catalyzes the dehydrogenation of acyl-CoA yielding *trans*-2-enoyl-CoA in the reductive half-reaction. ACO and ACD share the same catalytic dehydrogenation of acyl-CoA to the corresponding *trans*-2-enoyl-CoA in the reductive half-reaction. However, they differ completely in the oxidative half-reaction; ACO utilizes molecular oxygen to reoxidize reduced flavin produced in the reductive half-reaction while ACD passes electrons to electron-transferring flavoprotein to regenerate oxidized flavin. The crystal structure of rat liver ACO-II has been solved by MIR method[1]. The overall folding of ACO-II less C-terminal 221 residues is similar to that of medium-chain acyl-CoA dehydrogenase (MCAD). The flavin ring of FAD resides at the active site with its *si*-face attached to the b-domain, and is surrounded by active-site residues in a mode similar to that found in MCAD. However, the pyrimidine moiety of flavin is exposed to the solvent and can readily be attacked by molecular oxygen, while that in MCAD is protected from the solvent. The three-dimensional structure of ACO-II in complex with a C12-fatty acid was solved by molecular replacement method. The crystalline form of the complex was obtained by cocrystallization of ACO-II with lauroyl-CoA. The crystalline complex possessed, in the acitve-site crevice, only the fatty acid moiety that had been formed by hydrolysis of the thioester bond. The overall dimeric structure and the folding pattern of each subunit are essentially superimposable to those of uncomplexed ACO-II. The active-site including the flavin ring of FAD, the crevice embracing the fatty acyl moiety, together with adjacent amino acid side chains are superimposably conserved with an exception of Glu421, whose carboxylate group is tilted away to accomodate the fatty acid. The one of the carboxyl oxygens of the bound fatty acid is hydrogen-bonded to amide hydrogen of Glu421, the presumed catalytic base, and to ribityl 2'-hydroxy group of FAD. This hydrogen-bonding network relates to the substrate recognition/activation in MCAD. The binding mode of C12-fatty acid suggests that the active-site does not close upon substrate-binding and remains spacious during the entire catalytic process holding the oxygen accessibility in the oxidative half-reaction.

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