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Crystal structures of human sulfotransferase 1A1: from broad to narrow specificity

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Human Sulfotranferase 1A1 (hSULT1A1) catalyzes the transfer of a sulforyl group from a 3'-phosphoadenosine 5'-phosphosulfate (PAPS) donor to a variety of substrates (acceptors) containing either an amine or a hydroxyl group, leading to modification of the acceptor's biological activity. In order to gain insight into the molecular mechanism underlying the broad specificity of hSULT1A1, we have crystallized the enzyme and determined its structure in the presence of 3' phosphoadenosine 5'-phosphate (PAP) alone, PAP and 3-Cyano-7-Coumarin (3CyC), and PAP and 2-Napthol (2NAP). These structures demonstrated high plasticity of the acceptor binding site which was mainly attributed to substantial movements of the gating loop (residues 86-90) that enabled the binding of large and elongated phenol substrates. We were also interested in increasing the SULT1A1 specificity to para-nitrophenol (pNP) on the account of the other substrates through the use of directed evolution methodology. This method allowed the generation of a hSULT1A1 variant mutated at position D249G which exhibits a marked increase in activity toward pNP while decreasing its activity toward 3CyC and 2NAP. The determined crystal structure of D249G revealed the effect of the mutation on surface electrostatic potential and loop stability in the proximity of the active site pocket.

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DNA recognition by restriction endonuclease AgeI

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Type II restriction endonuclease AgeI recognizes 6 bp sequence A/CCGGT („/" denotes the cleavage site) [1] and belongs to a family of evolutionary related restriction enzymes which contain CCGG tetranucleotide in their target sites. These enzymes share a variation of PD-(D/E)XK catalytic sequence motif and use a conserved R-(D/E)R sequence motif for the recognition of the CCGG tetranucleotide [2]. We used X-ray crystallography to determine the structural mechanism of the target site recognition by AgeI. Crystals of AgeI complexes with 11 and 13 bp DNA oligonucleotides belong to 3 different space groups and diffract X-rays to 1.5-2.7 Å resolution. Crystal structure of AgeI complex with 13 bp oligonucleotide solved at 2.4 Å resolution reveals structural determinants for the target site recognition. Structure of AgeI complex with 11 bp oligonucleotide (resolution 1.5 Å) presumably represents semi-specific complex, because only a part of the protein-DNA contacts, observed in the 2.4 Å resolution structure, are found. The structures of AgeI-DNA complexes revealed that AgeI is the most diverged member of the family, since it exhibits variations both in the composition of the active site and in CCGG recognition.

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Crystal structure of the branching enzyme I (BEI) from *Oryza sativa* L

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Branching enzyme (BE) is the enzyme that catalyzes the formation of branch points by cleaving the α-1,4-linkage in polyglucans and reattaching the chain via an α-1,6 glucan linkage. Hence, the enzyme plays an important role in the biosynthesis of starch in plants and of glycogen in animals and bacteria. To date, bacterial glycogen BEs (GBE) have been extensively studied and some structures have been reported on the enzymes from *Escherichia coli* (*Eco*GBE) and *Mycobacterium tuberculosis (Mtu*GBE*)*. In this study, we tried to determine the crystal structure of the starch branching enzyme I (BEI) from *Oryza sativa*.

We determined the crystal structure of BEI at a resolution of 1.9 Å by molecular replacement using the *Eco*GBE structure as a search model [1]. BEI is roughly ellipsoidal in shape with two globular domains that form a prominent groove which is proposed to serve as the α-polyglucan-binding site, though sequence analysis of BEI indicated a modular structure in which the central α-amylase domain is flanked on each side by the N-terminal carbohydrate binding module (CBM48) and the α-amylase C-domain. Amino acid residues Asp344 and Glu399, which are postulated to play an essential role in catalysis as a nucleophile and a general acid/base, respectively, are located at a central cleft in the groove. The BEI structure was compared with that of the bacterial GBE, *Mtu*GBE. The two molecules could be superimposed with an rmsd of 1.85 Å for 643 common Cα atoms. Despite the overall similarity, there is a significant difference between BEI and *Mtu*GBE in the N-terminal region, where BEI folds into three consecutive α-helices (α1, α2 andα3), while *Mtu*GBE has a β-sandwich domain (N1 domain) in addition to the N-terminal CBM48 domain (N2 domain). The CBM48 domain itself is known to have glycogen/starch-binding functions and is believed to determine the length of the carbohydrate branches transferred. Hence, the structural difference observed in the N-terminal portion of BEI and *Mtu*GBE might be attributable to a difference in substrate.

Subsequently, the mutant E399Q, in which Glu399 was replaced with Gln, was prepared and crystallized in complex with maltopentaose. The crystallographic analysis revealed that the maltopentaose bound the N-terminal CBM48 module but was not detected at the possible active site on the prominent groove.

Finally, the BEI structure was compared with that of the *Klebsiella pneumoniae* pullulanase in a complex with maltotetraose. The structural comparison revealed that in BEI, extended loop structures cause a narrowing of the substrate-binding site, whereas shortened loop structures make a larger space at the corresponding subsite in the *K. pneumoniae* pullulanase, which hydrolyse α-1,6 glycosidic linkage. This structural difference might be attributed to distinct catalytic reactions, transglycosylation and hydrolysis, respectively, by BEI and pullulanase.