Crystal structures of human sulphotransferase 1A1: from broad to narrow specificity
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Human Sulphotransferase 1A1 (hSULT1A1) catalyzes the transfer of a sulfuryl 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 crystalized 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 SUL1T1A1 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.

Keywords: sulphotransferase, ligand, specificity

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 glycoconjugates in animals and bacteria. To date, bacterial glycoconjugates (GGE) have been extensively studied and some structures have been reported on the enzymes from Escherichia coli (EcOGE) and Mycobacterium tuberculosis (MtUSGGE). 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 EcOGE 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 CBM48 domain (N2 domain). Despite the overall similarity, there is a significant difference between BEI and MtUSGGE. The two molecules could be superimposed on an rmsd of 1.85 Å for 643 common Cα atoms. However, this structural difference observed in the N-terminal region, where BEI folds into three consecutive α-helices (α1, α2 and α3), while MtUSGGE 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 function, though sequence analysis of BEI shows high sequence similarity, but the structural difference observed in the N-terminal portion of BEI and MtUSGGE might be attributed to a difference in substrate.

Subsequently, the mutant E399Q, in which Glu399 was replaced with Gln, was prepared and crystallized in complex with maltotetraose. The crystallographic analysis revealed that the maltotetraose bound to the bacterial GBE, MtUSGGE. The two molecules could be superimposed on an rmsd of 1.85 Å for 643 common Cα atoms. Despite the overall similarity, there is a significant difference between BEI and MtUSGGE.

Finally, the BEI structure was compared with that of the Klebsiella pneumoniae pullulanase in a complex with maltotetraose. The crystallographic analysis revealed that the maltotetraose bound to the N-terminal CBM48 module but was not detected at the possible active site on the prominent groove.

In conclusion, the BEI structure provides the structural basis for understanding the branching enzyme activity and its possible role in the biosynthesis of starch in plants and of glycoconjugates in animals and bacteria.

Keywords: protein, DNA, recognition

DNA recognition by restriction endonuclease Agel
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Type II restriction endonuclease Agel recognizes 6 bp sequence A/CCGGT (‘’A’’ 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)XXK 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 Agel. Crystals of Agel 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 Agel complex with 13 bp oligonucleotide solved at 2.4 Å resolution reveals structural determinants for the target site recognition. Structure of Agel 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 Agel-DNA complexes revealed that Agel 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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