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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.

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Structural analysis of chondroitin polymerase from *Escherichia coli* K4

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Chondroitin, heparin/heparan and hyaluronan are the primary glycosaminoglycans found in humans and are linear polysaccharides consisting of an amino sugar and an uronic acid. Chondroitin chains range from 40 to over 100 repeating units of the disaccharide (GlcUA β (1–3)–GalNAc β (1–4)). Sulfated chondroitins are involved in the regulation of various biological functions such as central nervous system development, wound repair, infection, growth factor signaling, and morphogenesis, in addition to its conventional structural roles. Elongation of glycosaminoglycan chains is catalyzed by bi-functional glycosyltransferases. The bacterial chondroitin polymerase K4CP catalyzes elongation of the chondroitin chain by alternatively transferring the GlcUA and GalNAc moiety from UDP-GlcUA and UDP-GalNAc to the nonreducing ends of the chondroitin chain [1].

We previously reported the crystal structure of K4CP in the presence of UDP and UDP-GalNAc as well as with UDP and UDP-GlcUA [2]. The structures consisted of two GT-A fold domains in which the two active sites were 60Å apart. UDP-GalNAc and UDP-GlcUA were found at the active sites of the N-terminal and C-terminal domains, respectively.

Here, we present the crystal structure of K4CP complexed with chondroitin hexamer (CH6) and UDP at 3.5Å resolution. CH6 was found at the active sites of the N-terminal domain. In addition, we generated docking model of the C-terminal domain with chondroitin pentamer. These structural information revealed molecular basis of catalysis and substrate recognition, and provided the structural basis for further investigating the molecular mechanism of biosynthesis of chondroitin chain.

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FAD binding may inhibit a FMN-dependent Nitroreductase from *Idiomarina loihiensis* L2TR

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Nitroreductases are NAD(P)H-dependent flavoenzymes that catalyze the reduction of nitro groups on nitroaromatic or nitroheterocyclic compounds. The enzyme utilizes NAD(P)H to reduce, via Flavin mononucleotide (FMN), a wide variety of substrates bearing nitro groups to hydroxylamino or amino groups. The structure of an oxygen-insensitive NAD(P)H-dependent nitroreductase (YP_156458.1) from *Idiomarina loihiensis* L2TR has been determined at 1.9 Å resolution. Although very similar in structure to other nitroreductases, we report the first observation of the Flavin-adenine dinucleutide (FAD) cofactor bound to a nitroreductase. In addition, this is also the first structure in which both prosthetic groups, FMN and FAD, are bound to different molecules in the crystal asymmetric unit. There is no conformational change in the protein structure upon binding FAD vs. FMN, however, FAD adopts a novel binding mode in which the adenosine moiety folds back over its flavin moiety occupying the substrate binding pocket. The binding mode of FAD helps explain the reduced activity of FAD bound nitroreductase compared to FMN bound nitroreductase.

Keywords: nitroreductase, FMN, FAD

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Understanding the role of RecN in the DSB repair pathway of *Deinococcus radiodurans*

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Deinococcus radiodurans is well known for its ability to withstand high doses of ionizing radiations. Such treatments induce several hundreds of Double Strand Breaks in the DNA and *D. radiodurans* has therefore developed an highly efficient repair mechanism in order to restore the integrity of its genetic material. Multiple copies of the genome in concert with homologous recombination (HR) pathway lead to the complete repair of the DNA damages in only 3 hours [1].

Homologous recombination in *E. coli* is accomplished by RecBCD enzyme. In *D. radiodurans* no homologue of RecB and RecC proteins are encoded and the DNA repair is mainly carried out through the RecFOR pathway, which is responsible to load RecA protein onto the DNA.

The whole mechanism by which the RecFOR pathway repairs the DNA lesion is not fully understood yet. We are especially interested in understanding how the DSB recognition occurs inside the cell and how then the entire process takes place. In *D. radiodurans* the DNA damage could be detected by RecN protein, based on previous *in vivo* studies carried out in *Bacillus subtilis* [2].

RecN is an SMC-like protein that is made up of a "head" domain, structurally similar to the Nucleotide binding Domain (NBD) of ABC proteins, with which the protein hydrolyzes the ATP. The N- and C-termini of RecN are then connected by an unusually short coiled-coil domain, which is supposed to contain a dimerization site. We determined the crystal structures of both domains using Se-Methionine