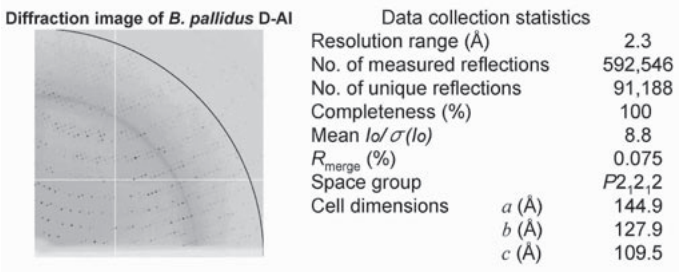

Keywords: isomerase, rare sugars, X-ray structure

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Structures of NADH and NAD$^+$ bound 3 α -hydroxysteroid dehydrogenase from *Pseudomonas* sp. B-0831

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Bacterial 3α-hydroxysteroid dehydrogenase (3αHSD) catalyzes the reaction of the reversible inter-conversion between 3α -hydroxysteroid and 3-ketosteroid in a coenzyme dependent manner. We reported the structure of 3αHSD of *Pseudomonas* sp. B-0831 (Ps3αHSD) complexed with NADH (*JBC*, 2006, **281**, 31876-84). The crystal which was obtained by co-crystallization with NADH, contained a dimer in an asymmetric unit of which one is apo-form and the other is holo-form. There was a distinct difference in the so-called substrate-binding loop (185-207) between these two subunits i.e. it consists of two α-helices in the holo-form while it is in disordered form in the apo-form. Here we obtained the complex by co-crystallization with NAD$^+$, which contained a dimer in an asymmetric unit as well. In this case, the coenzyme was bound to the both monomers in the dimer. It is noteworthy that there was a conformational difference between these subunits. One of them has two α-helices in the so-called substrate-binding loop region, while the other, even though NAD$^+$ was bound, takes a disordered form similar to the NADH complex. In these two alpha helices, there was a difference found in their mutual spatial arrangements between the respective complexes with NADH and NAD$^+$. This difference which accompanies a shift of the hydrogen bond partner of Tyr200 from His150 in the complex with NADH to Val74, whereas with NAD$^+$ results in loss of hydrogen bonding found between Tyr153 and NADH. This conformational change might play an important role in the coenzyme recognition of Ps3αHSD depending on the redox state.

Keywords: short-chain dehydrogenase reductases, cofactors, dehydrogenase steroid nucleotide

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High-resolution X-ray diffraction study of the hMTH1 mutant

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Human MutT homolog-1 (hMTH1) hydrolyzes a variety of oxidized nucleoside triphosphates to their corresponding monophosphates and prevents replicational and transcriptional errors caused by their misincorporations into DNA and RNA. hMTH1 has broad substrate specificity for several oxidized purine nucleotides such as 8-oxo-dGTP, 2-oxo-dATP and 8-oxo-dATP. Recently, we have determined the crystal structures of hMTH1 complexed with 8-oxo-dGTP and 2-oxo-dATP, respectively. These complex structures have revealed that hMTH1 recognizes the different oxidized purine nucleotides, 8-oxo-dGTP and 2-oxo-dATP, by the exchange of the protonation site in the neighboring Asp residues. To our knowledge, this is a brand new mechanism for the broad substrate specificity of enzymes. In order to completely establish this mechanism by the protein crystallography, it is essential to identify the protonation states of these two Asp residues by ultrahigh-resolution crystal structures of hMTH1. We have succeeded to obtain crystals which diffract to better than 1.12 Å resolution using the hMTH1 mutant with a homogeneous N-terminus and collect the diffraction data at 1.23 Å resolution. In this structure, two molecules in the asymmetric unit interact with each other through their mutated N-terminal regions, and a crystallization reagent, imidazole, is bound to Trp, Asp and Asn residues which are important for the substrate recognition. The high-resolution structure of hMTH1-imidazole complex has revealed the detailed recognition scheme of imidazole by the stacking interaction with Trp and hydrogen bonding interactions with Asp and Asn. Now the refinements with anisotropic thermal parameters using *SHELX* are in progress.

Keywords: DNA repair enzymes, high-resolution protein structures, substrate binding

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Tertiary structure of the catalytic and chitin-binding domains of hyperthermophilic chitinase

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A chitinase, engineered from the genes PF1233 and PF1234 of *Pyrococcus furiosus*, is a hyperthermophilic glycosidase that effectively hydrolyzes both α and β crystalline chitin. This chitinase contains two catalytic domains (AD1 and AD2) and two chitin-binding domains (ChBD1 and ChBD2). We determined the crystal structures of ChBD2 and AD2 (to the resolution at 1.7 Å and 1.5 Å, respectively), which are important for the activity of the chitinase toward crystalline chitin. The structure of ChBD2 comprised a typical β-sandwich architecture, which consists of two four-stranded β -sheets, and was similar to that of other carbohydrate-binding module 2 family proteins, despite low sequence similarity. The chitin-binding

surface identified by NMR was flat and included a strip of three solvent-exposed Trp residues flanked by acidic residues. The overall structure of the AD2 was a TIM-barrel fold, which is a common fold in family 18 chitinases. The active site of the AD2 was in the groove-like cleft and was open to the solvent due to the lack of an additional small domain, which is observed in other family 18 chitinases.

Keywords: catalysts, enzyme structure, domains

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Successful expression of archaeal STT3/AglB membrane protein in *E.coli* cells

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Oligosaccharyltransferase (OST) is an enzyme that catalyzes the transfer of the oligosaccharide from a lipid donor to the side chain of an Asn residue within a consensus sequence of Asn-X-Thr/Ser, where X can be any amino acid residue except for Pro. Asn-glycosylation is widespread not only in eukaryotes but also in archaea and some eubacteria. OST catalyzes the co-translational transfer of an oligosaccharide to Asn residues in nascent polypeptide chains. The OST enzyme is a membrane-associated multisubunit protein complex in eukaryotes. STT3 is the most conserved subunit in the OST complex in the three domains of life. The STT3 is called AglB for archaea and PglB for bacteria. All STT3/AglB/PglB proteins are characterized by 11-13 transmembrane helices in the N-terminal half of the amino acid sequence and a globular domain in the C-terminal half on the luminal/out side of the membranes. We reported that the OST of *Pyrococcus furiosus*, a thermophilic archaeon, is composed of the STT3 protein alone, and catalyzes the transfer of a heptasaccharide onto peptides in an Asn-X-Thr/Ser-motif-dependent manner[1)]. We determined the crystal structure of the C-terminal soluble domain of *P. furiosus* STT3 (PfSTT3) [1,2)]. Here, we expressed the full-length PfSTT3 in *E. coli*, and found that the membrane fraction of *E. coli* cells had the OST activity. We then optimized *E. coli* strains and culture conditions to minimize the degradation of the full-length PfSTT3, and succeeded to obtain homogenous PfSTT3 after several purification steps, including heat treatment and His-tag affinity chromatography. The PfSTT3 is estimated to be monomeric from the elution volume of gel filtration in the presence of various detergents.

1) EMBO J., **27**, 234-43 (2008)
2) Acta Cryst., **F63**, 798-801(2007)

Keywords: membrane proteins, purification, quaternary association of proteins

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X-ray structure of HIV-1 protease-product peptides complex: Insights into the reaction mechanism

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Inhibitors based on the correct mechanism are likely to provide an alternate set of drugs against AIDS. To investigate the mechanism of HIV-1 protease, we have undertaken crystal structure analyses of HIV-1 protease complexed with substrate oligo-peptides, corresponding in sequence to natural cleavage sites of this enzyme. The crystalline complexes were prepared by soaking hexagonal crystals of unliganded enzyme in solutions of substrate peptides under different conditions of pH. We have earlier reported structure of HIV-1 protease complexed with the oligopeptide HKARVL*nFEAnLS ('*' is the site of cleavage, nF-nitrophenylalanine and nL is nor-Leucine), which was found to be trapped as a tetrahedral intermediate. The present study has been carried out on a complex with the same oligopeptide, but at a pH value of 6.2 as against the value of 2.0 used in the earlier study. X-ray diffraction data to 1.8Å resolution has been collected on the FIP beamline at ESRF. The crystal structure, solved using difference Fourier methods, has been refined to crystallographic *Rw/Rf* values of 21.3% / 25.5%, to 1.8Å resolution. In the refined structure, the substrate peptide is found cleaved, and the product peptides are not yet fully dissociated from the enzyme active site. On the basis of comparison of structures of the present product complex with those of Michaelis complex and tetrahedral intermediate complex, a mechanism is proposed for the action of HIV-1 protease. An important and novel feature of this mechanism is invocation of bifurcated hydrogen bonds as a means to influence proton migration rates during different steps of the cleavage reaction.

Keywords: HIV-1 protease, X-ray crystallography, reaction mechanism

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Crystal structure of *Helicobacter pylori* spermidine synthase suggests a distinct active site

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Spermidine synthase (putrescine aminopropyltransferase, PAPT) catalyzes the transfer of the aminopropyl group from decarboxylated S-adenosylmethionine to putrescine during spermidine biosynthesis. *Helicobacter pylori* PAPT (HpPAPT) has a low sequence identity with other PAPTs and lacks the signature sequence found in other PAPTs. The crystal structure of HpPAPT, determined by multiwavelength anomalous dispersion, revealed an N-terminal β-stranded domain and a C-terminal Rossmann-like domain. Structural comparison with other PAPTs showed that HpPAPT has a unique binding pocket between two domains, numerous non-conserved residues, a less acidic electrostatic surface potential, and a large buried space within the structure. HpPAPT lacks the gatekeeping loop which facilitates substrate binding in other PAPTs. PAPTs are essential for bacterial cell viability; thus, HpPAPT may be a potential antimicrobial drug target for *H. pylori* due to its characteristic PAPT sequence and distinct conformation.

Keywords: *Helicobacter pylori*, spermidine synthase,