catalyzed by different way among species. Higher eukaryotes, such as human, and some archaea use a single enzyme, AIR carboxylase (class II PurE). Other organisms such as plants, yeasts and prokaryotes use two enzymes, N^5- aminoimidazole ribonucleotide (N5-CAIR) synthetase (PurK) and CAIR mutase (class I PurE). N^5-CAIR synthetase converts AIR, ATP and bicarbonate to N5-CAIR, ADP, and Pi. Here, we determined crystal structures of PurK of *Aquifex aeolicus VF5*, *Thermus thermophilus* HB8 and *Sulfolobus tokodaii* strain7. The space group, maximum resolution and *R*-value (free *R*-value) for each structure is as follows: *A. aeolicus* (2Z04), $P1$, 2.35 Å, 23.2% (26.2%), *T. thermophilus*, $P4_1$, 2.51 Å, 23.2% (28.2%) and *S. tokodaii*, $P6_3$, 2.00 Å, 19.4% (22.8%). The structures for *T. thermophilus* and *S. tokdaii* were solved as complex with AMPPNP. We compared these three structures and *E. coli* structure, which has already been determined [1], to each other. These four PurK share similar overall structure, including dimer conformation, and consist of three domains, A, B, and C. However, PurK of *A. aeolicus*, *T. thermophilus* and *S. tokodaii* have extra α-helix and β-sheet in A-domain compared with that of *E. coli* and it is possible that this extra α-helix and β-sheet are responsible for thermostability of these proteins.

[1]Thoden, J.B., Kappock, T.J., Stubbe, J., and Holden H.M. Biochemistry, 38, 15480-15492 (1999)

Keywords: nucleoside metabolism, N5-CAIR synthetase, thermophilic proteins

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Structural titration of two mobile loops in trigonal soybean β-amylase crystal with maltose

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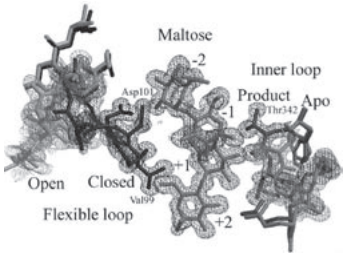
Soybean β-amylase has two mobile loops in the active site, a flexible loop (residue 96-103) and an inner loop (residues 340-346). The flexible loop moves about 11 Å from open to closed form to make interactions with substrate. Though the movement is relatively small (about 3 Å), two different conformations of the inner loop (apo and product forms) have been found. In the trigonal β-amylase crystal, these two loops can move without symmetry interactions. In order to elucidate the relationship between the structural states of these loops and the catalytic mechanism, the structures of the wild and mutant (D101E and D101N) soybean β-amylases were refined in the different maltose concentration (0-200 mM) at 1.0-1.5 Å resolutions with SHELXL. The refined structures of wild enzyme showed that the conformational changes of the flexible and inner loops correspond to the binding of maltose at subsites +1 to +2 and -2 to -1, respectively. It was found that the flexible loop of the inactive mutants moved abnormally without maltose binding to subsites -2 to -1. These results were in good agreement with the solution experiments of the enzyme.



Keywords: beta-amylase, enzymatic structure-activity relationships, enzyme ligand complexes

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The structure of the exo-arabinanase complex with arabinobiose

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Penicillium chrysogenum exo-arabinanase (Abnx) hydrolyzes the α-1,5-L-arabinofuranoside linkage of arabinan which is widely distributed in plant walls. The crystal structures of three arabinanases have revealed that the enzymes have a common unique fold consisting of five β-sheets, each of which is made up of four antiparallel β-strands. However, Abnx has a completely different primary structure from other arabinanses so far isolated. We have initiated an X-ray structure analysis of Abnx to clarify the three-dimensional structure and molecular mechanisms of the novel enzyme. The recombinant Abnx was expressed in *E.coli*. The purified enzyme was crystallized by 1.8 M MPD as a precipitant using the hanging-drop vapor diffusion method. The crystal of Abnx complexed with arabinobiose was obtained by soaking method and diffracted up to 1.04 Å resolution using synchrotoron radiation at PF. The crystal belong to $P2_12_12_1$ with unit cell parameters of a = 67.0, b = 77.1 and c = 79.6 Å. The structure of the complex was solved by the molecular replacement. The enzyme forms six-bladed β-propeller fold. Arabinobiose is located in the cleft formed across one face of the propeller. The center of the cleft is surrounded by three acidic residues, Glu42, Glu152 and Glu224, which are estimated to be active residues of Abnx.

Keywords: exo-arabinanase, complex, arabinobiose

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Crystallization and preliminary X-ray analysis of D-arabinose isomerase from *Bacillus pallidus*

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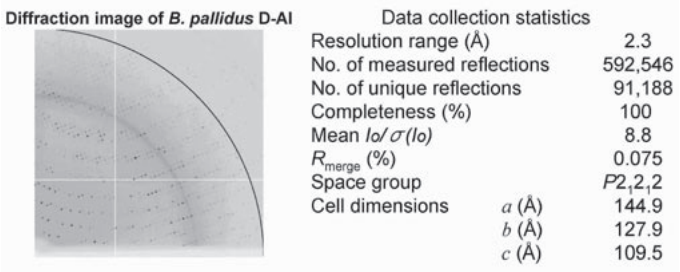
Rare sugars are referred to as sugars which exist in an extremely scanty amount in nature and they have significance not only in food industries but also pharmaceutical industries. *Bacillus pallidus* D-arabinose isomerase (B. pallidus D-AI) can catalyze the isomerization between rare sugars, D-arabinose and D-ribulose. B. pallidus D-AI has a broad substrate specificity and it can also catalyze various sugar conversions. Therefore, it is very useful for the production of rare sugars from natural sugars. Recombinant *B. pallidus* D-AI was successfully overexpressed using Escherichia coli and purified. Crystals of B. pallidus D-AI were grown by the vapor diffusion method using a protein solution (10 mg/ml *B. pallidus* D-AI in 10 mM HEPES (pH 8.0)) and a reservoir solution (20% (w/v) polyethylene glycol 3,000, 100 mM citrate buffer (pH 6.0), 1 M potassium sodium tartrate). X-ray diffraction data were collected on the BL-5A beam line in the Photon Factory (Tsukuba, Japan) at a resolution of 2.3 Å. The initial phases were successfully determined by molecular replacement method using the structure of E. coli L-fucose isomerase (PDB code: 1FUI).



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