aureus and shown that it differs from that described for E. coli (4,5). Moreover, we are also analysing Dsb systems in bacteria containing an extended array of Dsb proteins and results from this work also suggest divergent redox mechanisms. This research is not only providing a comprehensive picture of the process of oxidative protein folding in vivo, but also, given the role of Dsb proteins in the pathogenicity of microbes, the investigated proteins represent putative targets for the development of antimicrobials with a novel mechanism of action.

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Keywords: enzyme structure determination, protein crystallography, bacterial pathogenesis

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Crystal structure of Dxp reductoisomerase from *Geobacillus stearothermophilus*

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Isopentenyl diphosphate (IPP) is an essential compound for living organisms as a precursor of isoprenoids, such as hormones, cholesterols and carotenoids. Mammals use the mevalonic acid pathway, on the other hand, many eubacteria, plastid and malaria parasites use the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway. Inhibitors of the MEP pathway, therefore, are considered as effective antibacterial, antimalarial drugs and herbicides, which are harmless to human. In this pathway, we have focused on 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), which is responsible for the second step in the pathway. To characterize this enzyme, several DXR structures from *Escherichia coli*, *Zymomonas mobilis* and *Mycobacterium tubercrosis* have been reported with or without cofactors/substrate/inhibitors. The primary sequences of DXRs from those bacteria are highly homologous, however, based on the comparison of those crystal structures, we could observe the differences in the binding manner of inhibitors in the active sites. In order to analyze the inhibition mechanism further, we have started to study the DXR structure from *Geobacillus stearothermophilus*. We have successfully obtained crystals by the hanging drop vapor diffusion method with NADPH and Mg^{2+} for cocrystallization. We originally tried to solve the structure by the molecular replacement method without success, thus we performed the Se-SAD method to determine the phase using the program SHARP. The structure of GsDXR was refined at 1.9 Å resolution. The overall structure of GsDXR shows no significant differences with those of other DXRs. The electron density of the flexible loop region covering the active site was not observed clearly, and we are trying to obtain the complex structure with the inhibitor.

Keywords: MEP pathway, antimalarial drug, SAD

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Crystal structure of the thermostable mutant of hygromycin phosphotransferase from *Escherichia coli*

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Aminoglycoside antibiotics, such as hygromycin, kanamycin, neomycin, spectinomycin, and streptomycin, inhibit protein synthesis by acting on bacterial and eukaryotic ribosomes. These antibiotics are widely used for selection of transformants in molecular biology with the combination of the corresponding resistant genes. These selection markers, however, had been available at normal temperature except one for kanamycin/neomycin. We have recently obtained the thermostable mutant of hygromycin B phosphotransferase (Hph) (EC 2.7.1.119) from *Escherichia coli* by the directed evolution method. This mutant (Hph5) increased its thermostablity at 16 ℃ compared to the wild type and can be used as a selection marker for *Thermus thermophilus*. Hph from *E. coli* converts hygromycin B to 7 "-*O*-phosphohygromycin using the phosphate moiety from ATP, resulting in the loss of its cell-killing activity. In order to analyze the mechanism of its catalytic activity and thermostablity, we have crystallized the Hph5 protein for the first time by the hanging-drop vapour diffusion method. The crystal provides diffraction data to a resolution of 2.1 Å and belongs to space group $P3_221$ with unit-cell parameters $a = b = 71.0$, $c = 125.0$ Å. We also obtained the crystal complexes of Hph with hygromycin B and AMP-PNP or ADP in the same crystal form as that of the apoprotein. The structure was composed of N-terminal β-sheet domain and C-terminal α-helix domain, which is similar to that of protein kinases. Base on the comparison of apo and holo structures, Hph does not seem to show a conformational change according to the substrate binding or modification, which is typical in case of protein kinases.

Keywords: aminoglycoside antibiotics, kinase, thermostability

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Crystal structures of N^5-CAIR synthetase (PurK) from *A. aeolicus*, *T. thermophilus* and *S. tokodaii*

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The 6th reaction in the purine nucleotide biosynthetic pathway is the conversion from 5-aminoimidazole ribonucleotide (AIR) to 4-carboxy-5-aminoimidazole ribonucleotide (CAIR). This reaction is

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.

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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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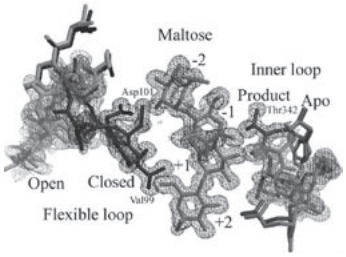
Soybean β-amylase has two mobile loops in the active site, a flexible loop (residue 96-103) and an inner loop (residues 340-346). The flexible loop moves about 11 Å from open to closed form to make interactions with substrate. Though the movement is relatively small (about 3 Å), two different conformations 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).