auraeus 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.


Keywords: enzyme structure determination, protein crystallography, bacterial pathogenesis

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

Shunsuke Yajima1, Daisuke Iino1, Yasuyuki Sasaki1, Ryota Kawakami1, Takayuki Hoshino2, Kanju Ohswawa1, Akira Nakamura2

1Tokyo University of Agriculture, Department of Bioscience, Sakuragaoka 1-1-1, Setagaya-ku, Tokyo, 156-8502, Japan, 2Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan, E-mail: yshun@nodai.ac.jp

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 thermostability at 16 °C 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 thermostability, 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 P3121 with unit-cell parameters *a* = *b* = 71.0 Å, *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

**P04.02.111**


**Crystal structures of N4-CAIR synthetase (PurK) from A. aeolicus, T. thermophilus and S. tokodaii**

Hiroyuki Taka1, Satoko Tamura2, Satoshi Tsunoda1, Kiyoshi Okada1, Seiki Baba3, Mayumi Kanagawa1, Mihoko Manzoku1, Yukiko Utsumiya1, Masami Nishida1, Noriko Nakagawa3, Akio Ebihara1, Seiki Kuramitsu2, Gota Kawai2, Gen-ichi Sanpei3

1The University of Electro-Communications, Applied Physics and Chemistry, 1-5-1 Chofugaoka, Chofu-shi, Tokyo, 182-8585, Japan, 2Faculty of Engineering, Chiba Institute of Technology, 2-17-1 Tsdanuma, Narashino-shi, Chiba, 275-0016, Japan, 3Riken SPRing-8 Center, Harima Institute, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5148, Japan, 4Japan Synchrotron Radiation Research Institute, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5198, Japan, 5Graduate School of Science Osaka University, 1-1 Yamadaoka, Suita, Osaka, 565-0871, Japan, E-mail: give_t_up@penguin.pc.uec.ac.jp

The 6th reaction in the purine nucleotide biosynthetic pathway is the conversion from 5-aminomimidazole ribonucleotide (AIR) to 4-carboxy-5-aminomimidazole 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, \(N^+\)-aminimidazole ribonucleotide (N5-CAIR) synthetase (PurK) and CAIR mutase (class I PurE). \(N^+\)-CAIR synthetase converts AIR, ATP and bicarbonate to N5-CAIR, ADP, and Pi. Here, we determined crystal structures of PurK of \textit{Aquifex aeolicus} VF5, \textit{Thermus thermophilus} HB8 and \textit{Sulfobolus tokodaii} strain7. The space group, maximum resolution and R-value (free R-value) for each structure is as follows: \textit{A. aeolicus} (2204), P1, 2.35 Å, 23.2% (26.2%), \textit{T. thermophilus} P4\(_1\), 2.51 Å, 23.2% (28.2%) and \textit{S. tokodaii}, \textit{P6}(_3)\(_2\), 2.00 Å, 19.4% (22.8%). The structures for \textit{T. thermophilus} and \textit{S. tokodaii} were solved as complex with AMPNP. We compared these three structures and \textit{E. coli} structure, which has already been determined [1], to each other. These PurK share similar overall structure, including dimer conformation, and consist of three domains, A, B, and C. However, PurK of \textit{A. aeolicus}, \textit{T. thermophilus} and \textit{S. tokodaii} have extra \(\alpha\)-helix and \(\beta\)-sheet in A-domain compared with that of \textit{E. coli} and it is possible that this extra \(\alpha\)-helix and \(\beta\)-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 \(\beta\)-amylase crystal with maltose**

\textbf{Bunzo Mikami, Aiko Tanabe, Youna Kang, Shigeru Utsumi}

Graduate School of Agriculture, Kyoto University, Division of Applied Life Science, Gokasho, Uji, Kyoto, 611-0011, Japan, E-mail: mikami@kais.kyoto-u.ac.jp

Soybean \(\beta\)-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 \(\beta\)-amyolase 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 \(\beta\)-amyolases 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 arabinobiase**

\textbf{Yuri Sogobe, Takayoshi Kinoshita, Asako Yamaguchi, Tatsui Sakamoto, Yoshiji Tada}

Osaka Prefecture University, Graduate School of Science, 1-1 Gakuuencho, Naka-ku, Sakai, Osaka, 599-8531, Japan, E-mail: sogabe06@b.s.osakafu-u.ac.jp

\textit{Penicillium chrysogenum} exo-arabinanase (Abnx) hydrolyzes the \(\alpha\)-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 \(\beta\)-sheets, each of which is made up of four antiparallel \(\beta\)-strands. However, Abnx has a completely different primary structure from other arabinanases 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 \textit{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 arabinobiase was obtained by soaking method and diffracted up to 1.04 Å resolution using synchrotron radiation at PF. The crystal belong to \(P2_12_12_1\) with unit cell parameters of \(a = 67.0, b = 77.1, c = 79.6\) Å. The structure of the complex was solved by the molecular replacement. The enzyme forms six-bladed \(\beta\)-propeller fold. Arabinobiase is located in the cleft formed across one face of the propeller. The center of the cleft is surrounded by three acyclic residues, Glu42, Glu152 and Glu224, which are estimated to be active residues of Abnx.

Keywords: exo-arabinanase, complex, arabinobiase

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**Crystallization and preliminary X-ray analysis of D-arabinose isomerase from \textit{Bacillus pallidus}**

\textbf{Kosei Takeda}\(^1,2\), Hiromi Yoshida\(^1\), Goro Takada\(^2\), Ken Izumori\(^2\), Shigehiro Kamitori\(^1\)

\(^1\)Kagawa University, Life Science Research Center, 1750-1, Ikoube, Miki-cho, Kita-gun, Kagawa, 761-0793, Japan, \(^2\)Kagawa University, Rare Sugar Research Center, 2393, Ikoube, Miki-cho, Kita-gun, Kagawa 761-0795, Japan, E-mail: k_hornet0711@hotmail.com

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. \textit{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 \textit{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 \textit{E. coli} L-fucose isomerase (PDB code: 1FU1).