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Crystal structures of GAR synthetase (PurD) from *A. aeolicus*, *G. kaustophilus* and *T. thermophilus*

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Glycinamide ribonucleotide (GAR) synthetase (PurD) catalyzes the second step of the purine nucleotide biosynthetic pathway. GAR synthetase converts phosphoribosylamine, glycine, Mg^{2+} and ATP to GAR, ADP and P_i. PurD consists of four domains, N, A, B and C, and the crystal structure of PurD from *E. coli* has already determined and it was suggested that the structure of PurD is similar to those of D-alanine: D-alanine ligase, biotin carboxylase, and glutathione synthetase, despite low sequence similarity [1]. In the present study, we determined crystal structures of PurD in apo form from *Aquifex aeolicus* VF5 (PDBID 2YYA, space group $P2_1$, max res. = 2.4 Å, R = 22.5%, (free R = 24.9%)), *Geobacillus kaustophilus* HTA426 (2YS7, $P2_12_12_1$, 2.2 Å, 20.8%, (25.1%)) and *Thermus thermophilus* HB8 (2IP4, $P2_12_12_1$, 2.8 Å, 21.9%, (23.8%)). Moreover, we also determined a structure of GAR synthetase from *A. Aeolicus* in complex with ATP (2YW2, $P1$, 1.8 Å, 20.3%, (22.7%)). Overall structures were found to be similar to each other. However, orientation of B domain was slightly different among the structures in apo form. In fact, B-factor values of the B domain are higher than those of other domains, especially for *A. Aeolicus*. ATP was inserted between A and B domains, and B-factor values of B domain in ATP complex were lower than those in apo form. The X-ray diffraction data of *A. aeolicus* and *G. kaustophilus* proteins were efficiently collected at BL26B1, BL26B2 of SPring-8 using sample auto-changer SPACE [2, 3]. We also report this automated data collection system.

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Keywords: nucleoside metabolism, GAR synthetase, automated data collection

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Structural genomics on the purine nucleotides biosynthetic pathway

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The purine nucleotide biosynthesis proceeds by a 14-step branched pathway (Fig. 1). This pathway is common to most organisms, and all reactions are concerned with the formation of C-N bond. And moreover we can observe some similar reactions in this pathway. Thus, it is important to compare the structure and reaction mechanisms to each other by determining of 3D structure of the enzymes, when the genesis of this pathway is considered. We have determined 22 structures of the enzymes in this pathway from several thermophilic bacteria including *T. thermophilus* HB8, *A. aeolicus* VF5, *G. kaustophilus* HTA426, *Symbiobacterium toebii*, *Methanococcus jannaschii*, and *S. tokdaii* strain7; PurD, PurN, PurS, PurM, PurK, PurE, PurC and GuaA. In addition to the structure determination, we started biochemical analysis as well as molecular dynamics calculations of several enzymes in this pathway. Furthermore, for T. thermophilus, transcriptome analysis using tiling array are also undergoing to elucidate the mechanism of expression regulation of purine operons. Our strategy and recent results for understanding the dynamic status in whole this pathway will be discussed.

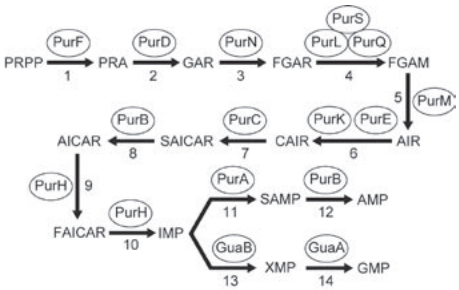

Fig.1 Purine nucleotide biosynthetic pathway

Keywords: nucleoside metabolism, structural genomics, thermophilic proteins

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Redox catalysis and protein folding in bacterial viruence

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The formation of disulfide bonds between cysteines is a key step in the biogenesis of many bacterial virulence factors such as fimbriae and toxins. To produce these virulence determinants, pathogens rely on protein-folding enzymes like Dsb proteins, which catalyse disulfide formation in bacteria. The importance of Dsb proteins in virulence is exemplified by the fact that mutation of dsb genes causes the same phenotypic effect as mutations in the genes encoding the virulence determinants themselves (1). The mechanisms for disulfide catalysis have been described for E. coli, where Dsb proteins form an oxidative (DsbA-DsbB) and an isomerase (DsbC(G)-DsbD) pathway (2,3). However, this mechanism is not conserved and Dsb proteins can vary from one organism to another. Our research focuses on investigating Dsb systems from different pathogens. Through structural and functional studies we have recently described the oxidative folding machinery in the human pathogen Staphylococcus

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