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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.

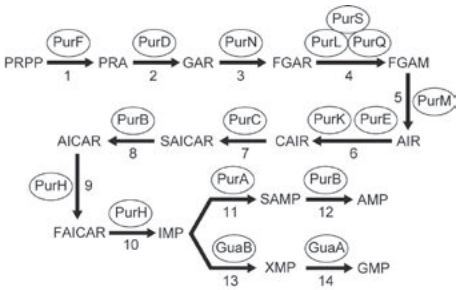

Fig.1 Purine nucleotide biosynthetic pathway

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