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Crystal Structure of the ATPPase Subunit and Its Substrate-Dependent Association with the GATase Subunit: A Novel Regulatory Mechanism for a Two-Subunit-Type GMP Synthetase from *Pyrococcus horikoshii* OT3

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Guanosine 5′-monophosphate synthetase(s) (GMPS) catalyzes the final step of the *de novo* synthetic pathway of purine nucleotides. GMPS consists of two functional units that are present as domains or subunits: glutamine amidotransferase (GATase) and ATP pyrophosphatase (ATPPase). GATase hydrolyzes glutamine to yield glutamate and ammonia, while ATPPase utilizes ammonia to convert adenyl xanthosine 5′-monophosphate (adenyl-XMP) into guanosine 5′-monophosphate. Here we report the crystal structure of PH-ATPPase (the ATPPase subunit of the two-subunit-type GMPS from the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3). PH-ATPPase consists of two domains (N-domain and C-domain) and exists as a homodimer in the crystal and in solution. The N-domain contains an ATP-binding platform called P-loop, whereas the C-domain contains the xanthosine 5′-monophosphate (XMP)-binding site and also contributes to homodimerization. We have also demonstrated that PH-GATase (the glutamine amidotransferase subunit of the two-subunit-type GMPS from the hyperthermophilic archaeon *P. horikoshii* OT3) alone is inactive, and that all substrates of PH-ATPPase except for ammonia (Mg^{2+}, ATP and XMP) are required to stabilize the active complex of PH-ATPPase and PH-GATase subunits.

Keywords: ATP pyrophosphatase; crystal structure; glutamine amidotransferase; GMPS; purine nucleotide biosynthesis

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Introduction

Guanosine 5′-monophosphate synthetase(s) (GMPS) is a widespread enzyme seen in all domains of life. GMPS is required for the final step of the *de novo* synthesis of guanine nucleotides, converting xanthosine 5′-monophosphate (XMP) into guanosine 5′-monophosphate (GMP), a precursor of DNA and RNA. GMPS is also involved in chromatin regulation[1] and axon guidance,[2] as shown in *Drosophila*. In addition, GMPS has been studied as an important chemotherapeutic target for immunosuppressive agents.[3]

GMPS belongs to the class I glutamine amidotransferase (GATase) family and consists of two catalytic units, GATase and ATP pyrophosphatase (ATPPase):

$$\text{GATase}: \text{L-glutamine} + H_2O \rightarrow \text{L-glutamate} + NH_3 \quad (1)$$

$$\text{ATPPase}: \text{XMP} + \text{Mg-ATP} \rightarrow \text{adenyl-XMP intermediate} + \text{pyrophosphate} + Mg^{2+} \quad (2)$$

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Abbreviations used: GMPS, guanosine 5′-monophosphate synthetase(s); GATase, glutamine amidotransferase; ATPPase, ATP pyrophosphatase; XMP, xanthosine 5′-monophosphate; GMP, guanosine 5′-monophosphate; PDB, Protein Data Bank; GDH, glutamate dehydrogenase; EDTA, ethylenediaminetetraacetic acid; adenyl-XMP, adenyl xanthosine 5′-monophosphate.

$$\text{adenyl-XMP intermediate} + NH_3 \rightarrow GMP + AMP \quad (3)$$

GATase hydrolyzes glutamine to generate glutamate and ammonia (Reaction (1)), and ATPPase adenylates XMP to form an adenyl xanthosine 5′-monophosphate (adenyl-XMP) intermediate in the presence of Mg^{2+}, ATP, and XMP (Reaction (2)). The highly reactive adenyl-XMP intermediate is then aminated by ammonia provided by GATase (Reaction (3)).[4–6] Overall, GMP is generated by the concerted reactions of these two catalytic units.[7,8]

The two catalytic units are either encoded by a single gene (two-domain type) in eukaryotes, bacteria, and some archaea, or encoded by two separate genes (two-subunit type) in other archaea. In two-domain-type GMPS, the GATase domain is located in the N-terminal half, and the ATPPase domain is located in the C-terminal half; in two-subunit-type GMPS, these two units exist as separate polypeptides (Fig. 1a). Almost all structural and functional studies on GMPS have been carried out for two-domain-type GMPS from humans,[11–13] rats,[14] mice,[15] *Escherichia coli*,[6,16–19] and *Plasmodium falciparum*.[20] So far, the crystal structures of three two-domain-type GMPS—EC-GMPS (the two-domain-type GMPS from *E. coli*) [Protein Data Bank (PDB) ID 1GPM],[21] TT-GMPS (the two-domain-type GMPS from *Thermus thermophilus*) (PDB IDs 2YWB and 2YWC; Baba *et al.*, unpublished results), and HS-GMPS (the two-domain-type GMPS from *Homo sapiens*) (PDB ID 2VXO; Welin *et al.*, unpublished results)—and two two-subunit-type GMPS—the GATase subunit from *Thermoplasma acidophilum* (PDB ID 2A9V; Joint Center for Structural Genomics, unpublished results) and *Pyrococcus horikoshii* (PDB ID 1WL8; Lokanath and Kunishima, unpublished results) (PDB ID 2D7J; Maruoka *et al.*[22]), and the ATPPase subunit from *P. horikoshii* (PDB ID 2DPL; Asada and Kunishima, unpublished results) (PDB ID 3A4I; this study)—have been deposited in the PDB. In the crystal structures of two-domain-type GMPS, the two active sites of the GATase and ATPPase domains are exposed to the solvent, and it has not been revealed yet how the putative ammonia channel, which efficiently couples the GATase and ATPPase reactions, is formed.[21]

To gain insights into the catalytic and regulatory mechanisms of GMPS, particularly two-subunit-type GMPS, we have determined the crystal structures of the GATase subunit [PH-GATase (the glutamine amidotransferase subunit of the two-subunit-type GMPS from the hyperthermophilic archaeon *P. horikoshii* OT3); PDB ID 2D7J][22] and the ATPPase subunit [PH-ATPPase (the ATPPase subunit of the two-subunit-type GMPS from the hyperthermophilic archaeon *P. horikoshii* OT3); this study] of PH-GMPS (the two-subunit-type GMPS from the hyperthermophilic archaeon *P. horikoshii* OT3) and analyzed the regulatory mechanisms of PH-GMPS. Our crystal structure has revealed that the ATPPase subunit of PH-GMPS (PH-ATPPase) is very similar to the ATPPase domains of EC-GMPS and TT-GMPS in terms of backbone fold, ATP-binding and XMP-binding residues, and dimerization mode, but is dissimilar with respect to the relative orientation of N-domain and C-domain. PH-ATPPase is also similar to the ATPPase domain of HS-GMPS in terms of backbone fold and ligand-binding residues, but is dissimilar in oligomerization mode because HS-GMPS exists as a monomer in solution.[12] We also performed activity assays

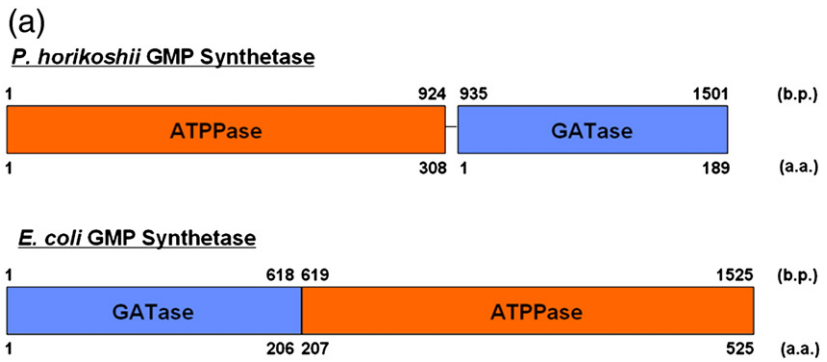

Fig. 1. (a) Gene and protein structure of GMPS from *P. horikoshii* and *E. coli*. *P. horikoshii* GMPS is encoded by two genes, *ph1346* (encoding GATase) and *ph1347* (encoding ATPPase), located 7 bp apart. *E. coli* GMPS is encoded by a single gene *guaA*, which encodes the GATase domain in the N-terminal half and the ATPPase domain in the C-terminal half. (b) Multiple sequence alignment of ATPPase subunits and domains. The proteins are derived from *P. horikoshii*, *T. thermophilus*, and *E. coli*. The secondary structure elements of PH-ATPPase are shown above the alignment. Sequence alignment was obtained with CLUSTAL W.[9] The figure was prepared with ESPript.[10] α-Helices, 3_{10}-helices, and β-strands are denoted as α, η, and β, respectively. Completely and highly conserved residues are boxed and indicated by white letters on a red background and red letters on a white background, respectively. Blue and green triangles indicate the putative binding residues for ATP and XMP, respectively. Red triangles indicate residues forming intermolecular hydrogen bonds in homodimers of PH-ATPPase, EC-GMPS, and TT-GMPS.