BL-5A/2019G015

# Crystal structure of galactose 1-phosphate uridylyltransferase

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## 1 Introduction

In the Leloir pathway of galactose metabolism, galactose is first phosphorylated to galactose 1-phosphate (Gal1P) by galactokinase. The second enzyme, galactose 1-phosphate uridylyltransferase (GalT; EC 2.7.7.12), catalyzes the transfer of the uridylyl group from UDP-glucose (UDP-Glu) to Gal1P to produce UDP-galactose (UDP-Gal) and glucose 1-phosphate (Glu1P). The third enzyme in the pathway, UDP-galactose 4-epimerase (GalE; EC 5.1.3.2), regenerates UDP-Glu from UDP-Gal to maintain the production of Glu1P, which enters the glycolytic pathway. These three enzymes, comprising the Leloir pathway, are widely distributed among both Bacteria and Eukarya, and structural and functional analyses have been reported. With regard to Archaea, the third domain of life, galactokinases from the hyperthermophilic archaea *Pyrococcus furiosus* and *P. horikoshii* have been characterized, and the molecular basis of their substrate recognition has been revealed. However, information about the other two enzymes involved in the Leloir pathway in Archaea remains limited.

Our database search revealed the presence of homologues of all three enzymes among phylogenetically distant archaeal species, including *Pyrobaculum* and *Pyrococcus*. This suggests that the Leloir pathway is functional in hyperthermophilic archaea, and that structural and functional analysis of its constituents could shed light on their features and diversity in these organisms. We previously identified a gene (Pcal_0885) encoding a GalE homologue within the genomic sequence of the hyperthermophilic archaeon *Pyrobaculum calidifontis* and confirmed the gene product to be an extremely thermostable GalE. We also solved the crystal structure of this enzyme and evaluated the structural features responsible for its high thermostability. We next identified a gene (PAE1184) encoding a GalT homologue within the genome of *Pyrobaculum aerophilum* (a species closely related to *P. calidifontis*) and performed the crystallization and preliminary X-ray analysis of the gene product. At that time, we confirmed that the gene product exhibits a high level of GalT activity, but were unable to obtain useful data for structure determination. In the present study, we solved the crystal structure of *P. aerophilum* GalT and found that its active site components are completely different than those observed in the two previously reported GalT structures. Here we present the first crystal structure of a novel type of GalT found in a hyperthermophile in the archaeal domain and report the formation of a unique active site in this enzyme.

## 2 Experiment

Data were collected under cryo conditions at the Beamline BL-5A at Photon Factory in Japan. The program Phaser-MR in the PHENIX was used for molecular replacement phase determination. The crystal structure of GalT from *Escherichia coli* (PDB ID: 1gup, amino acid sequence identity: 30%) was served as the search model. Model building was performed using the program Coot, and refinement was carried out using REFMAC5.

## 3 Results and Discussion

Two different crystal structures of *P. aerophilum* GalT were determined: the substrate-free enzyme at 2.33 Å and the UDP-bound H140F mutant enzyme at 1.78 Å. The main-chain coordinates of the *P. aerophilum* GalT monomer were similar to those in the structures of the *E. coli* and human GalTs, as was the dimeric arrangement. However, there was a striking topological difference between *P. aerophilum* GalT and the other two enzymes. In the *E. coli* and human enzymes, the N-terminal chain extends from one subunit into the other and forms part of the substrate-binding pocket in the neighboring subunit. By contrast, the N-terminal chain in *P. aerophilum* GalT extends to the substrate-binding site in the same subunit (Fig. 1). Amino acid sequence alignment showed that a shorter surface loop in the N-terminal region contributes to the unique topology of *P. aerophilum* GalT.

To determine the structure of the *E. coli* GalT/UDP-Glu complex, a catalytically inactive mutant, H166G, was prepared and cocrystallized with UDP-Glu. His166 in *E. coli* GalT is conserved as His140 in *P. aerophilum* GalT. Therefore, to solve the structure of *P. aerophilum* GalT with bound substrate, we prepared H140G, H140N, and H140F mutants of the enzyme and confirmed these three mutants were all inactive. We next endeavored to cocrystallize the three mutants with UDP-Glu, collect the diffraction data, and determine their structures. In the electron-density map for the structure solved with the H140F/UDP-Glu crystal, the electron density for the UDP

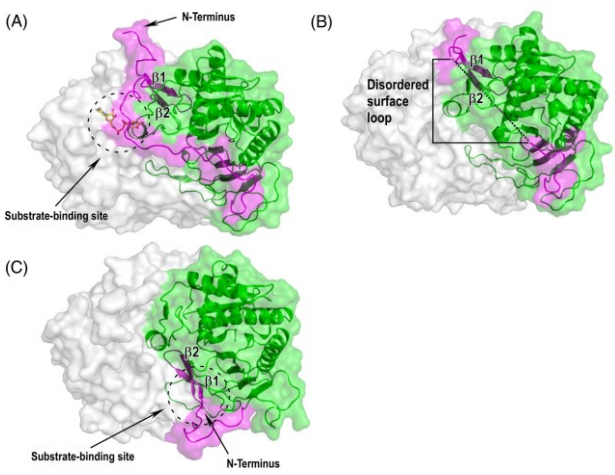


Fig. 1: Ribbon plots of the main-chain coordinates (green) of, A, *E. coli* GalT (PDB code: 1guq); B, human GalT (PDB code: 5in3); and C, *P. aerophilum* GalT (PDB code: 6k5z) monomers.

molecule was observed within the active sites of both subunits. Although the electron density for the α-phosphoryl group was discontinuous, the significant electron densities at the uridyl ribose, β-phosphoryl group, and uridine moiety enable us to deduce a plausible structure for UDP (Fig. 2). The structure was determined at a resolution of 1.78 Å. No density corresponding to the glucose moiety was detected, though the enzyme was cocrystallized with UDP-Glu. Within our model, the uridine moiety of UDP interacts with a backbone N atom of Arg51 (Asp78 in *E. coli* GalT). To accommodate the uridine moiety, the side chain of Arg51 in UDP-bound H140F rotates clockwise by about 80° around the Cβ atom of Arg51 relative to the substrate-free *P. aerophilum* GalT structure. The 2'- and 3'-hydroxyl groups of the uridyl ribose form hydrogen bonds with the side chain of Asn50 (Asn77 in *E. coli* GalT). The β-phosphoryl oxygen interacts with the side chains of Gln142 (Gln168 in *E. coli* GalT) and Tyr144. Nearly all of these interactions are conserved in the *E. coli* GalT/UDP-Glu complex, except for the interaction between the side chain of Tyr144 and the substrate, as this residue is replaced by Trp170 in *E. coli* GalT. In addition, UDP moieties show similar orientation in both the structures except for the situation of α- and β-phosphoryl groups. The N-terminal loop, including Arg19 and Arg22, is disordered in both subunits of the H140F/UDP structure. It is noteworthy that other conserved active site residues in H140F/ UDP are situated at positions similar to those in the substrate-free *P. aerophilum* GalT structure (rmsds [average] of 0.33 for the Cα atoms of Asn50, Asn127, Gly133, Ser135, Gln142, Lys280*, and Glu286*)(an asterisk indicates a residue in the neighboring subunit). This suggests that the binding of the glucose moiety of the substrate, but not the UDP moiety, gives rise to a large structural change around the active site, which in turn provides an appropriate environment for the enzyme reaction.

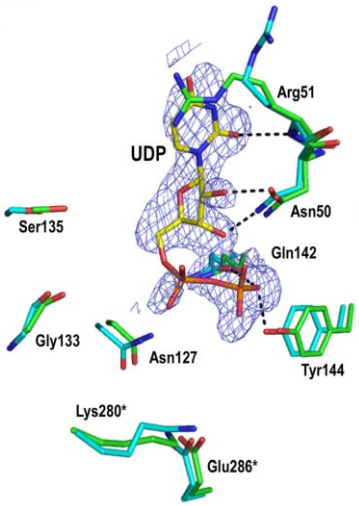


Fig. 2: Comparison of the UDP-binding site structures in substrate-free *P. aerophilum* GalT (green) and the UDP-bound H140F mutant (cyan). The UDP molecule in H140F is drawn in yellow.

Acknowledgement

We are grateful to the staff of the Photon Factory for their assistance with data collection, which was approved by the Photon Factory Program Advisory Committee.

Reference

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Research Achievements

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