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X-ray Crystallography of *N*-Acetylglucosamine-phosphate Mutase, a Member of the α-D-Phosphohexomutase Superfamily

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Introduction

N-Acetylglucosamine-phosphate mutase (AGM1) is an essential enzyme in the synthetic process of UDP-*N*-acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc is a UDP sugar that serves as a biosynthetic precursor of glycoproteins, mucopolysaccharides, and the cell wall of bacteria. Thus, a specific inhibitor of AGM1 from pathogenetic fungi could be a new candidate for an antifungal reagent that inhibits cell wall synthesis. AGM1 catalyzes the conversion of *N*-acetylglucosamine 6-phosphate (GlcNAc-6-P) into *N*-acetylglucosamine 1-phosphate (GlcNAc-1-P). This enzyme is a member of the α-D-phosphohexomutase superfamily, which catalyzes the intramolecular phosphoryl transfer of sugar substrates. We have determined the crystal structures of AGM1 from *Candida albicans* (CaAGM1) for the first time, both in the apo-form and in the complex forms with the substrate and the product, and discuss its catalytic mechanism.

Materials and Methods

Crystals were obtained within a week using a reservoir solution (pH 4.6) containing 200 mM $NH_4H_2PO_4$ and 14-20% (*w*/*v*) polyethylene glycol 3,350 [1]. Pt derivatives were prepared by soaking in the cryoprotectant with 10 mM K_2PtCl_4 for 7 min. Hg derivatives were prepared by soaking in the cryoprotectant with 50% saturated *p*-chloromercury benzoate for 1 day. The substrate and product complexes were prepared by soaking in the cryoprotectant with each ligand (25 mM) and 5 mM $ZnCl_2$ for 1 min. All crystals were cryocooled in an N_2 gas stream at 95 K. Diffraction data were collected using synchrotron radiation at the Photon Factory (BL5 with an ADSC Quantum 315 CCD detector and BL6A with an ADSC Quantum 4R CCD detector).

Results

The crystals belong to the primitive monoclinic space group $P2_1$, with unit-cell parameters of a = 60.2 Å, b = 130.2 Å, c = 78.0 Å and β = 106.7°. The processed dataset has shown an overall R_{merge} of 4.1% and a completeness of 94.9% at 1.93 Å resolution. The model was constructed using the program *O*. The model was refined to crystallographic *R*-factor of 19.2 % at 1.93 Å resolution using the program *CNS*.

The structure of CaAGM1 consists of four domains, of which three domains have essentially the same fold (Figure) [2]. The overall structure is similar to those of phosphohexomutases, but there are two additional β-strands in Domain 4 and a circular permutation occurs in Domain 1. The catalytic cleft is formed by four loops from each domain. The *N*-acetyl group of the substrate is recognized by Val-370 and Asn-389 in Domain 3, from which the substrate specificity arises. By comparing the substrate and product complexes, it is suggested that the substrate rotates about 180° on the axis linking C4 and the midpoint of the C5-O5 bond in the reaction.

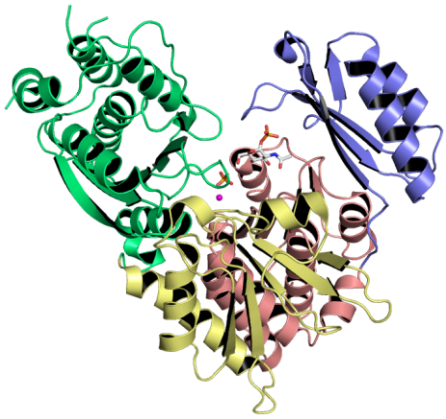

Figure. The overall structure of CaAGM1.

References

[1] Y. Nishitani, D. Maruyama, T. Nonaka, A. Kita, T. A. Fukami, T. Mio, H. Yamada-Okabe, T. Yamada-Okabe, and K. Miki, Acta Crystallogr. F62, 419 (2006).
[2] Y. Nishitani, D. Maruyama, T. Nonaka, A. Kita, T. A. Fukami, T. Mio, H. Yamada-Okabe, T. Yamada-Okabe, and K. Miki, J. Biol. Chem. 281, 19740 (2006).

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