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X-ray Crystallography of Uridine-diphospho-*N*-acetylglucosamine Pyrophosphorylase from *Candida albicans* and Catalytic Reaction Mechanism

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Introduction

Uridine-diphospho-*N*-acetylglucosamine (UDP-GlcNAc) is a precursor of the bacterial and fungal cell wall. It is also used in a component of *N*-linked glycosylation and the glycosylphosphoinositol anchor of eukaryotic proteins. It is synthesized from *N*-acetylglucosamine-1-phosphate (GlcNAc-1-P) and uridine-5'-triphosphate (UTP) by UDP-GlcNAc pyrophosphorylase (UAP). This is an S_N2 reaction; the non-esterified oxygen atom of the GlcNAc-1-P phosphate group attacks the α-phosphate group of UTP. We have determined crystal structures of UAP from *Candida albicans* (CaUAP1) without any ligands and also complexed with its substrate or with its product. The series of structures in different forms shows the induced fit movements of CaUAP1.

Materials and Methods

10 mg/ml CaUAP1 with its ligands was prepared in 50 mM Tris-HCl (pH 7.5) and 1 mM DTT, and co-crystallized under the condition of 100 mM sodium citrate (pH 5.5-6.0), 20-30% (*w*/*v*) polyethylene glycol 6000, 80-120 mM ammonium sulfate, and 5-15% (*v*/*v*) glycerol [1]. Their crystals grew to 0.05 x 0.05 x 0.3-5 mm (rod shape) or 0.05 x 0.2 x 0.3 mm (plate shape) at 20 °C within a week.

Diffraction data were collected at beamlines at the Photon Factory, Tsukuba, Japan. All datasets were collected at 95 K from flash-frozen crystals. The crystallization precipitant was used as a cryoprotectant. All images were indexed and integrated, and the dataset was phased with molecular replacement using the structure of human AGX2 (PDB 1JVD) as a search model.

Results

CaUAP1 consists of three domains, N-terminal, central, and C-terminal domains (Figure) [2]. The N-terminal domain is unique to the eukaryotic UAP. This domain consists of three α-helices in the N terminus and two anti-parallel β-sheets inserted to the central domain. The central domain is the catalytic domain of UAP and is referred to as the pyrophosphorylation domain, adopting an α/β-fold resembling the Rossmann fold, which consists of eight strands sandwiched by eight helices. The C-terminal domain consists of short β-strands and a long α-helix connected to the central domain.

Three loops approaching the ligand molecule close the active site when ligand is bound. In addition, Lys-421, instead of the metal ion in prokaryotic UAPs, is coordinated by both phosphate groups of UDP-Glc-NAc and acts as a cofactor. However, a magnesium ion enhances the enzymatic activity of CaUAP1, and thus we propose that the magnesium ion increases the affinity between UTP and the enzyme by coordinating to the α- and γ-phosphate group of UTP.

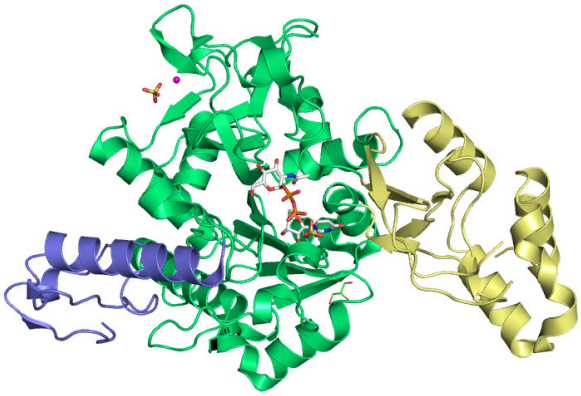

Figure The overall structure of CaUAP1.

References

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