Crystal Structure of Uridine-diphospho-N-acetylglucosamine Pyrophosphorylase from Candida albicans and Catalytic Reaction Mechanism*

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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 SN2 reaction; the non-esterified oxygen atom of the GlcNAc-1-P phosphate group attacks the α-phosphate group of UTP. We 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. 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-GlcNAc 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.

Uridine-diphospho-N-acetylglucosamine (UDP-GlcNAc) is a ubiquitous and essential metabolite in cellular processes. It is used as a precursor of chitin, lipopolysaccharide, peptidoglycan, proteoglycan, and glycoprotein. Lipopolysaccharide and peptidoglycan are essential for bacterial cell wall biosynthesis, and chitin is necessary for fungal cell wall biosynthesis (1–4). UDP-GlcNAc is also used as the N-acetylglucosamine moiety of N-linked glycosylation and the glycosylphosphoinositol anchor of eukaryotic proteins (5). As shown in Fig. 1A, UDP-GlcNAc is synthesized from fructose-6-phosphate via glucosamine-6-phosphate, glucosamine-1-phosphate, and N-acetylglucosamine-1-phosphate (GlcNAc-1-P)2 in prokaryotes, whereas it is synthesized via glucosamine-6-phosphate, N-acetylglucosamine-6-phosphate, and GlcNAc-1-P in eukaryotes (6–9).

UDP-GlcNAC pyrophosphorylase (UAP) is a key enzyme of the UDP-GlcNAc biosynthesis that synthesizes UDP-GlcNAc from uridine-5′-triphosphate (UTP) and GlcNAc-1-P in the cell cytoplasm. UAP catalyzes the following reversible reaction, GlcNAc-1-P + UTP ↔ UDP-GlcNAc + PP. The reaction proceeds as an S2,2 reaction (Fig. 1B) (10, 11). The non-esterified oxygen of the phosphate group of GlcNAc-1-P attacks the α-phosphate of UTP, and the β- and γ-phosphate groups are released as a pyrophosphate. The enzyme generally requires divalent cations such as magnesium ions (12).

UAP is essential for UDP-GlcNAc biosynthesis in both prokaryotes and eukaryotes. Prokaryotic and eukaryotic UAPs are not similar in the amino acid sequence, however, except for a “nucleotide binding motif” (L/M)_2GT_6PK (6, 13). UAP from prokaryotes, referred to as GlmU, is coded on glmU, and it is a bifunctional enzyme. The enzyme catalyzes glucosamine-1-phosphate, in addition to converting GlcNAc-1-P to UDP-GlcNAc (Fig. 1A). The crystal structures of GlmU have been determined from Escherichia coli (EcGlmU) (14, 15) and Streptococcus pneumoniae (SpGlmU) (11, 16). The crystal structure consists of two domains, i.e. the pyrophosphorylation and acetylation domains. In contrast to GlmU, the prokaryotic UAP enzyme, eukaryotic UAP enzymes do not catalyze the acetylation of glucosamine-1-phosphate. Eukaryotic UAPs are divided into two groups, mammalian UAPs and non-mammalian UAPs. The crystal structures of mammalian UAPs have been determined in humans (17) and Mus musculus (Protein Data Bank (PDB) 1VM8). These UAPs consist of three domains, a pyrophosphorylation domain and two other domains whose functions are unknown. Foldings of the pyrophosphorylation domain and two other domains whose functions are unknown.

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The atomic coordinates and structure factors (code 2YQC, 2YQH, 2YQJ, and 2YQS) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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2The abbreviations used are: GlcNAc-1-P, N-acetylglucosamine-1-phosphate; UAP, UDP-GlcNAC pyrophosphorylase; CaUAP1, UAP from C. albicans; PP, pyrophosphate; GlmU, N-acetylglucosamine-phosphate uridylyltransferase; EcGlmU, GlmU from E. coli; SpGlmU, GlmU from S. pneumoniae; DTT, dithiothreitol; PDB, Protein Data Bank.
tion domain are similar in enzymes of prokaryotes and eukaryotes, and they are also homologous to that of the structures of the SpSA GnT I core (SGC) superfamily (17–20). Members of the superfamily are nucleotide-binding proteins, some of which possess the nucleotide binding motif.

Non-mammalian UAPs have similar amino acid sequences to those of mammalian UAPs, although they lack a loop region, namely the "insertion loop," of more than 10 amino acid residues (Fig. 2). Because all known crystal structures of mammalian UAPs are dimers, the insertion loop region intrudes into the active site of another molecule and contacts the ligand inside (17). These contacts may not be observed in solution, because it is known that this enzyme functions as a monomer (17). In contrast to mammalian UAPs, non-mammalian UAPs are expected to exist as a monomer in their crystal structures because they possess no extra loop. Non-mammalian UAP structures are useful for characterizing the catalytic reaction mechanisms of eukaryotic UAPs, but to date the crystal structures of non-mammalian UAPs have not been determined.

Candida albicans is a fungus responsible for candidiasis (21). UAP from C. albicans, CaUAP1, is a 54.7-kDa protein identified as a homolog of UAP from Saccharomyces cerevisiae (6). The sequence identity between CaUAP1 and human UAP, AGX1, is 40%, and the active site residues that accommodate UDP-GlcNAc are almost identical (Fig. 2). We have determined the crystal structures of CaUAP1 in its ligand-free form and in complex with its substrate or with its product. Here, we discuss each step of the reaction on the basis of the crystal structures of CaUAP1. In the present study, we elucidated the roles played in these structures by metal ions. The present findings were suggestive of a novel catalytic mechanism for eukaryotic UAPs.

EXPERIMENTAL PROCEDURES

Expression, Purification, and Crystallization of CaUAP1—The CalUAPI gene was cloned into a pGEX-2T plasmid (Amersham Biosciences) and was expressed in JM109 cells as a glutathione S-transferase fusion protein (6). Methods of expression, purification, and crystallization were described previously (22). CaUAP1 crystallized in an apo-like form (Complex-1, see Table 1) was purified in a manner that differed slightly from our previously described method. Namely, Superdex 75 16/60 column (Amersham Biosciences) chromatography was applied instead of use of a GSTrap column (Amersham Biosciences) for the final chromatography step; the protein was prepared in gel filtration buffer containing 20 mM Tris-HCl, pH 7.5, 1 mM DTT, 10% (v/v) glycerol, and 0.5–1.0% (w/v) EDTA. CaUAP1 (10 mg/ml) 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. The co-crystallized ligands are summarized in Table 1. Their crystals grew to ~0.05 × 0.05 × 0.3–5 mm (rod shape) or 0.05 × 0.2 × 0.3 mm (plate shape) at 20 °C within a week.

FIGURE 1. UDP-GlcNAc synthesis reaction. A, the reaction pathway. The left and right routes in the middle pathway are those in prokaryotes and eukaryotes, respectively. B, S₉,₂ formation of UDP-GlcNAc. The oxygen atom of GlcNAc-1-P attacks UTP from the back side of the β- and γ-phosphates. UAP catalyzes both forward and reverse reactions.
FIGURE 2. Sequence alignment of eukaryotic UAPs. Conserved and homologous residues are shown in red and orange, respectively. The nucleotide binding motif and the insertion loop region are enclosed in magenta and black boxes, respectively. The residues bound to UDP-GlcNAc are indicated by blue triangles. The sequences were aligned with the program ClustalW (34). Secondary structures were assigned with the program DSSP (35). The figure was drawn with the program ESPript (36).
Data Collection, Structure Solution, and Refinement—Diffraction data were collected at beamlines at the Photon Factory, Tsukuba, Japan and at SPring-8, Hyogo, Japan (Table 2). 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 using the program HKL2000 (23). The dataset of Complex-1 was phased with molecular replacement by the program AMoRe (24) in the CCP4 program package (25) and using the structure of human AGX2 (an isoform of AGX, PDB 1JVD) (17) as a search model. The datasets of Complex-2, -3, and -4 were phased with molecular replacement by using the structure of Complex-1. The structures were constructed with the program O (26) and refined using the program CNS (27). The stereochemistry of all models was analyzed using the program PROCHECK (28) in the CCP4 package. The structures were superimposed on each other using the program LSQKAB (29) in the CCP4 package and the program LSQMAN (30). The figures illustrating these structures were prepared with the program PyMOL (31).
Enzyme Assay—For removing metal ions, CaUAP1 were dialyzed against chelating buffer (50 mM Tris-HCl, pH 7.5, 10 mM EDTA, and 1 mM DTT). The buffer was replaced with the stock buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 1 mM DTT). For using enzyme assay, the buffer was exchanged to 50 mM Tris-HCl, pH 7.5, in order to reduce the EDTA concentration, and the protein was concentrated to 10 μM. The substrates (2 mM GlcNAc-1-P and 2 mM UTP) and the protein (0.25 μM) were mixed with the assay buffer (50 mM Tris-HCl, pH 7.5), and 20 μl of mixture were incubated for 16 h on ice. The amount of the magnesium ion was adjusted by the addition of MgCl₂ (0.025, 0.13, 0.25, 0.50, 1.0, and 2.5 μM) or EDTA (1 mM). Some samples were incubated with 0.25 μM CaCl₂ or 0.25 μM ZnCl₂ to research the effect of the other divalent cation. To detect the product (UDP-GlcNAc), the reaction mixtures were separated by thin layer chromatography. They were loaded on a silica gel 60 F₃₅₄ plate (Fluka), which was developed with mixtures of 80 ml of 1-propanol, 9 ml of acetic acid, 11 ml of 5 M lithium chloride solution, and 80 ml of H₂O. The separated compounds on the plate, UDP-GlcNAc and UTP, were detected with UV light. The enzymatic activity was measured by the amount of produced UDP-GlcNAc and remaining UTP, which was estimated with the spot size.

RESULTS

Overall Structure of CaUAP1—We have determined CaUAP1 structures in four different forms, which we designated as apo-like (Complex-1), substrate binding (Complex-2), reaction-completed (Complex-3), and product binding (Complex-4) forms (Table 1). They were refined at 1.9, 2.3, 2.3, and 2.3 Å resolution, respectively. Other crystallographic statistics are summarized in Table 2. Overall structure of CaUAP1 is shown in Fig. 3A. All crystal structures of CaUAP1 do not form dimers like AGX1 and AGX2, and thus the crystal structures appear to reflect the active monomeric structure of this enzyme in solution.
Crystal Structure and Catalytic Mechanism of CaUAP1

![Diagram of CaUAP1](image)

**FIGURE 4. The binding network at the active site.** The same color codes were applied as those used in Fig. 3; the N-terminal, central (pyrophosphorylation), and C-terminal domains and the nucleotide binding motif are shown in orange, green, blue, and magenta, respectively. A, GlcNAc-1-P in the substrate binding form. B, the uridine group of UDP-GlcNAc in the reaction-completed form. C, the phosphate groups of UDP-GlcNAc in the reaction-completed form. D, the phosphate groups of UDP-GlcNAc in the product binding form. E, SO$_4^{2-}$-1 in the reaction-completed form. F, SO$_4^{2-}$-2 in the reaction-completed form.

CaUAP1 consists of three domains, N-terminal, central, and C-terminal domains (Fig. 3A). The N-terminal domain is unique to the eukaryotic UAP. This domain consists of three $\alpha$-helices in the N terminus and two anti-parallel $\beta$-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 $\alpha/\beta$-fold resembling the Rossmann fold (32), which consists of eight strands sandwiched by eight helices. The C-terminal domain consists of short $\beta$-strands and a long $\alpha$-helix connected to the central domain. Ser-216 was mutated to leucine due to the difference of codon usage in the host cell and C. albicans. This mutation does not appear to affect the enzymatic activity, as this residue is far from the active site (Fig. 3A). In the case of Complex-2, the nucleotide binding motif in one of the two crystallographically independent molecules was not built because these residues were highly disordered.

Ligand Binding of CaUAP1—The ligand molecules are located in the active site, at the bottom of the large cleft between the N-terminal and central domains (Fig. 3A). In the apo-like form structure, no ligand molecules can be located in the active site, because the electron density for the ligand is very weak and unclear (Fig. 3B). GlcNAc-1-P is found at the active site in the substrate binding form (Fig. 3C). UDP-GlcNAc is found at the active site in the reaction-completed and product binding forms (Fig. 3D and E). Complex-3 was co-crystallized with both GlcNAc-1-P and UTP, and therefore they were converted into the product while preparing or crystallizing the sample (reaction-completed form), whereas Complex-4 (co-crystallized with the product) is a product binding form. The co-crystallized molecules and the ligands in the crystal structures are summarized in Table 1.

The binding networks of CaUAP1 with its ligands are shown in Fig. 4. Residues of Asn-227, Gly-294, Glu-309, Tyr-310, Asn-335, and Lys-421 interact with GlcNAc-1-P in the substrate binding form and also GlcNAc-1-P moiety in the reaction-completed and product binding forms (Fig. 4A). The uridine group is bound to residues of Met-109, Gly-111, Gly-112, Gln-199, and Gly-226 in the reaction-completed and product binding forms (Fig. 4B). Residues of Arg-116, Tyr-310, and Lys-421 coordinate to the phosphate groups (Fig. 4, C and D). Lys-421 interacts with both the $\alpha$- and $\beta$-phosphate groups of UDP-GlcNAc in the reaction-completed form (Fig. 4C), whereas it does not interact with the $\alpha$-phosphate group in the product binding form (Fig. 4D). The position of the $\alpha$-phosphate group in the product binding form differs from that in the reaction-completed form. Because the reaction-completed form is considered to reflect the reaction state and the $\alpha$-phosphate group of UDP-GlcNAc is a part of UTP, Lys-421 of CaUAP1 is expected to bind to the $\alpha$-phosphate group of UTP.

One or two sulfate ions were also identified in the substrate binding, reaction-completed, and product binding forms (Table 1). One sulfate (SO$_4^{2-}$-1) exists at the active site in the reaction-completed and product binding forms. It binds to residues of Thr-115 and Arg-116 (Fig. 4E), and this site is positively charged. The other sulfate (SO$_4^{2-}$-2) binds to residues of Arg-285, Lys-321, and Arg-332 in the substrate binding and reaction-completed forms (Fig. 4F). These three residues are located at a different site from the active site and form the basic pocket.
Induced Fit Movement—Binding of GlcNAc-1-P triggers transformation from the apo-like form into the substrate binding form (Fig. 5A). By comparing the two structures, Loops-B and -C and the N-terminal domain move to close the entrance to the active site (Fig. 5A). The two loops, which have no atomic contacts with other molecules in the crystals, make contact with GlcNAc-1-P at Gly-294, Glu-309, and Tyr-310 (Fig. 5E). On the other hand, the change of the space groups in the present crystal structures seems to cause the movement of the N-terminal domain because the N-terminal domain does not have atomic contacts directly with GlcNAc-1-P but with the other CaUAP1 molecules in the crystals. However, because a linker between the N-terminal and central domains (Asn-227) comes into direct contact with GlcNAc-1-P (Fig. 4A), it is possible that binding the GlcNAc-1-P drives the N-terminal domain movement.

The substrate binding form is transformed into the reaction-completed form by binding of UTP and condensing GlcNAc-1-P with UTP (Fig. 5B). Loop-A, which consists of the nucleotide binding motif, makes contact with the uridine group of UDP-GlcNAc at Gly-111 and Gly-112, making the entrance to the active site more tightly closed (Fig. 5B, C, and F). Arg-116 is bound to the main chain of Lys-421 and the side chain of Asp-422 in the substrate binding form, but it is bound to the β-phosphate group of UDP-GlcNAc in the reaction-completed form.

FIGURE 5. Induced fit movement. The apo-like, substrate binding, reaction-completed, and product binding forms are shown in green, cyan, orange, and yellow, respectively. The arrows indicate the induced fit movement. The central domain is used for superimposing, which is carried out with the program LSQKAB (29). The color bars indicate the distance of each pair of Ca atoms. Overall movements from the apo-like form to the substrate binding form (A), from the substrate binding form to the reaction-completed form (B), from the reaction-completed form to the apo-like form (C), and from the reaction-completed form to the product binding form (D) are shown. E, movements around Loop-B and Loop-C from the apo-like form to the substrate binding form. F, movements of Lys-421, Asp-422, and Loop-A from the substrate binding form to the reaction-completed form. G, movements of Loop-B and Loop-C around SO₄²⁻ from the reaction-completed form to the apo-like form.

FIGURE 6. Effect of the metal ion on CaUAP1 activity examined by thin layer chromatography. A, lane 1 was incubated with 1 mM EDTA. Lanes 3, 4, 5, 6, 7, and 8 were incubated with 0.025, 0.13, 0.25, 0.50, 1.0, and 2.5 μM MgCl₂, respectively. B, lanes 2, 3, and 4 were incubated with 0.25 μM MgCl₂, CaCl₂, and ZnCl₂, respectively.

Induced Fit Movement—Binding of GlcNAc-1-P triggers transformation from the apo-like form into the substrate binding form (Fig. 5A). By comparing the two structures, Loops-B and -C and the N-terminal domain move to close the entrance to the active site (Fig. 5A). The two loops, which have no atomic contacts with other molecules in the crystals, make contact with GlcNAc-1-P at Gly-294, Glu-309, and Tyr-310 (Fig. 5E). On the other hand, the change of the space groups in the present crystal structures seems to cause the movement of the N-terminal domain because the N-terminal domain does not have atomic contacts directly with GlcNAc-1-P but with the other CaUAP1 molecules in the crystals. However, because a linker between the N-terminal and central domains (Asn-227) comes into direct contact with GlcNAc-1-P (Fig. 4A), it is possible that binding the GlcNAc-1-P drives the N-terminal domain movement.

The substrate binding form is transformed into the reaction-completed form by binding of UTP and condensing GlcNAc-1-P with UTP (Fig. 5B). Loop-A, which consists of the nucleotide binding motif, makes contact with the uridine group of UDP-GlcNAc at Gly-111 and Gly-112, making the entrance to the active site more tightly closed (Fig. 5, B and F). Arg-116 is bound to the main chain of Lys-421 and the side chain of Asp-422 in the substrate binding form, but it is bound to the β-phosphate group of UDP-GlcNAc in the reaction-completed form.
Because this arginine residue is conserved in UAP and the mutation of the residue decreases the enzymatic activity (6, 14, 17), we consider that this movement is important for the catalytic mechanism.

The three loops (Loops-A, -B, and -C) open the active site and release UDP-GlcNAc when the reaction-completed form returns to the apo-like form (Fig. 5C). The pocket to which SO$_4^{2-}$ is bound is located on one side of Loops-B and -C. When the active site is opened, the sulfate ion is released and Loops-B and -C close this pocket (Fig. 5G). Thus, if a molecule fills the pocket and keeps it open, it may prevent the induced fit movement of Loops-B and -C. These features could suggest a novel design for a specific inhibitor of CaUAP1, because these three residues are not conserved in human UAP.

The reaction-completed and the product binding forms are similar to each other (Fig. 5D) except for the position of the $\alpha$-phosphate group of UDP-GlcNAc (Fig. 4, C and D). Because UDP-GlcNAc is the substrate for the reverse reaction, the three loops close the active site by binding of UDP-GlcNAc.

**Effects of Magnesium Ion on CaUAP1 Activity**—Fig. 6 shows the result of the enzyme assay obtained by the thin layer chromatography measurements. The increase of the MgCl$_2$ concentration enhanced the amount of produced UDP-GlcNAc (Fig. 6A, lanes 2–8). Other metal ions such as calcium and zinc show similar enhancement (Fig. 6B). These results suggest that the activity of CaUAP1 depends on the concentration of the magnesium ion and that some metal ions including magnesium enhance the enzyme activity.

**DISCUSSION**

**Comparison with Other UAPs**—The structures of CaUAP1 and human AGX1 are essentially similar. Except for the insertion loop, the two structures can be reasonably well superimposed upon each other (Fig. 7A). GlmU, prokaryotic UAP, exhibits a different tertiary structure from that of eukaryotic UAP (Fig. 7B). The N-terminal domain of CaUAP1 does not exist in GlmU, and the acetylation domain of GlmU does not exist in CaUAP1. However, the central domain of CaUAP1, to which the ligand binds, is similar to that of GlmU.

Although all eukaryotic UAPs are known to function as a monomer in solution, all known crystal structures of mammalian UAPs form a dimer and their insertion loops approach to the active site of the other molecule in the crystal (Fig. 7C) (17). The insertion loop makes contact with the phosphate group of UDP-GlcNAc bound to the other molecule in the crystal (Fig. 7C). In contrast to mammalian UAPs, Arg-116 of CaUAP1 on the nucleotide binding motif binds to the $\beta$-phosphate group of UDP-GlcNAc as mentioned above (Figs. 4, C and D, and 7C). The insertion loop of the other molecule in the crystal prevents the corresponding residue of AGX1, Arg-115, from binding to the phosphate group (Fig. 7A).

Because both mammalian and non-mammalian UAPs are known to work as a monomer in solution, we propose that they recognize the phosphate group. Both Arg-116 of CaUAP1 and Arg-115 of AGX1 recognize the phosphate group.

**UTP and Metal Ion Binding Site**—The sulfate ion at the active site (SO$_4^{2-}$) may mimic the $\gamma$-phosphate group of UTP. The distance between the sulfate ion and the $\alpha$-phosphate group of UDP-GlcNAc is $\sim$5 Å, corresponding to the...
distance between the α- and γ-phosphate groups of UTP. The crystal structure of glucose-1-phosphate thymidlyltransferase has been determined as a complex with deoxythymidine-5′-triphosphate (dTTP) (33). Glucose-1-phosphate thymidlyltransferase belongs to the SpS Gly T1 core superfamily and has the nucleotide binding motif. The sulfate thymidylyltransferase belongs to the SpS Gly T1 core superfamily and has the nucleotide binding motif. The sulfate thymidylyltransferase binds to the α-phosphate group of dTTP (Fig. 8A) at the active site. CaUAP1 and glucose-1-phosphate thymidlyltransferase are shown in cyan and orange, respectively. B, the superposition of CaUAP1 (the substrate binding form) onto SpGlmU complexed with UDP-GlcNAc (PDB 1HM9) (16). CaUAP1 and SpGlmU are shown in cyan and orange, respectively. C, the superposition of SpGlmU complexed with UDP-GlcNAc (PDB 1HM9) onto CaUAP1 (the substrate binding form). SpGlmU and CaUAP1 are shown in orange and cyan, respectively. D, the hypothetical structural model of CaUAP1 binding to both UTP and GlcNAc-1-P.

FIGURE 8. A, the superposition of CaUAP1 (the substrate binding form) onto glucose-1-phosphate thymidlyltransferase complexed with dTTP (PDB 1G2V) (33) at the active site. CaUAP1 and glucose-1-phosphate thymidlyltransferase are shown in cyan and orange, respectively. B, the superposition of CaUAP1 (the substrate binding form) onto SpGlmU complexed with UDP-GlcNAc (PDB 1HM9) (16). CaUAP1 and SpGlmU are shown in cyan and orange, respectively. C, the superposition of SpGlmU complexed with UDP-GlcNAc (PDB 1HM9) onto CaUAP1 (the substrate binding form). SpGlmU and CaUAP1 are shown in orange and cyan, respectively. D, the hypothetical structural model of CaUAP1 binding to both UTP and GlcNAc-1-P. Thus, we propose that the magnesium ion may induce a triphosphate conformation suitable for the reaction.

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