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Crystal structures of an archaeal oligosaccharyltransferase provide insights into the catalytic cycle of N-linked protein glycosylation

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1 Introduction

Asparagine-linked glycosylation (N-glycosylation) of proteins is the most ubiquitous protein modification. The oligosaccharyl transfer from the donor substrate, the lipid-linked oligosaccharide (LLO), to the asparagine residues in the N-glycosylation sequon (Asn-X-Ser/Thr, X ≠ Pro) is catalyzed by a membrane-bound enzyme, oligosaccharyltransferase (OST). In eukaryotes, the reaction occurs on the luminal side of ER, whereas it occurs on the exterior surface of the plasma membranes in archaea and eubacteria. The catalytic subunit of the OST enzyme is a polypeptide chain referred to as STT3 in eukaryotes, AglB in archaea, and PglB in eubacteria, although they originated from a common ancestor. The eukaryotic OST is a multisubunit protein complex containing STT3, but the archaeal and eubacterial OSTs, are single subunit enzymes consisting only of the AglB and PglB proteins.

2 Experiment

Protein Expression and Purification. The DNA encoding *A. fulgidus* AglB-L (O29867_ARCFU) was amplified from the genomic DNA. The protein was expressed in *Escherichia coli* C43 (DE3) cells with a C-terminal His_{10}-tag after a thrombin cleavage site. The membrane fractions were prepared in Tris-Saline (TS) buffer (50 mM Tris·HCl, pH 8.0, 100 mM NaCl) containing 1% (wt/vol) DDM. The AglB-L protein was purified by affinity chromatography on nickel Sepharose resin in TS buffer containing 0.1% DDM. After removal of the C-terminal His tag, the protein was subjected to gel filtration chromatography, using a Superdex200 column in TS buffer containing 1% (wt/vol) OG or 0.06% (wt/vol) LDAO. The eluted protein was concentrated to 15 $mg \cdot mL^{-1}$, in 20 mM Tris·HCl buffer, pH 8.0, for crystallization.

Crystallization. In the presence of 1% (wt/vol) OG, crystals grew from a hanging drop with a 1:1 volume ratio (total volume, 2 μL) of the protein stock solution and the reservoir solution (0.2 M zinc sulfate, 0.1 M sodium acetate buffer, pH 4.6, 15% (vol/vol) polyethylene glycol 4000) at 293 K. In the presence of 0.06% (wt/vol) LDAO, crystals grew from a hanging drop with the reservoir solution (0.02 M Tris·HCl, pH 8.0, 22% (wt/vol) polyethylene glycol 550MME) at 293 K.

Structure Determination. The molecular replacement was performed with the structure of the C-terminal globular domain of *A. fulgidus* AglB-L [Protein Data Bank (PDB) 3WAI] as the search model [1].

3 Results and Discussion

We determined the crystal structures of the full-length AglB from a hyperthermophilic archaeon, *Archaeoglobus fulgidus* (Fig. 1) [2]. This is the second solved catalytic subunit structure of OST, after the eubacterial PglB.

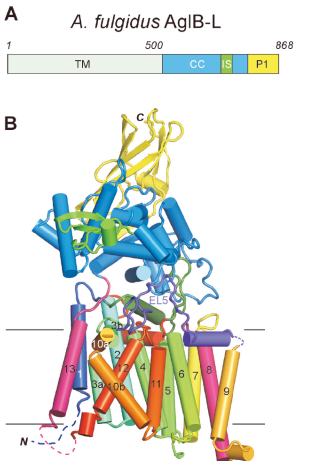

Fig. 1. (A) Domain organizations of the AglB protein. CC, central core; IS, insertion; P1, peripheral 1. (B) The AglB-L structure (OG form). The TM helices are numbered. Dashed lines indicate the disordered external and internal loops in the electron density maps.

The AglB protein consists of an N-terminal transmembrane region, which contains the catalytic center consisting of conserved acidic residues and a divalent metal ion, and a C-terminal globular domain, which contains a binding site for the Ser and Thr residues in the N-glycosylation sequon. In addition to the crystallographic data, NMR and biochemical studies suggested the essential flexibility of one long loop in the TM region and the Ser/Thr pocket in the C-terminal globular domain for the enzymatic activity [3]. It is likely that the dynamic nature facilitates the efficient scanning of a nascent polypeptide chain for the N-glycosylation sequons when coupled with ribosomal protein synthesis.

The atomic coordinates and structure factors have been deposited in the PDB (3WAJ and 3WAK).

References

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