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Crystal structure of the C-terminal globular domain of oligosaccharyltransferase from *Archaeoglobus fulgidus*

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1 Introduction

Asparagine-linked glycosylation (N-glycosylation) of proteins is widespread not only in eukaryotes, but also in archaea and some eubacteria. Oligosaccharyltransferase (OST) creates the oligosaccharide-asparagine bond by transferring glycan from a lipid-linked oligosaccharide (LLO) to asparagine residues in the N-glycosylation sequon, Asn-X-Ser/Thr (X ≠ Pro). OST is a multi-subunit membrane protein complex in higher eukaryotes, and a single-subunit membrane protein in lower eukaryotes, archaea and eubacteria. The catalytic subunit of the OST enzyme has a common evolutionary origin, but is referred to as STT3 in eukaryotes, AglB in archaea, and PglB in eubacteria. The STT3/AglB/PglB proteins consist of an N-terminal multi-span transmembrane region and a soluble C-terminal, globular domain. The STT3 proteins share more than 40% sequence identity, but exhibit limited sequence identity with the AglB and PglB proteins, typically less than 20%. Thus, a meaningful multiple sequence alignment across the three domains of life was almost impossible without referencing three-dimensional structures.

We previously determined the crystal structures of the C-terminal globular domains of *Pyrococcus furiosus* AglB [1] and *Campylobacter jejuni* PglB [2]. The structures facilitated the multiple sequence alignment in the C-terminal globular domain region, and unexpectedly led to the identification of a new, short motif within a characteristic, kinked helix. A comprehensive phylogenetic analysis revealed two conserved amino acid motif within the kinked helix, named DK and MI motifs, and suggested the existence of a third motif at the spatially equivalent position [2].

2 Experiment

Crystals grew from a hanging drop with a 1:1 volume ratio (total volume, 2 μl) of the protein stock solution (20 mg/ml, 20 mM Tris buffer, pH 8.0) and the reservoir solution (0.1 M MES buffer, pH 6.5, 12.5% polyethylene glycol 3350) at 293 K. Crystals were soaked in the reservoir solution containing 20% ethylene glycol for cryoprotection, and were cryo-cooled in liquid nitrogen.

The crystals belonged to the space group $P4_12_12$ with unit cell parameters, a = 47.05 Å, c = 159.82 Å, and diffracted to a resolution of 1.75 Å at beamlines 5A, PF and BL44XU, SPring8. The structure was refined to R/R_{free} of 0.223/0.254 using Se SAD phase. The atomic coordinates have been deposited in the Protein Data Bank, with the accession code 3VGP.

3 Results and Discussion

We determined the structure of the C-terminal globular domain of *Archaeoglobus fulgidus* AglB (*Af*AglB-S1), which possesses the third motif [3]. The crystal structure revealed that the kinked helix contained an unexpected inserted loop structure. A revised sequence alignment based on this finding identified that the third motif was actually a variant type of the DK motif. When taken together with the fact that the DK/MI motifs are involved in the formation of a binding pocket that recognizes the +2 Ser/Thr residue in the N-glycosylation sequons, this study defines the classification of OST: one group consisting of eukaryotes and most archaea possesses the DK-type Ser/Thr pocket, and the other group consisting of eubacteria and the remaining archaea possesses the MI-type Ser/Thr pocket. This classification provides a useful framework for future OST studies.

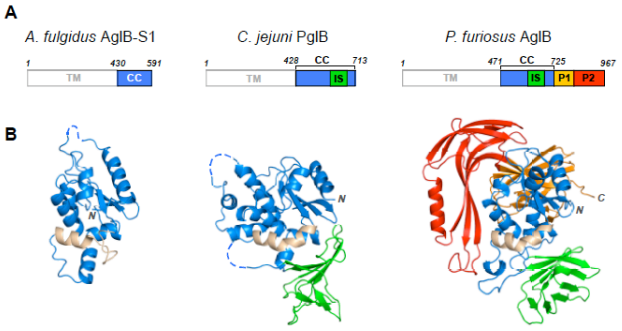

Fig. 1: Comparison of the structures of *Archaeoglobus fulgidus* AglB (this study), *Campylobacter jejuni* PglB, and *Pyrococcus furiosus* AglB. (**A**) Domain organization of the three AglB/PglB proteins. (**B**) Overall structures of the C-terminal globular domains. The characteristic kinked helix bearing the DK/MI motifs is highlighted in light brown.

References

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