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Crystallographic and NMR evidence for flexibility in oligosaccharyltransferases and its catalytic significance

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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. Multiple STT3/AglB/PglB proteins may be encoded in a single genome. As exemplified by the AglBs referred to in this study, *Archaeoglobus fulgidus* contains three paralogs. We discriminate among these archaeal paralogs with a letter plus an optional number, such as L (long) or S1 (short, number 1).

We previously determined the crystal structures of the C-terminal globular domains of *Pyrococcus furiosus* AglB-L [1], *Archaeoglobus fulgidus* AgB-S1 [2], and *Campylobacter jejuni* PglB [3]. The comparison of the three crystal structures unexpectedly revealed significant local variations in the conformations of about 25-residue segments in the C-terminal globular domains [2].

2 Experiment

The C-terminal domain of *P. horikoshii* AglB-L was crystallized in 0.1M bis-Tris propane-HCl (pH 7.5), containing 0.2 M sodium citrate and 15% w/v PEG3350, at 293 K in hanging drops within 4 days. The C-terminal domain of *A. fulgidus* AglB-S2 was crystallized in 0.1 M MES-NaOH (pH 6.0), containing 0.1 M $MgCl_2$ and 10% w/v PEG3350, at 293 K in hanging drops within 1 day. Crystals were soaked in the reservoir solutions containing 20% glycerol for *P. horikoshii* AglB-L and 20% ethylene glycol for *A. fulgidus* AglB-S2, for cryoprotection.

Structure determinations were performed by the molecular replacement. The *P. horikoshii* AglB-L crystals belonged to the space group $P2_12_12_1$ with unit cell parameters, a = 83.5 Å, b = 94.8 Å, c = 186.4 Å, and diffracted to a resolution of 2.7 Å at beamline 17A, PF. The structure was refined to R/R_{free} of 0.171/0.214. The *A. fulgidus* AglB-S2 crystals belonged to the space group $P3_1$ with unit cell parameters, $a = b$ = 111.2 Å, c = 36.7 Å, and diffracted to a resolution of 1.94 Å at beamline 17A, PF. The structure was refined to R/R_{free} of 0.188/0.219. The atomic coordinates have been deposited in the Protein Data Bank, with the accession code 3VU1 and 3VU0.

3 Results and Discussion

We have determined two crystal structures of the C-terminal globular domains of archaeal AglBs, *P. horikoshii* AglB-L and *A. fulgidus* AglB-S2 [4]. The one-to-one structural comparison of the five crystal structures indicated that the C-terminal globular domains of the AglB/PglB proteins contained a special plastic segment (Fig. 1), and identified the resting state conformation of the plastic segment, free of crystal contact effects. We characterized its dynamic properties in solution by ^{15}N NMR relaxation analyses. Intriguingly, the mobile region contains the binding pocket for the recognition of the +2 Ser/Thr residue in the consensus sequence. In agreement, the flexibility restriction forced by an engineered disulfide crosslink abolished the enzymatic activity, and its cleavage fully restored activity. These results suggest the necessity of multiple conformational states in the reaction. The dynamic nature of the Ser/Thr pocket could facilitate the efficient scanning of N-glycosylation sequons along nascent polypeptide chains

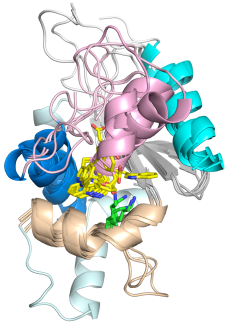

Fig. 1: Superposition of the CC structural unit in the five crystal structures of the C-terminal globular domains of the AglB/PglB proteins. The region that exhibits large plasticity includes the WWDYG motif (yellow side chains) and the following α-helical and loop regions (pink backbones). The overall structure of the *A. fulgidus* AglB-S2 was overlaid in pale cyan, as a visual aid.

References

[1] M. Igura *et al.*, EMBO J **27** (2008) 234.
[2] S. Matsumoto *et al.*, Biochemistry **51** (2012) 4157.
[3] N. Maita *et al.*, J. Biol. Chem. **285** (2010) 4941.
[4] J. Nyirenda *et al.*, Structure **21** (2013) 32.

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