Structural biology of the N-glycosylation reaction

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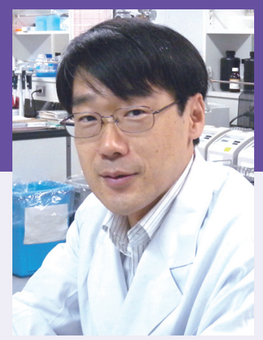

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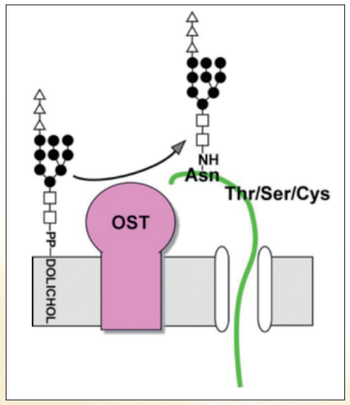
Profile

Daisuke Kohda received his Bachelor of Science in Biochemistry from the University of Tokyo in 1981. He obtained Ph.D. in Biochemistry from the same University in 1986. In the graduate course, he studied under the supervision of Prof. Tatsuo Miyazawa and Dr. Shigeyuki Yokoyama. After graduation, he joined the NMR laboratory led by Prof. Fuyuhiko Inagaki in the Tokyo Metropolitan Institute of Medical Science as a research scientist. During his 10 years in office, he spent one year at Oxford University, England, as a visiting scientist in Prof. Iain D. Campbell's laboratory. From January 1996, he joined Biomolecular Engineering Research Institute, Osaka, as a senior, then principal research scientist, and led an NMR group under the mentorship of Prof. Kosuke Morikawa. In 2002, Dr. Kohda accepted a position as Professor at Kyushu University, Fukuoka. He has been working in the field of structural biology using the NMR and X-ray crystallography. His targets include EGF, TGF-α, EGF receptor, SH3, PX, and oligosaccharyltransferases. The figure of the complex structure of Tom20 and a mitochondrial presequence is shown in four standard biochemistry textbooks, including Molecular Biology of THE CELL (5th edition).

Protein Asn-glycosylation is widespread not only in eukaryotes but also in archaea and some eubacteria. Oligosaccharyltransferase (OST) catalyzes the transfer of an oligosaccharide chain from a lipid donor to an asparagine residue in the N-glycosylation sequon, Asn-X-Ser/Thr (X ≠ Pro) (Figure 1). 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 (*St*aurosporine and *T*emperature sensitivity *3*) in eukaryotes, AglB (*A*rchaeal *Gl*ycosylation *B*) in archaea, and PglB (*P*rotein *Gl*ycosylation *B*) in eubacteria. The STT3/AglB/PglB proteins consist of an N-terminal multi-span transmembrane region (400 to 600 residues) and a soluble C-terminal, globular domain (150 to 500 residues). 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, with just one exception. The C-terminal globular domain contains a 5-residue motif, WWDYG, and its strong conservation enables multiple sequence alignment up to 100 residues, using the WWDYG motif as the pivot. As for the sequence alignment of the remaining regions, the three-dimensional structures are required as references. A comparative approach is very effective under this circumstance by finding common structural features among STT3/AglB/PglB proteins.

We determined crystal structures of the C-terminal globular domains of AglBs from *Pyrococcus* and *Archaeoglobus*[1)2)] and a PglB from *Campylobacter* (Figure 2) [1)2)]. These structures facilitated the multiple sequence alignment in the C-terminal globular domain region. Even though they are catalytically inactive, the comparison of the closely and distantly related AglB/PglB structures revealed the minimal structural unit as a common architecture, beyond sequence comparison. We identified new, short motifs within a characteristic, kinked helix. A comprehensive phylogenetic analysis revealed conserved amino acid motifs within the kinked helix at the equivalent spatial positions: M*XXIXXX*(I/V/W), D*XXK XXX*(M/I), and its variant, E<>*KXXX*(M/I/P), where <> denotes inserted 4 to 14 residue sequence. These motifs were referred to as MI, DK, and DKi, respectively[3)].

Recently, a crystal structure of the full-length *C. lari* PglB in a complex with an acceptor peptide was reported (Lizak et al, *Nature* (2011) 474, 350-355). Our *C. jejuni* PglB structure was used as a search model for the molecular replacement in the structure determination. This epochal structure revealed a special binding pocket that recognizes +2 Ser/Thr residue in the N-glycosylation consensus. The Ser/Thr pocket consists of the invariant Trp-Trp-Asp part of the conserved WWDYG motif and the second signature residue, Lys or Ile, of the DK/DKi/MI motif. Thus, the catalytic subunit of OST can be classified into two groups: one group consists of eukaryotes and most archaea, and the other group consists of eubacteria and the remaining archaea. The former STT3/AglB proteins possess the DK-type Ser/Thr pocket, and the latter AglB/PglB proteins possess the MI-type Ser/Thr pocket. This classification provides a useful framework for future OST studies.



Figuer 1. Oligosaccharyltransferase (OST) catalyzes the transfer of an oligosaccharide chain from lipid-linked oligosaccharide donors to the asparagine in a nascent polypeptide chain. This figure shows the N-glycan transfer by a eukaryotic OST.

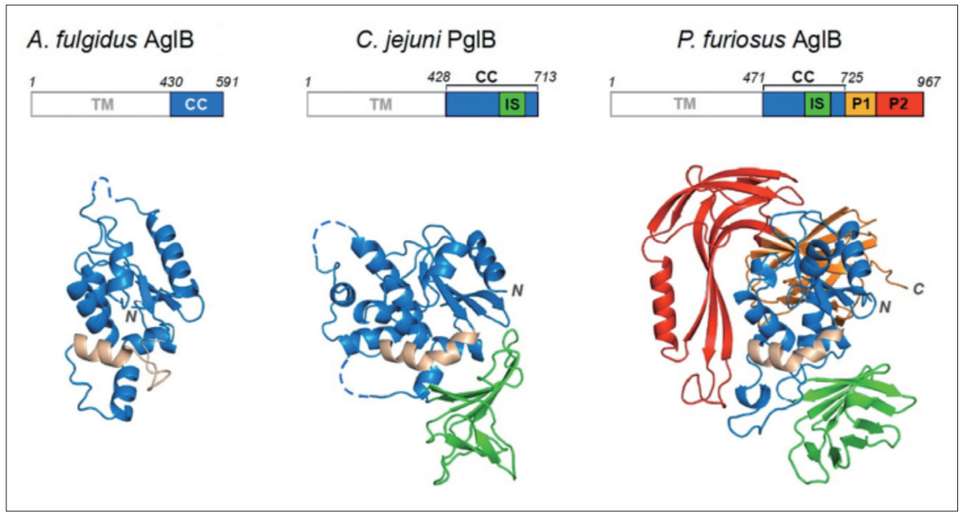


Figure 2. Overall structures of the C-terminal globular domain of *Archaeoglobus fulgidus* AglB, *Campylobacter jejuni* PglB, and *Pyrococcus furiosus* AglB. TM, transmembrane; CC, central core (*blue*); IS, insertion (*green*); P1, peripheral 1 (*orange*); P2, peripheral 2 (*red*). The TM region not included in the structure determination is outlined in gray. The characteristic kinked helix bearing the DK/DKi/MI motif is highlighted in light brown.

Detailed structural comparison of the crystal structures exhibited conformational differences of a segment including the WWDYG motif, due to crystal contact effects. This finding suggested an unusual plasticity in the C-terminal globular domain. ^{15}N NMR relaxation study using *A. fulgidus* AglB showed that this plastic segment was actually mobile in solution. Finally, we designed an engineered disulfide bond connecting two α-helices to restrict the flexibility in the C-terminal globular domain (Figure 3). The formation of the disulfide bond completely inhibited the enzymatic activity of *P. furiosus* AglB, while the activity was fully recovered upon the reduction of the disulfide bond. This indicates that the dynamic feature of the Ser/Thr pocket in the C-terminal globular domain is essential for the catalysis[4)]. Thus, we propose that the Ser/Thr pocket catches and releases the Ser and Thr residues in the N-glycosylation consensus, and its dynamic nature enables the efficient scanning of a nascent polypeptide chain when coupled with the ribosomal protein synthesis.

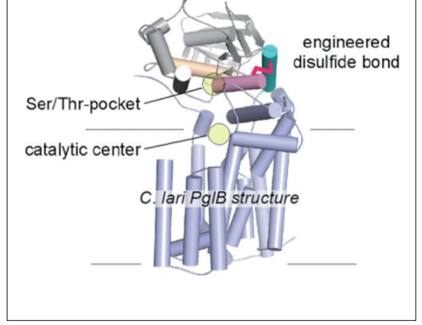


Figure 3. A disulfide bond was engineered in *P. furiosus* AglB to restrict the flexibility in the C-terminal domain. The position of the disulfide bond is schematically shown by magenta sticks in the crystal structure of *C. lari* PglB (PDB entry, 3RCE). The two shaded circles indicate the position of the +2 Ser/Thr pocket in the C-terminal globular domain, and that of the catalytic center in the N-terminal transmembrane region.

References

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