Decorating Proteins with "Sweets" Is a Flexible Matter

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In this issue of *Structure*, Nyirenda and colleagues use a combination of X-ray and NMR to demonstrate that ms timescale conformational dynamics of oligosaccharyltransferases are critical for N-linked protein glycosylation.

N-linked glycosylation is one of the most ubiquitous posttranslational protein modifications. This process occurs in all kingdoms of life, including bacteria, archaea, and eukaryotes, and is catalyzed by oligosaccharyltransferases (OSTs) (Kelleher and Gilmore, 2006). In glycosylation reactions, OSTs transfer lipid-linked polysaccharides to the asparagine of the conserved N-X-S/T sequon in acceptor proteins (Figure 1A).

OST is a monomer in archaea and bacteria, but it is a multi-subunit membrane protein complex in eukaryotes. The monomeric OST (AglB in archaea and PglB in bacteria) and the catalytic STT3 subunit of eukaryotic OST all contain an N-terminal multi-transmembrane domain and a C-terminal extracellular or periplasmic domain. Structural studies of STT3/AglB/PglB have played indispensable roles in uncovering the catalytic mechanism of OST. Several high resolution structures of the C-terminal domains of AglB or PglB from different species solved began to provide atomic, albeit partial, pictures of OSTs (Igura et al., 2008; Maita et al., 2010; Matsumoto et al., 2012). The breakthrough occurred when the full-length crystal structure of PglB in complex with a substrate peptide was solved by Lizak et al. (2011). In this structure, the transmembrane and periplasmic domains together form the binding pockets for both the N-X-S/T sequon-containing peptide substrate and the lipid-linked oligosaccharide (Figure 1A).

In this issue of *Structure*, by effectively combining X-ray crystallography and NMR spectroscopy, Nyirenda et al. (2013) report that the conformational flexibility of the N-X-S/T sequon binding pocket in the C-terminal globular domain is crucial for the enzyme activity of an archaeal OST. The authors began their work by reporting two crystal structures of archaeal OST C-terminal globular domains. They then carefully compared six C-terminal domain structures solved to date and identified a continuous stretch of amino acid residues within the domain consisting of a turn structure, an α-helix, and a loop structure (referred to as the turn-helix-loop [THL] segment), which display large conformational differences among the six structures. Such conformational differences point to a potential intrinsic dynamic property of the THL segment, although the crystallographic packing effects can complicate the interpretation. Interestingly, a highly conserved amino-acid sequence motif ("Trp-Trp-Asp" or "WWD"), which is directly involved in binding to the +2 Ser/Thr residue in the N-X-S/T sequon and thus is required for the activity of OSTs (Lizak et al., 2011; Wacker et al., 2002; Yan and Lennarz, 2002), is localized in the turn of the THL segment (Figure 1A). Following up on the hint from the crystal structures of the OCT C-terminal globular domains, Nyirenda et al. (2013) characterized the backbone dynamics of the C-terminal domain of *Archaeoglobus fulgidus* AglB-S2 in solution by NMR-based relaxation studies. They found that the THL segment in the AglB-S2 C-terminal domain displays ~0.5 ms time-scale conformation fluctuations. The authors hypothesized that such ms time-scale motion is important for the recognition of the +2 Ser/Thr of the N-X-S/T sequon and subsequent release of glycosylated products during OST's catalytic cycle so the enzyme can efficiently scan for the N-X-S/T sequons in a newly synthesized polypeptide.

To test their hypothesis, Nyirenda et al. (2013) introduced an artificial disulfide bond to the AglB C-terminal domain by mutating a pair of carefully selected amino acids to cysteines, which are designed to eliminate the plasticity of the THL segment upon disulfide bond formation. The engineered enzyme in its oxidized form is expected to be locked at the resting state that can efficiently bind to the +2 Ser/Thr of the N-X-S/T sequon of its peptide substrate (Figure 1B). One would predict that locking AglB in its resting state would slow down the glycosylated product from leaving the enzyme, thereby effectively lower the catalytic activity of the enzyme (Figure 1B). This engineered enzyme in its oxidized state indeed lost its catalytic activity in a glycosylation reaction assay. Importantly, reduction of the disulfide bond fully restored the enzyme activity of the engineered forms of AglB, indicating that the dynamics of the THL segment is critical for the glycosylation activity of the enzyme. Although the disulfide-induced rigidification of the engineered AglB is highly plausible, it remains to be seen whether the disulfide-linked enzyme lacks the slow time scale motion by NMR relaxation studies. It should also be pointed out that the X-ray and NMR-based study by Nyirenda et al. (2013) only deals with the C-terminal globular core domain of OSTs. It will be the part of future studies to examine if the dynamic properties of the C-terminal globular core of AglB (and other OSTs) are coupled with and mutually influenced by the transmembrane domain of the enzymes. The crystal structure of the full-length PglB in

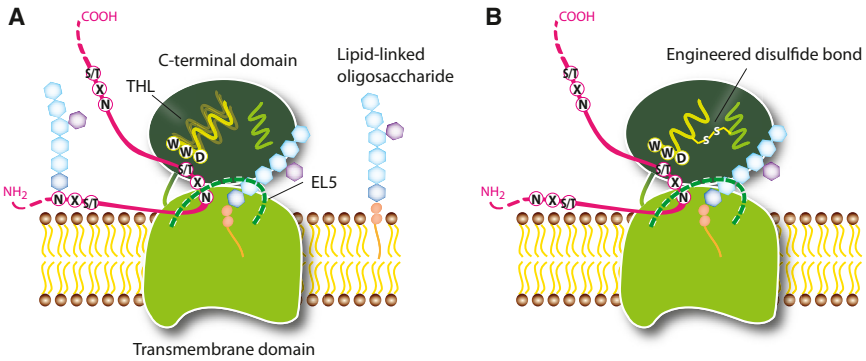

Figure 1. Dynamics of OSTs Is Important for Their Catalytic Activities

(A) The catalytic subunit of an OST contains an N-terminal transmembrane domain and a C-terminal globular domain. The enzyme scans through a nascent polypeptide from its N terminus to C terminus for the N-X-S/T sequon, where N is asparagine, S is serine, T is threonine, and X can be any amino acid but proline. The highly conserved WWD motif in the THL segment is involved in the binding of the +2 Ser/Thr in the sequon. Once the sequon is "caught" by the enzyme, the external loop 5 (EL5) of the transmembrane domain packs with the C-terminal domain to form the +2 Ser/Thr binding pocket and the catalytic site. The glycosylated sequon is released from the enzyme so that the next sequon can access the active site of the enzyme. The release of the glycosylated sequon requires dynamic motions of the THL segment and EL5. For simplicity, the lipid moiety is not drawn to the scale, and the position of the C-terminal end of the peptide substrate is arbitrary drawn.

(B) Engineering of a disulfide bond to lock the THL segment eliminated the catalytic activity of the enzyme, indicating that the dynamics of the THL segment is important for the catalytic activity of the enzyme.

complex with a substrate peptide showed that an extended loop (called EL5) connecting the 9th and 10th transmembrane segments packs with the C-terminal globular domain completing the +2 Ser/Thr binding pocket as well as forming the catalytic site of the enzyme (Lizak et al., 2011). This interaction also forms a narrow channel for the transfer of the polysaccharide to Asn in the substrate peptide (Figure 1A). As one can imagine, EL5 has to disengage from the C-terminal domain for the release of the glycosylated product, because otherwise, the bulky polysaccharide chain attached to peptide chain cannot cross the narrow channel. Thus, the reaction catalyzed by OST also requires the dynamics of EL5. Finally, most AglB/PglB proteins contain additional structural units besides the C-terminal globular domain and the N-terminal transmembrane domain. It would be interesting to know how these additional units are involved in the OST activity and if these structural units can tune the dynamics of the substrate binding pocket as well as the catalytic site of OSTs.

The critical role of dynamics in enzyme catalysis has been increasingly recognized (Nagel and Klinman, 2009). X-ray crystallography and NMR spectroscopy stand at the front of this exciting area, as both techniques can provide information on dynamic properties of proteins and enzymes at the atomic resolution. However, each of the techniques has its own intrinsic limitations. In theory, NMR spectroscopy is capable of obtaining motion properties of proteins in solution at a very broad time scale range (from picosecond to hours or even longer) (Boehr et al., 2006). In reality, both size limitations and behaviors of proteins in solution limit practical applications of NMR spectroscopy in studying detailed dynamics of a wide range of proteins. Although not limited by the molecular sizes of proteins and their complexes, limited dynamic information can be obtained from crystal structures. In crystals, dynamic regions of proteins are often invisible or artificially stabilized by inter-molecular crystal packing. As shown in the current study by Nyirenda et al. (2013) as well as in a number of other recent cases (see Fraser et al., 2009 and Masterson et al., 2010 for a few examples), combination of X-ray crystallography and NMR spectroscopy can be exceedingly powerful in addressing the functional roles of dynamics in proteins and enzymes. It is anticipated that combined uses of X-ray crystallography and NMR spectroscopy will uncover many more examples showing how dynamics can influence and even dictate the functions of broad range macro-biomolecules in the near future.

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