Structure
Preview

Xylosyltransferase 1 and the GAG Attachment Site

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Xylosyltransferase initiates glycosaminoglycan synthesis on the proteoglycan core protein. In this issue of *Structure*, Briggs and Hohenester (2018) determined the crystal structure of xylosyltransferase 1 and its structure in ternary complex with UDP-xylose donor and peptide acceptors, providing a mechanistic insight into the role of xylosyltransferase for glycosaminoglycan site selection.

Proteoglycans (PGs) are the most structurally complex biomacromolecules located in the extracellular matrix (ECM) and cell surface of virtually all animal cells and are comprised of a core protein to which one or more glycosaminoglycan (GAG) chain is covalently attached. PGs display many essential roles in biology, protecting the membranes from compressive loads, regulating cell migration, proliferation, and differentiation, facilitating the formation of morphogen gradients, and participating in specialized biological activities, such as coagulation, or wound repair. The biological functions of PGs depend on both protein core and attached GAG chains. The characterization of PGs is challenging due to their heterogeneity, highly charged acidic nature, structural complexity, variable occupancy of GAG attachment sites, and the diversity of GAG chains, such as the type of GAG chain and their length and sequence variability.

Although the GAG chain sequence of the simplest PG, bikunin, has been solved (Ly et al., 2011), and recent advances in understanding the sequence of the decorin GAG chain have been reported (Yu et al., 2017), advances in the sequencing of the GAG chains of more complex PGs, such as syndecan, are not yet on the horizon. An improved understanding of the mechanism of GAG biosynthesis could greatly accelerate progress in GAG chain sequencing. Also, altered PG biosynthesis is associated with numerous human diseases (Couchman, 2010). The introduction of GAG chains onto the core protein involves the controlled action of a number of glycosyltransferases (GTs). Yet, the mechanism, specificity, and control of GTs remain some of the major unsolved core questions in glycobiology. Glycosylation of core protein in post-translational modification begins in the endoplasmic reticulum. GAG assembly starts with the attachment of xylose from the uridine diphosphate (UDP)-xylose as donor onto a specific serine residue of the core protein by a xylosyltransferase (XT1 or XT2). The tetrasaccharide linkage region [glucuronic acid-β1-3-galactose-β1-3-galactose-β1-4-xylose-β1 (GlcA β1-3Gal β1-3Gal β1-4Xyl β1)] is built up from this Xyl residue through the action of β1, 4-galactosyltransferase I, β1, 3-galactosyltransferase II, and β1, 3-glucuronosyltransferase I, respectively. The linear GAG, based on repeating disaccharide units, is then assembled on this tetrasaccharide through the action of a bi-catalytic GTs called synthases. PGs fall into several major classes based on their GAG chains, chondroitin sulfate/dermatan sulfate, or heparan sulfate/heparin.

XT initiates the GAG attachment on the core protein. Yet, how XT selects the site for GAG attachment initiating tetrasaccharide linkage region synthesis and GAG chain extension remains controversial. Unlike the well-established consensus sequence or sequon in the *N*-glycan attachment site, the sequon for *O*-glycosylation in GAG chain attachment is less well understood. Generally, the motif Ser-Gly-X-Gly (where X can be any amino acid residue but proline) has been proposed as a sequon for xylosylation. In addition, the mutation of XT1 can result in severe diseases, suggesting the critical importance of the specificity and activity of this enzyme (Mis et al., 2014).

In this issue of *Structure*, Briggs and Hohenester (2018) describe the structure of XT1 involved in the first step of PG biosynthesis and its specificity for UDP-xylose donor and for the peptide sequence in the core protein acceptor. How XT1 selects the serine residue of the GAG attachment site in the core protein acceptor is of great interest to researchers in the PG field. Although enzymes can recognize subdomains and organize these in the GAG chain, our current understanding of biosynthetic control of GAG chain structure is still insufficient to understand how GAGs have a defined or limited number of sequences. Briggs and Hohenester (2018) report the structures of ternary complexes of XT1, polypeptide, and UDP-xylose. XT1 shows a stringent specificity for UDP-xylose donor but a relatively lax specificity for the polypeptide acceptor, which begins to explain the observed diversity of PGs (Figure 1). While there is a strong requirement for Ser at the 0 site (the GAG attachment site), the authors predict that a Thr at the 0 site might also act as acceptor, suggesting that there may be unreported GAG chains attached to threonine residues in core proteins and that researchers in the field might be on the lookout for such structures. Other interesting findings are the following: (1) all but one reported GAG attachment site have a Gly at position +1 (on the C-terminal side of the 0 site), and that while Ala is possible at +1 site, it might interfere with Xyl phosphorylation, resulting in a capped linker that is subsequently degraded or remains undetected due to its small size; (2) the −1 site has a large tolerance

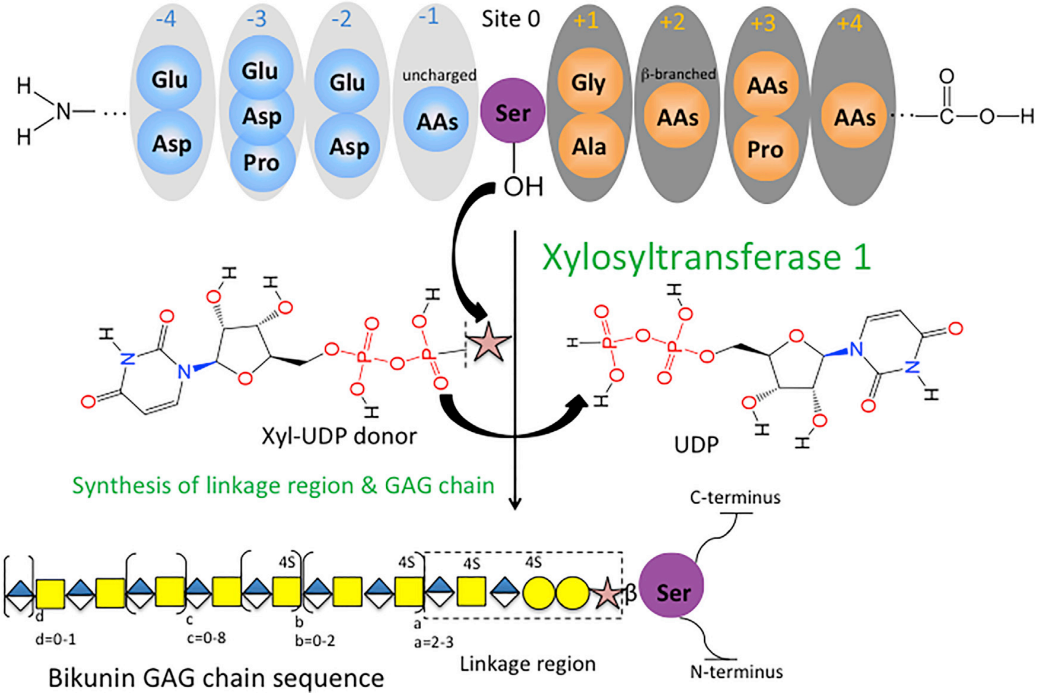

Figure 1. A Schematic Mechanism for the Biosynthesis of Bikunin PG in which Xylosyltransferase 1 Selects the Attachment Site in the Acceptor Peptide Sequence folowed by the Synthesis of the Glycosaminoglycan Chain

Saccharides are designated by their standard symbols: xylose (orange star), galactose (yellow circle), glucuronic acid (blue/white diamond), *N*-acetylgalactosamine (yellow square), and 4-*O*-sulfo group as 4S.

for uncharged amino acids; (3) the +2 site has a preference for β-branched amino acids; and (4) at least four residues on either side of the 0 site must be capable of assuming an extended conformation for GAG attachment—proline can be accommodated at the −3 and +3 sites, and acidic residues are preferred at the −2, −3, and −4 sites—consistent with most GAG chains being formed on surface loops or in unstructured domains.

There is tremendous diversity and structural variation of PGs due to a large number of core proteins, which have been identified using genomic and proteomic methods, and the different classes of substituted GAG chains. While some PGs, such as bikunin and decorin, contain only one GAG chain, others, such as aggrecan, have more than 200 GAG chains. Xylosylation is an incomplete process in some PGs, which may explain why PGs with multiple potential attachment sites often contain different numbers of GAG chains (Esko et al., 2009). The lack of a crystal structure has been a major impediment to investigating XT molecular mechanisms, understanding substrate recognition, and developing inhibitors (Lazarus et al., 2011). It is generally accepted that xylosylation of serine in the core protein is the critical step for determining GAG attachment. PGs can contain clusters of GAG attachment sites, where some always have GAG side chains and others have GAG chains present some of the time (Roch et al., 2010).

The Xylo_C domain in XT1 is non-catalytic, and while its role still remains unclear, the authors suggest it might be responsible for binding enzymes involved in subsequent steps of GAG biosynthesis. This domain is typically 169–183 amino acids length, and further functions in XT need to be characterized (Götting et al., 2004). A unique active-site flap in XT1 may provide a β-strand-like interface for the extended peptide substrate and shape the constriction at position +1, restricting it to occupancy by the small amino acid glycine.

The clarification of the XT1 ternary structure with its substrates provides an improved understanding of the specificity of this enzyme and the structure of PGs involved in many physiological and pathophysiological processes.

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