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Synthetic heparin and heparan sulfate oligosaccharides and their protein interactions

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Heparin and heparan sulfate bind a host of basic proteins that take advantage of the sugar's dense structural information. The significance of these interactions in various aspects of development, physiology, and disease stimulated keen interest in evaluating structure–activity relationships. The well-defined heparin and heparan sulfate oligosaccharides needed for these studies can be mainly accessed by chemical synthesis and, more recently by chemoenzymatic means. The various synthetic strategies available to chemical synthesis have recently enabled the acquisition of several regular and irregular sequences, including a number of dodecasaccharides, through improved coupling methods and judicial protecting group manipulations. Controlled chain elongation and critical application of modification enzymes allowed the generation of well-defined constructs via chemoenzymatic synthesis. Investigations of various protein interactions with the synthetic constructs delivered valuable information that could aid future drug development endeavors.

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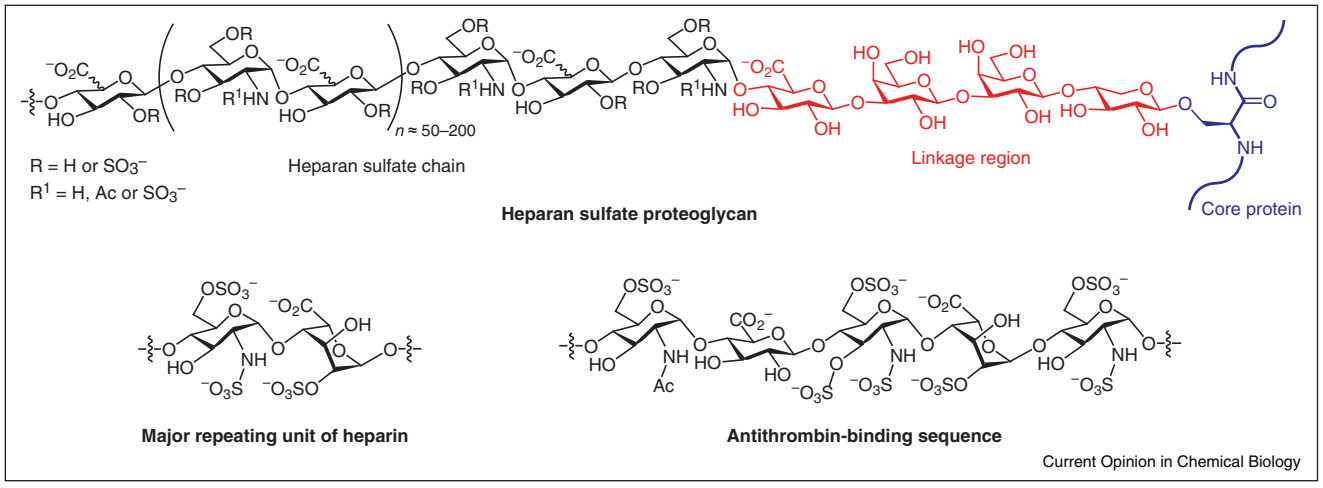
Introduction

Proteoglycans are vital components of cell surfaces and extracellular matrices of animal tissues [1,2]. They are complex macromolecules that comprise a core protein and one or more conjugated glycosaminoglycans (GAGs) — linear polymers with repeating disaccharide backbones. While the protein segments displayed notable activities [3,4], the vast majority of proteoglycan functions are associated with GAGs of which heparan sulfate (HS) is the most heterogeneous and most widespread [5]. Alternating 1 → 4-linked α-D-glucosamine (GlcN) and either β-D-glucuronic acid (GlcA) or α-L-iduronic acid (IdoA) make up the extended HS backbone (Figure 1). Potential sulfations may occur at C3 and C6 of GlcN and at C2 of the uronic acid (UA), and the GlcN amine function may be sulfonated, acetylated or unsubstituted. These variations account to 48 disaccharide possibilities within the chain. However, only about half of those were observed in *Nature*, likely due to biological restrictions that also granted tissue-specific sulfonation patterns and intermittent swatches of unsulfated regions [6]. Hundreds of basic proteins, implicated in fertilization, growth and development, bacterial and viral infections, wound healing, immune response, and cancer progression among others, take advantage of the rich structural diversity of HS [7]. HS grant proteins localized availability near the cell surface and facilitate various means of delivering intended functions. The biomedical significance of these interactions prompted intense investigations aiming to determine the structural features optimally required for function. The antithrombin activation by the HS analog heparin leading to the development of the anticoagulant fondaparinux has long inspired the study of HS–protein associations [8]. Sequestered *in vivo* by mastocytes, heparin is generated similar to HS and carries the same disaccharide variations. It is, however, more homogeneous with N-sulfonated and 6-O-sulfonated GlcN (GlcNS6S) and 2-O-sulfonated IdoA (IdoA2S) occupying most of the chain [9]. The binding of antithrombin with a distinct 3-O-sulfonated pentasaccharide sequence in heparin triggers the exposure of the protease reactive center loop capable of deactivating factors IIa and Xa of the coagulation cascade [10].

The heparin and HS fragments isolated from natural sources are typically unsuitable for structure–activity relationship evaluations because of their polydispersity and structural ambiguity. Chemoenzymatic alterations of such fragments and that of heparosan, the N-acetyl-D-glucosamine (GlcNAc)–GlcA copolymer harvested from *Escherichia coli* strain K5 [11,12], to afford certain defined features only supplied partial information on the structural requirements for binding [13]. Here, the non-uniform starting materials, the incomplete enzymatic transformations, and the difficulties in reaction monitoring and product purification are persistent concerns. Despite the considerable effort and resources involved, chemical synthesis remains the most common and reliable source of well-defined heparin and HS oligosaccharides [14–16,17•]. The chemical approach grew in

Figure 1



Structure of a proteoglycan with attached heparan sulfate chain and some notable structures found in heparin.

sophistication by adopting advances in mainstream carbohydrate chemistry and discovering novel ways in dealing with the challenges associated with the complex structure of heparin and HS. On the other hand, chemoenzymatic approach progressed by dealing with the problem of selectivity during enzymatic modifications [18]. The recent methodologies in generating defined heparin and HS oligosaccharides and the information obtained from their biological evaluations are the subject of the present review.

Chemical synthesis

As a synthetic target for many years, numerous strategies were disclosed addressing the challenges in the chemical synthesis of heparin and HS oligosaccharides [17•]. Orthogonal protecting groups at key positions played central roles in the transformations, ensuring the regioselectivity and stereoselectivity in glycosylation as well as the functional group pattern of the target constructs. Additionally, unnatural functional groups, such as linkers that are tailor-made for assay purposes, can be conveniently installed on the sugar chain. Novel and improved methodologies in recent reports contributed in increasing the variety and complexity of the synthesized structures.

The repeating disaccharide nature of heparin and HS motivated the generation of disaccharide building blocks to form longer skeletons (Figure 2). As enzymatic degradation of the natural compound delivers oligosaccharides with repeating UA–GlcN backbone, past syntheses often leaned toward disaccharide building blocks corresponding to this sequence. Notably, a higher number of recent efforts were developed using the GlcN–UA disaccharide precursor. The latter approach acknowledges the greater difficulty in α-glucosaminylation relative to 1,2-*trans* glycosylation involving the UA precursor. With azide masking the 2-amine function, α-glucosaminylation particularly relies on anomeric effect. α-Stereoselectivity is further enhanced by an acceptor with axially oriented hydroxyl nucleophile [19], the remote participation by acyl groups, and the steric influence of bulky groups. In particular, Hung showed that *tert*-butyldiphenylsilyl and *p*-bromobenzyl groups at the respective 6-O and 3-O positions of a glucosaminyl donor confer full α-stereoselectivity regardless of leaving group, activator, and acceptor [20•]. *tert*-Butyldimethylsilyl group at 4-O also provided similar effect on stereoselectivity [21•]. The UA precursor can be made with the carboxyl function already present before chain assembly. Conversely, oxidation may be carried out, typically using 2,2,6,6-tetramethyl-1-piperidinyloxyl free radical, until the relevant glycosidic bonds have formed to avoid reactivity and epimerization issues. The acquisition of the L-idose or IdoA derivative is another main concern. Fueled by the high price of the unprotected monosaccharide, several synthetic strategies were developed using cheaper starting materials [22]. There are recent updates concerning the formation of 1,6-anhydro-L-idose by Hung [23] and introduction of various protecting groups in the D-xylose-derived IdoA derivative by Seeberger [24]. Alternatively, Gardiner's group disclosed a new method via D-xylodialdose involving the stereoselective cyanohydrin formation at C5 in the L-*ido* configuration [25].

Glycosylations with the same disaccharide building block is a typical route in generating heparin and HS oligosaccharide. By this approach, different lengths can be readily prepared leading to compounds with regular repeating patterns. Elongations were achieved using