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Synthetic probes of glycosaminoglycan function

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Glycosaminoglycans (GAGs) participate in many critical biological processes by modulating the activities of a wide range of proteins, including growth factors, chemokines, and viral receptors. Recent studies using synthetic oligosaccharides and glycomimetic polymers have established the importance of specific structural determinants in controlling GAG function. These findings illustrate the power of synthetic molecules to elucidate glycan-mediated signaling events, as well as the prospect of further advancements to understand the roles of GAGs *in vivo* and explore their therapeutic potential.

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Introduction

Glycosaminoglycans (GAGs) are a class of highly charged, extracellular polysaccharides that display rich structural diversity [1,2]. The two most prevalent members of the family, heparin/heparan sulfate (HS) and chondroitin sulfate (CS), are composed of repeating disaccharide units of uronic acid (L-iduronic acid or D-glucuronic acid) and hexosamine (D-glucosamine or D-galactosamine) sugars (Figure 1a). Each sugar is differentially sulfated at the hydroxyl and/or amino positions by various sulfotransferase enzymes, giving rise to hundreds of potential sulfation sequences. This structural diversity leads to a wide range of protein-binding motifs and enables modulation of cellular signaling pathways. Indeed, GAGs participate in many important signaling events such as neuronal growth [2–5], tumor progression [6], inflammation [7], and development [8]. Their involvement in both normal and disease-related processes has sparked intense interest in understanding the mechanisms and structural determinants that control GAG activity. However, the chemical complexity and diversity of these polysaccharides present a formidable challenge to elucidating their structure–function relationships.

A major barrier to understanding GAGs has been the difficulty of accessing defined structures. Well-characterized, homogeneous GAG fragments are challenging to purify from natural sources due to their strong anionic character and assorted sulfation patterns. Purified structures are usually limited to the most abundant sulfation sequences and may lack the rare, highly sulfated epitopes that are often physiologically important. Chemically modified heparin/HS polysaccharides and CS motif-enriched polysaccharides typically contain a small percentage of other sulfated epitopes that can also exert biological activity. As such, caution must be used when interpreting results obtained with natural GAGs. From a purity and selectivity standpoint, these limitations make the use of defined synthetic structures crucial for studying the structure–function relationships of GAGs.

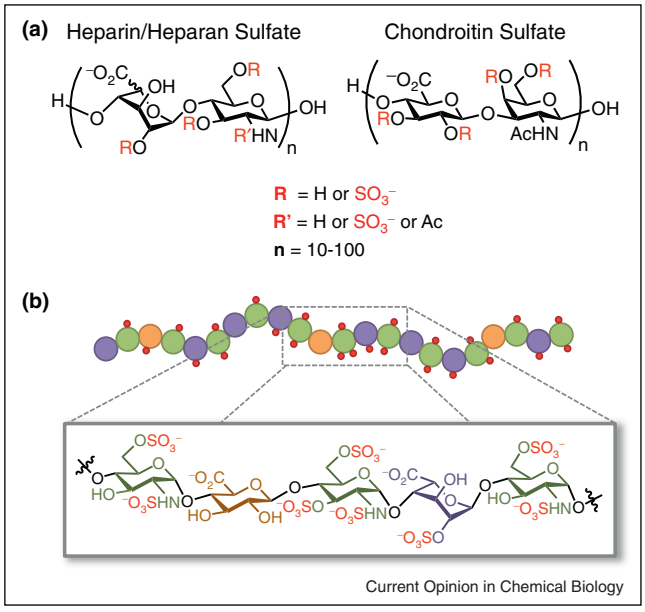
In this article, we describe some of the recent advances in the synthesis of GAG oligosaccharides and glycomimetic polymers. These advances include both synthetic organic and chemoenzymatic approaches to access defined oligosaccharides and polymerization techniques to develop simplified glycomimetics that emulate the natural multivalency of GAGs. We also highlight how these molecules have been used as probes to identify the roles of specific sulfation motifs in mediating key signaling events and cellular processes. Together, the studies suggest opportunities for the continued development of new chemical approaches to interrogate the functions of this ubiquitous, important class of polysaccharides.

Chemical synthesis of GAG oligosaccharides

The chemical synthesis of GAGs is notoriously challenging because it requires iterative, stereoselective formation of glycosidic bonds and sophisticated protecting group strategies to achieve regioselective sulfation [9–15,16•]. Many syntheses employ a modular, convergent approach in which a core oligosaccharide is assembled from a common disaccharide precursor. A late divergent approach is then exploited to differentially deprotect and regioselectively sulfate the core oligosaccharide and produce compounds with specific sulfation motifs.

A major challenge remains the generation of diverse libraries of GAG oligosaccharides with defined structures. To date, only a small proportion of the theoretically possible structures have been synthesized. However, new methods have emerged that may accelerate the generation of comprehensive collections of defined GAG oligosaccharides. For example, Jacquinet and coworkers developed efficient syntheses of CS tetrasaccharides and hexasaccharides bearing the CS-A, CS-C, CS-D,

Figure 1



The structural diversity of GAGs. **(a)** Representative GAGs heparin/HS and CS, with potential sites of sulfation indicated. Ac = acetyl. **(b)** Bioactive sulfation sequences such as the Arixtra pentasaccharide are found within the heterogeneous structure of GAG polysaccharides.

CS-E, CS-K, CS-L, and CS-M sulfation motifs using a precursor derived from natural CS polysaccharides [17–19]. Their method exploited the controlled hydrolysis of commercially available CS to rapidly and inexpensively obtain a fully protected disaccharide building block. More recently, Seeberger and coworkers reported a solid-phase synthesis of CS oligosaccharides [20]. By attachment of the oligosaccharides to Merrifield resin, many of the arduous purification steps associated with carbohydrate synthesis were avoided. The growing oligosaccharides were elongated by the sequential addition of monosaccharide phosphate donors and were selectively deprotected and sulfated over the course of three days to produce CS-A and CS-C hexasaccharides with an average yield of 86–88% per step. These methods may help expedite the development of much needed structural libraries that encompass a larger proportion of the molecular diversity found in GAGs.

The joint venture of chemical synthesis and biological studies can have an enormous impact on our understanding of GAGs and their roles in physiological processes. The quintessential illustration of the power of this combined approach is demonstrated by the pioneering studies of Petitou, Sinaÿ, Choay, Lindahl and others, whose investigations in the late 1970s and early 1980s led to the discovery of a specific pentasaccharide within heparin that is responsible for its anticoagulant activity (Figure 1b) [21–23]. Their work provided critical insights into heparin's mechanism of action and facilitated the development of the pentasaccharide drug Arixtra (GlaxoSmithKline).

More recently, de Paz *et al.* studied the interactions of various synthetic heparin oligosaccharides with chemokines, a large family of proteins involved in injury, inflammation, and atherosclerosis [24•]. Various monosaccharides, disaccharides, tetrasaccharides, and hexasaccharides with natural and non-natural sulfation motifs were synthesized with amine-terminated linkers to allow for the generation of glycan microarrays. The authors rapidly screened the affinities of various chemokines for 12 oligosaccharides, revealing patterns of binding strengths that depended on individual sulfate groups. Notably, different chemokines displayed preferences for different sulfation patterns. For example, CCL21 exhibited robust binding to tetrasaccharides and hexasaccharides containing at least two sulfate groups per disaccharide, whereas CXCL13 and CCL19 showed weak or no binding to the same structures. This study demonstrates that synthetic oligosaccharides, when combined with glycan microarrays, can be used to rapidly assess the role of individual sulfation patterns in mediating GAG–protein interactions. Further development of comprehensive libraries of synthetic GAGs may lead to elucidation of the exact structural requirements for HS recognition by different chemokines.

The effects of HS sulfation pattern and chain length on cytokine-dependent angiogenic functions were examined by Cole *et al.* using a series of synthetic oligosaccharides [25•]. Compounds ranging in length from 7 to 12 sugar units and containing a repeating disaccharide unit of 2-*O*-sulfated iduronate with or without *N*-sulfated glucosamine (2S or 2SNS) were tested for their ability to compete with HS for binding to fibroblast growth factor 2 (FGF-2) and vascular endothelial growth factor 165 ($VEGF_{165}$) (Figure 2a). A strong correlation was found between the oligosaccharide structures, their affinity for the cytokines, and their ability to inhibit specific cytokine-dependent functions such as endothelial cell migration and tube formation. The 2SNS decasaccharide and dodecasaccharide showed the most potency overall, whereas the 2SNS heptasaccharide and all 2S oligosaccharides had no significant effect. This work represents one of the most systematic structure–function studies to date using synthetic HS oligosaccharides, and the results suggest that important cytokine-mediated cell functions depend highly on the fine structure of HS.

Hu *et al.* recently studied the role of specific HS sulfation motifs in herpes simplex virus type 1 (HSV-1) infection using synthetic oligosaccharides [26•]. HSV-1 attachment and entry into host cells is mediated by the binding of several viral envelope glycoproteins to HS chains on the host cell surface. One glycoprotein, gD, binds specifically to HS modified with the rare 3-*O*-sulfate motif [27]. In this