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Using structurally defined oligosaccharides to understand the interactions between proteins and heparan sulfate

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Heparan sulfate (HS) is widely present on the animal cell surface and in the extracellular matrix. HS achieves its biological functions by interacting with proteins to change proteins' conformation, oligomerization state and cellular location. The challenging question to study HS is how to dissect the relationship between the structures of HS and the biological activities. In the past several years, crucial techniques have been developed to overcome this challenge. A novel chemoenzymatic method to synthesize structurally defined HS oligosaccharides has offered a key access to this class of sulfated carbohydrate molecules. Recent rapid progress of HS microarray technology allows screening of the interaction of a target protein with a large number of HS oligosaccharides. The improved availability of HS oligosaccharides and HS microarray analysis will undoubtedly accelerate the investigation of the contribution of the specific sulfated carbohydrate structures of HS in a wide range of biological contexts.

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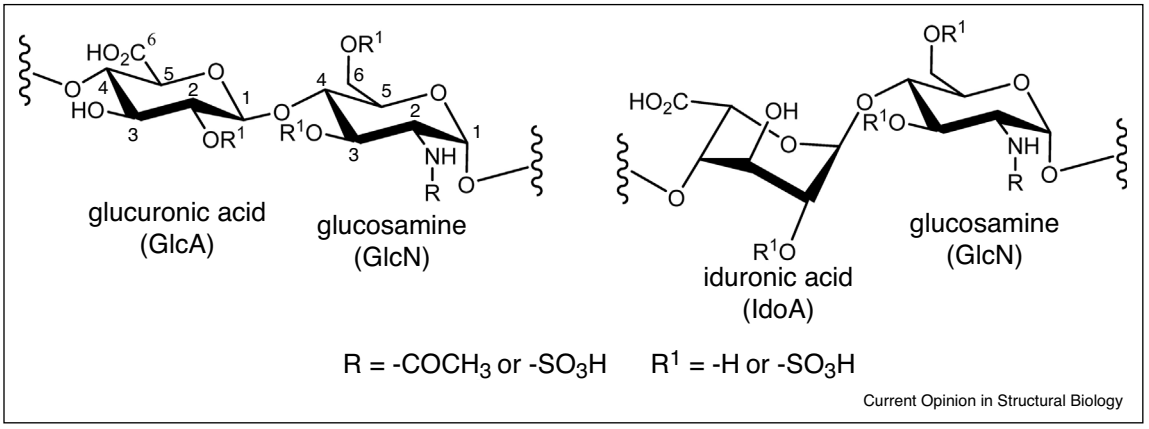
Heparan sulfate (HS), a linear sulfated polysaccharide, plays essential physiological roles and binds to hundreds of HS-binding proteins (HSBPs). Present on the cell surface and in the extracellular matrix space, HS interacts with proteins on the cell surface as well as the proteins around the cells, notably in the extracellular matrix. The interaction of HSBPs and HS adds a layer of regulations, that is, altering oligomerization forms for unique functions, gaining binding affinity to different binding patterns, and positioning proteins at specific cellular locations. Appearing early in the metazoan evolution, HS displays a wide range of variations in sulfations and the content of iduronic acid (IdoA) in both invertebrates and higher organisms. One group of studies suggest that the HS from vertebrate cells has a higher level of sulfation and iduronic acid than the HS from *Caenorhabditis elegans* and Drosophila [1,2]. However, other studies on HS from different invertebrates and chordates show that there is no clear structural difference in the HS between mammals and lower organisms [3,4].

HS polysaccharides consist of the disaccharide repeating unit of glucosamine (GlcN) and glucuronic acid (GlcA) or IdoA (Figure 1). The disaccharide units are repeated 50–200 times in a HS polysaccharide chain to form a macromolecule with molecular weight of 10 000 to 80 000 Da. Each GlcN unit is capable of carrying *O*-sulfo groups at C_3 and C_6 positions or an *N*-sulfo group at C_2 position, and GlcA or IdoA unit may carry an *O*-sulfo group at the C_2 position. Epimerase converts GlcA to an IdoA residue, while sulfo groups are transferred by four different classes of HS sulfotransferases. There are around 21 commonly identified disaccharide species. In principle, a large number of saccharide sequences can be built from 21 disaccharide building blocks (Table 1); however, only a limited number of oligosaccharides have been confirmed to be present in HS [5].

HSBP cover a wide range of biological functions. For example, HS binds to fibroblast growth factors (FGFs) and fibroblast growth factor receptors to control cell proliferation and differentiation [6]. It also binds to antithrombin, a protease inhibitor that regulates the blood coagulation process [7], as well as chemokines to modulate inflammation responses [8]. To understand the biological significance of individual HS–HSBP interaction, it is of paramount importance to dissect the structural detail of the interaction. In one aspect, there is a need to identify the contribution of the side chains of HSBPs amino acid residues to the HS binding event. This can be accomplished through site-directed mutagenesis studies of the protein. In another aspect, there is a need to identify the contribution of sulfation patterns and the size of the HS oligosaccharide. The latter is a much more difficult task because of the unavailability of structurally defined HS oligosaccharides. In the past, a HSBP-binding domain was isolated using a protective digestion approach, a

Figure 1



Structures of disaccharide repeating units of HS.

Table 1

Commonly observed HS disaccharides.

	Abbreviated structure	Disaccharide code[a]
1	GlcA-GlcNAc	G0A0
2	GlcA-$GlcNH_2$	G0H0
3	GlcA-GlcNS	G0S0
4	GlcA-GlcNAc6S	G0A6
5	GlcA-$GlcNH_2$6S	G0H6
6	GlcA-GlcNS6S	G0S6
7	GlcA-GlcNS3S6S	G0S9
8	GlcA2S-GlcNAc6S	G2A6
9	GlcA2S-GlcNS	G2S0
10	GlcA2S-GlcNS6S	G2S6
11	IdoA-GlcNAc	I0A0
12	IdoA-GlcNAc6S	I0A6
13	IdoA-GlcNS	I0S0
14	IdoA-GlcNS6S	I0S6
15	IdoA2S-GlcNAc	I2A0
16	IdoA2S-GlcNS	I2S0
17	IdoA2S-$GlcNH_2$6S	I2H6
18	IdoA2S-$GlcNH_2$3S6S	I2H9
19	IdoA2S-GlcNS3S	I2S3
20	IdoA2S-GlcNS6S	I2S6
21	IdoA2S-GlcNS3S6S	I2S9

[a] Structure codes are used during the disaccharide analysis of HS. The definitions of these codes are described in a paper by Lawrence and colleagues [10]: G, glucuronic acid; I, iduronic acid; A, glucosamine; H, glucosamine with free amine; S, *N*-sulfated glucosamine; 0, zero *O*-sulfation; 2, 2-*O*-sulfation; 6, 6-*O*-sulfation; 3, 3-*O*-sulfation and 9, 3-*O*-sulfation and 6-*O*-sulfation.

technique used for studying the interactions between DNA and proteins. In this experiment, a mixture of HS isolated from cells was incubated with HSBP to allow complex formation, followed by heparin lyases digestion [9]. Because HSBP would protect bound HS fragment from the digestion of heparin lyases, the size and structure of the bound HS oligosaccharide could be further analyzed by gel filtration and disaccharide composition analysis, respectively. Although this method can reliably determine the size of the resultant HS oligosaccharide, the compositional analysis only reveals the identity of the disaccharide components of an oligosaccharide [10], but no saccharide sequence information can be obtained. As a result, for most of the HSBPs, the structural requirement for binding at the oligosaccharide level is entirely lacking. Recently, an advanced mass spectrometry method has been reported to study the binding of HS and HSBP; however, the analysis requires the use of structurally defined HS oligosaccharides [11].

A better way to identify the structures of HS oligosaccharides that are responsible for binding to HSBPs would be to prepare a panel of structurally defined oligosaccharides and test their binding affinity to HSBPs [12]. This method was successfully used to prove the structure of antithrombin-binding pentasaccharide in heparin, and the pentasaccharide was subsequently developed into an anticoagulant drug known as fondaparinux [13]. The design of structurally defined pentasaccharides was built on a large amount of work from the isolation and characterization of naturally occurring polysaccharides as reviewed by Ricard-Blum and Lisacek [14]. This approach however has not been possible as a general method to prove relationship between the binding affinity of a HSBP and the structure of HS due to the lack of structurally defined HS oligosaccharides. Despite extensive efforts [15–19], it is technically challenging to synthesize sufficient amount of HS oligosaccharides using a purely chemical synthetic approach for biological studies, especially those larger than octasaccharides with complex sulfation patterns. In recent years, an alternative chemoenzymatic approach has emerged to offer a shorter synthetic route to synthesize HS oligosaccharides [20–22,23•]. The newly developed chemoenzymatic approach demonstrates the ability to make diverse oligosaccharides with sufficient quantities for a wide range of biological analysis [24••]. The chemoenzymatic method