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Combinatorial Enzymatic Synthesis of Heparan Sulfate

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Abstract

Escherichia coli K5 heparosan was enzymatically modified by Chen and colleagues [10] to construct a library of heparan sulfate polysaccharides for evaluation, leading to the discovery that a 2-*O*-sulfo-iduronic acid residue is not essential for antithrombin-mediated anticoagulant activity in larger oligosaccharide and polysaccharide structures.

Heparan sulfate is an anionic polysaccharide biosynthesized in the Golgi of eukaryotic cells through the sequential action of up to 30 biosynthetic enzymes [1]. A specific highly sulfated version of this glycosaminoglycan produced in mast cells, called heparin, is commercially and therapeutically important as a clinical anticoagulant drug [2]. The sequence, within heparin and heparan sulfate, primarily responsible for anticoagulant activity was elucidated through the elegant work of the Lindahl and Rosenberg laboratories. The structure of the antithrombin binding site responsible for this activity is a pentasaccharide sequence of the structure →4) β-D-6-*O*-sulfo *N*-acetyl glucosamine (1→4) β-D-glucuronic acid (1→4) β-D-3-*O*-sulfo 6-*O*-sulfo *N*-sulfo glucosamine (1→4) α-L-2-*O*-sulfo iduronic acid (1→4) β-D-6-*O*-sulfo *N*-sulfo glucosamine (1→. The chemical synthesis of this pentasaccharide [3] and subsequent structure-activity relationship (SAR) studies on this pentasaccharide demonstrated that presence of each of the functional groups critical for antithrombin-binding and anticoagulant activity [4].

Since the original synthesis of the heparin pentasaccharide many advances have been made in the chemical synthesis of heparin related glycosaminoglycan oligosaccharides [3–5]. These improvements allow more rapid and higher yield syntheses and have been applied to the preparation of small libraries for oligosaccharide screening. Unfortunately, chemical synthesis is limited to relatively small oligosaccharides (no bigger than 8–10-mer) and requires numerous synthetic and purification steps. More recently, chemoenzymatic approaches for the synthesis of heparin and related glycosaminoglycan oligosaccharides have been reported [6–9]. Early attempts at chemoenzymatic synthesis relied on bio-synthetic enzymes purified from cell culture media and, thus, afforded very small amounts of product [6]. Other approaches have utilized chemical persulfation followed by selective de-sulfation and, while scaleable afford unnatural structures as side-products [7]. More recently, Liu and coworkers chemoenzymatically synthesized heparan sulfate

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polysaccharides with and without anticoagulant activity using both solution and solid phase approaches [8, 9].

In the current study by Chen, Jones, and Liu [10], a collection of recombinant *Escherichia coli* expressed enzymes is used to modify *N*-sulfo heparosan, which had been chemoenzymatically prepared from *E. coli* K5 polysaccharides (Figure 1, adapted from Figure 1B of [10]). Eight heparan sulfate polysaccharides were prepared by exposure of *N*-sulfo heparosan to selected biosynthetic enzymes in the presence of the sulfo donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS). The resulting polysaccharides were characterized by disaccharide analysis. Surprisingly, a heparan sulfate polysaccharide containing a repeating unit consisting of →4) β-D-glucuronic acid (1→4) α-D-6-*O*-sulfo, *N*-sulfo glucosamine (1→ called "Recomparin" showed antithrombin-mediated anticoagulant activity. This activity was observed despite the absence of a 2-*O*-sulfo iduronic acid residue found previously to be critical for pentasaccharide binding to antithrombin. Interestingly, the conformation of the glucuronic acid residue presents in "Recomparin" residue in a $^{4}C_{1}$ conformation and is less flexible than the iduronic acid residue of the pentasaccharide, residing in an equilibrium mixture of $^{1}C_{4}$ and $^{2}S_{o}$ conformers. Thus, it appears that the flexibility presumed to be important in pentasaccharide-antithrombin interactions [2] is less important in polysaccharide-antithrombin interactions. Finally, Liu's group examined the effect of chain length on antithrombin binding (Figure 2, adapted from Figure 3 of 10). These data clearly demonstrate that for small oligosaccharides, tetrasaccharide to octasaccharide or decasaccharide, the presence of 2-*O*-sulfo iduronic acid residues plays a major role in antithrombin binding but for larger oligosaccharides, decasaccharide or dodecasaccharide to full-length polysaccharide, little if any difference in antithrombin-mediated activity can be observed between structures with glucuronic acid and 2-*O*-sulfo iduronic acid residues.

In conclusion, Liu and coworkers have given the scientific community pause when performing SAR studies using oligosaccharides, suggesting it might be necessary to examine both sequence and molecular size effects on binding and activity.

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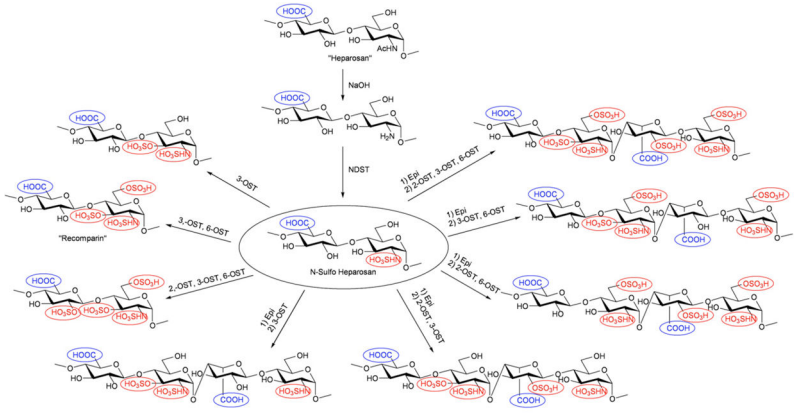

Figure 1. Chemoenzymatic Synthetic Scheme of Polysaccharide Variants of Heparan Sulfate from *E. coli* Heparosan

NDST is *N*-deacetylase *N*-sulfotransferase; OSTs are *O*-sulfotransferases; and Epi is C5 epimerase catalyzing the conversion of glucuronic acid to iduronic acid. PAPS is required as a sulfate donor for NDST and OST enzymes.

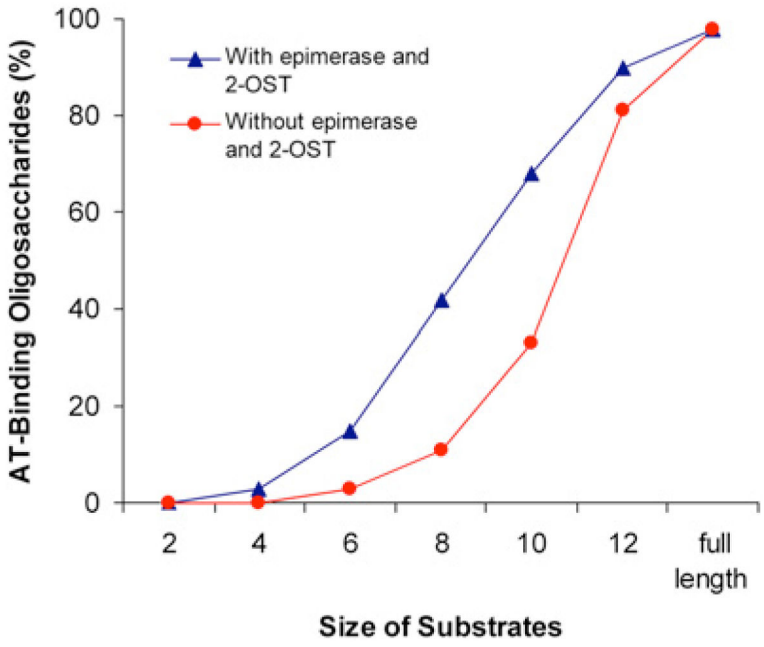

Figure 2.

Antithrombin Binding of Oligosaccharides and Full-Length Heparan Sulfate Polysaccharides as a Function of the Number of Saccharide Residues