DRUG DISCOVERY INTERFACE

Study of Physico-Chemical Properties of Novel Highly Sulfated, Aromatic, Mimetics of Heparin and Heparan Sulfate

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ABSTRACT: Heparin (H) and heparan sulfate (HS) play major roles in a number of biological processes. Yet, H/HS-based pharmaceutical agents are also associated with multiple adverse effects. This has led to the concept of designing noncarbohydrate, aromatic mimetics that modulate H/HS function. In this work, we study a library of synthetic, aromatic H/HS mimetics for their capillary electrophoretic profiles, the acid and base stability, and aqueous–organic partitioning property. The nonsugar H/HS mimetics exhibit electrophoretic properties similar to sulfated oligosaccharides suggesting that the mimetics can be rapidly and quantitatively analyzed. Stability studies show that the mimetics are essentially stable under neutral and basic conditions in a manner similar to the heparins, but are considerably unstable under acidic conditions in contrast to heparins. The measurement of partition coefficients show major differences within the sulfated mimetics as well as between the measured and calculated log*P* values. Understanding these physico-chemical properties is expected to have significant implications in the pharmaceutical development of this growing class of molecules. J Pharm Sci 99:1207–1216, 2010

Keywords: capillary electrophoresis; sulfated lignins; heparin mimetics; heparan sulfate mimetics; heparinoids; charge-to-mass ratio; chemical stability; partition coefficient

INTRODUCTION

Heparin (H) and HS, two common GAGs, play major roles in a number of physiological and pathological processes. These functions arise from their interaction with numerous proteins such as serpins (e.g., antithrombin and heparin cofactor II[1]), growth factors and their receptors (e.g., FGF-1, -2, and others[2]), coagulation proteinases (e.g., thrombin, factor Xa, factor IXa[3]), chemokines and cytokines[4,5] (e.g., platelet factor-4, interleukin-8), and viral cell envelope glycoproteins (e.g., gD, gB, gC of HSV[6]). Both GAGs are linear, alternating copolymers of uronic acid (UA*p*) and glucosamine (GlcN*p*) residues, which are decorated with numerous sulfate and carboxylate groups.[7–9] Whereas H is heavily sulfated and has a high negative charge density (~3.7 per disaccharide), HS is much less sulfated. The multitude of sulfate groups that exhibit a pK_a of ~0.2 make H the strongest acid in human physiology.

Abbreviations: CE, capillary electrophoresis; DHP, dehydropolymers; EOF, electroosmotic flow; GAG, glycosaminoglycan; H, heparin; HS, heparan sulfate; LMWH, low molecular weight heparin; P, partition coefficient; RMSD, root mean square deviation.

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A major reason for the multiple biological activities is the phenomenal structural diversity of H and HS. The cellular biosynthetic apparatus builds the H/HS chain from nearly 23 different UA*p*(1 → 4)GlcN*p* building blocks to generate massive structural complexity in the polymer.[9] Of these, only a handful of these sequences are expected to recognize a target protein specifically. For example, the interaction of heparin pentasaccharide H5 (Fig. 1) with antithrombin, a plasma glycoprotein, and a major regulator of the coagulation cascade, is known to be highly specific.[8] Another example is that of a specific H/HS octasaccharide that appears to mediate the penetration of HSV-1 into cells.[10] Yet, the polymeric H/HS structures are likely to be associated with multiple side effects, as widely noted for anticoagulant heparin. Coupled with these are concerns about the animal origins of H/HS, which may result in pathogenic contamination. Also, the structural complexity of heparin also affords avenues for doping as demonstrated by recent events in which a contaminant present in pharmaceutical heparin led to several deaths in the US.[11]

The expectation that only selected structures would be biologically active coupled with the well-established side effects of the longer chains has led to the concept of designing noncarbohydrate, aromatic mimetics that modulate H/HS function. Our efforts in this direction has resulted in the synthesis of several small and large H/HS mimetics including sulfated flavonoids,[12,13] benzofurans,[14] isoquinolines,[15] and sulfated DHPs of lignin type[16] (Fig. 2). These small and large aromatic molecules mimic the binding of H to several proteins including antithrombin, thrombin, and factor Xa, to antagonize clotting. For example, the sulfated DHPs display plasma and blood anticoagulation similar to that of LMWHs, although they utilize a new mechanism of inducing anticoagulation.[17]

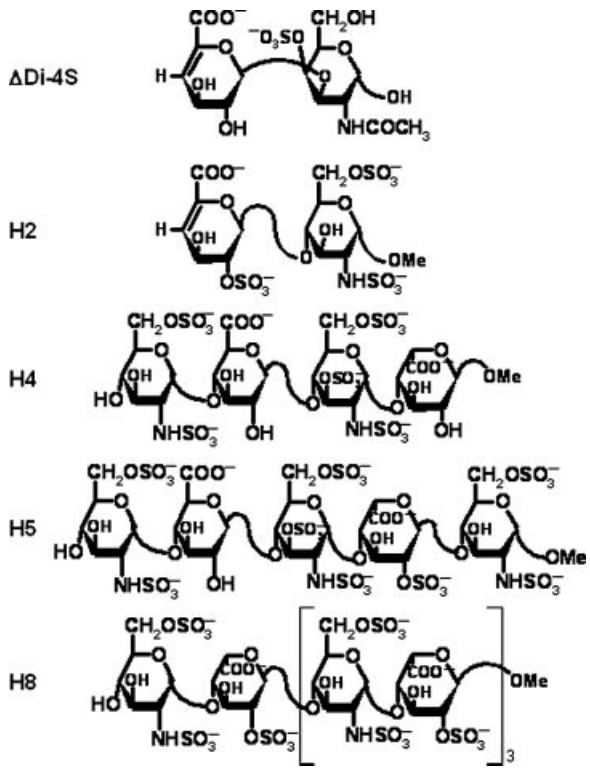

Figure 1. Structures of heparin oligosaccharides H2, H4, H5 and H8, and chondroitin sulfate disaccharide ΔDi-4S studied in this work.

The designed heparin mimetics represent unique and interesting structures. They contain both hydrophobic (aromatic rings) and hydrophilic (sulfates, carboxylates) groups that introduce an ability to recognize both hydrophobic and electropositive domains on protein surfaces as well as high water solubility. Yet, such structures have been minimally studied. The possibility of mimicking H/HS implies that understanding the physico-chemical characteristics of these novel scaffolds is important.

In this work, the capillary electrophoretic profile, the acid and base stability, and aqueous–organic partitioning property of selected designed small and large aromatic H/HS mimetics have been studied. The nonsugar H/HS mimetics exhibit electrophoretic properties similar to sulfated oligosaccharides suggesting that the mimetics can be rapidly and quantitatively analyzed. Stability studies show that the mimetics are stable under neutral and basic conditions in a manner similar to the heparins but are considerably unstable under acidic conditions in contrast to heparins. Finally, measurement of partition coefficients indicates major differences from the heparins reflecting the role of the aromatic scaffold in these novel molecules. These properties are expected to have major implications in drug development process.

MATERIALS AND METHODS

Chemicals and Reagents

Pentasaccharide (DEFGH or H5), tetrasaccharide (DEFG), and disaccharide (H2) were gifts from Professors Steven Olson (University of Illinois,