TOWARDS A CHEMOENZYMATIC SYNTHESIS OF NATURAL AND UNNATURAL GLYCOSAMINOGLYCAN OLIGOSACCHARIDES

By

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ABSTRACT

Glycosaminoglycans (GAGs) are anionic, linear polysaccharides that regulate a number of physiological processes. Many of them are found in the extracellular matrix of the cell, covalently bound to a core protein. The Linhardt lab is particularly interested in a GAG called heparin that interacts with a variety of proteins through its negatively charged sulfo (S) groups. The most commonly known biological activity of heparin is its ability to inhibit the blood coagulation cascade by binding to antithrombin III (AT). Although the specific structure that interacts with AT is well-known, the role of the number and positions of the sulfo groups is poorly understood due to the lack of an efficient method to synthesize structurally defined heparin oligosaccharides.

The conventional route of preparing oligosaccharides is by chemical synthesis. However, chemical synthesis of sulfated carbohydrates is especially difficult due to many protection/deprotection steps and laborious purifications. To obtain even a few milligrams of an oligosaccharide is challenging and time-consuming.

In an effort to overcome these issues, this thesis will describe a chemoenzymatic approach for the synthesis of heparin and related oligosaccharides. Enzymes can be a powerful tool in synthesis as they function in a regioselective and stereoselective manner in an aqueous environment. By using heparin biosynthetic enzymes to add monosaccharide building blocks and sulfo groups, structurally defined heparin oligosaccharides can be prepared without protection chemistry, significantly shortening the number of synthetic steps.

We chemoenzymatically synthesized activated monosaccharide building blocks of heparin, or uridine diphosphate (UDP)-sugars. In addition to its natural ones, we have synthesized unnatural and isotope-enriched UDP-sugars. Natural UDP-sugars can be used to synthesize naturally-occurring GAGs *in vitro*. On the other hand, unnatural UDP-sugars can be used for the selective chemical *N*-sulfation or for giving GAGs a unique function.

Using these UDP-sugars, we synthesized two ultralow molecular weight (ULMW) heparins with defined structures and sulfation patterns. They were synthesized from a disaccharide acceptor that was extended to a heptasaccharide, followed by regioselective epimerization and *O*-sulfation using heparin biosynthetic enzymes. The two ULMW heparins were characterized by one- and two-dimensional nuclear magnetic resonance (NMR) spectroscopy. Their bioactivities were determined by *in vivo* and *in vitro* studies, which showed that the two ULMW heparins exhibited comparable anticoagulant activity to Arixtra.

To facilitate purification steps, a fluorous-tagged disaccharide acceptor was chemically synthesized. At its reducing end, an α-configured *O*-methyl group was employed to mimic the structure of Arixtra. The disaccharide was synthesized through a chemical glycosylation reaction between a protected glycosyl acceptor and donor. The fluorous tag was successfully installed as a carbamate on the reducing end of the acceptor.

Another approach for the preparation of heparin oligosaccharides is through the enzymatic depolymerization of the intact polysaccharide. However, this method results in the formation of an unsaturated double bond in the nonreducing end. We used ozonolysis to cleave the double bond and to remove the sugar residue in the nonreducing end. Ozonolysis was applied to commercially available low molecular weight heparins (LMWH). *In vitro* anticoagulant activity assays showed that ozone-treated LMWH retained their anticoagulant activities.