Developing a solid-phase method for the enzymatic synthesis of heparan sulfate and chondroitin sulfate

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Introduction

- Despite the recent progress on the solution-phase enzymatic synthesis of heparan sulfate (HS) and chondroitin sulfate (CS), solid-phase enzymatic synthesis has not been fully investigated[1].
- Here, we describe the solid-phase enzymatic synthesis of HS and CS using specialized linkers.
- The enzyme-based methods to synthesize a new class of unnatural chimeric HS-CS oligosaccharides has been already reported[2].
- We demonstrate the use of immobilized HS linker to synthesize CS, and the use of immobilized CS linker to synthesize HS. The linkers were then digested with chondroitin ABCase and heparin lyases, respectively, to obtain the products. Our findings uncover a potential approach for accelerating the synthesis of structurally homogeneous HS and CS oligosaccharides.

Methods

- The oligosaccharides were immobilized on DBCO resin by click reaction in PBS 1X buffer at pH 7.4.
- Solid and liquid-phase enzymatic synthesis were carried out using the chemoenzymatic method already reported[3,4,5].
- A small fraction (1uL) of functionalized resin was digested using chondroitin ABCase. A ^{13}C-labelled disaccharide CS-0 standard was added to the digested material and analyzed using LC-MS/MS technique.
- *Compound characterization:* 1D ^{1}H-NMR and 2D NMR (^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, ^{1}H-^{13}C HSQC-TOCSY and ^{1}H-^{13}C H2BC) at 298 K on Bruker Avance III 850 MHz spectrometer. LC–MS performed on a Vanquish Flex UHPLC system (Thermo Fisher Scientific) coupled with TSQ Fortis triple-quadrupole mass spectrometer as the detector.

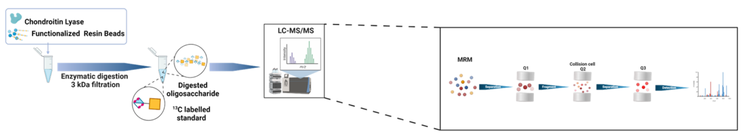

Figure 1. LC-MS/MS method applied to quantify the oligosaccharide bound to the solid support structures using ^{13}C labelled disaccharide standard.

Results

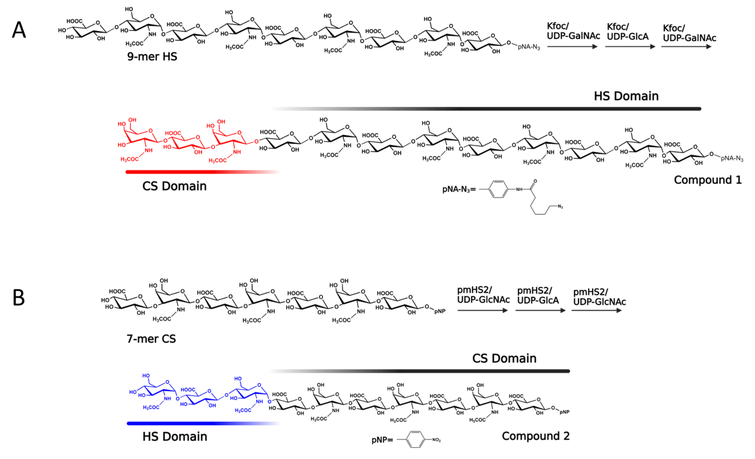

Figure 2. Synthetic schemes of HS/CS and CS/HS chimeric oligosaccharides using the chemoenzymatic method. ***Panel A*** shows the synthesis of CS/HS chimeric dodecasaccharides (12-mer, **Compound 1**). ***Panel B*** shows the synthesis of HS/CS chimeric synthesis of HS/CS decasaccharide (10-mer, **Compound 2**).

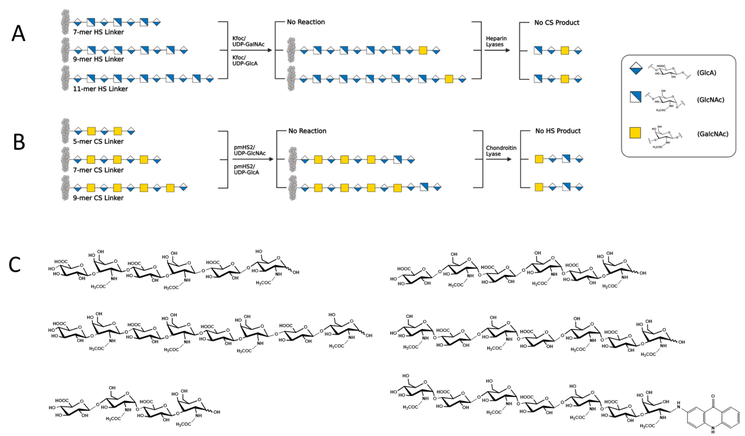

Figure 3. Schematic synthesis of CS and HS oligosaccharides using the solid-phase synthesis approach. ***Panel A*** shows the synthesis of CS oligosaccharides using different lengths of HS linkers. CS tetrasaccharides were obtained after cleavage with heparin lyases (mixture of heparin lyase I, II and III) only from HS 9-mer and 11-mer linkers. No product obtained when HS 7-mer was used as linker. ***Panel B*** shows the synthesis of HS oligosaccharides using CS 5-mer, 7-mer and 9-mer CS linkers. Both CS 7-mer and 9-mer linkers were capable to release HS tetrasaccharides after cleavage with chondroitin lyase (chondroitin ABCase). No product was observed using the CS 5-mer linker.
Panel C Structures of two CS oligosaccharides and four HS oligosaccharides synthesized using the solid phase chemoenzymatic approach.

Conclusion

- Our data demonstrate the feasibility of the chemoenzymatic synthesis of both HS and CS chimeric oligosaccharides using a solid phase approach.
- Although HS/CS chimeric polysaccharides have not been found in nature, our studies clearly demonstrate that the enzymes are fully capable of carrying out such synthesis if the right combination of enzymes, primers and sugar nucleotides is provided.
- Our findings provide a new tool for constructing HS and CS oligosaccharides linkers to keep the substrates away from the solid support to avoid steric hindrance[6]. Our rationale is that both HS and CS linkers have enough rigidity in biological buffers. The linkers are also degradable by either chondroitin ABCase or heparin lyases, offering an enzyme-based method to cleave the products from the solid support with high efficiency and selectivity.
- We demonstrate for the first time the ability of this solid phase chemoenzymatic method to synthesize milligrams of product.

Acknowledgement

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