

Solution Structure of Poly-amido-saccharides

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Statement of Purpose: The synthesis of novel chiral carbohydrate-based polymers will open up new avenues in biomaterials research. Today, natural and synthetic polysaccharides are used in a wide range of applications, including drug delivery and tissue engineering. Most polysaccharides are currently isolated from natural sources, which may require extensive purification and generate unwanted variations between batch-to-batch samples. Therefore, synthetic polysaccharides offer several benefits over their naturally-occurring counterparts, including strict control over molecular weight and distribution, and the ability to control the structure on the monomer level. Studies have shown that the solution structure of polysaccharides greatly influences their function, so an important step in the creation of new synthetic polysaccharides is the characterization of the solution structure. In our group, we have recently synthesized a new type of carbohydrate polymer using a ring-opening polymerization method, in which glucose is linked via an alpha-amide bond to give a poly-amido-saccharide (PAS). The conformational structures and secondary character of these PASs have been investigated through the use of circular dichroism and 2D NMR and compared to a computed model.

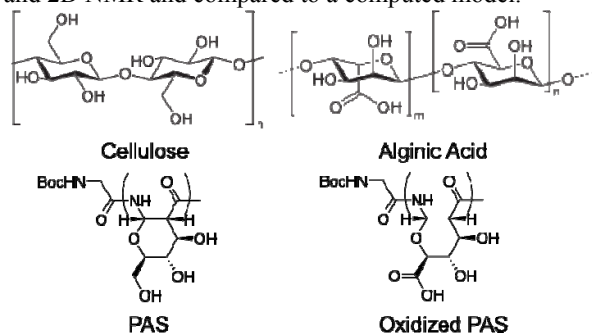


Figure 1. Structure of the PAS and oxidized PAS compared to their natural counterparts.

Methods: Poly-amido-saccharides were synthesized via the controlled anionic ring-opening polymerization of a beta-lactam sugar monomer to provide well-defined chiral polymers with molecular weights ranging from 2,000 to 20,000 g/mol with low dispersity. The polymers were then deprotected to increase their water-solubility, and the primary alcohol was selectively oxidized to a carboxylic acid, to provide a range of materials. Molecular modeling was performed on a representative 12-mer polymer based on gas phase minimization with MMFF94s in Avogadro. NMR experiments were performed on a Varian 500MHz VNMR in D₂O. Circular dichroism (CD) experiments were performed on an Applied Photophysics CS/2 Chirascan using a standard Xenon lamp. Samples were prepared in a 2.5mM phosphate buffer at a concentration of 0.25mg/mL under varying ionic strength, pH, temperature, and denaturing conditions.

Results: Poly-amido-saccharides were successfully synthesized over a wide molecular weight range and

oxidized to various degrees. Molecular modeling of the unoxidized 12-mer based on energy minimization revealed a left-handed helix with 3₁ symmetry. The CD spectrum in water showed a minimum at 221 nm and a maximum at 190 nm, consistent with a helical conformation as seen in previously reported oligo-beta-peptides, which are structurally similar. The CD results suggest that unoxidized PASs maintain their secondary conformation under varying ionic strength, pH, temperature, and denaturing conditions. On the other hand, oxidized PASs showed variation in their secondary conformation, changing based on degree of oxidation (Figure 2), pH, and time in solution. 2D ROESY and NOESY NMR experiments on the oxidized and unoxidized PASs offer further confirmation of a helical structure in solution, based on an NOE observed between two protons on opposite faces of the monomer unit.

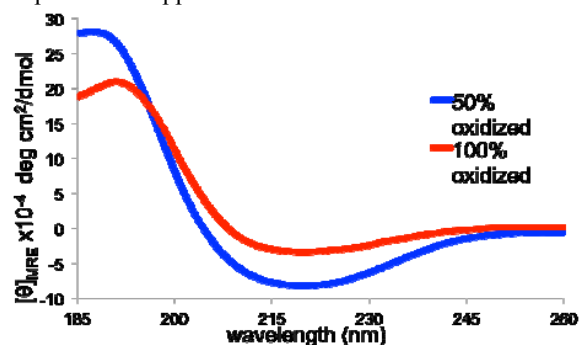


Figure 2. Circular dichroism (CD) spectra comparing degrees of oxidation.

Conclusions: Poly-amido-saccharides present a fascinating new class of synthetic carbohydrate polymers. Molecular modeling, circular dichroism, and 2D NMR indicate that these polymers adopt a helical conformation in solution. While the unoxidized PASs appear to be structurally robust and highly stable to changes in ionic strength, pH, temperature, and denaturing conditions, the secondary structure of the oxidized PASs appears to vary under several conditions. Obtaining crystal structures of these polymers will be a continuing goal for further research. Understanding the structure of these new polymers will indicate new directions of research for the use of these materials in biomedical applications.

References:

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