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Active fragments of natural polysaccharides

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Abstract L-Iduronic acid residues of glycosaminoglycans are in an equilibrium of different conformations, the relative proportion of conformers being a function of sulfation pattern and sequence. This unique conformational flexibility may provide an explanation for the remarkable and versatile biological activities of iduronic acid-containing glycosaminoglycans, such as heparin and dermatan sulfate.

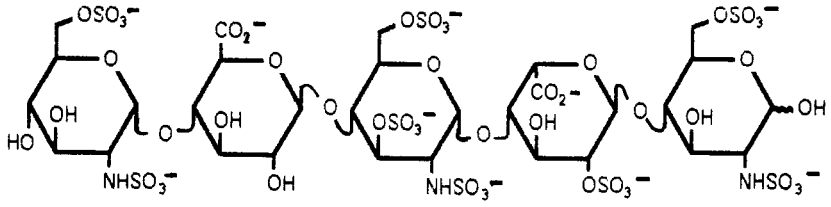
Glycosaminoglycans (GAGs) are anionic polysaccharides widely distributed in animal tissues. They are alternating co-polymers of an uronic acid and hexosamine. A number of the hydroxyl and amino groups of GAGs are sulfated. They may be classified on the basis of their major uronic acid. Whereas the uronic acid of chondroitin sulfates and hyaluronate is D-glucuronic acid (GlcA), the uronic acid of dermatan sulfate and heparin is largely L-iduronic acid (IdoA).

The amounts of these two uronic acid in heparan sulfates varies according to their biological origin. IdoA-containing GAGs have biological properties of current therapeutic interest. Heparin is used as an anticoagulant and antithrombotic agent and recently both heparan sulfate and dermatan sulfate have been shown to have antithrombotic properties in animal models. Several recent reviews have stressed the important role played by IdoA containing GAGs in a wide range of biological phenomena, such as cell growth and differentiation (ref. 1 to 5). The biological activities of these GAGs are associated with binding to plasma or tissue component. The anticoagulant activity of heparin is the consequence of a very specific interaction with antithrombrin III. Indeed a unique pentasaccharide is involved, as was unumbiguously demonstrated by total synthesis (ref. 6). This result clearly points out the importance of organic synthesis in the field. Several other interactions involving IdoA containing GAGs are thought to be nonspecific and related to charge density on the polysaccharide. However, at equal charge density, GlcA-containing GAGs display very weak binding properties and no known biological property. In order to provide an explanation for this remarkable observation, we decided to examine in detail the conformation of a IdoA residue within an oligosaccharide fragment-either natural or synthetic-of well defined structure. Careful analysis of vicinal interproton coupling constants for the synthetic heparin pentasaccharide corresponding to the antithrombrin binding site of heparin suggested that the conformational equilibrium of IdoA could not be accounted for by the classical two chair forms alone. Indeed, by combining the NMR data with the molecular models provided by force-field calculations (ref. 7), it was shown that the skew boat form $^{2}S_{0}$ is an important contributor (64 %) in the conformational equilibrium of sulfated IdoA. NMR studies have then been extended to IdoA in several other sequences. When the sulfated IdoA residue is at the non-reducing end of an oligo or polysaccharide chain, it is predominantly in the $^{1}C_{4}$ conformation. When inside a polysaccharide sequence, IdoA

residues are in equilibrium between only the two forms $^1C_4$ and $^2S_0$, in essentially the same proportions (60 : 40) for both nonsulfated and sulfated residues. A 3-O-sulfo group on the preceding aminosugar residue (as in the pentasaccharide sequence of the binding site for antithrombrin III), drives the equilibrium towards the $^2S_0$ form. Data collected for nineteen mono, di, tri, tetra, penta, hexa and polysaccharides clearly establish the influence of sequence and sulfation on conformational equilibrium of IdoA. At this stage, it is fascinating to speculate on the biological relevance of the conformational flexibility of IdoA. Indeed, the different interactions, either specific or non-specific, in which IdoA containing GAGs are involved with protein can be viewed as a consequence of this flexibility. It may be expected that the change in orientation of sulfate and carboxylate that accompagnies the change in conformation of IdoA residues facilitates interactions of these charged groups "receptors" on the proteins.

Energy calculations have shown that interconversion can easily take place without a major rearrangement of the rest of the chain (ref. 8), making it an easy process. It is striking that a significant change in the conformer populations (from a $^1C_4$: $^2S_0$ ratio 60 : 40 to about 40 : 60) is induced on the sulfated IdoA residues of heparin by 3-O-sulfation on the preceding glucosamine residue. We may speculate that this critical sulfate group, which is essential for eliciting anticogulant and antithrombotic properties of heparin and heparin fragments, modulates the conformation of the corresponding IdoA.

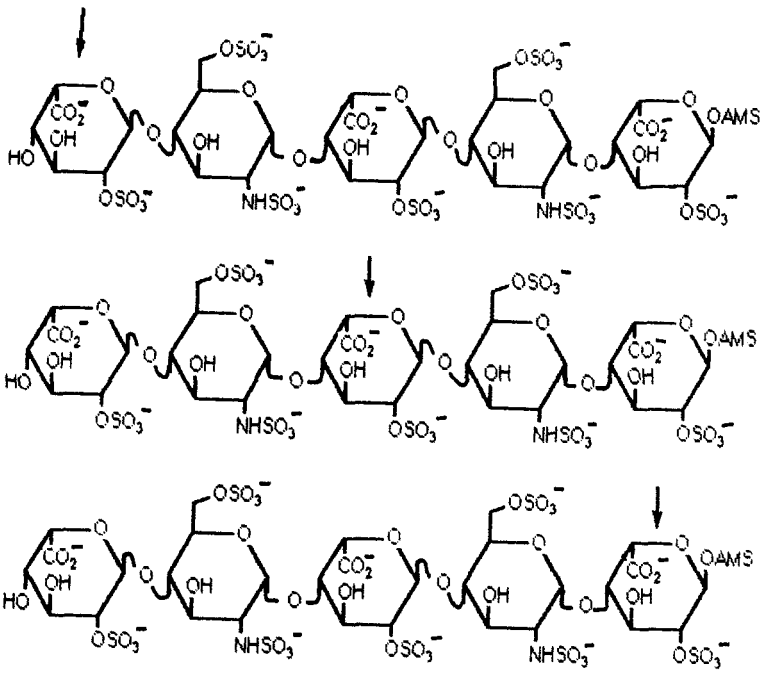
Interconversion of IdoA conformers (especially $^1C_4$ and $^2S_0$ forms) allows the 2-O-sulfo and carboxylate groups an energetically inexpensive search for sites on basic receptors. This could very well be at the basis of the high versatility of IdoA containing GAGs in binding to basic sites of interacting molecules. More precisely, we may propose an explanation for the highly specific interaction between the following unique pentasaccharide sequence (DEFGH) and antithrombrin III.



D E F G H

The DEF trisaccharide moiety of this sequence does not contain any IdoA and is thus a rather rigid structure which may be primarily designed for precise recognition and binding to antithrombrin III. The more flexible, IdoA containing moiety EF, is now in close proximity to a heavily charged zone of the receptor protein and is able to adjust its shape so that overall high affinity binding of DEFGH will result, together with elicitation of the anti-Xa-activity.

In the case of a regular sequence of heparin, made exclusively from IdoA, the lack of a localised rigidity may result in rather nonspecific but significant interaction between this sequence and a receptor protein. As shown in the following scheme, the conformational equilibrium of IdoA residues varies with their location within the chain.



	$^1C_4$	$^4C_1$	$^2S_0$
	95	5	-
	63	7	30
	52	-	48

We can thus appreciate the paramount importance of the biochemical epimerization of D-glucuronic acid to L-iduronic acid.

The possibility of modulating the shape of a polysaccharide chain through changes in the conformation of individual monosaccharide residues is a new concept for this class of biopolymers.

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