A stereochemical approach to pyranose ring flexibility: Its implications for the conformation of dermatan sulfate

(conformational flexibility/glycosaminoglycans/stereochemistry)

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ABSTRACT Glycosaminoglycans, such as heparin, heparan sulfate, and dermatan sulfate, are characterized by a disaccharide repeating unit of a uronate and a hexosamine and are increasingly understood to be important physiologically as soluble components of the extracellular matrix. The secondary structure of this class of acidic polysaccharides is believed to play a key role in determining the wide range of biological specificities. Central to the structural diversity of the glycosaminoglycans is the experimentally documented conformational flexibility of the iduronate residue. Here, we outline an approach to explore the iduronate conformational flexibility by imposing stereochemical criteria of nonbonded contact distances. By performing a complete search of all possible torsions that define the iduronate ring geometry, we eliminate any prior bias with regard to minimum energy conformers. The approach led to alternative feasible conformers for the iduronate ring that are stereochemically satisfactory and are consistent with the available physico-chemical data.

Glycosaminoglycans (GAGs), acidic polysaccharides that play a major role in defining the physical and chemical properties of the extracellular matrix, are characterized by a disaccharide repeating unit of uronic acid (L-iduronic or D-glucuronic) and hexosamine (1). Each uronate is either 1,4 linked to the hexosamine, as in heparin and heparan sulfate, or 1,3 linked as in dermatan and chondroitin sulfates. Further GAGs are highly heterogeneous due to variation in the sulfation of the sugar units (2). Understanding the structure and conformation of GAGs forms the basis of determining structure–function relationships of this important class of biopolymers, ranging from assembly with collagen in the extracellular matrix to binding to growth factors with a high degree of specificity, and regulation of growth factor activity (3–5).

Classical approaches of studying polysaccharide secondary structure have relied upon determining the molecular orientation of the monosaccharide units in relation to the polymer (6). These approaches used a global energy minimum geometry (such as the chair shape) for the monosaccharide units in a polysaccharide. However, in GAGs that contain the α -L-iduronate residue, it is seen that the secondary structure of the polysaccharides cannot be interpreted solely on the basis of the relative orientation of the monosaccharide residues in a disaccharide repeating unit (7). This is due to the fact that standard sugar geometries are unable to explain all the physicochemical data, resulting in controversy (8, 9). For example, in the case of dermatan sulfate, a 4C_1 chair conformation was indicated for both the iduronate and the galactosamine residues, in order to satisfy the repeat

distance of the helical chain obtained from fiber diffraction studies (10). However, both solid-state and solution NMR studies indicate a trans-diaxial orientation of the hydroxyl groups for the iduronate residue, as seen in a 1C_4 chair conformation (11). In the case of dermatan sulfate, the question of how to satisfactorily explain both the x-ray and the NMR data together still remains. Recent conformational analysis of GAG chains has been limited to exploring equienergetic minimum energy conformers (12–14). It has been proposed that in heparin the iduronate exists in an equilibrium between the equienergetic 1C_4 , 4C_1 , and 2T_0 [a twist-boat (15), formerly the skew-boat 2S_0] conformations (16). It is our belief that an inadequate solution to the iduronate conformational discrepancy stems from incomplete analysis of the conformational flexibility of the sugar. A more complete analysis of the sugar ring geometry can be performed by going beyond a global minimum energy conformer and exploring all feasible minimum energy conformers.

In this study, the feasible conformations of dermatan sulfate were determined by defining certain criteria to explore the conformational flexibility of the iduronate ring. We start with the x-ray diffraction evidence that the observed height per residue (h) of the disaccharide units in the helical chains lies between 0.92 and 0.944 nm and that the number of residues per turn (n) lies between two and three (10). The conformational spaces of the iduronate ring that are available for such structures were then determined. We imposed stereochemical criteria of nonbonded contact distances to eliminate disallowed conformers (17) and obtained energetically favorable conformers. This approach led to alternative feasible conformers for the iduronate ring in dermatan sulfate.

METHODS

A data base of stereochemically satisfactory conformations of the disaccharide pairs β -D-GalpNAc4S-(1 $\rightarrow$ 4)- α -L-IdoAp and α -L-IdoAp-(1 $\rightarrow$ 3)- β -D-GalpNAc4S, where IdoA is iduronic acid (Fig. 1), was created as follows. Two structural features were identified as target criteria—a distance of greater than 0.9 nm between the alternate bridge oxygen atoms of the disaccharide (O1 to O1' in Fig. 1) and an absence of short contacts between nonbonded atoms (17). By using the stereochemical criteria of nonbonded contact distances, a search was made by systematically varying a set of independent parameters that changed the conformation of the

Abbreviation: GAG, glycosaminoglycan.

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†The conformation notation used here was derived from ref. 15. The symbols *B* (boat), *C* (chair), *T* (twist-boat), *S* (screw-boat), *H* (half-chair), and *E* (envelope) are used.

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FIG. 1. Disaccharide repeat unit [β -D-GalpNAc4S-(1 $\rightarrow$ 4)- α -L-IdoA β -(1 $\rightarrow$ 3)] of dermatan sulfate (starting geometry taken from ref. 10). Torsions about bonds 1–9 are the independent parameters. The torsions are defined as follows: torsion 1, C1'-O1-C3-C2; torsion 2, O1-C3-C2-C1; torsion 3, C3-C2-C1-O4; torsion 4, C2-C1-O4-C4; torsion 5, C1-O4-C4-C3; torsion 6, O4-C4-C3-C2; torsion 7, C4-C3-C2-C1; torsion 8, C3-C2-C1-O1; torsion 9, C2-C1-O1'-C3'; endocyclic torsion 6 = torsion 6 - 116.7°; endocyclic torsion 7 = torsion 7; endocyclic torsion 8 = torsion 8 - 120°.

iduronate moiety, the relative disposition of the two sugars, and hence the O1–O1' distance.

The independent parameters chosen were the nine torsions of the repeating backbone of the disaccharide, shown in Fig. 1. The torsions about bonds 1 and 9 (1 $\rightarrow$ 3 linkage torsions) and bonds 4 and 5 (1 $\rightarrow$ 4 linkage torsions) vary the relative orientation of the hexosamine and the uronate residues. These torsions, referred to as ϕ_1 , ψ_1 and ϕ_2 , ψ_2 , respectively, were varied from 0° to 360° in discrete steps of 10°. Torsions about bonds 6–8 define the pucker of the iduronate ring. These torsions were allowed to vary in steps of 10° such that the corresponding endocyclic torsions varied from -80° to 80°. Torsions 2 and 3 define the galactosamine pucker. Experimental observation strongly suggests that the galactosamine adopts a 4C_1 -like conformation (18); furthermore, varying these torsions about 4C_1 does not change the O1–O1' distance significantly; torsions 2 and 3 were therefore kept invariant. The bond angles at the bridge oxygens were varied within experimentally observed values (Cambridge Database, Cambridge, U.K.). The O1–O1' distance was thus computed by simultaneously varying the seven torsions mentioned above, and only those torsions that resulted in a repeat distance of more than 0.9 nm were considered for further analysis. Of these torsions, the iduronate ring torsions (6, 7, and 8) for which ring closure was achieved were grouped in sets of defined geometries (15).

For each of the above-mentioned iduronate ring sets, atoms O4, C4, C3, C2, C1, and O1' of the iduronate were fixed (Fig. 1), and the positions of the remaining endocyclic and exocyclic atoms of the iduronate were determined using the CHARMM minimization procedure (Molecular Simulations, Burlington, MA) on a Silicon Graphics (Mountainview, CA) Iris workstation. Fifty steps of the steepest gradient minimization were followed by 500 steps of the adapted basis Newton–Raphson method. Coulombic terms (electrostatic energy) were not used in the energy calculations or during minimization. As a result, the minimized structure represents a stereochemically feasible iduronate ring conformer in that set of the defined geometry and does not reflect the overall global minimum structure. Bond lengths and bond angles of the minimized structures were within experimentally observed ranges (Cambridge Database).

By using the different iduronate conformers obtained after the above minimization and a 4C_1 galactosamine, disaccharide units were constructed. The nonbonded contacts between the atoms of the iduronate ring and those of the neighboring galactosamine were investigated by using stereochemical criteria (17) on the linkage torsions (ϕ – ψ maps). The ϕ – ψ maps of the 1 $\rightarrow$ 3 and the 1 $\rightarrow$ 4 linkage torsions for the galactosamine in the 4C_1 conformation and the iduronate in the different stereochemically feasible conformations were calculated using QUANTA (Molecular Simulations) on a Silicon Graphics Iris workstation. Contours of equal nonbonded energies as functions of ϕ and ψ were used to determine allowed regions of the linkage torsions.

For those allowed values of the linkage torsions (ϕ_1 , ψ_1 and ϕ_2 , ψ_2) that give rise to the experimentally observed n and h values (ref. 10; note that $h > 0.9$ nm), helical chains (of the different disaccharide repeat units) were built using QUANTA. For the three helices corresponding to the 4C_1 , 2T_0 , and 0T_2 iduronate ring, the positions of the exocyclic functional groups (sulfates, acetates, and carboxylates) were established using CHARMM minimization of the corresponding disaccharide units while keeping torsions 1–9 invariant.

The vicinal proton-coupling constants of the uronate residue in the different conformations were calculated using the equation of Haasnoot *et al.* (19). The effect of electronegative substituents on neighboring carbons was also accounted for by using this equation.

RESULTS AND DISCUSSION

Stereochemical Criteria and Feasible Conformers. Application of the O1–O1' distance criteria eliminated several disaccharide conformers. In the remaining disaccharide conformers, the iduronate residues were grouped into seven defined ring geometries (4C_1 , 2T_0 , 4H_3 , 0T_2 , ${}^{2.5}B$, $B_{2.5}$, and $B_{3.0}$). It should be noted that 1C_4 , screw-boat (S), and envelope (E) conformations did not satisfy the criteria.

The molecular energy of the seven conformers of the iduronate ring, minimized to relieve short contacts between the exocyclic atoms, resulted in three conformers, 4C_1 , 2T_0 , and 0T_2 , with very similar nonbonded energies. Furthermore, the bond lengths, the bond angles, and the torsion angles were well within the experimentally observed values (20). It

should be pointed out that the 4H_3 conformer is grouped with 4C_1 because it is a minor 4C_1 variant. A comparison of the ϕ - ψ plots (1 $\rightarrow$ 3 and 1 $\rightarrow$ 4 linkages) of the disaccharides with the iduronate in 4C_1 , 2T_0 , and 0T_2 shows some interesting features. The ϕ - ψ plots of the 0T_2 and the 2T_0 conformers are similar (data not shown). However, the 0T_2 conformer has greater allowed area, and both have allowed regions for the 1 $\rightarrow$ 3 linkage greater than that of the 1 $\rightarrow$ 4 linkage torsions (ϕ_2 , ψ_2) (only 15% of the entire area is allowed for the 1 $\rightarrow$ 4 linkage). The ϕ_2 - ψ_2 plot of the 4C_1 iduronate is similar to the corresponding plots of the 0T_2 and the 2T_0 conformers; the ϕ_1 - ψ_1 plots, however, are different (data not shown). The details of the effect of the sugar pucker on the sterically allowed regions for the torsions will be discussed elsewhere (unpublished observations).

Helical chains were built for 4C_1 , 2T_0 , and 0T_2 iduronate conformations with the linkage torsions (ϕ_1 , ψ_1 and ϕ_2 , ψ_2) varied in the allowed regions. For 4C_1 , 2T_0 , and 0T_2 conformations, the linkage torsions that are well within the allowed regions satisfy the n values of 2, 2.66, and 3 and the h values of 0.92, 0.94, and 0.944 nm observed in x-ray diffraction studies.

It should be pointed out that Ragazzi *et al.* (21) report that a 1C_4 conformer satisfies the distance criteria. However, our entire conformational search about the 1C_4 pucker is consistent with the earlier report (10) that the 1C_4 conformation of the iduronate does not provide the repeat length required for dermatan sulfate. Only 4C_1 , 2T_0 , and 0T_2 conformations for the iduronate in dermatan sulfate satisfy the x-ray diffraction criteria. However, others have interpreted NMR studies as indicating a distorted 1C_4 -like conformer for the α -L-iduronate (11).

NMR and Conformational Polymorphism. Similar to what is seen for heparin, the interproton coupling constants (J) from dermatan sulfate 1H NMR studies indicate that no single iduronate conformation explains the experimental data (22, 23). The J constant variations for the iduronate in heparin have been interpreted to indicate the existence of both 1C_4 and 2T_0 conformations in equilibrium (16). However, for dermatan sulfate, the 1C_4 and 2T_0 conformational equilibrium does not satisfy the experimentally observed J_{12} and J_{34} values (Table 1). While the interproton coupling constants clearly indicate iduronate conformational pluralism, 1H NMR studies, however, are limited in providing a unique solution for the following reasons: (i) the experimentally observed J values vary dramatically depending upon the sample preparation and the saccharide composition and length (16), and (ii) more than one possible combination of the different feasible conformers can explain the observed J values (Table 1).

The most compelling evidence that the iduronate conformer in dermatan sulfate adopts a 1C_4 -like conformer comes

from Winter *et al.* (11). Using x-ray diffraction and solid-state and solution ${}^{13}C$ NMR, they showed the similarity of dermatan sulfate conformations in both solid and solution states and the trans-diaxial orientation of hydroxyl groups for the iduronate, as in a 1C_4 -like conformation. They concluded that a distorted 1C_4 conformation may provide a satisfactory answer to all available physicochemical data. We therefore investigated the geometrical features of the two equienergetic non- 4C_1 conformers (2T_0 and 0T_2) and that of the 1C_4 conformer. In this context, we discovered a geometrical relationship between 4C_1 and 2T_0 , and 1C_4 and 0T_2 conformations (discussed below). Further, to the best of our knowledge, there has been no report of such geometrical similarity between the conformers.

Geometrical Similarity: Monosaccharide Conformers. The orientations of the exocyclic bonds of a 0T_2 conformer are nearly the same geometrically to the corresponding exocyclic

FIG. 2. The geometric projections of the sugar pucker: view a, sugar planar view; view b, view through C1-C4 atoms. (A) Projections of 0T_2 - β -D-glucuronate and 1C_4 - α -L-iduronate. Note the similar dispositions of the functional groups, namely the trans-diaxial orientation of the hydroxyl groups and the equatorial orientation of the carboxylate group. (B) Projections of 4C_1 - β -D-glucuronate and 2T_0 - α -L-iduronate, where the functional groups (hydroxyls and carboxylates) are all equatorially oriented. However, the striking difference between the conformers in a given set is the orientation of the pyranose oxygen atom with respect to the sugar plane.

Table 1. Vicinal coupling constants

Parameter	J_{12}	J_{23}	J_{34}	J_{45}
Experimental	3	6	3.5	3.3
Theoretical				
1C_4	3.1296	4.52	4.4553	0.2564
0T_2	0.98	4.5282	0.8887	8.6149
4C_1	7.8864	9.8976	9.6769	4.4774
2T_0	5.1194	9.3905	5.4085	4.3098
4H_3	3.8643	10.268	9.6769	4.4774
3H_4	1.6865	0.1455	6.7028	0.2564
1T_3	7.2554	7.0631	4.4553	0.2564

The table represents the experimentally determined vicinal coupling constants (22) for the dermatan sulfate iduronate and those calculated for the defined conformers using equation 3 from Haasnoot *et al.* (19). A combination of 1C_4 and 2T_0 for the iduronate does not satisfy the J values. The best-fit J values for dermatan sulfate iduronate are with a combination of 1C_4 , 0T_2 , and 2T_0 conformers.

bonds of a 1C_4 conformer, in particular the trans-diaxial orientations of the hydroxyl groups. Fig. 2A shows the projections of 0T_2 - β -D-glucuronate and 1C_4 - α -L-iduronate. The disposition of the hydrogen atoms for the two conformers is very similar, except for the hydrogen at the chiral carbon (C5) atom. The equatorial position of the C5—C6 bond of the 1C_4 - α -L-iduronate, however, is identical only to a β -D-glucuronate in the 0T_2 conformation, a property of the asymmetric carbon atom. Such a geometric similarity is also seen between 4C_1 and 2T_0 conformations (Fig. 2B). The orientation of all the exocyclic atoms (non-hydrogen atoms) in 2T_0 - α -L-iduronate is equatorial, as in a 4C_1 - β -D-glucuronate conformation. Moreover, the carboxylate group of 2T_0 - α -L-iduronate is in the equatorial position, similar to the one in 4C_1 - β -D-glucuronate. Fig. 2B shows that a 4C_1 - β -D-glucuronate is almost identical to a 2T_0 - α -L-iduronate. However, on scrutiny, this geometrical similarity does not extend to 4C_1 - α -L-iduronate and 2T_0 - β -D-glucuronate or to 1C_4 - β -D-glucuronate and 0T_2 - α -L-iduronate. Thus, a 0T_2 conformation is geometrically similar to a 1C_4 conformer, and a 2T_0 conformation is similar to a 4C_1 conformer.

Geometrical Similarity: Dermatan Sulfate Helices. The above geometrical similarity, seen at the monosaccharide

level, can be extended to the polysaccharide level. NMR studies on GAGs present in physiological settings have indicated that GAGs possess two-fold symmetry (24). Polysaccharide helical structures with two-fold symmetry were generated for the dermatan sulfate chain with the iduronate in the 2T_0 and 0T_2 conformations, respectively, and the galactosamine in a 4C_1 conformation (Fig. 3). Also shown for comparison in Fig. 3 is a 2-fold helix of chondroitin 4-sulfate with D-glucuronate in 4C_1 . The 2-fold helices constructed with the uronate in the 2T_0 -L- and 4C_1 -D-conformations have similar dispositions of all functional groups, especially the axial orientation of most of the CH groups that contribute to the hydrophobic patches (Fig. 3D). However, if the iduronate were in 0T_2 conformation, this hydrophobic patch would be eliminated because the CH groups would no longer cluster together (Fig. 3C). The sulfate and the carboxylate charges are clustered on the same face of the helix containing the iduronate in a 0T_2 conformation, whereas they are uniformly disposed at about 90° apart along the helix containing the iduronate in a 2T_0 conformation. Furthermore, in a helix containing the iduronate in a 2T_0 conformation, the carboxylate is equatorial to the plane of the sugar, whereas it is axial in a helix containing the iduronate in a 0T_2 conformation.

The high degree of similarity of a helix containing the iduronate in a 2T_0 conformation to a helix containing 4C_1 -D-glucuronate is unexpected, since the monomer orientation is preserved in the context of the helix in spite of the differences in the linkage torsions in the two helices. This similarity could have significance in the C5 epimerization reaction, where the uronosyl C5 epimerase could have either uronate isomer as substrate (25).

FIG. 3. Computer-drawn projections of 2-fold helical structures of GAG. (A) Chondroitin 4-sulfate helix containing 4C_1 - β -D-glucuronate. (B) Dermatan sulfate helix containing 0T_2 - α -L-iduronate in the repeat unit. (C) Dermatan sulfate helix containing 0T_2 - α -L-iduronate in the repeat unit. Note the overall similarities of the two helices in A and B, specifically in the dispositions of the sulfates and the carboxylates. Also, note the similarity in the orientation of the CH groups (●) and hence the hydrophobic patches as shown in D (Corey-Pauling-Koltun model of helices in A and B). The carboxylate in C is axial, at right angles to the plane of the paper, thus bringing this group closer to the sulfate group; however, in A and B, the carboxylate group is equatorial, along the plane of the paper, away from the sulfate. (E) Helices in A and B superimposed in a projection, to highlight the similarity of the two conformers.

FIG. 4. Computer-drawn models of the 2-fold (A), 83-fold (B), and 3-fold (C) helical structures of dermatan sulfate containing the iduronate in the 0T_2 conformation. (Upper) The structures viewed perpendicular to the helical axes. (Lower) Projected view down the helical axes.

In the case of chondroitin 4-sulfate, the experimentally observed intramolecular hydrogen-bonding scheme and the hydrophobic patch contributed by the axially oriented CH groups (Fig. 3) that aid in chondroitin 4-sulfate self-aggregation are consistent with a 4C_1 conformation for the glucuronate (26). However, NMR studies on dermatan sulfate in dimethyl sulfoxide indicate the presence of very weak hydrogen bonding, if any. Further, dermatan sulfate has limited self-aggregation properties (24). If the iduronate in dermatan sulfate were in a 2T_O conformation, its hydrogen-bonding schemes and self-aggregation properties would be very similar to those of chondroitin 4-sulfate. In other words, dermatan sulfate containing the iduronate in a 2T_O conformation would be very similar if not almost identical to that of chondroitin 4-sulfate (Fig. 3). The lack of self-aggregation properties of dermatan sulfate thus does not favor a 2T_O conformation for the iduronate in dermatan sulfate.

In addition to the 2-fold helix, GAGs exist in 8₃-fold and 3-fold helices (10). Fig. 4 shows the 8₃-fold and 3-fold helices for dermatan sulfate with the iduronate in a 0T_2 conformation. The cross-section view indicates that the 2-fold helix is more compact than the 8₃- or the 3-fold helices. The orientation of the carboxyl groups in relation to the 4-sulfate of the galactosamine in these helices is different. Interestingly, while the relative orientation of 2T_O -L- and 4C_1 -D-monosaccharide units are preserved in a 2-fold helix (Fig. 3), this feature is not preserved in the 3-fold helix.

Thus, we show by imposing stereochemical criteria of nonbonded contact distances alternate feasible conformers for the iduronate residue in dermatan sulfate—in particular, the 0T_2 conformer, which not only has nearly the same nonbonded energy as the 4C_1 and 2T_O conformers but also satisfies the experimental data for dermatan sulfate.

Earlier conformational analyses on sugars of nucleic acids and polysaccharides have been performed with rigid sugar geometries (6, 27, 28). However, conformational flexibility is increasingly seen in several biopolymers. Realization of the inherent conformational flexibility of the sugar geometry in nucleic acids was the key to the understanding of conformational polymorphism of DNA and its biological significance (28). In the case of acidic polysaccharides such as heparin and dermatan sulfate, the role of the carbohydrate moiety in providing a high degree of specificity to the range of physiological functions (29) may stem from its ability to be conformationally flexible. Addressing the conformational flexibility of the iduronate by adopting a stereochemical rather than a global minimum energy approach leads us to several feasible conformers; depending upon the environment (e.g., such as the counter ions or solvation effects), a structure among the feasible conformers may be preferred. A thorough stereochemical conformational search of heparin, therefore, could identify feasible saccharide conformers and their role in heparin's structure–function activities.

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