Gas-Phase and Solution Conformations of the α-L-Iduronic Acid Structural Unit of Heparin

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The IdoA2S structural unit of heparin (subunit G) may oscillate among the three conformations ($^4C_1$, $^1C_4$, and ^2S_o). Only the twisted boat conformation allowed the biologically active pentasaccharide unit of heparin (DEFGH) to bind to antithrombin. Our work reports, in detail, the results of systematic large-scale theoretical investigations of the three basic conformations ($^4C_1$, $^1C_4$, and ^2S_o) of the IdoA2S structural unit of heparin, its anionic forms, and its sodium salt using the B3LYP/6-311++G(d, p) and B3LYP/6-31+G(d) model chemistries. According to our calculations, the most stable structure of these molecules corresponds to the ^2S_o skew-boat conformation. This form is also the most stable in a water solution. The ^2S_o conformation of neutral molecules is not maintained in the anionic species. With anions, both $^1C_4$ and $^4C_1$ conformations are present. The relative stability of individual species of the substituted iduronic acid affects extra stabilization by means of intramolecular hydrogen bonds. The calculated macroscopic pK_a of 1,4-DiOMe IdoA2S are as follows: $pK_a = 0.25$ for the terminal $C(2)-OSO_3H$ group, $pK_a = 3.67$ for the terminal $C(5)-CO_2H$ group, and $pK_a = 14.00$ for the $C(3)-OH$ hydroxyl group. The computed Gibbs interaction energies, $\Delta G°$, for the reaction 1,4-DiOMe IdoA2S^{2-} + 2Na$^+$ ⇌ 1,4-DiOMe IdoA2SNa$_2$ ($^4C_1$, $^1C_4$, and ^2S_o conformations) are negative and span a rather small energy interval (from −1244 to −1290 kJ mol^{-1}).

1. INTRODUCTION

Heparin (HP) and structurally related heparan sulfate (HS) are a family of macromolecules (glycosaminoglycans) found in virtually all tissues in a wide variety of species.[1−4] HP and HS are chemically similar except for the content of *N*- and *O*-sulfo groups, the content of *N*-acetyl groups, and the ratio of the type of hexuronic acids.[3,5] Sulfo groups in HP appear to play an important role in various biological effects of this polymer.[5,6] Heparin is used in clinics for the prevention and treatment of thrombosis.[2] Its main antithrombotic activity is explained by its ability to potentiate the activity of serine protease inhibitor antithrombin III (AT-III), which inactivates a number of serine proteases—such as thrombin and factor Xa—in the coagulation cascade.[1,6] The action of anticoagulants starts when they bind to antithrombin through a group of five subunits (DEFGH). It was found that this unique pentasaccharide fragment (PS) constitutes the minimal binding domain for AT-III. It is now possible to synthetically produce the active subunit (DEFGH) of heparin,[7−9] which has been recently introduced on the market (fondaparinux sodium).[10] The preparation of PS and its many analogues led to the establishment of the structure−activity relationships of heparin-like pentasaccharides.[11] It was found that the subunit G of the pentasaccharide DEFGH oscillated among the three conformations ($^4C_1$, $^1C_4$, and ^2S_o; Figure 1). Only the twisted boat conformation allowed the pentasaccharide DEFGH to bind to antithrombin.[11] Despite these interesting properties, the glycosaminoglycans remain one of the struc-

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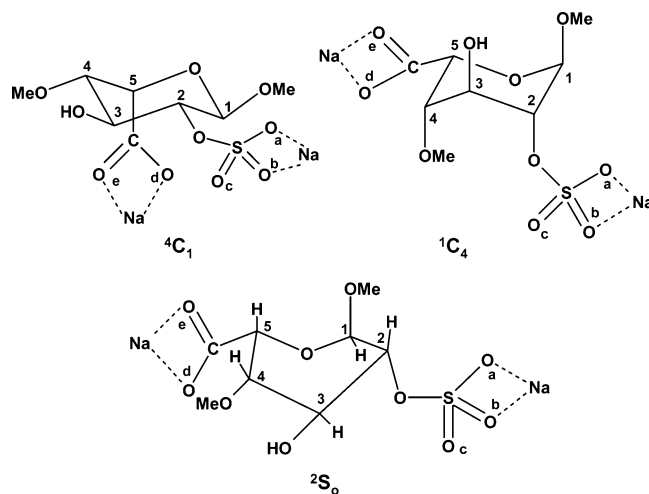† Comenius University.
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Figure 1. Structure and atom numbering of the 1,4-DiOMe IdoA2S species investigated.

turally less-well-characterized classes of saccharides. The available theoretical studies of heparin and heparin−protein interactions are limited to molecular mechanics and dynamics calculations.[6,12−15] However, there is still no molecular mechanics force field parametrization capable of adequately reproducing all polysaccharide conformational features.[15]

The present paper reports, in detail, the results of systematic large-scale theoretical investigations of the three basic conformations ($^4C_1$, $^1C_4$, and ^2S_o) of the IdoA2S structural unit of heparin (subunit G), its anionic forms, and its sodium salt. The actual calculations were performed with the 1,4-DiOMe IndoA2S derivative. Of particular interest are the molecular geometries, acidities, and sodium affinities. The

THE A-L-Iduronic Acid Structural Unit of Heparin

J. Chem. Inf. Model., Vol. 46, No. 3, 2006 **1195**

effect of solvent (water) on the structure and stability of the IdoA2S species was also investigated. These data are discussed and compared with the available experimental results for structurally related systems.

2. COMPUTATIONAL DETAILS

The geometry of 1,4-DiOMe IndoA2S, its anions, and its sodium salt (Figure 1) has been completely optimized with the Gaussian 03 and Jaguar 6.0 program systems,[16,17] using density functional theory[18,19] B3LYP/6-311++G(d, p) and B3LYP/6-31+G(d) methods.[20-22] The solvated systems were treated with a self-consistent reaction field method, using the Poisson–Boltzmann solver of Jaguar.[23,24]

The interaction enthalpy, ΔH^{298}, for the reaction of a sodium cation with Lewis bases (reaction A) is given by eq 1:

$$Na^+(g) + L^-(g) \rightarrow NaL(g) \tag{A}$$

$$\Delta H^{298} = \{E^{298}(Na^+\cdots L^-) - [E^{298}(L^-) + E^{298}(Na^+)]\} + \Delta(pV) \tag{1}$$

where $E^{298}(Na^+)$ and $E^{298}(L^-)$ are the energies of the sodium cation and ligand molecules, respectively, and $E^{298}(Na^+\cdots L^-)$ is the energy of the complex corrected for thermal energy at $T = 298.15$ K. For the work term in eq 1, we substituted $\Delta(pV) = -RT$.

The gas-phase acidity, $\Delta E(A)$, was defined as the energy of deprotonation, ΔE, for reaction A.

$$AH(g) \rightarrow A^-(g) + H^+(g) \tag{B}$$

The energy of deprotonation, ΔE, at $T = 0$ K was computed using eq 2:

$$\Delta E(A) = E(A^-) - E(AH) \tag{2}$$

where $E(AH)$ and $E(A^-)$ stand for the total energies of the stable conformations of the acid and its anion. The enthalpy of deprotonation, ΔH^{298}, was computed using eqs 3 and 4:

$$\Delta H^{298}(A) = \Delta E^{298}(A) + \Delta(pV) \tag{3}$$

$$\Delta E^{298}(A) = [E^{298}(A^-) + 3/2RT] - E^{298}(AH) \tag{4}$$

where E^{298} stands for the total energies of the stable conformations of the acids and their anions (including the thermal energy correction at $T = 298.15$ K). In eq 3, we substituted $\Delta(pV) = RT$ (one mole of gas is obtained in reaction B). The gas-phase Gibbs energy, ΔG^{298}, of the proton abstraction reaction may be calculated from

$$\Delta G^{298} = \Delta H^{298} - T\Delta S^{298} \tag{5}$$

The enthalpy of deprotonation was calculated using expression 3. The entropy contribution is given by

$$-T\Delta S^{298} = -T[S(A^-) + S(H^+) - S(AH)] \tag{6}$$

For $T = 298$ K at the standard pressure, the second term $TS(H^+) = 32.5$ kJ mol^{-1}.[25] Thus,

$$\Delta G^{298} = \Delta H^{298} - T[S(A^-) - S(AH)] - 32.5 \tag{7}$$

Notice that there is an inverse relationship between the magnitude of ΔG and the strength of the acid. The more

positive the value of ΔG, the weaker the acid. It has been shown[26] that Becke 3LYP triple-ζ calculations reproduce thermodynamic quantities of the cation–Lewis base complex within the targeted accuracy of about 10 kJ mol^{-1}. Hence, density functional theory is suitable as an alternative to traditional ab initio methods for studying larger complexes. Momany et al. has shown that the B3LYP/6-311++G(d,p) level of theory also gives reliable geometries and conformational energies of carbohydrates.[27-29]

3. RESULTS AND DISCUSSION

3.1. Geometry of 1,4-DiOMe IndoA2S and the 1,4-DiOMe IndoA2S Sodium Salt. An analysis of the harmonic vibrational frequencies at the B3LYP/6-311++G(d,p) and B3LYP/6-31+G(d) levels of theory of the optimized monomers and complexes revealed that these systems are minima (zero number of imaginary frequencies). The selected structural parameters of the fully optimized 1,4-DiOMe IndoA2S and 1,4-DiOMe IndoA2S sodium salt computed at the higher B3LYP/6-311++G(d,p) level of theory are given in Tables A and B of the electronic Supporting Information. The 1,4-DiOMe IndoA2S and 1,4-DiOMe IndoA2S sodium salt molecules are 2-substituted iduronic acids in the L form, and the starting conformations of the pyranose ring for optimization were set to the $^4C_1$ and $^1C_4$ chair-form and ^2S_o skew-boat conformations (Figure 1). These conformations were taken because they are the prevalent forms, depending on the substitution pattern of this residue and on its relative position in the chain, in heparin and its derivatives when observed in high-resolution NMR spectra.[30-33]

1,4-DiOMe IndoA2S. Substituted iduronic acid derivatives represent the simplest models for the L-iduronic acid residues in biologically important heparin, dermatan sulfate, and heparan sulfate.[34] The conformational structure of this residue in both neutral and ionized states was investigated using 1,4-DiOMe IndoA2S systems. The 1,4-DiOMe IndoA2S molecule models unit G of the typical fragment of heparin, in which two neighboring (units F and H) structural units of heparin bound by the (1−4) glycosidic bonds are substituted by the methyl groups. Some trends are apparent. An examination of the space models for the $^4C_1$, $^1C_4$, and ^2S_o conformations of 1,4-DiOMe IndoA2S indicate that these conformations are also preserved in the resulting fully optimized structures. Gas-phase conformers of this acid are stabilized via intramolecular hydrogen bonds. The $^4C_1$ conformation of 1,4-DiOMe IndoA2S is stabilized by the system of two intramolecular SO−H⋯OC(3) and C(3)O−H⋯OC(4) hydrogen bonds with lengths of 1.776 and 2.332 Å, respectively, which is less than the sum of the van der Waals radii[35] of hydrogen and oxygen atoms (2.7 Å). The same system of intramolecular hydrogen bonds is also present in the twisted ^2S_o conformer with bond lengths of the SO−H⋯OC(3) and C(3)O−H⋯OC(4) hydrogen bonds equal to 1.774 and 2.224 Å, respectively. The existence of such intramolecular hydrogen bonds is, because of stereochemistry, not possible in the $^1C_4$ conformer. This conformer is, however, stabilized by the intramolecular hydrogen bond C(3)O−H⋯OC(1) with an optimal hydrogen-bond distance of 2.043 Å.

A different hydrogen-bonding pattern was found in the optimized dianions of the substituted L-iduronic acid studied. The $^1C_4$ conformation is again stabilized by the intramo-

lecular hydrogen bond C(3)O−H⋯OC(1) with a slightly longer optimal hydrogen-bond distance of 2.163 Å. In the $^4C_1$ form, the unsubstituted C(3)O−H group takes part in the charged intramolecular C(3)O−H⋯$^-$O−S hydrogen bond. The strong electrostatic attraction in this hydrogen bond results in a much shorter optimal hydrogen-bond distance of 1.840 Å. The ^2S_o conformation of 1,4-DiOMe IndoA2S is, however, stabilized by the neutral five-membered intramolecular hydrogen bond C(3)O−H⋯OC(4) with an optimal hydrogen-bond distance of 2.099 Å. The observed large changes in the hydrogen-bond patterns of the $^4C_1$, $^1C_4$, and ^2S_o conformations in the 1,4-DiOMe IndoA2S species upon ionization are accompanied with the rotation of the C(3)O−H, −O−SO₃H, and −OSO₃⁻ groups {dihedral angles Φ[C(2)−O(2)−S−O(a)] and Φ[C(2)−C(3)−O−H], respectively}, see the Supporting Information. Thus, for the C(3)OH group, the existence of different conformations in the individual species studied is characteristic {dihedral angle Φ[C(2)−C(3)−O−H]}. Its relative stability depends on the form of the ring and on the character of neighboring substituents. The geometry of the 2-O-sulfo group of the 1,4-DiOMe IndoA2S species studied is different in the $^4C_1$ and $^1C_4$ chair-form and ^2S_o skew-boat conformations. The space orientation of this group is similar for the $^4C_1$ chair-form and ^2S_o skew-boat conformations of neutral substituted iduronic acid. The situation is different with anionic forms. The −OSO₃⁻ group in both gas-phase and solvated structures adopts, in the three conformations studied, different conformations, which results from the competition for electrostatic interaction between intramolecular interactions, and this allows the C(2)−OSO₃⁻ group to be nearly freely rotating rather than strongly directed toward the near C(3)−OH hydrogen-bond donor group.

The effect of a solvent (water) on the geometry of the 1,4-DiOMe IndoA2S species studied was examined using the Poisson−Boltzmann solver of Jaguar. The compounds investigated contain a small number of rotatable bonds, and thus, one does not expect large changes in geometry upon solvation. Selected bond lengths, bond angles, and dihedral angles of the most stable species obtained by the B3LYP/6-311++G(d,p) method and Poisson−Boltzmann model of the solvent are also presented in the Supporting Information. The optimal bond lengths and bond angles of the pyranose ring of the species studied computed with the self-consistent reaction field method and solvent water do not considerably differ from those obtained for isolated molecules. However, the functional groups of individual 1,4-DiOMe IdoA2S species in a water solution exhibit different conformational changes. The solvated $^4C_1$ structure is also stabilized by two intramolecular SO−H⋯OC(3) and C(3)O−H⋯OC(4) hydrogen bonds with lengths of 1.575 and 2.680 Å, respectively. The C(3)O−H⋯$^-$OS intramolecular hydrogen bonds with lengths of 1.840 and 1.892 Å respectively stabilize both the gas-phase and solvated anionic $^4C_1$ conformers. Solvation of the neutral $^1C_4$ conformer causes breaking of the C(3)O−H⋯OC(1) intramolecular hydrogen bond and considerable change of the Φ[C(2)−C(3)−O−H] dihedral angle (40.4° and −169.3° for the gas-phase and solvated system, respectively). In the $^1C_4$ anionic form, however, the intramolecular C(3)O−H⋯OC(1) hydrogen bond is preserved. Much larger changes of the equilibrium geometry upon solvation were found for the ^2S_o skew-boat conformer. While the gas-

phase conformer is stabilized by two intramolecular hydrogen bonds [SO−H⋯OC(3) and C(3)O−H⋯OC(4)], in the solvated ^2S_o conformation, only one "short" intramolecular SO−H⋯OC(3) hydrogen bond with a distance of 1.500 Å is present.

1,4-DiOMe IndoA2S Sodium Salt Species. An examination of the space models for the $^4C_1$, $^1C_4$, and ^2S_o conformations of the 1,4-DiOMe IndoA2S sodium salt indicate that these conformations are also preserved in the resulting fully optimized structures. The relevant structural parameters of three conformations of the 1,4-DiOMe IndoA2S sodium salt and their anionic species are presented in the Supporting Information. The ionization of the respective sodium salts results in structural rearrangements of anions. The sulfate group in the gas-phase species investigated is essentially tetrahedral with O−S−O angles ranging from 97° to 104°. The pattern of the bond lengths S−O(a) and S−O(b) in the sodium salt of 1,4-DiOMe IndoA2S allows the bonds to be recognized as S−O bond types with considerable resonance bond character. The S−O(c) bond in the sodium salt of 1,4-DiOMe IndoA2S is not involved in coordination to the sodium cation and may be recognized as a S=O bond type with optimal lengths in the range 1.44−1.45 Å. The S−O(c) bond length is similar to that of the typical S=O (1.514 Å) in (CH₃)₂S=O computed by us at the B3LYP/6-311++G-(d,p) level of theory. Thus, the coordination of the sodium cation to the O(a) and O(b) oxygen atoms of the sulfate group via the bifurcated bond in neutral sodium salts of 1,4-DiOMe IndoA2S results in an appreciable shortening of the free S−O(c) bond. For both carboxylate and sulfate sodium ion complexes, bidentate (direct) bonding was found in the relatively short O⋯Na separation range (2.2−2.5 Å). The O(a)⋯Na⋯O(b) angle of the "bifurcated" sodium bond lies within the relatively narrow interval of 60−65°. The sodium cation of the −OSO₃⁻⋯Na⁺ group is always tridentated in gas-phase complexes. In the $^4C_1$ and $^1C_4$ chair conformers, this Na⁺ atom is in coordination with the ether oxygen atom of the C(1)−OCH₃ group. In the ^2S_o skew-boat conformation, the sodium cation is coordinated to the oxygen atom of the C(3)OH group. The carboxyl group is always dicoordinated and almost symmetrically bonded to both oxygen atoms of the carboxyl group in the $^4C_1$, $^1C_4$, and ^2S_o conformations. Removal of the two sodium cations leads to a structural change of the −OSO₃⁻ and −COO− moieties {dihedral angles Φ[C(1)−C(2)−O(2)−S)], Φ[C(2)−O(2)−S−O(a)], and Φ[O(5)−C(5)−C(6)−O(d)]}. Ionization of the −OSO₃⁻⋯Na⁺ bond caused elongation of the O(2)−S bond (by about 0.05 Å) and shortening of the S−O(a) and S−O(b) bonds. Three S−O bonds of the −OSO₃⁻ group are geometrically equivalent and possess considerable double-bonded character. In the anionic species studied, the C(3) hydroxyl group forms intramolecular hydrogen bonds. In the $^4C_1$ and ^2S_o conformations, intramolecular hydrogen bonds C(3)O−H⋯O−C(4) with optimal lengths in the range 2.0−2.3 Å are present. This hydrogen bond is, because of stereochemistry, impossible in the $^1C_4$ conformation, and this chair form is stabilized via intramolecular hydrogen bond C(3)O−H⋯O−C(2) with an optimal length of 2.158 Å.

The geometries of the optimized sodium salt of 1,4-DiOMe IndoA2S and its anions in a water solution are also presented in the Supporting Information. Hydration of the $^4C_1$, $^1C_4$, and ^2S_o conformers caused appreciable geometry changes,

THE A-L-Iduronic Acid Structural Unit of Heparin

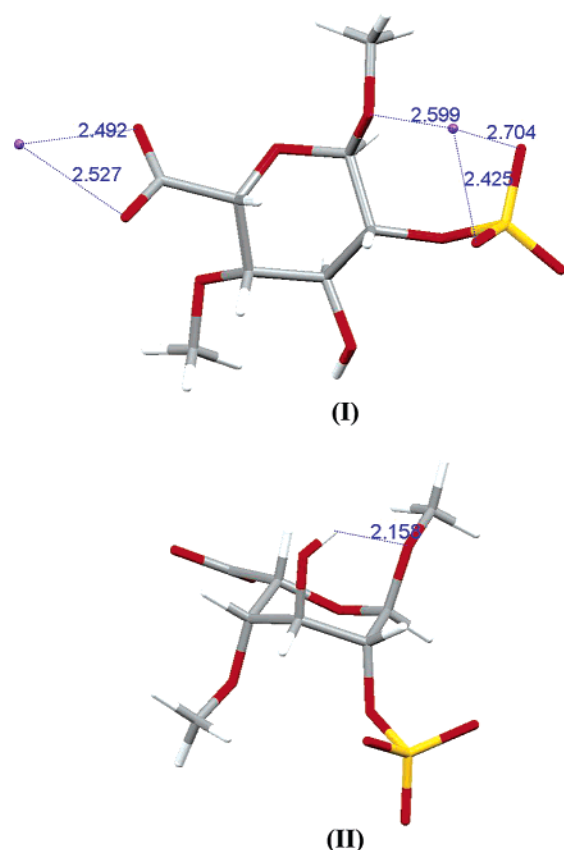*J. Chem. Inf. Model., Vol. 46, No. 3, 2006* **1197**

Figure 2. Solution structure of the $^1C_4$ conformation of the 1,4-DiOMe IdoA2S sodium salt (I) and its anion (II).

especially for substituents and hexopyranose rings. In most cases, considerable lengthening of the sodium $O^-\cdots Na^+$ bonds (by about 0.2−0.4 Å) upon hydration is observed. The tridentated sodium bonds of the $-OSO_3^-\cdots Na^+$ moiety are also preserved in the solvated $^4C_1$ and $^1C_4$ conformers. On the other hand, in the solvated ^2S_o conformer, the sodium cation forms two bonds ($S-O^-\cdots Na^+$ and $C(3)-O\cdots Na^+$) with lengths of 2.377 and 2.413 Å, respectively. The molecular structure of the solvated 1,4-DiOMe IndoA2S sodium salt (conformer $^1C_4$ I and its dianion II) is illustrated in Figure 2. Figure 3 shows differences in the geometries of the $^4C_1$, $^1C_4$, and ^2S_o conformations of substituted iduronic acid and its anions optimized in the gas phase and of the solvated structure that was optimized in solution.

An examination of the space model for the chair $^4C_1$ and $^1C_4$ conformations indicates that the solvent (water) treated within the self-consistent reaction field method does not considerably change their computed gas-phase equilibrium geometries. However, much larger changes of the equilibrium geometry were observed for the ^2S_o skew-boat conformer for both the neutral and anionic forms (Figure 3). According to the present theories of the binding of heparin and other iduronic acid-containing glycosaminoglycans,[36,37] it is the ^2S_o conformation of the iduronic acid moiety which represents the "active" residue structure responsible for its bonding to the interaction site of a protein. The necessary conformational change of the heparin by its interaction with proteins requires a certain "plasticity" of its chain. This flexibility of heparin in its interaction with proteins is mainly ascribed to the iduronic acid structural unit. Our calculations of the $^4C_1$, $^1C_4$, and ^2S_o conformations of substituted iduronic acid and its

dianions are in agreement with present theories of heparin biological activity.[36,37] The $^4C_1$ and $^1C_4$ chair conformers show a certain conformational rigidity. However, the biologically active ^2S_o form of the iduronic moiety exhibits, upon influence of the environment, considerable flexibility (Figure 3). Accordingly, it can adjust its shape for proper interaction with the receptor-bonding site of a protein.[36−39]

3.2. Relative Energies. The relative energies of individual conformations for the acid and sodium salt of 1,4-DiOMe IndoA2S with respect to the most stable neutral species computed for the gas-phase and solvated molecules are reported in Table 1. For solvated geometry optimizations, the solvation energy was computed as the difference between the energy of the optimized gas-phase structure and the energy of the solvated structure that was optimized in solution. Previous experimental NMR and theoretical molecular mechanics studies have shown that the iduronic acid pyranose bearing a sulfate group at C(2) is a rather flexible structure with the $^1C_4$ chair and ^2S_o skew-boat preferred conformations.[38−41] According to our calculations for 1,4-DiOMe IndoA2S and the 1,4-DiOMe IndoA2S sodium salt, the most stable structure corresponds to the ^2S_o skew-boat conformer. This conformer is also the most stable in a water solution. The relative stabilities of the individual conformations of 1,4-DiOMe IndoA2S decrease as follows: $^2S_o > {}^4C_1 > {}^1C_4$ (gas-phase and solvated structure). The same order of stability of the individual conformations was found also in the gas-phase 1,4-DiOMe IdoA2S sodium salt (Table 1). Hydration of the 1,4-DiOMe IdoA2S sodium salt changed the relative conformational stability of the hexopyranose ring conformations. The computed relative stability decreases in the order $^2S_o > {}^1C_4 > {}^4C_1$. The relative stability of the three 1,4-DiOMe IdoA2S conformations investigated is prevailingly influenced by the occurrence of the intramolecular hydrogen bonds formed by the C(3)OH and SOH groups and, in the case of the sodium salt, the selective coordination of the Na^+ cations to individual oxygen coordination centers of the conformers studied (as it is also discussed in the preceding section).

Because polyanionic species of heparin are biologically active forms, consequently, the most prominent type of interaction between heparin and proteins is ionic.[6] Thus, the situation with anionic species is more interesting. The most stable ^2S_o conformation of neutral molecules is not maintained in the anionic species. For isolated anions, both the $^1C_4$ and $^4C_1$ conformations may be present. The $^1C_4$ conformation was computed to be the most stable one in solvated anions (Table 1). The differences in the relative stability of individual dianions of the substituted iduronic acid and its salt are apparently caused by different extra stabilization by means of the intramolecular hydrogen bond. The $^4C_1$ chair dianion of the acid stabilizes the $C(3)O-H\cdots O-S$ hydrogen bond. Because of the negative charge on the sulfo group, this hydrogen bond is strong and is also preserved in the solvated anion. The $^1C_4$ and ^2S_o anions are stabilized by much weaker intramolecular hydrogen bonds. For the solvated ^2S_o anion, the equilibrium conformation without hydrogen bonds was found (Table 1). The results of our calculations are in good agreement with experimental findings about the stereochemistry of the pyranose ring of the iduronic acid. Unsubstituted iduronic acid exists predominantly in the $^1C_4$ form,[40,42] and the iduronic acid building unit of heparin bearing a sulfate group at C(2) prefers the $^1C_4$ and ^2S_o conformations.[36,38,41]
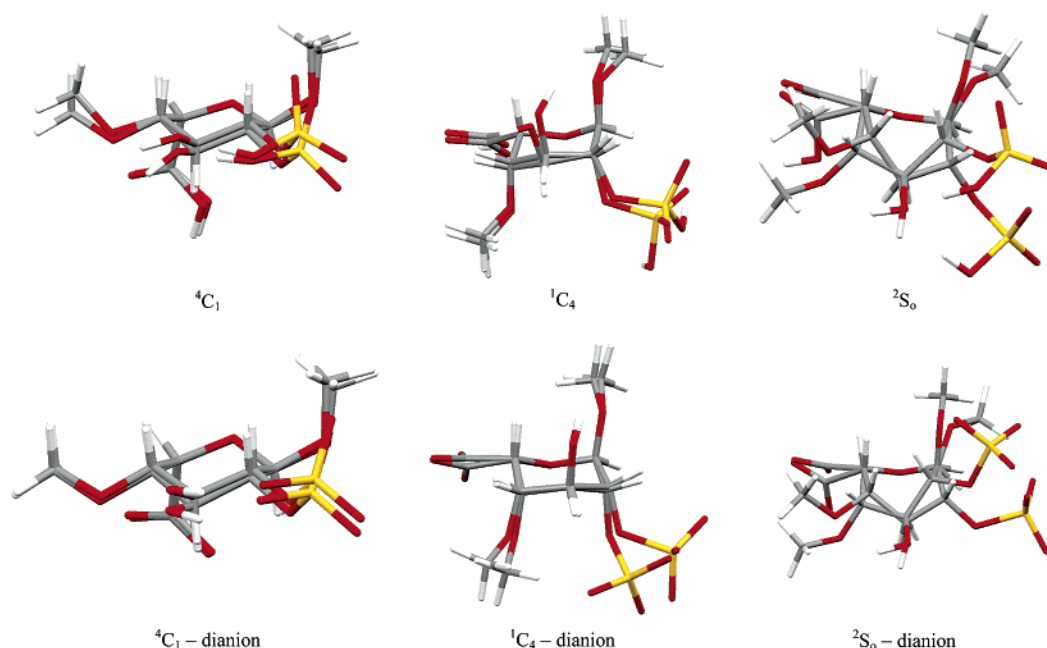
Figure 3. Molecular superimposition of the $^4C_1$, $^1C_4$, and ^2S_o conformations of 1,4-DiOMe IdoA2S and its dianions optimized in the gas phase and of the solvated structure that was optimized in solution.

Table 1. Relative Energies (kJ/mol) and Observed Intramolecular Hydrogen Bonds of the 1,4-DiOMe IdoA2S Species Studied

	B3LYP/6-31+G(d)		B3LYP/6-311++G(d,p)		
species	ΔE	ΔE_{solv}	ΔE	ΔE_{solv}	hydrogen bond
		1,4-DiOMe IdoA2S			
$^4C_1$ neutral molecule	6.2	4.2	5.6	1.2	SO−H···OC(3)[a,b]
					C(3)O−H···OC(4)[a,b]
$^4C_1$ anion	−31.2	−12.5	−31.0	−12.9	C(3)O−H···⁻O−S[a,b]
$^1C_4$ neutral molecule	38.5	7.8	20.6	7.8	C(3)O−H···OC(1)[a]
$^1C_4$ anion	−4.4	−28.4	−5.0	−28.7	C(3)O−H···OC(1)[a,b]
^2S_o neutral molecule	0	0	0	0	C(3)O−H···OC(4)[a]
					SO−H···OC(3)[a,b]
^2S_o anion	0	0	0	0	C(3)O−H···OC(4)[a]
		1,4-DiOMe IdoA2S sodium salt			
$^4C_1$ neutral molecule	27.2	39.4	25.3	40.0	
$^4C_1$ anion	45.6	13.3	44.2	−9.3	C(3)O−H···OC(4)[a,b]
$^1C_4$ neutral molecule	30.2	11.0	30.1	18.0	
$^1C_4$ anion	−4.4	−28.0	−4.9	−27.0	C(3)O−H···OC(1)[a,b]
^2S_o neutral molecule	0	0	0	0	
^2S_o anion	0	0	0	0	C(3)O−H···OC(4)[a]

[a] Hydrogen bonds in gas-phase conformations. [b] Hydrogen bonds in solvated system.

3.3. Gas-Phase Acidities, pK_a, and Sodium Affinities.
The substituted iduronic acid 1,4-DiOMe IdoA2S contains two (carboxyl and sulfate) acidic groups and, thus, may undergo deprotonation reactions. It is well-known[6] that the polyanion of heparin is bound to the protein active site and, therefore, represents the active species. Despite the great importance of these groups in heparin and other glycoaminglycans, no deprotonation of iduronic acid derivatives has been experimentally studied. Table 2 contains the acidities of 1,4-DiOMe IdoA2S computed at the two levels of theory. The acidities of individual conformations of 1,4-DiOMe IdoA2S and the sodium affinity of the sodium salt of 1,4-DiOMe IdoA2S are equally reproduced by both theoretical methods. The enthalpy (proton affinity) and Gibbs energy (acidity) of deprotonation was computed as the difference between the fully optimized neutral acid and that of the respective dianion. Thus, the acidities shown in the Table 2 represent the total acidity of 1,4-DiOMe IdoA2S by the dissociation of two acidic (CO₂H and OSO₃H) groups.

Table 2. Calculated Gas-Phase Interaction Enthalpies, $\Delta H°$; Gibbs Energies, $\Delta G°$ (in kJ mol⁻¹); and Entropies, $\Delta S°$ (in J K⁻¹ mol⁻¹), of 1,4-DiOMe IdoA2S[a] and the 1,4-DiOMe IdoA2S Sodium Salt[a] (at 298 K)

	B3LYP/6-31+G(d)			B3LYP/6-311++G(d,p)		
isomer	$\Delta H°$	$\Delta S°$	$\Delta G°$	$\Delta H°$	$\Delta S°$	$\Delta G°$
			1,4-DiOMe IdoA2S[a]			
$^4C_1$	2874.7	205.1	2813.6	2848.6	205.5	2787.4
$^1C_4$	2883.2	188.9	2826.9	2844.6	194.8	2786.6
^2S_o	2910.8	228.4	2842.8	2885.4	223.3	2818.9
			1,4-DiOMe IdoA2S sodium salt[a]			
$^4C_1$	−1356.7	−255.6	−1280.5	−1363.6	−255.7	−1287.4
$^1C_4$	−1302.1	−236.6	−1231.6	−1307.6	−213.3	−1244.0
^2S_o	−1336.2	−232.5	−1266.9	−1343.3	−226.8	−1275.7

[a] Enthalpy, entropy, and Gibbs energy of two bonds.

Different acidities were found for individual conformations. The acidities increase in the order ^2S_o < $^4C_1$ < $^1C_4$. In the gas phase, the $^1C_4$ chair form exhibits the highest acidity, which can be obviously explained by the extra

stabilization of the $^1C_4$ dianion via the intramolecular $C(3)O-H\cdots OC(1)$ hydrogen bond (Table 1).

In solution, the dissociation constant or the pK_a is a measure of the strength of an acid or a base. Therefore, this parameter is very useful in understanding the behavior of drug molecules at the site of action. Calculations of the macroscopic pK_a of 1,4-DiOMe IdoA2S were carried out using the Web-based tool SPARC[43] developed by Carreira et al.[44] Their values are as follows: $pK_a = 0.25$ for the terminal $C(2)-OSO_3H$ group, $pK_a = 3.67$ for the terminal $C(5)-CO_2H$ group, and $pK_a = 14.00$ for the $C(3)-OH$ hydroxyl group. This indicates that the $C(2)$-substituted sulfuric acid of 1,4-DiOMe IdoA2S is more acidic than the $C(5)$-terminal carboxyl group. However, at physiological pH = 7.4, both acids are completely ionized, and thus, 1,4-DiOMe IdoA2S is a strong acid at pH = 7.4. For comparison, the pK_a of the carboxyl group of 1,4-DiOMe IdoA2S was also computed using the pK_a module of the Jaguar program. The pK_a values obtained for the two most stable conformations ^2S_o and $^4C_1$ (3.9 and 3.5) fit the macroscopic pK_a computed by SPARC ($pK_a = 3.67$) well. The pK_a value (5.5) determined by Jaguar for the $^1C_4$ form is, by 2.17 pK_a units, larger. This conformation is also the least acidic in the gas phase (Table 1).

The calculated gas-phase Na^+ affinities (enthalpies) and Gibbs interaction energies of the 1,4-DiOMe IdoA2S sodium salt are given in Table 2. Thermodynamic parameters computed using the double-ζ 6-31+G(d) basis set are always, by 5−10 kJ mol^{-1}, higher than the more accurate calculations using the triple-ζ basis set. However, the relative trends in the individual stabilities of sodium complexes are equally reproduced by both methods. The computed Gibbs energies, $\Delta G°$, of the $^4C_1$, $^1C_4$, and ^2S_o conformations are negative and span a rather small energy interval (from −1244 to −1290 kJ mol^{-1}). The computed sodium affinities (Table 2) represent values for the dissociation of two sodium monocations from their binding sites (carboxyl and sulfate groups). As is evident from Table 2, the dissociation of the sodium cations from their coordination sites is thermodynamically much more favorable than the deprotonation of the corresponding acid (1,4-DiOMe IdoA2S). The ionization of the $^4C_1$, $^1C_4$, and ^2S_o forms of the sodium salt is associated with considerable conformational rearrangements of ionic species. These rearrangements cause additional energetic stabilizations of anionic species. In real molecular complexes, the tendency to associate is described by Gibbs energies. It is, therefore, important to know the role of entropy in the processes studied. Table 2 also lists the computed entropies for the reactions studied (the $^4C_1$, $^1C_4$, and ^2S_o conformations). The formation of a single cationic metal−ligand complex from a pair of species necessarily involves a loss of entropy. In the case of the sodium complexes of the 1,4-DiOMe IdoA2S sodium salt, the entropy change due to complexation is about −210 to −260 J mol^{-1} K^{-1}, and the calculated enthalpies and Gibbs energies follow the same trend in the affinity of the sodium cations studied. Medicinally important glycosaminoglycans such as heparin have been recognized to bind to the receptor proteins mostly through their anionic (carboxylate and *N*- and *O*-sulfate) groups.[6] Breaking of the sodium bonds in the glycosaminoglycans is costly in terms of enthalpy (Table 2) but results in an appreciable gain in entropy. It is assumed that much of the Gibbs energy of interaction of heparin and its derivatives with proteins should be derived (like with DNA) from the entropically favorable release of Na^+ ions.[6] However, the sodium ion affinity of glycosaminoglycans is greatly affected by the site's structural features, stereo effects, and the type of intramolecular noncovalent interactions that can be supported at or near the binding group.

4. CONCLUSIONS

This theoretical study set out to determine stable conformations ($^4C_1$, $^1C_4$, and ^2S_o forms) of 1,4-DiOMe IdoA2S and its sodium salt, for which a relatively small amount of experimental physicochemical data exist, considering their chemical and biological importance. When theoretical methods are used, the following conclusions can be drawn.

The most stable structure of the neutral molecules corresponds to the ^2S_o skew-boat conformation. This form is also the most stable in a water solution. The most stable ^2S_o conformation of the neutral molecules is not maintained in the anionic species. With anions, both the $^1C_4$ and $^4C_1$ conformations are present. The relative stabilities of individual species of the substituted iduronic acid are affected by the solvent and extra stabilization of the pyranose ring conformation by means of intramolecular hydrogen bonds.

Sodium coordination of the carboxylate and sulfate groups of 1,4-DiOMe IdoA2S results in corresponding changes in the optimum geometry of the parent species. Only two negatively charged oxygen atoms in the sulfate SO_3^- group are involved in coordination of the sodium cation. A third S−O bond of the 1,4-DiOMe IdoA2S sodium salt is not involved in coordination to the sodium cation and may be recognized as a S=O bond type with optimal lengths in the range 1.45−1.46 Å.

The computed total acidity of of the individual forms of 1,4-DiOMe IdoA2S increase in the order $^2S_o < ^4C_1 < ^1C_4$. In the gas phase, the $^1C_4$ chair form exhibits the highest gas-phase acidity, which can be obviously explained by the extra stabilization of the $^1C_4$ dianion via the intramolecular $C(3)O-H\cdots OC(1)$ hydrogen bond.

The computed macroscopic pK_a values of 1,4-DiOMe IdoA2S [$pK_a = 0.25$ for the terminal $C(2)-OSO_3H$ group, $pK_a = 3.67$ for the terminal $C(5)-CO_2H$ group, and $pK_a = 14.00$ for the $C(3)-OH$ hydroxyl group] indicate that both the sulfate and carboxyl groups are, at physiological pH = 7.4, fully ionized.

Our calculations have shown that the dissociation of the sodium cations from their coordination sites (carboxylate and *O*-sulfate groups) is thermodynamically much more favorable than the deprotonation of the corresponding acids of 1,4-DiOMe IdoA2S.

Supporting Information Available: B3LYP/6-311++G-(d,p) optimized relevant bond lengths (Å), bond angles (deg), and dihedral angles (deg) for the functional groups of the 1,4-DiOMe IdoA2S and 1,4-DiOMe IdoA2S sodium salt species studied (Tables A and B). This material is available free of charge via the Internet at http://pubs.acs.org.

1200 *J. Chem. Inf. Model., Vol. 46, No. 3, 2006*

REMKO AND VON DER LIETH

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