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Molecular dynamics and atomic charge calculations in the study of heparin conformation in aqueous solution

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Abstract—HF/6-31G** and molecular dynamics (MD) simulations were used to evaluate the performance of different atomic charge basis sets (i.e., Mulliken, Löwdin, and Electrostatic Potential Derived Charges—ESP) in heparin simulations. HF/3-21G calculations were also used to study the NMR conformation of the IdoA residue. The results thus obtained indicated that ESP and Löwdin charges gave the better results in heparin simulations, followed by Mulliken charges, and that the minimum-energy conformation of IdoA can be different from that observed by NMR spectroscopy by less than 1 Å. However, it was found that this small conformational modification is capable of inducing a change of almost 200 kJ/mol in the interactions of heparin with the surrounding environment, which is a meaningful amount of energy in the context of ligand–receptor interactions. This information can be potentially of great relevance in the design of heparin-derived antithrombotic compounds.

Keywords: Heparin; Molecular dynamics; Polysaccharide structure; GROMACS; Löwdin charges; NMR refinement

1. Introduction

Heparin was first identified and isolated from a preparation of dog liver in 1916, and it constitutes the first compound that was clinically used as an anticoagulant and antithrombotic agent. It is still today recognized as a powerful therapeutic drug and is currently in clinical use.[1] Heparin is a sulfated copolymer composed of disaccharide units containing (1→4)-linked residues of uronic acids (2-sulfated α-L-iduronic acid, IdoA, or non-sulfated β-D-glucuronic acid, GlcA) and glucosamine (GlcN) 2,6-disulfate.[1,2] The combination of disaccharide units form a hexasaccharide repeating unit, which is the most common chemical structure usually reported:[3] ΔUA2S(1→4)-α-D-GlcN*p*-S6S(1→4)-α-L-IdoA*p*-2S(1→4)-α-D-GlcN*p*-S6S-α-L-IdoA*p*-2S(1→4)-α-D-GlcN*p*-S6S. When bound to antithrombin III (AT), this polysaccharide accelerates the inhibition of thrombin, as well as of other serine proteases of the blood clotting cascade. This occurs when these molecules are bound in a ternary complex were heparin induces conformational changes in AT, thus inducing its best fit to the target protease, which increases several folds the affinity constants for the binding of the two proteins.[4]

The first conformational studies on heparin oligosaccharides highlighted several difficulties that are specific to heparin fragments: (a) compared to other pyranose sugars the iduronate ring displays great flexibility and can adopt a variety of conformations viz., $^4C_1$, $^1C_4$, and $^2S_0$, (b) parameterization for the *O*- and *N*-sulfo groups is not readily available in most force fields, and (c) the polyanionic character of the decasaccharide requires special consideration for solvent or counter-ion effects.[5] Considering that these polysaccharides have important biological activities due to their ability for complexation with target biomolecules, studies of the interaction of glycosaminoglycans (GAGs) with proteins is a challenging task because of the poor surface

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complementarity, the high charge density of the binding areas, and the highly flexible nature of the polysaccharide.[6]

Experimental data for the heparin structure present a great variance in the glycosidic linkage geometries (i.e., ϕ and ψ angles), because there is no homogeneity between different sources of data. Our previous work[7] compared different sources of heparin structures obtained by NMR spectroscopy, molecular dynamics (MD), and X-ray crystal methods.[8–11] The variations among these data can exceed 50° for the GlcN→IdoA ψ angle. However, using MD simulations we were able to represent the heparin structure in aqueous solution with a maximum deviation of 30° from the reference data that was used.[8]

The deviations observed in our previous work of heparin simulation[7] as compared to NMR solution conformation, appear to be derived from intramolecular interactions. It seems that the atomic charges of the interacting groups may have implications in the improvement of the description of the heparin structure. Thus there is a lack of comparative studies between different atomic charge basis sets to MD calculations. While ESP charges have performed well in simulations of carbohydrate structures in solution,[12] these present a conformationally dependent behavior.[13] At the same time Mulliken population analyses are known to be generally poor for reproducing intermolecular interaction energies.[13] On the other hand, very little information regarding the use of Löwdin atomic charges is available.

In this work we evaluated and compared different methodologies to calculate atomic charges (i.e., Löwdin, Mulliken, and electrostatic potential derived charges—ESP charges) for the reproduction of heparin conformation in aqueous solution. A refinement for representing the solution conformation of heparin[8] based on ab initio and MD calculations is proposed. These improvements based on computational methods can be useful in supporting the design of new antithrombotic agents based on heparin structure-derived compounds.

2. Experimental

2.1. Computational methods

2.1.1. Nomenclature and software. Recommendations and nomenclature symbols proposed by IUPAC[14] were used. The relative orientation of a pair of contiguous sugar residues (e.g., iduronic acid, glucosamine, and/or glucuronic acid) is described by two torsional angles at the glycosidic linkage, denoted ϕ and ψ. For a (1→4)-linkage the definitions are those shown in Eqs. 1 and 2,

$$\phi = \text{O-5–C-1–O-1–C-4}' \quad (1)$$

$$\psi = \text{C-1–O-1–C-4}'\text{–C-5}' \quad (2)$$

The decasaccharide topologies were generated with the PRODRG program,[15] and the ab initio calculations were performed using GAMESS.[16] Manipulation of structures was performed with the MOLDEN program,[17] and all MD calculations and analysis were performed using the GROMACS simulation suite and force field.[18,19]

2.1.2. Topology construction. The building of a heparin decasaccharide (Fig. 1) structure has already been described.[7] Briefly the heparin fragment was retrieved from protein data bank under code 1HPN.[8] This dodecasaccharide was reduced to a decasaccharide structure and then submitted to the PRODRG server.[15] The resultant topology was further submitted to refinements, for example, atomic charges and improper dihedrals in order to define the conformational state of IdoA residue ($^1C_4$ or $^2S_0$).[7]

2.1.3. Atomic charge calculations. The calculation of atomic charges suitable to heparin MD simulations has been previously described.[7] Briefly the monosaccharide residues GlcA, IdoA, and GlcN, in their negatively charged forms, were submitted to full geometry optimization using ab initio quantum-mechanical computations at the 3-21G level with GAMESS.[16] The conformations of IdoA and GlcN residues that were used were obtained directly from the 1HPN PDB file, including the endocyclic and exocyclic dihedral conformations, thus reproducing the solution conformation evaluated by NMR methods.[8] These minimal energy conformations were submitted to single-point ab initio calculations at the 6-31G** level in order to obtain the Löwdin, Mulliken, and electrostatic potential (ESP) derived charges. Hessian matrix analyses were employed to unequivocally characterize the conformations thus obtained as true minima potential energy surfaces.

2.1.4. Molecular dynamics calculations. The dodecasaccharide conformation determined by NMR methods[8] (PDB entry 1HPN) was the starting point for all MD simulations performed. This structure, reduced to a decasaccharide fragment of heparin, was solvated in a rectangular box using periodic boundary conditions and the SPC/E[20] water model, and 20 Na^+ ions were added in order to neutralize the simulated system (water plus the decasaccharide). The box size was adjusted to give a physiological sodium concentration, that is, ~145.0 mM. Sodium ions were added as counter-ions by manually changing the water molecule closest to each negatively charged group. The total system size comprised ~18,500 atoms for all MD simulations (runs 1,

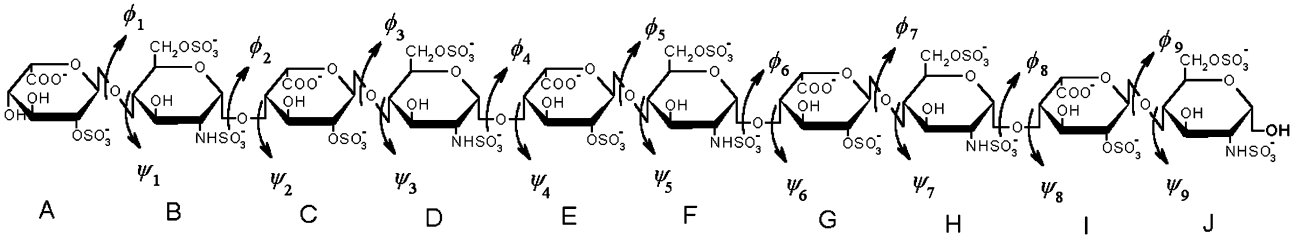


Figure. 1. Decasaccharide A–J fragment of heparin. This structure shows five disaccharide repeating units of GlcN and IdoA residues. The dihedral angles ϕ and ψ are indicated.

2, 3, and 4). These systems were submitted to a steepest descent minimization and MD simulations for 3 ns, applying the Particle-Mesh Ewald method,[21] at 310 K. The system was heated slowly from 50 to 310 K, in steps of 5 ps, each one increasing the reference temperature by 50 K. The thermalization, together with the first nanosecond, was considered as equilibration, and all analyses were performed over the remaining part of the trajectory. Data concerning the decasaccharide structure, for example, that of the glycosidic linkage and intramolecular hydrogen bonds, were obtained by averaging over four or five values for the same linkage located in five different positions in the decasaccharide. The average of these property values indicates not only deviations within a single linkage, but also contributions from different positions in the polysaccharide, as reported before.[11] It should be noted that when a hydrogen bond occurs between a donor and multiple acceptors, for example, carboxylate and sulfate groups, only the lowest distance of two or three possibilities was retrieved at each frame of MD. As a result we have an average distance representative of multiple interactions. A reference value of 3.5 Å between heavy atoms was considered for a hydrogen bond,[22] and a cutoff angle of 60° was used between donor–hydrogen–acceptor.[19]

3. Results and discussion

3.1. Atomic charges

We have previously determined a protocol to reproduce at a reasonable level of accuracy the heparin conformation in explicit aqueous solution using a non-carbohydrate force field.[7] More recently we applied such parameters in the study of the heparin–AT complex, being able to observe to some extent the conformational modifications induced in AT by heparin and to correctly predict the interaction energy in such a complex in an 8.0 ns simulation (Verli and Guimarães, manuscript in preparation).

In these studies, we have used atomic charges for heparin residues derived from a Löwdin population analysis.[7] In fact, this choice was motivated due to the tendency of Mulliken analysis to place all the charge on the oxygen atom.[23] However, studies describing the influence of distinct atomic charge schemes in polysulfated polysaccharide dynamics are, to our knowledge, so far missing.

In order to fulfill this lack of information, regarding the choice of methods to parameterize carbohydrate-based force fields, we proceeded to the evaluation of both Löwdin and Mulliken population analysis and electrostatic potential derived charges (ESP) in the representation of heparin conformation in solution using molecular dynamics simulations. The calculated values of such atomic charges are presented in Table 1.

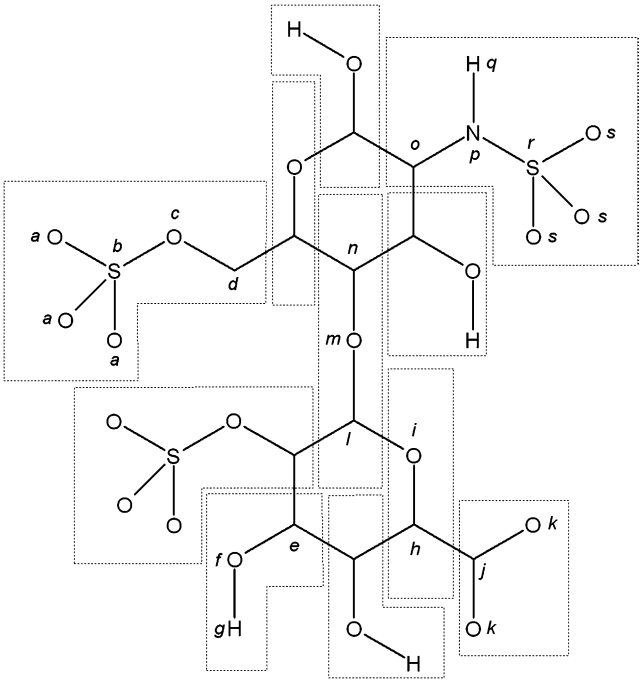
The data presented in Table 1 indicate that Mulliken analysis presents the greatest modular charge for 14 atoms in a total of 19, including oxygen atoms from sulfate and hydroxyl groups (Table 1), inducing an increase in the dipole of chemical bonds. The consequence of such a profile would be an increase in the electrostatic interactions within the molecule and with the surrounding environment. Regarding the comparison between Löwdin and ESP charges, these values are more similar between one another than with Mulliken charges. Furthermore, important charge differences can be observed in Table 1. For instance, the sulfate group is more charged in Löwdin charges than in ESP charges, while the carboxyl and hydroxyl groups are more charged in the ESP scheme than in the Löwdin scheme. In the sulfonamide group the charges are almost identical.

The unique characteristics observed in each group of atomic charges can be related to a previous works of Pérez et al., where several different parameterizations of force fields applied to carbohydrate simulation were compared.[24] In fact, it is possible that the accuracy of the data obtained from MD simulations using the above-mentioned charges depends on the system under study. We then decided to focus the application of these charges in heparin simulation in water, using the MD protocol previously described by our group,[8] and in the identification of which charges give better results describing such a system.

3.2. Conformation of IdoA

The internal iduronate residues can adopt an equilibrium between chair $^1C_4$ and skew-boat $^2S_0$ forms,[25] as this flexibility is proposed to contribute to the unique

Table 1. Schematic representation of the atomic charges calculated for heparin uronic acids and glucosamine residues[a]



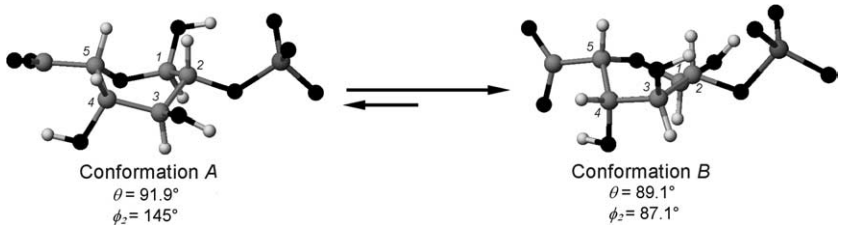
Atoms		Atomic charges[b]		
		Löwdin[c]	Mulliken	ESP charges
Sulfate	*a* (O)	−0.774	**−0.799**	−0.672
	b (S)	1.612	**1.783**	1.307
	c (O)	−0.515	**−0.763**	−0.431
	d (C)	0.225	**0.377**	0.140
Hydroxyl	*e* (C)	0.160	**0.356**	0.331
	f (O)	−0.400	**−0.759**	−0.735
	g (H)	0.240	0.403	**0.404**
Ether	*h* (C)	0.242	**0.420**	0.253
	i (O)	−0.242	**−0.420**	−0.253
Carboxyl	*j* (C)	0.172	0.650	**0.862**
	k (O)	−0.586	−0.825	**−0.931**
Glycosidic linkage	*l* (C)	0.254	**0.565**	0.285
	m (O)	−0.396	**−0.785**	−0.397
	n (C)	0.142	**0.220**	0.112
Sulfonamide	*o* (C)	0.095	**0.131**	0.095
	p (C)	−0.476	**−0.807**	−0.476
	q (H)	**0.192**	0.175	**0.192**
	r (S)	1.553	**1.790**	1.553
	s (O)	**−0.788**	−0.763	**−0.788**

[a] The charges are presented for each atom (designed from *a* to *s*), within each charge group of a disaccharide unit, using Mulliken, Löwdin, and ESP schemes.
[b] The largest modular values are presented in boldface type.
[c] Data obtained from Ref. 7.

heparin-binding properties as compared to the lower antithrombotic activity of glycosaminoglycans presenting more rigid uronate residues.[9] In this context, the observation of IdoA minimization by an ab initio 3-21G basis set prior to the HF/6-31G** (see methods for details), starting from the NMR obtained conformation, reveals some important features (Table 2).

As indicated in Table 2, the minimum-energy conformation of IdoA (conformation B) obtained by the HF/3-21G basis set unexpectedly showed a reorientation of some atoms during minimization, modifying the IdoA ring conformation related to the NMR conformation (conformation A, Table 2). While the interatomic distances within the residue did not change to a great extent

Table 2. Conformational modification of IdoA monomer during 3-21G minimization[a]



Conformation	Distances (Å)							
	H-1–H-2	H-1–H-3	H-1–H-4	O-1–O-4	H-1–H-5	H-2–H-4	H-3–H-4	H-4–H-5
A[b]	3.08	2.86	4.62	5.02	3.58	3.03	3.07	2.40
B[c]	3.00	2.88	3.93	4.25	3.74	3.64	2.64	2.47
Difference[d]	0.08	−0.02	**0.69**	**0.77**	−0.16	−0.61	0.43	−0.07

[a] The Cremer–Pople puckering parameters θ and ϕ_2 relative to conformations A and B are presented, as well as the distances between the atoms connected to positions-1 and -4 of the ring. Atoms are shown as follows: hydrogen in white, oxygen in black, sulfur in dark gray and carbon in light gray.

[b] Conformation obtained from NMR data, Ref. 8.

[c] Conformation obtained after minimization using the 3-21G ab initio method.

[d] The distance in conformation A subtracted from the distance in conformation B. Negative values correspond to distances that increases in conformation B, whereas positive values correspond to distances that decreases in conformation B.

(i.e., <1.0 Å), the greatest changes are located between the atoms connected to positions-1 and -4 of the saccharide residue (~0.7 Å), which are the positions responsible for the 1→4 glycosidic linkages of heparin (Table 2).

The importance of such apparently small conformational modifications can be highlighted looking at the internal dihedral angles of IdoA (Table 3). Some angles presented structural torsions of about ~60°, a modification capable of reorienting the saccharide side chains, for example, the sulfate and hydroxyl groups, and consequently modifying both intramolecular hydrogen bonds and the interactions with the surrounding medium, which has previously been suggested to contribute to the global heparin conformation.[7]

In order to study saccharide ring conformations the Cremer–Pople representation[26] was also considered, where the general definition of the ring puckering coordinates involves the calculation of phase angles ϕ_2 and θ. According to this representation, the ϕ_2 and θ angles were evaluated for both conformations A and B and are presented in Table 2.

Table 3. Internal dihedral angles of IdoA and its variation from the NMR-determined conformation (conformation A) and the HF/3-21G minimized structure (conformation B)[a]

Dihedral angle	Conformation A	Conformation B
C-1–C-2–C-3–C-4	−57.23	−30.25
C-2–C-3–C-4–C-5	21.96	−30.43
C-3–C-4–C-5–O-6	38.88	60.12
C-4–C-5–O-6–C-1	−72.10	−25.16
C-5–O-6–C-1–C-2	34.44	−32.95
O-6–C-1–C-2–C-3	29.74	64.33

[a] The structures of conformations A and B are presented in Table 2.

The phase angles obtained for conformation A, $\phi_2 = 145°$ and $\theta = 91.9°$, describes the $^2S_0$ geometry ($\phi_2 = 150°$ and $\theta = 90°$), as previously described,[8] while the phase angles of conformation B, $\phi_2 = 87.1°$ and $\theta = 89.1°$, represents another conformer of the boat–skew boat pseudorotational family,[27] matching to the $^5S_1$ conformation ($\phi_2 = 90°$ and $\theta = 90°$). In fact, initial research on the IdoA conformation had pointed to other possible conformations for this residue,[28,29] although NMR studies reinforced the $^2S_0$ conformation.[25] Such rationalization, based on the observed vicinal coupling constants, employed the empirical generalization of the Karplus equation proposed by Haasnoot et al.[30] This equation relates the vicinal coupling constants to the torsional angle between the coupling protons and accounts for the effects of electronegativity and the relative position of substituents attached to the H–C–C–H fragment. The application of such a relation requires that the H–C–C–H torsional angles be computed for geometrical models of each conformer, usually provided by molecular-mechanics calculations,[29] and this is the point where the results here presented differ from previous data regarding IdoA conformation. The lack of electron representation in force field calculations, together with the problems in adequately describing the properties of hypervalent groups (e.g., sulfonamide and sulfate),[31] remove important factors in conformational stabilization. As a consequence of the consideration of such stereoelectronic effects in ab initio calculations, one can expect that the energy map for the equatorial pseudorotational path of IdoA would be considerably different from previous data based on force field calculations,[29] and so would the relative stability of each conformation. In doing so, the use of those minimum-energy

conformations evaluated by quantum-mechanical methods in the rationalization of vicinal coupling constants is a necessary development.

In the context of the real importance of such conformational modification to heparin structure and conformation, it should be noted that experimental approaches based on the measurement of NMR coupling constants[32] or chemical shifts[33] usually lack precision[11] (NMR-based 3D structure determination has a resolution limitation of about 2–3 Å[34]), whereas angles derived theoretically that have been obtained from molecular mechanics energy calculations must be viewed with caution owing to their force-field dependence.[11] Moreover, the use of experimentally determined restraints during energy minimization can result in the formation of 'virtual conformers',[35] molecular conformations that are not at the global energy minimum. So next in the text we evaluate the effect of the atomic charge schemes (Löwdin, Mulliken, and ESP charges) and the two observed conformations of the IdoA residues (conformations A and B) in heparin dynamics using MD simulations.

3.3. MD simulations and IdoA conformations evaluation

Using the protocols previously described by our group in evaluating heparin conformation under the $^2S_0$ and $^1C_4$ conformations of IdoA,[7] we performed an MD simulation of a heparin decasaccharide considering the two accessed conformations of IdoA (conformations A and B), as well as the Löwdin, Mulliken, and ESP charges. The heparin conformation so obtained, described by its glycosidic linkages, is described in Table 4.

Related to the evaluation of distinct atomic charge schemes, the MD simulations indicate that the Löwdin and ESP charges give similar results in the description of heparin conformations (runs 2 and 4). The Mulliken basis set (run 3), however, presents a different behavior, showing a deviation of almost 15° in the IdoA→GlcN ϕ angle related to the Löwdin and ESP charges (Table 4).

Related to the evaluation of distinct conformations of IdoA, the simulation of heparin with the conformation A (run 1) produces an average geometry for the GlcN→IdoA ψ angle that deviates ~30° from the NMR reference conformation, as previously described.[7] A correct description of this dihedral angle was achieved only by the substitution of the experimental conformation (conformation A) by the HF/3-21G conformation (conformation B) of the IdoA residue, as the difference between the conformations A and B is smaller than 1 Å (Table 2). In fact, a so small conformational modification in IdoA being capable of generating such a meaningful effect in the heparin conformation must be carefully observed with regard to both the molecular simulation and biological activity aspects. It is important to note that run 2 (with IdoA residues in the conformation B) is generated from the last structure obtained in run 1 (with IdoA residues in the conformation A), which was submitted to energy minimization in order to modify the IdoA conformation. In the same way, by taking the last structure of run 2 and changing its IdoA residues to the NMR conformation A, the dihedral angles obtained in a 3.0 ns MD simulation are equal to the values of run 1, which confirms the reproducibility and reversibility of this process, and thus the reliability of the data so obtained.

As we previously suggested, the intramolecular hydrogen bonds may be the main forces responsible for, and allowing an accurate reproduction of heparin conformational structure by MD methods.[7] Thus, in order to elucidate the origins of such conformational modification we performed an analysis of such interactions in the MD simulations (Table 5).

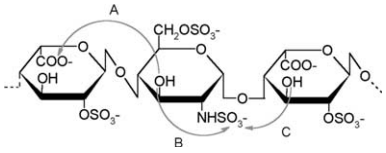
Our previous results suggested that the correct representation of the heparin structure under different conformations of IdoA (i.e., $^2S_0$ and $^1C_4$) has a large contribution from the intramolecular hydrogen bonds.[7] For example, a hydrogen bond between the carboxyl group from IdoA and the hydroxyl group of GlcN could be the main factor in determining the NMR conformation of the GlcN→IdoA ψ angle. This conclusion was based in the observation of the distance representing such interactions in run 1, which was about 1 Å larger than the distance described in the 3D structure of

Table 4. Average dihedral angles and NMR reference values for the $^2S_0$ conformation of IdoA MD simulations using different atomic charge basis sets (Löwdin, Mulliken, and ESP charges) and the conformations A and B of the IdoA residue

Dihedral angle	$^2S_0$ NMR[a]	Average dihedral (°)			
		Run #			
		Löwdin		Mulliken	ESP
		1[b]	2[c]	3[c]	4[c]
IdoA→GlcN ϕ	−55	−75 ± 11	−75 ± 15	−90 ± 14	−77 ± 15
IdoA→GlcN ψ	−107	−117 ± 11	−121 ± 11	−127 ± 11	−118 ± 10
GlcN→IdoA ϕ	109	97 ± 14	89 ± 13	93 ± 15	88 ± 12
GlcN→IdoA ψ	−158	−125 ± 15	−151 ± 15	−145 ± 13	−145 ± 15

[a] Data obtained from PDB code 1HPN, Ref. 8.
[b] Simulation performed with IdoA residues in conformation A (Table 2).
[c] Simulation performed with IdoA residues in conformation B (Table 2).

Table 5. Average MD intramolecular hydrogen bond distances and NMR reference values in heparin



Hydrogen bond	Distance (Å)				
	$^2S_0$ NMR[a]	Run #			
		Löwdin		Mulliken	ESP
		1[b]	2[c]	3[c]	4[c]
A	3.4	3.8 ± 0.5	4.2 ± 0.5	4.0 ± 0.4	4.2 ± 0.4
B	2.8	3.7 ± 0.6	4.8 ± 0.8	5.0 ± 0.8	5.2 ± 0.9

[a] Data obtained from PDB code 1HPN, Ref. 8.
[b] Simulation performed with IdoA residues in conformation A (Table 2).
[c] Simulation performed with IdoA residues in conformation B (Table 2).

heparin[8] (see Table 5). In other words, the increase in the distance between the carboxyl and hydroxyl groups would induce a weaker interaction and thus an extension of the glycosidic linkage at this point. However, looking at the data presented in Table 5, the distance relative to this interaction in run 2 that correctly predicts the geometry of the GlcN→IdoA ψ angle, is 2 Å larger than that from the NMR reference. This is an intriguing feature, suggesting that intramolecular hydrogen bonds may not be the only factors in determining heparin three-dimensional dynamics in solution. So we move in the direction of studying the intermolecular interactions, that is, the interaction of heparin with its counter ions and water. These interactions were analyzed by means of the average interaction energy and are described in Table 6.

In agreement with the idea of additional forces in the determination of heparin structure in solution, the observation of the atomic charges of the carboxyl group described by Löwdin and ESP charges basis sets indicates a 2-fold difference. In spite of this fact, such a modification in the electrostatic properties of the carboxyl group is not capable of modifying the intramolecular interactions nor the heparin conformation, since Löwdin (run 2) and ESP charges (run 4) generate the same heparin geometry. However, this difference is capable of inducing an increase of almost 70% in the interactions with the sodium ions on the system (Table 6), while the interaction with water does not present a change of the same magnitude.

As an example of the influence of the interaction energy between heparin and the surrounding medium it is the geometry of the IdoA→GlcN ϕ angle with Mulliken charges compared to Löwdin and ESP charges. The Mulliken method produces strong negative charges on the oxygen atoms of the sulfate and sulfonamide groups (Table 1). These negative charges increase the interaction energy of the heparin decasaccharide with sodium ions by almost 150% related to Löwdin atomic charges, which is correlated with the increase in the IdoA→GlcN ϕ angle. It must be noted, however, that the influence of atomic charge is not linear, and the system is flexible enough to accommodate its electrostatic properties to some extent without compromising the global structure of the molecule of the decasaccharide under study. Above such limits (those for heparin appear to be determined by the ESP adjusted charges), conformational modifications in the solute structure can be observed, as is being observed with Mulliken charges.

The data presented in Table 6 highlight the pitfalls in choosing atomic charge basis sets to parameterize molecular mechanics force fields in molecules presenting hypervalent groups. While the different charge schemes

Table 6. Average interaction energy between the heparin decasaccharide and sodium ions or solvent molecules[a]

Molecules interacting	Interaction energy (kJ/mol)			
	Run #			
	Löwdin		Mulliken	ESP
	1[b]	2[c]	3[c]	4[c]
Heparin–Na^+	−632.95 ± 256.65	−883.79 ± 149.62	−2074.32 ± 195.88	−1400.37 ± 289.23
Heparin–water	−6456.19 ± 284.82	−6279.39 ± 196.72	−6009.12 ± 214.35	−6373.51 ± 255.72

[a] Data obtained from PDB code 1HPN, Ref. 8.
[b] Simulation performed with IdoA residues in conformation A (Table 2).
[c] Simulation performed with IdoA residues in conformation B (Table 2).

can give similar descriptions of the three-dimensional structure of the molecule under study (Table 4), it can be observed at the same time differences in the interaction energy with the surrounding medium up to 1000 kJ/mol (Table 6).

The interaction energy between the heparin decasaccharide and the surrounding medium (Table 6) presents high variations, mainly as a consequence of the molecular diffusion in the system. At the same time, the standard deviation is considerably constant within the same atomic charge basis set, indicating an intrinsic property, dependent on the non-bonded parameters of the system. By comparing the results of runs 1 and 2 (Table 6), it can be noted that the change in IdoA conformation decreases in about ~200 kJ/mol the interaction energy between heparin and sodium ions, with a concomitant increase in the interaction with water (Table 6).

Apparently, the conformational modification of IdoA is capable of exposing its charged groups, that is, carboxylate and sulfate, increasing its interaction with the counter ions of the surrounding medium. While this modification is small when expressed in interatomic distances (<1 Å, Table 2), it is more meaningful when expressed in torsion angles of the IdoA ring (~60°, Table 3), suggesting the reorientation of the saccharide side chains. The intensification in intermolecular interactions due to the IdoA change in the HF/3-21G conformation has the consequence of modifying the geometry of the glycosidic linkage, mainly the GlcN→IdoA ψ angle, allowing a correction in the description of the representation of the heparin structure.

It is somewhat surprising that small structural modifications in the IdoA conformation, even smaller than 1 Å, induce a ~200 kJ/mol change in the intermolecular interactions within the simulated systems, indicating a unique sensitivity of these polysulfated molecules to small conformational modifications. An energy contribution of such magnitude to the ligand–receptor complex is, with no doubt, of great biological importance. For instance, this value corresponds to approximately one-third of the total energy interaction between the synthetic pentasaccharide and AT, estimated previously by MD simulations (Verli and Guimarães, manuscript in preparation).

4. Conclusions

The use of NMR experiments for conformational studies of oligosaccharides differs significantly from that of proteins, in part because distance geometry calculations for such molecules are complicated due to their flexible nature.[36] In this context, computational methods, mainly molecular mechanics and molecular dynamics, are required as additional aids to complement the experimental data.[36] However, there is still no molecular mechanics force field parameterization capable of adequately reproducing all polysaccharide conformational features, that is, high polar functionality, flexibility, and conformational/configurational changes, like the anomeric, exo-anomeric, and gauche effects.[6] In this context, the data presented here support the indication and use of molecular modeling techniques to refine experimental data, including ab initio calculations, thus improving their capabilities to describe biological and chemical phenomena by computational methods.

Here we present a molecular modeling study comparing different atomic charge basis sets, that is, Löwdin, Mulliken, and ESP charges, in heparin simulation using MD in order to determine the best parameters to describe the solution properties of such a polysaccharide. Based on MD simulations of heparin in aqueous solution, the use of Löwdin atomic charges appears to be an adequate choice for parameterization of molecular mechanics force fields, with similar results when compared to ESP atomic charges, without a conformationally dependent behavior.

Moreover, in the energy minimization process of heparin residues, we observed a conformational modification in the IdoA residue. This new conformation, corresponding to a $^5S_1$ form, was then inserted into MD simulations of heparin and was shown to be responsible for a 25° correction in the heparin GlcN→IdoA ψ angle description compared to the use of the NMR conformation in heparin MD, even based on an ab initio calculation without considering the solvent effect. Since the accuracy of a method can be judged by how well it reproduces known quantities,[37,38] we propose this new conformation as an alternative in refining the NMR heparin conformation data presented previously by Mulloy et al.[8], corresponding to a small torsion in the IdoA residues that induced changes in the distances within the residue atoms in less than 1 Å. These small changes were, however, able to change the interaction energy of heparin with the surrounding medium by about ~200 kJ/mol, thus indicating an unusual sensitivity of polysulfated carbohydrate polymers to small conformational modifications.

Throughout the fine tunings of polysaccharide structures as presented here for elucidation of molecular aspects of heparin interaction with its target proteins, we were able to find that small structural modifications even smaller than 1 Å can produce a large energy contribution to the system, thus affecting ligand–receptor complexes, so with a significant biological importance. It is concluded that the more frequent use of molecular modeling methodologies for GAGs and heparin-derived compounds can potentially contribute to the design of new antithrombotic agents.

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