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A molecular dynamics description of the conformational flexibility of the L-iduronate ring in glycosaminoglycans†

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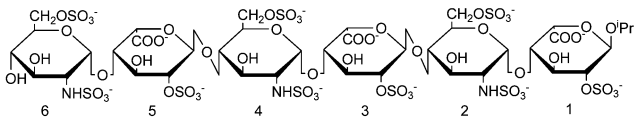
For a synthetic hexasaccharide model it is shown that the conformational flexibility of the L-iduronate ring in glycosaminoglycans can be adequately described by using the PME methodology together with simulation protocols suitable for highly charged systems.

The linear polysaccharides heparin and heparan sulfate are glycosaminoglycans (GAGs) composed of differently sulfated alternating units of D-glucosamine and an uronic acid (either D-glucuronic or L-iduronic).[1] These GAGs are involved in the regulation of extracellular matrix events through protein recognition processes.[2] It is widely accepted that the selectivity of these processes essentially depends on the spatially defined sulfate and carboxylate pattern, the shape, and the internal flexibility of the polysaccharide chains.[3,4] The internal L-iduronate units in these structures exhibit a conformational equilibrium, which depends on its own substitution pattern and on that of adjacent glucosamine units (Scheme 1).[5] This conformational flexibility is believed to play a key role in the wide range of specific interactions exhibited by these molecules.[5c] NMR data have indicated that, at least, a $^1C_4$ chair, evidenced by coupling constant values, and a ^2S_O skew boat conformation, detected through an exclusive 2-H/5-H NOE,[4,5] are present in the conformational equilibrium, but an additional fast pseudorotational equilibrium among skew boats has also been proposed on the basis of molecular dynamics (MD) calculations on an unsulfated iduronate model.[6]

As has been recently suggested, adequate methods for unrestrained modeling of these systems could be an useful tool for understanding the selectivity of specific GAG–protein interactions.[4] Acceptable descriptions of the overall shape of heparin have been published using restrained MM and MD techniques.[7] However, very few of such MD studies on these polysaccharide derivatives have been reported so far,[7] most likely as a result of the difficulties of modeling such highly charged and flexible systems. Therefore, the relevant problem of modeling the flexibility of the iduronate units has not yet been adequately considered. A MD study of a heparin-like hexasaccharide carrying 2-*O*-sulfo L-iduronate units in explicit water has been reported.[7b] However, the authors report *anomalous ring conformations* for D-glucosamine residues and *alternative iduronate conformations* and the description of the boat conformers is not given.[7b] These anomalous results are explained in terms of *electrostatic artifacts* and the use of more sophisticated protocols for further refinements are proposed.[7b]

We now have addressed this problem by performing molecular dynamics calculations on the heparin-like hexasaccharide **1**,[8] a suitable model of the regular region of heparin previously synthesized by us, the NMR spectrum of which provides independent data for each L-iduronate unit.[8] We have carried out these calculations[9] in explicit water with periodic boundary conditions and Particle Mesh Ewald method (PME)[13] for the evaluation of the electrostatic interactions. This is the methodology of choice when dealing with MD calculations of highly charged molecules such as nucleic acids but, to the best of our knowledge, it has never been applied to GAG-like structures. Concerning the iduronate flexibility, two types of transitions must be distinguished: the slow (millisecond) $^1C_4$–^2S_O interconversion and the faster pseudorotational interconversion along the skewboat space.[4,5d] We have used two different starting geometries with the three L-iduronate units in the $^1C_4$ and in the ^2S_O conformation respectively, a common approach imposed because this interconversion can not be sampled by these simulations.[4] However, the pseudorotational equilibrium can be adequately sampled by MD.[4,7,8] For comparison purposes we have also run MD simulations in explicit water with truncation of the electrostatic terms and *in vacuo* using $\varepsilon = 80$ and $\varepsilon = 5r$.



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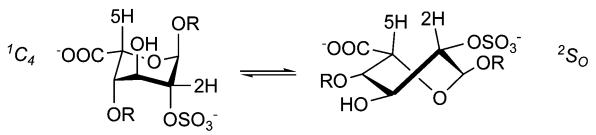
The results indicate that the behavior of the glycosidic linkages is roughly the same along the different simulations.[8] Regarding the pyranoid ring conformations, the D-glucosamine rings, in agreement with experimental data,[8] remain in the $^4C_1$ conformation in all cases with the exception of the simulation using explicit solvent and classic cut-off treatment where unacceptable distortions have been observed probably as described by Mikhailov *et al.*[7b] The conformational performance of the L-iduronate rings along the MD trajectories has been analyzed using the Cremer-Pople puckering co-ordinates. While the $^1C_4$ forms ($\theta = 180°$) are stable along all the simulations, the skew boat conformations ($\theta = 90°$) show different behavior depending on the electrostatic treatment (Figure 1). When using $\varepsilon = 80$ a large pseudorotational skew boat interconversion involving $^1S_5$, ^2S_O, $^5S_1$ and $^3S_1$ forms is observed but with $\varepsilon = 5r$ the equilibrium involves only the ^2S_O and the $^5S_1$ forms as previously reported.[4,6] Interestingly enough, this pseudorotational equilibrium is not detected in the PME simulations where only the conformational space corresponding to the ^2S_O form is covered (Figure 1).

The results of these simulations have been assessed through a comparison of the NMR coupling constants with those predicted by the MD data by using the Altona relationship.[14] The experimental coupling constants, calculated from 2D dqf-COSY,[8] have been least square fitted to those for the chair and skew-boat ensemble averages through the respective MD trajectory and to those predicted for the $^1C_4$ and ^2S_O canonical forms. The goodness of the fit has been assessed by the square errors summation per residue over the experimental coupling constants and their average for each simulation condition (Table



Scheme 1

† Electronic supplementary information (ESI) available: details of simulation protocols. See http://www.rsc.org/suppdata/cc/b3/b303386b/

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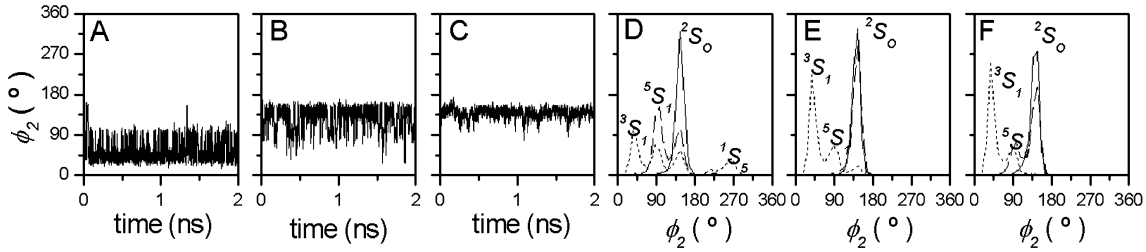

Fig. 1 Trajectories of the Cremer-Pople azimuthal puckering coordinate[4] ϕ_2 describing the skew boat forms for iduronate residue 5 using ε = 80 (A), ε = 5r (B) and PME (C) electrostatic treatment and distribution curves of the same coordinate ϕ_2 for iduronate residues 1 (D), 3 (E) and 5(F) along the MD simulations with ε = 80 (dotted lines), ε = 5r (dashed lines) and PME (solid lines) electrostatic treatment.

Table 1 Relative conformational populations of the iduronate rings

		Conformer population (%)					Errors	
	Residue	$^1C_4$	$^1S_5$	^2S_O	$^5S_1$	$^3S_1$	Σ squared	Averaged
Canonical								1.45
	IdoA-1	72		28			2.15	
	IdoA-3	69		31			0.86	
	IdoA-5	70		30			1.35	
ε = 80								3.93
	IdoA-1	52	7	10	14	17	0.59	
	IdoA-3	74			8	18	5.41	
	IdoA-5	87			3	10	5.78	
ε = 5r								1.56
	IdoA-1	69		12	19		2.62	
	IdoA-3	61		39			0.53	
	IdoA-5	60		27	13		1.53	
PME								0.56
	IdoA-1	63		37			0.28	
	IdoA-3	58		42			0.56	
	IdoA-5	62		38			0.84	

Table 2 Experimental H–H coupling constants for **1** and back calculated for MD ensemble averages using: a) ε = 80, b) ε = 5r, and c) PME

	IdoA-1				IdoA-3				IdoA-5			
	exp	a	b	c	exp	a	b	c	exp	a	b	c
$^3J_{1,2}$	3.4	3.7	3.5	3.6	2.9	3.1	3.6	3.7	2.7	2.4	3.8	3.7
$^3J_{2,3}$	–	–	–	–	5.6	2.6	5.4	5.4	5.7	2.6	5.0	5.3
$^3J_{3,4}$	3.4	3.3	2.7	3.4	3.9	2.9	3.5	3.3	3.5	2.9	3.2	3.3
$^3J_{4,5}$	2.9	2.2	1.4	2.4	2.7	1.8	2.1	2.3	2.8	1.7	1.7	2.0

1). The best agreement has been found for the simulations using the PME method. In this case, the conformational behaviour of the L-iduronate units corresponds to a dynamically stable ^2S_O form and no pseudorotational equilibrium is predicted (Figure 1). The averaged errors when using classical cut-off simulations are, in the best of cases (ε = 5r), comparable to those found for the fit to canonical forms, showing strong border effects due to the truncation of the electrostatic energy term.

In conclusion, we show in this paper that the use of the PME electrostatic treatment in MD simulations of heparin-like structures in explicit water lead to much better predictions of the conformational behavior than classical cut-off methods. This methodology,[13] whose application to this type of structure is here reported for the first time, considerably improves the performance and accuracy of the simulations with regard to the conformational equilibrium of the L-iduronate units providing excellent agreement with the experimental data (Table 2). According to these predictions, the 2-*O*-sulfo-L-iduronate rings present in synthetic compound **1**, which is a good model for the regular region of heparin,[8] are best described as existing as an equilibrium between flexible $^1C_4$ and ^2S_O forms without any other contribution from the skew boat pseudorotational ensemble. Additional simulations with other synthetic oligosaccharide models carrying different sulfation patterns and well defined NMR spectra are presently underway.

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