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Depiction of the forces participating in the 2-*O*-sulfo-α-L-iduronic acid conformational preference in heparin sequences in aqueous solutions

Laercio Pol-Fachin[a] and Hugo Verli[a,b,*]

[a]*Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Av Bento Gonçalves 9500, CP 15005, Porto Alegre 91500-970, RS, Brazil*
[b]*Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av Ipiranga 2752, Porto Alegre 90610-000, RS, Brazil*

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Abstract—2-*O*-Sulfo-α-L-iduronic acid (IdoA2S) is one of the main components of heparin, an anticoagulant and antithrombotic polysaccharide able to potentiate the inhibitory effect of antithrombin over plasma serine proteases. This monosaccharide unit adopts an equilibrium between chair ($^1C_4$) and skew-boat (^2S_O) forms as a function of heparin sequence size and composition. Although the prevalence of the $^1C_4$ chair conformation in monosaccharides is understood, the reasons for the increase in ^2S_O contribution in the whole polysaccharide chain are still uncertain. In this context, 0.2 μs molecular dynamics simulations of IdoA2S-containing oligosaccharides indicated that stabilization due to intramolecular hydrogen bonds around IdoA2S is highly correlated ($p \leqslant 0.001$) with the expected conformational equilibrium for this residue in solution. This behavior explains the known effect of different heparin compositions, at the monosaccharide level, on IdoA2S conformation in biological solutions.
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1. Introduction

Heparin, the first compound used clinically as an anticoagulant and antithrombotic agent,[1] is a sulfated polysaccharide composed of (1→4)-linked disaccharide units, mainly containing residues of uronic acids, 2-*O*-sulfo-α-L-iduronic acid (IdoA2S) or non-sulfated β-D-glucuronic acid, and 2,6-di-*O*-sulfo-glucosamine (GlcNS,6S).[2] Through the formation of a ternary complex with antithrombin (AT) and proteases from the blood clotting cascade, heparin potentiates the AT inhibitory effect on these proteases,[3] in a process that appears to be influenced by the unusual flexibility of IdoA2S residues. This monosaccharide unit is capable of adopting an equilibrium between the chair ($^1C_4$) and skew-boat (^2S_O) forms,[4] not causing the whole polysaccharide chain to bend and so contributing to unique binding properties.[5]

Although the knowledge of the IdoA2S conformational equilibrium has been known for more than 20 years,[6] an understanding of its driving forces is mostly unknown. Early force field calculations on heparin oligosaccharides pointed out that the 3-*O*-sulfo group on GlcNS residues is capable of promoting small stabilization (0.5 kcal mol^{-1}) of the skew-boat conformation on the subsequent IdoA2S residue.[4] However, the assessment of more refined computations capable of providing a comprehensive understanding of IdoA2S conformational equilibrium was, until recently, absent.

The major contribution in this direction was supplied by Hricovíni, using B3LYP/6-311++G** on methyl 2-*O*-sulfo-α-L-iduronate monosodium salt, in a study that was able to reproduce the relative stability expected to

* Corresponding author. Tel.: +55 51 3308 7770; fax: +55 51 3308 7309; e-mail: hverli@cbiot.ufrgs.br

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occur for this residue in aqueous solution,[7] that is, a great prevalence of the $^{1}C_{4}$ chair conformation in the monosaccharide. These preferences were not reproduced by other authors, who identified the $^{2}S_{O}$ conformation as the preferred form of IdoA2S in solution using the same basis set.[8] Although these results may be explained by the limited set of starting conformations that can be simulated by quantum-mechanical calculations, it points to the potential advantages in employing molecular dynamics (MD) simulations to access a biologically relevant ensemble of conformations, correlated with biological phenomena.

In previous work, our group applied MD simulations to estimate the enthalpic penalty in the interaction of heparin with AT due to conformational modifications of IdoA2S.[9] The results suggested an absence of major conformational requirements, at least at the enthalpic level, on IdoA2S interaction with AT, as both skew-boat and chair conformations contribute with similar enthalpies upon interaction with the target protein. Additionally, these studies suggest that AT may select the predominant IdoA2S solution conformation, as seen in crystallographic data.[10] In a synthetic pentasaccharide, this residue lies almost completely in the skew-boat form when in solution.[11] A similar dynamic recognition was already observed to occur between heparin and FGF-1.[12,13] Nevertheless, the conformational equilibrium of IdoA2S is indeed capable of inducing conformational changes in heparin sequences in solution.[14,15] However, there is still no connection between the recent studies at the monosaccharide level of this residue,[7] where chair forms predominate,[4] and the entire polysaccharide chain, where the skew-boat may contribute from ~40% to 60% of the total IdoA2S conformational preference, depending on the heparin sequence.[4]

In this context, the current work intends to identify the interactions responsible for the IdoA2S conformational equilibrium in heparin sequences. Two models for evaluation of IdoA2S forms were employed: compound **1**, which represents the usual heparin sequences with considerable contributions of the $^{2}S_{O}$ conformation and compound **2**, which represents a heparin sequence with a major contribution of the $^{1}C_{4}$ conformation (Fig. 1). The methodology used includes the calculation of energy contour plots, that is, relaxed maps, associated with heparin oligosaccharides, including IdoA2S in its different solution conformations. The resulting minimum-energy geometries were further refined using a series of 0.2 μs MD simulations in explicit solvent. The results obtained expand information about IdoA2S flexibility from the monosaccharide to the polysaccharide level, contributing to an understanding of the forces responsible for the conformational preferences of this residue in oligosaccharide sequences.

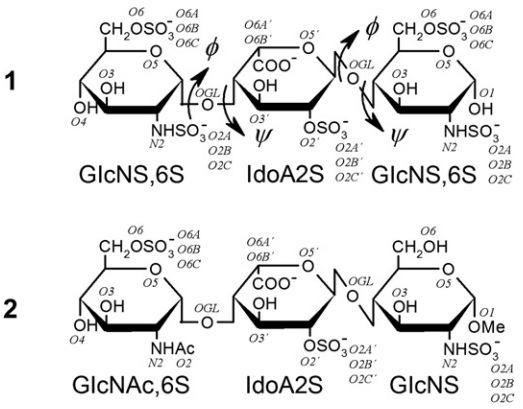

Figure 1. The two trisaccharide fragments of heparin, **1** and **2**, constructed based on the dodecasaccharide structure previously determined by NMR.[14]

2. Experimental

2.1. Computational methods

2.1.1. Nomenclature and software. The recommendations and symbols of nomenclature as proposed by IUPAC[16] are used. The relative orientation of a pair of contiguous carbohydrate residues 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:

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

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

All disaccharide topologies were generated with the PRODRG server,[17] manipulation of structures was performed with MOLDEN[18] and all the MD calculations and analysis were performed using GROMACS simulation suite [19] employing GROMOS96 force field as previously described.[9,15,20]

2.1.2. Topology construction. The heparin fragment under the 1HPN PDB code[14] was retrieved to be used on this work. The dodecassaccharide was reduced to the disaccharide units included in compounds **1** (GlcNS,6S–IdoA2S and IdoA2S–GlcNS,6S) and **2** (GlcNAc,6S–IdoA2S and IdoA2S–GlcNS), as illustrated in Figure 1. Although **1** shows a conformational distribution of its IdoA2S residue between $^{1}C_{4}$ and $^{2}S_{O}$ states in amounts of 60% and 40%, respectively,[4] compound **2** presents only the $^{1}C_{4}$ conformation in aqueous solutions.[21] These structures were then submitted to the PRODRG server and the initial geometries and crude topologies were retrieved. These two PRODRG topology files were used to describe properly the different conformational state of IdoA2S ($^{1}C_{4}$ or $^{2}S_{O}$) and GlcNS residues ($^{4}C_{1}$) through improper dihedrals.[15] Based on such an approach, it is possible to control the conforma-

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