Communication

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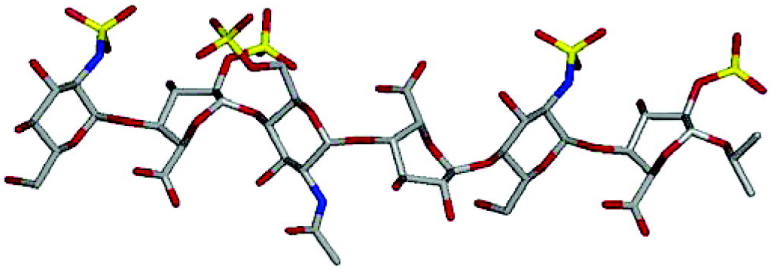

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Conformational Flexibility of a Synthetic Glycosylaminoglycan Bound to a Fibroblast Growth Factor. FGF-1 Recognizes Both the $^1C_4$ and ^2S_O Conformations of a Bioactive Heparin-like Hexasaccharide

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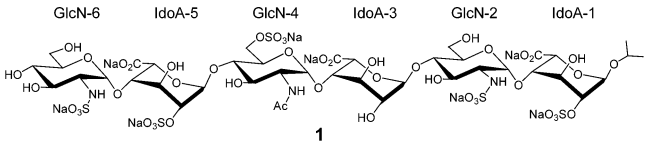
The study of the molecular recognition of carbohydrates by protein receptors at atomic level has attracted considerable interest during the past few years, due to their key role in a variety of relevant physiological processes.[1] In particular, major attention has been paid to the study of the biological, structural, and conformational details of the binding of glycosylaminoglycans (GAGs) to polypeptides of the fibroblast growth factor (FGF) family,[2] the chemokines,[3] and to antithrombin-III (AT-III).[4]

The structural basis of the biological activation of the different members of the FGF family is still a controversial issue.[5] There is convincing evidence that, at least in the case of both FGF-1 and FGF-2, GAGs induce the formation of a different sort of oligomers, a process that has been proposed to constitute an essential step in FGF-1-driven mitogenesis, prior to the recognition of the protein by the cell membrane receptors.[6] However, we have recently afforded data[7] that show that hexasaccharide **1**, in which the sulfate groups are exclusively oriented on a single side of the typical helical-like structure of GAGs,[8] only forms monomeric 1:1 complexes with FGF-1. Still, it induces a mitogenic activity of the order of heparin and is more active than a regular heparin-like octasaccharide, which contains the sulfate group pattern which has been proposed for high affinity recognition by FGF-1.[9]



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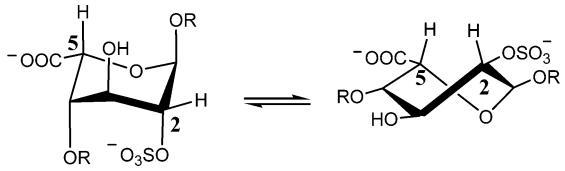
From the structural viewpoint, the extent of conformational mobility of GAGs in both the free and bound states has also been a matter of debate, especially focused on the chair-skew boat equilibrium of the iduronate rings[10] (Scheme 1). Although the features of this equilibrium are well documented in the free state, there are difficulties in deducing the six-membered shape in the bound states. In the AT-III case, Sinay *et al.* prepared skew boat conformationally locked compounds that keep the biological activity, thus providing direct evidence on the recognition of these conformers by AT-III.[11] However, the availability of direct evidence is sparse, and the possibility of having a conformational selection process or of binding different conformers, and thus of existence of a dynamic complex, has not been addressed. X-ray structures of complexes may not show enough resolution to deduce the fine conformational details,[12] and the application of NMR methods

Scheme 1. $^1C_4-^2S_O$ Conformational Equilibrium of Iduronate Rings Showing the NOE between H2 and H5 Exclusive for the ^2S_O Form



(especially exchange transferred NOE methods[13]) is hampered by the slow dissociation rate of the GAGs/FGF complexes, unless rather drastic experimental conditions are used.[14] Dimerization of the polypeptide in the presence of regular heparan sulfate (HS) sequences also poses experimental problems[15] for getting the key NMR parameters to answer these questions.

Within the context of a wider NMR study on the structural features of the complex between FGF-1 and **1**, we have shown that, according to sedimentation equilibrium data, the protein does not dimerize in the presence of the hexasaccharide and forms a well defined 1:1 complex.[16]

The access to a double-labeled [^{15}N,^{13}C] FGF-1 receptor[16] has allowed us to perform ^{13}C double-filtered NMR experiments.[17] Thus, all aliphatic TOCSY and NOESY cross-peaks of the ^{1}H−^{12}C pairs of the bound GAG have been assigned, without interferences from the ^{1}H−^{13}C protein protons.[18] The chemical shifts of the bound ligand signals are very similar to those observed for the free hexasaccharide,[8] suggesting strong structural similarities (Supporting Information). Moreover, the double-filtered NOESY (Figure 1) allowed the observation of key interglycosidic and intraresidue NOE peaks, permitting the definition of the bound 3D structure at the global and residue level. Indeed, the pattern of the NOE cross-peaks and their relative intensities are strikingly similar to those for the free sugar (Table 1).[8] Therefore, it can be safely concluded that the FGF-1-bound hexasaccharide keeps the typical helical shape present in heparin and HS.[19] A second essential aspect of the interaction of HS with FGFs is the role of the conformational equilibrium of the iduronate residues in the binding. NMR is particularly sensitive to the presence and extension of this equilibrium, due to the exclusive H2−H5 NOE for the ^2S_O conformer, incompatible with the $^1C_4$ form.[10] For the **1**/FGF-1 complex, the filtered NOESY experiments clearly show the H2−H5 cross-peaks for the two central iduronate rings, indicating the presence of an appreciable population of the ^2S_O conformers (Supporting Information). To get more quantitative information, intraresidual distances were determined by comparing the H2−H5 cross-peak relative intensities (at five different mixing times between 50 and 200 ms

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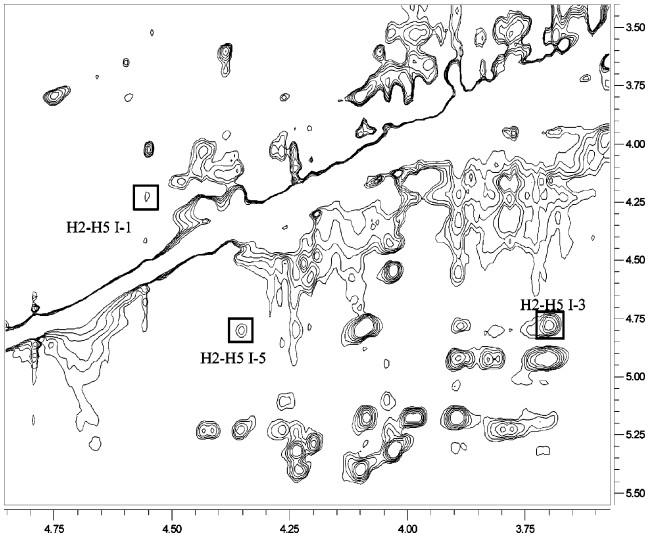

Figure 1. Expansion of the 600 MHz double ^{13}C filtered NOESY (100 ms mixing time) of the complex of **1** and FGF-1, showing the assignment of the ^2S_O exclusive H2−H5 NOE cross-peaks for the iduronic (I-1, I-3, I-5) moieties. The different intensities indicate the existence of skew boat conformers, but with different percentages for the different IdoA residues. The H2−H5 cross-peak for Ido-1 could not be integrated properly.

Table 1. Key Distances (from σ_{NOE} ratios at two magnetic fields) for **1** in the Free and FGF-1 Bound States[a]

proton	proton	free **1** (Å)	bound **1**
H1 GlcN-2	H3 IdoA-1	(2.6)	s (2.5)
	H4 IdoA-1	(2.4)	s (2.4)
H1 IdoA-3	H4 GlcN-2	(2.4)	s (2.4)
	H6R GlcN-2	(2.8)	m (2.7)
H2 IdoA-3	H5 IdoA-3	(2.7)	ms (2.6)
H1 GlcN-4	H3 IdoA-3	(2.3)	s (2.4)
	H4 IdoA-3	(2.5)	s (2.5)
H1 IdoA-5	H4 GlcN-4	(2.4)	s (2.4)
	H6R GlcN-4	(2.7)	m (2.7)
H2 IdoA-5	H5 IdoA-5	(2.8)	m (2.8)
H1 GlcN-6	H3 IdoA-5	(2.2)	s (2.3)
	H4 IdoA-5	(2.1)	s (2.2)

[a] NOEs at 200 ms mixing time and 600 MHz (bound) are given as strong (s), medium (m), or weak (w). The H2−H5 cross-peak for Ido-1 could not be integrated properly.

at 500 and 600 MHz) with those of the H4−H5, used as internal reference, since its corresponding distance (2.45 Å) basically does not change between both conformers. Interestingly, the $\sigma_{H2-H5}/\sigma_{H4-H5}$ ratios measured from the 600 MHz NOESY for the complex indicated the presence of $^1C_4$:^2S_O equilibrium, as for free **1**, ratios are 0.41 (bound) versus 0.36 (free) for Ido-5 and 0.69 (bound) versus 0.53 (free) for Ido-3. According to these data, the $^1C_4$:^2S_O ratios in the bound state for IdoA-5 and IdoA-3 are ca. 60:40 and 30:70, respectively, while those for the free state, based on *J* analysis, were 59:41 and 47:53.[20]

Thus, these results indicate unequivocally that within the complex with FGF-1, the iduronate rings of the bound oligosaccharide display a conformational equilibrium between the $^1C_4$ and ^2S_O forms, as for the free HS. This observation indicates that FGF-1 does not induce a conformer selection process, in contrast to that reported for AT-III, for example.

Therefore, this HS-like molecule displays conformational flexibility even bound to a key biological receptor, such as FGF-1. It has been shown that this conformational interconversion has a small effect on the global 3D shape of HS. Only the C-2 and C-3 Ido atoms swap their positions above and below the ring plane, changing accordingly the orientation of the sulfate groups at O-2.[21] This local dynamic might be possible in the bound state since the major interactions of the sulfate groups take place with Arg and Lys side chains,[22] which are flexible enough as to allow concerted motions.[23] Such local flexibility at both the receptor and ligand sides, together with the preorganization of the basic structure of the oligosaccharide, might be a mode for alleviating the entropic penalty associated with the restriction on the degrees of freedom of the side chains caused by the binding.

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Supporting Information Available: ^1H NMR assignment of the **1**/FGF-1 complex and NOESY spectra for free and complexed **1**. Three dimensional view of the hexasaccharide and of the complex. This material is available free of charge via the Internet at http://pubs.acs.org.

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