COMMUNICATION

Importance of the polarity of the glycosaminoglycan chain on the interaction with FGF-1

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Introduction

Fibroblast growth factor (FGF)-1 is a member of the fibroblast growth factor family that interacts with heparin/heparan sulfate (Hep/HS) polysaccharides and the membrane receptors fibroblast growth factor receptors (FGFRs). The formation of a ternary complex between FGF, FGFR and Hep/HS is the key step for the activation of the FGF signaling pathway. This is at the origin of different cellular essential functions as regulation of embryonic development, homeostasis and regenerative processes (Bernfield et al. 1999; Eswarakumar et al. 2005; Kreuger et al. 2006). Dimerization of the receptors and subsequent intracellular autophosphorylation activates a mitogenic response through an enzymatic cascade (Mohammadi et al. 2005; Pellegrini et al. 2000; Schlessinger et al. 2000).

The helical structure of Hep, a highly sulfated form of HS, directs the sulfate groups towards opposite sides of its longitudinal molecular axis (Mulloy et al. 1993). According to that the first crystallographic structures of the Hep and human FGF-1 complexes (pdb code: 1amx and 2amx) corresponded to dimeric structures linked by a regular Hep chain in its native helical structure (DiGabriele et al. 1998). However, nuclear magnetic resonance (NMR) data in solution corresponded to a 1 : 1 complex (pdb code: 2erm) (Canales et al. 2006). Remarkably, the dimers have two alternative symmetry relationships: while for 1amx the FGF-1 proteins are related by a center of symmetry, in the case of 2amx, the symmetry element is a plane along the binding site (DiGabriele et al. 1998). In addition, the ternary complexes of Hep and FGF with the extracellular domains of the membrane receptor FGFR are assembled into two different forms (pdb codes 1fq9 and 1e0o, respectively) (Pellegrini et al. 2000; Schlessinger et al. 2000; Pellegrini 2001).

The analysis of these structures indicates that the Hep/HS-binding site corresponds to a shallow depression on the surface of the growth factor that could be considered divided into two sub-binding sites (Digabriele et al. 1998). Consequently, as Hep does not much change its helical 3D structure upon binding, in the monomeric case as is the NMR complex (pdb code: 2erm) (Canales et al. 2006) some of its sulfate groups will be directed in the monomeric case as is the NMR complex (pdb code: 2erm) toward the solvent and will not interact with the FGF-1, as the structural studies have shown (Figure 1).

In order to analyze the Hep-FGF-1-binding mode, 2, a hexasaccharide with axially non-symmetric sulfate groups distribution and unable to form FGF dimers was prepared (Ojeda et al. 2002). The FGF-1-induced mitogenic activity of hexasaccharide 2 was higher than 1 (de Paz et al. 2001), which corresponds to the regular sulfation pattern of Hep (Angulo et al. 2004)
similar to the recently isolated from natural sources as hexamer (Smits et al. 2010). This result permitted to discard the dimerization of FGF-1 through a chain of bound Hep as a requirement for the FGF-1-mediated bioactivity. Interestingly, 3, which presents similar symmetry on the sulfate groups distribution than 2, with respect to the longitudinal axis, was inactive (de Paz and Martin-Lomas 2005). The substitution pattern of 3 was designed to fit with the requisites proposed by Pellegrini to maximize the interactions and symmetry in the ternary complex as it was deduced from the analysis of different crystallographic structures (Pellegrini 2001). Other synthetic oligosaccharides with diverse sulfation patterns prepared in our group lacking of sulfate groups in all the positions 6-\text{O} of glucosamines (4) or in all the 2-\text{O} of iduronates (5) were also inactive (Lucas et al. 2003). During the revision of this manuscript, a paper describing the synthesis of three hexasaccharides and examining their bioaffinities profiles for was published (Roy et al. 2014).

Recently performed was an in depth analysis of the 3D structure of 3 using NMR and molecular dynamics in order to search for any structural differences that might justify the loss of activity (Munoz-Garcia et al. 2013). From this analysis, it was concluded that 3 exhibits the same main structural features characteristics of Hep than the analogs 1 and 2, which are known to promote the interaction with FGF-1: (i) a well-defined rigid helical backbone with four residues per turn, (ii) a characteristic chair\textsuperscript{1C4}—skew boat\textsuperscript{2SO} conformational equilibrium for the iduronate residues and (iii) a rigid behavior of the glycosidic linkages. This structural analysis allows to discard any potential difference in the 3D structure that could justify the differences in the observed biological activity between 2 and 3 (e.g., modification of the glycosidic linkages geometry towards an anti-disposition) (Munoz-Garcia et al. 2013). Consequently, the main differences in affinity to FGF-1 among the hexasaccharides 1–5 would be due to the capacity of each sulfation pattern to interact with FGF-1 as a function of its spatial distribution along the chain (Figure 1).
Results

To investigate the ability of the five synthetic oligosaccharides, 1–5, to interact with human FGF-1, an inhibition assay was set up. The growth factor, either alone or coincubated with each of the five molecules to be analyzed, was injected over both a Hep-functionalized sensor chip and a streptavidin sensor chip, the latter being used as a control surface, and the interaction was followed by surface plasmon resonance (SPR) spectroscopy (Figure 2). Injection of 8.8 nM of FGF-1 over the Hep surface produced a binding response of 350 response units (RU) at equilibrium, whereas a response of 5 RU was observed over the streptavidin surface (data not shown). Analysis of the results showed that these oligosaccharides strongly differ in their ability to prevent FGF-1-Hep binding (Figure 2). The inhibitory activity of 1 was characterized by an IC\textsubscript{50} of 8.3 × 10\textsuperscript{-8} M, whereas 5 did not display binding activity in the range of concentrations tested indicating that, 2-O\textsubscript{sulfate} groups were essential for the interaction (Angulo et al. 2004). In contrast, 6-O\textsubscript{sulfate} sulfate groups, which are involved in the biological activity (Angulo et al. 2004), seem to be dispensable for the interaction with FGF-1, with an IC\textsubscript{50} = 4.7 × 10\textsuperscript{-7} M for 4.

Next, to investigate the importance of the presentation of the sulfate groups along the chain, the same assay was used with 2 and 3 since both have an asymmetric sulfate distribution. Interestingly, it was observed that, 2 features an IC\textsubscript{50} = 4.6 × 10\textsuperscript{-7} M, thus similar to 4, although 2 has five sulfate groups compared with six for the regular oligosaccharide. Finally, 3, which also displays six sulfate groups with an axially asymmetric distribution, has a much lower binding activity, with an IC\textsubscript{50} = 1.6 × 10\textsuperscript{-6} M.

Thus, while the mitogenic activity previously obtained was 2 > 1 >> 3, 4, 5 (Angulo et al. 2004), in the case of the binding affinity the order was 1 > 2, 4 >> 3 >> 5.

Discussion

Assuming a fundamental role for the electrostatic interactions, and considering the structural differences between the hexasaccharides, the sulfation pattern should be at the origin of the differences in the strength of the interaction and therefore in the activity. Apparent inconsistencies between the smaller IC\textsubscript{50} values for 1 compared with 2, measured by SPR experiments, and the induction of mitogenic activity, which is larger for 2, can be explained considering the assembly of the ternary complex. This is essential for the biological activity, and additional hidden requirements may play additional roles (Pellegrini et al. 2000; Schlessinger et al. 2000).

Compounds 2 and 3, as they have their sulfate groups directed towards one side of the molecular axis, only can interact with FGF using one of their half. On the contrary, as the 3D structures of 1, 4 and 5 have an axially symmetric distribution of sulfate groups, they have the possibility to interact with two molecules of FGF using two opposite sides of the oligosaccharide in a sandwich-like fashion, with two simultaneous binding events (de Paz et al. 2001; Lucas et al. 2003; Angulo et al. 2004). This observation might also explain the observed differences between the mitogenic activity measured by proliferation studies (Angulo et al. 2004) and the relative-binding strength to FGF-1, reported in this study.

Fig. 2. Inhibition of the FGF-1-HEP interaction by synthetic oligosaccharides. FGF-1 (8.8 nM) was preincubated with a range of concentrations of the different oligosaccharides and injected for 3 min over a HEP-activated sensor chip at 50 µL/min (see Supplementary data). The binding responses (in RU) were recorded as a function of time and corresponded to the FGF-1-HEP complexes in presence of 1 (A), 2 (B), 3 (C), 4 (D) and 5 (E). The oligosaccharide concentrations were (from top to bottom curves in each panel) 0, 0.0055, 0.0165, 0.15, 0.5, 1.33 and 4 µM.
The Hep binding site of FGF-1 could be divided into two spatially contiguous subsites (DiGabriele et al. 1998). The first one binds a trisaccharide GlcNS–IdoA2S–GlcN6S, interacting via three negatively charged sulfate moieties (Saxena et al. 2010). Such arrangement of charged groups displays the proper number and orientation of sulfate groups to establish a tight interaction with FGF-1. Recent studies have revealed key differences between FGF-1 and FGF-2 binding to GAG in this subsite. For the case of FGF-2, the trisaccharide that interacts in subsite a is the complementary one, Ido2S–GlcNS–Ido2S (Saxena et al. 2010). The second subsite interacts with a disaccharide, GlcN6S–IdoA2S. A central iduronate residue with a non-participating sulfonate group links both motifs. Remarkably, hexasaccharides 2 and 3 display simultaneously these two decorations for the interaction with FGF-1 but in reverse order if the polarity of the chain is considered.

In an attempt to find a satisfactory explanation to the lack of activity of 3 with respect to 2, a molecular modelling docking protocol was employed to analyze the molecular interactions from a structural perspective. Thus, the backbone of the most representative conformation of 3, taken from 500 ns of unrestrained molecular dynamics trajectory (Munoz-Garcia et al. 2013), was manually superimposed to the most representative structure of the NMR complex between FGF-1 and 2 (pdb 2erm). As the distances between the three sulfate groups directed towards the same side of the molecule were similar, two polarities for superimposition were used, from the reducing to non-reducing end and its reversed alternative. We have employed as first criteria, the alignment of the longitudinal axis of both carbohydrates. However, the positions of the sulfate and sulfamate groups of 3 were not adequate for the complete interaction with the complementary residues of the protein. After that, the non-reducing end trisaccharide of 3 was manually docked into the main subsite in the “reverse” orientation. In this case, the rest of the oligosaccharide did not fit in the complete binding pocket and pointed towards outside the complex. In addition, a steric clash was observed between the protein side chains and the GlcN–IdoA–Glc6N trisulfated trisaccharide of 3 (see Supplementary data for description of additional modes). The impossibility to assemble a complex with the complete set of charged interactions between the FGF-1 and the hexasaccharide 3 leads us to conclude that the correct polarity of the GAG chain is essential for the interaction with the growth factor (FGF-1).

We decided to perform docking calculations in order to get a deeper insight into the possible binding of 3 and FGF-1. We have used Glide, first using the Induced Fit Docking protocol with the standard conditions and then the results were subjected to a run of Single Precision Docking. In this case, the focus was put into the three saccharides of the triad, leading to a displaced sequence. A remarkable superimposition of the poses for this region was found in the solutions (see Supplementary data).

Interestingly, 2 (Figure 3A) has both sulfate clusters in the right disposition to interact simultaneously with both binding subsites, whereas 3 is only capable of interacting with the subsite a (Figure 3) (Canales et al. 2006). This can explain the value of IC50 for 3, 3.5-fold larger than 2. This difference can be explained considering that the polarity of the glycosaminoglycan chain is essential for the maximum number of interactions take place. While the subsite a is interacting with 2 through the trisaccharide GlcNS–IdoS–Glc6S starting at glucosamine in position i, the equivalent trisaccharide that binds the main subsite in the case of 3 is shifted to the GlcN at position i + 4. The secondary binding subsite does not establish any interaction with 3, thus explaining the lower affinity measured by SPR and the absence of mitogenic activity due to the failure to assemble of higher order complexes needed. Therefore, the interaction between FGF-1 and 3 should be weaker than 2. This should be the cause why 2 and 3 showed such dramatic
differences in their binding affinity and bioactivity in spite of bearing the same two binding motifs, but in opposite order.

Additional information can be extracted from the comparison between the affinity experiments and biological activity ones. For instance, the sulfation in position 6 of glucosamine that, according to our previous biological results, is essential for the FGF-1 mitogenic activity (Angulo et al. 2004), it is not for the interaction with FGF-1. That observation might be exploited in the design of potential inhibitors of the FGF-1-mediated mitogenic activity that being able to interact with the FGF-1, the absence of this key group prevent the assembly of the ternary active complex, and the subsequent biological activity. Another important conclusion that can be extracted from our work is the evidence of the strong influence of the polarity of the GAG chain on the binding. This also can be exploited for the design of inhibitors that interacting with the FGF-1 they do with the opposite polarity and they will not be able to form the active ternary complex.

In summary, we have demonstrated that the polarity of the oligosaccharide chain relative to FGF-1 is a critical factor for the strength of the binary interaction and further assembly of the ternary complex (Brown et al. 2013).

Materials and Methods

Syntheses of compounds 1–5 have been previously described (de Paz et al. 2001; Ojeda et al. 2002; Lucas et al. 2003; de Paz and Martin-Lomas 2005).

Size defined Hep (6 kDa) was immobilized on a Biacore sensor chip. For that purpose, Hep was biotinylated at its reducing end by coinubcation with 10 mM biotin/LC-hydrazine for 24 h at room temperature. The mixture was then extensively dialized against H2O to remove unreacted biotin and freeze-dried. Two flow cells of a CM4 sensorchip were then functionalized with ~2500 RU of streptavidin as described (Crublet et al. 2008) and biotinylated HP (5 µg/mL), in HBS-EP (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20, pH 7.4) was injected across one flow cells to obtain an immobilization level of 50 RU. The other flow cell was left untreated and served as negative control. For binding assays, 150 µL of FGF-1 (8.8 nM), co incubated with a range of concentration of the different oligosaccharides, were simultaneously injected, at a flow rate of 50 µL/min, over the control and the HP surfaces. The formed complexes were washed with running buffer for 3 min and the sensorchip surfaces were regenerated with a 3 min pulse of 2 M NaCl. Control sensorgrams were subtracted on line from HP sensorgrams.

The protein database structures 1amx, 2aam and 2erm were used for the preliminary studies of docking described in this paper, performed with Glide (Friesner et al. 2004). The monomer C from the 1amx complex was isolated from the rest of the aggregates and used to prepare the model of the hexasaccharide 3 with FGF-1 by superimposition of the trisaccharide of its reducing end with the one at the non-reducing end of the 1amx and/or 2erm complexes aligning the sulfate groups. Hydrogen atoms were added to the crystallographic structure when it was necessary using the Maestro protein preparation module. The corresponding hexa- and pentasaccharides were prepared and named consistently and using partial charges from GLYCAM (Kirschner et al. 2008), ligand preparation module was run and the structure was minimized. A grid (10 × 10 × 10 Å) centered in the glycosaminoglycan was constructed. We first run an Induced Fit Docking with the standard conditions keeping the GLYCAM charges. The resulting structures were submitted to a Single Precision Docking, with a 10 Å grid using GLYCAM partial charges with an electrostatic cutoff of 2.0. The minimization was performed using OPLS-2005 force field with a dielectric constant of 4r.

Supplementary Data

Supplementary data for this article are available online at http://glycob.oxfordjournals.org/.

Conflict of interest statement

None declared.

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Abbreviations

FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; Hep, heparin; HS, heparan sulfate; NMR, nuclear magnetic resonance; RU, response units; SPR, surface plasmon resonance

References


Directionality of glycosaminoglycan chain


