Hepatocyte growth factor/scatter factor and its interaction with heparan sulphate and dermatan sulphate

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Abstract

Hepatocyte growth factor (HGF)/scatter factor (SF) is a unique growth factor, in that it binds both heparan sulphate (HS) and dermatan sulphate (DS). The sequences in HS and DS that specifically interact with and modulate HGF/SF activity have not yet been fully identified. Ascidian DS, which uniquely possesses O-sulphation at C-6 (and not C-4) of its N-acetylgalactosamine unit, was analysed for HGF/SF-binding activity in the biosensor. The kinetic analysis revealed a strong, biologically relevant interaction with an equilibrium dissociation constant ($K_d$) of approx. 1 nM. An Erk activation assay also demonstrated stimulation of the MAP kinase pathway downstream of the Met receptor following addition of both HGF/SF and ascidian DS to the glycosaminoglycan-deficient CHO-745 mutant cell line. Furthermore, the activation of Met and the MAP kinase pathway by HGF/SF and ascidian DS leads to a cellular response in the form of migration.

Hepatocyte growth factor (HGF)/scatter factor (SF) is a pleiotropic growth factor that binds to and activates the tyrosine kinase receptor Met. Receptor activation leads to the stimulation of various signalling pathways, which result in cellular responses, such as cell proliferation, motility, angiogenesis, differentiation, invasiveness and protection from apoptosis.

Substantial clinical data show that various types of tumours have up-regulation of HGF/SF and/or Met. Indeed, the stage or invasiveness of the tumour often correlates positively with increased HGF/SF and/or Met activity. This is a likely consequence of Met activation leading to the stimulation of growth and motility of the tumour cells, as well as an increase in the secretion of matrix-degrading proteases facilitating the invasion of the surrounding stroma by the motile cells. Also, the potent angiogenic action of HGF/SF may further aid tumour growth by stimulating the development of a supporting vasculature. Inhibition of this up-regulated activity of the HGF/SF–Met system may be beneficial in the treatment of tumours.

HGF/SF contains a 54 kDa α-chain, disulphide-linked to a 26 kDa β-chain. The α-chain possesses a hairpin loop close to the N-terminus, followed by a sequence of four kringle domains. Binding of HGF/SF to the Met receptor requires the hairpin loop and the first kringle domain.

HGF/SF has also been shown to bind the glycosaminoglycan (GAG) chains of heparan sulphate (HS) [1] and dermatan sulphate (DS) [2] proteoglycans, although with lower affinity than to the Met receptor. GAGs bind HGF/SF primarily via the hairpin loop of the α-chain, with possibly the second kringle domain also being involved [3]. The GAG-binding site is thus located in very close proximity to the Met receptor-binding site on the α-chain. In fact, GAGs are a necessary co-receptor for Met activation [4], and evidence suggests that an active ternary complex forms between HGF/SF, Met and appropriate proteoglycans [5].

HS is composed of repeating disaccharide units that consist of N-substituted glucosamine linked to either glucuronic acid or iduronic acid. N-sulphation of the hexosamine and the epimerization of glucuronic to iduronate residues serve as prerequisites for O-sulphation of these modified disaccharide units, primarily at C-2 of iduronate and C-6 of the hexosamine. DS, in contrast, is composed of N-acetylgalactosamine linked to either glucuronic acid or iduronic acid residues. O-sulphation occurs on the hexosamine, primarily at C-4, and to a lesser extent on C-2 of iduronate.

Many in vitro studies have shown that specific sulphation patterns are necessary for GAGs to bind/activate a particular protein. A tetrasaccharide sequence in heparin is the minimum size for HGF/SF–Met activation [6]. The essential structural features required for HS or DS to bind HGF/SF are not precisely known. Lyon et al. [1] speculated that idurionate residues and 6-O-sulphation of glucosamine residues are important for binding of HGF/SF to HS, but not N-sulphates. Also, 2-O-sulphation of idurionate residues is not a requirement [7]. As DS contains iduronate and 4-O-sulphated rather than 6-O-sulphated hexosamine, it was maybe surprising that this GAG can also bind HGF/SF with high affinity [2]. Perhaps 6-O-sulphation of the hexosamine is, in fact, not important for HGF/SF binding, or perhaps...
the 4-O-sulphate on the N-acetylgalactosamine in DS can adopt a similar spatial position as the 6-O-sulphate on the N-sulphoglucosamine of HS in the solution structures of these two GAGs.

To try and answer this puzzle, a unique DS from the murine tunicate, *Ascidia nigra*, was employed [8]. This DS possesses 6-O-sulphation of the hexosamine residue, rather than the 4-O-sulphation that is common in mammalian DS. In addition, it contains substantial 2-O-sulphation of iduronate (Figure 1). If there is a strict spatial requirement for sulphate groups that is satisfied by 4-O-sulphation of the DS hexosamine or by 6-O-sulphation of the HS hexosamine for binding of HGF/SF, then the ascidian DS may be expected to not bind HGF/SF. Surprisingly, the ascidian DS does bind HGF/SF, and with high affinity. Moreover, binding leads to productive activation of the Met receptor, as detected by MAP kinase (ERK 1/2) activation, and a cellular response. Interestingly, HGF/SF bound the ascidian DS with slightly higher affinity compared with its binding to mammalian DS, and similar to that observed with mammalian HS (Table 1).

Perhaps the GAG-binding site in HGF/SF is relatively flexible in its hexosamine sulphation requirement, but with 6-O-sulphation being slightly preferred over 4-O-sulphation. Or maybe hexosamine O-sulphation is not actually such a major contributor to the interaction. The results with the ascidian DS highlight the need for further investigation of the requirement for specific sulphate groups on HS/DS for productive binding to HGF/SF. A programme of selective modification of these GAGs is presently under way in order to clarify these requirements. Elucidation of the minimum activatory sequence is necessary in order to fully understand the co-receptor function of GAGs, and to aid in the design of potential therapeutic inhibitors of HGF/SF activity in cancer.

**Table 1** | $K_d$ values derived from the binding of soluble HGF/SF to surface-immobilized GAGs by surface plasmon resonance

<table>
<thead>
<tr>
<th>HGF/SF-binding GAG</th>
<th>$K_d$ (nM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalian HS</td>
<td>$\sim 0.2-3.0$</td>
<td>Rahmoune et al. [9]</td>
</tr>
<tr>
<td>Mammalian DS</td>
<td>$\sim 20$</td>
<td>Lyon et al. [2]</td>
</tr>
<tr>
<td>Ascidian DS</td>
<td>$\sim 1$</td>
<td></td>
</tr>
</tbody>
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The predominant disaccharide structures of the HGF/SF-binding domains of HS and DS are shown in Figure 1.

**References**


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