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Cooperativity in Glycan-Protein Interactions

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Using FGF-2 HSGAGs as a model, de Paz and colleagues [1] have demonstrated the importance of multivalency in cooperative glycan-protein interactions to achieve specificity.

Heparin and heparan sulfate glycosaminoglycans (HSGAGs) are complex polysaccharides that are primary components on the cell surface and extracellular matrix (ECM). Due to their ubiquitous distribution at the cell-ECM interface, HSGAGs interact with numerous proteins and modulate their activity, and thus impinge on fundamental biological processes such as cell growth, development, inflammation, immune response, microbial infection, and cancer [2]. HSGAGs are composed of a disaccharide repeat unit of α-D-glucosamine linked 1→4 to α-L-iduronic (IdoA) or β-D-glucuronic acid (GlcA). Each repeat unit in the HSGAG backbone can be modified via sulfation at the 2-O position of the uronic acid, and 3-O and 6-O of the glucosamine. The N-position of glucosamine can be sulfated (GlcNS) or acetylated (GlcNAc). HSGAGs are synthesized as O-linked glycosylation at a consensus motif of Ser-Gly/Ala-X-Gly in a core protein (HSGAG and protein together are known as proteoglycan).

The biosynthesis of HSGAGs is a complex nontemplate driven process involving several enzymes which give rise to regions of distinct sulfation patterns [3, 4]. By utilizing this complex biosynthetic machinery, the cell maintains a diverse set of HSGAG chains on the cell surface and thus dynamically changes its environment in response to biochemical signals. It is generally believed that HSGAG biosynthesis results in stretches of highly sulfated (NS) domains that are intervened by undersulfated GlcNAc (NA) domains [5]. Thus, a large diversity in HSGAG sequences arising from their sulfation pattern and epimeric state of the uronic acid facilitates binding to a variety of proteins such as growth factors, enzymes, morphogens, and surface proteins on microbial pathogens in the extracellular environment. The emerging view is that the biological activity of many HSGAG-binding signaling molecules is mediated by the specificity of HSGAG-protein interactions [2]. Given the abundance of HSGAG in the cell surface-ECM interface it is important to understand what governs the specificity of glycan-protein interactions. It is generally viewed that short stretches of NS separated by the NA domains can provide the appropriate binding template for monomeric, as well as oligomeric, HSGAG binding proteins. The appropriate length and distribution of the NS and NA domains could thereby confer the needed binding specificity [5, 6].

The interaction between HSGAGs and the fibroblast growth factor (FGF) family (specifically FGF-1 and FGF-2) is one of the best-characterized systems. The overall mechanism of FGF signaling involves binding of FGF to cell surface HSGAGs which act as coreceptors to facilitate FGF oligomerization and binding of FGF to its tyrosine kinase receptors (FGFRs) on the cell surface, leading to FGFR oligomerization and subsequent activation of MAP kinase signaling pathway. Therefore, HSGAGs are involved in multivalent interactions with multiple FGF molecules, facilitating their oligomerization, and with FGFR molecules, facilitating formation of multimeric assembly of FGF-FGFR complexes.

The length of HSGAG chain, sulfation pattern, and conformation of the IdoA residue are critical determinants of HSGAG-mediated FGF signaling. Structural studies on unbound HSGAG oligosaccharides point to a helical structure with 2_1 symmetry. Binding to FGF-1 and FGF-2 induces a kink in the helical structure of the oligosaccharide and this kink spans a trisaccharide motif of the form -GlcNS, 6S-IdoA2S-GlcNS,6S- [2]. The minimal FGF-1 and FGF-2 binding motif in HSGAGs appears to be a tetrasaccharide which is composed of the trisaccharide spanning kink and an IdoA2S sugar at the reducing or nonreducing end of this trisaccharide [2, 7]. In both FGF-1 and FGF-2, the kink positions the NS and 2S groups to provide maximum contact with the HSGAG binding site. On the other hand, while the 6S group interacts with FGF-1, it does not make significant contact with FGF-2 [8, 9]. The positioning of these minimal FGF-binding tetrasaccharide motifs in the context of chain length (and distribution) plays a critical role in association of FGF oligomers, as well as FGF-FGFR complexes. In the case of FGF-1, the minimal tetrasaccharide motif binds to two FGF-1 monomers to form a HSGAG bridged FGF-1 dimer complex [8]. Unlike FGF-1, FGF-2 self-associates, even in the absence of HSGAGs, to form dimers and higher order oligomers that facilitate FGF-2 signaling. HSGAGs containing multiple FGF binding tetrasaccharide motifs within a sufficient chain length (octasaccharide at least) stabilize and facilitate these active FGF-2 oligomers. However, shorter HSGAG chains can bridge two FGF-2 monomers in an inactive state, sequestering them in the ECM.

In the diverse sequence space of HSGAGs at the cell-ECM interface, the presence of such defined tetrasaccharide motifs in the right context of chain length for biological specificity in FGF signaling is expected to be in

low abundance. Importantly, there is multivalency in both FGF-2 association and HSGAG presentation as multiple chains on one or more proteoglycans. This multivalency enhances the cooperativity of HSGAG-FGF-2 interactions particularly given the low molar abundance of defined protein binding oligosaccharide motifs. The positive effect of multivalency enhancing FGF signaling was demonstrated using an engineered construct of an FGF-2 dimer [10]. It was demonstrated both in vitro and in vivo that much lower doses of the dimeric form of FGF-2 were sufficient to attain the maximum signaling response in comparison with monomeric FGF-2.

While most of the studies on FGF-2-HSGAG interactions have focused on multivalency in FGF-2 association, the study by de Paz et al. [1] in this issue of *Chemistry & Biology* has provided important insights into effects of multivalency in the presentation of HSGAG chains. Amine functionalized end groups have been utilized to attach multiple oligosaccharides to the polyamidoamine dendrimers resulting in about eight oligosaccharides per dendrimer. Elegant coupling chemistries have been utilized to perform binding studies on heparin-coated microarrays and SPR analysis on CM5 gold chip. Dendrimer **1** used in this study [1] composed of multiple HSGAG hexasaccharides (containing the minimal FGF-2 binding tetrasaccharide motif) shows the strongest binding to FGF-2 in comparison with other dendrimers. Importantly this multivalent dendrimer shows significantly higher affinity to FGF-2 as compared to the monomeric form of the same hexasaccharide (compound **6**) used in this study [1]. The fact that dendrimers of disaccharides and monosaccharides show only weak to moderate affinity despite the multivalent presentation reflects the minimum requirement of tetrasaccharide chain length for optimal FGF-2 binding. In the cell culture experiments to measure FGF-2 signaling, dendrimer **1,** and not hexasaccharide **6,** caused an increased expression of phosphorylated ERK-1 (downstream FGF-2 signaling molecule). The inability of the hexasaccharide to activate FGF-2 signaling is consistent with other studies that demonstrate that longer HSGAG oliogsaccharides are required for FGF-2 signaling [11]. However, the multivalent presentation of the hexasaccharide in dendrimer **1** appears to circumvent this requirement by facilitating the assembly of FGF2-FGFR complexes for FGF-2 signaling. The positive control for binding used in this study [1] is a 5 kDa heparin which is composed of longer oligosaccharides up to hexadecasaccharide chain length. Such long chains are likely to have multiple FGF-2 binding tetrasaccharide motifs and this is reflected by the much higher affinity of the 5 kDa heparin to FGF-2 as compared to dendrimer **1**. These results point to distinct effects of the kind of multivalency—presentation of multiple FGF-2 binding tetrasaccharide motifs in a longer chain versus single motif on multiple shorter chains—on FGF-2 association, FGF-FGFR interactions, and FGF signaling.

Earlier studies have shown that different HSGAG affinities to FGF generally correlate with the degree of sulfation [11]. This has led to the notion that nonspecific charge interaction may also exist in the formation of an FGF-HSGAG-FGFR complex [6]. Challenges in the past have been primarily due to mimicking physiological multivalent interactions while performing binding studies using defined HSGAG oligosaccharides. The current study and others have demonstrated the need for multivalency to provide the appropriate specificity for HSGAG-protein interactions. However, the concept of multivalency has also confounded the views of biologists as it is challenging to grapple with the issue of specificity, given the fact that affinity can be altered by multivalency in biochemical experiments. The work reported by de Paz and colleagues has contributed toward the understanding of multivalent HSGAG-protein interactions.

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REFERENCES

1. de Paz, J.L., Noti, C., Böhm, F., Werner, S., and Seeberger, P.H. (2007). Chem. Biol. *14*, this issue, 879–887.
2. Sasisekharan, R., Raman, R., and Prabhakar, V. (2006). Annu. Rev. Biomed. Eng. *8*, 181–231.
3. Esko, J.D., and Selleck, S.B. (2002). Annu. Rev. Biochem. *71*, 435–471.
4. Sugahara, K., and Kitagawa, H. (2002). IUBMB Life *54*, 163–175.
5. Gallagher, J.T. (2006). Biochem. Soc. Trans. *34*, 438–441.
6. Kreuger, J., Spillmann, D., Li, J.P., and Lindahl, U. (2006). J. Cell Biol. *174*, 323–327.
7. Guerrini, M., Agulles, T., Bisio, A., Hricovini, M., Lay, L., Naggi, A., Poletti, L., Sturiale, L., Torri, G., and Casu, B. (2002). Biochem. Biophys. Res. Commun. *292*, 222–230.
8. DiGabriele, A.D., Lax, I., Chen, D.I., Svahn, C.M., Jaye, M., Schlessinger, J., and Hendrickson, W.A. (1998). Nature *393*, 812–817.
9. Faham, S., Hileman, R.E., Fromm, J.R., Linhardt, R.J., and Rees, D.C. (1996). Science *271*, 1116–1120.
10. Kwan, C.P., Venkataraman, G., Shriver, Z., Raman, R., Liu, D., Qi, Y., Varticovski, L., and Sasisekharan, R. (2001). J. Biol. Chem. *276*, 23421–23429.
11. Jastrebova, N., Vanwildemeersch, M., Rapraeger, A.C., Gimenez-Gallego, G., Lindahl, U., and Spillmann, D. (2006). J. Biol. Chem. *281*, 26884–26892.

