

Specificity in Heparan Sulfate-Protein Interactions: Tools and Strategies for Elucidating Biological Functions

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It is becoming clear that heparan sulfate (HS) has a role as a dynamic cell regulator which cells use to tune the activity of many proteins and thus control their behaviour and responsiveness to the extracellular environment¹. Developing knowledge of the specific (HS) saccharide sequences which confer functional interactions with these proteins is a key to understanding the role of HS in biological processes. A prototypic example has been studies on HS in signaling by fibroblast growth factors (FGFs), in which HS saccharides have been demonstrated to be receptor-specific and ligand-specific modulators of activity². In addition there is emerging evidence that HS structures with differential FGF signaling activities are dynamically varied during development³. Such studies indicate the need to explore in detail the degree of specificity in HS-protein interactions, and the extent to which such specificity is actually utilized *in vivo* for cell regulation.

A powerful approach for investigation of HS-protein interactions is to generate libraries of saccharides prepared from HS and heparin. After fragmentation saccharides can be purified by gel filtration and SAX-HPLC chromatography and tested in binding or bioassays, either *in vitro* or *in vivo*^{2,4}. Structures of interest can be analysed and sequenced⁵ to reveal the specific structural features required for a particular activity. In addition, it is possible to use optical biosensors to determine the affinity and kinetics of binding of proteins to heparin/HS poly- and oligo-saccharides, and in competition binding assays⁶. We have also used chemically modified heparins to determine the specificity of binding of HS to proteins including FGF receptors, prion protein, amyloid precursor protein and the Alzheimer's β -secretase. Recently we have developed a novel strategy for engineering libraries of HS saccharide analogues by selective chemical modifications of heparin. These libraries have proved very useful in structure-activity studies in a variety of assays including FGF signaling, angiogenesis and anticoagulation. In addition, we have developed a complementary microarray approach for highly parallel screening of the interaction of saccharide libraries with protein partners. Data will be described covering these different strategies and illustrating how they can be used to determine the specificity of HS-protein interactions and the relevance of this specificity to the biological functions of HS.

References:

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