Chemistry & Biology
Preview

Got Sulfate? Luring Axons This Way and That

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DOI 10.1016/j.chembiol.2007.02.001

Glycosaminoglycans are sulfated in complex and changing patterns that affect neural development. These sugars mediate interactions between macromolecules, and their biological contributions are of high interest. In this issue of *Chemistry & Biology*, Shipp and Hsieh-Wilson [1] describe microarrays to probe these complex modifications.

Sulfation has long been recognized as a crucial modification involved in cell function and survival. Initial studies focused on elucidating the structure [2] and synthesis [3] of the special carbohydrate component of proteoglycans, the glycosaminoglycan (GAG). Concomitantly, other studies identified sulfation of small molecules as integral to many different physiological processes, e.g., elimination of the end products of catabolism, inactivation of hormones, and bioactivation of xenobiotics [4]. Subsequently, studies focused on posttranslational sulfation of many secreted and membrane-bound proteins as a determinant in protein-protein interactions, mediating leukocyte adhesion, hemostasis, and chemokine signaling [5]. Recently, interest has refocused on sulfate modifications as molecular determinants in glycosaminoglycan chains [6].

Glycoaminoglycan chains comprise a core unsulfated tetrasaccharide covalently O-linked to a protein backbone via serine residues, to which is attached a series of repeating disaccharides of uronic acids and N-acetylhexosamines modified predominantly by sulfation (Figure 1). The GAG chains of proteoglycans are critical environmental modulators. They play important roles in cell differentiation and tissue morphogenesis via their interaction with or adhesion to cell or other matrix components, or by binding to growth regulators and differentiation factors. These roles are particularly striking in the development of the nervous system where proteoglycans may promote or inhibit neurite outgrowth, stabilize new synapses, or inhibit axon regeneration [7].

Because of the enormous structural diversity arising from the type and content of the glycosaminoglycan chains of proteoglycans, the isolation, identification, characterization, and elucidation of the functions of these complex macromolecules elaborated during neural development is a challenge. Of special consideration is the function of the sulfate groups which decorate the carbohydrate chains, and whether their specific patterns, sequences, or positions encode a molecular recognition system that modulates biological processes, i.e., a "sulfate code" [8].

This question has been advanced, biochemically by purifying and sequencing complex GAG structures [9], and genetically by targeting specific sulfotransferase genes to produce altered sulfation patterns [10]; each has its obvious limitations. Most recently, chemical approaches are being developed to assemble oligosaccharide backbones along which sulfate groups can be directed to specific sugar sites [8]. Although not a simple or straightforward approach (i.e., more than 40 chemical steps may be required to create a single saccharide chain), the ability to create well-defined oligosaccharide sequences has opened up a new field of the systematic investigation of the role of sulfation in biological phenomena. Shipp and Hsieh-Wilson [1] describe the use of well-defined oligosaccharide structures in probing the interactions of growth factors and chemotatic proteins, previously shown to be important for neural development, with GAG sequences found naturally in the nervous system.

Specifically, Shipp and Hsieh-Wilson report a new high-throughput array similar to those reported for DNA and protein microarrays to probe specific GAG modifications in an efficient and systematic manner [1]. Unlike previous arrays where each sulfate modification was chemically synthesized [8], this new array uses commercially available GAG chains with defined sulfate modifications. This new microarray approach takes advantage of the known strong noncovalent interaction between GAG chains and poly-L-lysine to immobilize the GAG chain onto a glass slide without any special chemical modifications. This array overcomes many hurdles associated with the previous carbohydrate arrays, including time and expertise in carbohydrate chemistry.

As a proof of principle, the authors used the known interaction of fibroblast growth factors (FGFs) with heparan sulfate (HS) to show that different members of the FGF family have specific affinities to unique sulfated GAG chains. The results of the array were in agreement with previous reports [11] about the affinity of FGFs for HS. Furthermore, these experiments highlight the validity of the arrays, establishing them as a new tool for understanding protein interaction with discrete sulfated GAG chains. This new array can be used by anyone interested in probing the specificity of their protein of interest to defined sulfated GAG structures.

The authors use the nervous system as a biological context to demonstrate

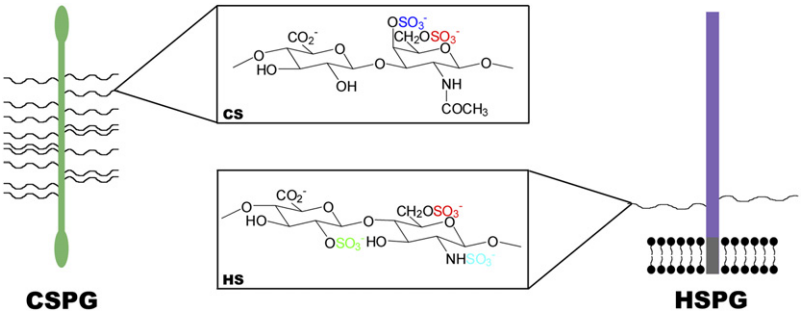

Figure 1. Schematic Representation Depicting Chondroitin Sulfate Proteoglycans (CSPGs) and Heparan Sulfate Proteoglycans (HSPGs)

The core proteins of CSPGs (green) tend to have multiple glycosaminoglycan (GAG) chains (black wavy lines) per core protein composed of repeating GalNAc units sulfated either at the 4 (blue, CS-A), or 6 (red, CS-C) positions. HSPGs' core proteins (purple) tend to have fewer GAG chains per core protein. They are composed of repeating GlcNAc units sulfated at 6 (red) position, and 2-N-sulfated (teal), and uronic acid units sulfated in the 2 position (green).

the application of their microarray technology. In the developing central nervous system, neuronal axons and migrating cells often must navigate long distances through a complex cellular landscape in order to reach their appropriate targets. Although several families of molecules that guide these axons and cells have been described, how the signaling of these molecules is modulated and integrated is not fully understood [12]. The expression of proteoglycan core proteins and their modifying enzymes are developmentally regulated and tissue specific [13]. Heparan sulfate and chondroitin sulfate (CS) proteoglycans have been found to be important modulators of the secreted molecules involved in both axon guidance and cell migration, but the mechanism and specificity of these interactions has not been examined [10]. Using the microarray approach, Shipp and Hsieh-Wilson present evidence that these interactions occur in a sulfation-dependent manner, and that different axon guidance molecules have distinct binding specificities for the pattern of HS or CS sulfation [1]. The authors examine the interaction of the secreted chemoattractants/repellents netrin, slit2, semaphorin5B, ephrinA1 and ephrinA5 with differently sulfated HS and CS chains (as well as dermatan sulfate, keratan sulfate, and hyaluronic acid). Interestingly, the authors found that while each of these molecules binds HS and double-sulfated chondroitin sulfate (CS-E) chains, their specificities for these chains were quite unique and dependent on the concentration of the sulfated chain. For example at high carbohydrate concentrations, slit2 shows a higher affinity for CS-E than for heparan, while ephrinA1 shows a higher affinity for heparan than for CS-E [1]. Additionally, it is demonstrated that it is not only the number of sulfate groups per disaccharide unit, but also their precise placement that is important for mediating the interaction between the guidance cue (attraction or repulsion) and the extracellular milieu. The authors validate the array data through the use of in vitro cell migration and axon outgrowth assays to illustrate the importance of specific patterns of HS and CS sulfation in slit2-mediated repulsion and netrin-mediated attraction. Therefore, this microarray approach provides a means to dissect whether the interplay of CS and HS may shape the gradients of these secreted guidance molecules along an axonal trajectory.

The ability of axon guidance molecules to interact with HS and CS in a concentration-dependent, sulfation-specific manner is intriguing, as it suggests a means by which the response of a growing axon to a myriad of guidance cues in its environment can be modulated via a "sulfate code." In the future, it will be important to examine the nuanced roles of specific HS and CS patterns in promoting axonal outgrowth versus serving a role in actual guidance (i.e., attraction and repulsion). There are already reports of the importance of appropriate sulfation in the guidance of axons in *C. elegans*, *Xenopus*, and mice, which have begun to shed light on the in vivo relevance of these interactions [14–16]. As we continue to develop an understanding of how guidance molecules can be modulated by the extracellular milieu, it will become clearer how the relatively small number of axon guidance molecules discovered to date can work together to pattern the numerous intricate connections of the central nervous system.

REFERENCES

1. Shipp, E., and Hsieh-Wilson, L. (2007). Chem. Biol. *14*, this issue, 195–208.
2. Rodèn, L. (1980). The Biochemistry of Glycoproteins and Proteoglycans, W.J. Lennarz, ed. (New York: Plenum Press), pp. 267–314.
3. Schwartz, N.B. (1982). Glycosaminoglycans and Proteoglycans in Physiological and Pathological Processes of Body Systems, R. Varma, ed. (Basel, Switzerland: S. Karger Ag), pp. 41–54.
4. Klaassen, C.D., and Boles, J.W. (1997). FASEB J. *11*, 404–418.
5. Kehoe, J.W., and Bertozzi, C.R. (2000). Chem. Biol. *7*, R57–R61.
6. Schwartz, N.B. (2005). Human Cytosolic Sulfotransferases, M.W. Coughtrie and G.M. Pacifici, eds. (London: Taylor and Francis Group), pp. 43–60.
7. Schwartz, N.B., and Domowicz, M. (2004). Glycoconj. J. *21*, 329–341.
8. Gama, C.I., and Hsieh-Wilson, L.C. (2005). Curr. Opin. Chem. Biol. *9*, 609–619.
9. Keiser, N., Venkataraman, G., Shriver, Z., and Sasisekharan, R. (2001). Nat. Med. *7*, 123–128.
10. Holt, C.E., and Dickson, B.J. (2005). Neuron *46*, 169–172.
11. Powell, A.K., Yates, E.A., Fernig, D.G., and Turnbull, J.E. (2004). Glycobiology *14*, 17R–30R.
12. Chilton, J.K. (2006). Dev. Biol. *292*, 13–24.
13. Bandtlow, C.E., and Zimmermann, D.R. (2000). Physiol. Rev. *80*, 1267–1290.
14. Bulow, H., and Hobert, O. (2004). Neuron *41*, 723–736.
15. Irie, A., Yates, E., Turnbull, J., and Holt, C. (2002). Development *129*, 61–70.
16. Pratt, T., Conway, C., Tian, N., Price, D., and Mason, J. (2006). J. Neurosci. *26*, 6911–6923.

