Synthetic sugars enhance the functional glycomics toolkit

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Glycosaminoglycan-protein interactions are an important frontier for discovering new mechanisms of cellular regulation by complex sugars. The integration of the 'chemical glycomics' strategies of synthetic chemistry, arrays and biological assays shows that the precise pattern of sugar sulfation dictates the specificity of a sugar's function.

Understanding the complex functions of the glycome—the repertoire of sugars expressed by cells, tissues and organisms—is a substantial challenge in the postgenome era. Glycosaminoglycans (GAGs) are an important class of complex sugars whose structurally diverse polysaccharide chains contain large amounts of information[1] that is linked to an extensive range of biological functions. However, an understanding of the mode of glycan specificity underlying these functions has proven elusive[1]. A paper by Gama *et al.* published in this issue of *Nature Chemical Biology* reports the synthesis of a set of chondroitin sulfate (CS) tetrasaccharides whose interactions and bioactivity provide evidence for a 'sulfation code' in which recognition of selected proteins is conferred by specific sequences[2]. The use of a unique combination of synthetic chemistry, microarray technology and biological assays indicates a way forward for future studies aimed at understanding the selectivity and functions of GAG structures.

Questions concerning the specificity of GAG-protein interactions abound and can only be definitively addressed using fully characterized compounds. The difficulty of obtaining such structures from nature is a significant bottleneck, and the development of tools and technologies to evaluate these molecules lags behind that of genomics and proteomics. Fortunately, the field is rapidly changing[3]. For instance, chemical synthesis avoids many of the purification problems that plague natural-products chemistry. In addition, large-scale interrogation of glycan-proteins is now possible, allowing the discovery of new molecular liaisons. In particular, recent studies describe new approaches for obtaining arrayed displays of both natural[4] and synthetic[5] GAG structures from the heparan sulfate–heparin family.

Initial efforts in glycosynthesis, such as those by Lopin *et al.*, demonstrated that chondroitin saccharides can be effectively synthesized in a highly convergent and efficient manner[6]. Gama *et al.*[2] extend these concepts by emphasizing the value of integrative science in addressing the highly complex problems being generated in glycobiology. The current work is among the first to undertake structure-activity relationship experiments for synthetic saccharides using a combination of binding assays, biological assays and specific blocking antibodies.

The authors focused on a small set of saccharides anticipated to effect neurite outgrowth based on previous studies involving natural structures[7]. The functionalized sugars were synthesized by highly convergent chemistry that allowed for incorporation of specific sulfation sequences on these carbohydrate templates as well as stereocontrol of the glycosylation reactions. This methodology also extends access to unnatural sequences such as their CS-R compound (2,3-di-O-sulfated; the sulfation pattern is altered with respect to the natural saccharide). The authors then displayed the structures via a versatile chemical handle in a microarray format, a new tool for glycomics[8]. They used this array system to show that a molecule displaying the natural CS-E sulfation motif bound best to the growth factors midkine and brain-derived neurotrophic factor (BDNF). Both midkine and BDNF are important for the development and repair of the nervous system. The CS-E structure was also the best at promoting neurite outgrowth (a measure of neuronal development), suggesting that specificity of sulfation is critical for function (**Fig. 1a**). The authors also used molecular modeling to begin to examine the spatial display of these sulfate groups.

Because correlations of binding activity with biological activities are often complex, data from bioassays are clearly of higher value in understanding functional selectivity. The neurite outgrowth studies confirm the results obtained by Bao *et al.* using libraries of natural saccharides[7]. Both groups also use blocking antibodies against selected epitopes[2,7], and these tools should prove very useful for investigating epitope expression and function *in vivo*. The new study demonstrates that integration of diverse experimental platforms provides an effective approach for investigating the ways in which GAGs encode functional information in a sequence-specific manner[2].

So what gaps still exist? Although the use of molecular modeling[2] has some utility, most structures of GAG saccharides bound to their protein receptors show distorted ring conformations not populated in their free solution state. Induced-fit binding energies are often derived from distortion of the ligand and/or receptor, so the solution structures need to be extended to bound conformations

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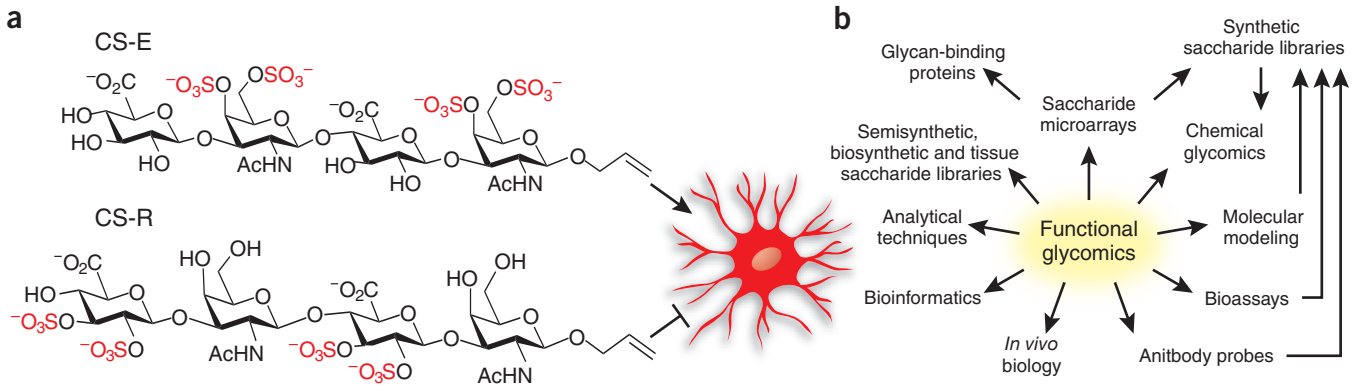

Figure 1 Synthetic tetrasaccharides having different impacts on neurite outgrowth provide a chemical dimension to functional glycomics. (**a**) Tetrasaccharides displaying the known sulfation motif CS-E bind midkine and BDNF growth factors and promote neurite outgrowth. The same sugar scaffold displaying a modified motif (CS-R), with an equivalent number but different pattern of sulfate groups, is not functional. Ac, acetyl. (**b**) Chemical glycomics enhance the functional glycomics toolkit. Synthetic sugars are an invaluable resource that can be used in chemical glycomics approaches (green boxes) for studying the functions of the glycome. They permit definitive structure-activity studies when integrated with methodologies such as microarrays, bioassays and molecular modeling. This generates new understanding of the ways in which glycans encode functional information in a sequence-specific manner. Chemical glycomics complements a variety of other multidisciplinary approaches that form a network comprising the emerging field of functional glycomics.

with protein partners to be of predictive value. Additionally, further development of glycoarray techniques[2,4,5,8] is clearly necessary. The microarraying and protein-binding interrogation of synthetic CS oligosaccharides reported by Gama *et al.*[2] is similar to that in use for synthetic heparins[5] and illustrates the utility of miniaturized interaction studies. However, there remains a need to optimize surface chemistries, coupling efficiencies, and densities, with the goal of producing quantitative data on binding affinities and kinetics. Glycoarrays could also be used with complex samples or cells and pathogens to derive information on binding and cellular responses. Thorough validation of all these formats will be needed to decide which approaches have real utility, and bioinformatics platforms will have to be developed to interpret and apply the huge amount of information that will be generated.

It is important to note that the concept of a sulfation code must be expanded to include other GAG structural features[9]. For example, the positioning of glucuronic acid and its epimer, iduronic acid, may be just as critical as sulfate spacing. Synthesis of iduronate residues is very challenging, yet most known specific GAG-protein interactions involve this residue. Small numbers of synthetic saccharides from the heparan sulfate–heparin family are emerging[5]. There is an evident need for GAG saccharides encompassing a wider range of sulfation and uronate variants, and in longer sequences composed of variant disaccharide building blocks. Extensive GAG libraries produced through modular, combinatorial synthetic approaches[10] are anticipated, with diversity to match and exceed that of natural structures.

Further development of chemical glycomics strategies will enhance the functional glycomics toolkit for exploring the glycome and its biological significance (**Fig. 1b**). Post-translational modifications of proteins, especially glycosylation, have become an important frontier for discovery of new mechanisms of cellular regulation. Understanding the detailed molecular mechanisms and specificity of GAG-protein interactions will underpin a diverse set of practical applications including drug design, diagnostics, biomaterials and tissue engineering. Advances in glycomic technologies may soon allow rapid progress in elucidation of the structure-function relationships of these highly complex and enigmatic molecules.

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