Carbohydrate Arrays as Tools for Glycomics

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Carbohydrates are the key components of glycolipid and glycoprotein cell-surface molecules responsible for recognition, adhesion, and signaling between cells.[1] These processes are essential for normal tissue growth and repair, bacterial and viral invasion of host organisms,[2] as well as tumor-cell motility and progression.[3] A better understanding of protein–carbohydrate interactions would greatly aid in the elucidation of intercellular signaling pathways, possibly leading to improved diagnostic and therapeutic tools.

DNA and protein microarrays arrays, prepared by attachment of the biopolymers to a surface in a spatially discrete pattern, have enabled a low-cost, high-throughput methodology for screening interactions involving these molecules. Oligonucleotide arrays were constructed in situ on glass slides by photolithographic methods[4] or by using ink-jet technology.[5] The polymerase chain reaction (PCR) allowed for quick amplification of libraries of complementary DNA (cDNA), to provide insight into cellular processes involved in the regulation of gene expression.[6] Protein arrays on glass slides, microwells,[7] and three-dimensionally modified gel-pad chips[8] have been used for high-throughput screening of protein activity and determination of protein–protein, protein–DNA, and protein–ligand interactions.[9]

The development of carbohydrate biosensors and arrays has recently received increased attention. Carbohydrates are by nature chemically more complicated substrates and several key challenges must be addressed to create carbohydrate arrays: 1) sugars must be displayed at the reducing end for successful protein recognition; 2) the many hydroxyl groups may be difficult to differentiate and linker systems have to take advantage of high-affinity interactions for surface attachment of the carbohydrate; and 3) access to carbohydrates of defined structure is difficult as both isolation and synthesis are very challenging.

Two linker systems have been devised for the attachment of carbohydrates to a surface. The first technique exploits the natural affinity of biotin for streptavidin,[10] while the second method relies on the formation of alkane thiolate monolayers on a gold surface.[11]

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Biotin–Streptavidin-Based Linkers

Initial efforts to understand lectin–carbohydrate binding utilized the biotin–streptavidin interaction for the display of oligosaccharides (Figure 1). The anomeric asparagine residues of twelve glycans were biotinylated and immobilized onto a 96-well titer plate coated with streptavidin to characterize the binding properties of six common lectins.[12]

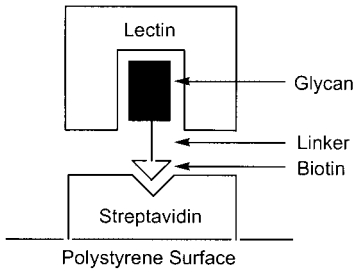

Figure 1. Surface display of carbohydrates for lectin-binding assays by interaction of biotin with streptavidin.

Characterization of lectin binding was simplified by inclusion of a 4-(biotinamido)phenylacetylhydrazine (BPH) chromophore, in the linker portion of the carbohydrate (Scheme 1)[13] and was monitored by surface plasmon resonance (SPR) spectroscopy.[14] This methodology was expanded to include a biotinyl-L-3-(2-naphthyl)-alanylhydrazide (BNAH) linker containing a chromophore with both UV-absorbing and fluorescent properties (Scheme 1).[15] The binding of monoclonal antibodies and selectins to surface-immobilized sialyl-Lewis^a (SLe^a) and sulfo-Le^a-related oligosaccharides was monitored.[16] Unexpectedly low binding signals between sulfo variants and L- and P-selectins highlighted the importance of the use of the correct ligand on the streptavidin matrix. The length of the spacer between the biotinyl tag and the oligosaccharide is a critical factor in the binding signal, as it limits both nonspecific ligand–ligand and ligand–surface interactions.

Most recently, biotinylated oligosaccharides have been used to probe poorly understood protein–carbohydrate interactions. A library of immobilized galactosyl ceramide (GalCer) analogues were individually probed for interaction with the HIV cell-surface glycoprotein gp 120.[17] A heparin sulfate glycosaminoglycan was similarly immobilized and interacted with a 3-*O*-sulfotransferase isoform-1 (3-OST-1)

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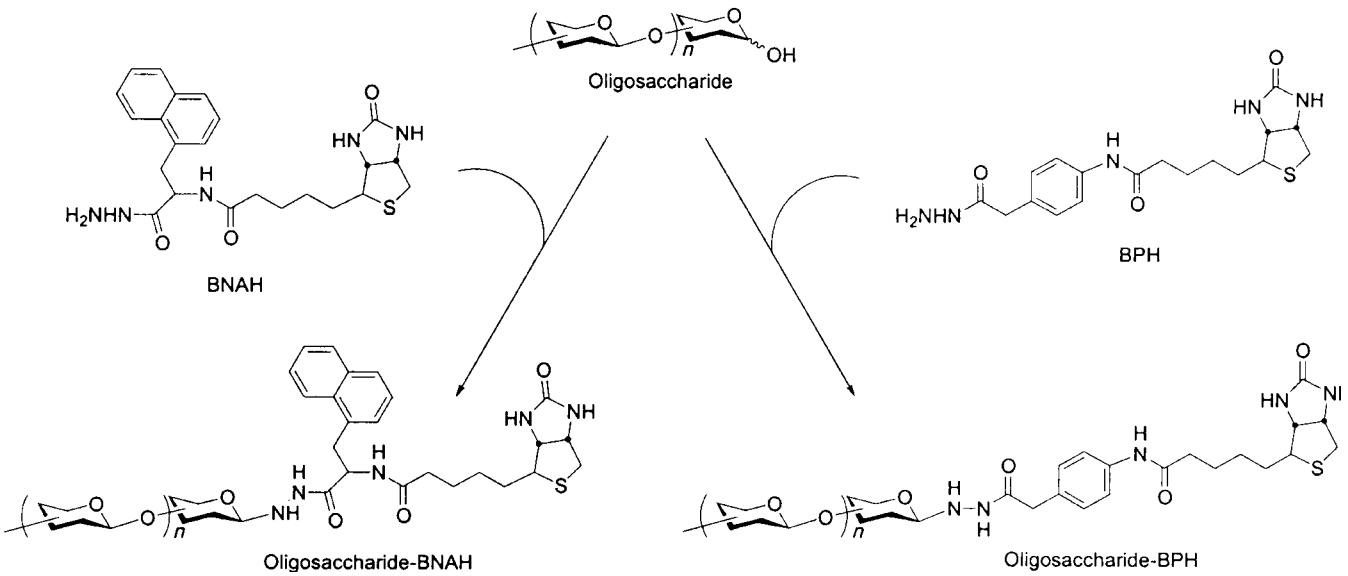


Scheme 1. Incorporation of chromophores into a linker for display of carbohydrates on a streptavidin-coated surface. The biotin-terminated linker is attached to the reducing end of an oligosaccharide through hydrazide formation under mild conditions.

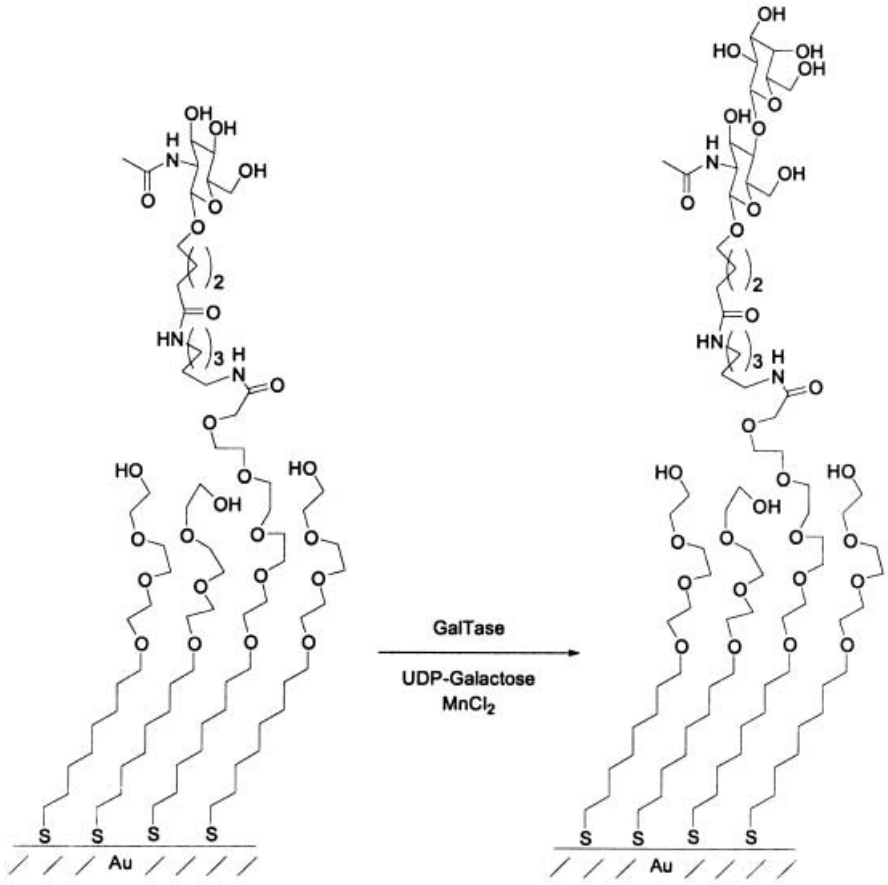
in an effort to improve antithrombin III binding.[18] These studies illustrate the potential of the biotin–streptavidin interaction to immobilize sugars for the screening of protein–carbohydrate interactions.

Carbohydrate Self-Assembled Monolayers (SAMs)

Self-assembled monolayers of alkanethiols on a gold-coated substrate allow for the display of densely packed structures on a surface.[11] The reactivity and properties of a monolayer depend entirely on the exposed functional groups, which may be elaborated to create defined surfaces. Hydroxylated surfaces present a readily derivatized moiety for the attachment of biomolecules, such as a thiol-terminated hexasaccharide.[19]

Subsequently, several SAMs were formed using thioglucose monosaccharides.[20] Characterization by reflection absorption infrared spectroscopy (RAIRS)[21] indicated that the orientation of the carbohydrates in each SAM was dependent on the anomeric thiol moiety. Thus, the nonreducing end of mannose was appropriately displayed to allow the lectins access to each monosaccharide unit. Interactions between this SAM and two lectins, concanavalin A (Con A) and *tetragonolobus purpureas*, were detected by using SPR spectroscopy. Con A exhibited clear selectivity for the mannose-containing SAM, as observed by SPR spectroscopy.[20]

Enzymatic modification of a carbohydrate-based SAM was demonstrated by Houseman and Mrksich.[22] A mixed SAM presenting both *N*-acetylglucosamine (GlcNAc) and tri(ethylene glycol) groups was subjected to bovine β-1,4-galactosyltransferase (GalTase; Scheme 2). The transfer of galactose was quantified by inclusion of [^{14}C]uridine diphosphogalactose ([^{14}C]UDP-



Scheme 2. A self-assembled carbohydrate monolayer. *N*-acetylglucoamine groups presented on the surface may be glycosylated when incubated with galactose and GalTase. Recognition of this glycosylation may be observed by lectin binding to the newly formed disaccharide.

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Gal) and showed that enzyme activity directly corresponds to the density of the monolayer.[22]

Carbohydrate-based SAMs were also used to correlate protein specificity and ligand density. Exposure of three SAMs displaying one of three disaccharides, a natural ligand, a synthetic ligand, and a control ligand, to *Bauhinia purpurea* lectin showed a change in the binding selectivity from the natural ligand to a synthetic ligand as the surface density increased.[23] These observations suggest that one protein may elicit more than one response by binding to several carbohydrate ligands. Thus, changes in the composition and density of cell-surface carbohydrates may substantially influence downstream regulatory pathways.

Carbohydrates and SAMs: The Development of a Carbohydrate Array

The ability to detect the presence of carbohydrates displayed on SAMs by a biological means made this technique a good candidate for the simultaneous display and detection of carbohydrate analytes. A cyclopentadiene-terminated linker, which enabled the attachment of biomolecules through a rapid and irreversible Diels–Alder reaction to a SAM displaying reactive quinone groups,[24, 25] immobilized ligands in a discrete location (Scheme 3).[26] Following initial proof-of-principle on a peptide chip,[27] a carbohydrate array was created by spotting solutions containing carbohydrate–cyclopentadiene conjugates onto specific regions of a monolayer displaying reactive benzoquinone groups.[28]

This multivalent carbohydrate array consisted of ten different monosaccharides and was probed by the addition of a series of five fluorescently labeled lectins.[28] Each lectin was observed to interact specifically with its known carbohydrate ligands, thus the array demonstrated utility in the selective identification of protein substrates. Arrays could also be enzymatically modified, as demonstrated by incubation with GalTase, followed by a lectin-binding assay.[22]

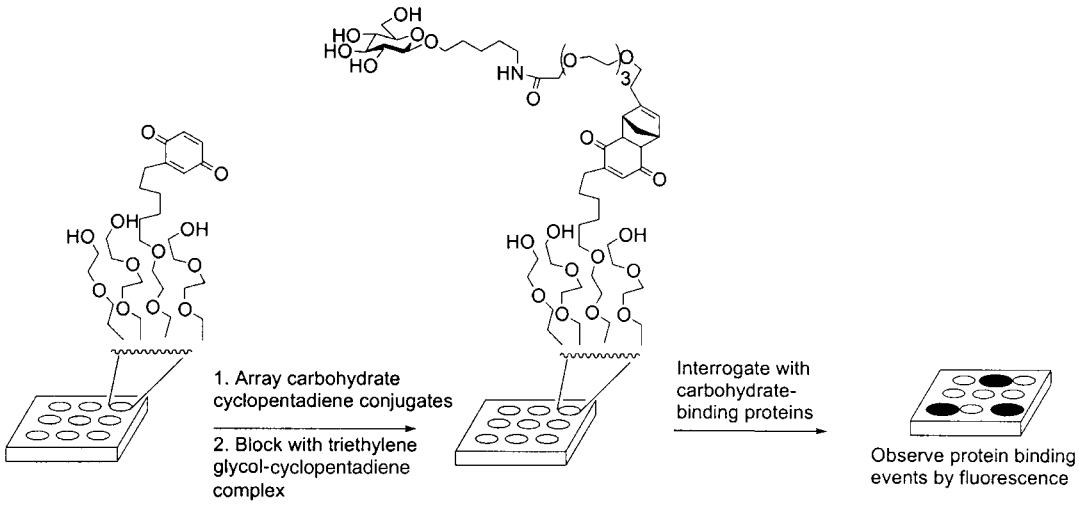
Houseman and Mrksich also used their surface chemistry to create a carbohydrate chip for the study of protein inhibition. A series of solutions containing Con A and various concentrations of α-methyl mannose (0–4 mM) were arrayed onto monolayers presenting either α-mannose, β-glucose, or α-*N*-acetylglucosamine.[28] After an incubation period, the amount of lectin binding for each concentration of soluble ligand was determined by fluorescence spectroscopy, thus the relative binding affinities of each carbohydrate for Con A were verified.

Another carbohydrate array for the detection of antibodies to a series of dextrans was recently reported.[29] Dextrans of varying glycosidic linkage composition and molecular weight were arrayed onto nitrocellulose-coated glass slides. The carbohydrate microarray was applied to detect human serum antibodies to microbial pathogens that typically express dextrans, including several strains of *Pneumococcus*, group *B Meningococcus*, and *Haemophilus influenzae*.

Comparison of Carbohydrate Microarrays and Outlook

Both biotin–streptavidin and SAM-based methods for biomolecule conjugation have been successfully employed for the presentation of carbohydrates on a surface. In each case, incubation of the surface with various lectins has confirmed known carbohydrate-binding specificities. While SAMs have recently shown potential in the synthesis of a carbohydrate array, biotin–streptavidin-based carbohydrate presentation has been limited to the display of a unique substrate on each individual surface. "Arrays" have been generated, but require the physical separation of different biomolecules, typically using a 96-well enzyme-linked immunosorbent assay (ELISA) plate. SAMs seem particularly suited to the construction of biochips owing to the ease of their construction and increased inertness to protein adsorption.[30] Furthermore, SAMs have demonstrated utility in conjunction with cell cultures;[31, 32] future generations of carbohydrate chips may be used in whole-cell assays.

The simultaneous detection of carbohydrate analytes by the binding of lectin in the context of a monosaccharide-based array is a powerful development in the emerging field of



Scheme 3. Generation of a carbohydrate array using self-assembled monolayers. A series of cyclopentadiene-functionalized monosaccharides were immobilized on a SAM displaying reactive quinone groups. An irreversible Diels–Alder reaction between the cyclopentadiene and quinone groups leads to localization of each monosaccharide.

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glycomics. However, use of array technology to understand the intricate role of carbohydrates in cell-signaling will require the immobilization of longer, more complex oligosaccharides. The development of automated methods for the synthesis of oligosaccharides[33, 34] in combination with the development of microcontact printing for the deposition of ligands on the surface of a SAM[35, 36] will make possible the rapid generation of chips containing entire classes of carbohydrates. Interrogation of these surfaces by systematic exposure to fluorescently labeled proteins, including bacterial and viral toxins, will ultimately lead to a better understanding of the role of carbohydrates in numerous signaling processes.

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Direct Electrochemical Aziridination of Alkenes under Metal-Free Conditions

Gerhard Hilt*

The generally accepted advantage of electrochemical conversions is the mass-free electron transfer between the electrode and the substrate, thereby eliminating the need for the waste disposal of a used redox reagent.[1] Since many chemical redox reagents are produced by electrochemical pathways, it would be an advantage to use the electrochemical power directly for the conversion of the starting materials in redox reactions. From this viewpoint, and because of increasing environmental problems, electrochemical methods will become more and more attractive in the future. Many classes of substrates could be successfully converted directly at the electrode under electrochemical conditions and the intermediates generated by electron transfer could be employed in synthetically useful follow-up reactions. Among these electrochemical conversions, applications on an industrial scale[2] and for the production of fine chemicals and laboratory-scale chemicals exist.[3]

The synthetic value of small, strained, and therefore highly reactive, ring systems is well established, and among these epoxides and aziridines are of great interest.[4] Indeed, the asymmetric synthesis of epoxides from allylic alcohols[5] was honored by the award of the Nobel prize to Sharpless in 2001.[20] Hydroperoxides are used as oxidants as an easily handled source of oxygen, even on a large scale.[5, 6]

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