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Oligosaccharide microarrays fabricated on aminooxyacetyl functionalized glass surface for characterization of carbohydrate–protein interaction

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Abstract

Carbohydrate–protein interactions play important biological roles in biological processes. But there is a lack of high-throughput methods to elucidate recognition events between carbohydrates and proteins. This paper reported a convenient and efficient method for preparing oligosaccharide microarrays, wherein the underivatized oligosaccharide probes were efficiently immobilized on aminooxyacetyl functionalized glass surface by formation of oxime bonding with the carbonyl group at the reducing end of the suitable carbohydrates via irreversible condensation. Prototypes of carbohydrate microarrays containing 10 oligosaccharides were fabricated on aminooxyacetyl functionalized glass by robotic arrayer. Utilization of the prepared carbohydrate microarrays for the characterization of carbohydrate–protein interaction reveals that carbohydrates with different structural features selectively bound to the corresponding lectins with relative binding affinities that correlated with those obtained from solution-based assays. The limit of detection (LOD) for lectin ConA on the fabricated carbohydrate microarrays was determined to be ~0.008 μg/mL. Inhibition experiment with soluble carbohydrates also demonstrated that the binding affinities of lectins to different carbohydrates could be analyzed quantitatively by determining IC_{50} values of the soluble carbohydrates with the carbohydrate microarrays. This work provides a simple procedure to prepare carbohydrate microarray for high-throughput parallel characterization of carbohydrate–protein interaction.

Keywords: Carbohydrate microarrays; Protein–carbohydrate interaction; Oxime bonding; Biochips; Oligosaccharide

1. Introduction

Specific protein–carbohydrate interactions underlie many aspects of important biological processes, including cell differentiation, cell adhesion, immune response, trafficking and tumor cell metastasis, which occurs through glycoprotein, glycolipid, and polysaccharide displays on cell surfaces and lectin, proteins with carbohydrate-binding domins (Bertozzi and Kiessling, 2001; Lis and Sharon, 1998; Smith et al., 2003). However, the functions of carbohydrates in biology have not been extensively studied due both to the more complex structures of oligosaccharides and to a lack of general methods for synthesizing and analyzing these molecules. Thus, tools for studying protein–carbohydrate interactions are important to gain an understanding of biological functions and the roles of these interactions in biological processes. The presentation of carbohydrates in a microarray format on solid surface can provide an efficient way to simultaneously monitor multiple binding events between immobilized carbohydrates and soluble proteins. However, due to the diverse structure and characteristics of carbohydrates, it is difficult to find a robust, general, and controlled strategy for fabrication of carbohydrates microarray. To date, there are few reports of the implementation of carbohydrate microarrays and no commercial slides surface is available for carbohydrates microarray fabrication (Feizi et al., 2003; Wang, 2003). An efficient slide surface for the construction of high-throughput carbohydrate microarray has become an attractive

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prospect, particularly in light of the many biological activities and potential medical applications of carbohydrates.

Few approaches have been developed, thus far, for the fabrication of carbohydrate microarrays on slide substrates. Polysaccharides and glycoproteins microarray have been fabricated on nitrocellulose-coated glass slides and hydrophobic black polystyrene substrate by non-covalent adsorption (i.e., massive adsorption) (Wang et al., 2002; Wiilats et al., 2002). Oligosaccharide and monosaccharide microarrays have been described by using derivatized oligosaccharide and monosaccharide candidates on functionalized surface. For example, Houseman et al. (2002) fabricated carbohydrate arrays on gold surfaces via Diels–Alder reactions between oligosaccharide–cyclopentadiene conjugates and benzoquinone functionalised surface. The results indicated that this immobilization method was highly efficient and oligo(ethylene glycol)-linked carbohydrate monolayers displayed minimal non-specific adsorption with proteins. Park and Shin (2002) and Park et al. (2004) constructed carbohydrate microarrays by covalent immobilization of maleimide-linked monosaccharides on glass slide functionalized with thiol groups. They also reported that linker lengths and immobilization concentrations of carbohydrates were important factors in governing protein binding to these microarrays. Importantly, the results showed that the specificity of protein–carbohydrate interactions on the solid surfaces resembled that observed in solution. Fazio et al. (2002) reported a alternative method to fabricate oligosaccharide microarrays by immobilization of azide forms of galactose and several neutral and sialic acid containing di- to tetra-saccharides on the plastic surface functionalized with long-chain (14-carbon) aliphatic alkyne by a 1,3-dipolar cyclo-addition reaction between the azide and alkyne groups. However, these approaches required multi-step modifications of monosaccharides or oligosaccharides moieties with chemical active groups or ligands, which are tedious and laborious.

In order to provide both excellent selectivity and quantitative performance, the development of oligosaccharide microarrays requires the attachment strategy to be simple, efficient. Ideally, no modification or minor modification of the carbohydrate species is required for the attachment, and the immobilized oligosaccharides are presented in a regular and homogeneous environment so that all immobilized ligands have equal activity towards soluble proteins and enzymes. We report here a convenient approach for constructing oligosaccharide microarray by direct immobilizing underivated oligosaccharides on aminooxyacetyl-functionalized monolayers. The oligosaccharide probes were immobilized on the aminooxyacetyl-functionalized surface by the formation of oxime bonding with the carbonyl groups at the reducing end of the oligosaccharide probes via irreversible condensation. Such surface chemistry, when combined with the currently robust spotting technologies, can provide a one-step direct immobilization of intact oligosaccharides for oligosaccharide microarray construction that eliminates the current tedious and laborious pretreatments of oligosaccharides.

2. Materials and methods

2.1. Reagents

Most reagents were purchased from Sigma-Aldrich-Fluka Chemical Corp. (Milwaukee, WI) and were used as received: (3-glycidyloxypropyl) trimethoxysilane (GPTS) (98%), Boc-aminooxy acetic acid, 1-ethyl-3-(3-dimethylaminopropylcarbodimide) (EDC), *N*-hydroxysuccinimide (NHS), succinic anhydride and oligosaccharide probes. Biotin labeled lectins of Concanavalin A (biotin-ConA), *E. cristagalli* (Biotin-EC), *Lotus tetragonolobus* (Biotin-LT) are purchased from Sigma or EY Laboratories (San Mmateo, CA). Cy3-labeled streptavidin, 5× Denhardt's solution (containing 1 mg/mL each of Ficoll, polyvinyl pyrrolidone, and bovine serum albumin), are also purchased from Sigma. Homobifunctional polyethylene glycol, α,ω-diamine-PEG (M_W = 2000 Da) was purchased from Rapp Polymere (Tubinggen, Germany). Frame-Seal incubation chambers (9 mm × 9 mm) was obtained from MJ Research Inc. (Watertown, MA).

2.2. Preparation of oxime-functionalized monolayer on glass surface (Scheme 1)

Microscope glass slide was cleaned with hot Piranha solution (H_2O_2:H_2SO_4/1:3 (v:v)), and then thoroughly rinsed with distilled water and HPLC grade ethanol. The cleaned slide substrate was immersed into a 1 mM of GPTS/toluene solution for 30 min to form a monolayer on the glass surface with epoxide functional groups towards the outside. The GPTS functionalized slide was then immersed in 1 mM of α,ω-diamine-PEG solution and left for 24 h with shaking at 60 °C (step 2, Scheme 1). After washing the amino-PEG functionalized slide was dipped into a 50 mM phosphate buffer solution (pH 6.0) containing 1 mM of Boc-aminooxyacetic acid, EDC and NHS and allowed to stand for 2.5 h (step 3, Scheme 1). The glass slide was then washed with water and immersed into 2 M HCl/acetic acid for 2 h to remove the Boc-group (step 4, Scheme 1). The glass slide was washed with ethanol and water and spun dry.

2.3. Fabrication of oligosaccharides microarray

Probe printing solutions were prepared by dissolving carbohydrate probes in solution of 50% H_2O, 25% DMSO, and 25% acetronitrite. 1 nL of the carbohydrates solution from a 384-well plate were printed onto an aminooxyacetayl-functionalized glass slide with a distance of 250 μm between the centers of adjacent spots by using a PixSys 5500 robotic printer (Cartesian Technologies, Inc., Irvine, CA) in 60% relative humidity followed by incubation for 12 h. Subsequently, the slides were left for incubation at room temperature for 12 h. Unbound carbohydrates were removed from glass by washing with PBST (containing 0.137 M sodium chloride, 0.00027 M potassium chloride, 0.008 M sodium phosphate

dibasic, and 0.002 M potassium phosphate monobasic, 0.1% Tween 20, pH 7.4) for 2 min followed by distilled H_2O for 1 min. Unreacted aminooxyacetyl on the substrate were inactivated by treatment of the slides with 10 mM succinic anhydride in *N*,*N*-dimethylformamide (DMF) overnight, the slides were carefully washed with DMF three times for 3 min each. The slide was then blocked with 5× Denhardt's solution (containing 1 mg/mL each of Ficoll, polyvinyl pyrrolidone, and bovine serum albumin) for 30 min, and washed with PBST buffer.

2.4. Probing microarrays

The carbohydrate microarray slides were incubated with individual or mixed biotinalyted lectins solution in PBST for 2 h. Following incubation, slides were washed twice in PBST buffer for 8 min each. For biotin-ConA binding, $MnCl_2$ and $CaCl_2$ were added at with final concentrations of 1 mM. Slides were then incubated with 50 μg/mL of Cy3-labeled streptavidin in a solution of PBST for 1 h. Slides were then washed twice in PBST for 10 min, and then rinsed briefly in deionized water to remove PBST buffer salts prior to being dried by centrifugation at 500 × *g*. For inhibition experiments, a solution of biotin-ConA (0.1 mg/mL in PBST) was mixed with a series of concentrations of inhibitor for 2 h. A 25 μL of each mixture was applied to the surface of carbohydrate microarray and then covered with frame seal. After 1 h incubation at room temperature, the slides were washed with PBST (2× 5 min) and then incubated with 25 μL of 10 μg/mL of Cy3-labeled streptavidin in a solution of PBST for 1 h. Following incubation, slides were washed as described above.

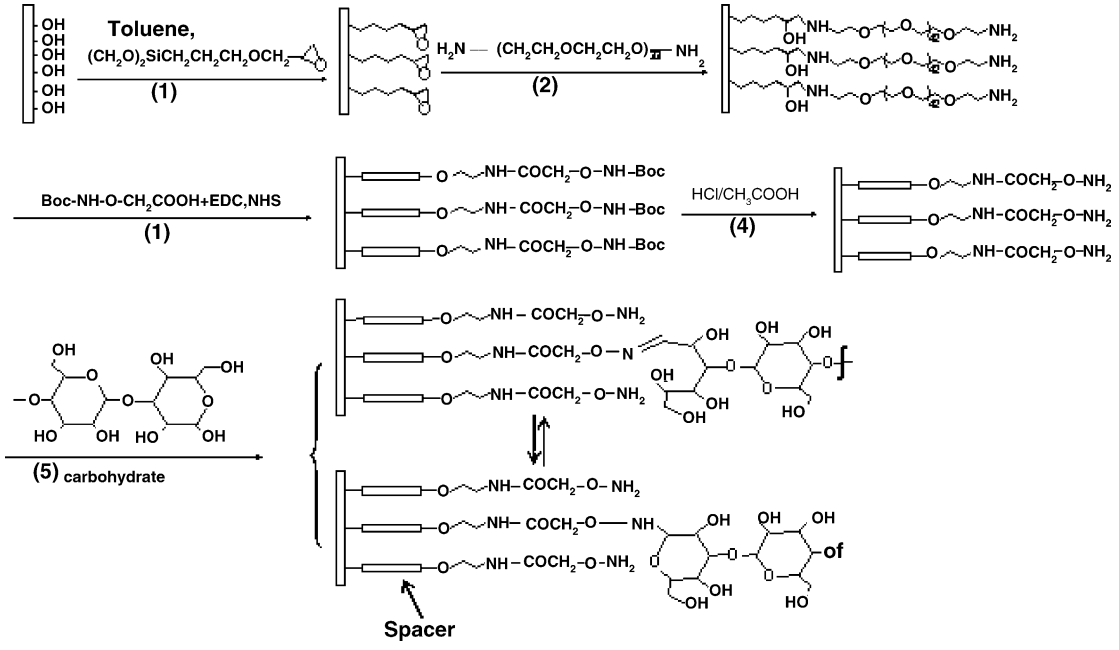
2.5. Microarray imaging and data analysis

Microarrays were scanned at 10 μm resolution with the scanning laser confocal fluorescence microscope of a ScanArray 5000 System. The emitted fluorescent signal was detected by a photomultilier tube (PMT) at 570 nm for Cy3. For all microarray experiments, the laser power was 85% and the PMT gain was 75%. The fluorescent signals were analyzed by quantifying the pixel density (intensity) of each spot using ImaGene 3.0 (Biodiscovery, Inc., Los Angeles, CA). The local background signal was automatically subtracted from the hybridization signal of each separate spot and the mean signal intensity of each spot was used for data analysis. Statistical analyses were performed using SigmaPlot 5.0 (Jandel Scientific, San Rafael, CA) or by Microsoft Excel®.

3. Results and discussion

3.1. Preparation of aminooxyacetyl-functionalized slide surface and immobilization of oligosaccharides

We employed self-assembled monolayers containing aminooxyacetyl groups on glass slide as a platform for immobilizing an array of oligosaccharide probes. The use of aminooxyacetyl-terminated self-assembled monolayers for the immobilization of carbohydrates takes advantage of the oxime formation reaction between a highly reactive amine group of the nucleophilic aminooxyacetyl group and the carbonyl group at the reducing end of suitable carbohydrates via irreversible condensation (Scheme 1) (Canne et al.,



Scheme 1. Scheme of the chemical approach for the preparation of aminooxyacetyl functionalized glass slide and the immobilization of oligosaccharides. The aminooxyacetyl groups react selectively with carbonyl group at the reducing end of carbohydrates via irreversible condensation in a contacting solution while the penta(ethylene glycol) groups serves as longer spacer arm and prevent the non-specific adsorption of protein to the monolayer.

1995; Rose, 1994). The oxime chemistry has recently been employed for preparing glycoconjugates (Mikola and Hanninen, 1992; Webb and Kaneko, 1990), polyuronic acid conjugates (Guillaumie et al., 2002) glycopeptides (Zhao et al., 1997), carbopeptides (Brask and Jensen, 2000), carboprotein (Brask and Jensen, 2001) and for immobilizing carbohydrates for ELISA detection of sugars (Mitchell et al., 1999; Forget et al., 2001). The efficiency of the method relies on the ease of formation and on the good stability of the oxime linkage up to pH 9. This methodology appears not to have been used for the construction of carbohydrate microarray on glass slide.

The aminooxyacetyl groups functionalized glass slides were prepared in four steps from (3-glycidyloxypropyl) trimethoxysilane (GPTS) self-assembled on glass slide surface (step1, Scheme 1). The glycidyl group of the GPTS monolayer was treated with diamino-PEG resulted in a PEG monolayer with amine as most outer groups on glass slide (step 2, Scheme 1). The amine groups were then coupled to carboxyl groups of the *N*-Boc-Aoa-OH that was activated with hydroxylsuccinimide group (step3, Scheme 1). Free aminooxyacetyl groups were then obtained upon treatment of the glass slide with HCl/acetic acid for the purpose to remove the Boc-group (step 4, Scheme 1). The aminooxyacetyl groups on the slide surface reacted with formyl groups of the reducing ends of the oligosaccharides to form oxime bonding (step 5, Scheme 1). In contrast to reductive amination, the sugar structure was preserved after coupling; equilibrium between the closed ring and open ring forms might occur at the surface of the support. This efficient technique requires only a few derivatization steps on slide surface treatments, which make it very attractive for preparing carbohydrate microarray in individual laboratories.

The poly(ethylene glycol) groups on the glass slides prevent the non-specific adsorption of protein to the substrate and ensure that only specific interactions between soluble proteins and immobilized ligands occur. The excellent control over unwanted adsorption with monolayers presenting oligo(ethylene glycol) groups has been validated in several studies (Mrksich and Whitesides, 1997; Mrksich, 2000; Chapman et al., 2001; Herrwerth et al., 2003). The poly(ethylene glycol) also functioned as longer spacer arm, which increase the accessibility of proteins to the binding site of the carbohydrates. It has been reported the maltooligosaccharides immobilized on slide surface with short spacer arm could not be detected by ConA (Satoh et al., 1999).

3.2. Binding capacity, dynamic ranges and detection limit

To demonstrate the utility of this oxime chemistry for the immobilization of carbohydrates and the use of the arrayed carbohydrates for parallel determination of protein–carbohydrate interactions, we printed 10 oligosaccharide probes on the aminooxyacetyl-functionalized glass slide in microarray format by robotic printer. The fabricated oligosaccharide microarrays were kept in a humidified chamber at room temperature for overnight, washed with water and dried. These conditions permitted near quantitative immobilization using minimal quantities of carbohydrate conjugates. After incubation and washing away the unbound oligosaccharide, the remaining aminooxyacetyl groups on the substrate were inactivated by treatment of the glass slides with succinic anhydride (10 mM in DMF) overnight, the slides were carefully washed with DMF three times to remove physically adsorbed succinic anhydride. To blocking the non-specific adsorption, the slide was further blocked with 5× Denhardt's solution for 30 min, and washed with PBST buffer. To investigate the carbohydrate–protein-binding properties of the fabricated oligosaccharide microarrays, identical microarrays were treated separately with each of three biotin-labeled lectins (with concentration of 2 μM in PBST) for 2 h, and then washed with PBST buffer for 8 min each. Detection of the bound analyte was subsequently achieved by incubating the microarray with Cy3-streptavidin at a final concentration of 5 μg/mL, and then imaged with a confocal array scanner after washing with PBST buffer for 10 min, as described in Section 2. In this experiment we used scanning laser confocal fluorescence microscope to image and quantitate the binding of lectins to the oligosaccharide probes on the carbohydrate microarrays. This technique is currently the most widely used method for interrogating arrays. The laser confocal fluorescence microscope examines microarrays by exciting fluorescent dye on the surface and gathering the fluorescence emission by converting a stream of photos into digital values through the photomultiplier tubes (PMT). The digital values, which reflect the fluorescence intensity can be displayed by assigning a color from a palette to each pixel based on the intensity value of that pixel. On the assessment of carbohydrate–protein interaction, the immobilized oligosaccharide probes on the carbohydrate microarrays participate in biospecific interactions with proteins and enzymes, whereas the penta(ethylene glycol) groups on the monolayers provide essentially complete resistance to unwanted protein adsorption and other non-specific interactions at the surface. Fig. 1 shows the multiple-analyte characterization conducted on the prepared carbohydrate microarray. The oligosaccharides were found to bind to their specific lectin proteins. For example, the carbohydrate microarray probed with ConA showed significant fluorescence intensity in the spots arrayed with mannose, glucose, and GlcNAc (Fig. 1 A). Analysis of the fluorescent intensity further reveals the binding of ConA to the oligosaccharide probes is in the order of mannose > glucose ≥ *N*-acetylglucosamine (GlcNAc). This affinity difference is consistent with previous report in which cyclopentadiene modified oligosaccharides were arrayed on benzoquinone functionalized gold surface (Houseman and Mrksich, 2002). Weak signal was obtained in the spots that arrayed with maltooligosaccharide which has 4–10 units of α-glucose. This could be because maltooligosaccharide was barely immobilized on the slide surface due to the reducing activity of the formyl groups of the maltooligosaccharide. In addition, no signal was observed in the spots

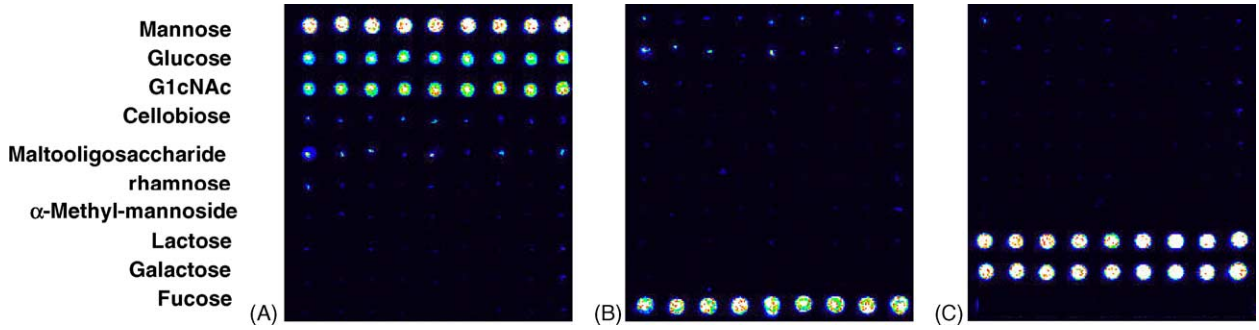


Fig. 1. Fluorescence image of oligosaccharide microarrays spotted with 10 oligosaccharide probes with identical carbohydrate chips were separately incubated with each of three biotin-labeled lectins (0.1 mg/mL in PBST) for 1 h, washed with PBST for three times for 5 min each, and stained with 5 μg/mL of cy3-strptavidin and evaluated by confocal fluorescence microscopy. Fluorescence images of oligosaccharide microarrays probed with (A) ConA, (B) LT, and (C) EC.

arrayed with methyl-α-mannoside, which the C1 position was substituted with methyl group. This result indicated that methyl-α-mannoside could be not immobilized on the glass surface. Probing the microarrays with each of the two other lectins also gave the expected results: *L. tetragonolobus* bound to the spots presenting α-fucose (Fig. 1 B), whereas, *E. cristagalli* bound only to spots presenting lactose and galactose (Fig. 1 C). The images also show that there is no non-specific adsorption on the spots arrayed with cellobiose and rhamnose and essentially no fluorescence in regions of the glass slide that without carbohydrate. The weak signal from the Glucose spots when the microarray was probed with *L. tetragonolobus* may be caused by the weak cross-reaction of Lectin *L. tetragonolobus*. It has been reported the protein–carbohydrate interaction has a very wide of affinity constant, varied from 0 to 10^6 (Kiessling and Cairo, 2002; Wang, 2003; Warkentin et al., 1995). However, this weak cross-interaction, which gave less than 8% of signal compared to the specific interaction would not affect the rapid determination of the presence or absence of specific carbohydrate epitopes. Overall, these results demonstrated that the binding of lectins with the prepared carbohydrate microarrays are specific and multiple-analyte characterization can be achieved on the aminooxyacetyl-functionalized slide with good selectivity. Furthermore, to confirm the above observed biospecific interactions were between the immobilized oligosaccharide probes and lectin proteins, we treated the fabricated oligosaccharide microarray with 1 M of $NaIO_4$ solution before incubation with the lectin solution, we found that no lectin binding was observed after the fabricated oligosaccharide microarray were treated with $NaIO_4$. This is because of the immobilized oligosaccharide probes were oxided and loss the structures for binding of lectin proteins. Together, these experiments confirm the presence of the immobilized oligosaccharide probes on the aminooxyacetyl functionalized surface and verify that the carbohydrate can participate in biospecific affinities with soluble proteins. It also demonstrates that the fabricated carbohydrate microarray is well suited for the selective identification of carbohydrate binding proteins in a high-throughput format.

To investigate the dynamic ranges of the fabricated carbohydrate microarrays, we incubated the carbohydrate microarrays with different concentrations of lectin solutions according to the typical protocol described in Section 2. Fig. 2 shows the dose-response curves of lectin ConA on mannose, glucose and G1cNAc, lectin EC on lactose and galactose, as well as lectin LT on fucose that spotted on the aminooxyacetyl-functionalized slide, respectively. It was apparent that an increase in lectin concentration resulted in a corresponding increase in the binding of lectin to the immobilized carbohydrate spots as seen from the increase of fluorescence intensities emitted from the arrayed spots, and saturation of affinities was obtained at high concentration of lectins. The calculated limit of detection (LOD, the concentration which gives fluorescent signal higher than the background + 3 S.D.) of the fabricated carbohydrate microarray was determined to be ∼0.008 μg/mL for ConA, which is lower than the microtiter plate assay developed by Hatakeyama et al. (1996).

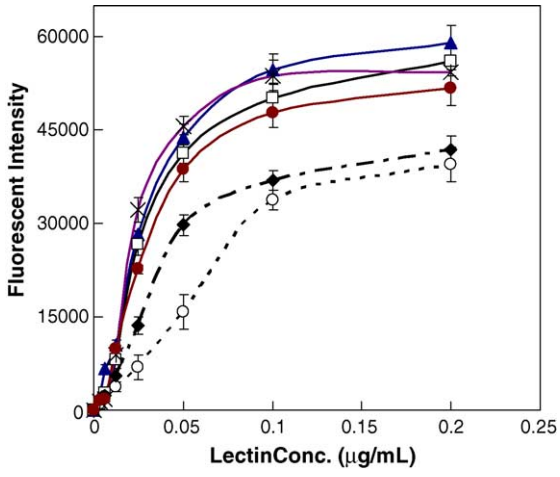


Fig. 2. Dose-response binding curve of lectins to the microarrayed spots on which oligosaccharides were immobilized, respectively. ConA binding on mannose (▲), glucose (♦) and G1cNAc (◯); EC on lactose (×) and galactose (□); LT on fucose (●). Each data point represents the average value of mean signal ± S.D. (standard deviation) of 18 replicate spots from two slides.

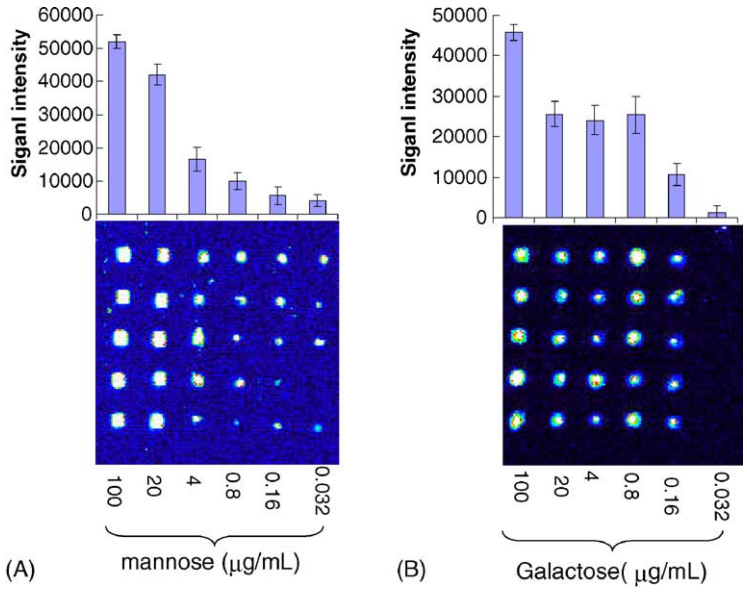


Fig. 3. Representative microarray images for the detection of oligosaccharide immobilized on aminooxyacetyl functionalized slide by lectins. Dilutions (0.1 mg/mL–0.032 μg/mL) of mannose and galactose are spotted on aminooxyacetyl functionalized side, respectively. The slides were then probed with 0.1 mg/mL biotin-ConA (A) and biotin-EC (B), respectively, followed by staining with cy3-streptavidin. Each data point represents the average value of mean signal ± S.D. (standard deviation) of 30 replicate spots from six slides.

On the other hand, the developed aminooxyacetyl-functionalized slide can also be used to detect the amount of oligosaccharides immobilized on the glass surface. To determine the detection limits of oligosaccharides binding to the aminooxyacetyl functionalized surface, we arrayed dilution series of selected oligosaccharides (0.1 mg/mL–0.032 μg/mL) on the aminooxyacetyl functionalized slide and quantified the signals after probing with their specific lectins. Fig. 3 shows the representative examples for the detection of the amount of mannose and galactose that were immobilized on the slide surface, respectively. As shown in Fig. 3, as low as 0.032 μg/mL of mannose was detected by spotting the mannose solution on the aminooxyacetyl functionalized surface and probing with lectin ConA (Fig. 3A), while as low as 0.16 μg/mL of galactose can be detected on the aminooxyacetyl functionalized surface by probing with Lectin EC (Fig. 3B). The ability to detect very low levels of carbohydrate materials arrayed at high density is a significant advantage of microarrays compared to existing methods of carbohydrate analysis, such as ELISA and immunodot assay. The reproducibility of oligosaccharide probes immobilized on the aminooxyacetyl-functionalized surface and storage time of the arrayed oligosaccharide probes were also tested. The intra-slide variation in fluorescence, determined as the coefficient of variation (CV) among the 30 replicates of mannose spots at each concentration from six separate slides after probing with ConA, ranged from 4.8% at 100 μg/mL to 9.6% at 0.032 μg/mL. Storage time was assessed by storing the arrayed slides dry for up to 3 months before probing with lectins and analysis. No significant qualitative differences in signals were obtained for stored carbohydrate microarrays compared to freshly prepared carbohydrate microarrays, which verified the immobilized oligosaccharide probes on the slides appeared to be stable during prolonged storage time.

3.3. Quantitative analysis of inhibition of binding with the carbohydrate microarrays

Quantitative analysis of protein–carbohydrate interactions is one of the important issues and carbohydrate arrays have the characteristics required for quantitative assays of multiple protein–carbohydrate interactions with minimal quantities of reagents. The results reported above suggested that these developed carbohydrate microarrays have the characteristics required for quantitative assays of protein–carbohydrate interactions. To assess the utilization of carbohydrate microarrays fabricated on aminooxyacetyl-functionalized slide for quantitative assays, we measured the inhibition of lectin ConA binding to the mannose spots immobilized on the aminooxyacetyl-functionalized slide by soluble α-methyl mannose. A series of mixed solutions containing biotin-labeled ConA (2 μM in PBST) and α-methyl mannose (0–4 mM in PBST) were applied on the carbohydrate microarray surfaces and incubated for 1 h at 25 °C, the carbohydrate microarrays were then rinsed with PBST buffer. The carbohydrate microarrays were then illuminated with 5 μg/mL Cy3-streptavidin and analyzed with the fluorescence array scanner to quantify the amount of ConA bound to the spots of mannose and glucose. The amount of lectin ConA that was bound to the mannose and glucose spots after the inhibition with different concentration of soluble ligand (i.e., α-methyl mannose) is shown in Fig. 4A and B, respectively. The IC_{50} was determined at the concentration of

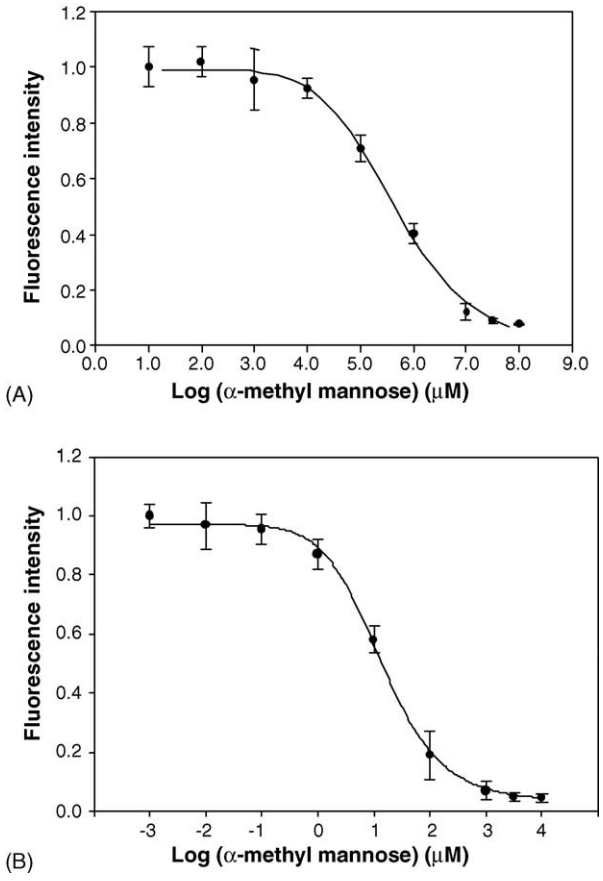


Fig. 4. Quantitative inhibition assays in oligosaccharide microarray. (A) Determination of concentration of soluble α-methyl mannose to inhibit 50% of ConA binding to the spotted mannose; (B) Determination of concentration of α-methyl mannose to inhibit 50% of ConA binding to the spotted glucose. Each data point represents mean ± S.D. for 10 spots from two independent experiments.

α-methyl mannose that was required to inhibit 50% of ConA binding to the mannose or glucose spots. The IC_{50} value can be used to compare the binding affinities of the lectin for the immobilized carbohydrates. As shown in Fig. 4A and B, the microarray spots of mannose (IC_{50} = 60 μM) competed more effectively with the soluble ligand (i.e., α-methyl mannose) for ConA than that of glucose (IC_{50} = 23 μM). The relative binding affinities of these carbohydrates for ConA is consistent with those obtained in previous studies (Houseman et al., 2002).

4. Conclusion

In summary, we have developed a simple and efficient method to prepare oligosaccharide microarray on aminooxyacetyl functionalized glass surface by oxime bonding formation with the carbonyl group at the reducing end of suitable carbohydrates via irreversible condensation. The arrays are highly reproducible, stable and can be fabricated with standard microarray equipment and simple probing procedures. Importantly, carbohydrates are immobilized on array format directly without the need for the modification to generate reactive groups. This methodology offers the advantages of including simplicity, the use of undervatized carbohydrates, inert surfaces, and the capability of quantitative analysis on the carbochip. This method may also be widely applicable for development other type biochips for the detection of the carbohydrate-binding activity in various sources. The aminooxyacetyl monolayer functionalized slides described here also has the advantage that they are compatible with all of the principal techniques used for analyzing chips. Although we only demonstrated here the fabrication of carbohydrate microarray with short oligosaccharides on the aminooxyacetyl-functionalized surface, the immobilization of long polysaccharides on the aminooxyacetyl functionalized surface would be achieved by treating the aminooxyacetyl-functionalized slides that spotted carbohydrate probes with microwave energy, which the research is ongoing in our lab.

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