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Rapid characterization of sugar-binding specificity by in-solution proximity binding with photosensitizers

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Cell-surface carbohydrates are known to participate in many important physiological and pathological activities by interacting with their corresponding proteins or receptors. Although several methods have been developed for studying carbohydrate–protein interactions, one major problem originates from the weak bindings of carbohydrates/proteins that are often lost during repeating wash steps. Herein, we established a homogeneous solution carbohydrate array in which polyacrylamide-based glycans are used for offering a multivalent environment. The method requires no wash step and can be carried out in a high-throughput manner. We characterized the carbohydrate-binding specificities of 11 lectins and 7 antibodies, the majority of which displayed the binding patterns in consistence with previous reports. These results demonstrate that our developed solution carbohydrate array provides a useful alternative that is better than or comparable with the current available methods.

Keywords: carbohydrates / lectins / microarray

Introduction

Glycosylation decorates more than one half of the proteins in eukaryotic cells (Varki 1993; Ratner, Adams, Disney, et al. 2004). This post-translational modification plays an indispensible role in many important biological events, especially on cell surface (Varki 1993; Fukuda 2000). Alterations in carbohydrate structures are known to correlate with the changes in protein stability and clearance, as well as various physiological functions including cell–cell adhesion, inflammation, tumor metastasis and infection of bacteria and viruses (Magnani et al. 1981; Kansas 1996; Geijtenbeek et al. 2000; Skehel and Wiley 2000; Sacchettini et al. 2001). Although glycosylation is essential for the formation and progression of various diseases, the study of this subject is hampered by lack of effective tools available to date, in addition to structural heterogeneity and complexity of carbohydrates.

A number of techniques have been developed to characterize the binding interactions between carbohydrates and proteins (Ratner, Adams, Disney, et al. 2004; Raman et al. 2005). The lectin blotting/binding assay has become a routine method to determine the interacting glycoepitopes of glycoconjugates (Wu 2001), but the use is restricted by relatively low sensitivity and the necessity of multiple wash steps that are time-consuming. The method based on surface plasmon resonance monitors the interactions in real time and in a quantitative manner (Smith et al. 2003; Ratner, Adams, Su, et al. 2004; Vila-Perello et al. 2005; Tian et al. 2008; Ofokansi et al. 2009; Sletmoen et al. 2009). The sensitivity is, however, relatively low toward the use of low-molecular-weight carbohydrates, though the problem can be overcome by labeling sugars with organoplatinum(II) (Beccati et al. 2005). Recently, carbohydrate microarrays that immobilize glycans to solid supports were developed and widely used to probe the carbohydrate-binding properties of proteins, cells or viruses (Fukui et al. 2002; Feizi et al. 2003; Shin et al. 2005; Huang et al. 2006). For instance, a high-content glycan array was developed by a robotic microarray printing technology in which amine-functionalized glycans are coupled to the succinimide esters on glass slides (Blixt et al. 2004; Wong 2005). These arrays were utilized for profiling the specificities of plant/animal lectins, antibodies and intact viruses. In addition, fluorescence-based techniques, such as fluorescence polarization and two-photon fluorescence correlation, were also applied to study the lectin–sugar interactions (Khan et al. 1981; Lee 2001; Sorme et al. 2004; Pohl et al. 2006).

We report herein a high-throughput, homogenous and sensitive method to characterize the protein–carbohydrate interactions and glycostructures by in-solution proximity binding with photosensitizers (Figure 1). The technology, also called AlphaScreen™, was first described by Ullman et al. (1994, 1996) and has been used to study interactions between biomolecules (Warner et al. 2004; Yi et al. 2009; Bouchecareilh

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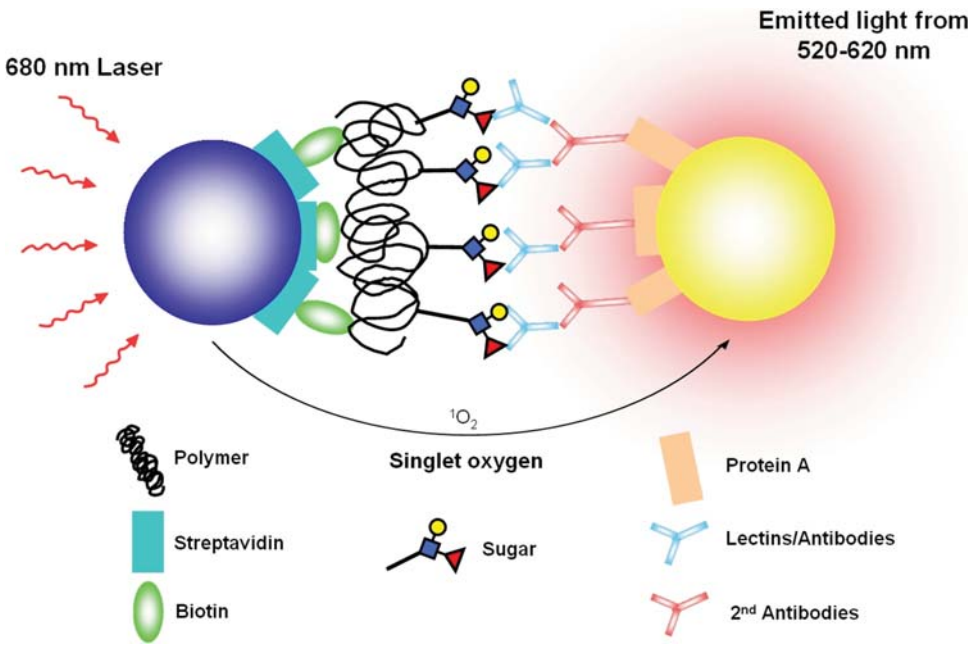


Fig. 1. In-solution proximity binding with photosensitizers: a new method has been developed to characterize the protein–carbohydrate interactions.

et al. 2010). In these assays, a light signal is generated when a donor bead and an acceptor bead are brought into proximity. This method usually provides good sensitivity with *femto*-mole detection under optimized conditions, relying on the binding affinity between analytes. All the procedures are carried out in 384-well microtiter plates, thus qualifying the protocol as high-throughput. Two particles of 200 nm are involved in this technology including streptavidin-coated particles (donor beads) and protein A-conjugated particles (acceptor beads). Biotinylated polyacrylamide (biotin-PAA)-based glycans that are immobilized on donor beads can be recognized by lectins or antibodies and connected with acceptor beads through specific antibodies (Figure 1). A number of carbohydrate-binding proteins, including 11 lectins and 7 antibodies, were profiled for their carbohydrate-binding specificity to validate the efficacy of this developed technology.

Results

Condition optimization

Owing to the features of non-washing and high sensitivity of our solution carbohydrate array, the concentration of each reagent should be controlled in a restricted range. First, the amounts of donor and acceptor beads were fixed at 500 ng/well. The concentration of rabbit anti-mouse immunoglobulin (Ig)G or IgM antibody (that was immobilized on protein A-conjugated acceptor beads) was fixed at 25 ng/well according to the binding analysis by using biotinylated rabbit anti-mouse antibody. We prepared and examined serial dilutions of biotin-PAA-Le^x (0–200 ng/well) and mouse monoclonal anti-Le^x antibody (3.12–200 ng/well). The optimal concentration of mouse monoclonal anti-Le^x antibody and the lowest concentration of biotin-PAA-sugar to generate the highest signal were then determined to be 40 and 20 ng/well, respectively (Supplementary data, Figure S1). Antigen/ligand excess effect was observed when the concentration of biotin-PAA-sugar was >20 ng/well. The linear range of biotin-PAA-Le^x was found to be 3–12.5 ng/well. Furthermore, all the secondary antibodies used in the study were not found to produce any signal. The resulting data were presented in relative intensities with respect to the highest fluorescence in the same batch of assays.

Carbohydrate-binding profiles of lectins and antibodies

Fifty-four biotin-PAA-sugars (Table I) were collected in total to examine 18 carbohydrate-binding proteins, including 11 lectins [three FITC-conjugated lectins: *Erythrina cristagalli* (ECA), *Maackia amurensis* (MAA) and *Sambucus nigra* Lectin (SNA); eight unlabeled lectins: *Canavalia ensiformis* (Con A), *Dolichos biflorus* (DBA), *Griffonia simplicifolia* I (GS-I), *Arachis hypogaea* (PNA), *Glycine max* (SBA), *Ulex europaeus* (UEA-1), *Wisteria floribunda* (WFA) and *Triticum vulgaris* (WGA)] and 7 antibodies (anti-Le^a, Le^b, Le^x, Le^y, sialyl Le^a, sialyl Le^x and blood group A). The resulting signals were indicated with bars as relative intensities (Figures 2 and 3). All the lectins showed nearly the same carbohydrate-binding preferences as those in previous reports, except for MAA (Supplementary data, Table S2). For instance, concanavalin A (Con A) bound preferentially to mannose (no. 3) and biantennary *N*-glycan (no. 53) and very weakly to 3- and 6-sulfated galactosides (nos 19, 23 and 25). DBA, a GalNAc-binding lectin, was in favor of binding to GalNAcα1-3Gal-containing epitopes (nos 11 and 39). ECA recognized LacNAc disaccharide, Galβ1-4(6-sulfo)GlcNAc and Galβ1-4(α1-2Fuc)GlcNAc (nos 17, 24, 31 and 47), in contrast to the weak binding of Le^c (Galβ1-3GlcNAc, no. 20). GS-I preferred binding with Gal/GalNAc that contains α1-3 or 1-4 linkage (nos 11, 13, 14, 16, 40 and 42). MAA, in our

Table I. Lists of PAA-linked carbohydrates

Number	Glycan name	Glycan number of CFG[a]	Number	Glycan name	Glycan number of CFG[a]
1	PAA-biotin		28	GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ, sp = -NHCOCH2NH-	173
2	β-GlcNAc-biotin		29	GlcNAcβ1-3Galβ1-4GlcNAcβ	164/165
3	α-Mannose	9	30	Fucα1-2Galβ1-3GlcNAcβ, Le^d (H type1)	63/64
4	β-GlcNAc	21	31	Fucα1-2Galβ1-4GlcNAcβ (H type2)	71/72
5	β-GalNAc	20	32	Galβ1-3 (Fucα1-4)GlcNAcβ (Le^a)	119
6	α-Fuc	11/12	33	Galβ1-4 (Fucα1-3)GlcNAcβ (Le^x)	135/136
7	α-NeuAc	14	34	3-HSO_3-Galβ1-4 (Fucα1-3)GlcNAcβ (3′sulfate Le^x)	34
8	α-NeuGc		35	NeuAcα2-3Galβ1-3GlcNAcβ (3′Sialyl Le^c)	225/226
9	Glcα1-4Glcβ	177	36	NeuAcα2-6Galβ1-4Glcβ (6′Sialyl Lactose)	249/250
10	GlcNAcβ1-4GlcNAcβ		37	NeuAcα2-3Galβ1-4Glcβ (3′Sialyl Lactose)	239/240
11	GalNAcα1-3Galβ	86	38	NeuAcα2-3(NeuAcα2-6)GalNAcα	213
12	Galβ1-4Glcβ (lactose)	154/155	39	GalNAcα1-3 (Fucα1-2)Galβ (Blood Group A)	84
13	Galα1-3Galβ	107	40	Galα1-3 (Fucα1-2)Galβ (Blood Group B)	99
14	Galα1-3GalNAcβ	102	41	3-HSO_3-Galβ1-3 (Fucα1-4)GlcNAcβ (3′sulfate Le^a)	31
15	Galβ1-3GalNAcβ	126	42	Galα1-4Galβ1-4Glcβ	111
16	Galα1-4GlcNAcβ (αLacNAc)	112	43	NeuAcα2-3Galβ1-4GlcNAcβ	236/237
17	Galβ1-4GlcNAcβ (LacNAc)	152/153	44	NeuAcα2-3Galβ1-3GalNAcα	202
18	Fucα1-2Galβ	74	45	Galβ1-3(NeuAcα2-6)GalNAcα	122
19	3-HSO_3-Galβ1-4GlcNAcβ	36/37	46	Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ	132
20	Galβ1-3GlcNAc (Le^c)	133/134	47	Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ	148
21	NeuAcα2-6GalNAcα	242	48	Fucα1-2Galβ1-3 (Fucα1-4)GlcNAcβ (Le^b)	57
22	NeuGcα2-6GalNAcα	262	49	Fucα1-2Galβ1-4 (Fucα1-3)GlcNAcβ (Le^y)	67/68
23	3-HSO_3-Galβ1-3GlcNAcβ	33	50	NeuAcα2-3Galβ1-3 (Fucα1-4)GlcNAcβ (sialyl Le^a)	217
24	Galβ1-4(6-HSO_3)GlcNAcβ		51	NeuAcα2-3Galβ1-4 (Fucα1-3)GlcNAcβ (sialyl Le^x)	230/231
25	6-HSO_3-Galβ1-4GlcNAcβ	44	52	$(NeuAc\alpha2\text{-}8)_{5\text{-}6}$	
26	NeuAcα2-3Gal	222	53	$(NeuAc\alpha2\text{-}6Gal\beta1\text{-}4GlcNAc\beta1\text{-}2Man)_2\alpha1\text{-}3,6Man\beta1\text{-}4GlcNAc\beta1\text{-}4GlcNAc\beta$	54
27	NeuAcα2-3GalNAcα	214	54	H_2O	

Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcNAc, *N*-acetylglucosamine; Man, mannose; Fuc, fucose; NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolylneuraminic acid.
[a]Printed array Ver. 2 from CFG.

study, bound mainly to 3′-sulfated Galβ1-3GlcNAc, 3′-sulfated Galβ1-4GlcNAc and LacNAc, but weakly to 3-sialylated galactosides (nos 26, 37 and 53). PNA mainly bound to Galβ1-3GalNAc structure (no. 15) and weakly bound to some galactosides (nos 12, 16, 20, 45 and 46). SBA preferentially bound to α-linked galactosides (nos 16 and 42) and *N*-acetylgalactosaminidase (no. 11). SNA, a well-known α2-6 sialoside-binding lectin, strongly bound with 6′-sialyl lactose and sialylated diantennary *N*-glycan (nos 36 and 53). UEA-1 specifically recognized Fucα1-2Gal-containing glycans (nos 18, 31 and 49). Although WFA was the only one lectin showing higher background signals than the others due to weak interaction with PAA. WGA recognized nearly half of the glycans on the list, such as GlcNAc- and NeuAcα2-3-Gal/NeuAcα2-6-Gal containing saccharides. Some minor signals indicated that WGA also bound to terminal Gal or GalNAc epitopes (GalNAcα1-3Gal, no. 11, and Galβ1-4(6HSO_3)GlcNAc, no. 24). Interestingly WGA appeared to bind better with chitotriose than with chitobiose and GlcNAc. Furthermore, the binding specificities of monoclonal anticarbohydrate antibodies also revealed interesting features, as shown in Figure 3. To our surprise, anti-Le^a antibody bound tightly with Le^x, but less with Le^b and sialyl Le^a, in addition to its native binding feature with Le^a. Anti-Le^b antibody is specific for both Le^b and Le^a, but less for Le^x and sialyl Le^x. Anti-Le^y antibody not only displayed the desired binding to Le^y, but also recognized lactose, Le^x, sialyl Le^x and H type 2 structures (though less efficiently). Meanwhile, anti-blood group A antibody showed higher binding signal to GalNAcα1-3Gal than its designated characteristic (Fucα1-2GalNAcα1-3Gal).

Additionally, the aforementioned lectin specificities were applied to characterize the glycan structures of six biotinylated proteins, including ovalbumin, porcine mucin, human serum albumin, human transferrin, fetuin and asialofetuin. Except for the non-glycosylated human serum albumin, the remaining five proteins generated distinctive glyco-patterns in our analysis (Supplementary data, Figure S2). Although the results were consistent with those obtained from previous lectin microarray or dot blot analysis (Kolarich and Altmann 2000; Karlsson and Packer 2002; Pilobello et al. 2005; Yang et al. 2006), further confirmation of these findings biochemically is necessary.

Discussion

Our initial trial to study the binding interactions of biotinylated fucose and Le^x with lectin and antibody failed to produce any positive signal. Interestingly, the replacement with PAA-supported fucose and Le^x was able to produce signals, which was attributed to the presence of multivalency (Sparks et al. 1993; Lees et al. 1994; Mammen et al. 1995; Sigal et al. 1996). The assay is performed in homogeneous solutions and does not require extra wash steps, preventing the loss of weak bindings that often occur in the repeating washes of glycan microarray. However, antigen/ligand excess effect may happen in the homogeneous solution assay if the

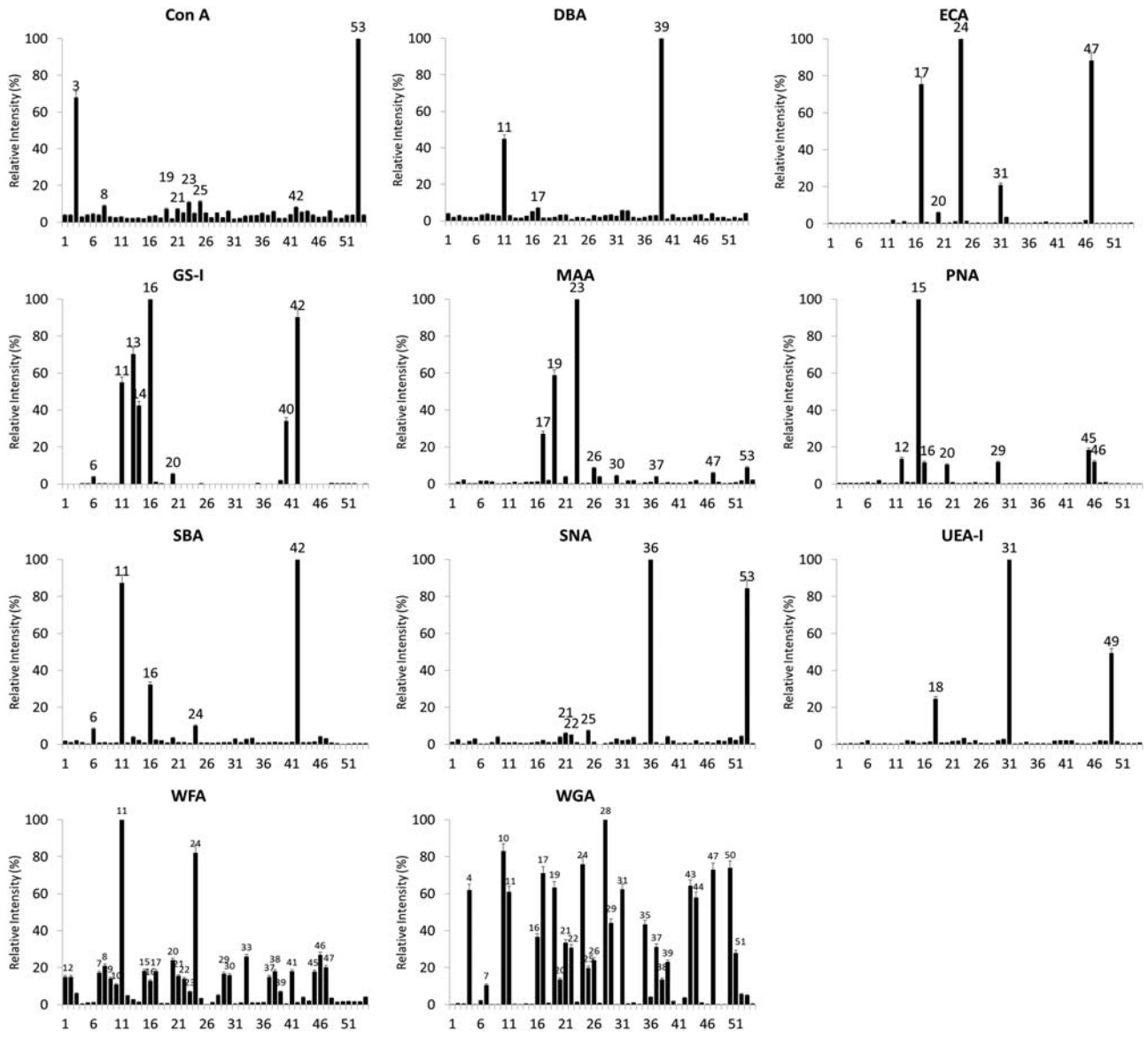
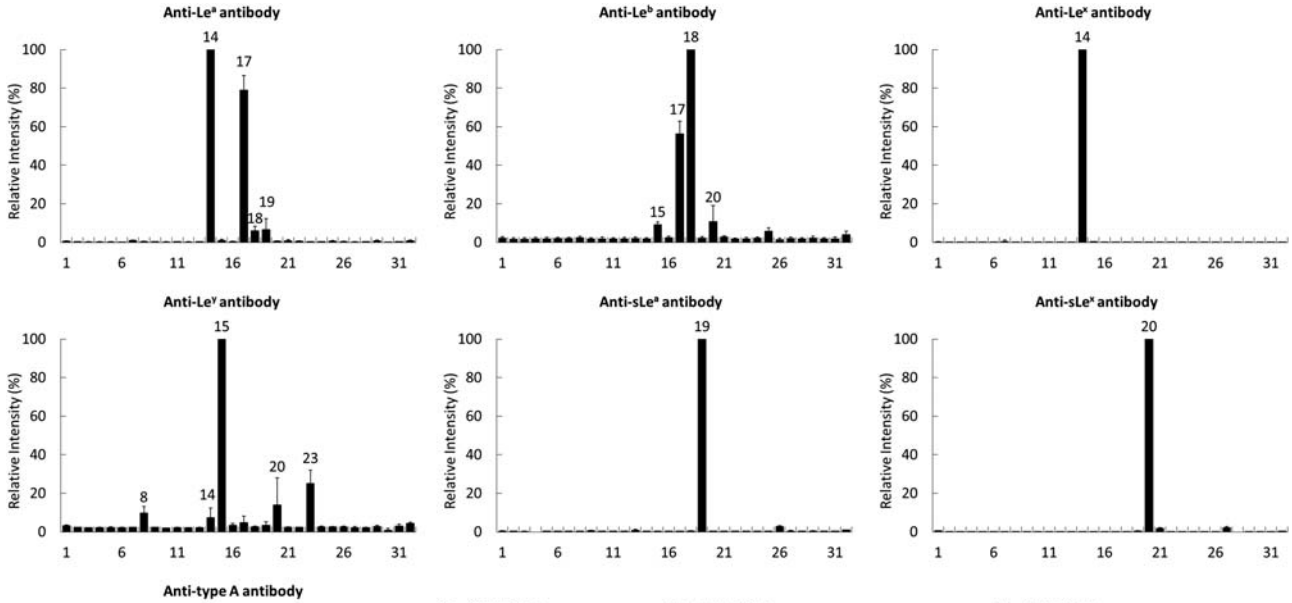


Fig. 2. Sugar-binding specificities of 11 lectins with 52 PAA-sugars (listed in Table I) are indicated by relative intensities of the highest fluorescence signal (*y*-axis). The carbohydrate-binding preference of the lectins reported by literature is listed in Supplementary data, Table S2. The sugar identities are designated by numbers (*x*-axis) and shown in detail in Table I. The measurement was carried out by EnVision. Each of the results was averaged from at least three independent assays.

concentrations of carbohydrate epitopes, proteins or antibodies are too high. We thus fixed the two particles at low concentrations to significantly diminish the background. The concentrations of biotin-PAA-sugars and rabbit anti-mouse IgG/IgM were then optimized. It should be noted that all the biotin-PAA-sugars were either purchased from GlycoTech (Gaithersburg, MD) or synthesized according to the known procedures (Lees et al. 1994; Mammen et al. 1995). One milligram of biotin-PAA-sugar can be applied for 50,000 assays because minimal amount of materials was needed in this array system (a range of nanogram is required per well). Although the detection limit of biotin-PAA-sugar was good (2 ng/well), the linear range was too narrow for quantitative application. Owing to the short half-life (4 μs) and heat-unstable feature of the generated singlet oxygen, the reaction should be kept in a temperature-constant and dark environment, and radical scavenger molecules are restricted to prevent the false-negative errors. In addition, the results were shown in relative strength of the highest fluorescence intensity in the same batch of assays.

In this study, we demonstrated that most of the lectins recognized their designated epitopes, but few lectins showed new binding preference. For instance, Con A was found to interact with 3- or 6-sulfated galactosides; PNA bound to some galactosides (nos 12, 16, 20 and 46); WFA recognized Galβ1-4(6HSO$_3$)GlcNAc (no. 24); WGA preferred binding with terminal Gal and GalNAc epitopes. We also found two factors critical to the interactions with lectins, including the



No.	Glycan Name	No.	Glycan Name	No.	Glycan Name
1	α-Mannose	12	Galα1-3GalNAcβ	23	Fucα1-2Galβ1-4GlcNAcβ (H type2)
2	β-GlcNAc	13	GalNAcα1-3Galβ	24	GlcNAcβ1-3Galβ1-4GlcNAcβ
3	GlcNAcβ1-4GlcNAcβ	14	Galβ1-4(Fucα1-3)GlcNAcβ (Le^a)	25	NeuAcα2-3Galβ1-3GlcNAcβ (3'Sialyl Le^c)
4	GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ	15	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ (Le^y)	26	Fucα1-2Galβ
5	Galβ1-4GlcNAcβ (LacNAc)	16	PAA-biotin	27	GalNAcα1-3(Fucα1-2)Galβ (Blood Group A)
6	Galα1-4GlcNAcβ (αLacNAc)	17	Galβ1-3(Fucα1-4)GlcNAcβ (Le^a)	28	Galα1-3(Fucα1-2)Galβ (Blood Group B)
7	Galβ1-3GlcNAc (Le^c)	18	Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ (Le^b)	29	$(NeuAc\alpha2\text{-}8)_{5\text{-}6}$
8	Galβ1-4Glcβ (Lactose)	19	NeuAcα2-3Galβ1-3(Fucα1-4)GlcNAcβ (sialyl Le^a)	30	Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ
9	Galα1-3Galβ	20	NeuAcα2-3Galβ1-4(Fucα1-3)GlcNAcβ (sialyl Le^x)	31	Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ
10	Galβ1-3GalNAcβ	21	3-HSO_3-Galβ1-4(Fucα1-3)GlcNAcβ (3'sulfate Le^a)	32	H_2O
11	Glcα1-4Glcβ	22	3-HSO_3-Galβ1-4GlcNAcβ		

Fig. 3. Sugar-binding profiles of seven antibodies with 30 PAA-sugars (listed below) are indicated by relative intensities of the highest fluorescence signal (*y*-axis). The sugar identities are designated by numbers (*x*-axis) and shown in detail in Table I. The measurement was carried out by EnVision. Each of the results was averaged from at least three independent assays.

linkage between the last two carbohydrates of the non-reducing end, and the sugar residue adjacent to the terminal saccharide. In other words, the specificity of most lectins is not limited to the non-reducing terminal monosaccharide. The unexpected binding profiles not only implicate previous mis-characterization of proteins or cells, but also suggest new biological functions of the lectins (Yakubov et al. 2007). Moreover, the carbohydrate-binding specificities are not influenced by FITC conjugation (owing to the aforementioned results of ECA, MAA and SNA). The results of seven blood group antibodies demonstrate that this AlphaScreen platform offers an effective way of easy operation to evaluate the quality of monoclonal carbohydrate antibodies. Additionally, the carbohydrate-binding preference of recombinant C-type lectin domain of thrombomodulin was explored by this solution microarray successfully (Shi et al. 2008).

We compared the binding profiles of 10 lectins with the results reported by Blixt and coworkers at Consortium for Functional Genomics (CFG) in which 264 different glycans were studied by using the printed array (Ver. 2; http://www.functionalglycomics.org/glycomics/publicdata/primaryscreen.jsp) (Figure 4). Forty-seven glycans are found to be identical in both analyses. Despite the different principles and procedures of the two systems, the binding patterns of nine lectins (such as Con A, DBA, ECA, GS-I, MAA, PNA, SBA, SNA and UEA-1) are nearly the same, except for a few minor differences. For instance, our characterized profiles of WFA and WGA display 90% similarity to the CFG data. Nevertheless, the bindings of SBA, WFA and WGA to β-GalNAc (no. 2) in the CFG's printed array were not observed in our system. Our method and the CFG printed array both indicate that MAA preferentially binds to sulfated glycans (Bai et al. 2001). Because of the observed consistency shown by the two very different methods, we thus conclude the lectin–sugar-binding interactions not to be affected by the PAA linker, the assay procedure (washing vs. non-washing) and the interacting microenvironment (two-dimension for printed array vs. three-dimension for our solution array). In addition, Galβ1-4(6HSO_3)GlcNAc (no. 24), which is not listed in the CFG's printed array, is shown to be recognized by ECA, WFA and WGA in our array. In order to verify whether washing steps affect the interactions between proteins with carbohydrates, we immobilized biotin-PAA-sugars on UltraBind membrane to confirm the binding patterns of eight lectins and four antibodies by western blot-like procedures (Supplementary data, Figure S3; Lao et al. 2011). Although the lectin-binding patterns of the two methods were similar, weak interactions detected by the aforementioned solution microarray were not found in the membrane method that requires additional multiple washing

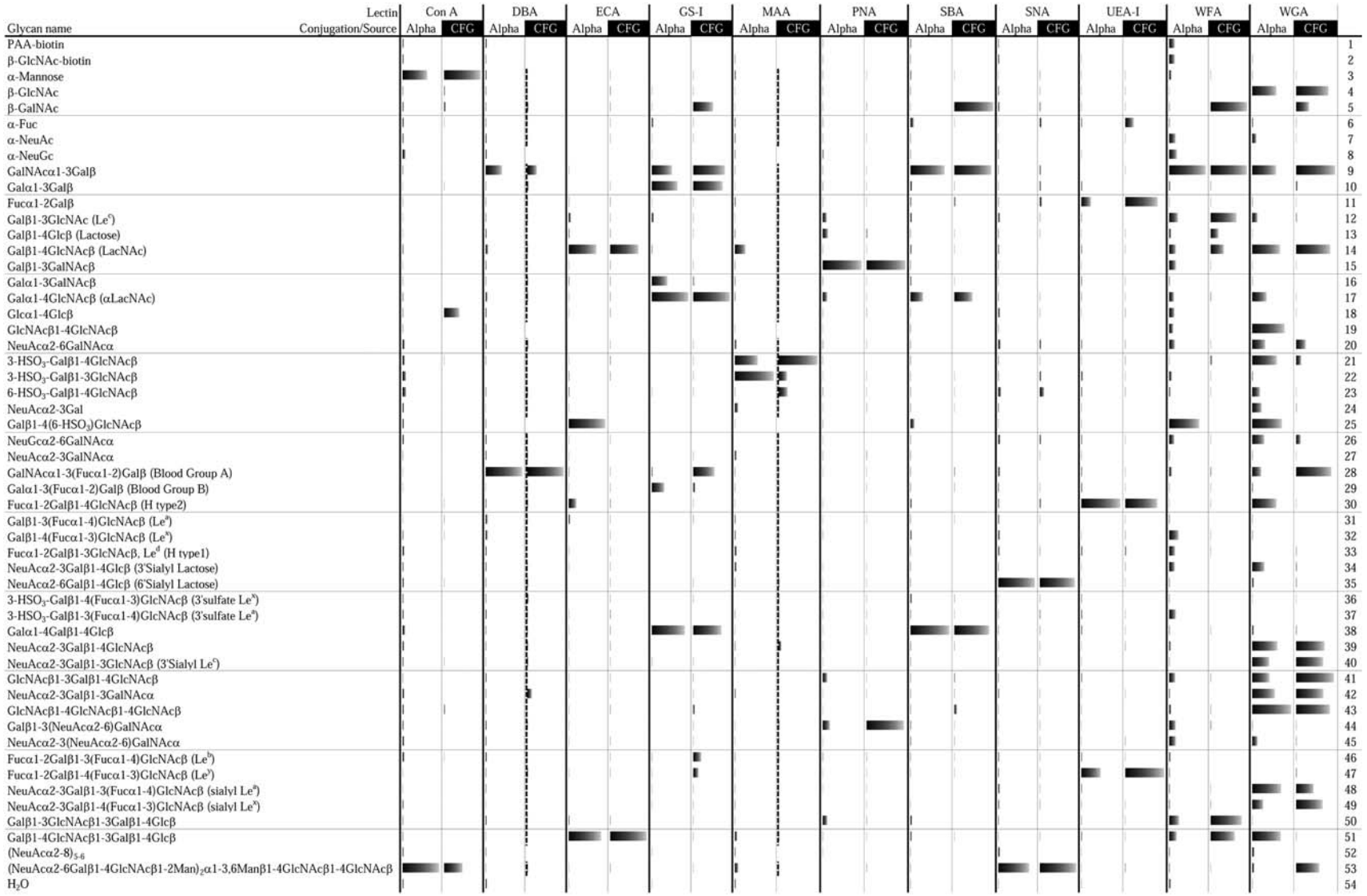


Fig. 4. The sugar-binding patterns of lectins are compared between the solution glycan microarray (designated as Alpha) and the printed glycan array developed by CFG. The degree of binding interactions is indicated the bar length that is shown by relative intensities of the highest signal in the same assay.

steps. In spite of the higher specificity observed by the membrane method when examining the binding of antibodies, it is not certain if the solution microarray detects extra weak binding or non-specific interactions (Supplementary data, Figure S4). Additional studies are required to address this issue.

In summary, our studies have demonstrated a rapid, highly sensitive, and reliable method to characterize carbohydrate–protein interactions with minimized materials (in the range of nanogram per well). Lectins and antibodies were evaluated and most of the results are coherent to previous reports, including those of CFG (Figure 4). This non-washing, homogeneous carbohydrate solution array offers another reliable alternative for characterizing sugar-binding features of lectins and proteins, as well as antibodies.

Material and methods

General information

AlphaScreen™ assays were carried out on a PerkinElmer Envision instrument. Streptavidin-coated donor beads, protein A-conjugated acceptor beads and ProxiPlate-384 assay plates were purchased from PerkinElmer Life Sciences, Inc. (Boston, MA). Lectins, including Con A, DBA, MAA, PNA, SBA, UEA-1, WFA, WGA, ECA, GS-I and related rabbit anti-lectin antibodies, were purchased from EY Labs, Inc. (San Mateo, CA). SNA was purchased from Vertor Laboratory Inc. (Burlingame, CA). Rabbit anti-mouse IgG, rabbit anti-mouse IgM and rabbit anti-FITC were purchased from Zymed, Inc. (South San Francisco, CA). Mouse anti-CD 15 was purchased from BioLegend, Inc. (San Diego, CA). Mouse anti-Le^a and mouse anti-sialyl Le^a were purchased from Biomeda, Inc. (Foster City, CA). Mouse anti-Le^b, mouse anti-Le^y and mouse anti-6 × His tag antibodies were purchased from Abcam, Ltd (Cambridge, UK). Mouse anti-sialyl Le^x was purchased from Chemicon International, Inc. (Temecula, CA). Mouse anti-blood group type A was purchased from Acris Antibodies GmbH (Hiddenhausen, DE). Biotin-PAA-based sugar polymers were purchased from GlycoTech. Reagents of the highest purity were purchased from Sigma–Aldrich (California, USA), Acros (Geel, Belgium), and Novabiochem (Darmstadt, Germany).

Evaluation of the carbohydrate microarray by 11 lectins and 7 blood group antibodies

All the procedures and incubations must be carried out in the dark. Donor beads (500 ng/well) and biotin-PAA-sugars (20 ng/well) mixed with lectins (10 ng/well) or antibodies (40 ng/well) were incubated at ambient temperature for 1 h (total 15 μL). The mixture of acceptor beads (500 ng/well), mouse anti-lectin antibody (50 ng/well) and rabbit anti-mouse IgG or IgM antibody (25 ng/well) was added into the reaction to a final volume of 25 μL. After 2 h of incubation, the binding signals were obtained on the PerkinElmer Envision instrument using the AlphaScreen™ program. Optimized concentrations of lectins or antibodies were measured and used for binding assay. The results were showed by relative intensities.

Supplementary data

Supplementary data for this article is available online at http://glycob.oxfordjournals.org/.

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Conflict of interest

None declared.

Abbreviation

Biotin-PAA, biotinylated polyacrylamide; Con A, *Concanavalin A*; DBA, *Dolichos biflorus*; ECA, *Erythrina cristagalli*; GS-I, *Griffonia simplicifolia* I; Ig, immunoglobulin; MAA, *Maackia amurensis*; PNA, *Arachis hypogaea*; SBA, *Glycine max*; SNA, *Sambucus nigra* Lectin; UEA-1, *Ulex europaeus*; WFA, *Wisteria floribunda*; WGA, *Triticum vulgaris*

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