Glycoconjugate microarray based on an evanescent-field fluorescence-assisted detection principle for investigation of glycan-binding proteins

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The extensive involvement of glycan-binding proteins (GBP) as regulators in diverse biological phenomena provides a fundamental reason to investigate their glycan-binding specificities. Here, we developed a glycoconjugate microarray based on an evanescent-field fluorescence-assisted detection principle for investigation of GBPs. Eighty-nine selected multivalent glycoconjugates comprising natural glycoproteins, neo-glycoproteins, and polycyclolamid (PAA)-conjugated glycan epitopes were immobilized on an epoxy-activated glass slide. The GBP binding was monitored by an evanescent-field fluorescence-assisted scanner at equilibrium without washing steps. The detection principle also allows direct application of unpurified GBPs with the aid of specific antibodies. Model experiments using plant lectins (RCA120, ConA, and SNA), galectins (3 and 8), a C-type lectin (DC-SIGN) and a siglec (CD22) provided data consistent with previous work within 4 h using less than 40 ng of GBPs per analysis. As an application, serum profiling of antiglycan antibodies (IgG and IgM) was performed with Cy3-labeled secondary antibodies. Moreover, novel carbohydrate-binding ability was demonstrated for a human IL-18 binding protein. Thus, the developed glycan array is useful for investigation of various types of GBPs, with the added advantage of wash-free analysis.

Keywords: evanescent/glycoconjugate/lectin/microarray/specificity

Introduction

Glycan-binding proteins (GBP), or lectins, exist as diverse families of proteins that bind specifically to glycans (Sharon and Goldstein 1998; Sharon and Lis 2004). Their ubiquitous occurrence in the biosphere has been established in viruses, bacteria, fungi, plants, and animals including humans, while their endogenous functions largely remain to be elucidated. However, there is accumulated evidence that they play regulatory roles in diverse biological processes, such as microbe infection, immune response, cell differentiation, and tumor-cell metastasis through specific binding to their glycan ligands (Robinson et al. 2006; Crocker et al. 2007; van Vliet et al. 2007). Lectins of known specificity have served as useful tools in biochemical research fields such as in cell typing, tissue staining, enrichment and purification of glycoproteins, western blotting, flow cytometry, and glycan profiling (Hirabayashi 2004; Wu et al. 2008). A lectin microarray provides a comprehensive approach to profiling diverse glycan structures displayed at the cell surface and in glycoconjugates (Kuno et al. 2005; Pilobello et al. 2005; Hsu et al. 2006; Tateno, Uchiyama, et al. 2007). The different specificities of lectins for complex glycans render them valuable tools for recognizing a particular type of glycan (epitope), and hence, fuel the search for yet more novel lectins (Goldstein 2002).

From an historical viewpoint, most of the known lectins have been identified through agglutination and binding assays using model cells (e.g., erythrocytes) and glycoproteins (e.g., asialofetuin), respectively (Boyd and Shapleigh 1954; Sharon and Lis 2004). In addition, there is a variety of approaches to the determination of detailed sugar-binding specificity: these include enzyme-linked immunosorbent assay (ELISA) (Blixt et al. 2003; Wu et al. 2008), equilibrium dialysis (Mega and Hase 1991), surface plasmon resonance (Shinohara et al. 1994), isothermal titration calorimetry (Dam and Brewer 2004), frontal affinity chromatography (FAC) (Tateno, Nakamura-Tsuruta, et al. 2007), and glycan microarray (Fukui et al. 2002; Feizi et al. 2003; Blixt et al. 2004; Manimala et al. 2006). Among the analytical systems developed, the glycan microarray may be the most promising approach for high-throughput investigation of GBPs and has now been established as an essential tool for functional glycomics. The glycan arrays developed in the Consortium for Functional Glycomics (CFG) (Blixt et al. 2004) and the UK Glycoarray Consortium (Fukui et al. 2002) have been providing enormous information on the glycan-binding properties of various types of GBPs. The current CFG glycan array (version 3.2) has now 406 of distinct glycan compounds, including a series of N- and O-linked glycans and glycolipid-type glycans. The library size of both of the glycan arrays is still expanding. However, there is still a room to improve the glycan array technology such as detection methods.

An evanescent-field fluorescence-assisted detection principle is one of the most sensitive detection methods, which was successfully applied for detection of sugar–protein interactions in the lectin array (Kuno et al. 2005). The system enables detection of interactions between glycans and immobilized lectins without any washing step. Thus, even weak interactions between sugars and lectins can be detected (Uchiyama et al. 2008). However, there has been no report on the application of the detection system for the glycan microarray.

Here we developed a glycoconjugate microarray with the evanescent-field fluorescence-assisted detection principle. To enhance the avidity of GBPs, glycoproteins and glycopolymer
displaying highly multivalent glycan ligands were immobilized on epoxy-activated glass slides. The resulting glycoconjugate array is capable of detection of carbohydrate-binding activities of diverse families of lectins and glycan-binding antibodies, even in crude samples. Thus, the detection system was proved to be versatile for investigation of sugar–protein interactions, in either formats of lectin microarray or glycan microarray.

Results

Strategy for specificity profiling of lectins

In general, lectins show much lower affinity for monovalent oligosaccharides than do antibodies, with $K_d$ values in the µM to mM range, whereas it is well known that they show much higher binding avidity for multivalent glycan ligands by “glycoside cluster effect” (Lee and Lee 2000; Gestwicki et al. 2002; Collins and Paulson 2004; Dam et al. 2007). Such monovalent interactions, if any, would not withstand the extensive washing steps adopted in general microarray techniques. Therefore, we adopted a strategy to immobilize multivalent rather than monovalent glycans on microarray glass slides: natural glycoproteins, neo-glycoproteins, and glycan epitope-conjugated polyacrylamide (PAA) polymers (Figure 1). In order to detect even weak interactions that should be eliminated by extensive washing steps, we took advantage of an evanescent-field fluorescence-assisted scanner, which was originally developed for the purpose of lectin microarray (Kuno et al. 2005).

The use of epoxy-activated glass slides allows covalent attachment of (neo)glycoproteins containing primary amines. As an extension of this approach, commercially available glycosides attached to the PAA backbone of 30 kDa, which have often been used as high-affinity probes for lectins (Bovin 1998; Collins et al. 2006; Tateno, Li, et al. 2007), were found to be efficiently immobilized on epoxy-activated glass slides under the same conditions (see Materials and methods). Varying concentrations of BSA or PAA conjugates of α-Fuc (0–500 µg/mL, Fuca-BSA and Fuca-PAA, respectively) were spotted on glass slides, and ligand coupling efficiency was measured by binding to a Cy3-labeled *Aleuria aurantia* lectin (AAL). As shown in Figure 2, binding of AAL was observed on the spots of both Fuca-BSA and Fuca-PAA with similar intensities in a concentration-dependent manner, whereas no signal was observed for control BSA and PAA. Uniform spot morphology and a high S/N ratio were obtained. The CV (%) of the signal intensity of the positive spots was low (2.2), demonstrating the reliability and reproducibility of the array. In this study, 61 glycoside-PAA conjugates containing representative terminal glycan structures (epitopes) of glycoproteins and glycolipids were spotted at a concentration of 0.1 mg/mL, whereas 28 (neo)glycoproteins expressing glycan structures of representative N- and O-glycans were spotted at 0.5 mg/mL. All samples were printed in triplicate in each 14-well glass slide (Figure 3 and supplementary Table I).

Validation study using established plant lectins

Sensitivity is one of the most important factors for developing a glycan array system, since the quantities of natural samples are usually limited. To validate the sensitivity of the developed glycoconjugate array, we first analyzed three representative plant lectins: a Gal-binding lectin (RCA120), a Man-binding lectin...
Fig. 3. Array design. Glycoproteins or glycoside-PAA were spotted in triplicate on an epoxy-coated glass slide attached with a silicone rubber sheet with 14 chambers, using a noncontact microarray printing robot (A). Glycan structures of glycoside-PAA spotted on the array are shown in the symbol nomenclature, whereas natural glycoproteins with heterogeneous glycans are shown in common name (B). Detailed information on the glycans used in this study can be found in supplementary Table 1.
Fig. 4. Specificity profiling of plant lectins. (A) Varying concentrations of Cy3-labeled RCA120, Con A, and SNA (0–40 ng/well, 0–1000 ng/mL) were applied on the array and binding was detected by the scanner. Scan images of each lectin are shown. (B) Scan images of the lectins (12 ng/well) were analyzed with the Array Pro analyzer ver. 4.5. The net intensity value for each spot was determined as the signal intensity minus the background value. Data are the average ± S.D. of triplicate determinations.

(Con A), and an α2-6Sia-binding lectin (SNA). Varying amounts of the Cy3-labeled plant lectins (0–40 ng/well, 0–1000 ng/mL) were applied to the array and incubated at 20°C for 3 h, and binding was directly detected by the evanescent-field fluorescence-assisted scanner without washing steps. As shown in Figure 4A, concentration-dependent signals were obtained, where 4–12 ng of lectins per well (100–300 ng/mL, 40 µL) gave signals (i.e., >10,000 net intensity) strong enough for analysis. The three plant lectins exhibited distinct binding profiles on the array as described in detail below.

RCA120, an R-type lectin isolated from the seeds of the common castor bean (Ricinus communis), exhibited binding to glycoproteins containing galactosylated N-glycans (23, 24, 36–39), but not to their agalactosylated forms (42–44) (Figure 4B, top). Among synthetic glycans tested, strong binding was observed for β-D-Gal (25), but not for its anomer, α-D-Gal (69), demonstrating that the protein is a βGal-specific lectin. Linkage specificity of RCA120 was also demonstrated on this array. It showed preference for type 2 (Galβ1-4GlcNAc, 31) over type 1 (Galβ1-3GlcNAc, 29) structures. Binding to type 2
the synthetic ligands tested, the lectin showed selective binding (N-terminal and C-terminal CRDs). A specificity profile of Gal-mannan (αbottom). Among the synthetic glycosides, SNA bound to not to their desialylated forms (36). β-tained (Figure 5, top). As described previously (Hirabayashi et al. 1987). interpretations are as follows.

In order to extend applicability of the system for specificity profiling, we analyzed a few members of the galectin family, a major group of animal lectins with β-galactoside specificity and an evolutionarily conserved carbohydrate-recognition domain (CRD). As shown in Figure 5, overall binding profiles of the C-terminal domain of galectin-3 (Gal-3C) and the N-terminal domain of galectin-8 (Gal-8N) are different despite the fact that they share basic specificity for β-galactoside. Detailed interpretations are as follows.

Gal-3 is a chimera-type galectin composed of an N-terminal collagenase-sensitive domain and a C-terminal CRD. Using 4 ng of Gal-3C, a characteristic specificity profile was obtained (Figure 5, top). As described previously (Hirabayashi et al. 2002; Stowell et al. 2008), Gal-3C exhibited strong affinity to blood group A (5) as well as blood group B antigens (6, 71). Gal-3C also displayed significant binding to both type 1 (Galβ1-3GlcNAc, 29) and core 1 (Galβ1-3Galβ1-4GlcNAc, 52) structures as well as to their 3′-O-sulfated forms (30 and 59, respectively), while binding to core 2 structure (Galβ1-3GlcNAcβ1-6GlcNAc, 53) was somewhat reduced. Binding to these O-glycans was confirmed for asialoglycophorin (Asialo-IgG and CD22-Fc fusion proteins, which represent C-type lectins and siglecs, respectively. Firstly, varying concentrations of these fusion proteins were premixed with Cy3-labeled goat antihuman IgG and incubated on the array at 20°C for 3 h, and their binding was directly detected by the scanner without washing. As shown in Figure 6A, concentration-dependent binding signals were obtained, where 4–12 ng of the lectins per well (100–300 ng/mL) gave stable signals, comparable to the case using Cy3-labeled lectins directly. Thus, indirect labeling via the fluorescent-labeled anti-Fc secondary antibody could be readily achieved. However, when the DC-SIGN-Fc and CD22-Fc were Cy3-labeled and analyzed by the array, much weaker binding signals could be obtained, indicating that antibody precomplexing also helps to enhance the binding signals of lectins with low affinity to glycans.

The method was then examined for its applicability to unpurified lectins. Expression vectors encoding DC-SIGN-Fc and CD22-Fc were transfected into CHO cells and HEK293T cells, respectively. The resulting cell culture supernatant was pre-incubated with the above detection probe (Cy3-labeled goat antihuman IgG) and applied to the array, and the binding was detected by the evanescent-field-type scanner without washing. As shown in Figure 6B, the control culture supernatant gave no detectable signals, whereas the culture supernatant (CS) of cells expressing either DC-SIGN-Fc or CD22-Fc gave stable profiles, which were similar to those obtained for their purified forms (Figure 6A). This result clearly indicates that glycan-binding activities of the recombinant lectins expressed in CS could be easily and precisely analyzed without the need for purification. Detailed explanations of sugar-binding specificities of the two recombinant lectins are as follows.

DC-SIGN, a member of the type 2 receptor subgroup of the C-type lectin family, is a dendritic cell-specific immune receptor implicated in viral and pathogen infections and cancer cell immune tolerance. As shown in Figure 6C (top), DC-SIGN-Fc displayed strong binding to both fucosylated (1, 3, 7–10) and 8N was obtained using 12 ng (300 ng/mL) of the recombinant protein (Figure 5, bottom). Gal-8N displayed particular preference for LacNAc substituted with Sia (15–17) or sulfate (30) at the 3-OH of Gal, although binding to neither type 1 (29) nor type 2 (31) LacNAc was detectable under this condition. Indeed, Gal-8N bound to α2-3Sia-containing glycoproteins such as fetuin (21) and α1-acid glycoprotein (AGP, 22), but not to their desialylated forms (36, 37). Gal-8N also exhibited binding to O-glycans such as sialyl T (Siaα2-3Galβ1-3GlcNAc, 65) and 3′-O-sulfo T (3-O-sulfate-Galβ1-3GlcNAc, 59), but not detectably to T antigen (Galβ1-3GlcNAc, 52), demonstrating the strong effect of substitutions with Sia or sulfate at the 3-OH of Gal on affinity enhancement (Hirabayashi et al. 2002).

Application to crude extracts of recombinant lectins: DC-SIGN and CD22

The above results confirmed that the developed system worked satisfactorily for elucidation of sugar-binding specificities of several established lectins. However, the main purpose of this study is to develop a rapid profiling system of extensive GBPs, and hence its direct applicability to crude samples is a critical factor in determining whether or not the method is of high utility. For this purpose, we carried out two-step experiments by using DC-SIGN-Fc and CD22-Fc fusion proteins, which represent C-type lectins and siglecs, respectively. Firstly, varying concentrations of these fusion proteins were premixed with Cy3-labeled goat antihuman IgG and incubated on the array at 20°C for 3 h, and their binding was directly detected by the scanner without washing. As shown in Figure 6A, concentration-dependent binding signals were obtained, where 4–12 ng of the lectins per well (100–300 ng/mL) gave stable signals, comparable to the case using Cy3-labeled lectins directly. Thus, indirect labeling via the fluorescent-labeled anti-Fc secondary antibody could be readily achieved. However, when the DC-SIGN-Fc and CD22-Fc were Cy3-labeled and analyzed by the array, much weaker binding signals could be obtained, indicating that antibody precomplexing also helps to enhance the binding signals of lectins with low affinity to glycans.

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and mannose-containing glycans (47, 48, 50, 84, 85), consistent with previous reports (Cambi et al. 2003; Guo et al. 2004). CD22, a member of the siglec family, is a negative regulator of B cell signaling and binds selectively to α2-6Sia (Powell and Varki 1994; Blixt et al. 2003). As expected, CD22-Fc exhibited binding to synthetic α2-6sialyllactose (20) as well as to α2-6Sia-containing glycoproteins (22–24), but not to their desialylated forms (37–39) (Figure 6C, bottom). CD22-Fc also bound to sialyl-Tn (Siaα2-6GalNAc, 63, 64) only at a high concentration (40 ng/well, 1 µg/mL) (Figure 6A). The binding of CD22 to sialyl-Tn was previously reported by Brinkman-Van der Linden et al. (Brinkman-Van der Linden and Varki 2000).

Challenge for investigating novel glycan-binding activities of GBPs

Having demonstrated that the system is valid for rapid and sensitive profiling of established groups of lectins, we finally investigated the glycan-binding activities of unidentified groups of GBPs, such as cytokine-binding proteins and antibodies. Among cytokine-binding proteins, poxvirus IL-18 binding proteins (IL-18BPs), which bind IL-18 and inhibit IL-18-mediated immune responses, were reported to have glycosaminoglycan (GAG)-binding activity (Xiang and Moss 2003; Esteban et al. 2004). However, there is no report on the glycan-binding activity of its human homolog, human IL-18BP. As shown in Figure 7 (top panel), human IL-18BP exhibited glycan-binding activity toward a series of GAGs (78–82), similar to that of poxvirus IL-18BPs. Interestingly, it also showed clear preference for a sulfated disaccharide, i.e., LacNAc with sulfate at the 6-OH of Gal (33), but not for its position isomer (LacNAc with sulfate at 3-OH of GlcNAc, 32) nor nonsulfated LacNAc (31). To our surprise, human IL-18BP also bound to α-L-rhamnose (α-L-Rha, 83) that is often seen in the outermost cell surface components of bacteria, but not in eukaryotes. Although the actual ligand(s) of human IL-18BP has not been identified, the present finding implies that human IL-18BP has sugar-binding function, which is related to glycosaminoglycans or bacterial polysaccharides.

The final attempt was to examine whether or not the developed microarray system was useful for rapid glycan-epitope profiling of human serum immunoglobulin. Human type H serum was diluted at 1000 times with a probing buffer and applied to the array. In order to detect IgM and IgG classes independently, the array was probed with either Cy3-labeled goat antihuman IgM or goat antihuman IgG antibodies, respectively. As shown in Figure 7 (middle and bottom panels), IgG and IgM gave distinct profiles from each other. Antiglycan IgM were found to be generated mostly against glycan epitopes representing microorganisms, such as β-D-Man (48), yeast invertase (50), β-D-Glc (75), α-L-Rha (83), and mannan from S. cerevisiae (84) and C. albicans (85) except for A and B blood group antigens (5, 6) and Galβ1-3Gal, a xenotransplantation epitope (70, 71). In contrast, antiglycan IgG exhibited more selective binding to particular structures, such as Tn (α-D-GalNAc, 51), melibiose (Galβ1-6Glc, 73), and mannan from C. albicans (85) but not that from S. cerevisiae (84). Binding to the A and B blood group antigens (5, 6) and yeast invertase (50) is common to both antiglycan IgM and IgG.

Discussion

In conventional hemagglutination assay, the approximate sugar-binding specificity of lectins is determined by hapten inhibition using a panel of free saccharides. Although the hemagglutination assay is the most common and convenient method for the detection of lectin activity, it has limitations: (i) it is applicable only for lectins with affinity to abundant glycans expressed at the
Glycoconjugate microarray

Fig. 6. Application to crude extracts of recombinant lectins: DC-SIGN and CD22. (A) Varying concentrations of purified DC-SIGN-Fc and CD22-Fc proteins (0–40 ng/well) precomplexed with Cy3-antihuman IgG (0.5 µg/mL) were applied on the array and binding was detected by the scanner without washing. Ctr: negative control. (B) Culture supernatants (CS) of CHO cells and HEK293T cells transfected with expression vectors of DC-SIGN-Fc and CD22-Fc, respectively, were recovered and mixed with Cy3-antihuman IgG (1 µg/mL). After incubation with the array at 20 °C for 3 h, binding was detected by the scanner. No signals were detected for control culture media (Control CS). (C) Data were analyzed with the Array Pro analyzer ver. 4.5. The net intensity value for each spot was determined as the signal intensity minus background value. Data are the average ± S.D. of triplicate determinations.

Materials and methods

Materials

A panel of glycoside-polyacryamide (PAA) conjugates synthesized by coupling spacered oligosaccharides and spacered biotin to poly(4-nitrophenyl acrylate) were purchased from Glycotech (MD) (Bovin et al. 1993). Fetuin from fetal bovine serum, α1-acid glycoprotein from bovine serum, transferrin from human serum, porcine thyroglobulin, mucin from bovine submaxillary glands, glycoporin from human blood type MN and its desialylated form, ovalbumin from chicken egg white, ovomucoid from chicken egg white, invertase from S. cerevisiae, and mannan from S. cerevisiae were purchased from Sigma (MO). Mannan from C. albicans was purchased from Takara (Shiga, Japan). Hyaluronic acid, chondroitin sulfate (CSA), dermatan sulfate (CSB), heparin sulfate (HS), keratin sulfate (KS), RCA120, and ConA were purchased from Seikagaku Co. (Tokyo, Japan). Sambucus nigra agglutinin (SNA) was obtained from Vector Laboratories (CA). Goat antihuman IgG (Fc-specific) and goat antihuman IgM (FcεRI-specific) were purchased from Jackson ImmunoResearch Laboratories, Inc. (PA). The recombinant human IL-18 binding protein was purchased from R&D Systems (MN). BSA-glycosaminoglycan conjugates (GAG-BSA) were prepared using N-ethoxy carbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) as previously described.
Fig. 7. Investigation of novel glycan-binding activities of GBPs. (Top) Cy3-labeled human IL-18 binding protein (IL-18BP) was applied on the array at 1 µg/mL (40 ng/well) and scanned. (Middle) Human blood group H serum (1:1000 dilution) were applied on the array and detected by subsequent overlay with Cy3-antihuman IgM (0.5 µg/mL). (Bottom) Human blood group H serum (1:1000 dilution) was applied on the array and detected by subsequent overlay with Cy3-antihuman IgG (0.5 µg/mL). Data were analyzed with the Array Pro analyzer ver. 4.5. The net intensity value for each spot was determined as the signal intensity minus background value. Data are the average ± S.D. of triplicate determinations.

(Nishioka et al. 2007). Asialo-glycoproteins were prepared by incubating in 0.1 N HCl at 80°C for 1 h. Agalactosylated glycoproteins were prepared by Streptococcus 6646K β-galactosidase (Seikagaku) treatment.

Preparation of CD22-Fc and DC-SIGN-Fc chimera
The human CD22-Fc chimeric construct (CD22-Fc) was generated by cloning the N-terminal three Ig domains (1-324 amino acids) into the EK-Fc/pcDNA3.1(−) vector (Angata et al. 2002). The DC-SIGN-Fc fusion construct (DC-SIGN-Fc) was generated by cloning the carbohydrate-recognition domain (CRD, 251-404 amino acids) into the pSecTag/FRT/V5-His vector containing the Fc portion of human IgG1. The CD22-Fc and DC-SIGN-Fc constructs were transfected into HEK293T cells cultured in OPTI-MEM/2.5% fetal calf serum (FCS) and CHO cells in F12/10% FCS media, respectively. Culture supernatants were recovered and CD22-Fc and DC-SIGN-Fc fusion proteins were then purified by Protein A Sepharose. Culture supernatants were also directly analyzed by the glycoconjugate microarray.

Preparation of human galectins
Expression plasmids for the C-terminal domain of Galectin-3 (Gal-3C) and the N-terminal domain of Galectin-8 (Gal-8N) were constructed using pET-27b (Hirabayashi et al. 2002). The plasmids were cloned into Escherichia coli (E. coli) BL21-CodonPlus (DE3)-RIL strain (Stratagene, CA). The recombinant proteins were produced by the addition of 1 mM isopropyl-β-D-thiogalactoside (IPTG) and purified by lactosyl-Sepharose.

Fabrication of the glycoconjugate microarray
Glycoproteins and glycoside-PAA conjugates were dissolved in a spotting solution (Matsunami Glass Ind., Ltd, Osaka, Japan) at a final concentration of 0.5 and 0.1 mg/mL (25 µL for 12 glass slides, 168 wells), respectively. After filtration using 0.22 µm pore size filter to remove insoluble particles, they were spotted on a microarray-grade epoxy-coated glass slide (Schott AG, Mainz, Germany) attached with a silicone rubber sheet with 14 chambers, using a noncontact microarray printing robot (MicroSys 4000; Genomic Solutions Inc., MI) with a spot diameter size of 220 µm spaced at a 260 µm interval. The glass slide was incubated in a humidity-controlled incubator at 25°C for 3 h to allow immobilization. After incubation, excess amounts of nonimmobilized materials were washed out with the probing buffer (25 mM Tris–HCl, pH 7.4 containing 0.8% NaCl, 1% (v/v) Triton-X, 1 mM MnCl2, 1 mM CaCl2) and blocked with 100 µL of TBS (25 mM Tris–HCl, pH 7.4 containing 0.8% NaCl) containing 1% BSA at 20°C for 1 h.

Binding assay
Cy3-labeled GBPs or GBPs precomplexed with Cy3-labeled antibodies in the probing buffer were applied to each chamber of the glass slides (40 µL/well) and were incubated at 20°C for 3 h, after which fluorescent images were immediately acquired using an evanescent-field activated fluorescence scanner, SC-Profiler (Moritex, Tokyo) under Cy3 mode without washing steps. Throughout experiments, scanning conditions of the cooled charge-coupled device (CCD) camera, i.e., resolution (5 µm), number of times for integration (4), and exposure time (200 msec) were fixed. Data were analyzed with the Array Pro...
**Supplementary Data**

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

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**Conflict of interest statement**

None declared.

**Abbreviations**

CFG, Consortium for Functional Glycomics; CS, culture supernatant; FAC, frontal affinity chromatography; FCS, fetal calf serum; GBP, glycan-binding protein; PAA, polyacrylamide.

**References**


