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A Fluorescent Lectin Array Using Supramolecular Hydrogel for Simple Detection and Pattern Profiling for Various Glycoconjugates

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Abstract: Because sugar and its derivatives play important roles in various biological phenomena, the rapid and high-throughput analysis of various glycoconjugates is keenly desirable. We describe herein the construction of a novel fluorescent lectin array for saccharide detection using a supramolecular hydrogel matrix. In this array, the fluorescent lectins were noncovalently fixed under semi-wet conditions to suppress the protein denaturation. It is demonstrated by fluorescence titration and fluorescence lifetime experiments that the immobilized lectins act as a molecular recognition scaffold in the hydrogel matrix, similar to that in aqueous solution. That is, a bimolecular fluorescence quenching and recovery (BFQR) method can successfully operate under both conditions. This enables one to fluorescently read-out a series of saccharides on the basis of the recognition selectivity and affinity of the immobilized lectins without tedious washing processes and without labeling the target saccharides. Simple and high-throughput sensing and profiling were carried out using the present lectin array for diverse glycoconjugates, which not only included a simple glucose, but also oligosaccharides, and glycoproteins, and, furthermore, the pattern recognition and profiling of several types of cell lysates were also accomplished.

Introduction

Recent advances in glycobiology and glycochemistry have revealed that sugar and its derivatives such as oligosaccharides, glycolipids, and glycoproteins play important roles in various biological phenomena.[1] It became clear, for instance, that the attachment of oligosaccharides to a protein surface is effective in protein folding and protein stability in many cases. It is also reported that a saccharide−protein recognition or sugar−sugar interaction may be crucial in cell adhesion processes and/or intercellular communication. Because these events are essential for the precise maintenance of diverse biological systems, it is now reasonably considered that the disorder in processes involving natural saccharides results in many diseases such as diabetes, tumors, and viral and/or bacterial infection (AIDS, influenza, E-coli O-157, etc.).[2] Therefore, the rapid and high-throughput analysis of various glycoconjugates as biomarkers in various events is keenly desirable not only for basic science advancement but also for diagnostic applications.[3]

In recent years, various types of sugar chips, which immobilized carbohydrates on a solid substrate, have been actively developed by many researchers to detect or estimate sugar−protein interactions, to assay the activity of the glycosylation-related enzymes or proteins, and so on.[4] In contrast to the significant progress in such sugar chips, the array tools suitable for sensing the complicated saccharide-conjugates have been poorly developed. To date, there are only three examples of arrays to detect saccharides, all of which use lectin,[5] a naturally occurring saccharide binding protein.[6] By use of the lectin array,

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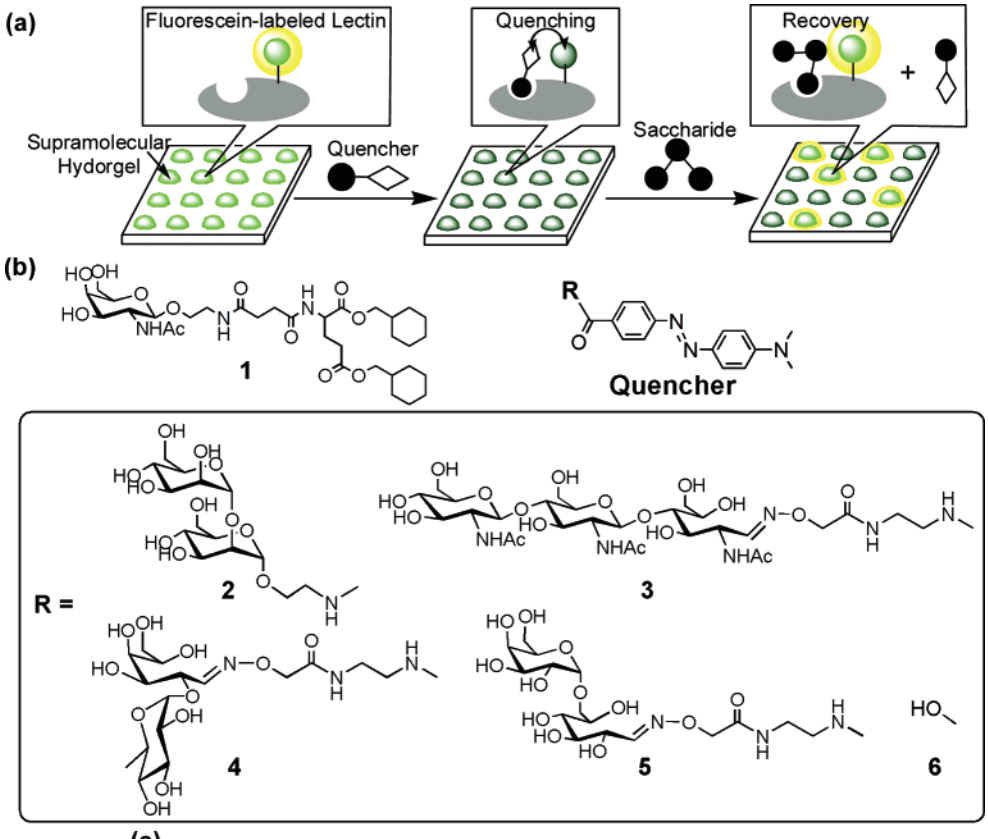

(c)

Lectin	Carbohydrate Specificity	Suitable Quencher
Con A	α-Man,α-Glc	2
WGA	β-GlcNAc, α2,3-NeuNAc	3
GSL-II	α,β-GlcNAc	
AAL	Fuc	4
UEA-I	α1,2-Fuc	
GSL-I	α–Gal,GalNAc	5

Figure 1. (a) Schematic illustration of BFQR fluorescent detection system on hydrogel chip. (b) Molecular structures of gelator **1** and quenchers **2**−**6**. (c) Carbohydrate specificities and suitable quenchers of fluorescein-labeled lectins.

Mahal's group succeeded in analysis of the bacterial glycans, Hirabayashi's group detected glycoproteins, and Smith's group analyzed the sugar chains of mammalian cell surface. Especially, in Hirabayashi's system using evanescent field fluorescence, there is no need to wash the array before detection. In these reports, however, the lectins must be covalently attached to the solid substrate by complicated manners. In addition, the labeling of all of the samples with fluorescent probes is inevitably required prior to the analysis, or the analyzable sample is limited to the cell lines in the other report due to the use of a special reading-out technique.

We describe herein a unique fluorescent lectin array using a supramolecular hydrogel matrix. We have noncovalently fixed fluorescent lectins in a hydrogel array.[7,8] Under the semi-wet conditions provided by the supramolecular hydrogel, protein denaturation was effectively suppressed so that the embedded lectin acts as a talented molecular recognition scaffold toward specific saccharides. More importantly, it is demonstrated that a bimolecular fluorescence quenching and recovery (BFQR) method, which is conventionally used in an aqueous solution system, was successfully operated under the semi-wet conditions. This enables one to fluorescently read-out a series of saccharides using the recognition selectivity and affinity of the immobilized lectin without labeling of the target saccharides. Simple and high-throughput sensing and profiling have been carried out using the lectin array for diverse glycoconjugates, not only a simple glucose, but also oligosaccharides, glycoproteins, and cell lysates.

Results and Discussion

Bimolecular Fluorescence Quenching/Recovery Method (BFQR) Useful for Reading-Out Saccharides Both in Aqueous Solution and in Supramolecular Hydrogel. With the aim of constructing a lectin chip using a supramolecular hydrogel, we initially confirmed a lectin-based fluorescence read-out

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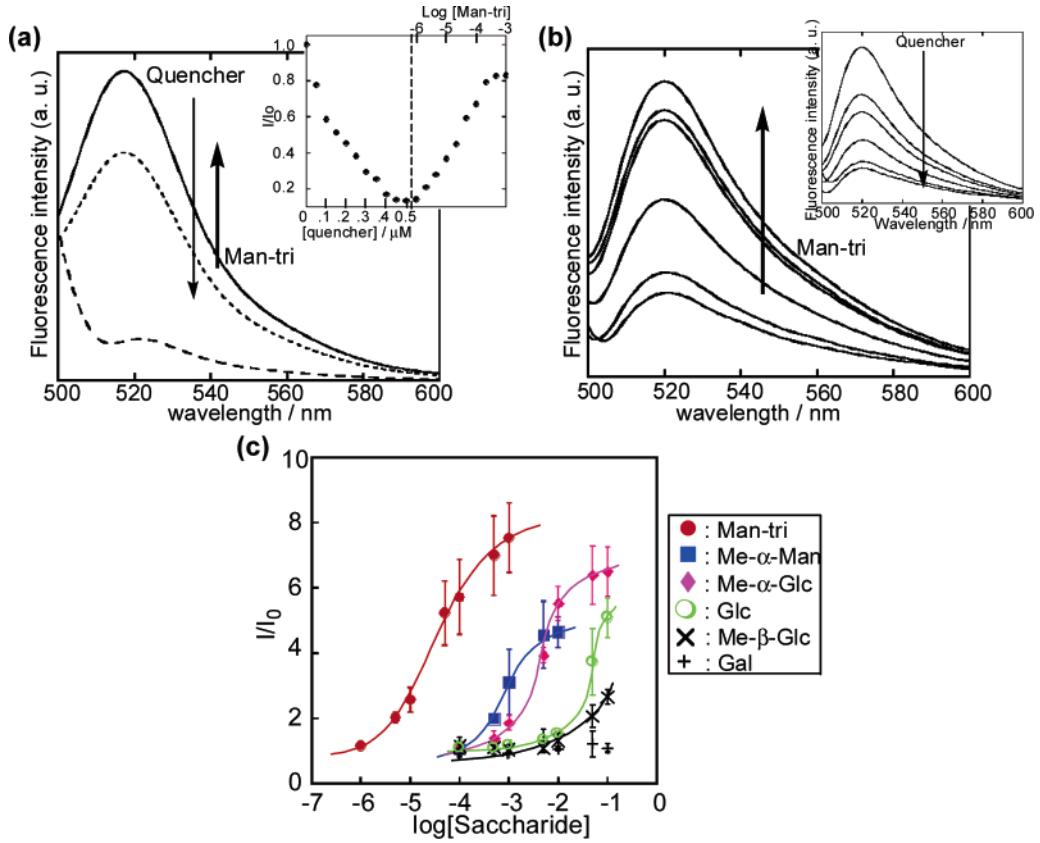

Figure 2. (a) Fluorescent spectra of F-Con A in the absence (solid line) or presence of **2** (0.5 μM, broken line) in aqueous solution. The dotted line shows the Man-tri induced fluorescence recovery of F-Con A in the presence of **2**. Inset: Titration curve with **2** and then with Man-tri. [F-Con A] = 0.1 μM, **2** (0−0.5 μM), Man-tri (0−1 mM) in 50 mM HEPES buffer (pH 7.5, containing 1 mM $MnCl_2$, 1 mM $CaCl_2$, and 0.1 M NaCl), 20 °C, λ_{ex} = 488 nm. (b) Fluorescence recovery of F-Con A immobilized in the hydrogel matrix by the addition of Man-tri (0−1 mM). Inset: Fluorescence quenching process of F-Con A upon addition of **2** (0−10 μM). [F-Con A] = 1 μM, 50 mM HEPES buffer (pH 7.5, containing 1 mM $MnCl_2$, 1 mM $CaCl_2$, and 0.1 M NaCl), in 0.25 wt % of **1**, λ_{ex} = 475 nm. (c) Fluorescence titration plots of the relative intensity (I/I_0) of F-Con A versus the saccharide concentration (log[saccharide]) in hydrogel **1** (0.25 wt %): F-Con A (0.5 μM), **2** (5 μM), Man-tri (●), Me-α-Man (■), Me-α-Glc (◆), Glc (○), Me-β-Glc (×), Gal (+). The error bar in the graph represents the standard deviation of three independent measurements.

method for a saccharide in aqueous solution,[9] which is a bimolecular fluorescence quenching/recovery (BFQR, Figure 1a) method including the fluorescein-labeled concanavalin A (F-Con A, a mannose and glucose binding lectin) and Man-2-appended dabcyl quencher (**2**, Figure 1b). As shown in Figure 2a, the fluorescence intensity of fluorescein at 516 nm significantly decreased by the addition of quencher **2** and recovered by Man-tri, the strongest ligand for Con A. The inset shows the fluorescence titration curve of F-Con A depending on the concentration of **2** or Man-tri. The effective quenching did not occur using dabcylic acid **6**, a quencher lacking the Man-2 unit, and the quenched fluorescence did not recover by inappropriate sugars such as galactose (Gal), which does not bind Con A. These results indicate that the present fluorescence quenching and recovery can be ascribed to the binding of **2** and its replacement by Man-tri, respectively, in the sugar binding pocket of Con A.

Subsequently, we applied the present BFQR method to the sugar detection in a hydrogel system comprised of a supramolecular hydrogelator **1**, which spontaneously formed a transparent hydrogel without any polymerization reagents/treatment.[10] As was recently reported by us, many proteins and enzymes can be immobilized in internal aqueous phases of the hydrogel matrix while retaining the natural activities that are shown in an aqueous solution. After the immobilization of Con A in the supramolecular hydrogel **1** on the 10 μL scale, quencher **2** was added to the microgel. The inset of Figure 2b shows that the fluorescence peak at 520 nm gradually decreased and leveled off by the increase in **2**. The reduced fluorescence then significantly recovered by the addition of Man-tri (Figure 2b), the behavior of which is perfectly identical to that observed in the aqueous solution. Figure 2c shows the titration curves of various saccharides monitored by the fluorescence recovery process using the Con A chip in the presence of quencher **2**. It is clear that the saccharides, which have an affinity to Con A, cause the fluorescence recovery in a manner similar to Man-tri, whereas Gal and Me-β-Glc, the sugars having no affinity to Con A, do not induce any significant fluorescence change even at a 100 mM concentration. The sensing selectivity is in the order of Man-tri > Me-α-Man > Me-α-Glc > Glc ≫ Gal, Me-β-Glc, which is in good agreement with the selectivity of the native Con A in aqueous solution.[6b] Although the minimum

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(10) The present β-GalNAc type of gelator does not interfere with the recognition of GSL-I, α-GalNAc binding lectin (see the evaluated binding constant in the Supporting Information). Because many gelators bearing various saccharide headgroups were developed in our group, it is possible to select a suitable gelator if such an interference was observed.[7d]

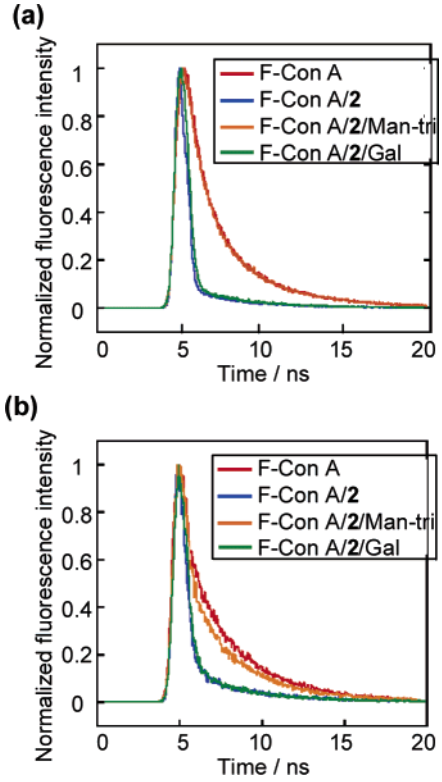

Figure 3. Fluorescent lifetime study of F-Con A in aqueous solution (a) and in hydogel **1** matrix (b). Time-dependent fluorescence decay curve of F-Con A (0.5 μM) was recorded from 500 to 540 nm after laser excitation at 337 nm in the absence (red line) or presence of **2** (5 μM, blue line). The decay of F-Con A was also measured in the presence of **2** and Man-tri (1 mM, orange line), or in the presence of **2** and Gal (1 mM, green line).

sensing concentrations are slightly higher than those of the native Con A due the competitive assay of the present BFQR method, the order in sensing agrees well with that evaluated in the aqueous system. The saturation curves also provided the apparent binding constants, by which we can evaluate the net binding constants for each saccharide as follows: 7.0×10^4 M^{-1} for Man-tri, 1.6×10^3 M^{-1} for Me-α-Man, 4.7×10^2 M^{-1} for Me-α-Glc, and less than 10 M^{-1} for Gal.[11] These values are almost identical to the values reported in the aqueous system, demonstrating that the original function of the native Con A is retained in the supramolecular hydrogel matrix.

Fluorescence Lifetime Study for BFQR Method. The BFQR events were also confirmed by the fluorescence lifetime measurement.[12] In the absence of quencher **2**, the decay curve of F-Con A in the hydrogel as shown in Figure 3b gave the fluorescence lifetime of 3.3 ns, the value of which is almost identical to that observed in aqueous solution (3.2 ns in Figure 3a). This suggests that the fluorescein moiety of F-Con A embedded in the hydrogel is located in almost the same microenvironment as in the aqueous solution. The lifetime was shortened to 0.4 ns by the addition of quencher **2** and then subsequently returned to 3.1 ns by the further addition of Man-tri, but not by the addition of Gal (0.4 ns). These are reasonably ascribed to the **2**-complexation-induced quenching of the

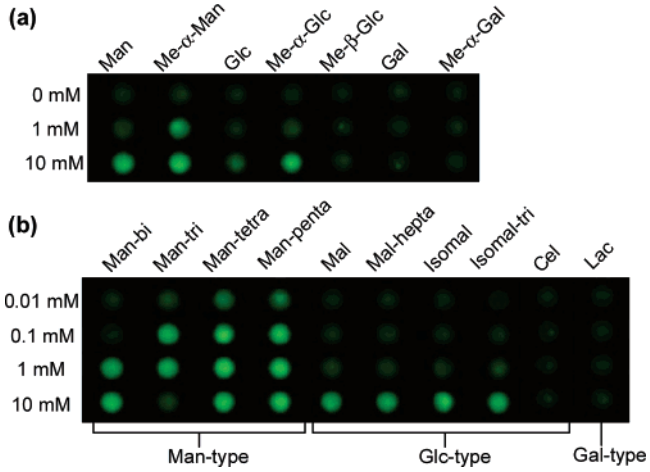

Figure 4. Fluorescence image of the Con A chip based on supramolecular hydrogel upon addition of (a) monosaccharides and (b) oligosaccharides. [F-Con A] = 0.1 μM, [quencher **2**] = 1 μM.

fluorescein of F-Con A and the fluorescence recovery by the replacement with Man-tri on the sugar-binding pocket of F-Con A, respectively. Such behaviors are again similar to those in aqueous solution (0.4 ns (in the presence of **2**), 3.2 ns (in the presence of **2** and Man-tri)). Therefore, it is concluded that the BFQR type of fluorescence read-out method, which has been usually used in an aqueous solution, can successfully operate under the semi-wet supramolecular hydrogel conditions.

Visualization of Glucose and Mannose Derivatives Using a Semi-wet Con A Chip. Because of the fact that the fluorescence intensity change in the present microgel is clear enough to be distinguished by the naked eye, a semi-wet Con A chip can be used for the rapid estimation of the sugar selectivity of lectin as well as for the convenient detection of a family of saccharides. Figure 4a displays a fluorescence image of the Con A array containing seven distinct monosaccharides at different concentrations (0−10 mM). According to the binding affinity of the native Con A, a strong green fluorescence appeared at 1 mM Me-α-Man, and at 10 mM Man or Me-α-Glc. Glc was also weakly detected at spots containing 10 mM. In a series of oligosaccharides, on the other hand (Figure 4b), the α-linked oligomannosides were more sensitively detected in the 1 mM concentration range for Man-bi, the 0.1 mM range for Man-tri,[13] and 0.01 mM for Man-tetra and Man-penta monitored by the fluorescence recovery image. In contrast, the glucose type of oligosaccharides is less sensitively detected (more than 1 mM concentration range) due to the lower affinity to the native Con A. Neither the β-linked saccharides, such as Me-β-Glc and cellobiose, nor the galactose-terminated derivatives, such as Me-α-Gal and lactose, are sensed by this Con A chip. These results imply that the saccharide selectivity of a lectin was rapidly and conveniently evaluated using the present semi-wet lectin chip.

As proof of the principle experiment to demonstrate the practical utility of the lectin chip, we next conducted the fluorocolorimetric imaging of glucose (Glc), an important analyte for the diagnosis of diabetes.[14] To produce a clear

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(13) We confirmed that the abnormal quenching of F-Con A reproducibly occurred only at the high concentration (10 mM) of Man-tri not only in gel spots, but also in aqueous solution. However, the fluorescence recovery up to 1 mM of Man-tri is reasonable. This is probably due to a small amount of impurity included in the commercialized Man-tri sample.

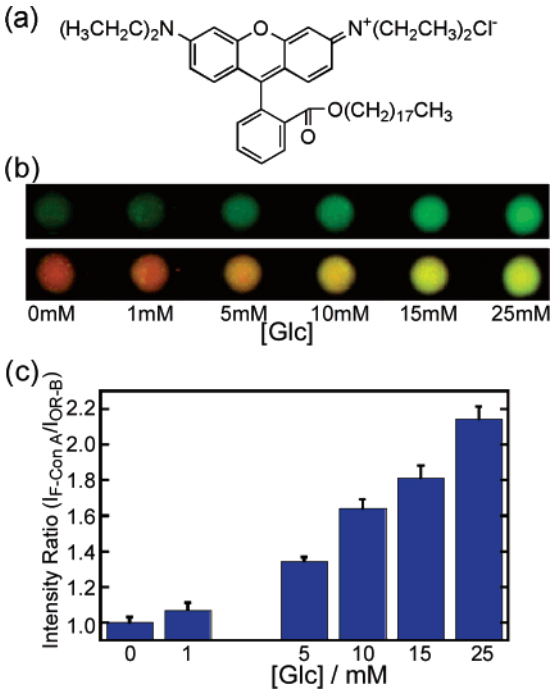

Figure 5. (a) Molecular structure of octadecyl rhodamine B chloride (OR-B) for staining a hydrogel fiber. (b) Clear image of glucose using the Con A chip containing OR-B. Top: Fluorescent image of F-Con A for various concentrations of glucose by fluorescein excitation. Bottom: Fluorescent merged image by fluorescein (green) excitation and by OR-B (red) excitation. (c) The graph of fluorescent ratio (F-Con A/OR-B) by the addition of various concentration of glucose. [F-Con A] = 0.1 μM, [quencher **2**] = 0.5 μM. The bar height and the error bar in the graph were estimated by the average and standard deviation of four spots on the same plate.

fluorescent color change, the present semi-wet Con A chip is equipped with a ratiometric mode. According to our previous finding, the present hydrogel matrix possesses many hydrophobic microfibers that can incorporate a hydrophobic fluorescent probe. On the basis of this unique feature, we designed a method to place octadesyl-rhodamin B (OR-B, Figure 5a), an additional fluorophore, in the hydrophobic fiber domain of the supramolecular hydrogel **1**. Because the BFQR event occurred in the aqueous cavity of the hydrogel, the fluorescence of the OR-B localized in the hydrophobic domain should be practically insensitive to the sugar binding of F-Con A, so as to become an internal standard in this system. When the Glc solution was added to the Con A chip containing both quencher **2** and OR-B, the intensity ratio of Fl over OR-B (Fl/OR-B) changed depending on the Glc concentration as shown in Figure 5c. It is known that the change in the Glc concentration in the range from 5 to 20 mM is critical for the diabetes diagnosis. In such a concentration range, it is clear that the ratio value (Fl/OR-B) linearly increased and, more interestingly, the fluorescent color change of the ratiometric Con A chip was clearly detected from reddish orange to yellowish green (the second lane of Figure 5b), in contrast to the case of the Con A chip using the simple intensity mode (the first lane of Figure 5b).

Fluorescent Detection and Profiling of Glycoconjugates Using Lectin Array. The BFQR method is so general that it can be applied to other fluorescein-tethered lectins showing distinct saccharide selectivities in the presence of a pair of corresponding sugar-appended dabcyl quenchers as shown in Figure 1b. In these five lectins (WGA, GSL-II, AAL, UEA-I, and GSL-I; the sugar selectivity is shown in Figure 1c), we confirmed that the quenching and recovery processes take place both in aqueous solution and in the hydrogel matrix (Figures s-1,2). Subsequently, six kinds of fluorescent lectins were arrayed on a glass plate to produce a lectin array as shown in Figure 6a. Without sugars, only a weak emission was detected from all of the gel spots in the presence of the corresponding quenchers (Figure 6b). By the addition of Me-α-mannose (Me-α-Man) to these spots, four spots containing Con A strongly emitted a green fluorescence, whereas the other spots containing other lectins having no affinity to Man remain dark. In the case of the addition of the *N*,*N*′-diacetyl chitobiose (NAG-2), the emission increased in the eight spots containing WGA or GSL-II, both of which display the affinity to the GlcNAc family. Similarly, the fucose addition induced the recovered emission at spots containing AAL and UEA-I, and melibiose did it at the spots of GSL-I.

Not only the simple saccharides, but also the branched sugar structures tethered to a glycoprotein surface can be roughly characterized by this lectin array. As shown in Figure 7a, the addition of ribonuclease B (Ribo B), which has a high mannoside branch (Man-5−8), induced the strong emission at the spots of Con A,[15a] and the Fetuin addition intensified the emission at the four spots of WGA due to the tethered sialic acid moiety.[15b] Unlike ribonuclease B and Fetuin, the fucose-appended Mucin caused the emission intensification at the spots of AAL and UEA-1,[15c] and ovalbumin having the terminal GlcNAc unit intensified the emission at the WGA and GSL-II spots.[15d] In sharp contrast, there are no considerable emission changes observed in the case of the addition of BSA undergoing no saccharides decoration.[16] Therefore, the comparison of the bar graph pattern for the emission recovery by the distinct glycoproteins (Figure 7b) clearly shows the difference among the types of glycoproteins on the basis of the structural difference of the attached saccharides.

For the more complicated analytes, cell lysates originating from different cell lines were subsequently profiled using the present lectin array. Figure 8a shows two typical emission images for the arrays analyzing the HepG2 human cell or NM522 bacteria. In the case of HepG2, a strong emission was observed at the spots of WGA and AAL.[18] The strong response in WGA spots (but not in GSL-II) can be reasonably explained by the existence of sialic acid derivatives on many animal cell surfaces.[19a] The strong response of AAL is consistent with the established profile that there are several kinds of fucosylated sugar chains such as N-linked and Lewis type saccharides in the higher organisms.[19b] In contrast, the AAL spots did not respond to the addition of NM522, but, instead, the emission

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(16) The response to the high concentration (40 μg/μL) of BSA is probably ascribed to nonspecific interactions of F-lectins with a hydrophobic pocket of BSA. Such nonspecific interactions are not negligible especially for the case of WGA, but not for other lectins. Thus, these data suggested that the considerable positive response was an over 10% recovery ratio for lectins other than WGA and over 20% for WGA in the glycoprotein analysis.

(17) Serious variability of the recovery ratio between spots on the same plate was not observed (see Figure s-4). Similarly, it is shown that the response patterns were practically conserved between independent arrays (see Figure s-5).

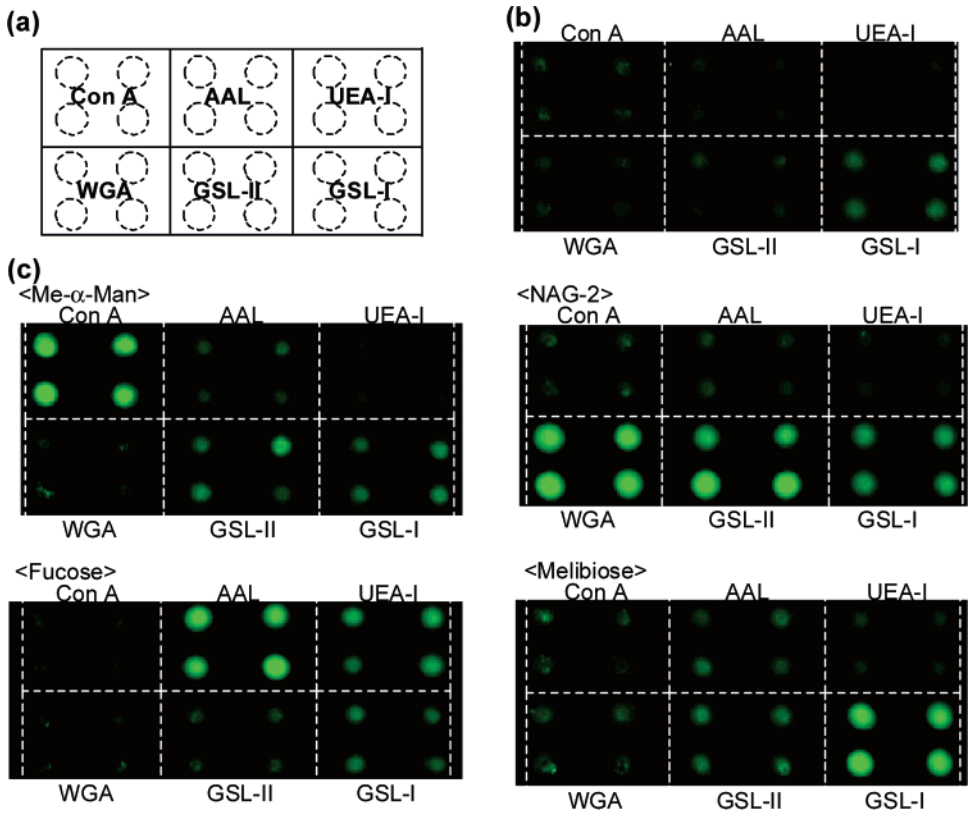

Figure 6. Mono- or oligosaccharides detection by lectin array. (a) Spatial position of six kinds of lectins on hydrogel chip. One lectin was arrayed at four spots, so that six lectins were arrayed in 24 spots. (b) Fluorescent image of the lectin array before the addition of analyte. (c) Fluorescent images after the addition of saccharides (Me-α-Man, NAG-2, fucose, melibiose (10 mM)): [F-Con A] = 0.1 μM, [quencher **2**] = 1 μM; [F-WGA] = 0.1 μM, [quencher **3**] = 2 μM; [F-GSL-II] = 0.1 μM, [quencher **3**] = 30 μM; [F-AAL] = 0.1 μM, [quencher **4**] = 1 μM; [F-UEA-I] = 0.1 μM, [quencher **4**] = 10 μM; [F-GSL-I] = 0.1 μM, [quencher **5**] = 30 μM.

intensification was induced at the GSL-II spots, suggesting that there are many GlcNAc units in this cell lysate. This is probably attributed to the fact that GlcNAc is the major component of the peptide-glycans located on the bacterial cell wall. From the bar graph for the eight different cell lines in Figure 8b, it is clear that the response pattern using the present lectin array is roughly classified into two categories, that is, the cell lines from the mammalian species and the cells from bacteria, by the main difference in the AAL and GSL-II spots. This is supported by the cluster analysis of these intensity-based data as shown in Figure 8c and d.[20] Among the mammalian cell lines, a subtle difference in the pattern was further observed; that is, MCF-7 cell showed the predominant response at AAL. It was supported by the previous report that there are richer fucosylated O-linked sugar chains in MCF-7 than in other cells.[21a] This pattern is apparently distinct from the response from the other five cell lines (L-6, HepG2, CHO, A549, and HeLa) and may be due to the major presence of the fucose component in MCF-7. In more detail, the cluster analysis showed us that the L-6 is secondarily distinguished from the other four, due to the enhanced presence of α-galactose (GSL-I) as well as fucose (AAL) and sialic acid (WGA). This was consistent with the histological findings that the skeletal muscle cells such as L6 cell are effectively stained by GSL-I.[21b] Thus, it is clear that the response pattern of cell lysates obtained by using this lectin chip is in good agreement with previous general findings and reports. However, the more subtle differences in other responses are not clearly assigned so far, because the high-throughput and systematic analysis for glycoconjugates has been poorly accomplished. On the other hand, a significant similarity between NM522 and JM109, both of which are bacterial strains, is observed in the cluster analysis of the response pattern. These results demonstrated that the present semi-wet lectin array is useful for profiling the cell lines on the basis of the characteristic difference in the sugar components of each cell. The more precise and fine profiling of the various cell lines can be envisaged by increasing the number and variety of the immobilized lectins and other binding scaffolds in this semi-wet hydrogel matrix.

Conclusion

In summary, the successful transfer of a BFQR method from a solution system to a semi-wet supramolecular hydrogel system

(18) WGA is able to bind both the β-GlcNAc derivatives and the NeuNAc (sialic acid) derivatives as shown in Figure 1c. In contrast, GSL-II can bind only GlcNAc derivatives, but not NeuNAc derivatives. As shown in Figure 8, the mammalian cell lines responded at the WGA spots, but not at the GSL-II spots. Thus, it is reasonable to conclude that these cell lines include NeuNAc derivatives in greater amount than GlcNAc. In the matter of AAL, the great difference between the AAL response and the UEA-I response is probably due to the difference in the affinity of AAL and UEA-I for fucose. As shown in Figure s-2, AAL shows an affinity to fucose that is stronger by 10-fold than UEA-I. Based on these data, the amount of the fucosylated species may be roughly speculated.

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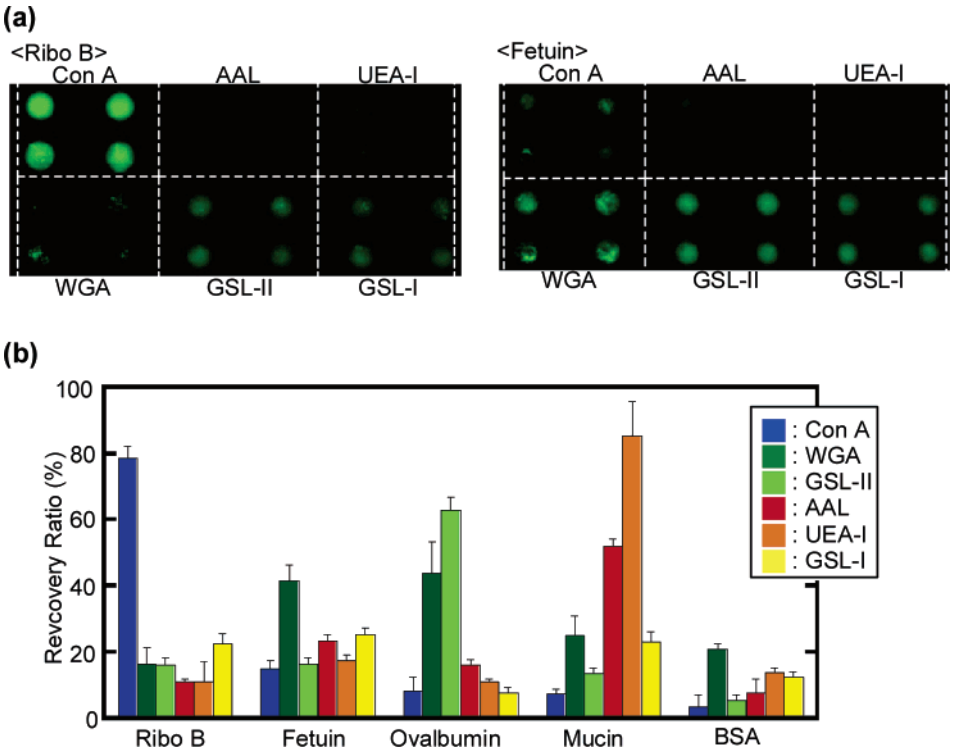

Figure 7. Pattern detection of glycoproteins by lectin chip. (a) Fluorescent images of lectin chip for Ribo B, a high mannose-branched protein, and Fetuin, a sialylated N-type glycan branched protein. (b) The graph of fluorescent recovery ratio by the addition of four different glycoproteins (Ribo B, Fetuin, Ovalbumin, Mucin) and no glycosylated protein (BSA): 40 μg/μL, Con A (blue), WGA (green), GSL-II (yellow-green), AAL (red), UEA-I (orange), GSL-I (yellow). The bar height and the error bar in the graph were estimated by the average and standard deviation of four spots on the same plate.[17]

can afford a unique lectin array that can fluorescently respond to the types and concentration of the sugars, by which a high-throughput and convenient sensing of various saccharide derivatives were carried out. In addition to an advantage that tedious washing processes are not necessary in this method, we do not need any labeling of the target glycoconjugates including not only monosaccharides such as glucose, but also oligosaccharides, glycoproteins, and even cell lysates, based on this single detection mechanism. It is also advantageous that the detection sensitivity can be tuned by the concentration of the quencher.[22] However, there are several disadvantages to this method. For example, the detection limit is suppressed because of the competitive quencher.[23] Furthermore, synthesis of the corresponding quenchers for each lectin and the subsequent optimization of the lectin/quencher pair are required for extending the arrayed lectins.[24] Although a perfect 1-to-1 type of discrimination is always ideal in analyzing a target molecule, it is difficult for the recognition of structurally complicated and diverse biological glycoconjugates. In such cases, we believe that the pattern recognition and profiling are valuable.[25] The present lectin array is very promising for many objectives, particularly for the throughput analysis of all glycoconjugates in various cell lines and organisms.

Experimental Section

General Comments. Gelator **1** was prepared according to the method reported previously by us.[7d] Quencher **2** was synthesized as shown in Scheme 1a, and quenchers **3**−**5** were synthesized as shown in Scheme 1b. Unless otherwise stated, all of the air- or moisture-sensitive reactions were performed under nitrogen atmosphere and using distilled solvents such as DMF, dichloromethane, and methanol.

Fluorescein-labeled lectins were purchased from the Funakoshi or Seikagaku Corp. and dialyzed by 50 mM HEPES buffer (pH 7.5, containing 1 mM $MnCl_2$, 1 mM $CaCl_2$, and 0.1 M NaCl) for 1 day before usage. Quenchers were dissolved in methanol and diluted by distilled water. The molar absorbance coefficients of F-lectins ($\epsilon_{494\ nm}$ = 68 000 (pH 7.5)) and quenchers ($\epsilon_{428\ nm}$ = 30 000 (in MeOH)) were calculated by the coefficient of FITC and dabcyl group,[26] and the concentration was determined via a UV−visible spectrometer (Shimazu UV-2550).

Quencher 2. Peracetyl-α-1-2-mannobiose (**7**) was synthesized by treating α-1-2-mannobiose with pyridine and acetic anhydride. Peracetyl-α-1-2-mannobiose (**7**) was treated with acetic acid and piperidine to remove the *O*-acetyl group at position 1. The crude hemiacetal was then transformed into the α-trichloroacetimidate by treatment with trichloroacetonitrile and cesium carbonate, followed by the glycosylation with 2-bromoethanol in the presence of BF_3–OEt_2. The 2-bromoethyl mannobioside (**8**), thus obtained, was treated with sodium azide, followed by hydrogenolysis of the azide group, to give the 2-aminoethyl

(22) The detection sensitivity is adjustable by modulating the quencher concentration. As a typical example, Table s-1 shows that we can tune the detection sensitivity of glucose from 5 to 24 mM.
(23) The detection limit for a monosaccharide (glucose) was 1 mM; a glycoprotein (Ribo B) was 0.5 μg/μL (30 μM). The typical pattern for a bacteria (NM522) lysate was detected at 0.5 μg/μL (Figure s-6).
(24) These may not be so problematic, because the corresponding quenchers were easily synthesized by the reaction of **13** with various saccharides, which have a reducing terminal using the aminooxy-method (Scheme 1b), and the pair can be universally used for various analytes once it is optimized.
(25) (a) Lavigne, J. L.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2001**, *40*, 3118. (b) Wright, A. T.; Griffin, M. J.; Zhong, Z.; McCleskey, S. C.; Anslyn, E. V.; McDevitt, J. T. *Angew. Chem., Int. Ed.* **2005**, *44*, 6375. (c) Zhang, C.; Suslick, K. S. *J. Am. Chem. Soc.* **2005**, *127*, 11548. (d) Zhou, H.; Baldini, L.; Hong, J.; Wilson, A. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **2006**, *128*, 2421. (e) Baldini, L.; Wilson, A. J.; Hong, J.; Hamilton, A. D. *J. Am. Chem. Soc.* **2004**, *126*, 5656. (f) Buryak, A.; Severin, K. *J. Am. Chem. Soc.* **2005**, *127*, 3700.
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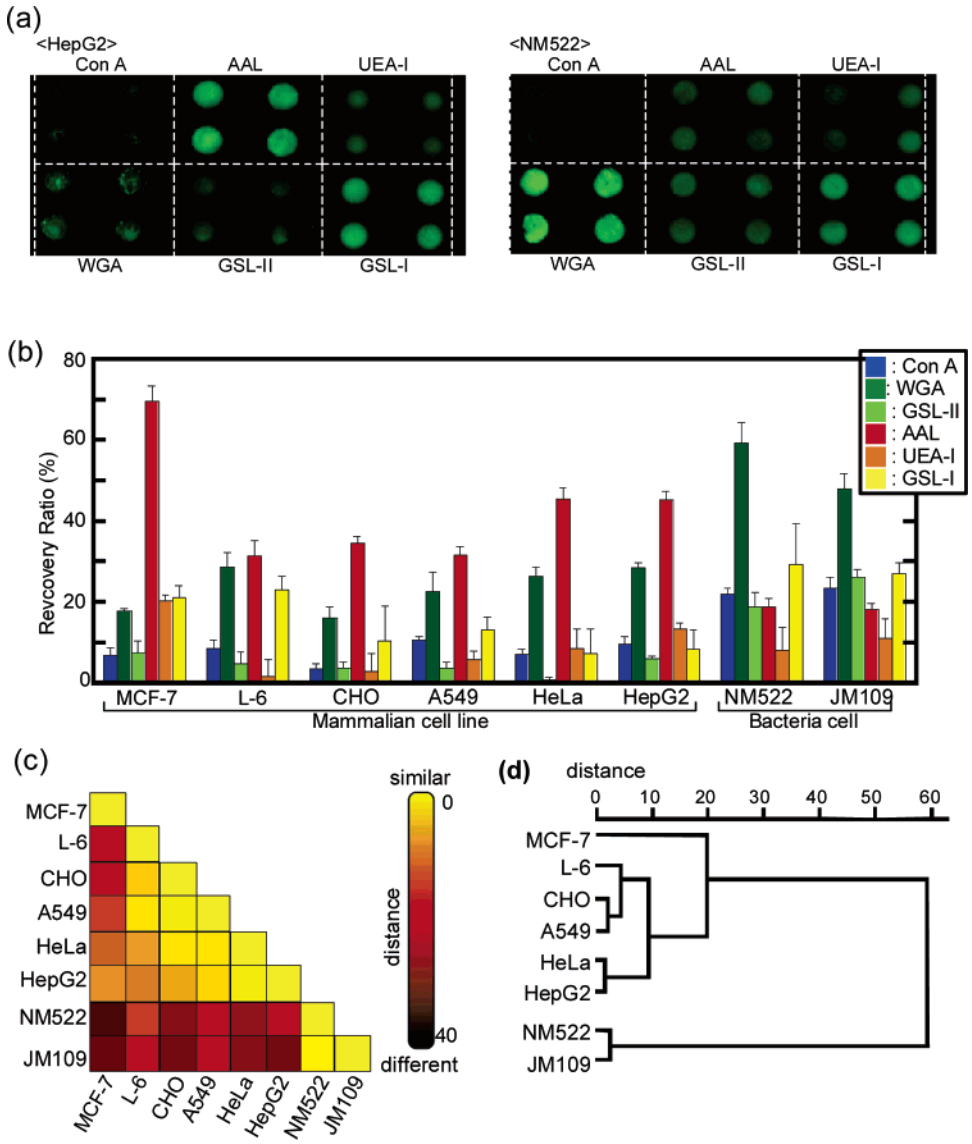


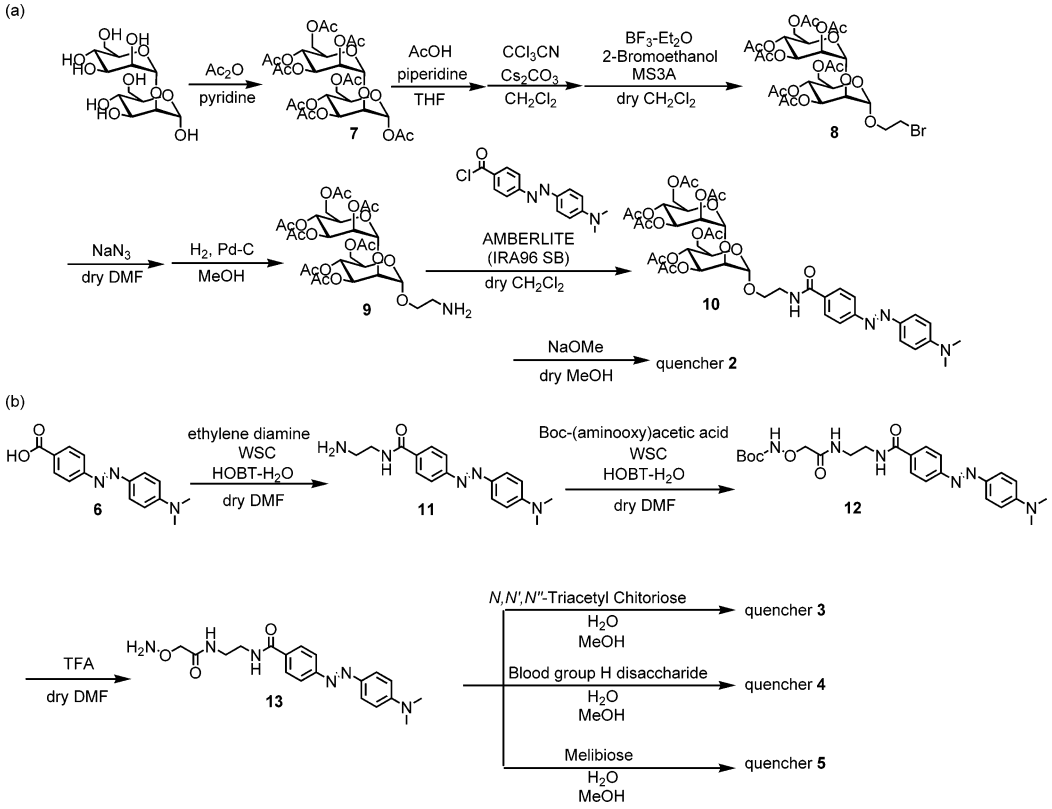
Figure 8. Pattern detection of carbohydrates in cell lysates by lectin chip. (a) Typical fluorescent images for lectin chip analyzing human cell line (HepG2) and bacteria cell (NM522). (b) The bar graph of the fluorescent recovery ratio by the addition of mammalian cell lines (MCF-7, L-6, CHO, A549, HeLa, HepG2: 10 000 cells/μL) and bacteria cells (NM522, JM109: 10 μg/μL by wet weight). The bar height and the error bar in the graph were estimated by the average and standard deviation of four spots on the same plate.[17] (c) The Euclidean distance matrix between the patterns of the different cell lines obtained by the lectin chip was represented by color coding (yellow for the highest similarity and black for the lowest). (d) The dendrogram of the response patterns for eight cell lines generated by the analysis of Euclidean distances. The horizontal axis represents the distances among the normalized lectin chip patterns (left for patterns with the highest similarity and right for patterns with the lowest similarity).

mannnobioside (**9**). The peracetyl form (**10**) was synthesized by condensation of 2-aminoethyl mannnobioside (**9**) with dabcylic acid chloride. Quencher **2** was obtained by deacylation of the peracetyl form (**10**) with NaOMe. ^{1}H NMR (400 MHz, CD_3OD), δ/ppm: 3.10 (s, 6H, N$-(CH_3)_2$), 3.56 (d, J_H = 4.4 Hz, 2H, $-CH_2-$), 3.58−3.98 (m, 14H, H-2,3,4,5,6, H′-2,3,4,5,6, $-CH_2-$), 4.97 (d, J_H = 1.6 Hz, 1H, H′-1), 5.16 (d, J_H = 1.2 Hz, 1H, H-1), 6.82 (d, J_H = 9.2 Hz, 2H, Ar−H), 7.83−7.86 (m, 4H, Ar−H), 7.95 (d, J_H = 9.2 Hz, 2H, Ar−H). HRMS (FAB) calcd for [M + Na^+] ($C_{29}H_{40}N_4O_{12}Na$), 659.2540; found, 659.2545. Anal. Calcd for peracetyl form **13** ($C_{43}H_{54}N_4O_{19}$): C, 55.48; H, 5.85; N, 6.02. Found: C, 55.88; H, 5.86; N, 6.12.

Quencher 3. 2-Aminoethyl dabcyl (**11**) was synthesized by the condensation of dabcylic acid (**6**) with ethylenediamine. 2-Aminoethyl dabcyl (**11**) was then condensed with Boc-(aminooxy) acetic acid using WSC, and the Boc group was deprotected by TFA. The treatment of 2-aminooxy ethyl dabcyl (**13**) with *N*,*N*′,*N*″-triacetyl chitriose yielded quencher **3**. Purification was conducted by HPLC (column; YMC-pack ODS-A, 250 × 10 mm). The HPLC conditions were as follows: CH_3CN/H_2O (containing 10 mM $(NH_4)HCO_3$) = 5/95−65/35 (linear gradient over 60 min), flow rate = 3 mL/min, detection by UV (432 nm). ^{1}H NMR (400 MHz, CD_3OD), δ/ppm: 1.94−2.00 (m, 9H, −NHAc), 3.11 (s, 6H, N$-(CH_3)_2$), 3.38−3.87 (m, 21H, H-3,4,5,6, H′-2,3,4,5,6, H″-2,3,4,5,6, $-CH_2-CH_2-$), 4.43 (d, J_H = 8.8, 1H, H″-1), 4,47 (s, 2H, $-CH_2-$), 4.53 (d, J_H = 8.8, 1H, H′-1), 4.92 (t, J_H = 6.0, 1H, H-2), 6.81 (d, J_H = 9.2, 2H, Ar−H), 7.65 (d, J_H = 5.6, 1H, H-1), 7.83−7.86 (m, 4H, Ar−H), 7.95 (d, J_H = 8.8 Hz, 2H, Ar−H). HRMS (FAB) calcd for [M + H^+] ($C_{43}H_{64}N_9O_{18}$), 994.4369; found, 994.4369.

Quencher 4. Quencher **4** was obtained in the same method as quencher **3** using 2-aminooxy ethyl dabcyl (**13**) and Blood Group H disaccharide (Fuc-α-1,2-Gal), and purified by HPLC (column; YMC-pack ODS-A, 250 × 20 mm). The HPLC conditions were as follows: CH_3CN/H_2O (containing 10 mM $(NH_4)HCO_3$) = 25/75−50/50 (linear gradient over 20 min), flow rate = 9 mL/min, detection by UV (432 nm). ^{1}H NMR (400 MHz, CD_3OD), δ/ppm: 1.16 (s, 3H, CH_3), 3.08 (s, 6H, N$-(CH_3)_2$), 3.47−4.05 (m, 13H, H-3,4,5,6, H′-2,3,4,5, $-CH_2-$-

Scheme 1. Synthetic Scheme of (a) Quencher **2** or (b) Quenchers **3**−**5**



CH_2-), 4.51 (s, 2H, $-CH_2-$), 4.63 (t, $J_H = 7.2$ Hz, 1H, H-2), 4.95 (d, $J_H = 9.2$ Hz, 2H, H′-1), 6.83 (d, $J_H = 9.2$ Hz, 2H, Ar−H), 7.72 (d, $J_H = 7.6$ Hz, 1H, H-1), 7.83−7.86 (m, 4H, Ar−H), 7.93 (d, $J_H = 8.8$ Hz, 2H, Ar−H). HRMS (FAB) calcd for $[M + H^+]$ ($C_{31}H_{45}N_6O_{12}$), 693.3095; found, 693.3091.

Quencher 5. Quencher **5** was obtained in the same method as quencher **3** using 2-aminooxy ethyl dabcyl (**13**) and melibiose (Gal-α1,6-Gal), and purified by HPLC (column; YMC-pack ODS-A, 250 × 20 mm). The HPLC conditions were as follows: CH_3CN/H_2O (containing 10 mM $(NH_4)HCO_3$) = 5/95−35/65 (linear gradient over 40 min), flow rate = 9 mL/min, detection by UV (432 nm). ^{1}H NMR (400 MHz, CD_3OD), δ/ppm: 3.01 (s, 6H, $N-(CH_3)_2$), 3.38−3.87 (m, 15H, H-3,4,5,6, H-2,3,4,5,6, $-CH_2-CH_2-$), 4.22 (t, $J_H = 6.8$ Hz, 1H, H-2), 4.41 (s, 2H, $-CH_2-$), 4.45 (d, $J_H = 6.8$ Hz, 1H, H′-1), 6.75 (d, $J_H = 9.2$ Hz, 2H, Ar−H), 7.54 (d, $J_H = 6.8$ Hz, 1H, H-1), 7.74−7.77 (m, 4H, Ar−H), 7.84 (s, $J_H = 8.0$ Hz, 2H, Ar−H). HRMS (FAB) calcd for $[M + H^+]$ ($C_{31}H_{45}N_6O_{13}$), 709.3045; found, 709.3055.

Preparation of Lectin Chip. The gel array was prepared according to a slight modification of the method reported previously by us.[7a] A suspension of 0.25 wt % gelator **1** in 50 mM HEPES buffer (pH 7.5, containing 1 mM $MnCl_2$, 1 mM $CaCl_2$, and 0.1 M NaCl) was heated to form a homogeneous solution. A 10 μL portion of the hot solution was spotted on a glass plate and incubated to complete gel gelation in a sealed box under the high humidity at room temperature for 1 h. One microliter of the mixed solution of F-lectins and quenchers was then dropped onto each resultant hydrogel spot. After 30 min, an analyte solution was dropped onto each spot of lectin chip, and left for 1 h before the measurement.

Fluorescence Measurement. In aqueous solution, F-lectin solution was diluted to 0.1 μM by 50 mM HEPES buffer (pH 7.5, containing 1 mM $MnCl_2$, 1 mM $CaCl_2$, and 0.1 M NaCl) and measured via a fluorescence spectrometer (Perkin-Elmer LS55). The slit widths for the excitation and emission were set to 15 and 10 nm. The excitation wavelength was 488 nm. In hydrogel matrix, the fluorescent spectral change of F-lectins in hydrogel was traced via a multichannel photo detector (MCPD; Otsuka Electronics, excitation at 475 nm) at room temperature.

Fluorescence Lifetime Study. The fluorescence lifetime was measured with a fluorescence limited instrument (Hamamatsu photonics, streak camera, model C4334, optically coupled to a change-coupled-devaice (CCD) array detector). A 0.5 μM F-Con A in aqueous solution [50 mM HEPES buffer (pH 7.5, containing 1 mM $MnCl_2$, 1 mM $CaCl_2$, and 0.1 M NaCl)] or in hydrogel matrix (0.25 wt %, gelator **1**) as samples and a N_2 laser (337 nm) were used for excitation.

Fluorescence Imaging of Lectin Chip. The fluorescence images of the resulting lectin chip were collected by Molecular Imager FX pro (BioRad) under FITC-mode. In the case of saccharide and glycoprotein analysis, the stock solutions dissolved in distilled water were prepared by the measurement of the weight and added 1 μL or 2 μL to the lectin chip. Bacteria cells were cultured in LB medium according to general methods and destroyed by the ultrasonic homogenizer after washing by TES. Mammalian cells were cultured in DMEM (HepG2, L-6, A549), MEM (MCF-7, HeLa), or F12 (CHO) according to general methods and were destroyed by the ultrasonic homogenizer after washing by PBS. The resultant lysate was 3 μL added to the lectin chip without any modifications. This lectin chip was stably stored for a few days and produces an almost constant response pattern for at least 3 days. Furthermore, we can convey these chips by car without problem.

Data Analysis of Lectin Chip. The fluorescent recovery ratio was calculated by the fluorescence intensity obtained in the gel image of the corresponding lectin chip. The intensity ($I_{quenching}$) at the spots containing the quencher in the absence of analytes is set at 0%, and the intensity ($I_{recovery}$) at the spots in the presence of the large amount of corresponding saccharides (the saturated value of Me-α-Man for Con A, NAG2 for WGA and GSL-II, fucose for AAL and UEA-I, melibiose for GSL-I) is set at 100%. Using these two values, the data were normalized. The recovery ratio of the analysis spots was calculated

by comparing the obtained intensity ($I_{analyte}$) with these two values according to:

$$\text{ratio (\%)} = (I_{analyte} - I_{quenching})/(I_{recovery} - I_{quenching}) \times 100$$

Euclidean distance analyses were conducted according to a modification of the method previously reported.[20]

Fluorescent Merged Imaging of Con A Chip. In the preparation of the lectin chip containing OR-B, the OR-B stock solution was added to the heated gelator solution before spotting, and the other manipulation was the same as for the simple lectin chip. The fluorescence images were collected under FITC-mode and TAMRA-mode. The intensity ratio was calculated by the comparison with both fluorescent images.

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Supporting Information Available: Quenching and recovery process of F-lectins by BFQR event in aqueous solution or in hydrogel matrix, the calculation method of binding constants in hydrogel matrix, the fluorescence images of lectin chip on the addition of glycoproteins or cells, complete ref 4g, and molecular structures of saccharides used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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