Screening of a Glycopolymers Library of GM1 Mimics Containing Hydrophobic Units Using Surface Plasmon Resonance Imaging

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ABSTRACT: Effective screening methods for the development of glycopolymers as molecular recognition materials are desirable for the discovery of novel biofunctional materials. A glycopolymers library was prepared to obtain guidelines for the design of glycopolymers for the recognition of cholera toxin B subunits (CTB). Glycopolymers with varying ratios of hydrophobic and sugar units were synthesized by reversible addition fragmentation chain transfer polymerization. N-tert-Butylacrylamide, N-phenylacrylamide, and N-cyclohexylacrylamide as hydrophobic units were copolymerized in the polymer backbone, and galactose, which contributes to CTB recognition, was introduced into the side chains by "post-click" chemistry. The thiol-terminated glycopolymers were immobilized on a gold surface. The polymer immobilization substrate was analyzed in terms of interaction with galactose recognition proteins (CTB, peanut agglutinin, and *Ricinus communis* agglutinin I) using surface plasmon resonance imaging. The polymers with high ratios of sugar and hydrophobic units had the strongest interactions with the CTB, which was different from the trend with peanut agglutinin and *Ricinus communis* agglutinin I. The binding constant of the CTB with the glycopolymer with hydrophobic units was $4.1 \times 10^6 \text{ M}^{-1}$, which was approximately eight times larger than that of the polymer without hydrophobic units. A correlation was observed between the log $P$ value and the binding constant, indicating that the hydrophobic interaction played an important role in binding. New guidelines for the design of recognition materials were obtained by our screening method.

INTRODUCTION

Carbohydrates are used as the energy sources for living organisms, and the carbohydrates on cell surfaces also play important roles in living systems. Carbohydrates mediate various biological phenomena including bacterial and viral infections and cancer metastasis by interaction with proteins. Generally, bioactive carbohydrates composed of oligosaccharides are expensive to produce because they are difficult to synthesize and extract. Thus, it is difficult commercially use oligosaccharides as biomaterials. Therefore, there is a demand for substitutes that can be obtained inexpensively and in large quantities.

Glycopolymers, which have sugar units on the side chains, have been used as scaffolds for the development of compounds mimicking oligosaccharides. Monovalent carbohydrates only bind weakly to carbohydrate recognition proteins (lectins); however, the interaction between multivalent carbohydrates and lectins can be amplified, which is termed as "the cluster glycoside effect." This effect is essential to provide glycan capability artificially and can be exhibited by glycopolymers. Glycopolymers that have high affinity and specificity to various target proteins have been developed, and this development provides guidelines for the design of biofunctional materials.

Many groups have investigated the interaction of glycopolymers with viruses, bacteria, and lectins, and various glycopolymers with different sugar densities and distances between sugars and linker lengths have been studied. However, the optimal design of glycopolymers for a specific target is difficult because the structures of the target proteins are complex and diverse. Recently, screening methods using glycopolymers libraries with various parameters have attracted attention. The Bradley group has reported the preparation of a glycopolymer library using a substrate and high-throughput analyses with lectins, showing the utility of the library method. *Vibrio cholerae* can cause serious diseases. The natural ligand of the cholera toxin secreted by *V. cholerae* is the oligosaccharide GM1, which is too expensive to produce for use in inhibitors and diagnostic medicine. To obtain a more readily available material for such applications, many groups have developed inexpensive GM1 functional mimics that strongly bind to cholera toxin, using glycopolymer screening methods. Our group has shown that a glycopolymer with galactose (Gal) and neuraminic acid (Neu5Ac) is advantageous for CTB recognition in an analysis using a glycopolymer library incorporating various sugar units. The Gibson group has prepared a glycopolymer library containing monomers incorporating hydrophobic groups that could facilitate improvements in the CTB recognition ability. However, both the synthesis and screening methods for these libraries were tedious and ineffective, requiring multistep synthesis, and...
the development of inexpensive GM1 mimics with sufficient affinity has not yet been achieved.

In the present study, hydrophobic monomers that are readily available were copolymerized into a polymer scaffold by reversible addition fragmentation chain transfer (RAFT) polymerization with the aim of further improving the affinity for CTB. Gal was attached to the polymer side chain by "post-click" chemistry because it has been reported that Gal is an essential structure for the GM1-recognition protein, CTB. In the high throughput screening of sugar−protein interactions, a sensitive screening method is essential, and optics are useful because a precise method is necessary. In this study, surface plasmon resonance imaging (SPRI), which is capable of rapidly screening large libraries with high sensitivity and does not require the labeling of proteins, was used. The glycopolymer was immobilized on a gold-deposited substrate using a terminal thiol. The sugar and hydrophobic unit ratios were analyzed for the effect on the ability of the glycopolymers to recognize the CTB and other Gal lectins, using SPRI of the prepared substrates.

### RESULTS

#### Preparation of Glycopolymers with Hydrophobic Groups.

4-Trimethylsilyl-3-butynyl acrylamide (TMS BtnAam) and acrylamide (AAm) were used as the alkyne monomer and spacer, respectively. N-tert-Butylacrylamide (TBAm), N-phenylacrylamide (PhAam), and N-cyclohexylacrylamide (CyHex), which have different degrees of hydrophobicity, were used as the hydrophobic monomers. These monomers were incorporated to evaluate the contribution of hydrophobic groups to CTB recognition. The monomers were polymerized in different ratios by RAFT polymerization.

Glycopolymers with 10, 30, and 50% sugar units were prepared to evaluate the interactions between the protein and the sugar groups in the glycopolymers. The ratios of the alkyne and functional groups in the obtained polymer backbones were confirmed by $^1$H NMR, and the values corresponded to the monomer feed ratios (Table 1, Figure 1). Size exclusion chromatography (SEC) analysis revealed that the $M_n/M_w$ values of the polymer backbones were low and the relative molecular weights were close to the theoretical values (Table 1). The results suggested that living radical polymerization was achieved successfully, and the glycopolymers were

### Table 1. Properties of the RAFT Polymerization of the Glycopolymers

| entry | TMS (%) | TBAm (%) | PhAam (%) | CyHex (%) | AAm (%) | conv (%) | alkyn ratio (%) | hydro. ratio (%) | $M_n$ ($\text{g/mol}$) | $M_w$ ($\text{g/mol}$) | $M_w/M_n$ |
|-------|---------|----------|-----------|-----------|---------|----------|---------------|---------------|----------------|----------------|-----------|
| T10   | 10      | 0        | 0         | 0         | 90      | 94       | 10            | 0             | 8500           | 6100         | 1.20      |
| T10T20| 10      | 20       | 0         | 0         | 70      | 80       | 9             | 21            | 9500           | 6100         | 1.25      |
| T10P20| 10      | 0        | 20        | 0         | 70      | 79       | 10            | 19            | 9700           | 15 000       | 1.44      |
| T30   | 30      | 0        | 0         | 0         | 70      | 98       | 28            | 0             | 9900           | 15 000       | 1.41      |
| T30T20| 30      | 20       | 0         | 0         | 50      | 90       | 31            | 21            | 10 900         | 11 200       | 1.48      |
| T30P20| 30      | 0        | 20        | 0         | 50      | 86       | 28            | 21            | 11 700         | 13 100       | 1.43      |
| T50   | 50      | 0        | 0         | 0         | 50      | 96       | 49            | 0             | 10 800         | 11 800       | 1.36      |
| T50T20| 50      | 20       | 0         | 0         | 30      | 93       | 53            | 25            | 12 600         | 13 900       | 1.47      |
| T50P20| 50      | 0        | 20        | 0         | 30      | 89       | 45            | 21            | 12 600         | 17 300       | 1.57      |
| T50C20| 50      | 0        | 0         | 20        | 30      | 64       | 52            | 23            | 12 600         | 13 600       | 1.49      |

aThe target degree of polymerization was set at 100 ([M]/[RAFT] = 100). The ratio of the initiator ([RAFT]/[2,2′-azobis isobutyronitrile (AIBN)]) was fixed at 250. [M] = 1.0 mol/L. bMonomer conversions were determined by $^1$H NMR. cAlkyne and hydrophobic unit ratios were determined by $^1$H NMR after purification. dTheoretical molecular weight was calculated by the following formula: $M_n,\text{th} = (MW_{\text{monomer}} \times \text{each unit ratio/100}) \times 100 \times \text{Conv.} + MW_{\\text{RAFT}}$. eRelative molecular weight and polydispersity index were determined by SEC analysis calibrated with a polystyrene standard. The eluent was 10 mM LiBr DMF. fRelative molecular weight and polydispersity index were determined by SEC analysis calibrated with a pullulan standard. The eluent was 10 mM LiBr DMSO because of poor solubility in water. gMonomer conversions and theoretical molecular weight were calculated from the yield because of the overlap of monomer and CyHex peaks.

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Figure 1. Synthetic scheme of the glycopolymer library and the compound list used in the study.
synthesized as designed. The TMS groups were deprotected in a tetrahydrofuran (THF) solution with tetrabutylammonium fluoride (TBAF) or in aqueous solution with KOH for activation of the alkyne groups in the polymer backbones. The deprotection of the TMS groups was confirmed by the disappearance of the peak at 0.13 ppm in the $^1$H NMR spectra.

The glycopolymers were obtained by postmodification of azide-terminated Gal into the polymer backbones. The progress of the copper-catalyzed azide−alkyne cycloaddition (CuAAC) reaction was confirmed by $^1$H NMR: the ratio of the integral values of the anomer peak at approximately 5.6 ppm and the peak from the alkyl group next to the triazole at approximately 8.0 ppm reached 1:2. The relative molecular weights of the glycopolymers were evaluated by SEC analysis (Table 2). The glycopolymers with PhAAm or CyHex hydrophobic units incorporated into the glycopolymers and the ratio of the incorporated sugar units, respectively. The z-axis shows the SPR reflection intensity change (SPRI signal) on the glycopolymer-immobilized surface after injection of the protein solution for 1 h. (G10P20, G30P20, G50P20, and G50C20) were dissolved in dimethyl sulfoxide (DMSO) because they were not soluble in water. The relative molecular weights of the glycopolymers with TBAm (G10T20, G30T20, and G50T20) were smaller than those of the glycopolymers without hydrophobic units (G10, G30, and G50). For example, the molecular weight of G50T20 with TBAm was smaller than that of G50 without hydrophobic groups. The results suggested that the glycopolymers incorporating hydrophobic groups were slightly aggregated in water.

**Analysis of the Interaction between the Glycopolymers and the Proteins.** The load concentrations of CTB, peanut agglutinin (PNA), and *ricinus communis* agglutinin I (RCA120) were 1 $\mu$M, 500 nM, and 50 nM, respectively (Figure 2). In Figure 3, the x- and y-axis represent the

![Figure 2](schematic_illustration.png)

**Figure 2.** Schematic illustration of the current investigation.

![Figure 3](SPRI_signals.png)

**Figure 3.** SPRI signals of the glycopolymer library with (a) CTB (1 $\mu$M), (b) PNA (500 nM) and (c) RCA 120 (50 nM).

### Table 2. Glycopolymers Prepared by the CuAAC Reaction

| entry | sugar unit ratio (%) | $M_{n,\text{th}}$ (g/mol) | $M_n$ (g/mol) | $M_w/M_n$ |
|-------|---------------------|-----------------------------|--------------|-----------|
| G10   | 8                   | 9500                        | 24700        | 1.22      |
| G10T20| 7                   | 10400                       | 8700         | 1.34      |
| G10P20| 10                  | 10800                       | 18500        | 1.25      |
| G30   | 27                  | 13100                       | 21700        | 1.55      |
| G30T20| 28                  | 14300                       | 8600         | 1.39      |
| G30P20| 28                  | 14800                       | 15600        | 1.24      |
| G50   | 49                  | 15000                       | 8000         | 1.40      |
| G50T20| 49                  | 17700                       | 10300        | 1.41      |
| G50P20| 46                  | 18900                       | 20000        | 1.14      |
| G50C20| 53                  | 19400                       | 15500        | 1.36      |

*The target degree of polymerization was set at 100 ([M]/[RAFT] = 50). The ratio of the initiator ([RAFT]/[AIBN]) was fixed at 250. bAlkyne and hydrophobic unit ratios were determined by $^1$H NMR after purification. cTheoretical molecular weight was calculated by theoretical molecular weight-based conversion. dRelative molecular weight and polydispersity index were determined by SEC analysis calibrated with a pullulan standard. eThe eluent was 100 mM NaNO₃ aq. fRelative molecular weight and polydispersity index were determined by SEC analysis calibrated with a pullulan standard. The eluent was 10 mM LiBr DMSO because of the poor solubility in water. gThe theoretical molecular weight was determined assuming the yield to be equal to the conversion because the conversion could not be calculated.
The SPRI signal changes after the addition of the proteins are shown in Figure 3. All the glycopolymers showed a SPRI signal change after the addition of the proteins. The SPRI intensities differed according to the ratios of Gal and the hydrophobic units incorporated in the polymers. The CTB binding was shown to be dependent on the Gal ratio. The glycopolymers with higher Gal ratios showed the largest SPRI signals. The glycopolymers with 50% Gal tended to show the highest adsorption of CTB. PNA and RCA120 showed a different tendency regarding the Gal incorporation ratio compared with CTB. The polymer with 30% Gal showed the highest amount of adsorption after the addition of PNA or RCA120. The effect of hydrophobicity was also investigated by comparing the signals for the polymers with the same sugar ratios (Figure 3). CTB binding was amplified by the addition of hydrophobic groups as the SPRI signals of the glycopolymers with TBAm and PhAAm (blue and green bars, Figure 3) were larger than those of the polymers without hydrophobic units (red bars, Figure 3). In contrast, the SPRI signals of the polymers, with and without hydrophobic units, were approximately the same for PNA and RCA120 binding.

Calculation of the Binding Constants for the Proteins. The binding constants for each glycopolymer were calculated from the SPRI screening results. The curves formed by the SPRI signal plots for the protein solutions for each concentration were analyzed by fitting based on eq 1. For the CTB solution, the binding constants for the surface-immobilized G50, G50T20, and G50P20 were 0.39, 2.7, and 4.1 × 10^6 M⁻¹, respectively (Figure 4). The binding constant of G50P20 with hydrophobic units was slightly larger than that of G50 without hydrophobic groups, but the increase in binding between G50P20 and G50 was only 1.3 times. In the case of RCA120, the binding constants for the surface-immobilized G50, G50T20, and G50P20 were 3.1, 2.5, and 2.5 × 10^8 M⁻¹, respectively. The binding constants of G50T20 and G50P20 with hydrophobic units were slightly smaller than that of G50 without hydrophobic groups.

The binding constants calculated in the previous section were evaluated using the partition coefficient of the log P value, which is a calculated value representing the hydrophobicity of chemical substances. The x- and y-axis represent the log P value of the hydrophobic group monomer (AAm, TBAm, PhAAm, and CyHex) in each glycopolymer and the binding constant for each immobilized glycopolymer, respectively (Figure 5). Glycopolymers incorporating highly hydrophobic monomers had large binding constants.

Inhibition Assay with Addition of the Natural Ligand GM1. An inhibition assay was performed to investigate the site of CTB that interacts with the glycopolymers (Figure 6). The vertical axis of the graph represents the SPRI signal after flowing the protein solution for 1 h. The left and right bars are the results for the CTB solution (250 nM) and a mixed solution with CTB (250 nM) and GM1 (10 μM), respectively. A SPRI signal appeared in all the polymer-immobilized substrates after the addition of the CTB solution. In contrast, no SPRI signal change was observed after the addition of the mixed solution.
DISCUSSION

Hydrophobic units have been frequently added to the Gal units in glycopolymers to enhance the molecular recognition with CTB. The Bundle group reported that the ligand library conjugated with multivalent polymer scaffolds assisted in identifying hydrophobic units to complement the affinity of Gal for CTB. The Gibson group has reported that the affinity and selectivity of glycopolymers toward CTB and PNA were changed by incorporation of monomers with a secondary hydrophobic motif for Neu5Ac recognition sites next to Gal.

In this study, an amplification effect on CTB binding was observed with glycopolymers having hydrophobic units and Gal residues. Inhibition assays indicated that the hydrophobic group performed a similar function to that of Neu5Ac in GM1, and that the hydrophobic unit and Gal interacted cooperatively with carbohydrate recognition domains. The results of the present study differ from Gibson’s report in that the interaction with the proteins had no correlation with the log P value of the hydrophobic groups in the glycopolymers, and the hydrophobic group might interfere with the interaction with PNA. The ligands on the side chains might have been able to reach the carbohydrate recognition sites because the polymers used in the present study are flexible compared with the polymers used by other groups. Although there are still some issues to consider, the introduction of Gal and hydrophobic units to a glycopolymer has the potential to be effective for enhancing CTB recognition.

The contribution of the hydrophobic groups to the CTB (PDB-ID: 3CHB) binding was investigated based on the crystal structure from the PDB. CTB was found to have hydrophilic amino acids on the surface, but PNA (PDB-ID: 2PEL) and RCA120 (PDB-ID: 1RZO) had hydrophobic groups scattered on the surface. CTB interacted strongly with the glycopolymers with hydrophobic units, and PNA and RCA120 did not interact with the hydrophobic glycopolymers, suggesting that the hydrophobic amino acid residues in Gal lectins contribute to the interaction only with the CTB. Unexpectedly, hydrophobic residues were found not only in CTB but also in PNA and RCA120; though a hydrophobic interaction was not observed with PNA or RCA120, one possibility is that some of the hydrophobic residues in CTB contribute to the recognition of Neu5Ac in GM1 or the hydrophobic unit in the glycopolymers and that the hydrophobic residues in PNA and RCA120 contribute to the protein folding. At the carbohydrate recognition sites in the CTB, Trp88, a hydrophobic amino acid, interact with Gal of GM1. The acetyl group of Neu5Ac in GM1 induced hydrophobic interaction with Glu11 and Tyr12, and Tyr12 form a hydrophobic cavity in the vicinity of Neu5Ac residue. The enhanced binding of CTB with molecules containing hydrophobic groups has been reported. Further studies are needed to determine whether hydrophobic groups act on this hydrophobic pocket. Elucidation of hydrophobic units interacting on the cavity requires further study.

The binding constant between G50P20 and CTB was 4.1 × 10^8 M^-1, which was the highest value in this study, and this affinity is comparable to antigen–antibody interactions; this suggested that G50P20 may be applicable as a biofunctional material for molecular recognition. However, this affinity was still much weaker than the natural ligand for CTB, GM1 (K_a = 10^10 to 10^12 M^-1). The strong binding of GM1 is achieved by multiple hydrogen bonding between the amino acids in the protein and the sugars in GM1, including Gal, GalNAc, and Neu5Ac. The binding constant of G50P20 is thought to be smaller than that of GM1 because only Gal in G50P20 functions as a sugar ligand with CTB. In addition, while the rigid structure of GM1 can suppress the entropy loss by binding with CTB, the flexible side chains of the glycopolymers G50P20 provide the potential advantage that the ligands can reach the carbohydrate recognition domains, but this advantage is canceled by an increased entropy loss.

We have previously reported another GM1 polymer mimic, containing Gal and Neu5Ac, which had a binding constant of 2.7 × 10^9 M^-1, which is much stronger binding than that observed for G50P20. A possible reason for the weaker binding of G50P20 is that G50P20 was composed of only 50% Gal, which is a lower amount than in the previous polymer; glycopolymers with higher sugar ratios might have better performance because of the cluster glycoside effect. The weak affinity of G50P20 indicated that the hydrophobic units could induce hydrophobic interactions but could not substitute for other interactions, such as electrical interactions and hydrogen bonds. Although hydrophobic groups have often been proposed to be substitutes for oligosaccharides and sialic acid in synthetic compounds, the design and scope of the application of hydrophobic groups require careful consideration.

CONCLUSIONS

The glycopolymers with high sugar ratios and strong hydrophobicity showed strong interactions with the CTB. For PNA and RCA120, the observed trends for glycopolymers that showed strong affinities were clearly different compared with the CTB; with these proteins, the hydrophobic units did not provide effective protein recognition. Quantitative binding constant analysis of the immobilized glycopolymers and CTB was performed to confirm the contribution of the hydrophobic units, and a correlation between the K_a values and the log P values of the introduced hydrophobic units was observed. Our analysis suggested that the hydrophobic units contributed to a hydrophobic interaction and functioned cooperatively with the Gal units. We were able to provide new design guidelines for glycopolymers for CTB recognition. This screening method using SPR could be a useful approach for developing new molecular recognition materials in the future. Research is in progress in our group on new development methods for glycopolymers as molecular recognition materials that are comparable to current drug-screening methods.

EXPERIMENTAL SECTION

Preparation of Polymer Backbones. All polymer backbones were prepared by RAFT polymerization (Figure 1). Briefly, AAm, TMS BtAAm, and hydrophobic acrylamide derivatives (TBAm, PhAAm, and CyHex) were dissolved in DMSO with 2-cyanoprop-2-yl butyl trithiocarbonate (CPBTC) and AIBN. Polymer structures are shown in the Supporting Information. The monomer feed ratio and target degree of polymerization were varied (Table 1). The solution was degassed by freeze–thaw cycles (three times) and placed in an oil bath at 70 °C for 18 h. The polymer backbones were purified by dialysis against a solvent mixture of MeOH and acetone (MeOH:acetone = 1:1, MWCO = 3.5 kDa) or Milli-Q water (MWCO = 3.5 kDa).

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Deprotection of TMS Groups from the Polymer Backbones. For G10–G30, the polymer (100 mg) was dissolved in a mixture of Milli-Q water (10 mL): KOHaq (1 M, 3 mL) (Figure 1). The solution was stirred at room temperature for 3 h. The product was obtained by freeze-drying. For TS0 and G30T20–G50C20, the polymer (100 mg) was dissolved in dry THF (5 mL), and TBAF solution (1 mL, 1.0 M in THF) was added into the solution. The solution was kept stirring at room temperature for 9 h. THF was removed under reduced pressure. The deprotected polymer was dialyzed against a mixture of MeOH and acetone (MeOH/acetone = 1:1, MWCO = 3.5 kDa).

Carbohydrate Addition by “Post-Click” Chemistry. The CuAAC reaction was conducted between the alkyne groups in the polymer backbone and azide-terminated Gal, where the molar amounts of the alkyne units were calculated based on the monomer ratio in the polymer. The polymer (20 mg), azide-terminated Gal (3 equiv to alkyne unit), and CuSO₄ (0.4 equiv to alkyne unit) were dissolved in Milli-Q water (1.5 mL). Tris(benzyltriazolylmethyl)amine (0.4 equiv to alkyne unit) in MeCN (1.5 mL) was added to the reaction mixture. Sodium ascorbate (1-Asc-Na) (2 equiv to alkyne unit) was added, and the reaction mixture was kept at 60 °C for 6 h with N₂ bubbling. The products were puriﬁed by dialysis (Spectra/Por 7; MWCO 3500) against water with hydrochloric acid (pH = 4) for 24 h. The solution was changed for pure water (pH = 7) and kept for 24 h, and the product was obtained by freeze-drying.

The glycopolymers used in this investigation are shown in Figure 1. We named glycopolymers as follows: the numbers following Gal show the incorporation ratio of Gal in the polymer, where G10 means 10% incorporation of Gal in the polymer. The letters and numbers represent the type and ratio of the hydrophobic group incorporated into the polymers, respectively.

SPRI Chips Preparation and SPRI Measurement. In all the experiments, the glass substrate of S-TIM35 (Vidtec, Fukuoka, Japan) was exposed to UV/O₃ light for 30 min. all the experiments, the glass substrate of S-TIM35 (Vidtec, Fukuoka, Japan) was exposed to UV/O₃ light for 30 min. For all the experiments, the glass substrate of S-TIM35 (Vidtec, Fukuoka, Japan) was exposed to UV/O₃ light for 30 min. For all the experiments, the glass substrate of S-TIM35 (Vidtec, Fukuoka, Japan) was exposed to UV/O₃ light for 30 min. The solution was kept stirring at 60 °C with N₂ bubbling. The products were purified by dialysis (Spectra/Por 7; MWCO 3500) against water with hydrochloric acid (pH = 4) for 24 h. The solution was changed for pure water (pH = 7) and kept for 24 h, and the product was obtained by freeze-drying.

Measurement of the Affinity between the Polymers and Proteins. SPRI chips were prepared in accordance with the method described above and set in the SPRI chip cell. Before the measurement, 10 mM PBS was flowed into the cell at 0.1 mL/min until the SPRi signal became stable. The protein solution (CTB, PNA, or RCA120) was injected with a flow rate of 0.1 mL/min, and the order of the injection was from the most dilute to the most concentrated solution. In the measurement, the SPRi signal was regarded as the amount of protein adsorption. The binding constants of each protein were calculated with the Langmuir isotherm using the SPRi signals:

\[
\Delta R = \frac{K_c \Delta R_{\text{max}} c}{1 + K_c c}
\]

where \(K_c\) and \(\Delta R_{\text{max}}\) are the SPRI signal, the maximum SPRi signal, the protein concentration, and the binding constant, respectively. Based on eq 1, the plots of the SPRi signals were analyzed by nonlinear regression to derive the binding constants.

Inhibition Assay for the Carbohydrate Recognition Sites of CTB. SPRI chips were prepared in accordance with the method described above and set in the SPRi chip cell. Before the measurement, 10 mM PBS was flowed into the cell at 0.1 mL/min until the SPR reflectivity became stable. Protein solutions were injected with a flow rate of 0.1 mL/min for 1 h. A mixture of CTB (250 nM) and GM1 (10 μM) was used. GM1 used was whole lipid and not only the saccharide unit. GM1 was soluble in a monomeric state which was confirmed by dynamic light scattering (Figure S44). The concentration was under the critical micelle concentration of GM1.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b02877.

H NMR spectra of the compounds and the detailed conditions for the polymerization (PDF)

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Notes

The authors declare no competing financial interest.

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