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# Analysis of GM3-Gg3 interaction using clustered gycoconjugate models constructed from glycolipid monolayers and artificial glycoconjugate polymers

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**Carbohydrate-carbohydrate interactions between clustered GM3 on the Langmuir monolayer and clustered Gg3 trisaccharide along a polystyrene chain were investigated using surface pressure-area ($\pi$-A) isotherms and surface plasmon resonance (SPR). The $\pi$-A isotherm of the GM3 monolayer was expanded substantially and specifically by the Gg3-trisaccharide-bearing glycoconjugate polystyrene [PN(Gg3)] even at $10^{-12}$ M. The PN(Gg3)-induced expansion of the GM3 monolayer required no calcium ion, and the expansion was strongly inhibited in the presence of urea and acetamido sugars. SPR studies of the GM3-Gg3 interaction were carried out to estimate the affinity constant and specificity of the interaction quantitatively. PN(Gg3) was adsorbed onto the GM3 monolayer strongly and specifically with an apparent affinity constant of $K_a = 2.5 \times 10^6$ M$^{-1}$. The mechanism of the GM3-Gg3 interaction was discussed on the basis of the relationship between affinity and structure. We found that the NHAc groups of *N*-acetylneuraminic acid in GM3 and of GalNAc in Gg3 play an important role in the GM3-Gg3 interaction and that PN(Gg3) recognizes not only some specified portions of GM3 but also the trisaccharide as a whole.**
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## Introduction

Increasing attention has been paid to the biological functions of oligosaccharide chains of glycolipids and glycoproteins at plasma membranes in various biological processes including cell-cell communications. Since Hakomori and coworkers reported that $Le^X$-$Le^X$ carbohydrate interaction played important roles in compaction in embryogenesis [1], carbohydrate-carbohydrate interactions have aroused much interest not only of biologists but also of chemists from the aspect of molecular recognition in cell-cell communications [2–5]. For example, GM3-Gg3 interaction was discovered in cell-cell adhesion between lymphoma and melanoma cells [6–8], and this interaction was recently reported to participate in triggering signal transduction [9,10]. Gal-3$SO_3$Gal interaction was reported to participate in formation of compacted myeline sheaths [11–14].

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In recent years, carbohydrate-protein interactions on cell surfaces have been fully elucidated by X-ray crystallographic and calorimetric analysis [15–17]. On the other hand, carbohydrate-carbohydrate interactions have scarcely been analysed quantitatively and the mechanisms are still unclear. According to an early attempt [18], the carbohydrate-carbohydrate interaction using a simple $Le^X$ model in water was too small to be detected by NMR spectroscopy. Geyer et al. devised a molecular assembly made of a $Le^X$-bearing lipid bilayer that interacts with a $Le^X$ trisaccharide in the presence of $Ca^{2+}$, but the affinity constant estimated by NMR spectroscopy was still low ($K_a = 2$ M$^{-1}$) [19].

It has been known that glycolipids on cell surfaces form a microdomain named glyco signaling domain (GSD) or raft [3,4,20,21] to acquire strong affinity to the complementary proteins. The importance of multivalency of the carbohydrate chains in carbohydrate-protein interactions has been well cited as the glyco-cluster effect [22–26]. Recently, a variety of synthetic models such as glycoconjugate polymers [27–32], dendrimers [33–36], DNA [37–42], and metal complexes [43,44] have been developed to

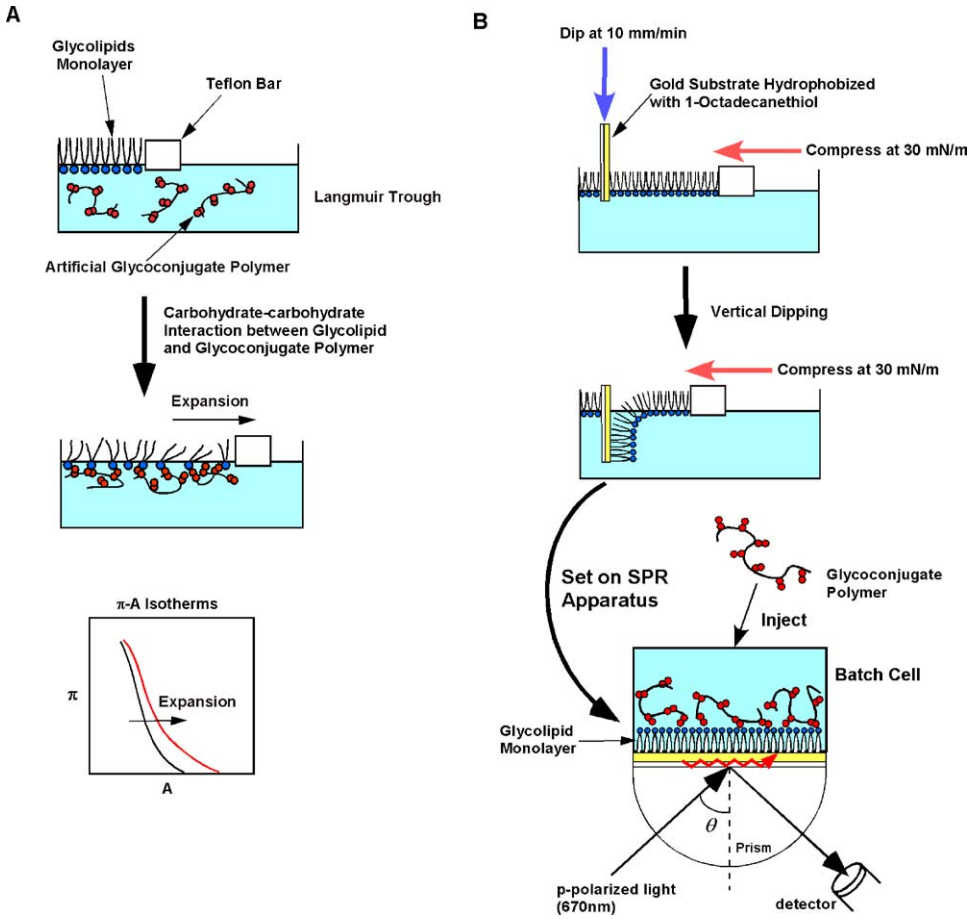


**Figure 1.** Schematic illustration of (A) highly sensitive detection of GM3-Gg3 interaction using surface pressure-area ($\pi$-A) isotherms and (B) quantitative estimation of the interaction by surface plasmon resonance (SPR).

enhance the binding affinity to specific lectins via the cluster effect.

We expected that carbohydrate-carbohydrate interaction would be sensitively detectable by applying the glyco-cluster effect and we proposed two glycoclustered model systems made of glycolipid monolayers and artificial glycoconjugate polymers (Figure 1). Recently, it is reported [45] that hydrogen bond-based interactions could be highly enhanced at the air-water interface and the molecular recognition of the lipid monolayer could be detected at the air-water interface by the surface pressure-area ($\pi$-A) isotherms. In addition, quartz crystal microbalance (QCM) [46–48] and surface plasmon resonance (SPR) [49,50] were reported to be useful to analyze quantitatively the interaction between lectins and glycolipid monolayers transferred on substrates. Thus, we considered that application of these useful techniques would provide highly sensitive and quantitative analysis of carbohydrate-carbohydrate interaction. We have evaluated GM3-Gg3 interaction by $\pi$-A isothems [51,52] and SPR [53,54] techniques using naturally-occurring and synthetic glycolipids and artificial glycoconjugate polymers as shown in Figure 2. This review describes our results with respect to the GM3-Gg3 interaction and discusses the mechanism.

## Results

### Synthesis and characterization of glycoconjugate polymers

*N*-Glycoside type glycoconjugate styrene monomers were synthesized by $\beta$-anomeric amination at the reducing terminal of carbohydrates with ammonium hydrogen carbonate followed by reaction with *p*-vinylbenzoyl chloride [28,52]. The polymerization was performed using a redox initiator (ammonium peroxodisulfate and *N*, *N*, *N*′, *N*′-tetramethylethylenediamine) in degassed water to afford the corresponding glycoconjugate polystyrenes. The polymers were soluble in water and dimethyl sulfoxide. The number-average molecular weights ($M_n$) were on the order of $10^4$ (Figure 2).

These glycoconjugate polymers consist of an amphiphilic structure unit bearing hydrophilic carbohydrate and a hydrophobic polystyrene backbone. Thus, it is expected that these glycoconjugate polymers take unimolecular micelle-like

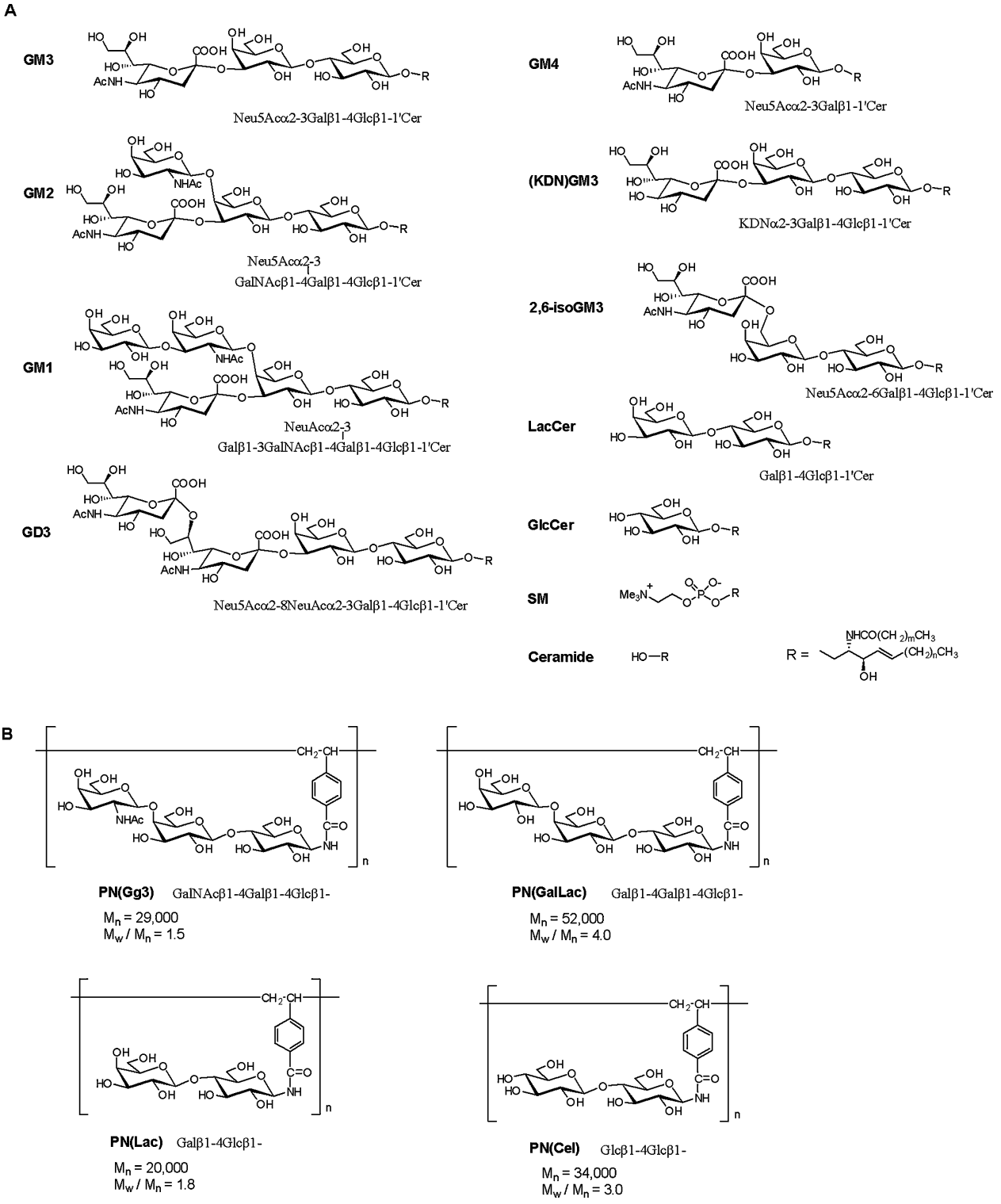


**Figure 2.** Structures and abbreviations of (A) lipids and (B) glycoconjugate polystyrenes used in this study. $M_n$ and $M_w$ are number and weight average molecular weight, respectively. The chain lengths of fatty acid ($m$) and sphingosine ($n$) moieties of the synthetic sphingolipids (GM4 and 2,6-isoGM3) are defined to $m = 16$ and $n = 12$, respectively. Natural sphingolipids (GM3, GM2, GM1, GD3, (KDN)GM3, LacCer, GlcCer, SM), however, have diverse distributions in the structures of fatty acid and sphingosine moieties. We consider that the structural distributions little affect the carbohydrate-carbohydrate interaction.

conformation in aqueous solution. In fact, small-angle X-ray scattering (SAXS) experiment [55] of lactose-carrying polystyrene (PVLA) synthesized by living radical polymerization [56] indicated that it takes cylindrical conformation consisting of a polystyrene chain at the interior and carbohydrates at the exterior. Since the polystyrene main chain would be flexible, the glycoconjugate polymers may be adsorbed on the hydrophobic surface. Analyses of adsorption behavior of the glycoconjugate polymers using a quartz crystal microbalance revealed that they could be adsorbed strongly on hydrophobic surfaces, but minimally adsorbed on hydrophilic surfaces [57,58].

### $\pi$-A Isotherm study of GM3-Gg3 interaction [51,52]

Figure 3 shows $\pi$-A isotherms of GM3 in the absence and presence of PN(Gg3) and its monomer (10 nM) in the subphase (the water phase in a Langmuir trough, see Figure 1) at 25°C. The $\pi$-A isotherm of the GM3 monolayer on pure water gave a monotonic and smooth curve to collapse at 57 mN m$^{-1}$ without passing through a phase of liquid-expanded film [59]. The limiting molecule-occupied area of the GM3 monolayer was calculated to be 62 Å$^2$. Addition of $1 \times 10^{-8}$ M PN(Gg3) into the subphase caused significant expansion of the GM3 monolayer over the whole range of surface pressure, and the apparent limiting molecule-occupied area of GM3 was increased to 88 Å$^2$. The expansion was observed even at the collapsing pressure at which the monolayer exists as a solid condensed film. Since the GM3 monolayer was minimally expanded by the Gg3 monomer, a large expansion of the GM3 monolayer with PN(Gg3) was induced by specific binding due to the carbohydrate-carbohydrate interactions between GM3 trisaccharides and clustered Gg3 trisaccharides along the polymer chain.

$\pi$-A Isotherms of the GM3 monolayer were measured in the absence and presence of various glycoconjugate polystyrenes

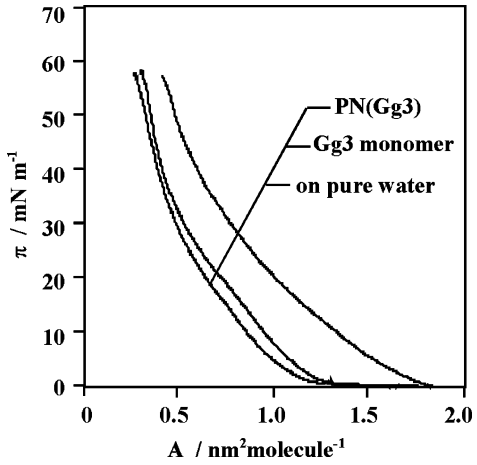


**Figure 3.** $\pi$-A Isotherms of GM3 in the presence of PN(Gg3) and its monomer in the subphase, and on pure water at 25°C. The concentration of oligosaccharide units in the subphase is $1 \times 10^{-8}$ M.

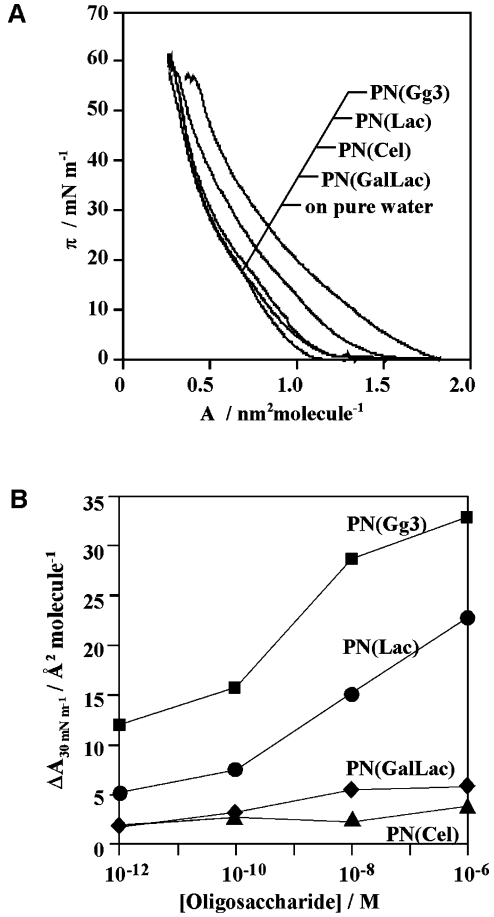


**Figure 4.** (A) $\pi$-A Isotherms of GM3 in the presence of PN(Gg3), PN(GalLac), PN(Lac), and PN(Cel) in the subphase, and on pure water at 25°C. The concentration of oligosaccharide units in the subphase is $1 \times 10^{-8}$ M. (B) Concentration dependence of the oligosaccharide units of glycopolymers on the expansion amount of the surface area of GM3 monolayers at 30 mNm$^{-1}$.

in a wide range of oligosaccharide concentrations ($10^{-12}$ M to $10^{-6}$ M) in the subphase. Figure 4A is a typical example of the $\pi$-A isotherms. The increments of area per molecule $\Delta$A of GM3 at 30 mN m$^{-1}$ are plotted against concentrations in Figure 4B. Since the surface pressure of plasma membranes is reported to be 30 mN m$^{-1}$ [59], the increment $\Delta$A is suitable as a measure to compare the extent of expansion clearly.

PN(Gg3) expanded the GM3 monolayer specifically, even at a very low concentration (1 pM). PN(Lac), in which GalNAc is deleted from PN(Gg3), also expanded the GM3 monolayer, but the increment of the area was about half that with PN(Gg3). On the other hand, PN(GalLac) and PN(Cel) expanded the GM3 monolayer only minimally or not at all. Therefore, the GM3 monolayer specifically recognized the carbohydrate structures in the glycoconjugate polystyrenes in the subphase. The essential role of the NHAc group in Gg3 for the carbohydrate-carbohydrate interaction is suggested. The weak interaction

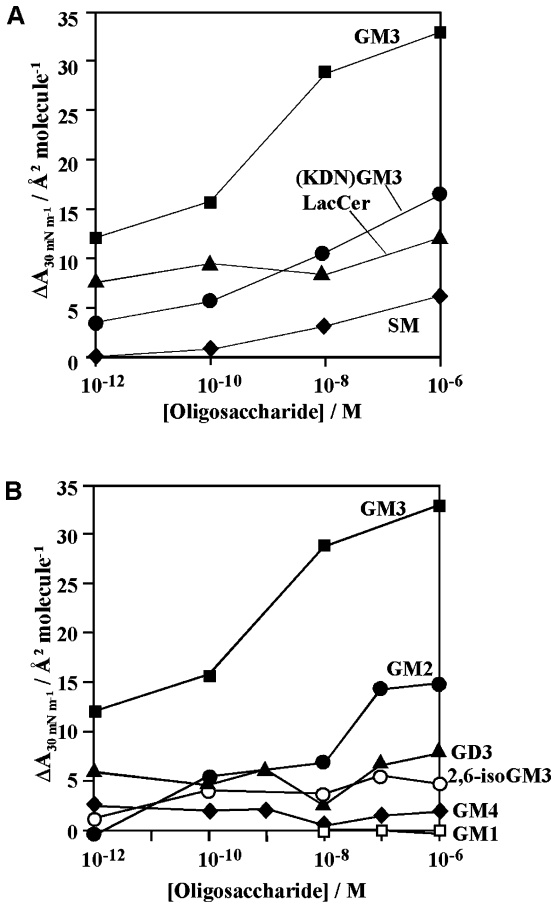


**Figure 5.** Increment of area per molecule of the monolayers of (A) various glycolipids and (B) gangliosides at 30 mNm$^{-1}$ in the presence of PN(Gg3) in the subphase.

between GM3 and lactosylceramide is consistent with the previous reports [7,8]. It is also important to note that the concentration dependency produced a gentle slope over a wide concentration range. This tendency is not consistent with the Langmuir-type isotherm, but suggestive of the interaction responsible for indefinite binding sites.

$\pi$-A Isotherms of various lipid monolayers were measured in the absence and presence of PN(Gg3). Figure 5A plots the increments $\Delta$A of various lipids at 30 mN m$^{-1}$ against the Gg3-concentration. The expansion of (KDN)GM3, which is substituted with OH instead of the NHAc group in Neu5NAc of GM3, was much smaller than that of GM3 and similar to that of LacCer over the entire range of concentrations of PN(Gg3). The sphingomyelin (SM) monolayer was slightly expanded by PN(Gg3) at 30 mN m$^{-1}$. Thus, not only the NHAc group in GalNAc of Gg3, but also the NHAc group in Neu5NAc of GM3 has an important role in the GM3-Gg3 interaction.

The expansion of monolayers of other gangliosides (GM1, GM2, GM4, GD3, and 2,6-isoGM3) by PN(Gg3) in the subphase was also examined and are summarized in Figure 5B. The GM2 monolayer was expanded by about half as much as the

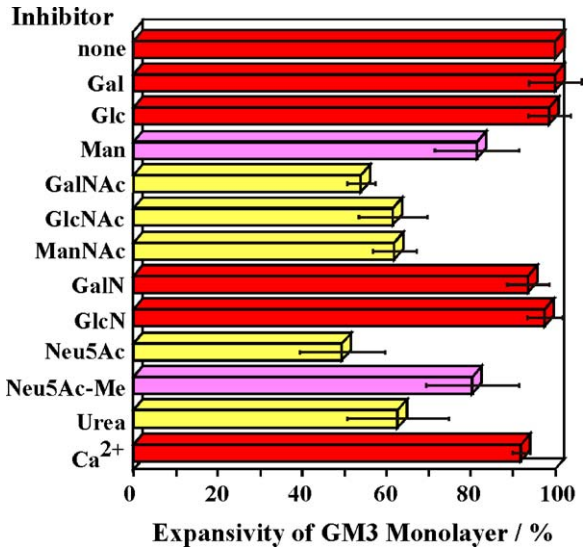


**Figure 6.** Effect of 1 mM of monosaccharides, urea, and $Ca^{2+}$ to the expansion ratio of the GM3 monolayer at 30 mN m$^{-1}$ in the presence of 10 nM of PN(Gg3). Neu5Ac-Me stands for *N*-acetylneuraminic acid methyl ester.

GM3 monolayer and similarly to the LacCer and (KDN)GM3 monolayers. The GM1 was not expanded at all, even at 1 $\mu$M of PN(Gg3). The interaction of GM3 with PN(Gg3) was decreased by substitution of the 3-position of Gal in GM3 with a monosaccharide, and further with a disaccharide. GM4, GD3, and 2,6-isoGM3 monolayers were only slightly expanded by PN(Gg3).

### Effect of additives on $\pi$-A isotherm expansion of GM3-Gg3 interaction [52]

$\pi$-A Isotherms of the GM3 monolayer induced by PN(Gg3) were influenced by adding calcium ion, urea, and various kinds of monosaccharide derivatives (1 mM) to the subphase. Figure 6 summarizes the expansivity of the GM3 monolayer at 30 mN m$^{-1}$ as a measure of the interaction. The GM3/PN(Gg3) interaction was strongly inhibited by urea and all the acetamido monosaccharides tested: GlcNAc, GalNAc, ManNAc, and Neu5NAc. In contrast, the interaction was affected little by Glc, Gal, GlcN, and GalN. The presence of an acetamido group is essential in the interaction. The interaction was inhibited intermediately by Man and the methyl ester of Neu5NAc. Participation of the carboxyl group of GM3 in the interaction is suggested, while the inhibition by mannose was unexpected. The expansion of the GM3 monolayer induced by PN(Gg3) was not affected by $Ca^{2+}$, indicating that $Ca^{2+}$ was not required for the GM3/Gg3 interaction in the present system.

### SPR study of GM3-Gg3 interaction [53,54]

SPR studies of the interaction between the GM3 monolayer and PN(Gg3) were carried out to estimate the affinity constant and

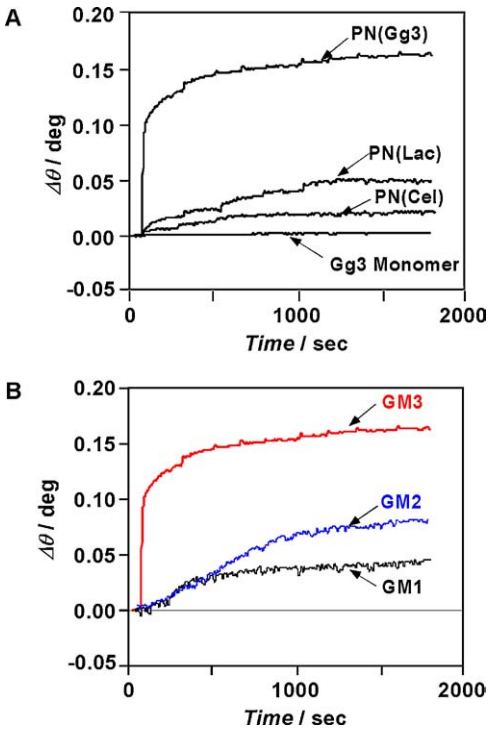


**Figure 7.** Typical SPR time courses of adsorption of (A) various glycoconjugate polymers onto GM3 and (B) PN(Gg3) onto various monolayer of gangliosides at 1 $\mu$M oligosaccharide concentration at 25°C.

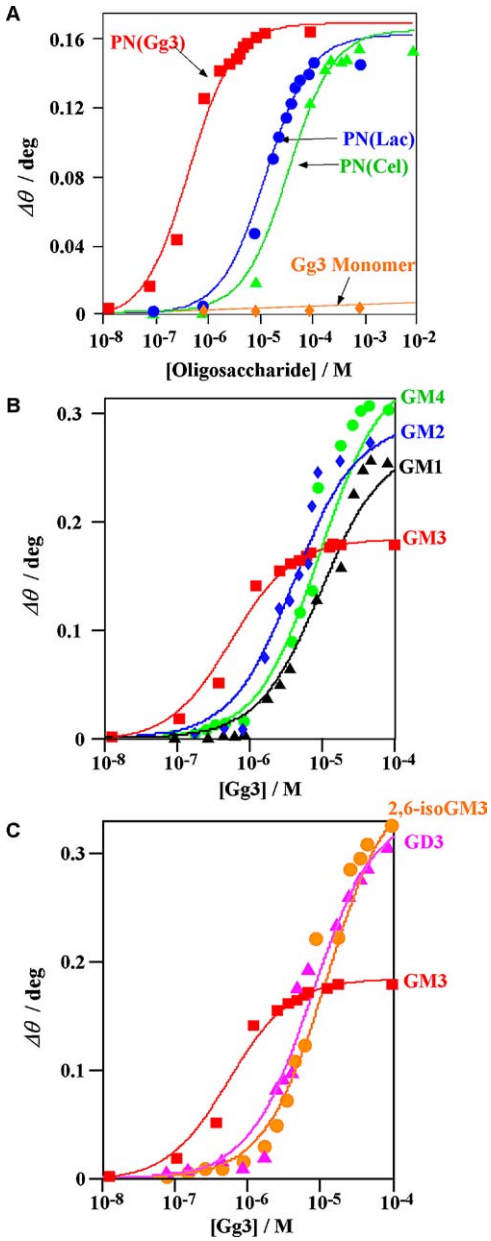


**Figure 8.** Binding isotherms of glycoconjugate polystyrenes to gangliosides monolayer depending on the concentrations of oligosaccharide unit of at 25°C. (A) Adsorption of glycoconjugate polystyrenes onto GM3 monolayer. (B) and (C) Adsorption of PN(Gg3) onto various ganglioside monolayers.

specificity of the interaction quantitatively. Figure 1B shows a schematic illustration of the SPR experiment. The Langmuir monolayer of a sphingolipid was compressed to 30 mN m$^{-1}$ and transferred by a vertical dipping method (dipping speed: 10 mm min$^{-1}$) onto a gold-deposited glass plate which had been hydrophobized with a self-assembled monolayer of 1-octadecanethiol. The glycolipid-immobilized gold substrate was set on an SPR apparatus and the change in the SPR spectrum by addition of glycoconjugate polymers was monitored.

Figure 7A shows the time courses of the resonance angle changes ($\Delta\theta$) of the GM3 monolayer responding to the addition of various glycoconjugate polymers and Gg3-carrying styrene monomer at 1 $\mu$M of oligosaccharide concentration. PN(Gg3) was adsorbed rapidly onto the GM3 monolayer to reach a large angle change in a few minutes. PN(Lac) and PN(Cel) were adsorbed more slowly to reach smaller angle changes. On the other hand, the corresponding Gg3-carrying styrene monomer was not adsorbed at all.

Figure 7B shows typical time courses of the resonance angle changes ($\Delta\theta$) of various monolayers (GM3, GM2, and GM1) responding to the addition of PN(Gg3) at 1 $\mu$M. PN(Gg3) was significantly adsorbed onto the GM3 monolayer and reached equilibrium within about 10 min, whereas PN(Gg3) was slightly adsorbed onto the GM2 and GM1 monolayers.

The $\Delta\theta_{eq}$ valuess at various oligosaccharide concentrations were determined and plotted against the concentration [oligosaccharide] to give typical Langmuir-type adsorption isotherms (Figure 8). PN(Gg3) was adsorbed onto the GM3 monolayer at even low concentration (about $10^{-7}$–$10^{-6}$ M), whereas PN(Lac) and PN(Cel) needed much higher concentration (Figure 8A). The isotherms of PN(Gg3) adsorption onto various ganglioside monolayers (Figure 8B and C) showed that not only the $\Delta\theta$ dependency on PN(Gg3) concentration but also the saturated maximum angle change ($\Delta\theta_{max}$) of PN(Gg3) varied with the structure of gangliosides. The difference in saturated maximum angle change may result from the difference

**Table 1.** Apparent affinity constants in binding of glycoconjugate polystyrenes to sphingolipids

| *Polymer* | *Monolayer* | $K_a$ [a] / $10^4$ $M^{-1}$ [b] | $\Delta\theta_{max}$ / deg |
|---|---|---|---|
| PN(Gg3) | GM3 | 250 | 0.168 |
| | GM2 | 11 | 0.420 |
| | GM1 | 6.1 | 0.337 |
| | GD3 | 12 | 0.338 |
| | GM4 | 11 | 0.342 |
| | KDN(GM3) | 25 | 0.232 |
| | 2,6-isoGM3 | 5.5 | 0.412 |
| | LacCer | 65 | 0.139 |
| | GlcCer | 24 | 0.142 |
| | Ceramide | 2.9 | 0.151 |
| PN(Lac) | GM3 | 7.7 | 0.152 |
| | KDN(GM3) | 5.9 | 0.101 |
| | LacCer | 7.7 | 0.026 |
| | GlcCer | 4.1 | 0.186 |
| | Ceramide | 0.01 | 0.223 |
| PN(Cel) | GM3 | 4.4 | 0.156 |
| | KDN(GM3) | 2.6 | 0.104 |
| | LacCer | 6.1 | 0.015 |
| | GlcCer | 3.9 | 0.077 |
| | Ceramide | 0.027 | 0.066 |

[a] Apparent affinity constants obtained from Langmuir Eq. (1).
[b] Molarity (mol $L^{-1}$) of the oligosaccharide unit.

in the stoichiometry of interaction or in the conformation of PN(Gg3) adsorbed on each monolayer [57,58].

Apparent affinity constants per oligosaccharide unit ($K_a$) and maximum angle change ($\Delta\theta_{max}$) were calculated from the slopes and intercepts according to Langmuir Eq. (1).

$$[\text{oligosaccharide}]/\Delta\theta = [\text{oligosaccharide}]/\Delta\theta_{max} + 1/\Delta\theta_{max} K_a \quad (1)$$

Table 1 summarizes these binding parameters in the carbohydrate-carbohydrate interaction. Comparison of $K_a$ in Langmuir Eq. (1) shows that PN(Gg3) was bound most strongly to the GM3 monolayer with $K_a = 2.5 \times 10^6$ $M^{-1}$. PN(Gg3) was bound to (KDN)GM3 monolayer more weakly than to GM3 by a factor of about 10, indicating the importance of the NHAc group. The affinity constants to monolayers of other gangliosides (GM2, GM1, GD3, GM4) were about 20–40 times smaller than that to the GM3 monolayer. The affinity constant of PN(Gg3) to the sphingolipids was decreased in the order of GM3 > LacCer > (KDN)GM3 ≈ GlcCer > GM2 ≈ GD3 ≈ GM4 > GM1 ≈ 2,6-isoGM3 > ceramide.

## Discussion

We have presented two model systems to amplify carbohydrate-carbohydrate interactions in biological recognition events. The model systems are constructed from clustered oligosaccharide chains along the polymer backbone and also in the glycolipid Langmuir monolayers and were applied for $\pi$-A isotherm and SPR experiments. Remarkable expansion of the $\pi$-A isotherm of GM3 monolayers was induced by specific interaction with PN(Gg3). The SPR experiments revealed that PN(Gg3) was selectively bound to the GM3 monolayer with $K_a = 2.5 \times 10^6$ $M^{-1}$. In contrast, its monomer was not adsorbed, indicating that the cluster effect is important to enhance the carbohydrate-carbohydrate interaction.

### Contribution of each structural unit in GM3-Gg3 interaction

The $\pi$-A isotherm and SPR experiments both gave a quite similar structural tendency on the GM3-Gg3 interaction and the following structural considerations can be deduced.

(1) LacCer and GlcCer: the removal of sialic acid and sialylgalactose from GM3 causes some decrease (about 1/4–1/10) of the affinity to Gg3.
(2) (KDN)GM3: the substitution of NHAc to an OH group in sialic acid of GM3 decreased the affinity (1/10) to Gg3. This result is in conflict with the liposome binding data [60]: binding of (KDN)GM3 liposome to Gg3 epitope was much stronger than that of GM3. The discrepancy may be caused by the differences of both determinations. Their determination is based on the binding of liposomes containing (KDN)GM3, phosphatidylcholine and cholesterol to Gg3Cer-coated polystyrene plates. Coating of lipids to polystyrene plates usually affords irregularly arranged surfaces, compared to those of our Langmuir-Blodgett monolayer. On the other hand, our models consist only of single glycolipid monolayer.
(3) GM4: the removal of glucose from GM3 significantly decreases (about 1/23) the affinity.
(4) GM2, GM1, GD3, and 2,6-isoGM3: the addition of a sugar moiety to GM3 and rearrangement of sialic acid of GM3 also significantly decrease (about 1/45–1/21) the affinity.
(5) ceramide: the lack of all of the sugar moieties further decreases (about 1/86) the affinity to Gg3. The measurement using ceramide was done only for SPR.
(6) PN(Lac), PN(Cel), and PN(GalLac) were bound to these glycolipids less strongly ($K_a \approx 10^4$ $M^{-1}$) and less selectively, although these polymers were minimally bound to ceramide ($K_a \approx 10^2$ $M^{-1}$).

These structural considerations suggest that PN(Gg3) recognizes not only some specified portions of GM3 but also the trisaccharide as a whole.

### Driving forces of GM3-Gg3 interaction

The presence of complementary hydrophobic surfaces between the respective trisaccharides may be a driving force of the GM3-Gg3 interaction [6]. The present study demonstrated the important role of NHAc groups in both GM3 and Gg3 and also the inhibition effect of urea and acetamido monosaccharides. It is

reasonable to assume that the hydrogen bonds of NHAc groups may also participate in addition to the hydrophobic interaction in the GM3-Gg3 interaction.

### The difference between $\pi$-A isotherm and SPR experiments

In the $\pi$-A isotherm experiment, expansion of the GM3 monolayer was observed even at the very low concentration ($10^{-12}$ M) of PN(Gg3), whereas the affinity constant of PN(Gg3) to the GM3 monolayer determined by the SPR experiment was $K_a = 2.5 \times 10^6$ $M^{-1}$. These results indicate that GM3-Gg3 interaction under the $\pi$-A isotherm conditions is incredibly stronger than that under the SPR conditions, although the exact affinity constant could not be calculated from the $\pi$-A isotherm experiment. The difference between these two experiments is ascribed to the physico-chemical environment of the interaction field: at the air-water interface in the $\pi$-A isotherm experiment and at the solid-water interface in the SPR experiment.

Hydrophobic molecules or functional groups may be accumulated at the air-water interface. Since glycoconjugate polystyrenes were adsorbed on hydrophobic solid surfaces [57,58], it is probable that a certain extent of PN(Gg3) may be concentrated near the air-water interface. Besides, it was recently reported that hydrogen bonding and electrostatic interaction are reinforced at the air-water interface rather than in the bulk water phase [45]. It is reasonable to assume that the carbohydrate-carbohydrate interaction at the air-water interface could be detected even at the very lower concentration ($10^{-12}$ M) due to the increase of effective concentration of PN(Gg3) and reinforcement of the hydrogen bond at the interface.

### Effect of $Ca^{2+}$ ions

$Ca^{2+}$ was not required for the GM3-Gg3 interaction in the present system. This is in contrast to Hakomori's report [6]: the interaction of GM3 liposomes with a Gg3-coated polystyrene surface is strongly promoted by $Ca^{2+}$ and inhibited by EDTA. As mentioned above, there is the possibility that the hydrogen bonds are reinforced at the air-water interface. Thus, it is probable that the hydrogen bonds in the GM3-PN(Gg3) interaction at the air-water interface are strong enough to cause the expansion of the monolayer, even in the absence of $Ca^{2+}$.

### Outlook on the physicochemical studies on carbohydrate-carbohydrate interactions

The present studies using the $\pi$-A isotherm and SPR experiments have addressed some important issues revolving around the GM3-Gg3 interaction. Recently, Basu et al. reported $\pi$-A isotherms study of carbohydrate-carbohydrate interaction between the GM3 monolayer and micelle of a lactose derivative [61]. Penadés et al. succeeded in highly sensitive detection of $Le^X$-$Le^X$ interaction by enhancing the SPR with $Le^X$-modified gold nanoparticles [62]. Other measurements also have been applied for probing carbohydrate-carbohydrate interaction. Boggs et al. detected Gal-3$SO_3$Gal interaction using mass spectroscopy and fluorescence resonance energy transfer (FRET) [13,14]. Surface force apparatus (SFA) [63] and atomic force microscopy (AFM) [64] are useful for direct evaluation of the force of carbohydrate-carbohydrate interaction.

No other detailed study has been reported on the GM3-Gg3 interaction, except that of liposome binding assay pursued by Hakomori et al. Two important differences have been disclosed between our and their results: the effect of $Ca^{2+}$ ions [6] and the contribution of (KDN)GM3 [60] as discussed above. Elucidation of these conflicting points is essential to deepen understanding of the real biological behaviors. Then, our next target is to extend the models to various mixed monolayers to simulate glycosignaling microdomains (GSD) of membranes and to realize the natural biological events concerned with carbohydrate-carbohydrate interactions.

Information on the relative strength of carbohydrate-carbohydrate interaction has been accumulated by the recent progress in physicochemical technologies, but the detailed mechanism of the interactions still remain open. We expect that detailed structural and thermodynamic analysis of carbohydrate-carbohydrate interactions will be advanced by the development of NMR, X-ray crystallography, and isothermal titration calorimetry (ITC).

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