A Quantitative Estimation of Carbohydrate-Carbohydrate Interaction Using Clustered Oligosaccharides of Glycolipid Monolayers and of Artificial Glycoconjugate Polymers by Surface Plasmon Resonance

Kazunori Matsuura,[†] Hiromoto Kitakouji,[†] Naoki Sawada,[‡] Hideharu Ishida,[‡] Makoto Kiso,[‡] Ken Kitajima,[§] and Kazukiyo Kobayashi*[,†]

Graduate School of Engineering, Nagoya University Nagoya 464-8603, Japan
Gifu University, Gifu 501-1193, Japan
Graduate School of Bioagricultural Sciences Nagoya University, Nagoya 464-8601, Japan

Received February 24, 2000

Oligosaccharide chains of glycoproteins and glycolipids at cell membranes have been revealed to interact not only with carbohydrate-binding proteins but also with complementary oligosaccharide chains.[1−5] Hakomori et al. have reported that the carbohydrate−carbohydrate interaction plays important roles in cellular recognition, for example, Le^x−Le^x interaction in compaction in embryogenesis and Gg3 (gangliotriaosylceramide)−GM3 interaction between lymphoma and melanoma cells.[1,2] Recently, the Gg3−GM3 interaction has been also suggested to initiate signal transduction through inhibition or activation of transducer molecules.[6] Although the importance of the carbohydrate−carbohydrate interaction has been widely accepted in the field of glycobiology and biochemistry, the mechanism has not yet been sufficiently clarified owing to the difficulty of quantitative analysis of the weak interaction. Attempts have been made to estimate the interaction in water[7] and on phospholipid bilayers[8] by NMR spectroscopy, but the interaction between simple oligosaccharide molecules is too small to elucidate the carbohydrate−carbohydrate interaction in biological recognition events. It is important to note that the carbohydrate−carbohydrate interaction in cellular recognition is expressed only when the glycolipids are arranged as clustered arrays in microdomain on the surfaces of cell membranes. [2]

We reported recently that surface pressure−area (π−A) isotherms of glycolipids in the Langmuir monolayer were expanded in the presence of structurally well-defined and complementary glycoconjugate polystyrenes in water.[9] These polymers possess highly concentrated oligosaccharide chains which are attached to all repeating units along the hydrophobic polystyrene main chains.[10] The clustered glycolipids in the Langmuir monolayer as well as the clustered oligosaccharide chains along the polymer chains could amplify the carbohydrate-carbohydrate interaction at the air−water interface. However, π−A isotherms are not quantitative enough to determine the affinity constant of the interaction. Recently, surface plasmon resonance (SPR) has been developed as a real-time analytical and quantitative method to investigate molecular recognition between various biomolecules.[11] In this contribution, we have applied SPR to investigate the carbohydrate−carbohydrate interaction using glycosphingolipids in Langmuir monolayers and glycoconjugate polystyrenes in aqueous solution.

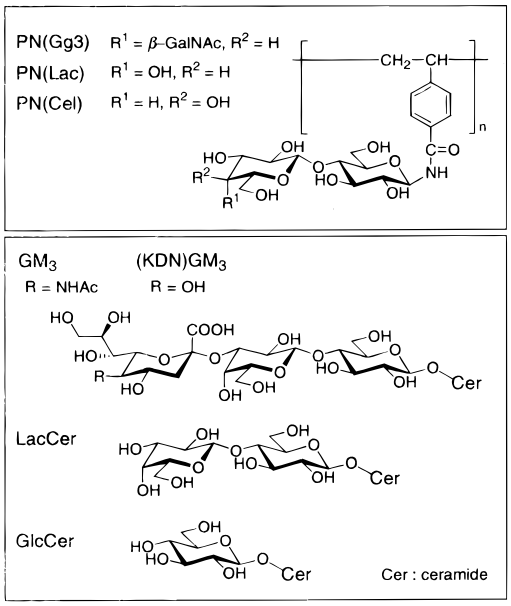

Figure 1. Structures and abbreviations of lipids and glycoconjugate polystyrenes used in this study.

Figure 1 illustrates the structures and abbreviations of sphingolipids and glycoconjugate polystyrenes[9,10a] used in this paper. PN(Gg3), PN(Lac), and PN(Cel) stand for polymers carrying *N*-glycosides of Gg3 trisaccharide (GalNAcβ1−4Galβ1−4Glcβ1−), lactose, and cellobiose, respectively. The synthesis of these polymers was described eleswhere.[9,10a] The number-average molecular weights (M_n) of PN(Gg3), PN(Lac), and PN(Cel) were estimated respectively to be 2.9×10^4 ($M_n/M_n = 1.5$), 2.0×10^4 ($M_n/M_n = 1.8$), and 3.4×10^4 ($M_n/M_n = 3.0$) by size exclusion chromatography (SEC) using pullulan as standards and water as eluent. KDN(GM3) was obtained from rainbow trout testis.[12] The sphingolipid was spread on pure water (>18 MΩ cm, purified with a Millipore Purification System) in a Miyata-type moving wall trough (Nippon Laser & Electronics Lab., Nagoya) and the Langmuir monolayer was compressed to 30 mN m^{-1} and transferred by a vertical dipping method onto a hydrophobized gold-deposited glass plate (transfer ratio = 1.0). The change in the incident angle ($\Delta\theta$) which provides a constant reflectance (about 0.5) responding to the addition of glycopolymers was recorded on a surface plasmon resonance apparatus (SPR 670, Nippon Laser & Electronics Lab., Nagoya) at 25 °C.

Figure 2 shows the time courses of the angle changes ($\Delta\theta$) of the GM3 monolayer responding to the addition of glycopolymers

* To whom correspondence should be addressed.
[†] Graduate School of Engineering, Nagoya University.
[‡] Gifu University.
[§] Graduate School of Bioagricultural Sciences, Nagoya University.

(1) Eggens, I.; Fenderson, B.; Toyokuni, T.; Dean, B.; Stroud, M.; Hakomori, S. *J. Biol. Chem.* **1989**, *264*, 9476−9484.
(2) (a) Kojima, N.; Hakomori, S. *J. Biol. Chem.* **1989**, *264*, 20159−20162. (b) Kojima, N.; Hakomori, S. *J. Biol. Chem.* **1991**, *266*, 17552−17558. (c) Kojima, N.; Shiota, M.; Sadahira, Y.; Handa, K.; Hakomori, S. *J. Biol. Chem.* **1992**, *267*, 17264−17270.
(3) Brewer, G. J.; Matinyan, N. *Biochemistry* **1992**, *31*, 1816−1820.
(4) (a) Hakomori, S.; Igarashi, Y.; Kojima, N.; Okoshi, K.; Handa, B.; Fenderson, B. *Glycoconjugate J.* **1991**, *8*, 178. (b) Stewart, R. J.; Boggs, J. M. *Biochemistry* **1993**, *32*, 10666−10674. (c) Koshy, K. M.; Boggs, J. M. *J. Biol. Chem.* **1996**, *271*, 3496−3499.
(5) Song, Y.; Withers, D. A.; Hakomori, S. *J. Biol. Chem.* **1998**, *273*, 2517−2525.
(6) Iwabuchi, K.; Yamamura, S.; Prinetti, A.; Handa, K.; Hakomori, S. *J. Biol. Chem.* **1998**, *273*, 9130−9138.
(7) Wormald, M. R.; Edge, C. J.; Dwek, R. A. *Biochem. Biophys. Res. Commun.* **1991**, *180*, 1214−1221.
(8) Geyer, A.; Gege, C.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **1999**, *38*, 1466−1468.
(9) Matsuura, K.; Kitakouji, H.; Tsuchida, A.; Sawada, N.; Ishida, H.; Kiso, M.; Kobayashi, K. *Chem. Lett.* **1998**, 1493−1494.
(10) (a) Kobayashi, K.; Tsuchida, A.; Usui, T.; Akaike, T. *Macromolecules* **1997**, *30*, 2016−2020. (b) Matsuura, K.; Tsuchida, A.; Okahata, Y.; Akaike, T.; Kobayashi, K. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2973−2977.
(11) (a) Shinohara, Y.; Sota, H.; Gotoh, M.; Hasebe, M.; Tosu, M.; Nakao, J.; Hasegawa, Y.; Shiga, M. *Anal. Chem.* **1996**, *68*, 2573−2579. (b) Mann, D. A.; Kanai, M.; Maly, D. J.; Kiessling, L. L. *J. Am. Chem. Soc.* **1998**, *120*, 10575−10582.
(12) Yu, S.; Kitajima, K.; Inoue, S.; Khoo, K.-H.; Morris, H. R.; Dell, A.; Inoue, Y. *Glycobiology* **1995**, *5*, 207−218.

10.1021/ja000672p CCC: $19.00
Published on Web 07/18/2000

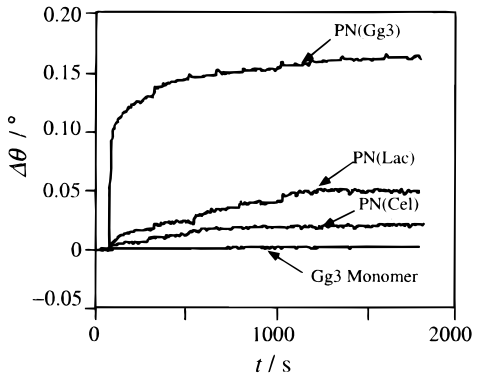

Figure 2. Typical time courses of the angle change ($\Delta\theta$) of SPR responding to the addition of glycoconjugate polystyrenes and a monomeric substance (1.0 μM) from an aqueous solution to the GM3 monolayer at 25 °C.

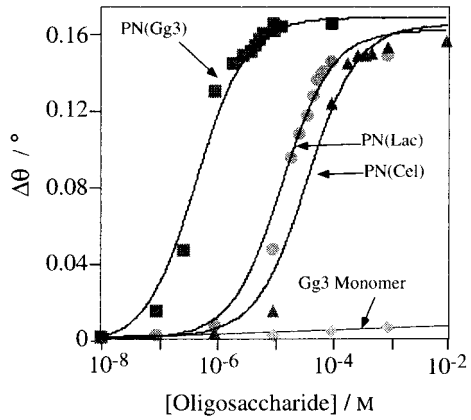

Figure 3. Saturation binding curves of glycoconjugate polystyrenes and a monomeric substance to the GM3 monolayer depending on the oligosaccharide concentrations at 25 °C. The solid curves are theoretical ones, according to the Langmuir equation using the parameters in Table 1.

and Gg3-carrying styrene monomer at the same oligosaccharide concentration (1.0 μM). 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.[13] The GM3 monolayer recognized strongly the Gg3-trisaccharide clusters of glycopolystyrenes in water.

In Figure 3 are plotted the angle changes ($\Delta\theta$) at the equilibrium binding to the GM3 monolayer against the concentration per oligosaccharide unit of glycopolymers. All of these glycopolymers gave typical Langmuir-type binding isotherms and similar maximum angle changes. It is suggested that these glycopolymers are adsorbed as monolayers in similar morphologies, although their affinities are quite different. Apparent affinity constants per oligosaccharide unit (K_a) and maximum angle changes ($\Delta\theta_{max}$) were calculated from the slopes and intercepts according to eq 1

$$\frac{[S]}{\Delta\theta} = \frac{[S]}{\Delta\theta_{max}} + \frac{1}{\Delta\theta_{max}K_a} \quad (1)$$

where [S] stands for the concentration of the oligosaccharide unit of the glycopolymers. Although the interaction of these polymers with the monolayer is probably complex mechanistically, the present data can be fit to the simple Langmuir-type equation with a single affinity constant. Hence, such a simple treatment is actually valid to evaluate the apparent affinity in the present multivalent carbohydrate-carbohydrate interaction as reported for several polymeric recognition system.[14]

Table 1. K_a, $-\Delta G$, and $\Delta\theta_{max}$ in Carbohydrate−Carbohydrate Interaction between Glycolipid Monolayers and Glycopolystyrenes at 25 °C

monolayer	polymer	K_a 10^4 M^{-1} [a] (10^6 M^{-1})[b]	$-\Delta G$ kcal mol^{-1} [a]	$\Delta\theta_{max}$ mdeg
GM3	PN(Gg3)	250 (110)	8.7	168
	PN(Lac)	7.7 (3.2)	6.7	152
	PN(Cel)	4.4 (3.2)	6.3	156
LacCer	PN(Gg3)	65 (28)	7.9	139
	PN(Lac)	7.7 (3.2)	6.7	26
	PN(Cel)	6.1 (4.4)	6.5	15
GlcCer	PN(Gg3)	24 (10)	7.3	142
	PN(Lac)	4.1 (1.7)	6.3	186
	PN(Cel)	3.9 (2.8)	6.3	77
(KDN)GM3	PN(Gg3)	25 (11)	7.4	232
	PN(Lac)	5.9 (2.5)	6.5	101
	PN(Cel)	2.6 (1.9)	6.0	104
ceramide	PN(Gg3)	2.9 (1.2)	6.1	151
	PN(Lac)	0.012 (0.005)	2.8	223
	PN(Cel)	0.027 (0.019)	3.3	66

[a] On the basis of the concentration of oligosaccharide unit. [b] On the basis of the polymer concentration.

Table 1 summarizes K_a, $-\Delta G$ (= $RT \ln K_a$ at 25 °C), and $\Delta\theta_{max}$ values in various combinations between glycolipid monolayers and glycopolymers. K_a values calculated on the basis of the polymer concentration are represented in parentheses, which emphasize the amplified polyvalent interaction of the glycopolymer molecules. The following discussion is made on the apparent K_a values on the basis of the concentration of the oligosaccharide unit. PN(Gg3) was bound to the GM3 monolayer with $K_a = 2.5 \times 10^6$ M^{-1}, whereas PN(Lac) and PN(Cel) were bound to the GM3 monolayer with $K_a = 7.7 \times 10^4$ M^{-1} and 4.4×10^4 M^{-1}, respectively. Thus, the affinity of PN(Gg3) to the GM3 monolayer was about 30 and 60 times those of PN(Lac) and PN(Cel), respectively. The difference in affinity constants corresponded to 2.0−2.4 kcal mol^{-1} of free energy gap at 25 °C. The affinity constant of PN(Gg3) to the lipids was decreased in the order of GM3 > LacCer > GlcCer ≈ (KDN)GM3 ≫ ceramide. The order of GM3, LacCer, and GlcCer was the same as those of carbohydrate−carbohydrate interaction estimated qualitatively with use of liposomes and cells by Hakomori et al.[2a] It is interesting to note that PN(Gg3) was bound to the (KDN)GM3 monolayer more weakly than to GM3 by a factor of about 10, and its binding strength was similar to that of the combination of PN(Gg3)−GlcCer. This result suggests that the NHAc group of *N*-acetylneuraminic acid in GM3 is essential in carbohydrate−carbohydrate interaction between GM3 and Gg3. PN(Lac) and PN(Cel) were bound to these glycolipid monolayers less strongly ($K_a \approx 10^4$ M^{-1}) and less selectively. Therefore, the strong adsorption of PN(Gg3) to the GM3 monolayer is attributable to complementary carbohydrate−carbohydarate interaction.

In conclusion, we have achieved for the first time the quantitative estimation of carbohydrate−carbohydrate interaction between the GM3 monolayer and Gg3-containing polymer by SPR. PN(Gg3) was selectively bound to the GM3 monolayer with $K_a \approx 10^6$ M^{-1}, whereas its monomeric substance was not adsorbed and PN(Lac) and PN(Cel) had little selectivity to the glycolipid monolayers. It was revealed that the NHAc group of *N*-acetylneuraminic acid in GM3 plays an important role in carbohydrate-carbohydrate interaction between GM3 and Gg3. This methodology will be useful to elucidate the mechanism of carbohydrate−carbohydrate interaction.

JA000672P

(13) Poly(ethylene glycol) little adsorbed onto GM3 monolayer even at 100 μM/monomeric unit.

(14) Sigal, G. B.; Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 3789−3800.