Carbohydrate-Carbohydrate Interaction between Glycolipids and Glycoconjugate Polystyrenes at the Air-water Interface

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Carbohydrate-carbohydrate interaction at the air-water interface was investigated by π-A isotherms of GM3 (ganglioside monosialate 3, NeuAcα2-3Galβ1-4Glcβ1-Cer) and LacCer (lactosylceramide, Galβ1-4Glcβ1-Cer) in the presence of glycoconjugate polystyrenes bearing Gg3 oligosaccharide (GalNAcβ1-4Galβ1-4Glc), lactose, and cellobiose.

Oligosaccharide chains of glycoproteins and glycolipids at cell membranes participate in cell-cell communications via their binding to proteins such as lectins, antibodies, toxins, and glycosyltransferases.[1] Hakomori *et al.* suggested that not only carbohydrate-protein interaction but also carbohydrate-carbohydrate interaction play an important role in cellular recognition, particularly Le^x-Le^x interaction in compaction in embryogenesis and Gg3 (gangliotriaosylceramide)-GM3 interaction between lymphoma and melanoma cells.[2] Attention has been paid recently to the investigation carbohydrate-carbohydrate interactions by applying electrospray ionization mass spectroscopy[3] and NMR titration[4] using sugar-bearing model substances.

We have developed glycoconjugate polystyrenes which are useful as cell-specific culture substrata, artificial antigens, and targetted drug delivery systems.[5] These polymers possess highly concentrated oligosaccharide chains which are attached to every repeating units along the hydrophobic polystyrene main chains. Numerous multi-antennary or clustered oligosaccharide chains were reported to enhance the binding to various proteins.[6] We expect that the clustered oligosaccharide chains along these glycoconjugate polystyrenes can amplify carbohydrate-carbohydrate interaction. This paper reports the interaction at the air-water interface between glycosphingolipids in Langmuir

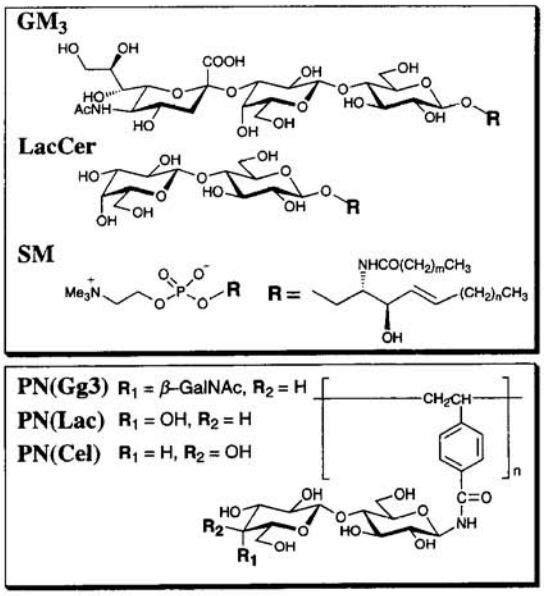

Figure 1. Structures and abbreviations of lipids and glycoconjugate polystyrenes used in this study.

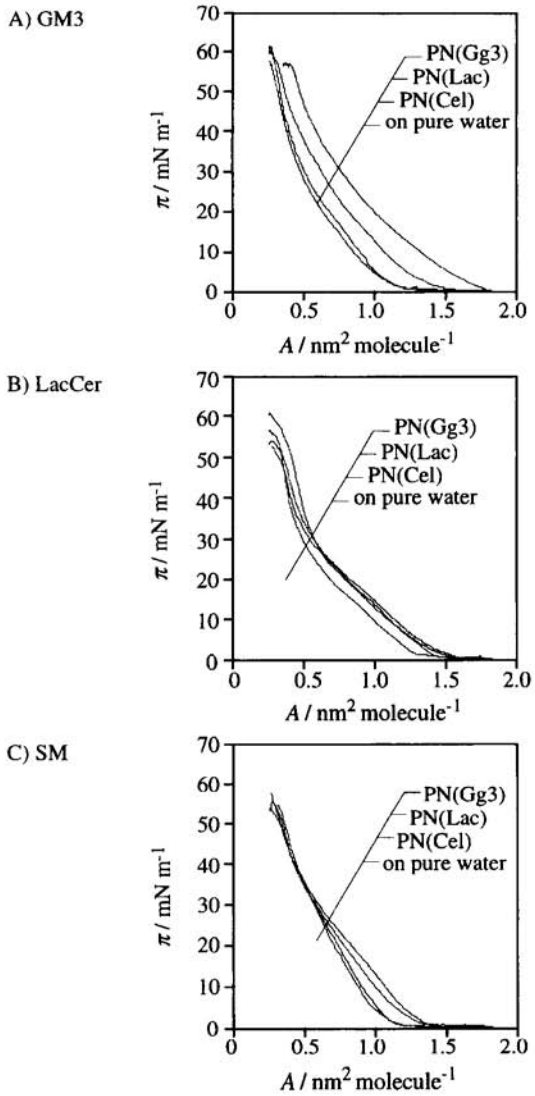

Figure 2. π-A isotherms of GM3(A), LacCer(B), and SM(C) in the absence and presence of glycoconjugate polystyrenes (1×10^{-8} M) in subphase at 25 °C.

monolayer[7] and glycoconjugate polystyrenes in subphase using π-A isotherms and quartz crystal microbalance (QCM). These were known as useful procedures for molecular recognition at air-water interface.[8]

Figure 1 illustrates the structures and abbreviations of sphingolipids and glycopolymers used in this paper. Gg3 trisaccharide was preparated by deprotection of the 2-(trimethylsilyl)ethyl glycoside derivative.[9] Poly(*N*-β-glycosyl-4-vinylbenzamide)s [PN(Gg3), PN(Cel), and PN(Lac)] were prepared by the previously reported method.[5b] The molecular weights of PN(Gg3), PN(Cel), and PN(Lac) were estimated to be $M_n = 2.9 \times 10^4$ ($M_w / M_n = 1.5$), $M_n = 3.4 \times 10^4$ ($M_w / M_n = 3.0$), and $M_n = 2.0 \times 10^4$ ($M_w / M_n = 1.8$) respectively by size exclusion chromatography (SEC) using pullulan as standards and water as eluent. π-A Isotherms of sphingolipids in the absence and presence of glycopolymer at the concentration of 1×10^{-8} M were measured with a Miyata-type moving wall trough (Nippon Laser & Electronics Lab., Nagoya).

Figure 2A shows that GM3 monolayer was expanded significantly by PN(Gg3) and PN(Lac), but little by PN(Cel) over the whole range of surface pressure. The increment of molecular area by PN(Gg3) was about twice the increment by PN(Lac). According to Figure 2B, LacCer monolayer was expanded by these glycopolymers to similar extent under 35 mN/m, and then above 35 mN/m by PN(Gg3) > PN(Lac) > PN(Cel), although the increments were smaller than those of GM3. However, sphingomyelin(SM) monolayer used as a control lipid was not expanded by these glycopolymers above 30 mN/m (Figure 2C). These π-A isotherms were reproducible.

These glycoconjugate polymers had little surface activity themselves at the concentration of 1×10^{-8} M. Thus it is reasonable to assume that the expansion of the monolayer membranes was caused by the carbohydrate-carbohydrate interaction between the clustered oligosaccharides chains on the glycosphingolipid membrane and the clustered oligosaccharides chains of glycopolymers in subphase. GM3 monolayer interacted strongly with PN(Gg3), weakly with PN(Lac), and scarcely with PN(Cel). LacCer monolayer was bound nonspecifically to these polymers at low surface pressure, while specifically and weakly to PN(Gg3) at higher surface pressure. It is suggested that, since the surface pressure of plasma membranes is about 30 mN/m,[7b] Gg3 recognized not only GM3 glycolipid cluster, but also LacCer glycolipid cluster in vivo. These results are consistent with carbohydrate-carbohydrate interaction using cells and liposomes reported by Hakomori *et al.*[2b,c]

Adsorption amount of the glycoconjugate polymers on GM3 was estimated with a quartz-crystal microbalance (QCM) which is useful to detect molecular adsorptions onto substrates in ng-order.[7b] QCM employed was a commercially available 9 MHz AT-cut quartz (diameter 9 mm) deposited with Au electrodes on both sides. The GM3 monolayer was transferred onto the QCM plate by horizonal lifting method at a surface pressure of 30 mN/m in the presence and absence of the glycopolymers (1×10^{-6} M) in the subphase after equilibration for 20 min. The frequency change of QCM was measured in air. The amount of polymers adsorbed to GM3 and also the molar ratio of the oligosaccharides in glycopolymer and GM3 were calculated and summarized in Table 1. PN(Gg3) and PN(Lac) were adsorbed on GM3 monolayer but little PN(Cel) was adsorbed. The adsorption amount as well as the ratio of PN(Gg3) was much larger than those of PN(Lac). In agreement with the π-A isotherm experiments, it is confirmed that the expansion of GM3 monolayer caused by PN(Gg3) and PN(Lac) at the air-water interface was attributable to the specific adsorption via carbohydrate-carbohydrate interaction.

Table 1. The amount of glycoconjugate polymers adsorbed on GM3 monolayer at 30 mN/m

Polymer [a]	Adsorption amount [b] / ng	[Adsorbed polymer] [c] / [GM3]
PN(Gg3)	74	0.78
PN(Lac)	26	0.11
PN(Cel)	3	0.001

[a] [Polymer] = 1×10^{-6}M of oligosaccharide unit.

[b] Adsorption amount was evaluated by subtracting frequency change in the absence of polymer from that in the presence of polymer in subphase after equilibration for 20 min.

[c] Carbohydrate molar ratio of adsorbed polymers and GM3 monolayer.

In conclusion, we could detect carbohydrate-carbohydrate interaction between glycolipids and glycoconjugate polystyrenes at the air-water interface by π-A isotherms and QCM. The interaction was decreased in the order of GM3-PN(Gg3) > GM3-PN(Lac) > LacCer-PN(Gg3) combinations. These methods will be useful to investigate the quantitative analysis, elucidation of mechanism, and detection of novel carbohydrate combination in carbohydrate-carbohydrate interaction.

References

1 M. Fukuda and O. Hindsgaul, "Molecular Glycobiology," IRL, Oxford(1994).

2 a) I. Eggenst, B. Fenderson, T. Toyokuni, B. Dean, M. Stroud, and S. Hakomori, *J. Biol. Chem.*, **264**, 9476(1989). b) N. Kojima and S. Hakomori, *J. Biol. Chem.*, **264**, 20159(1989). c) N. Kojima, M. Shiota, Y. Sadahira, K. Handa, and S. Hakomori, *J. Biol. Chem.*, **267**, 17264(1992).

3 K. M. Koshy and J. M. Boggs, *J. Biol. Chem.*, **271**, 3496(1996).

4 J. J. Barbero, E. Junquera, M. M. Pastor, S. Sharma, C. Vicent, and S. Penades, *J. Am. Chem. Soc.*, **117**, 11198(1995).

5 a) K. Kobayashi, H. Sumitomo, and Y. Ina, *Polym. J.*, **17**, 567(1985). b) K. Kobayashi, A. Tsuchida, T. Usui, and T. Akaike, *Macromolecules*, **30**, 2016(1997). c) A. Kobayashi, T. Akaike, K. Kobayashi, and H. Sumitomo, *Macromol. Chem. Rapid Commun.*, **7**, 645(1986). (d) K. Kobayashi, A. Kobayashi, S. Tobe, and T. Akaike, in "Neoglycoconjugates: Preparation and Applications," ed by Y. C. Lee and R. T. Lee, Academic Press, San Diego(1994), p. 262.

6 a) "Neoglycoconjugates: Preparation and Applications," ed by Y. C. Lee and R. T. Lee, Academic Press, San Diego(1994). b) T. Furuike, N. Nishi, S. Tokura, and S. Nishimura, *Macromolecules*, **28**, 7241(1995). c) D. Zanini and R. Roy, *J. Am. Chem. Soc.*, **119**, 2088(1997). d) D. D. Manning, X. Hu, and L. L. Kiessling, *J. Am. Chem. Soc.*, **119**, 3161(1997). e) S. Choi, M. Mammem, and G. M. Whitesides, *J. Am. Chem. Soc.*, **119**, 4103(1997).

7 a) B. Maggio, *Prog. Biophys. Molec. Biol.*, **62**, 55(1994). b) T. Sato, T. Serizawa, and Y. Okahata, *Biochem. Biophys. Res. Commun.*, **204**, 551(1994).

8 K. Ariga and T. Kunitake, *Acc. Chem. Res.*, **31**, 371(1998)

9 K. Hotta, S. Komba, H. Ishida, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, **13**, 665(1994).