Selective Bindings of a Lectin for Phase-separated Glycolipid Monolayers

Mineo Hashizume, Toshinori Sato,* and Yoshio Okahata*
Department of Biomolecular Engineering, Tokyo Institute of Technology, Nagatsuta 4259, Midori-ku, Yokohama 226-8501

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Phase separation was observed by measuring surface pressure (π)-area (A) isotherms of the mixed monolayer of lactosylceramide (LacCer) and dioleoylphosphatidylcholine (DOPC). Bindings of *Allomyrina dichotoma* (allo A) lectin to LacCer in mixed monolayers were measured by means of quartz-crystal microbalance (QCM) that was attached horizontally to the monolayers. Allo A showed the high binding affinity for the LacCer phase-separated in the DOPC monolayer, but showed the low binding affinity for the LacCer homogeneously mixed with dipalmitoylphosphatidylcholine (DPPC) matrix.

Specific recognitions of lectins, antibodies, and viruses against oligosaccharides of glycolipids or glycoproteins on biomembranes, are very important part in cell events. Factors that modulate the carbohydrate recognitions of glycolipids are sugar sequences of glycolipids, matrix lipids surrounding the glycolipids, and so on. In the previous papers,[1-3] we have demonstrated that bindings of WGA lectin and influenza virus to ganglioside GM_3 were affected by the chemical structures of hydrophilic part of matrix lipids.

Tillack *et al.* demonstrated from immunoelectron microscopy that glycosphingolipids (globosides) are existed as clusters on cell surface membranes.[4] If the phase separation of glycosphingolipid from matrix phospholipid can be confirmed by a model membrane, it is possible to give a useful support for a domain formation of glycosphingolipids on the cell surface. In this communication, we investigated the molecular miscibility of LacCer in DOPC or DPPC matrix monolayers, and the specific binding of allo A lectin to LacCer in the phase-separated or the homogeneously-mixed monolayer.

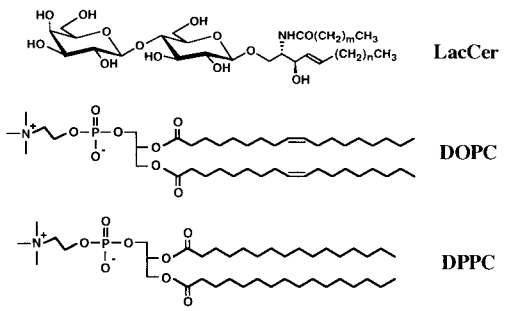

Figure 1. Chemical structures of lipids employed in this study.

LacCer and DPPC were obtained from Snow Brand Milk Products Co., Ltd. and Nichiyu Liposome Co., Ltd., respectively. DOPC was purchased from Sigma Co., Ltd. (Figure 1). Allo A lectin from beetle (*Allomyrina dichotoma L.*, Mw = 67000) was purchased from COSMO BIO Co., Ltd. Those were used without further purification. Each lipid was dissolved in a mixed solvent (spectrum grade) of chloroform:ethanol = 4:1.

Measurements of π-A isotherms of LacCer/DOPC and LacCer/DPPC mixed monolayers were carried out by using a Teflon-coated trough with a microcomputer-controlled Teflon barrier (USI Co., Fukuoka). Figure 2 shows π-A isotherms of the LacCer/DOPC mixed monolayers in 10 mM phosphate buffer (pH 7.2) at 20 °C. Figure 3 shows the dependence of the LacCer content on the collapse pressures of the mixed monolayers. If LacCer and DOPC or DPPC were

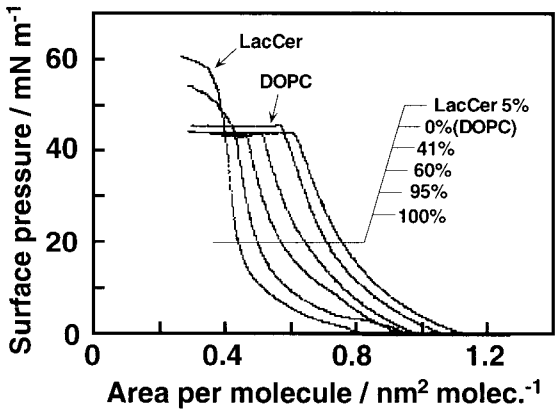

Figure 2. π-A isotherms of LacCer/DOPC mixed monolayers.

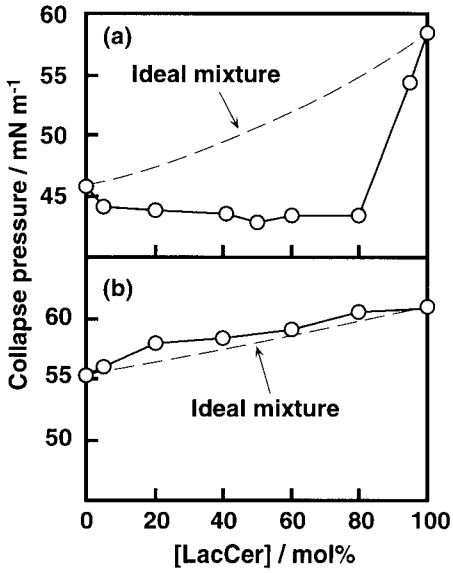

Figure 3. Collapse pressures of mixed monolayers as a function of LacCer content. Dashed lines represent theoretical values in the case of ideal surface mixtures (Reference 5). (a) LacCer/DOPC and (b) LacCer/DPPC mixed monolayers.

homogeneously mixed, the collapse pressures of the mixed monolayers will be represented as dashed lines.[5] In Figure 3a, however, the observed collapse pressures of the LacCer/DOPC mixed monolayers were deviated from the theoretical values, and were almost same values with that of simple DOPC monolayer when LacCer content was less than 80 mol%. This result suggested that LacCer and DOPC were phase-separated in the mixed monolayer. On the other hand, collapse pressures of the LacCer/DPPC mixed monolayers were very close to theoretical values, which indicated LacCer was homogeneously mixed with the DPPC matrix (see Figure 3b).

Binding behaviors of allo A lectin to LacCer in the mixed monolayers were investigated by a QCM method that was described previously (see an illustration of Figure 4).[1-3] A 27 MHz QCM (Showa Crystals Co., Chiba) was attached horizontally to the mixed monolayer at a surface pressure of 30 mN m^{-1}. Then frequency changes of the QCM responding to the addition of 20 ppm (3×10^{-7} M) allo A were followed with time. The binding amount (Δm) was obtained from the frequency decrease. Figure 4 shows the Δm of allo A for the LacCer/DOPC and the LacCer/DPPC mixed monolayers as a function of LacCer contents. The Δm values for the phase-separated LacCer/DOPC mixed monolayers were higher than those for the homogeneously-mixed LacCer/DPPC monolayers, independent on the LacCer contents. It is considered that the allo A lectin showed high affinity for the clustered LacCer. This finding about the binding properties of allo A may be supported by the results that allo A showed the maximum binding amount for 100 mol% LacCer monolayer.

Allo A is known as the lectin that binds specifically β-D-galactose residue.[6] In the present study, it was found that allo A strongly recognized the clustered galactose residues. These results suggest that allo A multivalently binds to galactose residues on a membrane surface.

Many studies on the effect of hydrophobic part of matrix lipids on recognition of glycolipids have been carried out by using liposome techniques.[7] However, the correlations between membrane structures and molecular recognition have not been discussed. In the present study, π-A isotherms gave information about molecular miscibility of the two kinds of lipids in the mixed monolayers. Furthermore, lectin binding affinity was quantitatively evaluated by a QCM method. By using these methodologies, we could demonstrate that LacCer was phase-separated from phospholipids having unsaturated fatty acids, and the phase separation of LacCer strongly affected its receptor function against lectin. These phenomena are expected to be an important knowledge for the receptor function of glycosphingolipids on cell surface.

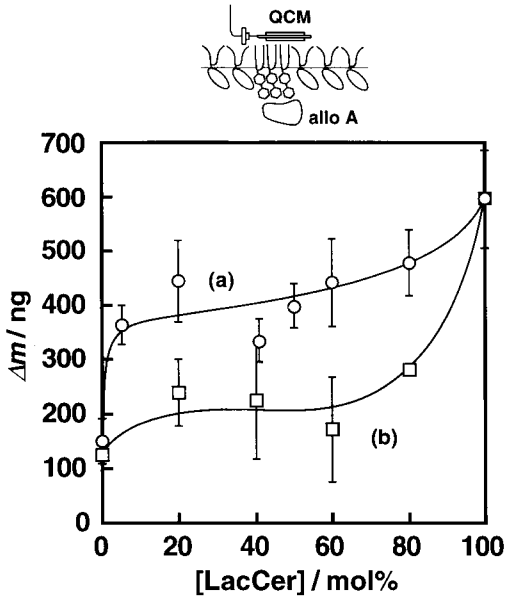

Figure 4. Binding amount (Δm) of allo A to (a) LacCer/DOPC and (b) LacCer/DPPC mixed monolayers as a function of LacCer contents.

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