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Small-Angle X-Ray Scattering Study of Ganglioside / Dipalmitoylphosphatidylcholine Mixture

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By using a small-angle X-ray scattering technique, we have studied the structural properties of ganglioside/dipalmitoylphosphatidylcholine (DPPC) mixture in solutions. We have obtained the following evidences. A minor presence of ganglioside in the mixture strongly induces a structural change from a multilamellar vesicle to a unilamellar vesicle. The increase of ganglioside molar fraction in the mixture tends to stabilize and prevent the deformation of the vesicle structures against temperature elevation from 10 °C to 65°C, Combined with our previous results that the elevation of temperature induces the dehydration and bending of the ganglioside oligosaccharide chains, the present results suggest that ganglioside molecules are mostly located at the external side of the ganglioside/DPPC vesicle to reduce the radius of the vesicle and that in the temperature elevation process the bendings of the ganglioside sugar heads compete with chain-melting of the hydrophobic region of the vesicle to suppress a deformation of its structure.

Keywords: small-angle X-ray scattering; thermotropic behavior; ganglioside; dipalmitoylphosphatidylcholine

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INTRODUCTION

Gangliosides, most abundant sialoglycosphingolipids in the plasma membrane of nerve cells, have been shown to play an important role in various cell surface events such as self-organization of tissues, immune response and cell differentiation through a numerous variety of the oligosaccharide chains in the hydrophilic polar heads of ganglioside molecules [1]. Such physiological functions are assumed to be attributable to physicochemical characteristics of ganglioside molecules. Hence we have been studied the structural properties of ganglioside micellar aggregates under various conditions [2-6] and a specificity of the complexions of gangliosides with proteins [7]. As shown in our previous reports, the elevation of temperature (10-65 °C) sensitively changes the conformations of the oligosaccharide chains of gangliosides, which accompanies the changes in both the surface charge and hydration of the micelles with a thermal reversibility [5, 6]. These results suggest that ganglioside aggregates can modulate both cell-surface charge and hydrophilicity, which are quite interesting because such a function is closely related to the physiological functions of gangliosides in cells.

Recent studies show that gangliosides form microdomains called "raft" structures in outer cell membranes, which is assumed to be important for physiological functions of gangliosides such as inducers of signal transduction [8, 9]. Ganglioside/phospholipid mixtures can be regarded as a model system of cell membrane in vitro, and many studies have been done on thermotropic phase behaviors of the mixtures using differential scanning calorimetry [10-12]. To elucidate directly structural properties of ganglioside/phospholipids mixtures, we have carried out small-angle X-ray scattering experiments of ganglioside/dipalmitoylphosphatidylcholine mixtures.

EXPERIMENTAL

Ganglioside used was Type-III ganglioside (containing 20% sialic acids) from Sigma Chemical Co. [13]. The compositions of ganglioside molecular species in Type-III were checked by thin-layer chromatography [4]. L-α-dipalmitoylphosphatidylcholine (DPPC) was purchased from Avanti Polar Lipids. Ganglioside/DPPC vesicles were prepared according to the method described by Sillerud *et al.* [8] with some modifications. The Type-III ganglioside powder was mixed with the DPPC powder in the chloroform/ methanol mixture solvent (1:1, v/v). The mixture solutions were dried under a nitrogen flow and kept *in vacuo* for 9 hour at 45 °C. The dried mixtures were suspended again in 50 mM Hepes buffer (pH 7.0), warmed to ~50 °C, and vortexed at this temperature. Here we employed two different sonication techniques. One was a moderate method using a bath ultra-sonicator (Model SUS-103 of SIMAZU Co.) for 30 minutes at 25 °C at 100 W. The other was done by using a probe-type ultra-sonicator (Model UH-50 of SMT Co.) for 20 minutes at 50°C at 50 W. These sonicated solutions were incubated for 2 hours at 45 °C, and then annealed at 4 °C for ~24 hours before scattering measurements. The molar fraction of [ganglioside]/[DPPC] ≡ [G]/[D] in the mixture was varied from 0/1 to 0.3/1, where the DPPC concentration was fixed at 1 % w/v.

Synchrotron radiation small-angle X-ray scattering (SR-SAXS) measurements were performed by using a solution scattering spectrometer installed at the Photon Factory of the High Energy Accelerator Research Organization, Tsukuba, Japan [15]. The X-ray wavelength, the sample-to-detector distance and the exposure time were 1.49 Å, 190 cm and 480 seconds, respectively. The temperature of the samples was elevated stepwise from 10 °C to 65 °C with the interval of 5 °C, which was monitored by using a thermocouple device attached directly to the sample cell.

RESULTS AND DISCUSSION

Figure 1 shows the scattering curves of ganglioside/DPPC mixtures with various molar fractions, where 1(A) and 1(B) correspond to the mixtures prepared by using the bath and probe-type sonicators, respectively. Under the moderate sonication condition in Figure 1(A), evident two peaks exist at around $q = 0.1$ Å^{-1} and 0.19 Å^{-1} in the scattering curve of (h), which are attributed to the DPPC multilamellar packing. Above the molar fraction of

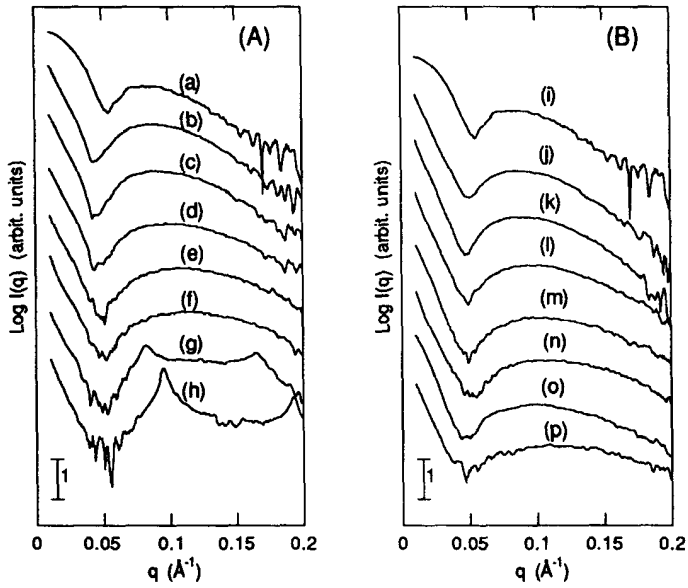

Figure 1. Variation of scattering curves of the ganglioside/DPPC mixture by using bath-type sonicator (A) and probe-type sonicator (B), respectively. The molar fractions of [ganglioside]/[DPPC] mixture are (a) and (i), pure ganglioside; (b) and (j), 0.3/1; (c) and (k), 0.2/1; (d) and (l), 0.1/1; (e) and (m), 0.04/1; (f) and (n), 0.02/1; (g) and (o), 0.01/1; (h) and (p), pure DPPC.

[G]/[D] = 0.02/1, these peaks disappear completely, indicating that even a minor presence of ganglioside strongly induces a drastic change of a multilamellar structure to form a unilamellar vesicle. In Figure 1(B) using the probe-type ultra-sonication method, the DPPC dispersion already forms unilamellar vesicles. Except for the mixtures with low molar fractions of gangliosides, the dependence of the scattering curve on the [G]/[D] ratio is almost same in both the sonication methods. Namely, with increasing the molar fraction of gangliosides, the vesicle radius of the mixture becomes smaller gradually to approach to a ganglioside micellar radius.

Figure 2 shows the temperature dependence of the scattering curve of the pure DPPC unilamellar vesicle. In Figure 2 the scattering curve starts to change in the range of 30-35 °C, and drastically changes at ~40 °C. These transition temperatures well agree with the previous evidences that

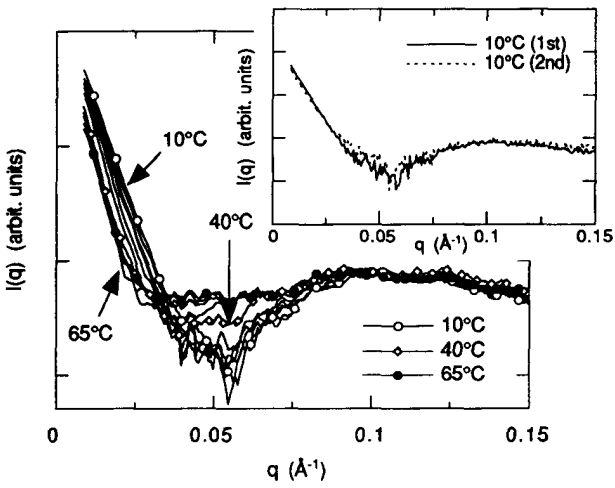

Figure 2. Thermotropic change of the scattering curve of the pure DPPC vesicle. The insert shows the comparison between the scattering curves at 10 °C before and after heating.

the bilayer and the multilamellar vesicle of DPPC indicate the phase transitions at 33.5 °C (pre-transition) and at 41.5°C (main-transition) in water [15, 16]. In the range of 45-65 °C the minimum at q = ~0.05 Å^{-1} disappears, indicating an occurrence of some polydispersity in the vesicle system. The insert of Figure 2(a) shows a high thermal reversibility of the structural transition. Some fusion seems to cause a serious thermal hysteresis of the vesicle structure, therefore, the polydispersity would occur mainly in the vesicle shape, not in the vesicle size. Our dynamic light scattering measurements also support the above result. The z-average hydrodynamic diameter and size distribution of the same sample mostly hold over the temperature range from 10 °C to 65 °C (data not shown). Thus, the change of the scattering curve in Figure 2 indicates that the thermal deformation of the vesicles is greatly enhanced by the hydrocarbon chain motions especially above the chain-melting temperature.

Figure 3 shows the temperature dependence of the scattering curves of the mixtures with various [G]/[D] fractions which were prepared using the probe-sonicator. We can recognize an evident difference in the changing tendencies of the scattering curves depending on [G]/[D] fraction. The temperature elevation induces a drastic change of the scattering curve between 40-45 °C of [G]/[D] = 0.04/ 1 and 0.1/1. On the other hand, the scattering curve of [G]/[D] = 0.3/1 varies rather gradually. Alternatively, the increase of [G]/[D] fraction significantly smears the transition temperature and reduces a drastic change of the vesicle structure. The presence of the evident hollows in the scattering curves around q = 0.05 Å^{-1} at 10 °C reflect a high monodispersity in both size and shape of the particles in solutions [2]. According to our dynamic light scattering measurements, the z-averaged diameters of the above mixtures are slightly increased with elevating temperature (data not shown), which is reflected in the shifts of the hollow positions to a small-q region with elevating temperature. In the temperature elevation process, the chain-melting transition of the hydrocarbon chains of DPPC and ganglioside molecules

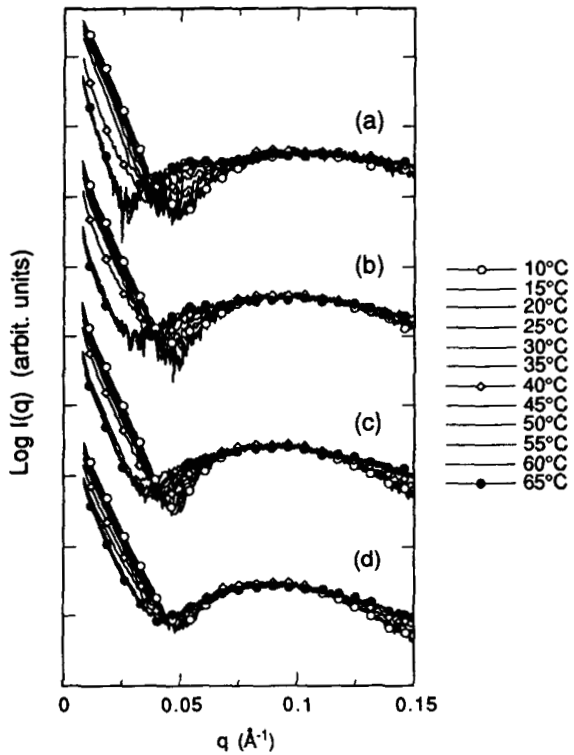

Figure 3. Thermotropic changes of scattering curves of the ganglioside/DPPC mixture. The molar fractions of [ganglioside]/[DPPC] mixture are (a) 0.04/1; (b) 0.1/1; (c) 0.2/1; (d) 0.3/1.

would tend to increase the vesicle radius. On the other hand, the dehydration and bending of the gangliosides sugar heads, which we found previously [2-6], affect to reduce the vesicle radius. These two different effects would be in competition with each other and modulate the thermal phase transitions. It should be mentioned a quite important difference in the transitions between the pure DPPC and mixture vesicles. For all cases of

the ganglioside/DPPC mixtures, even at far above the main transition temperature an extent of the deformation of the vesicle structure is smaller than that of the pure DPPC vesicles. In other words, it shows that the presence of the gangliosides acts to maintain the vesicle structure at an initial state against temperature variation.

In conclusion, the present results show that gangliosides significantly affect a vesicle curvature and strongly stabilize vesicle structure against temperature variation. These effects of gangliosides on the vesicle structures are essentially attributable to the low values of the packing parameters of ganglioside molecules owing to the presence of large sugar heads. According to the present and our previous results [2-6], we can assume that the gangliosides are favorably located at the external side of the ganglioside/DPPC vesicle so that the gangliosides are subject to formation of "raft" in outer cell membranes. The formation of "raft" containing amount of gangliosides would not only modulate a local hydrophilicity and charge distribution of a cell surface but also stabilize a membrane structure.

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References

[1] L. Svennerholm, A.K. Asbury, R.A. Reisfeld, K. Sandhoff. K. Suzuki, G. Tettamanti. G. Toffano, *Biological Function of Gangliosides*,Elsevier, Amsterdam (1994).

[2] M. Hirai, T. Takizawa, S. Yabuki, Y. Nakata and K. Hayashi, *Biophys. J.*, **70**, 1761 (1996).

[3] M. Hirai, T. Takizawa, S. Yabuki, T. Hirai and K. Hayashi, *J. Phys Chem.* **100**, 11675 (1996).

[4] M. Hirai, T. Takizawa, S. Yabuki and K. Hayashi, *J. Chem. Soc., Faraday Trans.* **92**, 4533 (1996).

[5] M. Hirai and T. Takizawa, *Biophys. J.* **74**, 3010 (1998).

[6] M. Hirai, H. Iwase and T. Hayakawa, *J. Phys. Chem. B* **103**, 10136 (1999).

[7] M. Hirai, H. Iwase, S. Arai, T. Takizawa, K. Hayashi, *Biophys. J.* **74**, 1380 (1998).

[8] K. Simons, and E. Ikonen, *Nature*, **387**, 569 (1997).

[9] S. Hakomori, S. Yamamura, and K. Handa, *In* Sphingolipids as signaling modulators in the nervous system; R.W. Ledeen, S. Hakomori, A. Yates, J.S. Scheider, R.K. Yu, Eds.; The New York Academy of Sciences, New York, 1 (1998).

[10] L.O. Sillerud, D.E. Schafer, R.K. Yu and W. Konigsberg, *J. Biol. Chem.*, **254**, 10876 (1979).
[11] B. Maggio, T. Ariga, J.M. Sturtevant and R.K. Yu, *Biochim. Biophys. Acta*, **818**, 1 (1985).
[12] M. Masserini and E. Freire, *Biochemistry*, **25**, 1043 (1986).
[13] D.B. Gammack, *Biochem. J.*, **88**, 373 (1963).
[14] T. Ueki, Y. Hiragi, M. Kataoka, Y. Inoko, Y. Izumi, H. Tagawa and Y. Muroga, *Biophys. Chem.*, **23**, 115 (1985).
[15] H.J. Hinz and J.M. Sturtevant, *J. Biol. Chem.* **247**, 6071 (1972).
[16] S.C. Chen, J.M. Sturtevant and B.J. Gaffney, *Proc. Natl. Acad, Sci. USA*, **77**, 5060 (1980).