Morphology transition of raft-model membrane induced by osmotic pressure: Formation of double-layered vesicle similar to an endo- and/or exocytosis

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Abstract. The effect of osmotic pressure on the structure of large uni-lamellar vesicle (LUV) of the lipid mixtures of monosialoganglioside (GM1)-cholesterol-dioleoyl-phosphatidylcholine (DOPC) was studies by using wide-angle X-ray scattering (WAXS) method. The molar ratios of the mixtures were 0.1/0.1/1, 0/0.1/1, and 0/0/1. The ternary lipid mixture is a model of lipid rafts. The value of osmotic pressure was varied from 0 to 4.16x10^5 N/m^2 by adding the polyvinylpyrrolidone (PVP) in the range from 0 to 25 % w/v. In the case of the mixtures without GM1, the rise of the osmotic pressure just enhances the multi-lamellar stacking with deceasing the inter-lamellar spacing. On the other hand, the mixture containing GM1 shows the structural transition from a uni-lamellar vesicle to a double-layered vesicle (a liposome including a smaller one inside) by the rise of osmotic pressure. In this morphology transition the total surface area of the double-layered vesicle is mostly as same as that of the LUV at the initial state. The polar head region of GM1 is bulky and highly hydrophilic due to the oligosaccharide chain containing a sialic acid residue. Then, the present results suggest that the existence of GM1 in the outer-leaflet of the LUV is essentially important for such a double-layered vesicle formation. Alternatively, a phenomenon similar to an endo- and/or exocytosis in cells can be caused simply by a variation of osmotic pressure.

1. Introduction
In this decade, the formation of lipid microdomain in mammalian plasma membrane, so-called a lipid raft [1], has been attracting intensive interests since lipid rafts are assumed to have functions as platforms of membrane-associated events such as signal transduction, cell adhesion, lipid/protein sorting and so on [1-3]. Although the steady-state existence, size, and shape of lipid rafts (liquid-ordered microdomains) in plasma membranes still remain the subject of debate, lipid rafts can be considered essentially as a dynamic assembly of a variety of lipids and proteins. As knowledge about lipid raft functions in cellular signaling has been accumulated, the present agreement has been reached on the fact that raft domains coalesce upon cross-linking to form signaling and sorting platforms [4]. A common feature of lipid rafts is their peculiar lipid composition, being rich in glycosphingolipids (GSLs), sphingomyelin and cholesterol. Gangliosides, major components of GSLs, are acidic lipids composed of a ceramide linked to an oligosaccharide chain containing one or more sialic acid residues, which are abundant in central nervous systems. Functions of lipid rafts are assumed to relate closely to the peculiar features of GSL molecules both in ceramide and oligosaccharide portions that can form complex hydrogen bonding networks (hydrogen bond donor and acceptor) [5].
By using small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS) and neutron spin-echo (NSE), we studied functional properties of gangliosides and aggregates of gangliosides with other lipids including raft-model membranes (ganglioside/cholesterol/phospholipid) under various conditions. In the case of ganglioside micelles the hydration of the polar head region sensitively changes depending on temperature with accompanying the conformational change of sugar chains [6-10] and the change of dissociation degree of sialic acids [11, 12]. Ganglioside-cholesterol binary mixtures show a maximum miscibility of cholesterol molecules against gangliosides, the cholesterol-dependent micelle-to-vesicle transitions [13], and the Ca\textsuperscript{2+}-induced vesicle-to-lamellar transitions accompanying the formation of an interdigitated structure between the sugar heads of gangliosides in the opposing bilayers [14]. In the case of small uni-lamellar vesicle (SUV) consisting of ganglioside-cholesterol-phospholipid ternary mixtures gangliosides and ganglioside-cholesterol rich regions show an asymmetric distribution at the outer leaflets of the vesicle bilayers [15, 16]. On the dynamics of the lipid aggregates observed by the NSE experiments, the dehydration and bending of the ganglioside sugar heads suppress the undulation of the micellar structure of gangliosides [17]. In the case of the SUVs of ganglioside-cholesterol-phospholipid ternary mixtures (as a model of intact neuronal cell membrane including lipid rafts), the bending modulus takes the smallest value at the lipid composition of [ganglioside]/[cholesterol]/[glycerophospholipid] \( \approx 0.1/0.1/1 \) that is a similar composition as in intact neuronal cell membrane including rafts [18]. The coexistence of ganglioside and cholesterol enhances the permeability of water across the bilayer by K\textsuperscript{+} ions [19]. The above results suggest that lipid rafts (especially ganglioside-cholesterol rich microdomains) can afford specific properties to membrane structure and dynamics that would affect functions of membrane proteins through the interaction between lipid and protein (hydrophobic coupling between a protein and a surrounding lipid bilayer) [20].

On the other hand, traffics in cells through a local deformation of membranes are known to take place in a highly crowded molecular environment where the total macromolecular concentration in a cytoplasm, for example, is approximately 350 mg/ml [21]. Structural stability and morphology transition of model biomembranes have been studied under a crowded molecular environment by using the so-called "osmotic stress" method. This method changes the osmotic pressure by adding a high molecular weight neutral polymer (osmolyte) into lipid aggregates to discuss about the hydration force between lipid bilayers [22-27]. As one of high molecular weight osmolytes, the use of PVP is well established [24] as well as dextran [23]. PVP is also used as a nontoxic additive in various industrial products such as medicines, cosmetics, etc. In the present study, we employed the osmotic stress method using PVP to investigate the effect of the change of osmotic pressure on the structure of large uni-lamellar vesicle (LUV) composed of monosialoganglioside (G\textsubscript{M1}) / cholesterol / dioleoylphosphatidylcholine (DOPC). We found the transition from the uni-lamellar vesicle to the double-layered lamellar vesicle depending on osmotic pressure. The present results indicate that under a crowded molecular environment such as a cytoplasm lipid-rafs are able to respond dynamically to a local perturbation of the osmotic pressure, suggesting a new insight on the functions of lipid-rafs from a physical point of view.

2. Material and methods

2.1. Materials

According to the method described elsewhere [28-31], G\textsubscript{M1} was extracted from bovine brain and purified by using a liquid column chromatography. A single band of G\textsubscript{M1} on the thin layer chromatography (TLC) plate (Kiesel gel 60; E. Merck, Darmstadt) was confirmed by a fluorometric method reported previously [31]. The purity determined by the TLC-densitometry of G\textsubscript{M1} used for the experiments was >95%. Cholesterol, DOPC, and polyvinylpyrrolidone (PVP, Mt. 40,000), purchased from SIGMA Chemical Co., were used without further purification. All other chemicals used were of analytical grade or better.
2.2. Preparation of LUV solutions

G_{M1}-cholesterol-DOPC LUVs were prepared as follows. G_{M1}, DOPC and cholesterol were separately dissolved in the chloroform/methanol mixture solvent (1/1 (v/v)). These solutions were mixed with the required molar ratios. After mixing, to remove the organic solvent, the lipid mixture solutions were dried up under a nitrogen stream and annealed in vacuo for overnight at 45 °C. The dried mixtures were dissolved in 10 mM HEPES (N-(2-hydroxymethyl) piperazine-N'-(2-ethane-sulfonic acid)) buffer (pH 7.0) to become 5 % w/v of total lipid concentration, and were vortexed for several minutes. Large unilamellar vesicle (LUV) solutions were prepared by an extrusion method using the Liposofast Basic extruder system (Avestin, Canada) with a polycarbonate filter (pore diameter of 50 nm from Nucleopore, Plesanton, USA). The above suspensions were subjected to ~40-times passes through the filter. The LUV suspensions with an odd number of passes were used for the X-ray scattering measurements to avoid a kind of contamination such as large multi-lamellar vesicles. The PVP solutions with different contents (10, 20, 30, 40, 50 % w/v) were also prepared by using the same HEPES buffer. The above G_{M1}-cholesterol-DOPC (or DPPC) LUV solutions and the PVP solutions were mixed by 1:1 in v/v. Finally, we obtained the LUV solutions served for the X-ray measurements. The values of the osmotic pressure at 5 %, 10 %, 15 %, 20 %, and 25 % PVP (w/v) are 1.27 x 10^{4}, 4.3 x 10^{4}, 1.09 x 10^{5}, 2.28 x 10^{5}, 4.16 x 10^{5} (N/m^{2}), respectively.

2.3. X-ray scattering measurements and analysis

Wide-angle X-ray scattering (WAXS) measurements were performed by using the spectrometer installed at BL-40B2 beam port of the 8 GeV synchrotron radiation source (SPring-8) at the Japan Synchrotron Radiation Research Institute (JASRI), Harima, Japan. Scattered X-ray was recorded by an imaging plate system of R-AXIS IV from RIGAKU Co. The X-ray wavelengths and the sample-to-detector distances were 0.75 Å for 51 cm camera length and 1.0 Å for 4089 cm camera length, respectively. Sample cells composed of a pair of thin-quartz windows with 1 mm path length were used. While the measurements, the sample solutions were moved oscillatory and slowly to avoid radiation damage on the sample due to the small beam size (~0.1 mm^{3}). Under the present measurement conditions, some radiation damages were negligible as reported previously [32, 33]. Small-angle scattering (SAXS) measurements were also performed by using the synchrotron radiation source (PF) at the High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. The X-ray wavelength used was 1.49 Å and the sample-to-detector distances were 80 cm and 190 cm. Scattered X-ray was recorded by the one-dimensional position-sensitive proportional-counter (PSPC) from RIGAKU Co. The exposure time for each measurement was 4 minutes at PF and 30 seconds at SPring-8. In the above SAXS and WAXS measurements, the temperatures of the samples were controlled at 25 °C.

The background correction of WAXS data was described in detail in elsewhere [33]. The distance distribution function p(r) was obtained by Fourier transform of the observed scattering intensity I(q) as

\[ p(r) = \frac{1}{2\pi^2} \int_0^\infty r q I(q) \sin(qr) dq \]  

where \( q = (4\pi\lambda)\sin(\theta/2) \), \( \theta \) and \( \lambda \) are the scattering angle and the X-ray wavelength. In the present model-fitting analysis, we used the scattering function \( I(q,R) \) of a multi-shelled spherical particle with a size-distribution as shown previously [16].

\[ I(q) = \int_{R_{\text{min}}}^R I_s(q,R)D(R)dR \]  

where \( R_{\text{min}} \) is a lower limit of particle radius, namely, in the present case the bilayer thickness of the LUV; \( D(R) \) is the number distribution function of the particle radius \( R \); \( I_s(q, R) \) is the spherical averaged scattering function of an ellipsoidal particle with the radius \( R \) composed of \( n \) shells (\( i \)-th shell
with average excess scattering density \( \bar{\rho}_i \) (so-called contrast), radius \( R_i \) and \( V_i \). \( I_s(q, R) \) and \( D(R) \) are given as follows.

\[
I_s(q, R) = \int_0^1 \left[ \bar{\rho}_1 V_1 j_1(q R_1)/(q R_1) + \sum_{i=2}^n (\bar{\rho}_i - \bar{\rho}_{i-1}) V_i j_i(q R_i)/(q R_i) \right]^2 dx
\]

(3)

where \( j_i \) is the spherical Bessel function of the first rank. \( R_i \) is defined by

\[
R_i = r_i \left( 1 + x^2 \left( v_i^2 - 1 \right) \right)^{1/2}
\]

(4)

where \( r_i \) and \( v_i \) are the semi-axis and its ratio of \( i \) th ellipsoidal shell, respectively. For a spherical-shelled particle \( (v_i = 1, R_i = r_i) \), Eq. 3 is simplified as

\[
I_s(q, R) = 9 \bar{\rho}_1 V_1 j_1(q R_1)/(q R_1) + \sum_{i=2}^n (\bar{\rho}_i - \bar{\rho}_{i-1}) V_i j_i(q R_i)/(q R_i)
\]

(5)

As a number distribution function of particle radii \( D(R) \), we adopted the following Gaussian distribution function which is used in many cases of SUV systems [34].

\[
D(R) = \frac{1}{\sqrt{2\pi}\sigma} \exp \left\{ -\frac{(R - \bar{R})^2}{2\sigma^2} \right\}
\]

(6)

In Eq. 6, \( \bar{R} \) and \( \sigma \) are the average radius and the standard deviation, respectively.

3. Results and discussion

3.1. WAXS curves depending on PVP concentration

Fig. 1 shows the osmotic pressure dependence of the wide-angle X-ray scattering (WAXS) curves of LUVs at 2.5 % w/v DOPC, pH 7.0, 25 °C, where A, \([G_{M1}]/[\text{cholesterol}]/[\text{DOPC}] = 0.1/0.1/1\); B, \([\text{cholesterol}]/[\text{DOPC}] = 0.1/1\); C, DOPC only. The osmotic pressure was raised from 0 to 4.16x10^5 N/m^2 by adding the PVP in the concentration range from 0 to 25 % w/v. In Fig. 1A, the change of the

![Fig. 1. Osmotic pressure dependence of WAXS curves of LUVs at 2.5 % w/v DOPC, pH 7.0, 25 °C, where A, \([G_{M1}]/[\text{cholesterol}]/[\text{DOPC}] = 0.1/0.1/1\); B, \([\text{cholesterol}]/[\text{DOPC}] = 0.1/1\); C, DOPC only. The osmotic pressure was raised from 0 to 4.16x10^5 N/m^2 by adding the PVP in the concentration range from 0 to 25 % w/v.](image)
WAXS curve below \( q = -0.02 \ \text{Å}^{-1} \) mostly reflects the structural change in the shape of the LUV. The saturating profile of the scattering intensity below \( q = -0.005 \ \text{Å}^{-1} \) except PVP = 5 % w/v indicates that the particle takes a globular shape. The shoulder at \( q = -0.01 \ \text{Å}^{-1} \) is ascribed to the spherical shape of the particle. The change of the WAXS curve in the \( q \) range from 0.04 Å\(^{-1}\) to 0.2 Å\(^{-1}\) depends on the bilayer structure (thickness) of the membrane. At the initial state (PVP = 0 % w/v), there exists a single broad hump with the maximum at \( q = ~0.11 \ \text{Å}^{-1} \). With raising the PVP concentration, the single hump becomes to be separated into two humps. Figs. 2A and 2B show the Guinier plot (\( \ln I(q) \) vs. \( q^2 \)) and the radius of gyration, \( R_g \), depending on the osmotic pressure, respectively. The \( R_g \) value decreases after having increased once at PVP = 5 % w/v. The above results show that the application of the osmotic pressure results in the structural transition from the LUV to another type LUV that is a double-layered lamellar vesicle (a liposome including a smaller one inside). The broad peak at \( q = ~1.4 \ \text{Å}^{-1} \) evidently shows that the hydrocarbon-chain packing of lipid molecules is at a liquid crystalline phase (fluid phase).

Fig. 2. Osmotic pressure dependence of Guinier plot (\( \ln I(q) \) vs. \( q^2 \)) and the radius of gyration, \( R_g \), of \([G_{M1}]/[\text{cholesterol}]/[\text{DOPC}] = 0.1/0.1/1 \) LUV as shown Fig. 1A.

Fig. 3. Change of the high-\( q \) peak and its half width corresponding to the hydrocarbon chain packing depending on osmotic pressure. A, peak profiles of \([G_{M1}]/[\text{cholesterol}]/[\text{DOPC}] = 0.1/0.1/1 \) LUV fitted using the log-normal function; B, half widths of the half maximum of the peaks \([G_{M1}]/[\text{cholesterol}]/[\text{DOPC}] = 0.1/0.1/1, 0/0.1/1, \) and 0/0/1 depending on the PVP concentration.
Fig. 3A shows the peak profiles of the $[G_{M1}]/[\text{cholesterol}]/[\text{DOPC}] = 0.1/0.1/1$ LUV in the high-$q$ region of Fig. 1A. The solid lines in Fig. 3A are the fitting curves obtained by using the so-called log-normal function. The half width of the peak is plotted in Fig. 3B. As shown in Fig. 3B, the half-width indicates the decreasing tendency depending on the increase of the osmotic pressure, suggesting that the chain packing becomes to be tighter. In the cases of $[\text{cholesterol}]/[\text{DOPC}] = 0.1/1$ and $0/1$, although the hydrocarbon-chain packing of lipid molecules is at a liquid crystalline phase in the whole PVP concentration range, the phenomena observed in the $q$ range below $0.2\,\text{ Å}^{-1}$ is quit different from that on Fig. 1A. The rise of the osmotic pressure induces the multi-lamellar stacking. As shown in Fig. 4A, the lamellar spacing mostly holds at PVP = 5 % w/v and begins to decrease gradually from for $62.8\,\text{ Å}$ to $57.3\,\text{ Å}$ for $[\text{cholesterol}]/[\text{DOPC}] = 0.1/1$ and from $61.9\,\text{ Å}$ to $56.5\,\text{ Å}$ for $[\text{cholesterol}]/[\text{DOPC}] = 0/1$. With the rise of the osmotic pressure, the full width of the half maximum (FWHM) of the first lamellar peak at $q = \sim 0.1\,\text{ Å}^{-1}$ decreases (Fig. 4B), and the third lamellar peak appears above PVP = 15 % w/v. Thus, it indicates that the lamellar ordering becomes higher with the rise of the osmotic pressure.

**3.2. Distance distribution function depending on PVP concentration**

Fig. 5A shows the PVP concentration dependence of the distance distribution function $p(r)$ of $[G_{M1}]/[\text{cholesterol}]/[\text{DOPC}] = 0.1/0.1/1$ LUV obtained by using Eq. 1. The $p(r)$ function of LUV is characterized by the following viewpoints. In the short distance region ($0-100\,\text{ Å}$) in the insert of Fig. 5A, the profile of the $p(r)$ function at PVP = 0 % w/v has a small peak at $\sim 16\,\text{ Å}$ followed by a dent at $\sim 40\,\text{ Å}$. This is ascribed to the presence of the negative average excess scattering density (so-called negative contrast) region of the bilayer against the average scattering density of the solvent namely, the negative contrast of the hydrophobic tail region within the bilayer. With raising the osmotic pressure, the dent becomes gradually shallow and changes to show a positive value followed by a shoulder at $\sim 100\,\text{ Å}$. In the long distance region above $150\,\text{ Å}$, the $p(r)$ functions at PVP = 0 % w/v have broad rounded maxima at $\sim 450-550\,\text{ Å}$ and show mostly bell-shapes, which are typical characteristics of $p(r)$ functions for globular particles. With the rise of the osmotic pressure, the shoulder or hump appears at $\sim 100\,\text{ Å}$, suggesting a generation of double-layer of the bilayer membranes. This understanding is supported by the following modeling analyses. Fig. 5B shows the maximum diameter $D_{\text{max}}$ of the solute particle estimated from the intercept of the $p(r)$ functions at $p(r) = 0$ for $r > 100\,\text{ Å}$. The $D_{\text{max}}$ value decreases after having increased once at PVP = 5 % w/v, which agrees well with the change of the $R_g$ in Fig. 2B. The $p(r)$ profiles at PVP = 5 and 10 % w/v deviate
from a symmetrical bell-shape, suggesting the deformation of the LUV structure from a spherical shape due to the osmotic pressure (below 4.3 x10^4 N/m^2). In other words, the LUV structure once deforms from a sphere and recovers a spherical shape as a double-layer vesicle, as suggested by the following modeling analyses.

![Graph](https://example.com/graph.png)

Fig. 5. Distance distribution function \( p(r) \) and maximum diameter \( D_{\text{max}} \) of \([G_{M1}]/[\text{cholesterol}]/[\text{DOPC}] = 0.1/0.1/1 \) LUV depending on osmotic pressure. A, \( p(r) \); B, \( D_{\text{max}} \). The insert in A expands the short distance region of \( p(r) \). \( D_{\text{max}} \) values are obtained from the intercepts of the \( p(r) \) functions at \( p(r) = 0 \) for \( r > 100 \) Å since the intercepts below \( r = 100 \) Å are attributable not to the particle sizes but to the internal structure of the bilayer as explained in Fig. 7.

### 3.3. Modeling analyses

#### 3.3.1. Basis of structure modeling of lipid aggregates and characteristics in scattering functions

When analyzing structures of particles in solutions, at first variations in shape and size of particles have to be considered. Fig. 6 shows the scattering functions of particles with different shapes, where we assumed three kinds of representative structure that lipid molecular aggregates could take, namely, spheroid, disk and rod. In Fig. 6, the volumes of the particles are constant, and the axial ratios of the spheroid model are set to be 1 for sphere, 2 for prolate ellipsoid, and 0.5 for oblate ellipsoid, respectively. The radius of the sphere is given as 368.6 Å that is obtained from the shell distribution functions given by Eq. 6. In Fig. 7, the structural parameters of the bilayer are as same as those obtained from the shell-model fitting in Fig.8, namely, a

![Diagram](https://example.com/diagram.png)
lipid bilayer forming a vesicle is given to consist of six shells with different scattering densities and widths; 1st, 2nd, 4th, and 5th shells correspond to the head and backbone portions of the outer and inner leaflet within the bilayer; 3rd shell, the tail portion. The most inner shell, the sixth shell, is a core of water pool that radius is variable depending on the vesicle size. With increasing the polydispersity, the ripple profile in the scattering function becomes to be smeared to show a shoulder at ~0.01 Å⁻¹ and a broad rounded peak at ~0.04-0.2 Å⁻¹. It is clear that the rounded peak results from the internal structure of the bilayer. Such smearing occurs in the long distance region of \( p(r) \) function, whereas, the short distance region is not affected by the polydispersity. Alternatively, it can be understood that the short distance region \( p(r) \) function reflects well the internal structure of the bilayer even under the presence of polydispersity.

Fig. 6. Model scattering functions depending on particle shapes such as spheroids (sphere, prolate ellipsoid, oblate ellipsoid), disk and rod. The volumes of the particles are set to be constant. Sphere, radius 368.6 Å; prolate ellipsoid, short axis 292.5 Å, axial ratio 2.0; oblate ellipsoid, short axis 464.4 Å, axial ratio 0.5; disk, radius 577.8 Å, height 200 Å; rod, radius 200 Å, height 1669.3 Å.

Fig. 7. Model scattering functions of multi-shelled spherical particles and those distance distribution functions depending on size distribution functions. A, scattering functions \( I(q) \); B, distance distribution functions \( p(r) \). The insert in A shows the size distribution functions \( D(r) \) used for the calculations. The insert in B expands the short distance region of \( p(r) \). The spherical particle used is a model of lipid liposome consisting of six shells as same as in Fig.8.
3.3.2. Structure determined by multi-shell model fitting

The above simulations shown in Figs. 6 and 7 suggest that the scattering curves below ~0.02 Å⁻¹ and in the range of ~0.04 Å⁻¹ - 0.2 Å⁻¹ are dominated by the vesicle shape and size and the intra-membrane structure, respectively. The experimental scattering curves can be reproduced by the scattering functions of the multi-shelled vesicle model structure with a size-distribution function. Fig. 8 shows the theoretical scattering functions and size-distribution functions of the multi-shell models that were optimized to describe the experimental data of [Gₘ₁]/[cholesterol]/[DPPC] = 0.1/0.1/1 LUV by the iteration fitting using Eqs. 2-6. In Fig. 8, A and B correspond to the LUVs at the PVP concentrations of 0 % w/v and of 25 % w/v, respectively. In Fig. 8A, the experimental data of the LUV at PVP = 0 % w/v was fitted by using a single-bilayer model consisting of six shells with different scattering densities and widths as explained in the above paragraph. The agreement between the theoretical scattering function and the experimental data is good enough. The reasonability of the present single-bilayer modeling is also supported by the previous results of the similar lipid mixture [15, 16]. On the other hand, the experimental data of the LUV at PVP = 25 % w/v was not able to be reproduced by a single-bilayer model in spite of the evidence that the LUV takes a spherical shape as shown in Fig. 5A. Only a double-bilayer model can qualitatively reproduce the experimental data in Fig. 8B. The agreement of the theoretical scattering function of the LUV at PVP = 25 % w/v with the experimental one is not so good. However, as shown in Fig. 9A, the characteristics of the obtained model scattering functions can qualitatively reproduce those of the experimental p(r) functions in Fig. 5A, especially in the short distance region from 0 to ~100 Å. Namely, at PVP = 0 % w/v, the model p(r) function has the small peak at ~16 Å, the negative dent at ~40 Å followed by the broad rounded sub-maximum. The model p(r) function at PVP = 25 % w/v has the small peak at ~18 Å, the positive dent at ~40 Å, the second small peak at ~50 Å that is seen as a shoulder in the insert of Fig. 5A, and the broad rounded maximum at ~450 Å. Such qualitative agreements would support the present modeling analysis. Fig. 9B summaries the contrast profiles of the optimized LUV models of [Gₘ₁]/[cholesterol]/[DOPC] = 0.1/0.1/1 at PVP = 0 & 25 % w/v. At PVP = 0 % w/v, the internal bilayer structure indicates the asymmetric distribution of lipid components due to the preferential location of Gₘ₁ molecules at the outer-leaflet of the membrane, which agrees with the previous results [15, 16]. The most outside region is resulted from the oligosaccharide portions of Gₘ₁ molecules. On the other hand, at PVP = 25 % w/v, there exists two bilayers across the thin solvent layer. The outer bilayer shows the similar asymmetry as at PVP = 0 % w/v. The width of the inner bilayer is smaller than that of the outer one.

![Fig. 8. Model scattering functions and size-distribution functions of [Gₘ₁]/[cholesterol]/[DOPC] = 0.1/0.1/1 LUV obtained by the shell-model fitting. A, at PVP = 0 % w/v; B, at PVP = 25 % w/v. The inserts in A and B show the size-distribution function D(r). The experimental data shown in Fig. 1A are also plotted by the marks.](image-url)
Thus, it can be assumed that G\(_{\text{M1}}\) molecules locate at the outer-leaflet of the outer-bilayer in the transition process to the double-layered vesicle. It should be mentioned that the total surface area of the hydrophobic tail regions of the double-layered vesicle is mostly as same as that of the LUV at PVP = 0. In addition, we have already confirmed that the above transition proceeds reversibly against the change of the osmotic pressure (data not shown).

4. Conclusion
The present results indicate that a small quantity of ganglioside molecules can afford a LUV of lipid mixtures an ability of the reversible morphology transition from a liposome to a double-layered liposome depending on the osmotic pressure, which is reasonably supported by multi-shell model simulation and fitting analyses. In the previous study [35], we found that the small uni-lamellar vesicles (SUVs) of \([G_{\text{M1}}]/[\text{cholesterol}]/[\text{DOPC}] = 0.1/0.1/1\) LUV (at PVP = 0 % w/v and 25 % w/v) obtained by the shell-model fitting. The experimental functions in Fig. 5A are plotted by the broken lines. A, distance distribution functions; B, contrast profiles. The insert in A expands the short distance region of \(p(r)\).

Fig. 9. Distance distribution functions obtained from the model scattering functions in Fig. 8A and the contrast profiles of \([G_{\text{M1}}]/[\text{cholesterol}]/[\text{DOPC}] = 0.1/0.1/1\) LUV (at PVP = 0 % w/v and 25 % w/v) obtained by the shell-model fitting. The experimental functions in Fig. 5A are plotted by the broken lines. A, distance distribution functions; B, contrast profiles. The insert in A expands the short distance region of \(p(r)\).

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References
[1] K. Simons, E. Ikonen, Nature 387 (1997) 569.
[2] K. Simons, E. Ikonen, Science 290 (2000) 1721.
[3] S. Hakomori, Trend. Glycosci. Glycotech. 13 (2001) 219.
[4] R.G. W. Anderson, K. Jacobson, Science. 296 (2002) 1821.
[5] I. Pascher, Biochem. Biophys. Acta 455 (1976) 433.
[6] M. Hirai, T. Takizawa, S. Yabuki, Y. Nakata, K. Hayashi, Biophys. J. 70 (1996) 1761.
[7] M. Hirai, T. Takizawa, S. Yabuki, T. Hirai, K. Hayashi, J. Phys. Chem. 100 (1996) 11675.
[8] M. Hirai, S. Arai, T. Takizawa, S. Yabuki, Y. Nakata, Thermochim. Acta. 308 (1998) 93.
[9] M. Hirai, T. Takizawa, Biophys. J. 74 (1998) 3010.
[10] T. Hayakawa, M. Hirai, Eur. Biophys. J. 31 (2002) 62.
[11] M. Hirai and T. Takizawa, S. Yabuki, K. Hayashi, J. Chem. Soc. Faraday Trans. 92 (1996) 4533.
[12] M. Hirai, T. Takizawa, S. Yabuki, K. Hayashi, J. Phys. Chem. B. 103 (1999) 1013.
[13] T. Hayakawa, M. Hirai, Mol. Cryst. and Liq. Cryst. 367 (2002) 631.
[14] T. Hayakawa, M. Hirai, J. Appl. Cryst. 36 (2003) 489.
[15] M. Hirai, H. Iwase, T. Hayakawa, M. Koizumi, H. Takahashi, Biophys. J. 85 (2003) 1600.
[16] M. Hirai, H. Hirai, M. Koizumi, K. Kasahara, K. Yuyama, N. Suzuki, Physica B 385&386 (2006) 868.
[17] M. Hirai, H. Iwase, T. Hayakawa, J. Phys. Soc. Jpn. 70 (2001) 420.
[18] M. Hirai, M. Koizumi, H. Hirai, T. Hayakawa, K. Yuyama, N. Suzuki, K. Kasahara, J. Phys.: Condens. Matter, 17 (2005) s2965.
[19] M. Hirai, T. Onai, M. Koizumi, H. Hirai, K. Kasahara, K. Yuyama, N. Suzuki, K. Inoue, J. Appl. Cryst. 40 (2007) s159.
[20] J.A. Lundbaek, P. Birn, A.J. Hansen, R. Sogaard, C. Nielsen, J. Girshman, M.J. Bruno, S.E. Tape, J. Egebjerg, D.V. Greathouse, G.L. Mattice, R.E. Koepe, and O.S. Andersen, J. Gen. Physiol., 123 (2004) 599.
[21] D.S., Goodsell, Trend Biochem. Sci., 16 (1991) 203.
[22] D.M. LeNeven, R.P. Rand, Biophys. J. 18 (1977) 209.
[23] L.J. Lis, M. McAlister, N. Fuller, R.P. Rand, Biophys. J. 37 (1982) 657.
[24] T.J. McIntosh, S.A. Simon, Biochemistry 25 (1986) 4058.
[25] R.P. Rand, V.A. Parsegian, Biochim. Biophys. Acta 988 (1989) 351.
[26] T.J. McIntosh, S.A. Simon, D. Needham, C.H. Huang, Biochemistry 31 (1992) 2020.
[27] S.T. Nagle, H.I. Petrache, J.F. Nagle, Biophys. J. 75 (1998) 917.
[28] L. Svennerholm, P. Fredman, Biochim. Biophys. Acta 617 (1980) 97.
[29] Y. Hirabayashi, T. Nakao, M. Matsumoto, J. Chromatogr. 445 (1988) 377.
[30] T. Momoi, S. Ando, Y. Nagai, Biochim. Biophys. Acta 441 (1976) 488.
[31] T. Hayakawa, M. Hirai, Anal. Chem. 75 (2003) 6728.
[32] M. Hirai, M. Koizumi, T. Hayakawa, H. Takahashi, S. Abe, H. Hirai, K. Miura, and K. Inoue, Biochemistry 43 (2004) 9036.
[33] M. Hirai, H. Iwase, T. Hayakawa, K. Miura, K. Inoue, J. Synchrotron Rad. 9 (2002), 202.
[34] P. Balgavy, M. Dubnickova, N. Kucerka, M. A. Kiselev, S. P. Yaradaikin, and D. Uhrikova, Biochim. Biophys. Acta. 1512 (2001) 40.
[35] T. Onai, M. Hirai, J. Physics: Conference Series 83 (2007) 012016.