Rheological characterization of thermal phase behavior of anionic lipid DMPG dispersions

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Received: 31 August 2016 / Accepted: 19 October 2016
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Abstract  We study thermo-structural behavior of anionic lipid 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG) dispersed systems. Continuous chain-melting transition of DMPG induces anomalous intermediate phase between gel and fluid phases. We found that the intermediate phase can be divided into two domains; anisotropic phase with high orientational order and isotropic viscous phase. Anisotropic phase would be identified to perforated lamellae or bicelle formed by rupturing the perforated vesicles under shear. Isotropic viscous phase, on the other hand, might be identified to densely packed perforated vesicle dispersions formed by rearranging extended bilayer membranes.

Keywords  lipid bilayer, phase behavior, DMPG

1. Introduction

Lipid vesicles show a variety of morphologies depending on external stimulus such as temperature, osmotic pressure, pH and so on [1]. Expression of such variety is significantly related to the mobility of lipid molecules through the phase behavior of lipid systems. Increasing temperature, the lipid undergoes a chain-melting transition from gel to fluid phase at lipid’s inherent melting transition temperature. In the gel phase, the hydrocarbon chains of the lipids are fully extended and closely packed. The lipid bilayers in the gel phase exist as a solid state. In the fluid phase, on the other hand, the hydrocarbon chains are randomly oriented and behave as a fluid. One can thus expect high deformability of the vesicles. The rheological behavior of the lipid vesicles is therefore affected by the deformability of the bilayer membranes. The effects of temperature and flow are strongly correlated so that a very unique rheological response could be observed as the gel / fluid transition is approached. In this paper, we focus on the structural transition of a particular lipid vesicle system with anomalous chain-melting transition: anionic lipid 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG), one of an important constituent of biological membranes.

DMPG dispersions exhibit an anomalous melting (gel / fluid transition) behavior over a wide ranges of the temperatures, instead of a definite melting transition [2–4]. In the gel and fluid phases, DMPG forms spherical vesicles with low viscosity. However, in the chain-melting “regime”, the system shows specific viscous property, which clearly suggests the existence of an intermediate structure between gel and fluid phases [2, 4, 5]. The phase behavior of DMPG dispersions in the quiescent state have been intensively studied by differential scanning calorimetry (DSC) and small angle X-ray scattering (SAXS) measurements [2–10]. Absence of the definite chain-melting transition is attributed to continuous melting of DMPG monomers [7]. In spite of that the existence of the intermediate structure was well accepted, structural character was a controversial issue for a decade. One hypothesis proposed by Schneider et al. was that the intermediate structure consists of three dimensional bilayer network, i.e., a sponge phase [5]. They suggested that three dimensional connection of the bilayer membranes enhances the viscosity. However, electron spin resonance (ESR) experiments demonstrated that the lipid monomers were not exchanged between vesicles, which did not support the existence of the sponge phase [11]. Other proposed model raised by SAXS data analysis suggests that new lamellar phase with pores or porous spherical vesicle is the most plausible structure [3, 7, 10, 12, 13]. Appearance of such intermediate structure during the chain-melting transition motivated us to characterize the phase behavior of DMPG systems by the use of a rheo-physical method, a
combination of rheology and small angle X-ray scattering.

In this paper, we focus on thermo-structural behavior of DMPG systems under shear flow. Our experimental results reveal that the intermediate phase in semi-dilute concentration can be classified into two domains; anisotropic structure such as lamellae or bicelles and viscous isotropic structure such as densely packed vesicles.

2. Experimental

We used anionic lipid 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG) in this study. Anionic phospholipid DMPG was purchased as a powder from Avanti Polar Lipids and used without further purification. Following the sample preparation of Schneider et al., DMPG dispersed systems with different lipid concentration were prepared [5]. Relevant amount of lipids were dispersed in a 2 mM Hepes (Sigma) 1 mM EDTA (Fluka) buffer. The value of pH in the buffer was adjusted at pH 7.5 by 1M NaOH aqueous solution. The dispersions were then incubated at 60°C for several minutes and vortexed after incubation. The ionic strength of the buffer generally is given in concentrations of Na-ions including counter ions of the buffer (2 mM Hepes, 1 mM EDTA, pH 7.5).

Viscosity measurements were performed using a strain-controlled rheometer, ARES-G2, equipped with a cone-plate shear cell which has a diameter of 40 mm and a cone angle of 1 degree. Temperature was controlled within 0.05 K by the use of circular device attached to the rheometer. Small angle X-ray scattering (rheo-SAXS) measurements were conducted using synchrotron radiation at beam line BL-10C of the Photon Factory at the Institute of Materials Structure Science of the High Energy Accelerator Research Organization in Tsukuba, Japan [14]. The wavelength used was \( \lambda = 1.506 \text{ Å} \) and the camera length was 200 cm. The scattering pattern was obtained in the \( q \)-range from 0.025 to 0.25 Å\(^{-1}\). A self-made shear cell with couette geometry driven by a stepping motor was equipped on the beam line. The gap of the couette shear cell is 1 mm. SAXS images were obtained after the shear rate of \( \dot{\gamma} = 10\text{s}^{-1} \) was applied for 600 s. In the SAXS measurement, a radial configuration where the beam passes the sample along the velocity gradient direction, allowing the 2D scattering pattern in the flow-neutral plane to be obtained, was used.

3. Results and Discussion

3.1. Rheometry

We first studied the temperature dependence of viscosity at fixed shear rate of \( \dot{\gamma} = 10\text{s}^{-1} \). The temperature dependences of the viscosity at different DMPG concentrations are shown in Fig. 1. As has been reported [2], the chain-melting regime spreading over the wide range of temperatures is characterized by high viscosity. The temperature dependences of the viscosity in Fig. 1 also reveal the same characteristic feature which proves the existence of the intermediate phase during the chain-melting transition. Onset and offset of the chain-melting regime, \( T_{\text{m}}^{\text{on}} \) and \( T_{\text{m}}^{\text{off}} \), can be thus estimated from the viscometry. As the DMPG concentration is increased, \( T_{\text{m}}^{\text{on}} \) and \( T_{\text{m}}^{\text{off}} \) come close [2], and the viscosity drops and shows a minimum in the middle of the intermediate phase. The drop of the viscosity becomes significant as the DMPG concentration is increased. The minimum viscosity divides the intermediate phase into two domains.

Rheological phase diagram prepared by the viscometry is shown in Fig. 2. By eyes, the intermediate region is rather transparent compared to the gel and fluid phases where spherical vesicles with μm-scale yields turbidity [2, 4, 7, 8]. Transparency in the intermediate phase indicates change in surface properties associated with the reflective index of the bilayer membrane or mesoscale transformation of the structure such as a rupture of the vesicle and a ripple phase. In any case, conformation change of a head group and acyl
chain in DMPG monomer due to the chain-melting transition would play a role on variable morphology \[6, 8, 15, 16\]. Complex endotherm profiles with multi-peaks in the same DMPG system indicate that the chain-melting continuously proceeds, and thus a chain packing in the membrane also changes continuously \[2, 7\]. In the intermediate phase, therefore, coexistence of the fluid membrane with high curvature and rigid domains would be expected \[6\]. The minimum viscosity in the intermediate phase would be associated with the change of the morphology and rearrangement of the bilayer membranes under shear. We should mention that the minimum viscosity was also observed in dynamic viscoelastic measurements at fixed angular frequency and amplitude, which means that the intermediate phase is thermal equilibrium phase formed in the quiescent state but not induced phase by shearing. The results in the dynamic viscoelastic measurements will be presented in anywhere else.

3.2. Rheo-SAXS

In this section, we highlight the presence of characteristic structures in the intermediate phase. Rheo-SAXS patterns at different temperatures are shown in Fig. 3. Rheo-SAXS measurements under shear flow of \( \dot{\gamma} = 10 \text{ s}^{-1} \) were performed only for the systems with DMPG concentration of 50 mM. SAXS patterns in the gel and fluid phases show an isotropic ring suggesting that DMPG forms spherical vesicles as expected \[3, 7, 17\]. Broad peak is a characteristic of single bilayer membranes \[3, 18\]. In the intermediate phase at \( T = 22 \) and \( 24 \text{°C} \), on the other hand, anisotropic rheo-SAXS patterns are obviously observed. For the reference, SAXS pattern in a quiescent state at \( T = 22 \text{°C} \) is compared. Before shearing, one can see the isotropic ring pattern indicating that the anisotropic profile of the intermediate phase is yielded by shear flow. Such anisotropy is indicative of the orientation of the bilayer membranes.

To obtain information about the orientation of the bilayer membranes, the SAXS profiles in Fig. 3 are plotted as an azimuthal angle dependence of the intensity at specific wavelength at which the SAXS profile shows the peak in the intensity (Fig. 4). In the azimuthal intensity profile, the peak intensity in the \( q \)-range from 0.1 to 0.15 Å\(^{-1} \) was aver-
aged. We follow the method of Picken et al. [19] and quantify a degree of orientation of the bilayer membranes. Picken et al. described the azimuthal intensity profile by the following relation, which assumes a Maier-Saupe type of orientation distribution function

\[ I(\theta) = I_0 \exp\left[ \alpha (P_2(\cos \theta) - 1) \right] + I_c, \quad (1) \]

where \( P_2(x) \) is the Legendre polynomial of variable \( x \) and \( \alpha \) is the distribution parameter that characterizes the width of the intensity profile [19–21]. \( I_0 \) and \( I_c \) are the maximum intensity and background contribution to the azimuthal profile, respectively. The orientational parameter \( S \), degree of the orientation, of the intermediate phase is determined as the numerical integration of

\[ S = \frac{\int_{-1}^{1} \exp\left[ \alpha P_2(\cos \theta) \right] P_2(\cos \theta) \, d\cos \theta}{\int_{-1}^{1} \exp\left[ \alpha P_2(\cos \theta) \right] \, d\cos \theta}. \quad (2) \]

The azimuthal intensity profiles were well described by eqn. (1) as can be seen in Fig. 4. The orientational parameter \( S \) estimated by numerical integration of eqn. (2) is plotted as a function of temperature in Fig. 5. In the same plot, the temperature dependence of \( S \) was compared with that of the viscosity measured for the system with DMPG concentration of 50 mM at \( \dot{\gamma} = 10 \, \text{s}^{-1} \).

Orientational parameter \( S \) was estimated only in the intermediate phase, while in the gel and fluid phases we could not estimate the value of \( S \) because of the isotropic azimuthal profiles in SANS. In the intermediate phase, \( S \) shows a peak at \( T = 23^\circ \text{C} \), which corresponds to the peak of the viscosity in lower temperature side. The first viscosity peak in lower temperature side correlates with appearance of the orientational order. On the other hand, the viscosity also has a second peak at \( T = 27^\circ \text{C} \) in higher temperature side, where \( S \) decreases and shows small value. Small value of \( S \) in the second viscosity peak indicates less orientation under shear flow. Actually, isotropic ring of the SANS profile in the second peak in Figs. 3 and 4 indicates rather isotropic orientation of the bilayer membranes such as a spherical vesicle. These results suggest that the intermediate phase consists of different structures at lower and higher temperature side. The first peak at lower temperature side is obviously associated with high orientation of bilayer membranes such as lamellae or bicelle-like structure, while the second peak at higher temperature might be attributed to repulsive interaction between isotropic structures such as the spherical vesicle. The intermediate phase can be thus divided into two domains; the anisotropic phase with high orientational order under shear flow and the isotropic viscous phase even under shear flow.

### 3.3. Structural transition mechanism

First we briefly summarize previous works and discuss the structural transition mechanism. Here we should remark that the minimum viscosity does not appear at low DMPG concentration. In earlier works, perforated membrane formation due to a repulsive interaction among DMPG head groups was proposed as a model of the intermediate phase [2, 4, 5]. Especially at low DMPG concentration, the formation of pores in the bilayer membranes expands the radius of gyration of DMPG vesicles in order to accommodate holes [8, 12, 13]. Actually threefold increase of the DMPG

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Fig. 4 SAXS intensity as a function of the azimuthal angle \( \theta \) in the radial configuration at several shear rates. Here, \( \theta = \pi/2 \) corresponds to the \( \nu \) direction. The azimuthal intensity profile was obtained from the SAXS image shown in Fig. 3. The solid lines are the best fit obtained using eqn. (1).

Fig. 5 Orientational parameter \( S \) (filled symbol) as a function of the temperature. Orientational parameter was estimated by the use of eqn. (2). Viscosity (solid line) was also plotted for the comparison of their temperature dependences.
vesicle size has been experimentally observed in the intermediate phase [13]. Besides the perforated vesicles, the absence of other intermediate structures has been recognized in the chain-melting regime at dilute DMPG concentration. In the semi-dilute concentration above 70 mM, on the other hand, the formation of a perforated lamellar phase was proposed by detailed analysis of the SAXS profiles [3, 9, 10].

Here we should also refer that similar complex temperature dependence of the viscosity has been reported for semi-dilute bicellar systems in lipid mixtures [22]. They have observed two peaks in the temperature dependence of the viscosity. They concluded that the first peak at low temperature side was attributed to the formation of a stacked bicellar phase and the second one at high temperature side to a densely packed multilamellar vesicle formation. From the structural point of view, their observation is likely applicable to interpret rheo-SAXS data in this study. Stacked bicelles will yield anisotropic SAXS patterns under shear as could be identified to perforated lamellae or bicelles. The isotropic viscous phase might be attributed to densely packed perforated vesicles. Further study on the structural transformation as a function of the shear rate will give more insights on the difference between two intermediate structures.

On the basis of previous works mentioned above, the following mechanism in the semi-dilute concentration is proposed. As $T_{m}^{\infty}$ is approached from the gel phase, many pores will be formed in the DMPG vesicles and open up to the lamellae or bicelle-like structures by local rupture of the perforated vesicles under shear. As the temperature is increased further, perforated bilayer membranes will coalesce into more extended structures which interact with each other. The interaction may cause morphological transformation possibly to the perforated vesicles by rearranging the extended bilayer conformation under shear flow. In densely packed vesicles, repulsive interaction between expanded porous vesicles would result in the second peak of the viscosity. As the chain-melting transition is completed at $T_{m}^{\infty}$, the perforated vesicles will shrink by closing pores, which results in the DMPG vesicle dispersion with low viscosity in the fluid phase.

We believe that this study contributes to add new insights on the thermal phase behavior of the DMPG dispersions. Structural rearrangements in the intermediate phase under shear flow are expected to correlate with fluctuation of the bilayer membranes, perforation dynamics, and inter-vesicle repulsive interaction. However, the mechanism mentioned above is speculative. The detailed transition mechanisms, especially the dynamics of the rupture of the perforated vesicles, structural transformation among two intermediate phases in connection with the minimum viscosity, and the formation of the perforated vesicles by rearrangement of bilayers are still missing. Studying structural transformation process of the perforated vesicles under shear flow will contribute to a better understanding of the anomalous phase behavior in the intermediate phase.

4. Conclusion

We performed rheological characterization of anionic DMPG lipid dispersions, which shows anomalous thermoostructural behavior in the semi-dilute concentration. We found that the continuous chain-melting transition induces two distinguished intermediate phases under shear; the anisotropic phase with high orientational order and the isotropic viscous phase. By comparison with previous works, the anisotropic intermediate phase under shear would be identified to perforated lamellae or bicelles. The isotropic viscous phase might be attributed to densely packed perforated vesicles. Further study on the structural transformation as a function of the shear rate will give more insights on the difference between two intermediate structures.

Acknowledgements SF acknowledge support by KAKENHI (Grant-in-Aid for Scientific Research) for Young Scientists (B) Grant No. 23740315 and for Scientific Research (B) No. 25287107 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References


