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nm-Scale Assembly of Phospholipid and Detergent Mixture

Kenji Kubota, Tomohiko Sato, Hideomi Kawai, Yoshihiko Inaoka, and Kaori Wakamatsu
Faculty of Engineering, Gunma University, Kiryu, Gunma 376-8515, Japan
Fax: 81-277-30-1447, e-mail: kkubota@bce.gunma-u.ac.jp

Mixture of phospholipid, L-α-dimiyristoylphosphatidylcholine (DMPC), and detergent, 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO), forms a unique molecular assembly of mixed micelle depending on the composition, total concentration, temperature, and so on. Among various types of mixed micelles, the disc shaped mixed micelle which maintains the bilayered structure composed of phospholipids, denoted as bicelle, is of great importance and is available for the research of membrane proteins because of its mimic nature for the biomembrane system. The assembled structure of DMPC and CHAPSO mixture was investigated by the scattering measurements (light scattering and small angle X-ray scattering) over the wide range of the composition, total concentration, and temperature, focusing on the structural variation (shape transformation) from the disc-shaped one (bicelle) to the rod-shaped one. It was ascertained that the assemblies take the rod-shaped structure at the conditions of high molar ratio of [DMPC] to [CHAPSO], low total concentration, and high temperature. Similarly to the case of bicelle, the scattering functions of SAXS measurements showed the regular arrangement of electron densities for the rod-shaped assemblies. Molar mass of those assemblies was determined in order to clarify their packing structures and to establish the model structure. The model structure for the bicelle and rod-shaped assemblies assuming the bilayered phospholipid arrangement reproduced well the scattering functions obtained by SAXS measurements. CHAPSO molecules are likely to be arranged with the steroid pseudoplane parallel to the circumference surface of the rod portion. As for the temperature dependence, the assembly structure exhibits a change near the phase transition temperature of lipid chain packing in the DMPC bilayer, and the size distribution of assemblies becomes very broad corresponding to the formation of rod-shaped assemblies.
Key words: DMPC, CHAPSO, bicelle, mixed micelle, light scattering, small-angle X-ray scattering

1. INTRODUCTION

Disc-shaped mixed micelle having bilayered structure, denoted as bicelle, is a unique molecular assembly composed of phospholipids. One of the well-known examples is the one formed by the mixture of long-chain and short-chain phospholipids, typically the mixture of L-α-dimyristoylphosphatidylcholine (DMPC) and L-α-dihexanoylphosphatidylcholine (DHPC) [1-9]. Assembly of DMPC molecules arranged in a bilayered disc-shaped manner is stabilized by the regular alignment of DHPC on the periphery of the disc. The most important characteristic is the formation of plane bilayered structure by phospholipids similar to the biomembrane.

Because bicelles align parallel (more rigorously, the disc normal being perpendicular) to the magnetic field, they have been utilized in the NMR studies to help the alignment of proteins for the residual dipolar coupling measurements. Moreover, plane bilayer of phospholipids is a mimic of biomembrane (cell membrane) and is suitable for embedding the membrane proteins. Therefore, bicelles can be used as a useful medium for the study of membrane proteins. In this point, bicelle composed of DMPC and DHPC mixture is restricted for such purposes due to various problems. For example, bicelles composed of DMPC and DHPC are physically unstable, because DHPC is susceptible to hydrolysis.

Since the short-chain phospholipid is introduced to stabilize the disc-shape arrangement, other compounds are possible to substitute. In fact, it has been known that some detergent molecules having steroid group form bicelles at an appropriate condition. Mixture of lecithin and bile salt has been investigated because of its unique shape of assembly [10-23]. CHAPSO, 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate, is a twitterionic type detergent analogous to cholesterol, and it has been established that CHAPSO is available to form bicelles in the mixture with DMPC, DPPC, and so on. Detergent molecules have been used for the extraction of various membrane proteins and stabilize them well. Therefore, bicelles composed of phospholipids and detergent is quite suitable for the structural research of the membrane proteins [24-29]. For such studies, stable and monodisperse bicelles, whose size is comparable to the targeting protein, are quite desirable.

In our previous report [29], it has been clarified that the mixture of DMPC and CHAPSO forms bicelles satisfying those requirements. However, it was found that the stability of bicelles becomes worse when the molar ratio [DMPC]/ [CHAPSO] increases, the total concentration [DMPC] + [CHAPSO] decreases, and the temperature increases: that is, the size distribution becomes much broader in case of very large dimension. Therefore, it is essential to find the optimum conditions to form bicelle and to clarify their stability with respect to the molar ratio, the total concentration, and the temperature. From the viewpoint of colloid science, too, the shape transformation (or, structural transition) of micellar aggregation is a very interesting subject.

In the present report, the assembly structure formed by the mixture of DMPC and CHAPSO was characterized employing the scattering techniques to clarify the

packing of DMPC and CHAPSO molecules over the wide range of [DMPC] and [CHAPSO] focusing on the shape transformation. In addition, the structural change of the mixed micelles near the phase transition temperature of DMPC was confirmed.

2. EXPERIMENTAL

L-α-dimyristoylphosphatidylcholine (DMPC) was purchased from Avanti Polar Lipids Inc. and was used without further purification. Detergent, 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO) was purchased from Sigma. Solvent was Milli-Q water. A necessary amount of CHAPSO was dissolved in Milli-Q water, and a necessary amount of DMPC was dispersed in this solution so as to make the final solution. Ultrasonication was also employed. The composition and concentration of the samples were identified by q (= [DMPC] / [CHAPSO]) and C_t (= [DMPC] + [CHAPSO]). The sample solutions were made clean optically by passing through the membrane filter of pore size 0.2 and 0.45 μm in the clean box. Dynamic and static light scattering measurements were carried out by use of a homemade spectrometer with Ar ion laser (wavelength = 488.0 nm) as a light source. Correlation functions were obtained by ALV-5000 multiple-tau digital correlator to determine the hydrodynamic radii R_h and the distribution function of them by CONTIN method (regularized Laplace inversion) [30]. Scattering functions were also obtained to determine the molar mass of the assemblies and the radius of gyration (if possible, for the large assemblies). Molar mass was determined by the conventional Zimm plot. Small angle X-ray scattering measurements were performed (wavelength = 0.149 nm) at the photon factory of High Energy Accelerator Research Organization (BL-10C, KEK-PF). Radii of gyration were determined by the Guinier-plot of the scattering functions. The observable scattering angle range with sufficient S/N ratio was up to ca. 3 nm^{-1}.

3. RESULTS AND DISCUSSION

It has been reported that the hydrodynamic radii R_h exhibit a rapid increase with increasing q value near q ~ 1, and the size distribution becomes much broader [29]. Figure 1 shows one of the typical examples of such a tendency observed for the total concentration C_t equal to 30 mM at 30°C. Similar behaviors were observed in the dependence of R_h on the total concentration and temperature, too. In the region where large R_h values (e.g., > 10 nm) were observed, the characteristic ratio R_g/R_h became fairly larger than unity (almost equal to ca. 1.8). The ratio of R_g/R_h is sensitive to the spatial mass distribution of the scattering particles, and the magnitude fairly larger than unity suggests that the mixed micelle has an extended rodlike shape. On the other hand, the scattering function in the region of very low C_t is represented well by the model of spherical shell with the thickness equal to the lipid bilayer. This fact suggests that the mixed micelle forms a vesicle in that region. That is, the mixed micelles of DMPC and CHAPSO form assemblies of various shapes, and the transformation takes place with respect to the composition, concentration, and temperature.

The local regular arrangements are almost unchanged

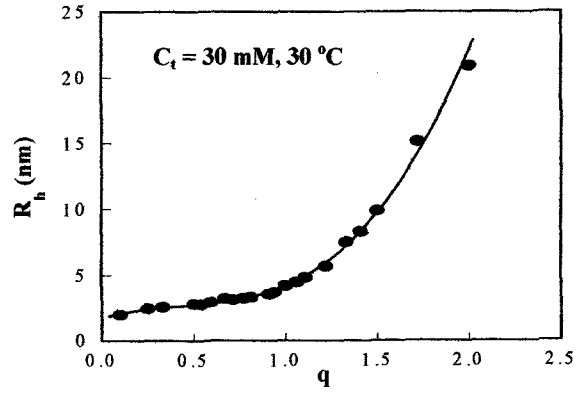

Fig. 1. Composition dependence of hydrodynamic radii at C_t = 30 mM and 30°C.

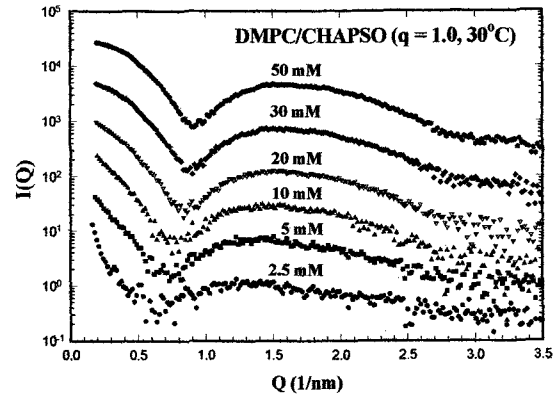

Fig. 2. Scattering functions (SAXS) at q = 1 and 30 °C for various C_t.

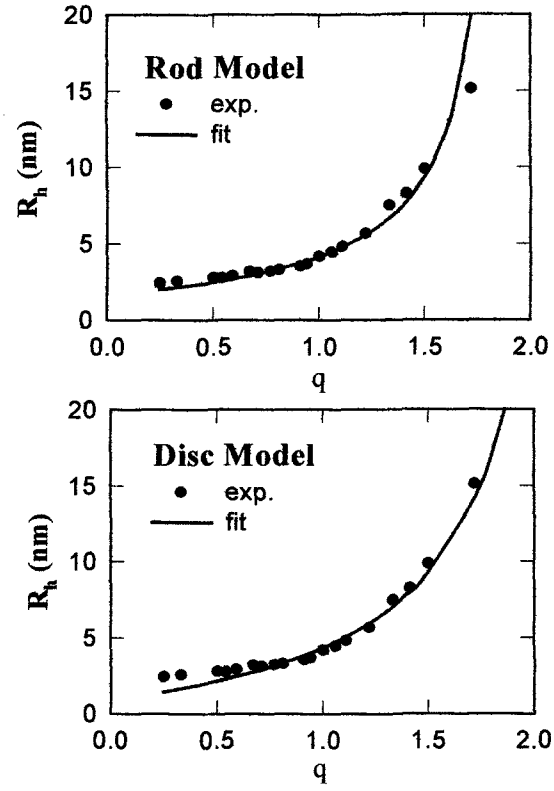

Fig. 3. Model calculations of R_h by the rod and disc model with constant lipid bilayer arrangement.

even though a large amount of size change (R_g and R_h) occurs and global structure varies with q, C_t, and temperature (Fig. 2 against the total concentration). In order to analyze the structure of those mixed micelles, rod and disc models, which maintain the lipid bilayer structure, have been introduced. Figure 3 shows the calculated curves for those model structures. For the rod model, the diameter was set 4.5 nm and the constant ratio of phospholipid to detergent molecules in the rod portion was assumed. On the other hand, the disc thickness was set 4.5 nm and the constant ratio of them in the disc surface was assumed for the disc model. Rh was calculated by the equation [17]

$$R_h = (3/4)D / \{[1 + (L/D)^2]^{1/2} + (D/L) \ln[L/D + (1 + (L/D)^2)^{1/2}] - L/D\} \quad (1)$$

Here, D and L are the diameter and thickness (length), respectively. As shown in Fig. 3, both models reproduced well the increase of R_h and are indistinguishable. This fact also suggests that the coexistence of disc (bicelle) and rod may occur. Moreover, it is probable that the ratio of phospholipid to detergent in the disc surface (or, rod portion) may not be constant against q. Then, it is necessary to characterize the packing condition in the mixed micelle assembly in detail (up to the molecular level of phospholipid and detergent) to clarify the assembly structure and stability of them The knowledge of molar mass of the assembly should be obtained in order to determine the numbers of DMPC and CHAPSO molecules assembled in one mixed micelle. When membrane proteins are embedded in the mixed micelle, such knowledge is important to avoid excessive empty micelles and to keep proper mixing.

In the mixture, however, there exist molecularly dispersed CHAPSO molecules as well as those assembled into the mixed micelles. In the dilution process, CHAPSO molecules assembled into the mixed micelles should dissociate partly, and as a result, shape transformation should occur due to the effective increase of q. However, especially for the bicellar assembly, since the size is very small (a few nm) and the molecularly dispersed CHAPSO molecules are in the equilibrium state with those in bicelles, it is very difficult to determine the concentration of molecularly dispersed CHAPSO by the dialysis method. Therefore, we tried to determine the phase map from the magnitudes of R_h and the variance of R_h distribution of the mixtures. It is expected that the boundary lines and/or regions between different assembly structures are astringent at molecularly dispersed CHAPSO concentration at very low DMPC concentration. The molecularly dispersed CHAPSO concentration thus evaluated was 2.5 mM, much lower than the CMC (8 mM) of CHAPSO. Weight-averaged molecular weight of the mixed micellar assembly was illustrated in Fig. 4. Though the molecular weights at 20 °C increase only slightly with q, they increase rapidly over the q value larger than unity in accordance with Fig. 1. That is, the assembled structure should indeed depend on q. Moreover, the continuous increase of the molecular weight and its size suggest that the shape transformation does not occur discretely, but does continuously with the existence of coexisting region of the two shapes (rod and disc).

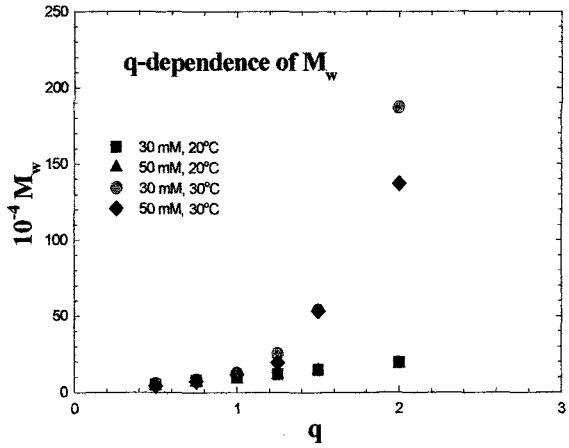

Fig. 4. q-dependence of molar mass of mixed micelle assembly for C_t = 30 and 50 mM and at 20 and 30 °C.

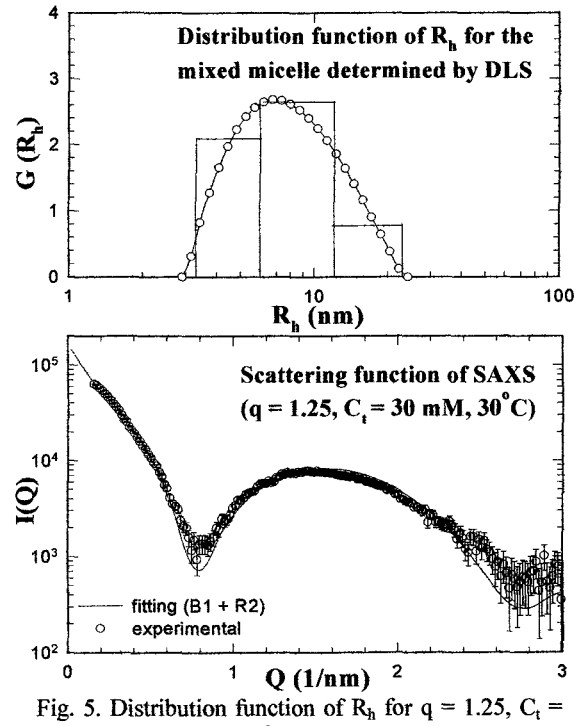

Fig. 5. Distribution function of R_h for q = 1.25, C_t = 30 mM, and at 30 °C (upper panel). Scattering function and the calculated one (solid curve).

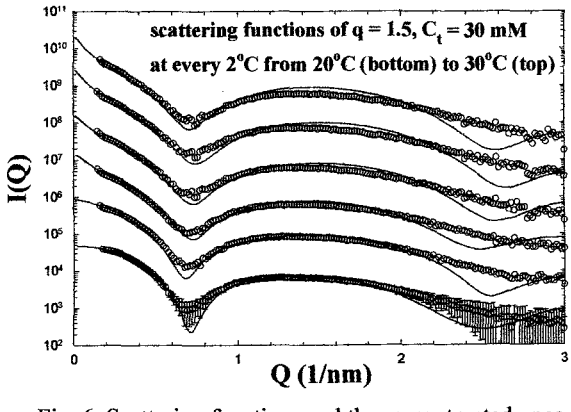

Fig. 6. Scattering functions and the reconstructed ones including coexistence of bicelle and polydisperse rod.

In the region of bicelle formation (typically, $0.5 < q < 1$ at C_t = 50 mM and 30 °C), packing structure in the assembly was examined using the disc diameter and the molecular weight. It is assumed that the disc surface is composed of the mixture of DMPC and CHAPSO. CHAPSO molecules coat the periphery of the disc. The resultant mixing ratio in the disc surface is not a constant, but is dependent upon q value, and the CHAPSO content decreases with q. This fact means that the essential factor determining the disc diameter is not the mixing ratio itself. Theoretical framework for the disc formation reported so far is based upon the idea that the disc diameter is determined so as to keep constant the mixing ratio in the disc surface with the variation of q [10]. On the contrary, it is rather suggested that the mechanical stability (bending stability) as a function of mixing ratio is the essential factor for the disc diameter [20]. If the ratio of DMPC increases and exceeds a threshold level, shape transformation may occur. In this scheme, the formation of vesicle might be due to the decrease of CHAPSO content to supply molecularly dispersed CHAPSO molecules. Meanwhile, such a situation could be a special case of the combination of DMPC and CHAPSO. So, this picture is still not confirmed thoroughly, and more detailed analyses are necessary.

Figure 5 shows the typical result for q = 1.25, C_t = 30 mM, and at 30 °C. Molecular weight is 25.1 x 10^4. Distribution function of the hydrodynamic radii is very broad in accordance with the coexistence of disc and rod. Then, the distribution function was divided into three parts, one disc (bicelle) and two rods, as drawn by histogram. Bicelle and rods with bilayered arrangement of phospholipids and the disc diameter and rod length, respectively, which give the average hydrodynamic radius of the respective histograms, were modeled. Reconstructed scattering function was calculated introducing the proper values of electron densities for the head and lipid chain of phospholipid, for CHAPSO, and for the mixing part of those. Fitting is reasonably good enough. However, only bicelles or only rods model failed to reproduce the experimental scattering function.

Similarly, temperature variation of the scattering function for q = 1.5 and C_t = 30 mM was analyzed. Figure 6 depicts the scattering functions at every 2 °C from 20 to 30 °C (bottom to the top). Respective curves are shifted to one order to make easy to distinguish. The experimental uncertainty is shown for the result at 20 °C. The fitted curves are shown by the solid curves, and they are constructed by the sum of bicelle and rods. Although monodisperse bicelle is able to fit the scattering function at 20 °C, one bicelle and one rod are necessary at 22 °C. At further higher temperature, more rods than one is needed and only 4 rods is the best choice at 30 °C. Containing the polydisperse rod (in rod length) means that the rod length is not determined uniquely by the composition ratio similarly to the case of bicelle formation. However, the coexistence of disc (bicelle) and rod-shaped assemblies is clearly ascertained. The electron densities of head group and lipid chain part of phospholipid molecules in the disc surface of coexisting bicelle decrease gradually with temperature, and a change of them is detected between below and over the phase transition temperature of lipid chain packing in the bilayer (ca 23 °C). This fact suggests that the disc-shaped bicellar assembly composed of phospholipid with higher phase transition temperature (e.g., DPPC) is more stable and is advantageous for the complex formation with membrane proteins at high temperature.

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