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Scattering Study of Assembled Structure of Phospholipid and Detergent Mixture

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Bicelle is a bilayered disc-shaped molecular assembly composed of phospholipids and detergent, and is of importance as a model membrane system. In order to characterize the bilayered mixed micelle of the mixture of phospholipid and detergent, DMPC and CHAPSO which is known to form bicelle in water, dynamic light scattering (DLS) and small angle X-ray scattering (SAXS) measurements were carried out. Hydrodynamic radius as a function of the molar ratio of respective components q, [DMPC] / [CHAPSO], increased slightly and was about 3-4 nm with almost monodisperse size distribution in the range of q = 0.5-1.0. Above this q range, hydrodynamic radius increased markedly indicating the structural change of the assembly. The detailed analyses on the internal structure of the assemblies were carried out by SAXS, and the spatial distribution of phospholipid and detergent molecules in the assembly was investigated. The formation of bilayered structure in this q-region of 0.5-1.0 was thus clearly demonstrated. The conditions to form stable bicelles were examined against the total concentration of phospholipid and detergent, too.

Key words: Bicelle, Phospholipid, Detergent, Dynamic light scattering, Small angle X-ray scattering

1. INTRODUCTION

Bilayered mixed micelle, known as bicelle, is a molecular assembly in which the long-chain phospholipid-rich disc-shaped bilayered structure is stabilized by the regular alignments of surfactant (short chain phospholipid or detergent) on the periphery of the disc. Therefore, bicelle is a suitable model membrane system and is useful for the physicochemical and biochemical studies of membrane proteins. Mixture of long and short chain phospholipids, DMPC (dimyristoylphosphatidylcholine) and DHPC (dihexa-noylphospha-tidylcholine), is the most popular bicelle forming system [1-4]. Since such bicelles spontaneously align parallel to the magnetic field at high magnetic field strength and thus help to align protein molecules coexisting in the solution, bicelles have been utilized in the NMR study of various proteins to obtain dipolar-coupling information, which is quite useful to determine the three-dimensional structures of proteins [5,6]. On the other hand, since the bilayer structure in the middle of bicelles provides the model system for cell membranes, membrane proteins may be reconstituted into bicelles keeping the intact functionalities as those in cell membranes. When the bicelle has a similar small diameter as that of membrane proteins, the fast isotropic tumbling motion of the small bicelles containing membrane proteins will facilitate detailed structural analyses of the protein by various solution NMR techniques, because sharp and well-resolved resonances are expected.

Mixtures of phospholipids (e.g., egg yolk phosphatidylcholine) and cholesterol (deriva- tives, e.g., bile salts) have been studied from the viewpoints of metabolism of lipids and the role of cholesterol. The structure of the mixture has been studied mainly by scattering methods (DLS, SAXS, and SANS) [7-11]. The mixture was found to form assemblies with various shapes and structures in a complicated manners depending upon the molar ratio of phospholipids to cholesterol derivatives. Here, the latter substance plays an solubilizing role for phospholipids, and the mixture forms the bicelle under some conditions, but exhibits structural transitions to tubular structure under another condition [9,10].

CHAPSO is a twitterionic detergent having the steroid group similar to bile salts, and its solubility is fairly high with CMC being ca.8mM. Though it has generally been expected that the mixture of DMPC and CHAPSO forms bicelles, the structure of phospholipid-CHAPSO mixture has not been studied in detail. CHAPSO is chemically more stable and less expensive than DHPC, and should be more useful than DHPC for the studies of membrane proteins [11,12]. Therefore, it is of importance to characterize the assembled structure of DMPC and CHAPSO mixture under various conditions. We have carried out DLS and SAXS measurements of this mixture under various conditions (molar ratio and total concentration), and found that stable bicelles can be formed under appropriate conditions. The size of assemblies (hydro- dynamic radius) was determined by DLS. The detailed structure of bicelles containing CHAPSO was determined by SAXS measurements combined with model calculations of scattering functions taking into account the spatial variation of electron densities.

2. EXPERIMENTAL

L-α-Dimyristoylphosphatidylcholine (DMPC) used for the preparation of the mixed micellar solutions were purchased from Avanti Polar Lipids Inc. (Alabaster, AL) and was used without further purifications. Detergent, (cholamidopropyl)dimethylammonio-2-hydroxy-1-propa-

nesulfonate (CHAPSO) was obtained from DOJINDO (Kumamoto, Japan), and was of 96 % purity. The respectively dispersed solutions of DMPC and CHAPSO were mixed to make the final solutions for the measurements by employing ultrasonication. Solvent was Milli-Q water (Millipore). The solutions were made optically clean by passing through the membrane filter of 0.2 μ pore size in a clean box. Dynamic light scattering measurements were carried out using a homemade spectrometer and an ALV-5000 multiple-tau digital correlator to obtain the correlation functions of scattered light $g^{(2)}(t)$. Vertically polarized Ar ion laser operated at the wavelength of 488.0 nm was used as the incident beam. Hydrodynamic radius was calculated by using the Einstein-Stokes equation. Small angle X-ray scattering measurements were performed (λ_0 = 1.49Å) by enzyme diffractometer (BL-10C) of the Photon Factory of High Energy Accelerator Research Organization (KEK-PF) at Tsukuba. The angular range of observation with sufficient S/N ratio was upto about 3 nm^{-1}. All the measurements were carried out at 30°C.

3. RESULTS AND DISCUSSION

<Light Scattering>

Figure 1 shows the hydrodynamic radius of the mixture at various molar ratios q (= [DMPC] / [CHAPSO]) at the total concentration of 30 mM and 30°C. The hydrodynamic radius R_h of bicelle shows only a slight dependence on q-value in the range of q = 0.1-1.0. When the q becomes larger than 1.0, the hydrodynamic radius increased markedly suggesting that structural transition occurs at q > 1.0. The structural transition at q > 1.0 is in fact demonstrated by the static light scattering measurement (angular dependence of scattered light intensity), which gives the radius of gyration R_g. The value R_g / R_h is known to be useful because of the strong dependence on the overall structure: R_g / R_h generally takes the value of 0.78 for the spherical structure, about 1.5 for the coiled structure, 2 - 3 for the stiff chain, and 1.0 for the vesicle, on the contrary. For the present mixtures the R_g / R_h value is found to be less than 1.0 at q = 0.1-1.0, 1.51 at q = 1.41, and 1.64 at q = 2.0. That is, the mixture forms the expected disk shape at q < 1.0, whereas wormlike structure at q >1.4.

Figure.2 shows the decay time distribution function of the correlation functions obtained for the mixtures of Fig.1 by CONTIN program, that gives the regularized Laplace inversion of the correlation function. At q = 0.25-1.0, the distribution function spanned in the short time range (0.03-0.1 ms) and shows the very narrow distribution. But at q > 1.5 it became to have a broader size distributions. The values of variance, the indicator of broadness of the distribution, are also shown in Fig. 2 for the numerical comparison. These results of Figs. 1 and 2 suggest that the assembled structures are definitely different between q > 1.0 and q < 1.0, and that the compact and stable assembly is spontaneously constituted in the range q < 1.0.

Figure 3 shows the variation of decay rate distribution functions against the total concentration for the mixture of q = 1.0 and at 30°C. Although the distribution functions for 30 mM is essentially the same as that for 50 mM, those for 10 mM and 5 mM are obviously different; that is, the distribution function shifts to the longer time

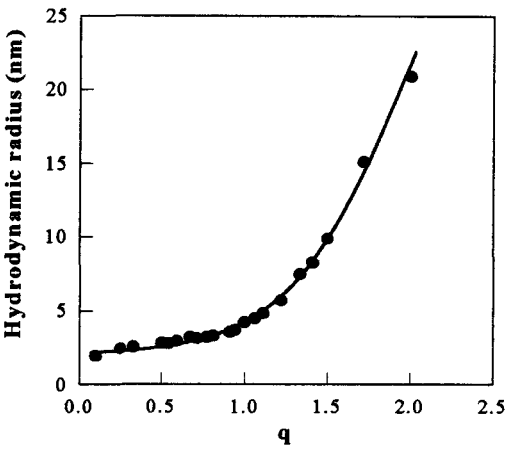

Fig. 1. Dependence of R_h on the molar ratio q, [DMPC]/[CHAPSO] at the total concentration of 30 mM and the temperature of 30℃

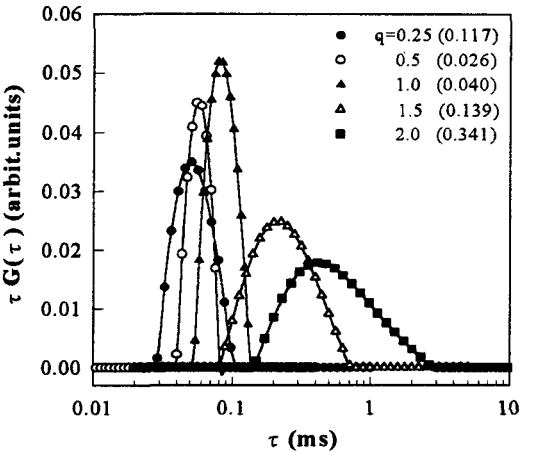

Fig. 2. Decay time distribution function at various q values of [DMPC]/[CHAPSO]. The numerals in the parenthese indicate the variance of distribution.

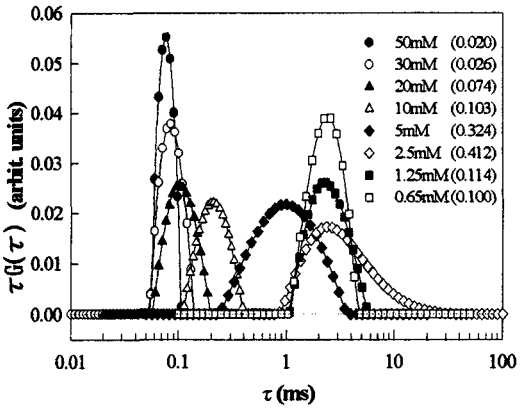

Fig.3. Decay time distribution function of the mixture of DMPC/CHAPSO for various total concentration (q=1.0 at 30℃).

range accompanied by broadening. For the total concentration smaller than 2.5 mM, the distribution becomes to have the similar (but longer than that at high concentration) time ranges again. From these observations, it can be deduced that the similar aggregates were formed in this low concentration range. The structure of the aggregate at this low total concentration is assigned to be vesicles composed essentially of DMPC alone, since R_g/R_h ratio is 0.98-0.99 (almost unity). This is because the critical micellar concentration of CHAPSO in water is 8 mM and that for the mixture of q = 1.0 is ca. 6 mM (lower than in pure water due to mixed micelle formation) at 30°C, thus essentially no CHAPSO molecules are available for the mixed micelle formation. The structure of the aggregate formed at this low total concentration is considered to be stable, since the decay time distribution function is fairly narrow. At the intermediate total concentration of 5 and 10 mM, some transient structures (rodlike or prolate ellipsoidal one) may be formed because the R_g/R_h ratio is 1.57 and 1.76, respectively, as large as those expected for the stretched structure. Under these transient conditions, the spatial distribution of CHAPSO in the assembly should be different qualitatively from those at high total concentration. In fact, the assembly became a little unstable at 20mM as evidenced by the broad distribution. This is because of the largeness of effective q value due to unnegligible contribution of intermicellar molecular dispersion of CHAPSO. It should be added that the assembly at q = 1.0 at lower temperature (20°C rather than 30°C) became more stable (data not shown).

<Small Angle X-ray Scattering>

Static light scattering measurements cannot afford any valuable information for the particles whose size is much smaller than the wavelength of the incident light. Therefore, SAXS was employed to investigate the detailed structure of the compact assemblies formed in the region of $q \leqq 1.0$ and at high total concentration. Typical result of the scattering function (q = 1.0, 50 mM, and at 30°C) is shown in Fig. 4. Here, the solid curve is calculated for a model structure of DMPC/CHAPSO bicelle by introducing the spatial variation of electron densities (details are described below). By the method of Guinier plot, radius of gyration R_g was determined at the small scattering vector region. R_g was 3.39 nm for Fig. 4.

Figure 5 shows the distance distribution function P(r) obtained for Fig. 4 by introducing the damping factor for the sake of finite observation of angular range. P(r), obtained by the indirect Fourier transform of scattering function, is defined as

$$P(r) = \frac{1}{2\pi^2}\int_0^\infty qrI(q)\sin(qr)dq \qquad (1)$$

When the scattering particle is spherical and has homogeneous electron density, P(r) has a parabolic shape. However, molecular assemblies composed of amphiphilic molecules that have heterogeneous electron densities like phospholipid, hydrophobic alkyl chains with low electron density and hydrophilic polar head groups with high electron density, exhibit a distinct effect of local regularity of electron density, if exists, on the scattering

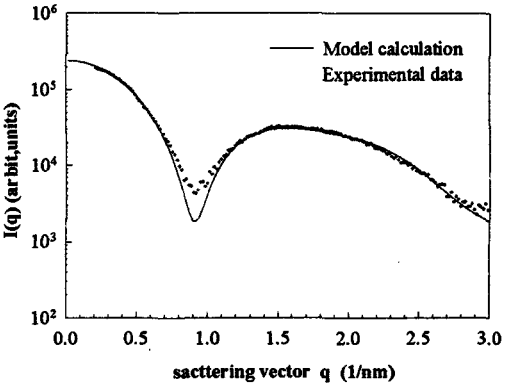

Fig. 4. Typical example of scattering function obtained by SAXS. (q = 1.0, 50 mM, 30°C)

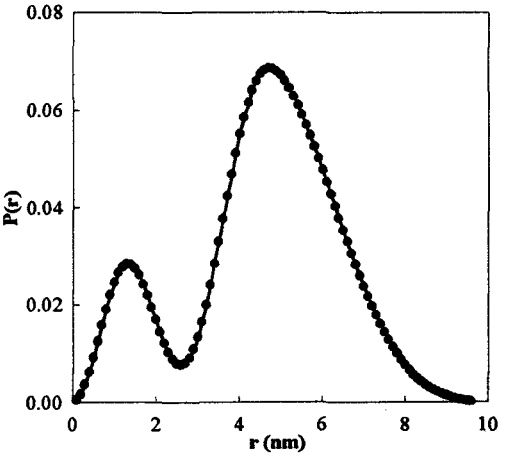

Fig.5. Distance distribution function P(r) of the solution of q = 1.0, 50 mM, and at 30°C.

function, and its effect could be expressed properly by the distance distribution function. The resultant distance distribution function has two peaks separated definitely. In the present case, it was necessary to simulate the scattering function based on the model structure of the assemblies to determine the detailed three-dimensional structure [8]. Scattering functions were calculated for several model structures, such as a bicelle (bilayered disc) with three or five layers of electron density variation along the disc normal and periphery (rim), rotational ellipsoid, sphere (micelle or vesicle), rod (or cylinder), and so on. The solid curve in Fig. 4 is thus obtained for the model of the bicelle structure with five layers of electron densities. The fitting is quite good with this model, and calculations with other model failed to give such an agreeable result. The resultant thickness of the bicelle composed of DMPC and CHAPSO is 4.4 nm in good agreement with the reported values for the lamellar thickness [8,11]. Respective thickness along the disc normal is 1.0, 0.5, 1.4, 0.5, and 1.0 nm with the electron densities of 390, 360, 285, 360, and 390 e/nm^2, respectively. The thickness of the rim is 0.25 nm with 380 e/nm^2. Such a spatial profile suggests that the bilayered assembly of DMPC is edge-stabilized by two

CHAPSO molecules aligned in the direction of the disc normal, and that CHAPSO molecules are located also in the bilayered surfaces with the sterol pseudoplane oriented parallel to the surface. The three OH groups of sterol ring which are located on one face of the pseudoplane are considered to direct toward the outside of the bicelle with the other (hydrophobic) face oriented toward the hydrophobic interior. The electron density of respective regions in the bicelle is indeed in good agreement with this picture. That is, the mixture of DMPC and CHAPSO forms the commonly expected bicelle structure at q = 1.0. The schematic model structure is illustrated in Fig. 6. This model picture is similar to that of phospholipid/bile salt mixture, although the packing state of CHAPSO molecules in the present bicelles differs from those proposed so far [7,8,10].

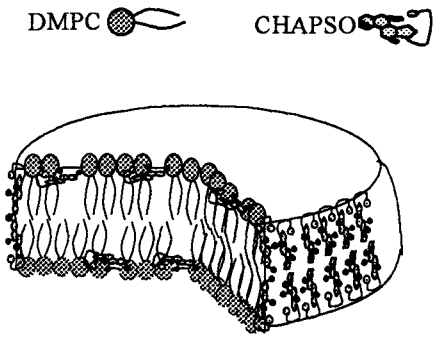

Fig.6. Schematic illustration of the model structure of the bicelle composed of DMPC and CHAPSO.

4. CONCLUSION

We found the conditions where the mixture of DMPC and CHAPSO forms stable disk-shaped bicelle structures at 30°C: the molar ratio $q \leqq 1.0$ and the total concentration (DMPC plus CHAPSO) > 20 mM. The diameter of the bicelles thus formed is sufficient for embedding many membrane proteins and membrane-acting peptides of physiological and pharmacological importance. For example, the DMPC/CHAPSO bicelle at q = 1.0 has a diameter of ca. 8 nm and accommodates a rhodopsin molecule (with ca. 4 nm diameter), a typical G-protein-coupled receptor. Hence the DMPC/CHAPSO bicelle system should be useful for the structural analyses of membrane proteins by solution NMR In fact, a membrane-acting peptide bound to the present bicelle gives well-resolved NMR resonances with high sensitivity (data not shown). Other combinations of phospholipids and detergents not reported in the present study are also being searched. The transition phenomena from bicelle to rod, wormlike chain, and vesicles, are of physicochemical interest as well; the redistribution of CHAPSO molecules in the assembly should occur at the transition. Detailed studies focusing on these points are now in progress.

5. ACKNOWLEGEMENTS

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