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# Disc-shaped Mixed Micelle Formation Constituted of Phospholipids with Different Acyl Groups

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Mixtures of phospholipids, L-α-dipalmitoylphosphatidylcholine (DPPC) and L-α-dioleoylphosphatidylcholine (DOPC) or L-α-1-palmitoyl-2-oleoylphosphatidylcholine (POPC), with detergent 3-[(3-cholamidopropyl) dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO) form nm-size disc-shaped mixed micelles holding the bilayered membrane structure (bicelle) composed of phospholipids. Mixed micelles were characterized being focused on the arrangements of respective molecules on the membrane surface using small angle X-ray scattering and dynamic light scattering techniques. Molar mass were determined by static light scattering being taken into account of the molecularly dispersed CHAPSO concentration. With the increase of DOPC and POPC fraction, radii and molar masses of bicelles increased. Number of CHAPSO molecules on the membrane surfaces increased, and surface area occupied by one CHAPSO molecule decreased with the increases of DOPC and POPC fraction. Packing state of CHAPSO molecules in the membrane varies depending upon the composition of phospholipids, from the state that the normal of steroid ring is perpendicular to the disc surface to the state being parallel. It was ascertained that molecular packing of phospholipids becomes more scarce by unsaturated C=C bonds in the acyl chains of phospholipids (especially, POPC), and CHAPSO molecules are arranged so as to fill such scarce regions.
Key words: Unsaturated phospholipid, detergent, mixed micelle, bicelle, molecular packing

## 1. INTRODUCTION

Phospolipids are able to form various molecular assemblies depending on the solution conditions. Among those assemblies, disc shaped mixed micelle holding bilayered membrane structure composed of phosphorlipids, denoted as bicelle, is a unique molecular assembly [1-5]. One of the typical examples is the one composed of the mixture of long-chain and short-chain phospholipids, the mixture of L-α-dimyristoylphosphatidylcholine (DMPC) and L-α-dihexanoylphosphatidylcholine (DHPC). Due to the large difference of acyl chain length, DHPC and DMPC molecules are arranged at the rim of disc and disc surfaces, respectively, and the mixed micellar assembly (bicelle) thus stabilized has nm scale size [6-10]. Utilizing the nature that bicelles align the disc normal 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 [11-16].

We have studied the structure of bicelles composed of DMPC and 3-[(3-cholamidopropyl) dimethyl ammonio]-2-hydroxy-1-propanesulfonate (CHAPSO) by employing SAXS and LS, and it was clarified that the mixture of DMPC and CHAPSO forms bicelles, but the stability of bicelles becomes worse when the molar ratio [DMPC]/ [CHAPSO] (= q) increases, the total concentration [DMPC] + [CHAPSO] (= $C_t$) decreases, and the temperature increases: that is, the size distribution becomes much broader in case of very large dimension [17,18]. In such conditions, structural transition from disc- to rod-shaped assemblies occurs. In fact, coexistence of disc- and rod-shaped assemblies was observed. Because the plane bilayer of phospholipids is a mimic of biomembrane and is suitable for embedding the membrane proteins, and bicelles can be used as a potent medium for the study of membrane proteins [19,20]. Therefore, the stability of disc-shaped assemblies is important for such applications.

So far, almost all the investigations about bicelles have been carried out using phosphatidylcholines with saturated chains for both acyl chains (mainly, DMPC). However, actual biomembranes are composed of not only by saturated one, but also by unsaturated ones. In fact, phosphatidylcholines with unsaturated acyl chain(s) are major components of biomembranes. Unsaturated C=C bond brings about disorder of the arrangement of phospholipid molecules in the membrane and should related to the fluidity of bilayered membrane. Therefore, it is quite important to characterize the structure and properties of bicelles composed of both saturated and unsaturated phosphatidylcholines [21-24].

In this report, we studied the effect of phospholipids with unsaturated acyl chains as a component and the arrangement of CHAPSO molecules in the membrane.

## 2. EXPERIMENTAL

All the phospholipids, DPPC, DOPC, and POPC, were 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 necessary amounts of phospholipids were dissolved into the aqueous CHAPSO solution. The composition ratio q of phospholipids to CHAPSO was set 0.5 and the total concentration was set 30 mM in all of the experiments, in order to form stable bicelles. The ratios of [DPPC] to [DOPC] and [POPC] were varied as 3:0, 2:1, 1:2, and 0:3. To make sure complete dispersion, mild heating

and/or sonication were employed. Sample solutions were made optically clean by passing through membrane filter of 0.45 μm in a clean box.

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 analyses of the scattering functions at low scattering angles. The observable scattering angular range with sufficient S/N ratio was up to ca. 3 $nm^{-1}$. All the measurements were carried out at 30°C.

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 regularized Laplace inversion method. Scattered intensities were also obtained to determine the molar mass of the assemblies, and the molar mass was determined by the conventional Zimm plot. Increments of refractive index were determined by Brice type refractometer. In those measurements, the effect of molecularly dispersed CHAPSO was taken into account.

## 3. RESULTS AND DISCUSSION

Figure 1 shows a typical scattering function of the SAXS measurements of phospholipids and CHAPSO dispersions. Characteristic broad peak appears near Q = 1.5 $nm^{-1}$ in the scattering function. Such a profile of scattering function was observed in all the mixture. Distance distribution functions obtained by the fourier transform of scattering function also showed two modes. Those facts are due to the local regular arrangements of phospholipids and reflecting relatively high electron density of head group of phospholipid molecules. Radii of gyration of particles $R_g$ were evaluated by Guinier-plots and were 3.00 nm (DPPC) to 3.30 nm (DOPC) and 3.35 nm (POPC). On the other hand, hydrodynamic radii $R_h$ were calculated by means of the Stokes-Einstein equation to the translational diffusion coefficients obtained by dynamic light scattering measurements. Those values were vary close to $R_g$ for all the mixtures, and the ratios Rg / Rh were in the range of 0.9 ~ 1. Those values are quite consistent with those expected for the disc particles. Therefore, it was ascertained that nm-size mixed micelles with bilayered phospholipid membrane (bicelle) are constituted in all the mixtures irrespective to the acyl chain structure. The size distributions of bicelles were quite narrow, and almost monodiesperse assemblies are formed.

In order to characterize the molecular arrangements in bicelles in detail, scattering functions were analyzed based on the model structure with electron density distribution as depicted in Fig. 2. Details of the model have been described elsewhere [17,18]. Calculated scattering functions based on this model structure (electron density profile) were fitted to the experimental scattering functions, and the result for the mixture of [DP]:[DO] = 2:1 is shown by the solid curve in Fig.1. Because of the characteristic Q dependence of the experimental scattering functions, determination of the fitting parameters was not difficult. Except for the data at large Q region, where the experimental uncertainty is

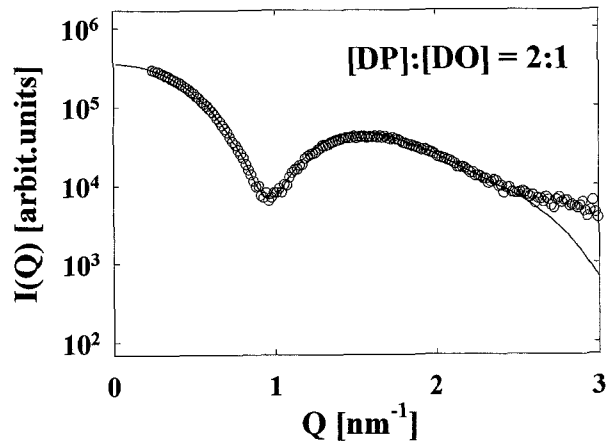


Fig. 1. Typical scattering function of SAXS measurements ([DPPC]:[DOPC] = 2:1). Q is the magnitude of scattering vector.

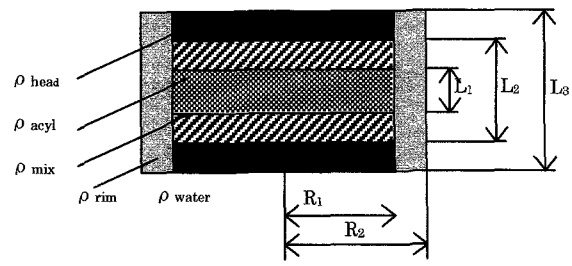


Fig. 2. Model structure of the electron density profile of bicelle. L, R and ρ means thickness, radius and electron density of respective portions. Mix means that both phospholipids and CHAPSO molecules are coexist.

large, fittings were very good enough, and validity of bicelle structure for the present mixed micellar assemblies was confirmed again. For example, values of disc thickness ($L_3$) were 4.90 (DPPC), 5.10 (DOPC), and 5.20 nm (POPC), respectively. Those magnitudes are consistent with the bilayered structure of phospholipids. And, radii were 2.90, 3.10, and 3.20 nm, respectively. Mixtures of phospholipids gave intermediate values, and DOPC and POPC mix homogeneously with DPPC in the membrane surface of the bicelles.

In order to analyze the distribution and packing state of phospholipids and CHAPSO molecules, numbers of them composing one bicelle particle are necessary, and the molar mass of bicelle must be determined. Simple dilution of the bicelle solutions by addition of water results in the variation of assembly condition due to the finite molecularly dispersed CHAPSO concentration, and dilution of CHAPSO solution with that concentration is needed [25-31]. Dynamic light scattering measurements for phospholipid (fixed at 1 mM) solutions with various CHAPSO concentrations were carried out, and the decay time distributions for respective phospholipids thus obtained were illustrated in Fig. 3. Decay time corresponds to the hydrodynamic radius. As shown in figure, the distribution profile varies markedly when CHAPSO concentration exceeds some critical concentration, and over those concentrations distribution

profiles are sharp and unvaried. Previous studies clarified that micellar assemblies with low CHAPSO concentration take rodlike shape and have a broad size (length) distribution [16]. The critical CHAPSO concentrations needed for bicellar assemblies were determined as 6, 7, and 7 mM for DPPC-, DOPC-, and POPC-CHAPSO mixture systems, respectively. Then, aqueous CHAPSO solutions with those concentrations (interpolated for the phospholipids mixtures) were used to dilute the original solution with $C_t$ = 30 mM and 1 = 0.5, and static light scatterined measurements were carried out. Conventional Zimm-plot was employed to determine the molar mass of bicelles. The results are depicted in Fig. 4. Molar mass increases monotonically with the increase in the fraction of DOPC and POPC.

As for the molecular packing in the bicellar assembly, assuming that all the phospholipid molecules are incorpolated in bicelles, the numbers of respective molecules can be calculated. Area occupied by one head group of phospholipid (choline group) was set as 0.717 $nm^2$, and the width of CHAPSO molecule along the circumference was set as 1 nm, as reported by Müller [22]. CHAPSO molecules are placed in two lines at the rim. Thus, the number of CHAPSO molecules located in the membrane surfaces and surface area per one CHAPSO molecule were obtained. In Fig. 5 surface areas per one CHAPSO molecule, S/n, are shown as functions of DOPC and POPC fractions. S/n decreases with the increase of DOPC and POPC (C=C double bond in acyl group), especially for POPC. However, the number of CHAPSO molecules located in the disc surfaces, which were evaluated by the fitting of scattering functions of SAXS measurements, increases.

Relating to those findings, electron density variations at the region of acyl chains as functions of DOPC or POPC fractions, $\rho_{acyl}$, are shown in Fig.2. Although $\rho_{acyl}$ of DPPC/DOPC mixture is almost constant, that of DPPC/POPC decreases with the increase of POPC. Moreover, $\rho_{mix}$ increases with both the increase of DOPC or POPC. CHAPSO has a pseudo plane molecular structure and can be located as the plane being perpendicular to the disc normal of the bicelle or parallel to that, and the occupying surface area is larger in the former than the latter. Those facts clearly indicate that the conformational variation of the CHAPSO distribution occurs with the increase of acyl groups having C=C double bond and more CHAPSO molecules are arranged as the plane parallel to the disc normal of bicelle. Both DOPC and POPC molecules have unsaturated and distorted acyl chains, and the molecular packing of those molecules in the bicelle becomes bulky and scarce. CHAPSO molecules are arranged to fill such scarce portions, and as a result the pseudo plane of CHAPSO molecule (steroid ring) becomes perpendicular to the disc normal. Therefore, more CHAPSO molecules contribute to the mix region and $\rho_{mix}$ increases. For DOPC, both acyl chains are unsaturated, good symmetricity is held resulting that the efficient packing of CHAPSO molecules there. Therefore, $\rho_{acyl}$ does not change. On the other hand, for POPC, one chain is saturated and has straight form, but the other chain is unsaturated and distorted asymmetrically. The packing of CHAPSO becomes worse and inefficient. So, $\rho_{acyl}$ decreases with the increase of POPC fraction.

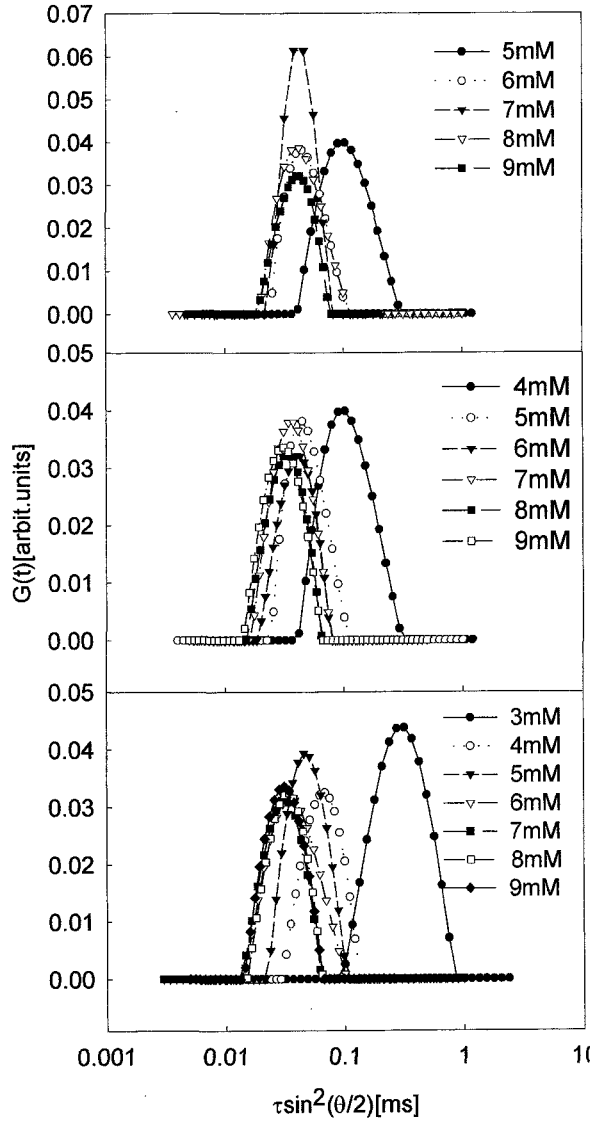


Fig. 3. Decay time distribution at various CHAPSO concentrations. DPPC (upper), DOPC (middle), and POPC (lower).

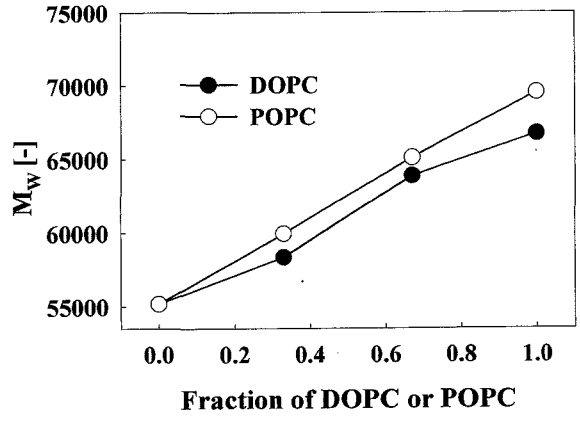


Fig. 4. Molar mass of bicellar assemblies determined by static light scattering measurements as functions of DOPC or POPC mol fractions.

Such a membrane structure with CHAPSO being perpendicular to the normal of membrane surface could be likely to be distorted furthermore and leads to good flexibility and fluidity. In biological systems, e.g. biomembranes, acyl chains of substantial phospholipids are unsaturated, and it could be related with making use of this nature. In case that membrane protein is embedded in bicelles, those flexible nature should become an important factor. Present results suggest that the phospholipids with unsaturated acyl chain are much more effective than those with both unsaturated acyl chains for those purposes.

In conclusion, packing structure of bicelles constituted of mixed phospholipids with detergent CHAPSO was characterized employing SAXS and LS measurements in detail. Incorporation of unsaturated acyl chain results in the variation of packing state of CHAPSO molecules dependent on the acyl chain structure of constituent phospholipids molecules.

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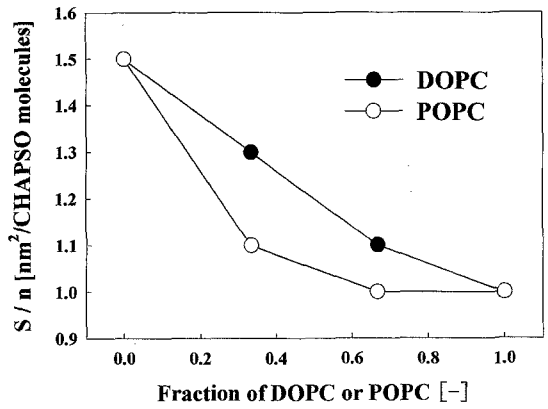


Fig. 5. Surface area occupied by one CHAPSO molecule as a function of DOPC or POPC fraction.

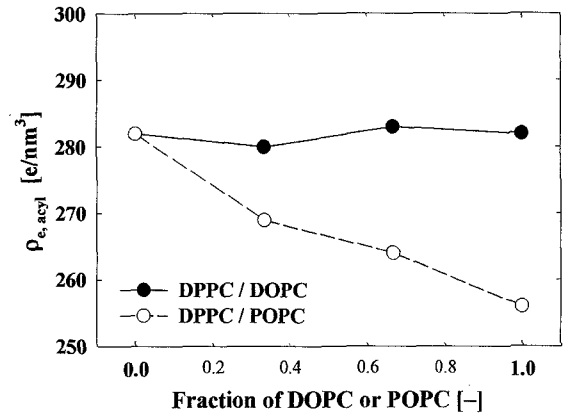


Fig. 6. Electron density variation with the fraction of DOPC or POPC.

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