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Morphology study of DMPC/DHPC mixtures by solution-state ^{1}H, ^{31}P NMR, and NOE measurements

Megumi Shintani [a], Nobuyuki Matubayasi [b,c,*]

[a] Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan
[b] Division of Chemical Engineering, Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531, Japan
[c] Elements Strategy Initiative for Catalysts and Batteries, Kyoto University, Katsura, Kyoto 615-8520, Japan

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ABSTRACT

The temperature and mixing-ratio dependencies are studied by solution-state NMR for the morphology of aggregates composed of dimyristoylphosphatidylcholine (DMPC) and dihexanoylphosphatidylcholine (DHPC). The ^{1}H and ^{31}P NMR and nuclear Overhauser effect (NOE) measurements were performed at 25–60 °C for molar ratio of $q = 1$–5 (q = [DMPC]/[DHPC]). For the $q = 1$ and 2 systems, the turbidity and viscosity of the sample are unchanged and the ^{1}H and ^{31}P NMR spectra are sharp over the whole temperature range examined. Combined with the NOE measurement and compared to the results for small unilamellar vesicle system, the $q = 1$ and 2 aggregates are found to form the spherical mixed micelle and/or small bicelle over 25–60 °C. When q is larger and is 2.5 or 3, the lipid-mixture system exhibits two morphological changes with temperature variation. At low temperature, the DMPC/DHPC system is of spherical mixed micelle and/or small bicelle, as evidenced from the visual observation, the ^{1}H and ^{31}P NMR spectra, and the NOE measurement. In the intermediate temperature range, the aggregate is the magnetically oriented bicelle since the solution is observed to be opaque and viscous with the ^{1}H signal broadening and the ^{31}P signal splitting into two peaks. When the temperature is high and above ~50 °C, the sample becomes milky with sharp ^{1}H and singlet ^{31}P signals. The NOE measurement shows that the lipid dynamics of the aggregate is faster than of the large unilamellar vesicle with low curvature. These show the formation of cylindrical mixed micelle.

1. Introduction

Bilayered micelle (bicelle) is a form of aggregation composed of two phospholipids with different lengths of alkyl chains, typically, dimyristoylphosphatidylcholine (DMPC) and dihexanoylphosphatidylcholine (DHPC) (Fig. 1). It is a disk-shaped micelle with planar bilayer of DMPC and curved rim of DHPC. Bicelle is often regarded as a minimal model of biomembrane, and is utilized to investigate protein structure and orientation in membrane. [1–4] Upon introduction of functional molecule such as protein, a membrane may change its form as observed in Bin/Amphiphysin/Rvs (BAR) domain and melittin [5–7]. Depending on the total lipid concentration, the molar ratio of component lipids, temperature, and salt concentration, furthermore, lipids and especially lipid mixtures can aggregate into a variety of morphological forms. For example, a two-component mixture of lipid has been reported to form spherical mixed micelle, cylindrical mixed micelle, and spherical vesicle [1,8–11]. The morphology of the aggregate reflects sensitively the intermolecular interaction, and thus a morphological analysis of lipid aggregate is desired to be conducted at atomic resolution.

The molar ratio of the two lipids q (=[DMPC]/[DHPC]) is an important factor determining the shape of the bicellar aggregate. [1,8–11] At low q condition ($q \leq 1$), the aggregate is a spherical mixed micelle and/or small bicelle and the form is almost independent of temperature. At high q condition ($q \geq \sim 3$), on the other hand, the morphology may strongly depend on temperature. It is proposed that a spherical mixed micelle forms at low temperature and that a perforated multilamellar vesicle (MLV) forms at high temperature [8–22]. However, a general agreement on the morphology for high q systems is not established, particularly at high temperature. In addition, many studies have been conducted for lipid systems containing buffer, salt, and charged lipids, and the morphology of lipid-only system is not clear. In this work, we focus on the temperature dependence of the aggregate form of the pure lipid systems without additional species.

The morphology of the DMPC/DHPC systems has been studied by the cryo-transmission electron microscopy (cryo-TEM) [10,12], x-ray scattering [22], small-angle neutron scattering (SANS) [11,16–18,20–22], dynamic light scattering (DLS) [10,14,19], and NMR measurements [8,10,12–15,23–26]. The microscopy and scattering studies have proposed the overall shape of the lipid mixture as described in the preceding paragraph. The magnetically oriented form of large bicelle has attracted much attention, and NMR studies have been mainly performed with ^{31}P. The ^{1}H NMR is not of common use for the morphology study on

* Corresponding author at: Division of Chemical Engineering, Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531, Japan.
E-mail address: nobuyuki@cheng.es.osaka-u.ac.jp (N. Matubayasi).

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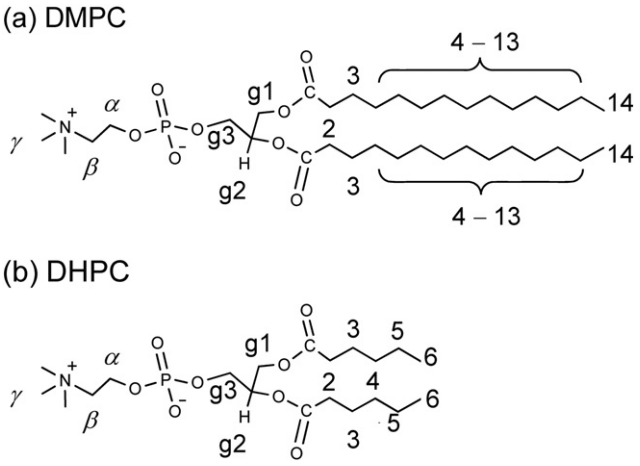

Fig. 1. Molecular structures of (a) DMPC and (b) DHPC.

DMPC/DHPC system due to signal broadening for magnetically oriented bicelle. In order to obtain the structural information of lipid aggregate at atomic level, though, the ^{1}H NMR can also be an important measurement since ^{1}H is the most sensitive nucleus abundant in both the hydrophilic and hydrophobic portions of lipid molecule.

Among ^{1}H NMR schemes, the ^{1}H-nuclear Overhauser effect (NOE) measurement provides structural and/or dynamic information about lipid aggregate at atomic resolution. The NOE measurement has been utilized for the study on static and dynamic properties of spherical vesicles [27–32]. To the best of our knowledge, however, there are no studies of the quantitative NOE measurement for the morphology analysis of the DMPC/DHPC system. In this study, we examine the morphology of DMPC/DHPC system from the ^{1}H and ^{31}P measurements and the quantitative ^{1}H-NOE analysis at solution state. In a previous work [33], we proposed a ^{1}H-NOE sequence combined with the spin-echo (SE) sequence (transient NOE-SE scheme), with which the broad signals are suppressed by the spin-echo part and the residual sharp signals can be detected quantitatively. It was then observed for lipid micelle and vesicle that the NOEs depend on the curvature of aggregate. The curvature reflects, in turn, the difference among a variety of forms of lipid mixture such as spherical micelle, bicelle, cylindrical micelle, and spherical vesicle. We also discuss the morphology of lipid mixture from NOE observations through comparison to vesicle systems with a wide range of curvature.

2. Methods

2.1. Experimental procedures

Dimyristoylphosphatidylcholine (DMPC) and dihexanoylphosphatidylcohline (DHPC) with purities of 99% were purchased as dry powders from Wako Pure Chemical Industries. Heavy water (D_2O; 99.9% D) was obtained from Euriso-top (Saint Aubin, France). DMPC with tail portion fully deuterated (DMPC-d_{54}) at deuteration purity of 98% was purchased from Cambridge Isotope Laboratories, Inc. 1-myristoyllysophosphatidylcholine (MyLPC) with purities of 99% was obtained from Avanti Polar Lipids. The molecular structures and the proton numberings are shown in Fig. 1. DHPC is hygroscopic, thus, the DHPC stock solution was prepared in dry box under N_2 atmosphere. The concentration of the DHPC stock solution was determined by ^{1}H NMR. The appropriate amount of the DHPC stock solution was mixed with DMPC dry powder and D_2O at room temperature. The inhomogeneous DMPC/DHPC mixture was heated to 60 °C. It was then vortexed, cooled by ice, and vortexed again for 1–2 min. This cycle was repeated more than 10 times until the homogeneous solution was obtained. The total lipid concentration was 300 mM (~15 *w*/*v*%). The molar ratio of DMPC to DHPC in the mixture is denoted as q (=[DMPC]/[DHPC]) and was set to 1, 2, 2.5, 3, 3.5, 4, and 5. The spherical micelle of MyLPC with diameter of 5 nm and the spherical vesicles of DMPC with diameters ranging from 30 to 200 nm were prepared using the procedures in Ref. [33]. The concentrations of MyLPC and DMPC in these cases were 20 mM.

All the NMR measurements were performed by using a JEOL ECA600 NMR spectrometer. The ^{1}H frequency was 600 MHz. The chemical-shift values of the ^{1}H and ^{31}P signals were set by referring to the deuterium lock signal. [34] The signals of DMPC and DHPC were overlapped except for the terminal methyl protons of alkyl chains. The signal assignments were carried out using DMPC-d_{54} deuterated at the alkyl chains. The NMR measurements were conducted when the thermal equilibrium was attained at 30 min after the sample setting. The temperature examined was 25–60 °C (above the gel-liquid crystal phase transition temperature of DMPC; 23 °C), and its uncertainty was ±1 °C. The ^{1}H signal width of impurity HDO can indicate the quality of shimming, and its full width at half height ($\nu_{1/2}$) was ~4 Hz (~0.007 ppm) for all the samples examined.

To obtain well-resolved NOE signals, we employ the transient NOE-SE method [33]. In this method, the conventional transient NOE scheme is combined with the spin echo (SE) scheme. The broad components are suppressed by the spin-echo part of the transient NOE-SE sequence and the sharp signals are detected against apparently flat baseline. The details and performance of the transient NOE-SE method are described in Ref. [33]. In the present study, the delay time of the spin echo τ_{SE} was 1 ms, and the field gradient for suppression of the solvent signal was not applied since the signal intensity of HDO was not stronger than of the lipid signals.

The NOE enhancement factor η is determined from

$$\eta(\tau_m) = \frac{I(\tau_m) - I_{SE}}{I_{SE}}, \tag{1}$$

where I_{SE} is the integrated intensity of the reference spectrum with spin echo and without NOE, $I(\tau_m)$ is the integrated intensity of the sample spectrum modified by NOE, and τ_m is the mixing time. The cross relaxation rate constant σ was determined from η through

$$\eta(\tau_m) = 2\sigma\tau_m. \tag{2}$$

This is the linear approximation, and is ensured by small enough τ_m. At the same time, τ_m is desirable to be large enough to have η with good S/N. A τ_m value of 100 ms was adopted; with this choice of τ_m, the conditions of the high S/N and of the linearity of η against τ_m are both satisfied. In Appendix A, our choice of τ_m = 100 ms is validated and the absence of spin diffusion along the hydrocarbon chains of lipids is shown. The cross relaxation rate constant σ is expressed as

$$\sigma \propto \frac{1}{r^6}\tau_c, \tag{3}$$

where r is the radial distance between the proton pair of interest and τ_c is the correlation time. See Refs. [35,36] for the details of theoretical expression for NOE.

3. Results and discussion

In the following, the proton sites of DMPC and DHPC are represented by referring to the character or number in Fig. 1. The ^{1}H signals of DMPC and DHPC are almost overlapped except for the hydrophobic terminals. The terminal methyl protons of alkyl chains are expressed as DMPC14 and DHPC6. For the overlapped signals, they are denoted with an abbreviation PC. For example, PCγ stands for the choline methyl protons of DMPC and DHPC. The assignments of ^{1}H signals are shown in Fig. 2. The hydrophobic terminal methyl protons of DMPC14 and DHPC6 are identified by utilizing DMPC-d_{54} (red line).