Bicelle Composed of 1,2-Dipalmitoyl-sn-Glycero-3-Phosphatidylcholine and Sodium Cholate

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Abstract
A mixture of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) phospholipid and sodium chelate (SC) formed bicelles (DPPC–SC bicelle) at around 50 °C. In ²H nuclear magnetic resonance (NMR) measurement of the dispersion of DPPC–SC bicelle, quadrupole splitting of D₂O, an index of the ability to orient molecules, was observed.

Keywords
bicelle, sodium chelate, DPPC, NMR, magnetic alignment

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A bicelle is a disk-shaped lipid–surfactant assembly in which the lipid bilayer is edge-stabilized with surfactants. Bicelles form a new class of membrane models comparable to natural membranes with a structural simplicity similar to lipid–surfactant mixed micelles. In addition, bicelles can spontaneously align under a magnetic field because of sufficient lateral size, making them useful as alignment media for nuclear magnetic resonance (NMR) analysis that gives 3-dimensional structural information on biomolecules.¹–⁴ In recent years, the potential of bicelle transdermal drug delivery systems has been revealed because of their high transdermal permeability.⁵ However, conventional bicelles are composed of a mixture of artificial molecular surfactants and phospholipids. For example, the widely used 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine bicelles are stabilized at the edge by nonnatural surfactants such as 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propane sulfonate (CHAPSO) and 1,2-dihexanoyl-sn-glycero-3-phosphatidylcholine.⁶ However, it is still a challenge to create bicelles composed of all naturally occurring products that can be expected to have high biocompatibility in view of the applications to biomaterials. We have been developing bicelles using cholic acid-derived compounds.⁷ In the course of these studies, we found that a bicelle was formed between 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) and sodium chelate (SC) (Figure 1) at 50°C.

As a typical preparation for the DPPC/SC bicelle, DPPC powder (17.5%, w/w) and SC powder (2.5%, w/w) were dispersed in water (total content = 20% w/w, [DPPC]/[SC] = 4/1) and subsequently heated to 60°C, which is higher than the phase transition temperature of DPPC (T_m = 41°C) and then cooling to room temperature. This heating–cooling process was repeated 3 times. When bicelles were formed in solutions, a negative peak shift would be observed in the ³¹P NMR spectrum from the 0 ppm peak derived from isotropic aggregates.⁸ We increased the temperature of the DPPC/SC mixture from 25°C to 70°C and measured the ³¹P NMR spectrum in periods of the temperature change (Figure 2a). From 25°C to 40°C, we observed peaks at 0 ppm. However, a strong negative peak appeared at 50°C indicating the formation of DPPC/SC bicelles. Upon further increasing the temperature to 70°C, a peak at 0 ppm was again observed. ²H quadrupole splitting of the solvent signal is a reliable probe for evaluating the ability of bicelles to align with surrounding molecules, which is crucial for NMR studies on biomolecules by observing residual dipole coupling and chemical shift anisotropy. When we measured the ²H NMR spectrum of the DPPC/SC mixture at 50°C, splitting was observed of the signal at

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27.0 Hz of the $^2$H quadrupole because of the formation of DPPC/SC bicelles (Figure 2b).

Since a high concentration of salt is present in biological environments, it is necessary for DPPC/SC bicelles to maintain their disk structure in the presence of salt in view of applications to biomaterials. Therefore, we next evaluated the stability of DPPC/SC bicelles in the presence of salt. For this purpose, we prepared DPPC/SC bicelles in the presence of NaCl (100 mM) and measured the temperature-dependent $^{31}$P NMR profile (Figure 3a). Although a broad NMR signal was observed at 40°C, a negative peak shift indicative of bicelle formation was still observed at 50°C (Figure 3b), as was the case in the absence of NaCl. Notably, the $^2$H quadrupole splitting signal was also sufficiently observed even in the presence of NaCl. This result indicates that the DPPC/SC bicelle could be used as an alignment medium for NMR measurements of proteins, which are usually used in salt-containing buffer conditions.

Interestingly, when the mixture of DPPC and SC was diluted to 5% (w/w), a negative peak shift was observed in the $^{31}$P NMR spectrum at 25°C, indicating a magnetically alignable bicelle was partially formed, although bicelles were not formed at 50°C (Figure 4a). Also, $^2$H quadrupole splitting was confirmed in the sample of DPPC and SC (Figure 4c). This bicelle formation was characteristic when we utilized SC as a surfactant, whereas a peak at 0 ppm at 25°C, indicating isotropic assembly, was observed when we utilized conventional CHAPS as a surfactant (Figure 4b). At room temperature, the DPPC membrane is known to form a kinetically stable gel phase, suggesting that DPPC/SC would exhibit high stability even under physiological conditions where the system is diluted.

In this study, we have reported a DPPC/SC bicelle composed of DPPC and SC. Since both of them are naturally occurring compounds and the concentration of the sample can be chosen as we should like in transdermal delivery, the DPPC/SC bicelle has a potential for use in transdermal drug delivery systems by tuning temperature in the bicelle formation.

**Experimental**

**General:** NMR measurement was performed on a Bruker model ACANCE-500. DPPC was purchased from Wako and SC from TCI.

**Sample Preparation:** As a typical procedure for the preparation of DPPC–Chol bicelle, DPPC powder (17.5%, w/w) and sodium cholate (2.5%, w/w) were dispersed in water...
(total content = 20%, w/w; [DPPC]/[SC] = 4/1) and subsequently heated to 60°C and cooled to 25°C, 3 times.

**Declaration of Conflicting Interests**

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