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Requirement of charged lipids for the hexadecanol-induced gelation in the phospholipid bilayer system

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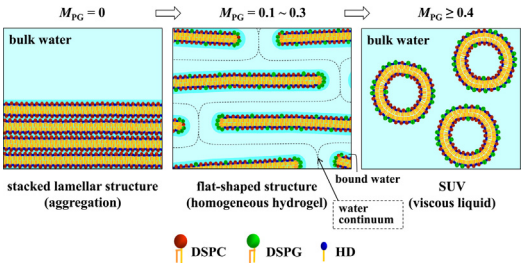
HIGHLIGHTS

- The gelation is induced by the homogeneous distribution of the flat-shaped structures.
- Hexadecanol flattens the bilayer, raising the chain packing density and the bilayer elasticity.
- Phosphatidylglycerol increases the charge density and the spontaneous curvature of the bilayer.
- The continuous water layers between flat-shaped structures work as a network in the hydrogel.
- Charged phospholipids in a crude lecithin may play a crucial role in the hydrogel formation.

GRAPHICAL ABSTRACT



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ABSTRACT

We investigated the formation mechanism of the new type of hydrogel we found previously in the lipid system composed of a crude lecithin mixture (PC70) and hexadecanol (HD) [1] by examining purified lipid systems systematically and quantitatively. On the basis of a working hypothesis that the charged lipids included in PC70 play a crucial role in the formation of the homogeneous hydrogel, we analyzed the physicochemical properties and structures of the simplified ternary bilayer system composed of HD, distearoylphosphatidylcholine (DSPC) and distearoylphosphatidylglycerol (DSPG) as a negatively charged lipid by rheometry, freeze-fracture electron microscopy, synchrotron X-ray diffraction and differential scanning calorimetry. We found that the simplified system is able to form a hydrogel though the composition of the system must fall in a fairly narrow range. Structural analyses suggested that the necessary conditions for the gelation are that the fairly rigid bilayer sheets or vesicles with a flat shape are homogeneously distributed in the aqueous solution under an interaction potential without a secondary minimum.

1. Introduction

Hydrogels composed of plural amphiphilic molecules are widely used in the cosmetic and pharmaceutical industries. For example, ternary systems consisting of surfactant, fatty alcohol and water are used for the formulations of hair conditioners [2,3] and skin moisturizing creams [4–8]. In hair conditioners, cationic surfactants are added to make the hair flexible and reduce the friction between fibers by their adsorption on the hair surface [2,9,10]. Nonionic surfactants are suitable for skin moisturizing creams because they give less irritation to the skin [11]. The structures and physicochemical properties of these ternary systems have been investigated for industrial applications by using various techniques such as

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rheometry [4,7,12–19], differential scanning calorimetry [8,20,21], light and electron microscopy [17,22,23], X-ray diffraction [5,6], conductivity and dielectric analyses [24–28], laser Raman spectroscopy [29], and NMR [30]. In these systems, it is well-known that the gelation is induced by the formation of convoluted bilayer network throughout the solution and their viscoelastic properties sometimes exhibit a long-term relaxation relating to the change in bilayer morphology [31,32].

Recently, we have found conditions for the hydrogel formation from a crude mixture of phospholipids (PC70), natural ingredients suitable for health care products, instead of surfactant [1,33]. Crude mixtures of phospholipids extracted from soybean or egg contain many kinds of phospholipids [34–38] and are widely used as liposome materials [39–42] and emulsifiers [43] in the cosmetic and pharmaceutical industries mainly because of their high cost performance. PC70 used in that study is hydrogenated soybean lecithin containing a variety of phospholipids such as phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylglycerol (PG) in addition to ~70% phosphatidylcholine (PC) and in common use in the cosmetic industry as a commercial raw material.

The addition of an appropriate amount of hexadecanol (HD) into PC70 bilayers induced the formation of a homogeneous aqueous gel. Incidentally, substitution of pure PC for PC70 abolished the gelation, in spite of similarity in phase behaviors of PC70/HD bilayer and pure PC/HD bilayer systems [1]. We had proposed a new gelation mechanism that the water continuum surrounding homogeneously distributed charged bilayer sheets works as a network. In order to use the PC70/HD hydrogel efficiently as a commercial product, we need to clarify the effect of charged lipids on the gelation and fully understand its mechanism. In this study we assumed as a working hypothesis that the charged lipids in PC70 may play a crucial role in the formation of the homogeneous hydrogel. To verify the hypothesis, we investigated the effect of HD on a simplified lipid system composed of distearoylphosphatidylcholine (DSPC) and an acidic lipid, distearoylphosphatidylglycerol (DSPG) by rheometry, freeze-fracture electron microscopy, synchrotron X-ray diffraction and differential scanning calorimetry (DSC).

2. Materials and methods

2.1. Sample preparation

Distearoylphosphatidylcholine (DSPC) and distearoylphosphatidylglycerol (DSPG) were purchased from Avanti Polar Lipids, Inc. and hexadecanol (HD) was obtained from Sigma–Aldrich Co. Other reagents used were of analytical grades. All materials were used without further purification. Samples were prepared as follows: All lipids including HD were dissolved in chloroform/methanol (1:1, v/v) to be mixed in a desired molar ratio at 50 °C. The solvent was first removed by blowing dry nitrogen and subsequently with a high vacuum pump for about 2 h. The remained film was dispersed in purified water. The sample solution was heated up to 80 °C and then cooled down to 30 °C in 5 min under continuous vortexing. The final lipid concentration was 50 mg/ml. We denote the molar fraction of HD, M_H, as moles of HD per moles of HD plus phospholipids and the molar fraction of DSPG, M_{PG}, as moles of DSPG per moles of phospholipids.

2.2. Rheological measurements

Rheological measurements were performed using a Physica MCR301 (Anton Paar GmbH) with a cone-plate geometry (25 mm diameter, 0.0174 radian cone angle). We used the strain sweep from 0.01% to 100% at the frequency of 1 Hz to determine the linear region for complex viscosity (η^*) measurements. A solvent trap was used to minimize the drying-out effects (water evaporation) and dust contamination. All measurements were carried out at 25 °C.

2.3. Freeze-fracture electron microscopy

The specimen frozen rapidly in liquid nitrogen slush was placed in a freeze-fracture apparatus (JFD-9010, JEOL Ltd.), and a replica film for the fractured surface was obtained according to a conventional method described elsewhere [44]. The replicas were observed with a transmission electron microscope (JEM-1400, JEOL Ltd.) operating at 100 kV.

2.4. X-ray diffraction

Synchrotron X-ray diffraction experiments were performed at Station BL40B2 of SPring-8, Japan. An aliquot of specimen solution was placed between kapton films kept parallel with a washer as a spacer and equilibrated at the room temperature before the measurement. Two-dimensional diffraction pattern was recorded with an imaging plate detector (R-AXIS VII, Rigaku). The wavelength λ was 0.1 nm, and the exposure time was 30 s. The camera length was set to be about 500 mm so as to be appropriate for simultaneous detection of small and wide-angle diffraction and calibrated using cholesterol powder crystals. One-dimensional intensity profiles as a function of the modulus of scattering vector $s = 2\sin\theta/\lambda$ (2θ is the scattering angle) were obtained by integrating the two-dimensional diffraction patterns along the azimuthal direction and divided by $2\pi r$, where r is the distance from the beam center.

2.5. Differential scanning calorimetry

The phase behavior of the DSPC/DSPG/HD mixed bilayers was examined by differential scanning calorimetry (DSC). The sample solution containing about 1 mg lipids was loaded into an aluminum pan and set in a DSC apparatus (DSC6100, SII Nano Technology Inc.). The temperature was increased from 15 °C to 85 °C at the scanning rate of 1 K/min. The starting (T_s) and end (T_e) temperatures of chain melting were measured to evaluate the phase behavior of the ternary bilayer system.

3. Results

3.1. HD-induced gelation in the purified phospholipid bilayer system

We visually investigated the effect of increasing molar ratio of HD on the dispersibility and fluidity of the DSPC/DSPG/HD bilayer system with two different DSPC/DSPG molar ratio (DSPC:DSPG = 10:0, 9:1). Fig. 1 shows a photograph of the aqueous solutions of DSPC/HD (DSPC:DSPG = 10:0) with the HD molar fraction M_H = 0, 0.2, 0.66. In this system, all sample solutions were fluid, containing sedimentation or aggregation. In contrast, the aqueous solutions of (DSPC:DSPG = 9:1)/HD with M_H from 0 to 0.8 were homogeneous (Fig. 2). In addition, prominent decrease in fluidity, i.e., gelation, was clearly seen between M_H = 0.60 and 0.66.

3.2. Requirement of DSPG in the hydrogel formation

In order to evaluate the charge density effect, we visually observed the dispersibility and fluidity of the DSPC/DSPG/HD bilayer system with various molar ratios of DSPC/DSPG. Fig. 3 shows a photograph of the aqueous solutions of DSPC/DSPG/HD with various molar fractions of DSPG (M_{PG}) at a fixed HD molar fraction M_H = 0.66. Here, M_{PG} is defined as the molar fraction of DSPG in the phospholipids. Therefore, this system with M_{PG} = 0