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Possibility of Vitamin C to Induce the Formation of Lecithin Organogel

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We investigated the formation of a new lecithin organogel composed of reverse worm-like micelles. The organogel is a mixed organic solution containing lecithin and vitamin C (i.e., ascorbic acid). The highly viscoelastic reverse worm-like micelles were formed upon addition of a small amount of ascorbic acid.

Surfactants are used in a variety of commercial products, such as cosmetics, medicines, foods, and ink. In combination with a solvent, such as water or oil, surfactants can form a variety of molecular assemblies, such as spherical micelles, rod-like micelles, lamellar liquid crystals, and hexagonal liquid crystals.

Recently, there have been many active studies attempting to control the structure of reverse micelles.[1–5] In particular, reverse worm-like micelles, one type of self-assembled structures that form in organic solvent, have attracted the interest of many researchers due to their structural and functional properties. Reverse worm-like micelles are cylindrically elongated reverse micelles. The typical system for forming reverse worm-like micelles is a three-component system composed of lecithin, water, and oil.[6–8] Lecithin is a zwitterionic phospholipid with two alkyl tails that forms spherical or ellipsoidal reverse micelles when added to oil. When trace amounts of water are added to this solution, the water molecules bind to the phosphate groups of neighboring lecithins and induce the formation of reverse worm-like micelles. The micelles can form a transient three-dimensional network in solution, thus turning the solution into an organogel (also called a lecithin organogel). In this system, water is a key ingredient for forming reverse worm-like micelles. Substances which can be used as a substitute for the key ingredient of water have been reported. Examples of such substances are glycerol, ethylene glycol, and formamide reported by Shchipunov et al.[9,10] and bile salt and multivalent cations of inorganic salts such as Ca^{2+}, Mg^{2+}, La^{3+}, and Ce^{3+} reported by Raghavan et al.[11,12] We also reported that urea,[13] sucrose fatty acid esters,[14] D-ribose and its reducing sugar,[15] and polyglycerols[16] can induce the formation of lecithin organogels. Recently, Shrestha et al.[17] reported a new strategy for the formation of reverse worm-like micelles in a nonaqueous system using sucrose-based nonionic surfactants instead of lecithin, which is also very interesting.

In this study, we discovered that vitamin C (ascorbic acid) can induce the formation of lecithin organogel composed of reverse worm-like micelles. It is well known that vitamin C, which is a water-soluble vitamin, is an essential nutrient for humans and certain animals. In living organisms, vitamin C not only plays a significant role in the synthesis of collagen, but also acts as an antioxidant to protect the body against oxidative stress. Lecithin and vitamin C are widely available, low-cost, food-grade materials. Therefore, lecithin/vitamin C/oil gels are likely to be biocompatible and nontoxic.

The required amounts of lecithin (Soy PC (95%); Avanti Polar Lipids, Inc.) and ascorbic acid (L-(+)-ascorbic acid; Wako Pure Chemical Industries, Ltd.) were dissolved in methanol in a vial. The solvent was completely removed using a desiccator equipped with a vacuum pump, and then *n*-decane (Kanto Chemical Co., Inc.) was added to the vial and mixed using a magnetic stirrer. The vial was then kept at 25 °C for a few days to allow for equilibration. Phase diagrams of the lecithin/ascorbic acid/*n*-decane system were obtained by visual observation through crossed polarizers, and by a small-angle X-ray scattering (SAXS) system (Nano-STAR; Bruker AXS Inc.) using Cu Kα radiation (45 kV/120 mA). Steady and dynamic rheological measurements of the solutions were performed using a stress-controlled rheometer (HAAKE MARS III, Thermo Fisher Scientific Inc.) equipped with cone-plate geometry (two sizes: 60 and 35 mm diameters, each having cone angles of 1, 2, and 4°) and a Peltier-based temperature control set at 25 °C.

Figure 1 shows the visual observations of the lecithin/ascorbic acid/*n*-decane solutions at a fixed lecithin concentration of 10 wt % and with increasing concentration of ascorbic acid. At low ascorbic acid concentration (Figure 1a), the solution has a low viscosity. However, the viscosity of the solution monotonically increased with the addition of ascorbic acid, and the solution finally changed into a gel at an ascorbic acid concentration of 1.3 wt % or higher (Figure 1b). Further addition of ascorbic acid (1.7 wt % or greater) caused the solution to separate into two coexisting liquid phases (Figure 1c); the upper phase had a low viscosity similar to that of *n*-decane, and the lower phase had a high viscosity similar to that of a reverse worm-like micellar solution. Similar phase behaviors have often been confirmed in other systems.[11,14–16]

To identify the structure formed in these solutions, we carried out SAXS measurements. Figure 2 shows the SAXS spectrum (intensity $I(q)$ vs. wave vector q) of the lecithin:ascorbic acid:*n*-decane = 2:0.26:97.74 (wt %) sample, which was obtained by 5-fold dilution of the lecithin:ascorbic acid:*n*-decane = 10:1.3:88.7 (wt %) sample to eliminate the

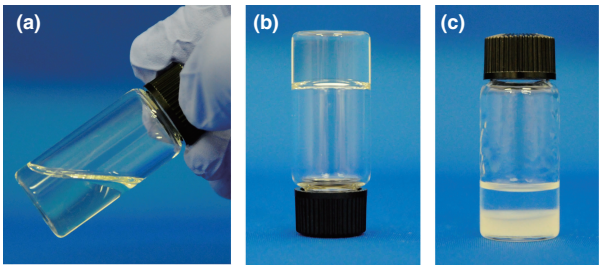

Figure 1. Visual observations of (a) lecithin:ascorbic acid:*n*-decane = 10:0.5:89.5 (wt %), (b) lecithin:ascorbic acid:*n*-decane = 10:1.3:88.7 (wt %), and (c) lecithin:ascorbic acid:*n*-decane = 10:2.5:87.5 (wt %).

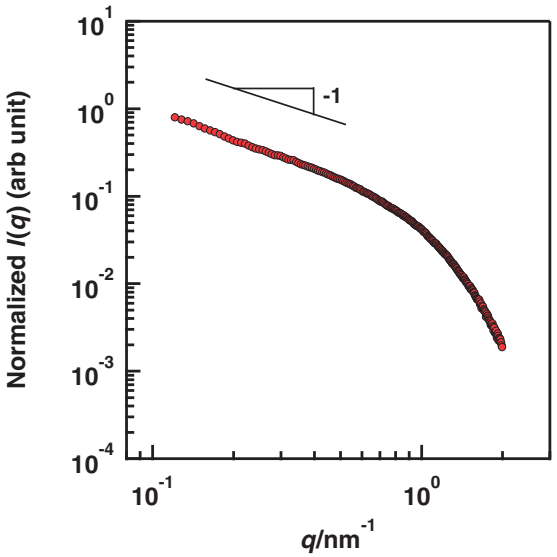

Figure 2. SAXS spectrum for the lecithin:ascorbic acid:*n*-decane = 2:0.26:97.74 (wt %) sample.

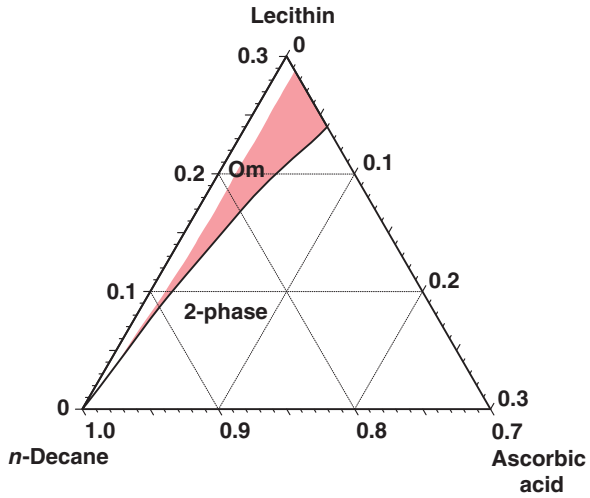

Figure 3. Partial phase diagram of the lecithin/ascorbic acid/*n*-decane system in a dilute region at 25 °C. The notation Om represents the reverse micellar phase. The region of high viscosity within the Om phase is shown by shading.

effect of structure-factor.[13,14] In the low-q region of the SAXS spectrum, the slope of the log–log plot was −1, which indicates the existence of rod-like structure, i.e., reverse worm-like micelles.

We next identified the phase state of the lecithin/ascorbic acid/*n*-decane system by visual observation through crossed polarizers and SAXS analysis. Figure 3 shows the phase diagram in the dilute region of the lecithin/ascorbic acid/*n*-decane system. As shown by the phase diagram, it was found that reverse micelles (Om) formed upon addition of a small amount of ascorbic acid, and highly viscoelastic region (area colored in red) was confirmed within the Om region. Furthermore, both regions enlarged with increasing lecithin concentration. It is also noteworthy that this reverse worm-like micellar

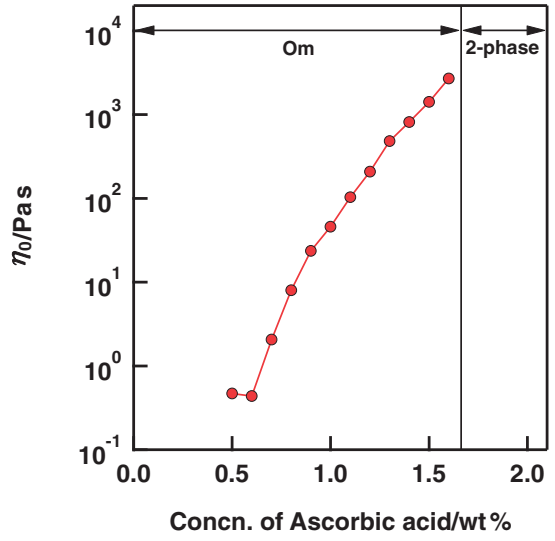

Figure 4. Zero-shear viscosity (η_0) of the lecithin/ascorbic acid/*n*-decane system as a function of ascorbic acid concentration at 25 °C. Lecithin concentrations were fixed at 10 wt %.

system maintained its stability without phase separation although this system contained ascorbic acid.

Ascorbic acid causes the formation of reverse worm-like micelles as follows. It is well known that lecithin forms spherical or ellipsoidal reverse micelles which have a large interface curvature in oil. For these reverse spherical micelles to grow into reverse worm-like micelles, the interface curvature of the molecular assembly must decrease slightly. The ascorbic acid we used in this study can bind to the phosphate groups of neighboring lecithins as well as other substances[6–16] because they have hydroxy groups, and the gaps between the head groups of the neighboring lecithin molecules become wider. Thus, the interface curvature of the molecular assembly decreases, causing reverse spherical micelles to transform into reverse worm-like micelles.

We next investigated the rheological behavior of the reverse micellar solutions. We first carried out steady-flow viscosity measurements. Figure 4 shows the relationship between the zero-shear viscosity (η_0), given by the steady-flow viscosity measurements, and the ascorbic acid concentration. The η_0 increased monotonically until phase separation occurred, and the maximum zero-shear viscosity of this solution was 3.5×10^6 times larger than that of *n*-decane. It follows from the above result that the reverse worm-like micelles grew upon addition of ascorbic acid, and a small amount of ascorbic acid can induce the formation of reverse worm-like micelles with enough length to entangle each other.

We next carried out dynamic viscoelasticity measurements to characterize the viscoelasticity of these reverse worm-like micelles. Figure 5 shows the frequency (ω) dependence of storage modulus (G') and the loss modulus (G'') for the lecithin/ascorbic acid/*n*-decane system. Here, the G' and G'' values represent elasticity and viscosity, respectively. It was found that these solutions exhibit viscous-body behavior ($G'' > G'$) at low frequencies (below the intersection of the G' and G'' curves), and elastic-body behavior ($G' > G''$) at high frequencies (above the intersection of the G' and G'' curves). These dynamic

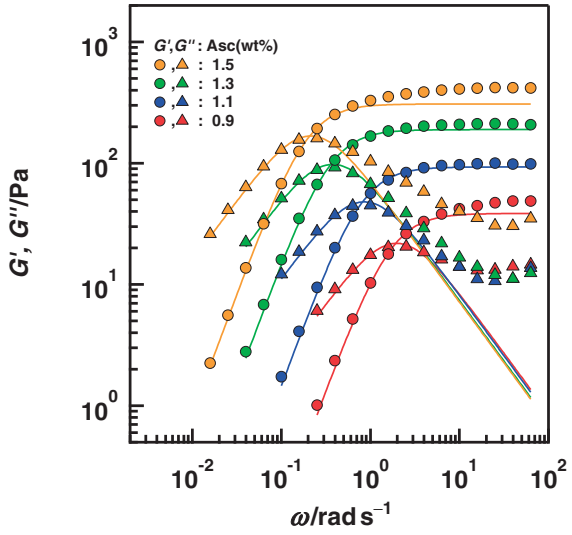

Figure 5. Dynamic viscoelasticity measurements (storage modulus G' and loss modulus G'' as functions of frequency ω) for lecithin/ascorbic acid (Asc)/*n*-decane system at 25 °C. Lecithin concentrations were fixed at 10 wt %. The Maxwellian fittings to the experimental data are shown by the solid lines.

viscoelastic behaviors follow that of the single Maxwell model. As shown in Figure 5, the entire frequency spectrum moved to the upper left with increasing ascorbic acid concentration. We have observed similar results in our previous studies.[13–16] Ascorbic acid not only induces the growth of reverse worm-like micelles, but also induces a greater number of entangled micelles.

In conclusion, the present results indicate that ascorbic acid is a useful key ingredient because it can induce the formation of lecithin organogel. Areas for future study include applied research in a variety of industrial fields, such as medicines, foods, cosmetics, and ink.

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