Effect of Lecithin on Organogel Formation of 12-Hydroxystearic Acid

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ABSTRACT: Optically active 12-D-hydroxystearic acid (12-HSA) gives a thermally reversible organogel in several organic solvents. When a small amount of lecithin coexists with 12-HSA in organic solvents, the mechanical strength of the organogel is remarkably reduced. Interaction of lecithin with 12-HSA was studied by using infrared (IR) spectrometry, nuclear magnetic resonance (NMR) spectrometry, and scanning electron microscopy (SEM). Incorporating lecithin into 12-HSA reduced the absorbance in IR spectra, derived from hydrogen bonding between the hydroxyl groups and the carboxyl groups of 12-HSA molecules. Based on NMR measurements, the polar head groups of lecithin associate with the carboxyl groups of 12-HSA in a 1:1 molar ratio. SEM showed that the shape of the fibrous aggregates varied from a helically extended form to a spherical form. These results suggest that intermolecular 1:1 complexes were formed between lecithin and 12-HSA, which caused a structural change in the fibrous network in the 12-HSA organogel and consequently induced gel deformation.
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KEY WORDS: Aggregate, gel, gel formation, gelling agent, gel structure, 12-hydroxystearic acid, organogel, IR, lecithin, NMR.

A large number of low-molecular weight organic substances, such as organometallic complexes (1,2), 12-hydroxystearic acid (12-HSA) (3–7), dibenzylidene sorbitol (8), various alkylamide derivatives (9–11), androstanyl derivatives (12,13), anthracenyl derivatives (14,15) and cholesterol-based derivatives (15,16), are known to form organogels with network structures in various organic liquids. These substances are widely utilized as regulators of viscoelastic properties for greases and cosmetics, as solidifier for used cooking oil, and as reagents for the recovery of ocean-spilled oil. The disposal of crude or waste oils has been identified as a serious problem with respect to pollution of the environment. Thus, development of these gelling agents is important.

12-HSA is usually utilized as a solidifier for waste edible oils after cooking because of the exceptional stiffness of the organogel that can be achieved with a small amount of 12-HSA (*ca.* 1%). The helical structures of the fibrous aggregates that constitute the gel network are caused by unidimensional hydrogen bonding of carboxyl groups with the hydroxyl groups on the chiral carbon at the C_{12} position of the 12-HSA molecule (3–5). The critical gel-forming concentration of 12-HSA shifts to a considerably lower concentration range, and the helical structure of the fibrous aggregate disappears in the gel when a small amount of alkali metal ion coexists with 12-HSA in soybean oil (7). In contrast, organogel formation was inhibited by the presence of various contaminants, such as polyvalent metal ions or alcohols. In particular, evidence was obtained that lecithin, which was a contaminant from egg yolk in edible oil, caused remarkable lowering of the stiffness of organogels.

However, lecithin forms organogels with many organic solvents when a slight amount of water is added (17–21). Although the water-to-lecithin molar ratio at which maximum viscosity is observed differs depending on the kind of organic solvent, most of these causes have a ratio below 10. It is generally believed that formation of a viscoelastic organogel in the system lecithin/organic solvent/water is due to the water-induced formation of flexible cylindrical reverse micelles by lecithin molecules and subsequent formation of a transient network of entangled micelles (19). These reports have suggested that the interaction of the polar head group in the lecithin molecule with water is the most significant factor for gel formation in organic media. In the present work, we studied the inhibition mechanism of lecithin in 12-HSA organogel formation by spectroscopic techniques.

EXPERIMENTAL PROCEDURES

Materials. 12-HSA was purified from a commercial product from Kawaken Fine Chemical Co., Ltd. (Tokyo, Japan) according to the method of Uzu and Sugiura (22). Egg yolk lecithin (NC-10S; >95% phosphatidylcholine) was obtained from Nihon Fat & Oil Co., Ltd. (Tokyo, Japan) and used without further purification. Cyclohexane, chloroform, and methanol of extra-pure grade were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan).

Preparation of organogels. The 12-HSA and lecithin were dissolved in organic solvents at 70°C, where both materials were homogeneously soluble, and the solution was left standing at room temperature to form gels. Appearance of the gels was monitored in a quartz cell (1 mm thickness) and pho-

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tographed. Gel stiffness was evaluated from the minimum stress to destroy the gel according to a previous paper (4).

Spectroscopy. Infrared (IR) spectra were measured with a Perkin-Elmer model-200 Fourier transform IR spectrophotometer (Perkin-Elmer Co., Ltd., Norwalk, CT). The measurements were carried out with a gel sample (0.2 mm thickness) supported between parallel BaF plates at 25°C.

The ^{13}C nuclear magnetic resonance (NMR) spectra were measured at 75.45 Mhz with a Jeol model JNM-LA300 spectrometer (Jeol Co., Ltd., Tokyo, Japan) at 30°C. For each ^{13}C NMR measurement, the organic solvent signals were used as internal references for chemical shifts (cyclohexane, 26.6 ppm; chloroform, 77.7 ppm; and methanol, 49.8 ppm).

Scanning electron microscope (SEM) observation. SEM was carried out as previously described (7) with a Hitachi model S-520 SEM (Hitachi Co. Ltd., Tokyo, Japan).

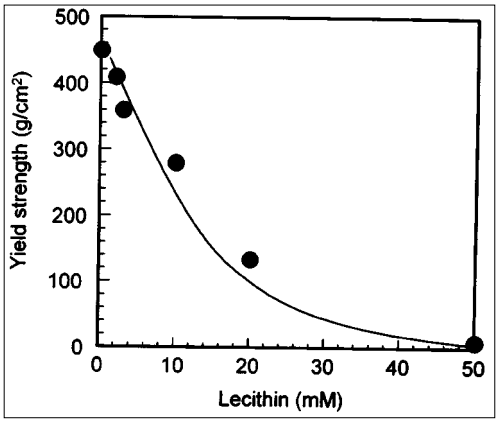

FIG. 2. The effect of lecithin concentration on the yield strength of a 12-HSA/cyclohexane gel. The concentration of 12-HSA was 100 mM. See Figure 1 for abbreviation.

RESULTS AND DISCUSSION

Gel formation. The change in the appearance of a 12-HSA/cyclohexane gel (the concentration of 12-HSA was 100 mM) with the addition of lecithin was observed. The 12-HSA/cyclohexane system forms a slightly turbid gel with high viscosity; the 12-HSA/benzene and soybean oil systems form transparent viscous gels (7,23,24). The slightly turbid appearance was attributed to the somewhat rectangular shape of the cross-section of the gel fibers (24). The degree of dipole-dipole interaction in the solvent seemed to determine the network structure, which varied from almost cylindrical fibers to ribbon-like fibers and lamellae. As shown in Figure 1, appearance of the 12-HSA/cyclohexane gels varied from homogeneously turbid to heterogeneously suspended, up to 50 mM lecithin; then they became completely transparent at 100 mM.

As shown in Figure 2, the yield strength of the 12-HSA/lecithin gels decreased linearly with increased lecithin concentration, up to 20 mM, and approached zero yield strength in the higher concentration range. The tendency of gel strength to decrease with the addition of lecithin was consistent with the change in appearance of the gel shown in Figure 1. The 12-HSA/cyclohexane gel no longer retained macroscopic gel strength beyond a concentration of 50 mM lecithin, where residual solid masses were still present in the medium.

Interaction of 12-HSA with lecithin observed by IR. It is well established that optically active hydroxyl groups, which exist in 12-HSA molecules, can additionally bind to the hydrogen-bonding head groups of the 12-HSA dimer by intermolecular interaction in the crystalline phase (25). IR spectra were measured to obtain information on the inhibitory effect of lecithin on gel formation. Figure 3 shows the change in the IR spectra of 12-HSA/cyclohexane systems with addition of various amounts of lecithin. The absorbance of the band at 3200 cm^{-1}, corresponding to hydroxyl groups bound to 12-

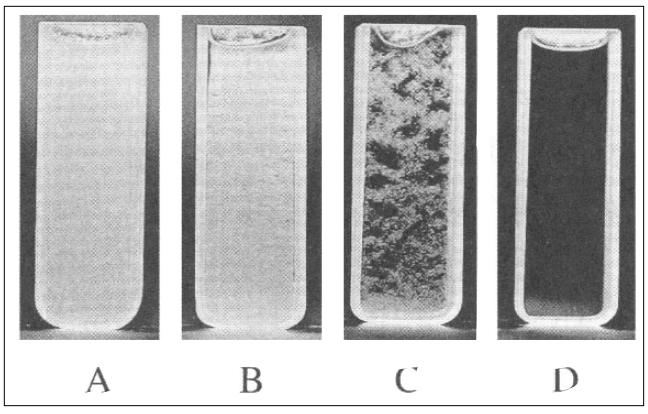

FIG. 1. Change in the appearance of a 12-hydroxystearic acid (12-HSA)/cyclohexane gel (100 mM 12-HSA) by the addition of lecithin. The concentrations of lecithin were (A) 0 mM, (B) 20 mM, (C) 50 mM, and (D) 100 mM.

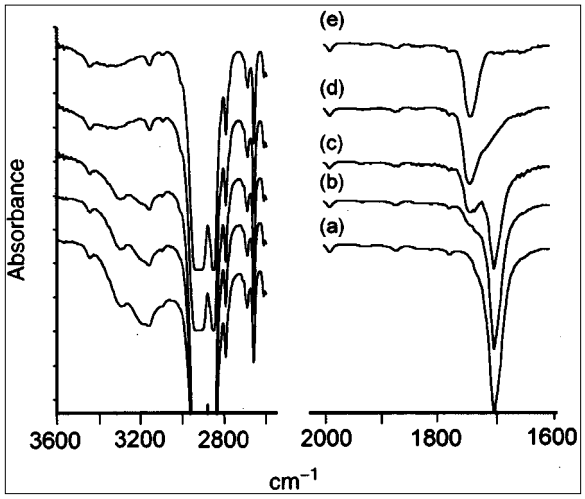

FIG. 3. Change in infrared spectra of a 12-HSA/cyclohexane system (100 mM, 12-HSA) with the addition of lecithin. The concentrations of lecithin were (A) 0 mM, (B) 10 mM, (C) 50 mM, (D) 100 mM, and (E) 100 mM without 12-HSA. See Figure 1 for abbreviation.

HSA dimers, decreased with increasing lecithin concentration. Consistent results were obtained in the absorption band at 1700 cm^{-1}, which corresponds to dipole-dipole interactions between the carboxylic groups of 12-HSA. No attempt was made to explain the change in 1700 cm^{-1} absorption because the absorption band at 1740 cm^{-1} corresponds to lecithin overlaps with the band at 1700 cm^{-1}. However, the results clearly show that lecithin molecules affect hydrogen bonding of both the hydroxyl and carboxylic groups of 12-HSA molecules.

Interaction of 12-HSA with lecithin observed by NMR. The interaction of 12-HSA with lecithin was studied by monitoring the chemical shifts in the peaks of both the carbonyl carbon of the 12-HSA molecule and the ester carbon of lecithin. Figure 4 shows the ^{13}C NMR spectra of a 100-mM chloroform solution of 12-HSA with the addition of lecithin. It was convenient to use 12-HSA/chloroform systems of low viscosities because NMR measurements were technically difficult in 12-HSA/cyclohexane systems that formed highly viscous gels. The signal at 179.3 ppm for a lecithin-free system (Fig. 4A), assigned to the carbonyl carbon of 12-HSA dimers, shifted upward with increasing lecithin concentration (Fig. 4B–E). The chemical shift nearly reached a constant value (176.3 ppm) at the molar ratio of 12-HSA/lecithin of 1:1 (Fig. 4D). No further chemical shift was observed at the molar ratio of 12-HSA/lecithin of 1:2 (Fig. 4E). These results suggest that 12-HSA and lecithin form a complex with a 1:1 molar ratio in organic media. Results of the chemical shifts of the carbonyl carbon for 12-HSA and stearic acid in the presence and absence of lecithin are shown in Table 1. In the chloroform system, the upfield chemical shift in the NMR signal ($\Delta\delta_c$) of stearic acid, caused by lecithin, was similar to that of 12-HSA (–3.0 ppm). No difference was observed in the chemical shifts between 12-HSA and stearic acid with the addition of lecithin in cyclohexane. Furthermore, the $\Delta\delta_c$ value of stearic acid in cyclohexane (–4.9 ppm) was substantially the same as that in chloroform (–3.6 ppm). These results indicate that 12-HSA or stearic acid molecules interact directly with lecithin molecules in cyclohexane. On the contrary, the resonance line of 12-HSA was observed at 177.4 ppm in methanol, and it was substantially unchanged by the addition of lecithin. Thus, the dipole-dipole interaction between the carbonic acids was interpreted to be that of a hydrogen donor solvent, such as methanol interacts directly with the carboxylic acid. These results suggest that 12-HSA directly interacts with lecithin in an equimolar complex.

TABLE 1
^{13}C NMR Chemical Shifts of Carbonyl Carbon of 12-HSA and Stearic Acid in the Presence and Absence of Lecithin[a]

Solvent	Lecithin (mM)	δ_c (ppm)	$\Delta\delta_c$ (ppm)
12-HSA (100 mM)			
Cyclohexane	0	—	
	100	174.9	—
Chloroform	0	179.3	
	100	176.3	–3.0
Methanol	0	177.4	
	100	177.3	–0.1
Stearic acid (100 mM)			
Cyclohexane	0	179.6	
	100	174.7	–4.9
Chloroform	0	179.9	
	100	176.3	–3.6

[a]12-HSA, 12-Hydroxystearic acid.

Furthermore, complex formation of lecithin with carboxylic acid was monitored by using the spectra of the polar head group of lecithin. Figure 5 shows ^{13}C NMR spectra of a 100-mM solution of lecithin in cyclohexane with the addition of 12-HSA (A) and stearic acid (B) in the 50–80 ppm range. There was a remarkable change in the resonance line of the

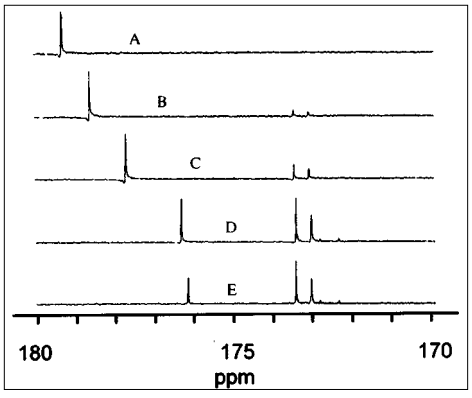

FIG. 4. Change of ^{13}C nuclear magnetic resonance spectra of 12-HSA/chloroform system [100 mM 12-HSA (A)–(D), 50 mM 12-HSA (E)] with the addition of 170–180 ppm lecithin. The concentrations of lecithin were (A) 0 mM, (B) 10 mM, (C) 30 mM, (D), and (E) 100 mM. See Figure 1 for abbreviation.

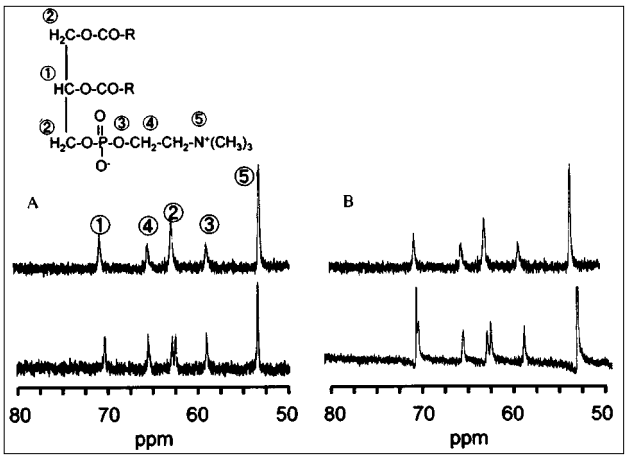

FIG. 5. Change in ^{13}C nuclear magnetic resonance spectra of a lecithin/cyclohexane system (100 mM lecithin) on the addition of 12-HSA and stearic acid in the 50–80 ppm range. (A) The concentrations of 12-HSA were 0 mM (upper part) and 100 mM (lower part). (B) The concentrations of stearic acid were 0 mM (upper part) and 100 mM (lower part). See Figure 1 for abbreviation.

carbons of the triglyceride part. The singlet assigned to the methylene carbons in the glyceride moiety (resonance ②) split into a double on the addition of equimolar 12-HSA, and its intensity became practically identical with that of other lecithin methylenes. At the same time, splitting of the singlet into a multiplet was also observed for the methyne carbon (resonance ①) because of its slight intensity loss observed on the addition of lecithin. On the other hand, no significant change was observed in both singlets assigned to the methylene carbons in the phosphatidylcholine moiety (resonances ③ and ④). The results undoubtedly suggest that the triglyceride moiety interacts with the carboxyl group in 12-HSA or stearic acid. Capitani *et al.* (9) studied organogel formation in a lecithin/cyclohexane system by NMR. Steep increases in the viscosity of the system were observed on the addition of small amounts of water. Considerable line broadening on the ^{13}C of methylene and methyne in the triglyceride moiety and the ^{31}P in phosphatidylcholine were also observed, which indicates resistance to molecular motion. On the contrary, our results showed that 12-HSA added to lecithin had no effect on the line width of methylene carbons because the formed complex was highly soluble in organic media. Summarizing the two complementary results in NMR observations suggests that the polar head groups of lecithin can bind to the carboxylic moiety of 12-HSA in a 1:1 complex, which permits the fibrous gel network of 12-HSA to dissolve in organic media.

SEM observation. The morphological effect of lecithin on the gel formed by 12-HSA was observed by SEM. Figure 6 shows changes in the SEM images of 12-HSA/cyclohexane gel with the addition of lecithin. Pure 12-HSA formed a network structure that was made up of tape-like thin fibrils that are entangled with each other (Fig. 6A). When 5 mM lecithin was added, however, a tightly packed network structure appeared partially with most still being in an inherent fibrous 12-HSA network. Upon further addition of lecithin, the fibrous structures completely disappeared, and spherical aggregates appeared and formed large clusters of spherical aggregates (Fig. 6C,D). The high-magnification image in Figure 6E clearly reveals that linerly branched crystals were elongated in all directions from the center of each spherical aggregate. Within the range of 20 mM lecithin concentration excess 12-HSA existed in the medium. Failure to observe fibrous aggregates in the gel suggests that the 1:1 complex between 12-HSA and lecithin may nucleate and condense with excess 12-HSA molecules to form spherical aggregates. In conclusion, the intermolecular complexes between lecithin and 12-HSA cause the structural change from fibrous networks of 12-HSA to clusters of spherical aggregates composed of 12-HSA/lecithin, which results in the rupture of the rather stiff organogel.

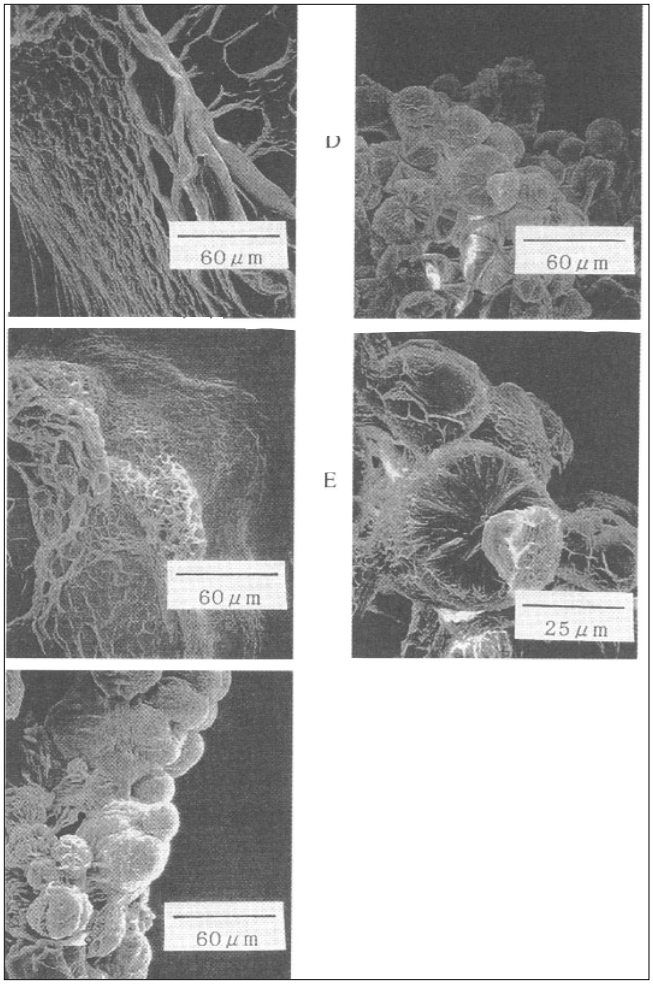

FIG. 6. Change in scanning electron microscopy images of the 12-HSA/cyclohexane gel structure (100 mM 12-HSA) with the addition of lecithin. The concentrations of lecithin were (A) 0 mM, (B) 5 mM, (C) 10 mM, and (D) 20 mM. A higher magnification image of (D) is displayed in (E). See Figure 1 for abbreviation.

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