

Helical Aggregate of Oleic Acid and Its Dynamics in Water at pH 8

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It is found that helical structures can be formed by a simple achiral fatty acid, oleic acid, in the pH range of 8.0–8.1 at room temperature. Moreover, we succeeded in the first observation of spontaneous dynamics of these helical aggregates in water by optical microscopy.

Helical structures are one of the fundamental building blocks of supramolecular system in a living body, such a protein α helix, a DNA double helix, etc., and they are invested with primary functions in living cells.¹ Fuhrhop et al. claimed that helices are formed from lamella structures of chiral amphiphilic molecules.^{1–3} One of the outstanding examples is formation of a helical structure by winding a myelin-like tube made of cardiolipin and phosphatidylcholine, being induced by the addition of calcium ions.² Here we report the first demonstration that a helical structure is formed even from a simple achiral fatty acid, such as oleic acid, in the specific pH range of 8.0–8.1. Another characteristic of this system is that the composition of oleic acid and oleate in the helix is closer to a nonlamellar structure rather than the bilayer lamella. Moreover, these helices turned out to exhibit spontaneous winding/rewinding dynamics in water.

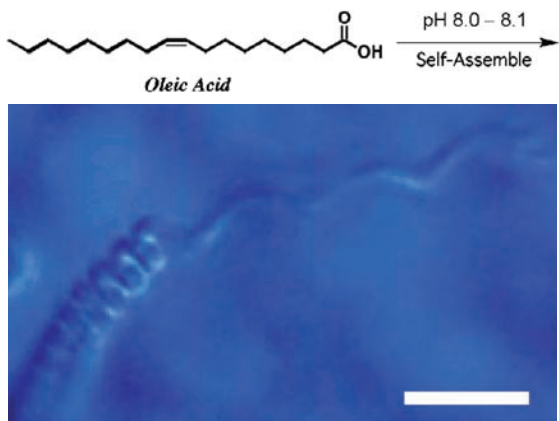


Figure 1. Differential interference microscopic (DIC) images of a right-handed helical structure of oleic acid in Bicine buffered solution. This helical structure wound, changing its pitches, in at least 30 min. Scale bar = 30 μ m.

Nonvesicular aggregates of oleic acid were prepared in a buffered aqueous solution by the following method.⁴ Sodium oleate (10 mg) was dissolved in 2.0 mL of Bicine buffer (70 mM, pH 7.8) at 25 ± 1 °C, and the solution was sonicated for 10 min, the pH of the solution being in the range of 8.0–8.1. Immediately after sonication, the suspension became turbid, and only oily particles with diameters of about 1 μ m (max \approx 5 μ m) were observed by differential interference contrast mi-

croscopy (BX51, Olympus, Japan). After 1 h, however, various aggregates appeared. More than half of the aggregates turned out to be helices (Figure 1), although other types of aggregates coexisted in the dispersion, such as glittering rods and droplets of oily streaks. The pitches of the helices ranged from 0.2 to 3.2 μ m and were positively correlated with their diameters, which ranged from 1 to 4 μ m. The ratio of left-handed to right-handed helices was ca. 1:1. Judging from the manner of their thermal movements, the helices were flexible, and some long and slender ones even meandered in the water. These structures existed for about two months, and eventually collapsed into nonhelical structures of oily streaks.

In fact, we directly observed morphological changes of oleic acid aggregates from glittering rods to helical structures under an optical microscope. To our surprise, some of the helical structures showed outstanding dynamics: tube-like structures wound into helices, helical structures changed their pitches or their handedness (switching from right- to left-handed or vice versa), or two helical structures coiled up around each other, as shown in Figure 2.

In order to clarify the nature of the helical structures of oleic acid in the buffered dispersion, we changed of parameters for the preparation of dispersion; pH value, temperature, buffer, and lipid. When the pH of the Bicine buffered solution of sodium oleate was adjusted to pH > 8.1 or pH < 8.0, no helical aggregates was observed. The Bicine buffered dispersion of sodium oleate, which was prepared at 10 °C (below T_c of oleic acid in water, i.e. 11 °C) in the range of pH = 8.0–8.1, the oleic acid/oleate system gave only water-insoluble solid aggregates. Neutralization of the sodium oleate with HCl aq (9 mM, 2.0 mL) to adjust the pH value in the range of 8.0–8.1 did not enable to form helices.

On the other hand, helical aggregates were prepared in other buffered solution: 50 mM Tris-HCl and 33 mM Na_2HPO_4 - KH_2PO_4 . Some counter ions of those buffers may interact with the hydrogen-bonded network of the surface of the helical structure. Several successful conditions were also found to form helical aggregates in the dispersion (16.4 mM) of sodium salts of other unsaturated fatty acids, *cis*-12-octadecanoic acid and linoleic acid, with the Na_2HPO_4 - KH_2PO_4 buffer (33 mM) in narrow range of pH value. To the contrary, no helical or rod structures were observed in the same buffered dispersion of sodium salts of *cis*-6-octadecanoic acid and linolenic acid.

The structure of helical aggregates of oleic acid in the dispersion was examined by means of polarized light microscopic images (BX50, Olympus, Japan) and cryo-scanning electron microscopic ones (S-3000N, HITACHI, Japan). It was confirmed that the helices are generated through the twisting of rod structures which are composed of liquid-crystalline multilayer tubes of closely packed streaks.⁵

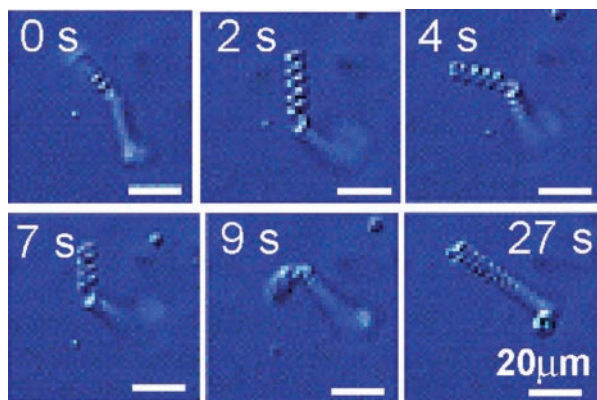


Figure 2. Sequential differential interference contrast microscopic images of rewinding of the helical structure, recorded every second. The helical structure consists of a right-handed part and a left-handed part. The right-handed part gradually rewound to the left as the kink point which migrated along the tube from bottom to head at a speed of ca. $5 \mu\text{m s}^{-1}$.

Because helical structures exist only in a narrow pH range (8.0–8.1), the degree of dissociation of oleic acid in the aggregates must play an intrinsic role in the formation of helical structures. Therefore, the degree of dissociation was evaluated by ^{13}C NMR.⁶ For the NMR measurements, samples were prepared by dispersing $1\text{-}^{13}\text{C}$ oleic acid sodium salt (16.4 mM) in deuterium oxide (D_2O) in the presence of 33 mM $\text{KD}_2\text{PO}_4\text{-Na}_2\text{DPO}_4$ buffer. Helical structures were formed when the pD of the suspension was in the range of 8.60–8.64 (23 °C), and the carbonyl chemical shift (δ) of oleic acid/oleate was 182.72 ppm at pD = 8.60 and 182.78 ppm at pD = 8.64. As references, the carbonyl chemical shift (δ_{COOD}) of protonated (deuteronated) oleic acid in D_2O was observed at 181.3 ppm in a 16.4 mM solution at pD = 2,⁷ and the chemical shift (δ_{COO^-}) of fully ionized oleic acid (80 mM) in D_2O at pD 10.84 appeared at 185.42 ppm. From these chemical shift values, we determined the degree of dissociation (α) of oleic acid in deuterium oxide was 0.62–0.68. Here, the α value was calculated by the following equation:⁸

$$\alpha = (\delta_{\text{COOD}^-} - \delta) / (\delta_{\text{COO}^-} - \delta_{\text{COOD}}).$$

From the α value evaluated by NMR measurements, it was found that the molar ratio of carboxylate to carboxylic acid in the helical aggregates was ca. 3:2.

This evaluated value of ratio is comparative with the value of the 1:1 acid–soup aggregation of sodium oleate in previous reports, while the ratio of carboxylate to carboxylic acid ranged from ca. 1:1 to 1:3 in the lamellar phase of oleic acid/oleate system.^{9,10} Namely, the result indicates that this rod aggregates must be formed as an intermediate stage between the lamellar liquid-crystalline phase and nonlamellar liquid-crystalline phase (e.g. Hex_{II} or cubic phase).¹¹ While the rod aggregates exist in the dispersion of oleic acid, their spontaneous dynamics is probably derived from a nonequilibrated condition. It is plausible that the curvature of each layer of the multilayer tube structure varies depending on the local fluctuation in the ratio of carboxylic acid to carboxylate, since the physical properties of self-aggregation of fatty acids in water are influenced by the ionization state of carboxylate group.¹⁰ A network of hydrogen bond on the surface of each layer of the multilamellar tube of oleic acid may waver locally,¹² and then a cooperative release of stress percolates be-

tween layers, giving rise to drastic deformations of multilayer tube, such as winding itself up into helices and changing their helical morphologies.

These findings on such a self-aggregate which exhibits dynamic behaviors as a whole will open a new horizon of supramolecular science of lipids.^{1,13}

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References and Notes

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- 4 We succeeded in formation of helices in another method with oleic acid. Oleic acid (1 μL) was gently mixed with 135 μL of Bicine buffer solution (70 mM, pH 8.1) containing cetyltrimethylammonium bromide (CTAB, 0.4 mM) at 23 ± 1 °C. The structure-forming process in this suspension was followed at 23 °C by differential interference contrast microscopy. Helical and stick-like structures, as well as vesicles, appeared 10 min after the mixing. The shapes of the helical structures were the same as in suspensions prepared by the method in the main text.
- 5 The polarized light microscopic image of helical aggregates was observed under a cross-polarizer. Helical structures appeared dark when the axis of the helix coincided with the extinction position, but turned bright when the axis was in off-diagonal positions. The inner packing structure of the helical tubes was also observed by cryo-scanning electron microscopy, using a cut-off specimen of the frozen dispersion that was obtained by rapid-freezing with liquid N_2 .
- 6 Although this buffered solution contains other types of aggregates in addition to helices as minor components, it is not probable that transitions between these forms of aggregates are in equilibrium with the exchange rate faster than the NMR time scale ($> 10^3 \text{ s}^{-1}$). Accordingly, the observed carbonyl chemical shift of the main peak can be assigned mainly to oleic acid in the helical structures.
- 7 For determining δ_{COOD} , cetyltrimethylammonium bromide (CTAB) was added to the acidic suspension to keep the oil emulsion of oleic acid stable. The δ_{COOD} value was estimated by extrapolating the straight line to the zero concentration of CTAB in the plot of $\log(\delta - \text{const.})$ against the concentration of CTAB.
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