Spontaneous Formation of Superhelical Strands

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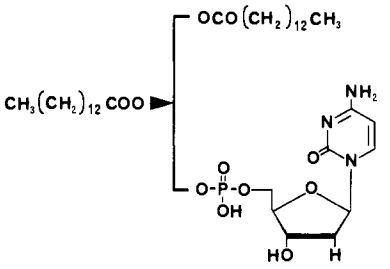
Abstract: We have developed a new class of helical structures that self-assembled spontaneously in aqueous solution. A phospholipid–nucleoside conjugate containing two long alkyl chains and a nucleotidyl residue, dimyristoyl-5′-phosphatidyldeoxycytidine (**1**), has been synthesized, and its self-organization, morphology, and physicochemical properties have been investigated. **1** spontaneously assembled to form two types of helical strands. One consisted of a duplex with a diameter of ≈110 Å and helical pitch of ≈240 Å. The duplex further formed a superhelical structure with a helical pitch of ≈1100 Å. The helix was right-handed. The other consisted of a double duplex whose diameter and helical pitch were much the same as those of the former. The double duplex also formed a right-handed superhelical structure. The superhelical strands were gradually converted into gels at 25 °C. We described the formation process of the superhelical strands and proposed a possible model for their structures.

Molecular helicity is the most fundamental property displayed by biological polymers such as nucleic acids,[1] proteins,[2] and starch.[3] DNA, which is a genetic information store, consists of double-helical polynucleotides formed by linking 3′- and 5′-positions of adjacent sugar residues by a phosphodiester bond.[4] Phospholipids,[5] which are quantitatively dominant constituents in biological membranes,[6] self-assemble to form micelles, monolayers, and bilayer vesicles in aqueous media.[7] This tendency to aggregate and to form organized structures is derived from the architecture and amphoteric character of the lipid molecules. A mononucleotide unit noncovalently linked through the hydrophobic groups of lipids should be able to self-assemble in aqueous media and form helical strands like DNA. Great interest in the construction of superstructures similar to DNA by self-assembly of a mononucleotide unit has prompted us to make phospholipid–nucleoside conjugates having two long alkyl chains and a nucleotidyl group in a molecule.[8] Recently we discovered that the phospholipid–nucleoside conjugates dipalmitoyl-5′-phosphatidylcytidine and dipalmitoyl-5′-phosphatidyldeoxycytidine spontaneously assemble to form circular and linear helical strands.[9] We concluded that stacking and hydrogen bonding between nucleic acid bases, and hydrophobic interactions between the long alkyl-chain moieties of phospholipid–nucleoside conjugates, are necessary for the formation of the helical strands. We expected to construct higher helical structures by controlling hydrophobic interactions between alkyl-chain moieties of phospholipid–nucleoside conjugates. Consequently, we have synthesized dimyristoyl-5′-phosphatidyldeoxycytidine (**1**) with the shorter chain length alkyl groups. We report here the superhelical structure of **1** in aqueous solution.



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Results and Discussion

Formation of Superhelical Structures from 1. After sonication at 50 °C for 45 min, an aqueous solution of **1** produced vesicles mainly with diameters of 200–1600 Å. The vesicles were slowly transformed into superhelical strands after aging at 25 °C for 1 day. Figure 1a shows the formation of two types of helical strands, i.e., a thin helical strand (strand 1) and thick helical strands (strands 2–4). Image processing showed that strand 1 consisted of a duplex with a diameter of ≈110 Å and a helical pitch of ≈240 Å (Figure 1b). The duplex further formed a superhelical structure with a helical pitch of ≈1100 Å. The helix was right-handed. Strands 2–4 consisted of a double duplex whose diameter and helical pitch were much the same as those of strand 1 (Figure 1, parts c and d). Each duplex also formed a right-handed superhelical structure with a helical pitch of 950–1100 Å. Among strands 2–4, the regularity of superhelical structure increased in the order strand 2, strand 4, and strand 3. Strand 2 seems to be a metastable form of strands 3 and 4.

At much early stage of aging, the vesicles budded out to form helical strands (Figure 2a). After aging for 10 h, the vesicles were completely transformed into linear double-helical strands (Figure 2b). Short superhelical structures then appeared (Figure 2c). Some linear helical double strands twisted to form incomplete double-duplex strands, corresponding to strand 2 in Figure 1. The incomplete strands further arranged to complete double-duplex superhelical structures (Figure 2d), corresponding to strand 3 in Figure 1. Many superhelical structures were formed after 1 day (Figure 2e). All of the superhelical structures were converted into gels after 1 week. The gels consisted of a number of thick strands formed from linearly extended helical strands (Figure 2f).

CD Spectra of 1. To understand the physicochemical properties of **1**, we have examined its CD spectrum in aqueous solution. A solution of **1** in 0.05 M Tris-HCl (pH 8.0) showed a drastic change in the CD spectrum when cooled from room temperature to 15 °C (Figure 3). In particular, the CD spectrum of **1** at 15 °C showed a striking increase in the positive Cotton effect with a peak at 277 nm. In contrast, only a weak single peak with a maximum at 274 nm in the CD spectrum was observed at 25 °C. The CD profile of **1** at 15 °C highly resembled that of helical poly(C),[10]

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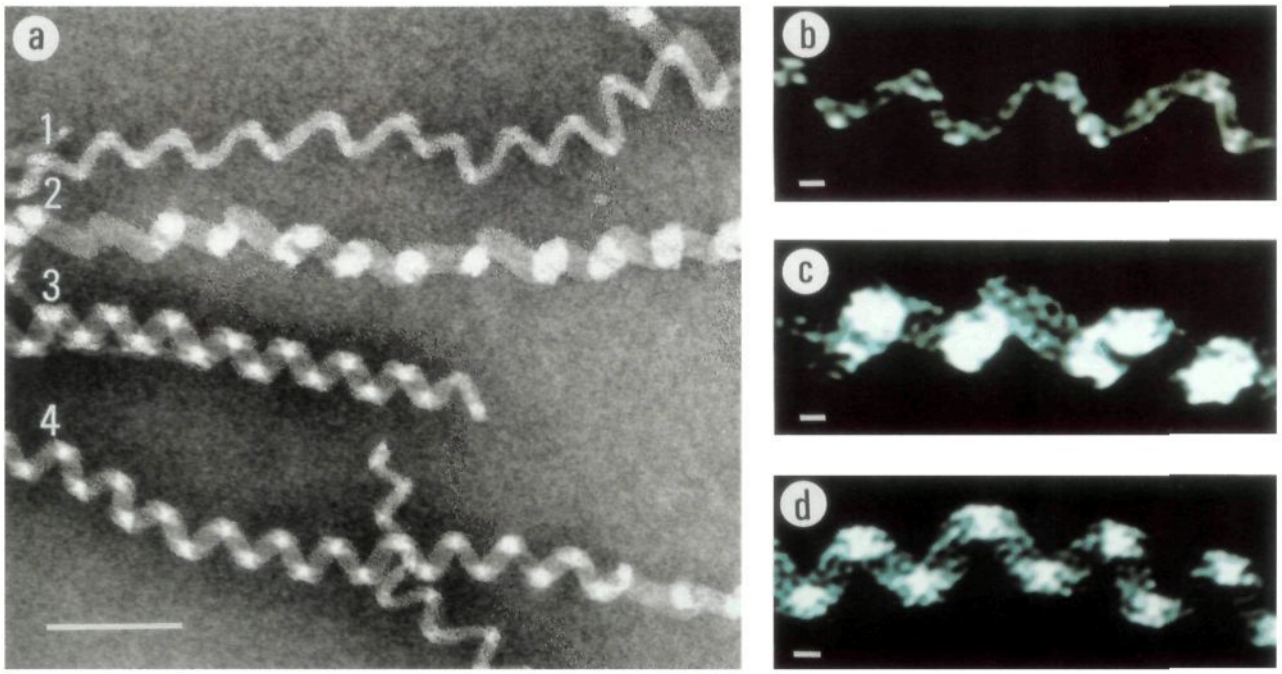

Figure 1. (a) Electron micrograph of superhelical strands formed from **1** after aging at 25 °C overnight (scale bar, 1000 Å). 1, strand 1; 2, strand 2; 3, strand 3; 4, strand 4. (b–d) Fourier-transformed images of strands 1, 2, and 4, respectively (scale bars, 100 Å).

but not poly(dC),[11] at identical pH and temperature. At neutral pH, poly(C) or poly(dC) exists as a nonprotonated, single-stranded helical structure. The properties of single-stranded poly(C) can be calculated fairly well from a consideration of nearest-neighbor base-stacking interactions.[12] Therefore, the helical aggregates formed easily from **1** at 15 °C seem to take a conformation similar to poly(C). The positive Cotton effect of **1** shown in Figure 3 was very sensitive to temperature and the formation of the helical aggregates. The melting temperature of the aggregates was determined to be 21 °C from the variation curve of the 277-nm peak with temperature (see the inset in Figure 3). The melting temperature was nearly in accord with the transition temperature (21 °C) of the gel–liquid crystal of **1**. The cooling profile was reversible upon heating. These results indicate that **1** has essentially the molecular characteristics of helicity which is stabilized by both hydrophobic interactions between the long alkyl-chain moieties and stacking between the nucleic acid bases.

A Possible Molecular Structure. Among resulting strands, a double-helical strand is the most fundamental structure unit. The electron micrograph in Figure 1 revealed that a double-helical strand has a diameter of 110 Å. Figure 4 shows a CPK molecular model for possible conformations of **1**. **1** might be able to take both bent and extended conformations. The molecular length can be estimated from their CPK molecular model. The molecular length of **1** was 26 Å if it takes a bent conformation and 36 Å for an extended conformation. In phospholipid–nucleoside conjugates, a hydrophobic alkyl-chain moiety and nucleic acid base moiety were necessary for the formation of helical strands.[9] Considering this fact and the formation process of helical strands reported here, we assumed a possible formation mechanism and molecular structure for the helical strands produced from **1**. **1** would first assemble by interaction between hydrophobic alkyl-chain moieties to form bilayer vesicles, which would then rearrange to bilayer cylinders by stacking between nucleic acid base moieties. The stacking effect is the only possible driving force to form helical structures because the conjugate with no nucleic acid base did not form helical strands. The existence of stacking effects was also supported by CD spectral data of **1**. The length of the bilayer cylinder was estimated to be 52 Å for a bent conformation and 72 Å for an extended conformation, respectively. The former length nearly accorded with half the diameter (≈110 Å) of a double strand which was measured by electron microscopy. Therefore, the single strand produced from **1** may well consist of a helical bilayer cylinder structure (Figure 5) having a bent conformation. Since the nucleic acid base moiety is a hydrophobic group, it can interact with the hydrophobic alkyl-chain moieties. Therefore, the interaction may be also a driving force for taking a bent conformation. Recently, Kunitake et al. reported that bent ammonium amphiphiles were advantageous for the formation of fibrous and tubular aggregates.[13] In addition, Fuhrhop et al. recently found that *N*-octylaldoamides with a bent conformation formed micellar cylinders and helices.[14] Therefore, bent amphiphiles appear to be advantageous for the formation of fibrous structures.

The results described above provide a general way for generating double-helical structures of phospholipid–nucleoside conjugates. The basic features of the present system allow us to imagine numerous extensions into a variety of directions.

From the point of view of general molecular features, phospholipid–nucleoside conjugates offer an entry into a design of systems displaying self-organization; they provide an opportunity for studying mechanism, thermodynamics, and kinetics of the formation of a double helix. Furthermore, variations in the structure of nucleoside and alkyl-chain moieties of phospholipid–nucleoside conjugates may lead to changes in their morphology and function.

From the biological point of view, the helical strands produced from phospholipid–nucleoside conjugates may undergo multiple binding to DNA or RNA and change their structures and functions. From the point of view of the origin of life, the helical strands can be conceived as models for primitive templates, and prebiotic assemblies, which may lead to spontaneous polymerization of nucleic acids. In addition, the structural and physical features of the phospholipid–nucleoside conjugates could have been

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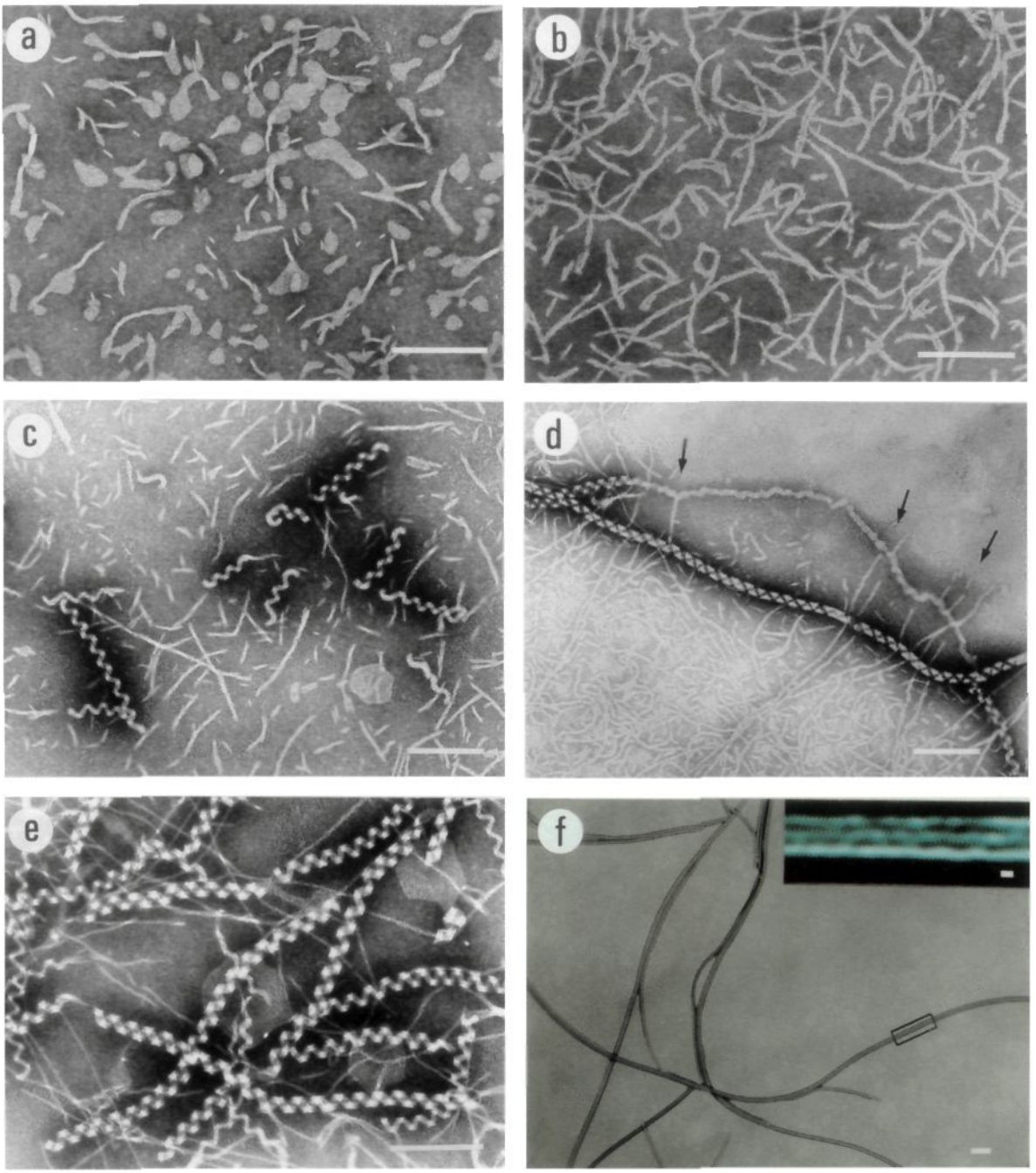

Figure 2. Formation process of superhelical strands. The electron micrographs were taken after aging for 5 h (a), 10 h (b), 15 h (c and d), 1 day (e), and 1 week (f), respectively. All scale bars represent 2000 Å. Arrows in Figure 2d represent positions where strands are twisting. Inset in Figure 2f is the Fourier-transformed image (scale bar, 100 Å) of the segment boxed by a rectangle.

of use in the formation of a molecular device for catalysis.

In summary, we have developed a new class of helical structures that self-assembled spontaneously in aqueous solution. We are further extending this approach to other phospholipid–nucleoside conjugates.

Experimental Section

General Procedures. ^{1}H NMR spectra were recorded on a JEOL FX90Q (89.95-MHz) spectrometer. All chemical shifts for ^{1}H NMR spectra are reported in parts per million relative to tetramethylsilane (Me_4Si, δ 0.00). Infrared spectra were recorded on a Hitachi 260-50 infrared spectrophotometer. Ultraviolet absorption was measured with a Jasco 660 spectrophotometer. FAB mass spectra were taken on a JEOL HX100 spectrometer by using glycerol as a matrix. Melting points were taken on a Yanaco micro melting point apparatus and are uncorrected. CD spectra were recorded on a Jasco J-40A automatic recording spectropolarimeter. Phase-transition temperature (T_c) was determined with a Daini Seikosha SSC-560 differential scanning calorimeter. A suspension of **1** (1.5 mg in 55 μL of 0.05 M Tris-HCl, pH 8.0) was encapsulated in a calorimeter aluminum pan. The heating rate was 0.5 °C/min.

Materials. 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine was purchased from Sigma (St. Louis, MO). *Streptomyces* phospholipase D[15] was kindly provided by Dr. S. Shuto of Toyo Jozo Co., Ltd. (Shizuoka, Japan). Deoxycytidine was obtained from Yamasa Shoyu Co., Ltd. (Chiba, Japan). All other chemicals used were of reagent grade.

Synthesis of Dimyristoyl-5′-phosphatidyldeoxycytidine (1). **1** was enzymatically synthesized from 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine and deoxycytidine by using *Streptomyces* phospholipase D as

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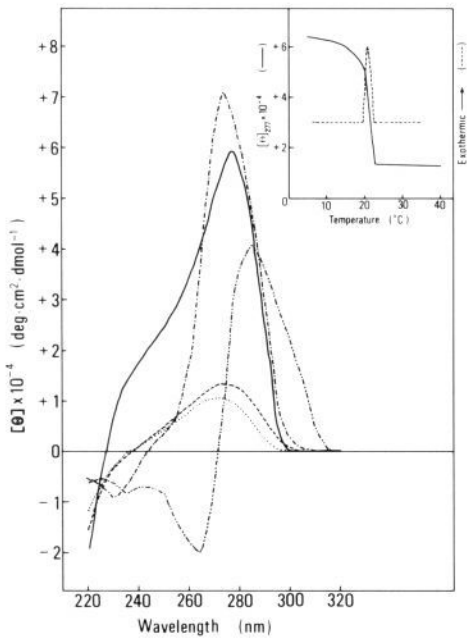

Figure 3. CD spectra of **1**, poly(C), poly(dC), and 5′-dCMP in 50 mM Tris-HCl buffer (pH 8.0). **1** at 15 °C (—); **1** at 25 °C (- - -); poly(C) at 15 °C (-·-); poly(dC) at 15 °C (-··-); 5′-dCMP at 15 (···). Path length, 0.21 mm. Inset: temperature–CD and temperature–differential scanning calorimetry profiles for **1**.

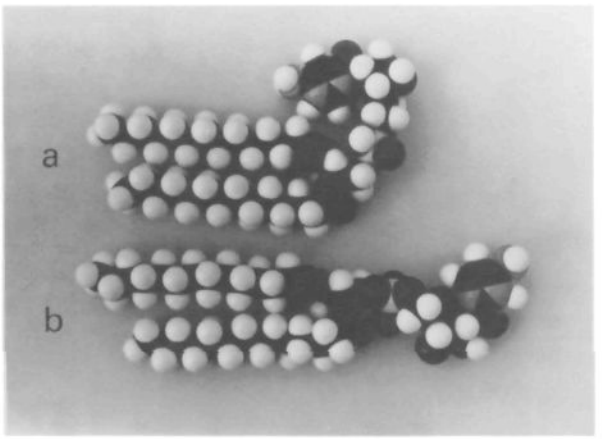

Figure 4. CPK molecular models for bent (a) and extended (b) conformations of **1**.

described previously[8c] and was obtained in 40% yield. Analytical, magnetic, and spectroscopic data of **1**: white crystal, mp 160–163 °C; ^{1}H NMR (90 MHz; $CDCl_3$–CD_3OD, 3:1) δ 8.37 (d, H-6, J = 8.1 Hz), 6.07 (m, H-5 and H-1′), 5.22 (m, glycerol CH), 4.52–3.90 (m, H-2′, H-2″, H-3′, H-4′, H-5′, H-5″, glycerol CH_2), 2.32 (m, CH_2CO), 1.60–1.28 (m, myristoyl CH_2), 0.89 (t, CH_3, J = 7.0 Hz); IR (KBr) ν 3340, 2930, 2860, 1740, 1680, 1470, 1280, 1195 cm^{-1}; UV_{max} ($CHCl_3$–MeOH, 95:5) 284 nm (ϵ 1.30 × 10^4); mass spectrum, m/z 802 $(M + 1)^+$. Anal. Calcd for $C_{40}H_{72}N_3O_{11}P{\cdot}H_2O$: C, 59.31; H, 9.21; N, 5.19. Found: C, 59.40; H, 9.22; N, 5.21.

Preparation of Superhelical Structures from 1. **1** (free-acid form, 1 μmol) was dissolved in 2 mL of chloroform–methanol (20:1 v/v), evaporated to dryness at 40 °C under reduced pressure, and dried at room temperature for 1 h in vacuo, then 0.1 mL of 20 mM NaOH was added to ionize the phosphate group, and the mixture was vortexed for 15 min. Then 0.1 mL of 0.4 M KCl–0.1 M Tris-HCl buffer (pH 8.0) was added and the mixture was vortexed for 10–15 s. The mixture was put into a Pyrex glass tube [5 mm (i.d.) × 110 mm], sonicated (Branson Sonifier B-1200; 300 W) at 50 °C for 45 min, and aged at 25 °C.

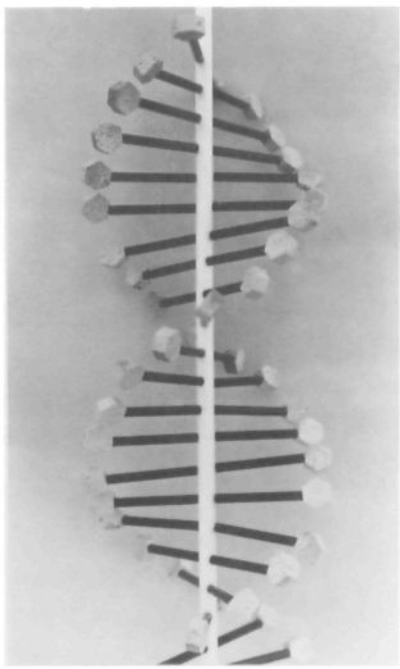

Figure 5. A molecular arrangement in the micellar bilayer cylinder structure. A black rod and hexagon represent alkyl-chain moieties and a nucleic acid base moiety of **1**, respectively. A central rod represents vertical interface of the alkyl chains.

Electron Microscopy. For negatively stained electron microscopic observation, a drop of the sample was placed on a carbon-coated collodion grid, the excess was removed with filter paper, 1% phosphotungstate was applied to the grid, and it was immediately examined under a JEM 1200EX electron microscopy at 80 kV.

Image Processing. Electron microscope images were read through a microdensitometer (Joyce-Loebl, Model 6) and stored in a disk on a Micro-Vax computer. The image had 512 × 512 points with 25-μm resolution (0.5-nm resolution for the original image taken by the magnification of 50 000×). Image processing was performed by the image processing language "Semper".[16,17] Noise reduction was performed using a Fourier transformation method which we call C(circular)-MASK. The steps of the C-MASK process are as follows: 1. Fourier transform of the image (FOURIER). 2. Display the diffractogram obtained above (DISPLAY) to determine the radius of the MASK. The selection of the radius is very important. 3. Values outside the circle on the diffractogram are reset to the boundary average (MASK). 4. Inverse Fourier transformation of the 3 (IMAGE).

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