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Spontaneous Formation of a Network of Helical Strands from Ditetradecanoyl- and Didodecanoyl-5'-phosphatidylcytidine

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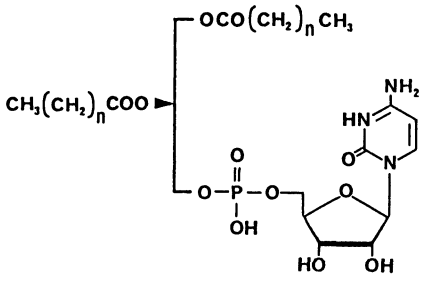
Phospholipid-nucleoside conjugates containing two tetradecanoyl or dodecanoyl groups and a cytidyl group spontaneously assemble to form a network structure of helical strands in aqueous solution. The network structures consisted of a double helical strand and two single helical strands. The network structures were gradually converted into linearly extended double helical strands.

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 two right-handed 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] 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. Recently we have discovered that a phospholipid-nucleoside conjugate, dihexadecanoyl-5'-phosphatidylcytidine (**3**) spontaneously assembles to form circular and linear strands.[8] We concluded that stacking and hydrogen bonding between 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 diacyl-5'-phosphatidylcytidines (**1**–**7**) with the different chain length of alkyl groups. We report here network structures of ditetradecanoyl-5'-phosphatidylcytidine (**4**) and didodecanoyl-5'-phosphatidylcytidine (**5**) in aqueous solution.

Diacyl-5'-phosphatidylcytidines[9] were enzymatically synthesized from the



(1) n=18 Diicosanoyl
(2) n=16 Dioctadecanoyl
(3) n=14 Dihexadecanoyl
(4) n=12 Ditetradecanoyl
(5) n=10 Didodecanoyl
(6) n= 8 Didecanoyl
(7) n= 6 Dioctanoyl

corresponding 1,2-diacyl-sn-glycero-3-phosphocholines and cytidine.[10] The network structures of diacyl-5'-phosphatidylcytidines were prepared in aqueous solution, and examined under an electron microscopy and by automatic image processing as described previously.[8]

After sonication at 50 °C for 45 min, aqueous solutions of diacyl-5'-phosphatidylcytidines produced vesicles mainly with diameter of 400-1500 Å. Among the vesicles, the vesicles formed from **4** and **5** were slowly transformed into network structures after ageing at 25 °C overnight (Fig. 1). Image processing showed that a juncture of the network structures of **4** consisted of a double strand with a diameter of ≈90 Å and a helical pitch of ≈190 Å, and two single strands with a diameter of ≈60 Å and a helical pitch of ≈100 Å (see the insert in Fig. 1a). The network structures of **4** and **5** were gradually converted into linearly extended strands at 25 °C (Fig. 2). As shown in Fig. 2b, the linear strands of **4** consisted of a double helical strand with a diameter of ≈110 Å and a helical pitch of ≈180 Å which nearly accorded with those of a double strand of the network structures. However, diacyl-5'-phosphatidylcytidines with longer chain lengths (n=18 and 16) such as **1** and **2** and with shorter chain lengths (n=8 and 6) such as **6** and **7** were not transformed into network structures.

To understand the physicochemical properties of **4**, we have examined its CD spectrum in aqueous solution. A solution of **4** in 0.05 M Tris-HCl(pH 8.0) showed a drastic change in the CD spectrum when cooled from room temperature to 13 °C (Fig. 3). In particular, the CD spectrum of **4** at 13 °C showed a tremendous increase in the positive Cotton effect with a peak at 279 nm. In contrast, only a weak single peak with a maximum at 278 nm in the CD spectrum was observed at 25 °C. The CD profile of **4** at 13 °C highly resembled that of helical poly(C) at identical pH and

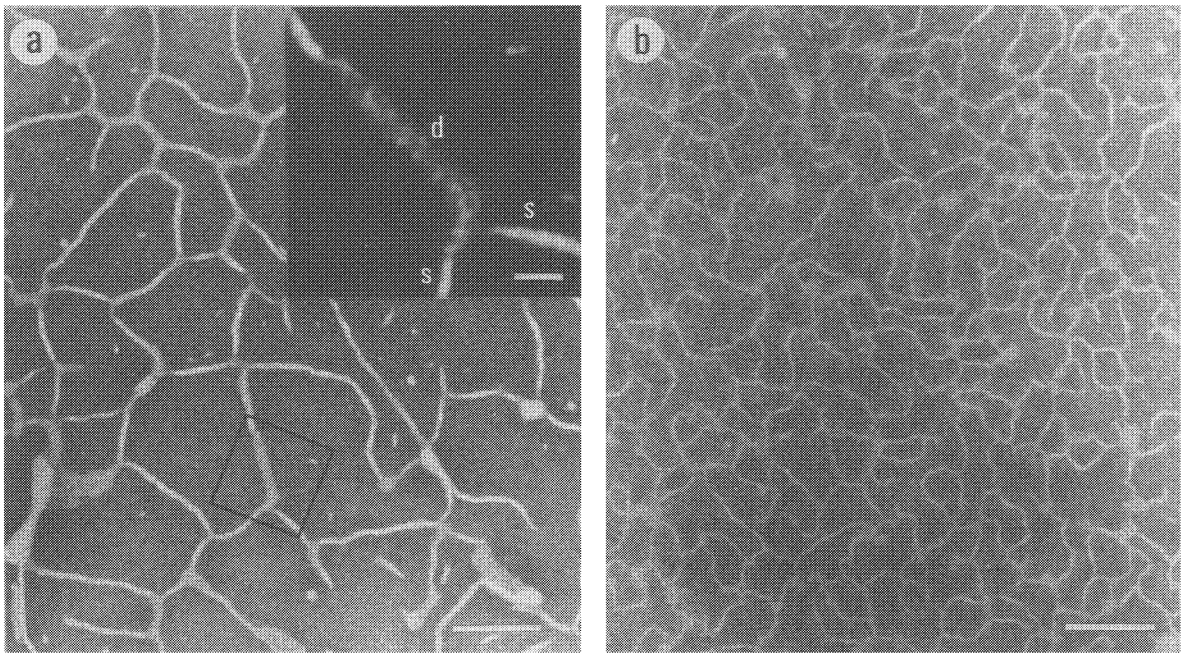


Fig. 1. a and b. Electron micrographs (scale bar, 1000 Å) of network structures formed from **4** and **5** after ageing at 25 °C overnight, respectively. Insert in Fig. 1a, Fourier transferred image (scale bar, 200 Å) of the part boxed by a square. d and s represent parts of a double helical strand and a single helical strand, respectively.

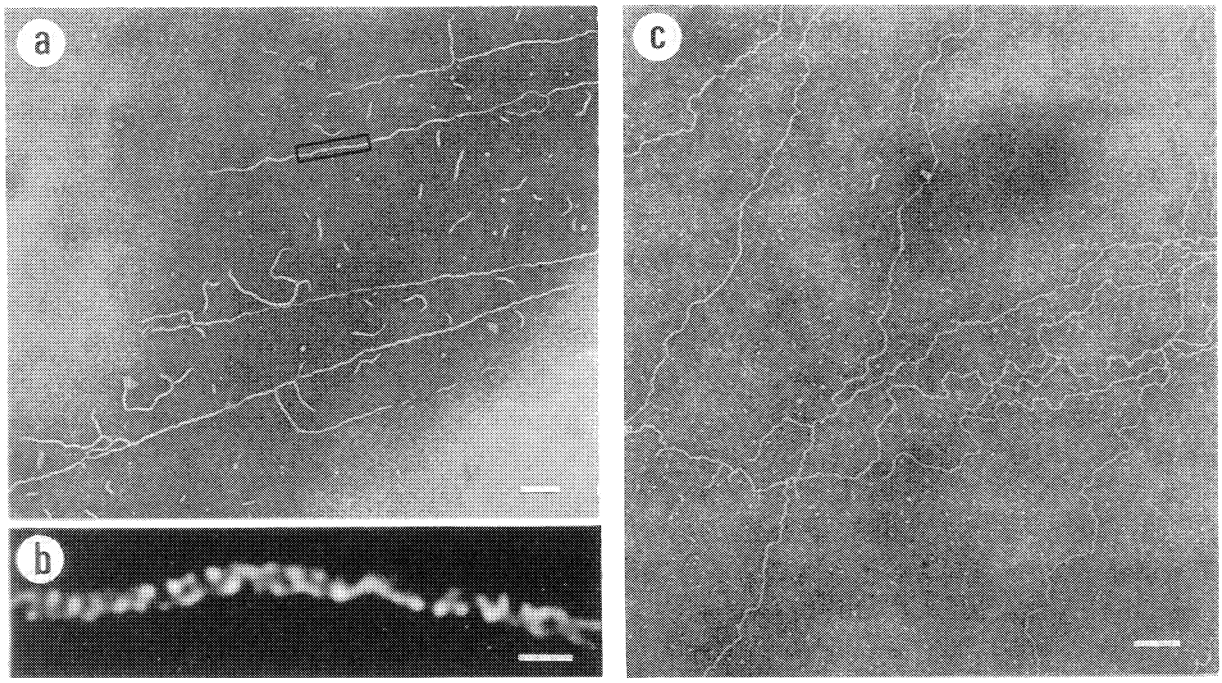


Fig. 2. a and c. Electron micrographs (scale bar, 1000 Å) of linearly extended helical strands formed from **4** and **5** after ageing at 25 °C for 20 days, respectively. b. Fourier transferred image (scale bar, 200 Å) of the part boxed by a rectangle in Fig. 2a.

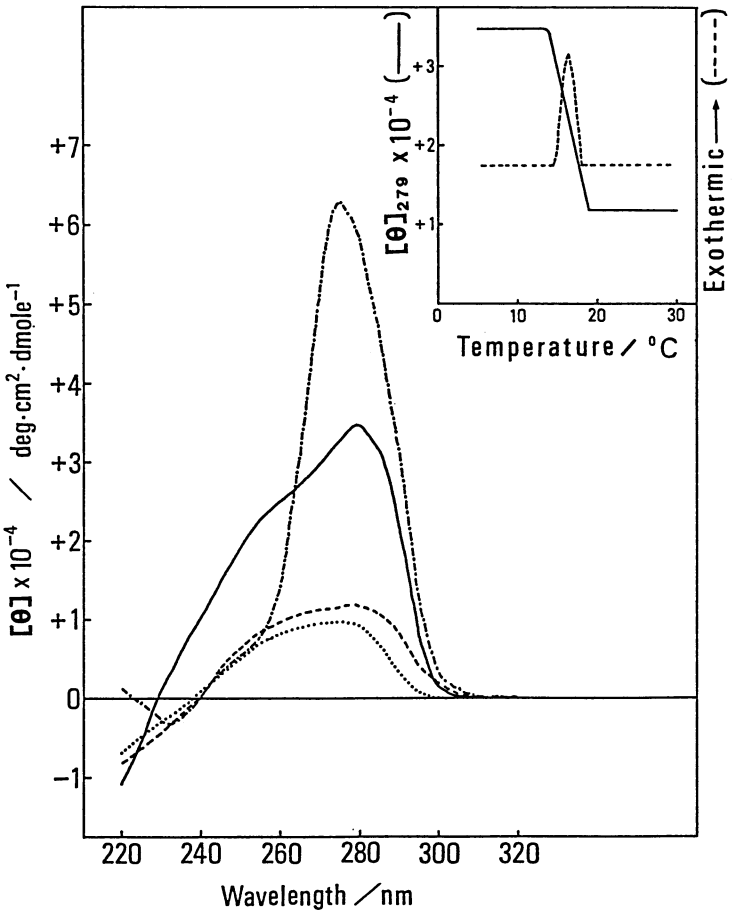


Fig. 3. CD spectra of **4**, poly(C), and 5'-CMP in 50 mM Tris-HCl buffer(pH 8.0). **4** at 13 °C(——); **4** at 25 °C(----); poly(C) at 13 °C(-•-•-); 5'-CMP at 13 °C(••••). Path length, 0.21 mm. Insert, temperature-CD and temperature-differential scanning calorimetry profiles for **4**.

temperature.[11] At neutral pH, poly(C) 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 **4** at 13 °C seem to take a conformation similar to poly(C). The positive Cotton effect of **4** shown in Fig. 3 was very sensitive to temperature and the formation of the helical aggregates. The melting temperature of the aggregates was determined to be 16.5 °C from the variation curve of the 279 nm peak with temperature (see the insert in Fig. 3). The melting temperature was nearly in accord with the transition temperature (16.3 °C) of gel-liquid crystal of **4**. The cooling profile was reversible upon heating. These results indicate that **4** has essentially the molecular characteristics of helicity which is stabilized by both hydrophobic interactions between the long alkyl chain moieties and stacking between the bases.

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

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9) Analytical, magnetic, and spectroscopic data of **4**: white crystal, mp 181-184 °C. Anal. Found: C, 57.37; H, 9.07; N, 4.97%. Calcd for $C_{40}H_{72}N_3O_{12}P \cdot H_2O$; C, 57.47; H, 8.92; N, 5.03%. ^{1}H NMR, (90 MHz, $CDCl_3$-CD_3OD, 3:1) δ 8.20 (d, H-6, J=8.0 Hz), 6.24 (d, H-5, J=8.0 Hz), 5.92 (s, H-1'), 5.28 (m, glycerol CH), 4.56-3.72(m, H-2', H-3', H-4', H-5', H-5", glycerol CH_2), 2.40 (t, CH_2CO), 1.60-1.30 (m, tetradecanoyl CH_2), 0.90 (t, CH_3, J=7.0 Hz); IR $\nu_{max.}$(KBr) 3300br, 2940s, 2870m, 1750m, 1710m, 1470w, 1190w, and 1090m cm^{-1}; UV $\lambda_{max.}$($CHCl_3$-MeOH, 95:5) 284 nm (ε 1.37 x 10^4); MS, m/z 818 (M^++1).
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