Articles

Spontaneous Formation of Helical Structures from Phospholipid–Nucleoside Conjugates

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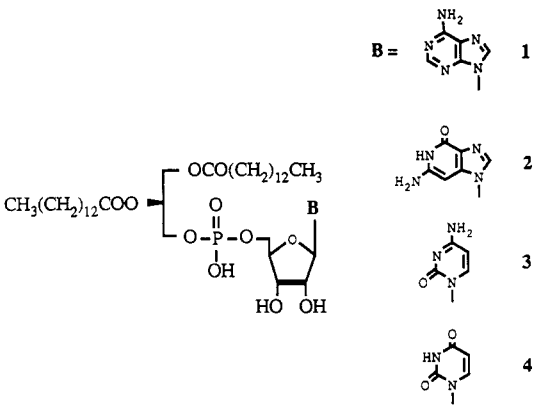
Received September 3, 1991; Revised Manuscript Received December 5, 1991

ABSTRACT: Phospholipid–nucleoside conjugates containing two myristoyl groups and a nucleotidyl group, collectively designated as dimyristoyl-5′-phosphatidylnucleosides, were enzymatically synthesized and their self-organization, morphology, and physicochemical properties investigated. The dimyristoyl-5′-phosphatidylnucleosides spontaneously assembled to form various types of helical strands. Neutral and alkaline solutions of dimyristoyl-5′-phosphatidyladenosine (DMPA) produced multihelical strands. The multihelical strand consisted of several single helical strands of ≈50 Å in diameter and helical pitch ≈100 Å. DMPA produced cigar-like scrolls (tubular structures) in acidic solution, which consisted of many double-helical strands aligned parallel to each other. Diacyl-5′-phosphatidyladenosine with a shorter chain length as long as an alkyl group, dilauroyl-5′-phosphatidyladenosine (DLPA), didecanoyl-5′-phosphatidyladenosine (DDPA), and dioctanoyl-5′-phosphatidyladenosine (DOPA) formed extended tape structures having double-helical strands aligned parallel. Dimyristoyl-5′-phosphatidylcytidine (DMPC) produced network structures at an early stage, which were slowly transformed into multihelical strands. The multihelical strands contained some single-helical strands of ≈55 Å in diameter and helical pitch ≈150 Å. DMPC produced no definite helical structure in acidic solution but rather large lamellar structures. Dimyristoyl-5′-phosphatidyluridine (DMPU) produced crystalline platelet structures of ≈1000 Å in width in both alkaline and acidic solution. A 1:1 mixture of DMPA and DMPU formed a new hybrid helical strand having a wide and thick ribbon structure of ≈300 Å in diameter and helical pitch ≈2000 Å. The formation of different helical strands and effects of chain lengths of alkyl groups and a nucleotidyl group in phospholipid–nucleoside conjugates on that of helical strands in aqueous solution are discussed.

Biological polymers such as nucleic acids (Watson & Crick, 1953), proteins (Pauling et al., 1951), and starch (Imberty et al., 1988) possess molecular helicity as their most basic property. DNA, a store of information, consists of double-helical polynucleotides formed by linking the 3′- and 5′-positions of adjacent sugar residues by phosphodiester bonds (Saenger, 1983). These helices are held together by hydrogen bonds between heterocyclic units. Phospholipids (Vance, 1984), which have long hydrophobic fatty acyl chains and a compact polar head group consisting of a phosphate group, self-assemble to form micelles, monolayers, and bilayer vesicles in an aqueous environment (Saier, 1984). This tendency to assemble and to form organized structures is derived from the amphipathic character of the lipid molecules.

A mononucleotide unit covalently linked to hydrophobic groups of phospholipids should be capable of self-assembly in aqueous solution to form helical strands as in the case of DNA and RNA. A great interest in the construction of a superstructure similar to DNA and RNA by self-assembly of the mononucleotide unit has stimulated us to provide phospholipid–nucleoside conjugates such as 5′-phosphatidylnucleosides having two long alkyl chains and a nucleotidyl group in a molecule as shown in Chart I. 5′-Phosphatidylnucleosides could be enzymatically synthesized from 1,2-diacyl-*sn*-glycero-3-phosphocholine and the corresponding nucleoside using *Streptomyces* phospholipase D (Shuto et al., 1987). The phospholipid–nucleoside conjugate, dipalmitoyl-5′-phosphatidylcytidine, was recently found to spontaneously assemble to form linear and circular helical strands (Yanagawa et al., 1988a, 1989a) and the phospholipid–deoxynucleoside conjugate, dimyristoyl-5′-phosphatidyldeoxycytidine, to form superhelical strands each consisting of a double and double duplex of ≈110 Å in diameter and helical pitch ≈240 Å (Yanagawa et al., 1989b). 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

Chart I



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0006-2960/92/0431-4757$03.00/0

helical strands. It should thus be possible to construct different types of higher helical structures through a hydrogen bonding and stacking between nucleic acid base moieties of phospholipid–nucleoside conjugates. Dimyristoyl-5′-phosphatidylnucleosides (**1–4**) with different nucleic acid bases such as adenine, guanine, cytosine, and uracil have already been successfully produced. The spontaneous formation of different helical structures from dimyristoyl-5′-phosphatidylnucleosides in alkaline and acidic solution is discussed in the following.

Materials and Methods

General Methods. 1,2-Diacyl-*sn*-glycero-3-phosphocholine and adenosine were purchased from Sigma (St. Louis, MO). *Streptomyces* phospholipase D (Imamura et al., 1982) was kindly provided by Dr. S. Shuto of Toyo Jozo Co., Ltd. (Shizuoka, Japan). Cytidine, uridine, and guanosine were obtained from Yamasa Shoyu Co., Ltd. (Chiba, Japan). All other chemicals used were of reagent grade. ^{1}H NMR spectra were recorded on JNM-GSX 500 (500-MHz) and Bruker AC 250 (250-MHz) spectrometers using Me_4Si as the internal standard at 25 °C. Infrared spectra were recorded on a Hitachi 260-50 infrared spectrophotometer. FAB-MS[1] was taken on a JEOL HX 100 spectrometer using glycerol as the matrix. Ultraviolet spectra were measured on a Gilford RESPONSE II spectrophotometer. CD spectra were recorded on a JASCO J-600 automatic recording spectropolarimeter. T_c was determined with a Daini Seikosha SSC-560 differential scanning calorimeter. A suspension of phospholipid–nucleoside conjugate (1.5 mg in 0.055 mL of 0.05 M Tris-HCl, pH 8.0) was encapsulated in a calorimeter aluminum pan. The heating rate was 0.5 °C/min.

Synthesis of Dimyristoyl-5′-phosphatidylnucleosides. DMPA, DLPA, DDPA, DOPA, DMPC, and DMPU were enzymatically synthesized from 1,2-diacyl-*sn*-glycero-3-phosphocholine and the corresponding ribonucleosides using *Streptomyces* phospholipase D according to the method of Shuto et al. (1987). Reactions were monitored by TLC on 0.2-mm-thick silica gel 60 F254 plastic sheets (Merck). Compounds were detected by UV and spraying with Dittmer–Lester reagent (Ryu & MacCoss, 1979). DMPG could not be enzymatically synthesized from 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine and guanosine. Reaction conditions and yields of DMPA, DLPA, DDPA, DOPA, DMPC, and DMPU and their spectroscopic data are as follows.

DMPA: This compound was prepared from an enzymatic reaction of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine and adenosine in a mixed solvent of 0.2 M acetate buffer (pH 6) containing 0.25 M $CaCl_2$ and chloroform (1:2) at 45 °C and 6 h in 30% yield: TLC (chloroform–methanol–water, 65:35:3) R_f 0.40; ^{1}H NMR (500 MHz) ($CDCl_3$–CD_3OD, 3:1) δ 8.37 (1 H, s, 8H), 8.01 (1 H, s, 2H), 5.92 (1 H, d, 1′H, $J_{1'-2'}$ = 4.5 Hz), 5.11 (1 H, m, glycerol CH), 4.34 (1 H, t, 2′H, $J_{2'-3'}$ = 4.5 Hz), 4.27 (1 H, t, 3′H, $J_{3'-4'}$ = 3.5 Hz), 4.26 (1 H, q, 4′H, $J_{4'-5'}$ = 3.0, 7.0 Hz), 4.11 (2 H, m, glycerol CH_2), 4.04 and 3.87 (2 H, A–B pattern, 5′H, $J_{5'}$ = −12.0 Hz), 3.84 (2 H, m, glycerol CH_2), 2.15 (4 H, m, $COCH_2$), 1.44 (4 H, m, $COCH_2CH_2$), 1.12 (40 H, m, alkyl CH_2's), 0.72 (6 H, t, CH_3); UV_{max} ($CHCl_3$–MeOH, 95:5) 261 nm (ϵ 1.31 × 10^4); FAB-MS, m/z 840 $(M - 1)^+$; IR (KBr) ν 3330, 3190, 2930, 2870, 1740, 1650, 1470, 1240, 1100 cm^{-1}.

DLPA: This compound was obtained from 1,2-dilauroyl-*sn*-glycero-3-phosphocholine and adenosine in 30% yield under the same condition as described for DMPA: TLC (chloroform–methanol–water, 75:45:3) R_f 0.40; ^{1}H NMR (250 MHz) ($CDCl_3$–CD_3OD, 3:1) δ 8.33 (1 H, s, 8H), 8.09 (1 H, s, 2H), 5.97 (1 H, d, 1′H, $J_{1'-2'}$ = 3.2 Hz), 5.17 (1 H, m, glycerol CH), 4.34–3.87 (2′H, 3′H, 4′H, 5′H + glycerol CH_2), 2.22 (4 H, m, $COCH_2$), 1.50 (4 H, m, $COCH_2CH_2$), 1.17 (32 H, m, alkyl CH_2's), 0.79 (6 H, t, CH_3); UV_{max} ($CHCl_3$–MeOH, 95:5) 260 nm (ϵ 1.15 × 10^4); FAB-MS, m/z 784 $(M - 1)^+$; IR (KBr) ν 3330, 3150, 2920, 2860, 1730, 1650, 1470, 1230, 1080 cm^{-1}.

DDPA: This compound was obtained from 1,2-didecanoyl-*sn*-glycero-3-phosphocholine and adenosine in 22% yield under the same condition as described for DMPA: TLC (chloroform–methanol–water, 75:35:2) R_f 0.30; ^{1}H NMR (250 MHz) ($CDCl_3$–CD_3OD, 3:1) δ 8.31 (1 H, s, 8H), 8.08 (1 H, s, 2H), 5.95 (1 H, d, 1′H, $J_{1'-2'}$ = 3.7 Hz), 5.13 (1 H, m, glycerol CH), 4.34–3.85 (2′H, 3′H, 4′H, 5′H + glycerol CH_2), 2.18 (4 H, m, $COCH_2$), 1.47 (4 H, m, $COCH_2CH_2$), 1.14 (24 H, m, alkyl CH_2's), 0.76 (6 H, t, CH_3); UV_{max} ($CHCl_3$–MeOH, 95:5) 261 nm (ϵ 0.96 × 10^4); FAB-MS, m/z 728 $(M - 1)^+$; IR (KBr) ν 3330, 3160, 2920, 2850, 1730, 1650, 1460, 1230, 1080 cm^{-1}.

DOPA: This compound was prepared from 1,2-dioctanoyl-*sn*-glycero-3-phosphocholine in 12% yield under the same condition as described for DMPA: TLC (chloroform–methanol–water, 75:35:2) R_f 0.40; ^{1}H NMR (250 MHz) ($CDCl_3$–CD_3OD, 3:1) δ 8.30 (1 H, s, 8H), 8.02 (1 H, s, 2H), 5.95 (1 H, d, 1′H, $J_{1'-2'}$ = 3.0 Hz), 5.15 (1 H, m, glycerol CH), 4.33–3.75 (2′H, 3′H, 4′H, 5′H + glycerol CH_2), 2.23 (4 H, m, $COCH_2$), 1.48 (4 H, m, $COCH_2CH_2$), 1.16 (16 H, m, alkyl CH_2's), 0.77 (6 H, t, CH_3); UV_{max} ($CHCl_3$–MeOH, 95:5) 260 nm (ϵ 1.05 × 10^4); FAB-MS, m/z 672 $(M - 1)^+$; IR (KBr) ν 3330, 3155, 2920, 2850, 1730, 1650, 1470, 1230, 1080 cm^{-1}.

DMPC: This compound was obtained from an enzymatic reaction of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine and cytidine in a mixed solvent of water (pH 4.5) and chloroform (1:5) at 45 °C for 6 h in 70% yield: TLC (chloroform–methanol–water, 65:35:3) R_f 0.40; ^{1}H NMR (500 MHz) ($CDCl_3$–CD_3OD, 3:1) δ 8.06 (1 H, d, 6H, J_{5-6} = 8.0 Hz), 6.04 (1 H, d, 5H), 5.70 (1 H, d, 1′H, $J_{1'-2'}$ = 4.5 Hz), 5.09 (1 H, m, glycerol CH), 4.24–3.88 (2′H, 3′H, 4′H, 5′H + glycerol CH_2), 2.16 (4 H, m, $COCH_2$), 1.44 (4 H, m, $COCH_2CH_2$), 1.11 (40 H, m, alkyl CH_2's), 0.72 (6 H, t, CH_3); UV_{max} ($CHCl_3$–MeOH, 95:5) 283 nm (ϵ 1.13 × 10^4); FAB-MS, m/z 816 $(M - 1)^+$; IR (KBr) ν 3190, 2930, 2860, 1740, 1610, 1480, 1220, 1070, 950 cm^{-1}.

DMPU: This compound was obtained from an enzymatic reaction of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine and uridine in a mixed solvent of 200 mM acetate buffer (pH 6) and chloroform (1:6) at 45 °C for 6 h in 70% yield: TLC (chloroform–methanol–water, 65:35:3) R_f 0.35; ^{1}H NMR (500 MHz) ($CDCl_3$–CD_3OD, 3:1) δ 7.55 (1 H, d, 6H, J_{5-6} = 8.0 Hz), 5.62 (1 H, d, 1′H, $J_{1'-2'}$ = 4.0 Hz), 5.56 (1 H, d, 5H), 5.02 (1 H, m, glycerol CH), 4.18–3.46 (2′H, 3′H, 4′H, 5′H + glycerol CH_2), 2.11 (4 H, m, $COCH_2$), 1.39 (4 H, m, $COCH_2CH_2$), 1.06 (40 H, m, alkyl CH_2's), 0.66 (6 H, t, CH_3); UV_{max} ($CHCl_3$–MeOH, 95:5) 260 nm (ϵ 8.57 × 10^3); FAB-MS, m/z 817 $(M - 1)^+$; IR (KBr) ν 3400, 2930, 2860, 1690, 1460, 1250, 1100 cm^{-1}.

Preparation of Helical Structures from DMPA, DMPC, and DMPU. A dimyristoyl-5′-phosphatidylnucleoside (free acid form, 1 μmol) was dissolved in 2 mL of chloroform–

[1] Abbreviations: DMPA, dimyristoyl-5′-phosphatidyladenosine; DMPC, dimyristoyl-5′-phosphatidylcytidine; DMPU, dimyristoyl-5′-phosphatidyluridine; DMPG, dimyristoyl-5′-phosphatidylguanosine; DLPA, dilauroyl-5′-phosphatidyladenosine; DDPA, didecanoyl-5′-phosphatidyladenosine; DOPA, dioctanoyl-5′-phosphatidyladenosine; FAB-MS, fast atom bombardment mass spectrometry; CD, circular dichroism; T_c, transition temperature of a gel–liquid crystal.

methanol (20:1 v/v), evaporated to dryness under reduced pressure, and dried at room temperature for 30 min in vacuo; 0.1 mL of 20 mM NaOH was added to neutralize the phosphate group, and the mixture was vortexed for 15 min. Then 0.1 mL of 50 mM Tris-HCl buffer (pH 8.0) was added followed by additional vortexing for 10–15 min. The mixture was placed into a Pyrex glass tube [5 mm (i.d.) × 110 mm], sonicated (Branson Sonifier B-1200, 300 W) at 45 °C for 45 min, and aged at 40 °C overnight and at 25 °C for 2 days. Helical structures were prepared in acidic solution as follows. After sonication in 10 mM Tris-HCl buffer (pH 8.0) at 45 °C for 45 min, 0.1 mL of 100 mM acetate buffer (pH 4–6) was added to acidify the solution, followed by aging at 40 °C overnight and 25 °C for 2 days.

Electron Microscopy. For negatively stained electron microscopic observation, a drop of the sample was placed on a carbon-coated collodion grid and the excess was removed with filter paper, 1% phosphotungstate (pH 8.0) was applied to the grid, and the sample was immediately examined under a JEOL 1200 EX electron microscope at 80 kV. For scanning electron microscopy of the helical structures, their aqueous solution was placed on a clean glass cover slips and freeze-dried. The dried specimen was coated with platinum/palladium (80/20) in an Eiko IB-3 ion coater and then was examined under a Hitachi S-800 scanning electron microscope operated at 20 kV. Some of the morphologies of aggregates are defined as follows: A stacked disk, multihelical strand, and extended ribbon are designated when the aggregates are converted into a pile of disk structures, a helical bundle of a number of thin strands, and a stretched ribbon strand, respectively.

Image Processing. The helical structures were examined in detail by automatic image processing. The original image taken at a magnification of 30000–50000× was extended by about six times, and a suitable area was read by a video camera of 1 cm/56 pixel resolution. Image processing was conducted according to the image processing language "Semper" (Saxton & Koch, 1982; Yanagawa et al., 1988b).

Measurement of CD Spectra. CD spectra were measured in the range of 200–320 nm with a JASCO J-600 spectropolarimeter at 0–80 °C under nitrogen flush, using a quartz cell with a light pathlength of 0.13 mm. Each sample was dissolved in 50 mM Tris-HCl buffer (pH 8.0).

Results

Multihelical Strand and Cigar-like Scroll Formation from DMPA. An alkaline solution of DMPA in 50 mM Tris-HCl (pH 8.0) produced stacked disk structures immediately following sonication at 45 °C for 45 min (Figure 1a). The stacked disk structures were gradually transformed into multihelical strands of ≈160 Å in diameter and helical pitch ≈1700 Å during aging at 40 or 25 °C (Figure 1d). This process appeared to proceed to completion in 1 day at 40 °C or in 2 days at 25 °C. The geometry of the resulting multihelical strands was an 80–20 left-handed–right-handed mixture. They consisted of several single-helical strands of ≈50 Å in diameter and helical pitch ≈100 Å (Figure 2a). At a very early stage of aging, the stacked disk structures became thin linear helical strands (Figure 1b), which subsequently twisted with each other to produce multihelical strands. Many stacked disk structures attached to the growing multihelical strands (Figure 1c). The strands gradually became extended ribbon structures during aging at 40 or at 25 °C (Figure 1e). After aging at 40 °C for 1 week or at 25 °C for 1 month, they were completely converted into gels. A gel solution contained extended ribbon structures (Figure 1f) in which many single-helical strands appeared to be aligned parallel to each other. For this to have occurred, the strands may possibly have unwound to give rise to the extended ribbon structures. Morphological changes of the helical strands of DMPA depended on aging temperature. Morphologies of the helical strands changed more rapidly at 40 °C than at 25 °C.

For clarification of the physicochemical properties of DMPA, its CD spectrum in aqueous solution was examined. An aqueous solution of DMPA in 50 mM Tris-HCl (pH 8.0) showed the characteristic CD spectra after sonication at 45 °C for 45 min and aging at 40 °C for 5 h. The CD spectrum measured immediately following sonication showed a peak at 259 nm along with two troughs at 215 and 280 nm (Figure 3, curve 1). The spectrum measured after aging at 40 °C for 5 h had a peak at 260 nm and two troughs at 218 and 280 nm (Figure 3, curve 2). Amplitude shapes and peak and trough positions of the two CD spectra were much the same. An aqueous solution of DMPA in 50 mM Tris-HCl (pH 8.0) showed a drastic change in its CD spectrum after aging at 40 °C for 1 day (Figure 3, curve 3). A striking increase was noted in the positive Cotton effect with a peak at 266 nm. The CD profile resembled that of helical poly(A) at identical pH and temperature (Figure 3, curve 4). An alkaline solution of DMPA showed a much larger peak at 266 nm after aging at 25 °C for 1 month. The magnitudes of [Θ] were about 2.5 times that after aging at 25 °C for 1 day. The intensity of the positive Cotton effect at 266 nm was quite sensitive to helical aggregate morphology. A solution containing multihelical structures (Figure 1d) showed a strong positive Cotton effect at 266 nm. In addition, a solution containing extended ribbon structures (Figure 1f) showed a stronger positive Cotton effect at 266 nm. In the course of aging, the stacked disk structures were more rapidly transformed into the multihelical structures and extended ribbon structures at 40 °C than at 25 °C, showing a stronger positive Cotton effect at 266 nm.

The transition temperature (T_c) of a gel–liquid crystal of DMPA was 16.3 °C. The positive Cotton effect at 266 nm of a solution containing multihelical structures increased with decrease in temperature in the range of 16.3–0 °C and decreased with increase in temperature from 16.3 to 60 °C. At 60 °C a positive peak at 266 nm was observed to become a strong negative peak with a minimum at 260 nm (data not shown). The multihelical structures completely converted into stacked disk structures at 60 °C. These results may indicate that the interaction between the resulting multihelical structures increased with decrease in temperature below the T_c; on the contrary, it decreased with increase in temperature above the T_c.

DMPA formed cigar-like scrolls at pH 4–5 (Figure 4d). None were produced at pH 6; only a thick multihelical strand was observed. At pH 5.5, a mixture of cigar-like scrolls and thick multihelical strands were formed. At less than pH 5, only a cigar-like scroll, tightly aggregated for the most part, was noted.

At a much earlier stage of aging, the stacked disk structures from an acidic solution of DMPA gradually became double-helical strands which were aligned parallel to each other (Figures 4a and 2b). The aligned structures twisted with each other to produce ribbon structures (Figure 4b). With additional twisting, cigar-like tubular structures were obtained (Figure 4c). These were formed from amphiphiles such as synthetic amphiphile (Yamada et al., 1984), polymerizable lecithin (Yager et al., 1985), and *N*-octylaldamide (Fuhrhop et al., 1988).

The shape of the CD spectrum of an acidic solution (pH 5.5) of DMPA was noted to clearly differ from that of an

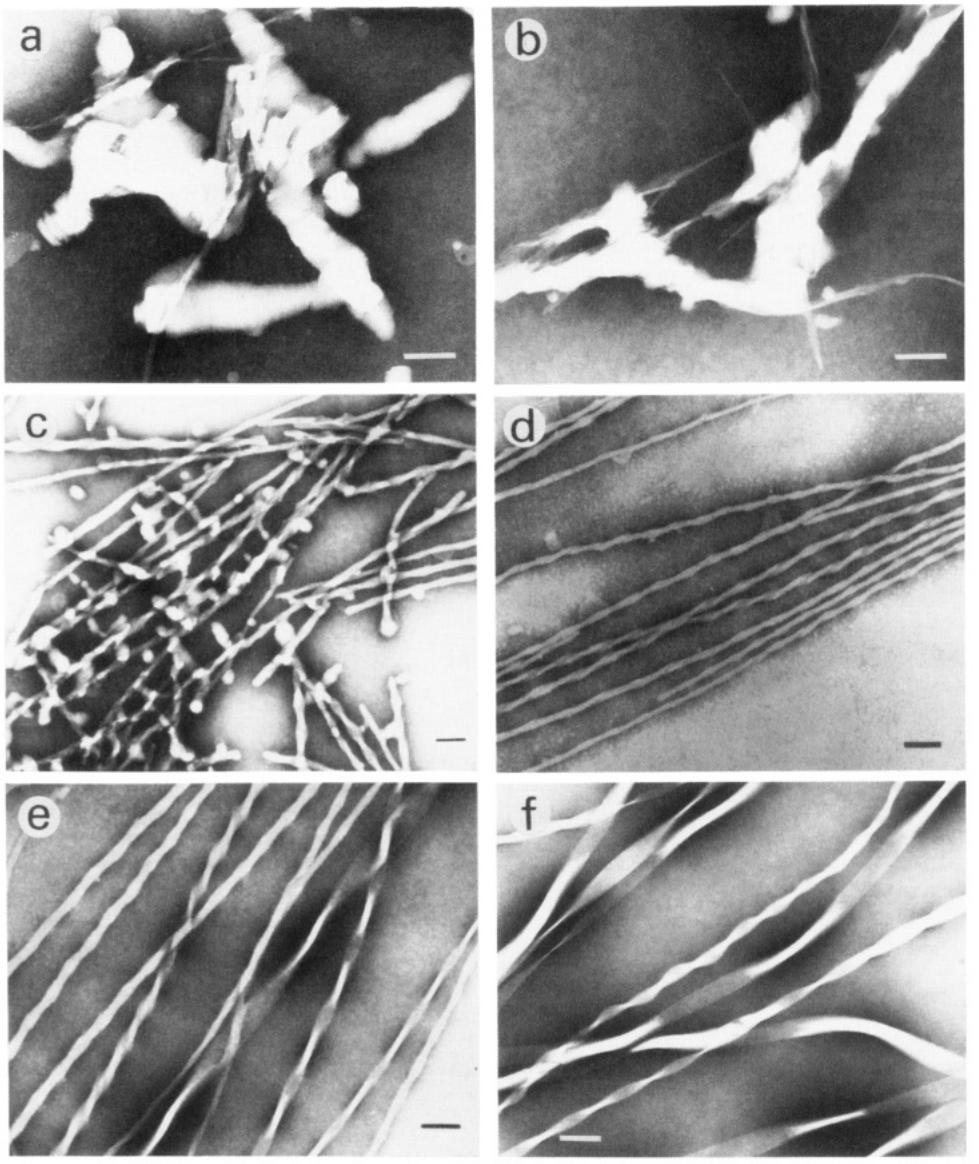

FIGURE 1: Electron micrograph of different helical structures from DMPA in 50 mM Tris-HCl buffer (pH 8.0): (a) stacked disk structure immediately following sonication at 45 °C for 45 min; (b) linear thin helical strand from disk structure; (c) incomplete multihelical strand from linear thin helical strand; (d) multihelical strand formed after aging at 40 °C for 1 day; (e) extended ribbon structure after aging at 25 °C; (f) ribbon structure after aging at 25 °C for 1 month. All scale bars represent 0.1 μm.

alkaline solution of DMPA, only a large positive peak at 285 nm being noted for the former (Figure 5, curve 1). This fact may be attributable to morphological and structural differences between the resulting multihelical strands in alkaline solution and cigar-like scrolls in acidic solution.

Formation of Helical Strands from DLPA, DDPA, and DOPA. To construct higher helical structures through manipulation of hydrophobic interactions between the alkyl chain moieties of phospholipid–nucleoside conjugates, diacyl-5′-phosphatidyladenosine with different chain lengths of alkyl groups, such as DLPA, DDPA, and DOPA, was synthesized.

Following sonication at 45 °C for 45 min, an aqueous solution of DLPA in 50 mM Tris-HCl (pH 8.0) produced vesicles. These resulting vesicles slowly became extended tape structures (Figure 6a) whose double-helical strands were aligned parallel (Figure 2c). These structures were also formed from DDPA and DOPA under the same conditions (data not shown).

Acidic solutions (pH 4–5) of DLPA, DDPA, and DOPA formed cigar-like scrolls similar to those from the DMPA acidic solution (Figure 6b).

Network Structure and Multihelical Strand Formation from DMPC. After sonication at 45 °C for 45 min, an aqueous solution of DMPC in 50 mM Tris-HCl (pH 8.0) produced vesicles of diameter usually from 200 to 600 Å (Figure 7a). They slowly became network structures after aging at 40 °C for 1 day (Figure 7b). These structures may possibly have been produced by budding out of the vesicles. They were gradually converted into linearly extended thin single-helical strands and irregular multihelical strands during aging at 25 °C (Figure 7c). This process was completed after aging at 25 °C for 1 week (Figure 7d). Image processing indicated the multihelical strands to consist of single-helical strands of ≈55 Å in diameter and helical pitch ≈150 Å (Figure 2d).

In acidic solutions (pH 4–5) of DMPC, mostly aggregated large lamellar structures were produced along with slightly cigar-like scroll structures similar to those formed in an acidic solution of DMPA (data not shown).

Platelet Structure Formation from DMPU. An aqueous solution of DMPU in 50 mM Tris-HCl (pH 8.0) showed the formation of network structures immediately following sonication at 45 °C for 45 min (Figure 8a), each being comprised

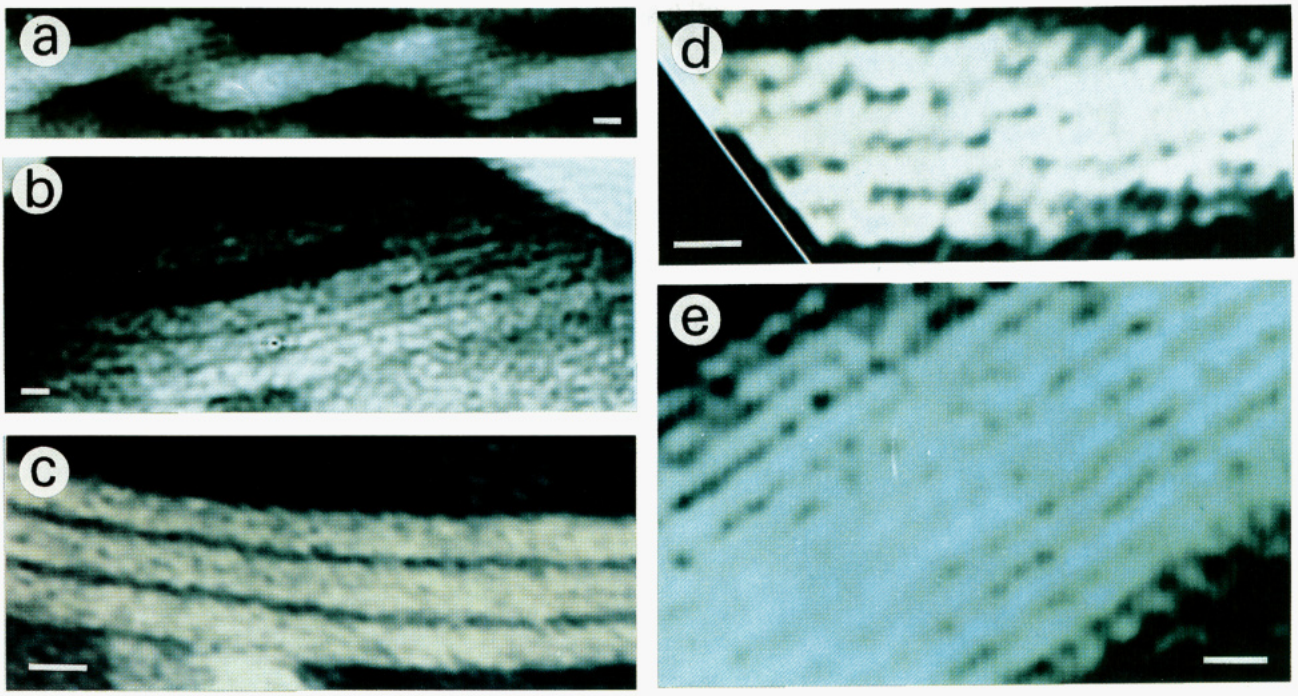

FIGURE 2: Fourier-transformed images of different helical strands: (a) multihelical strand from an alkaline solution (pH 8.0) of DMPA; (b) double-helical strand from an acidic solution (pH 5.0) of DMPA; (c) extended tape structure from an alkaline solution (pH 8.0) of DLPA; (d) multihelical strand from an alkaline solution (pH 8.0) of DMPC; (e) platelet structure from an alkaline solution (pH 8.0) of DMPU. All scale bars represent 100 Å.

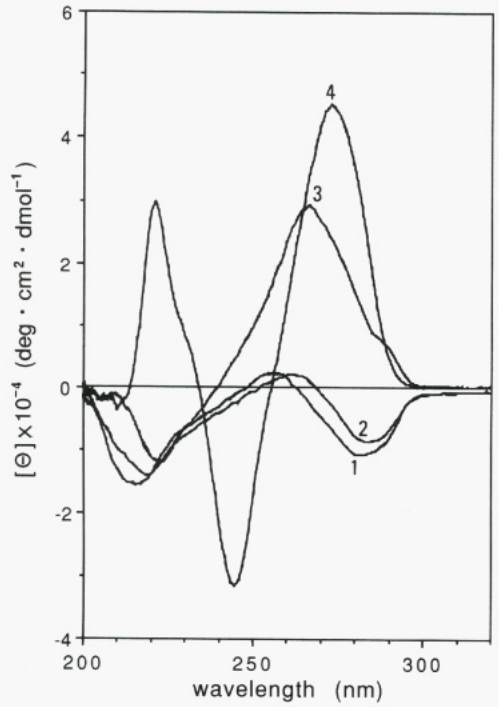

FIGURE 3: CD spectra of DMPA and poly(A) in 50 mM Tris-HCl buffer (pH 8.0): curve 1, DMPA immediately following sonication at 45 °C for 45 min; curve 2, DMPA after aging at 40 °C for 5 h; curve 3, DMPA after aging at 40 °C for 1 day; curve 4, poly(A).

of irregular sheet structures. The networks were gradually transformed into platelet structures of ≈1000 Å in width (Figure 8b). Scanning electron micrography indicated the platelet structures to be crystallike, as shown in Figure 8c. In Figure 8b, these structures can be seen to differ considerably from the helical structures from DMPA and DMPC. An image-processed photograph showed them to consist of several helical strands aligned parallel (Figure 2e). The helical strands were single helices of ≈50 Å in diameter and helical pitch ≈130 Å. From an acidic solution (pH 5) of DMPU, platelet structures longer than those in alkaline solution were obtained (Figure 8d).

Thick Ribbon Structure Formation from a Mixture of DMPA and DMPU. From a 1:1 mixture (pH 8.0) of DMPA and DMPU, a new hybrid helical strand was formed. As shown in Figure 9, these strands were wide and thick ribbon structures which subsequently produced superhelical structures of ≈300 Å in diameter and helical pitch ≈2000 Å. The ribbon structures had the characteristics of strands from both DMPA and DMPU. While DMPA formed thick multihelical strands, DMPU formed extended sheet structures. No definite helical strands could be detected in a 1:1 mixture (pH 5) of DMPA and DMPU.

From a 1:2 mixture (pH 8.0) of DMPA and DMPU, the hybrid helical strands could not be obtained but rather only short platelet structures. In a 2:1 mixture (pH 8.0) of DMPA and DMPU, the same ribbon structures as those in a 1:1 mixture (pH 8.0) of DMPA and DMPU were produced.

A 1:1 mixture (pH 8.0) of DMPA and DMPU showed a characteristic CD spectrum (Figure 10). A negative Cotton effect was noted at 284 nm and a positive effect at 225 and 246 nm. The spectrum differed considerably from those of DMPA and DMPU, indicating the strong possibility of new hybrid strand formation by DMPA and DMPU. The CD spectrum of this mixture resembled to some degree that of poly[r(A-U)] (Michaela et al., 1990), having two positive bands at 230 and 260 nm and a negative one at 290 nm.

A 1:1 mixture (pH 8.0) of DMPA and DMPC failed to give rise to any new hybrid strands, the one cancelling out the effect or action of the other in this regard. A mixture of DMPA and DMPC would thus not produce any definite helical strands.

A 1:1 mixture (pH 8.0) of DMPU and DMPC did not produce a new hybrid strand but rather platelet structures similar to those from DMPU alone; multihelical strands the same as those from DMPC were not formed. Multihelical

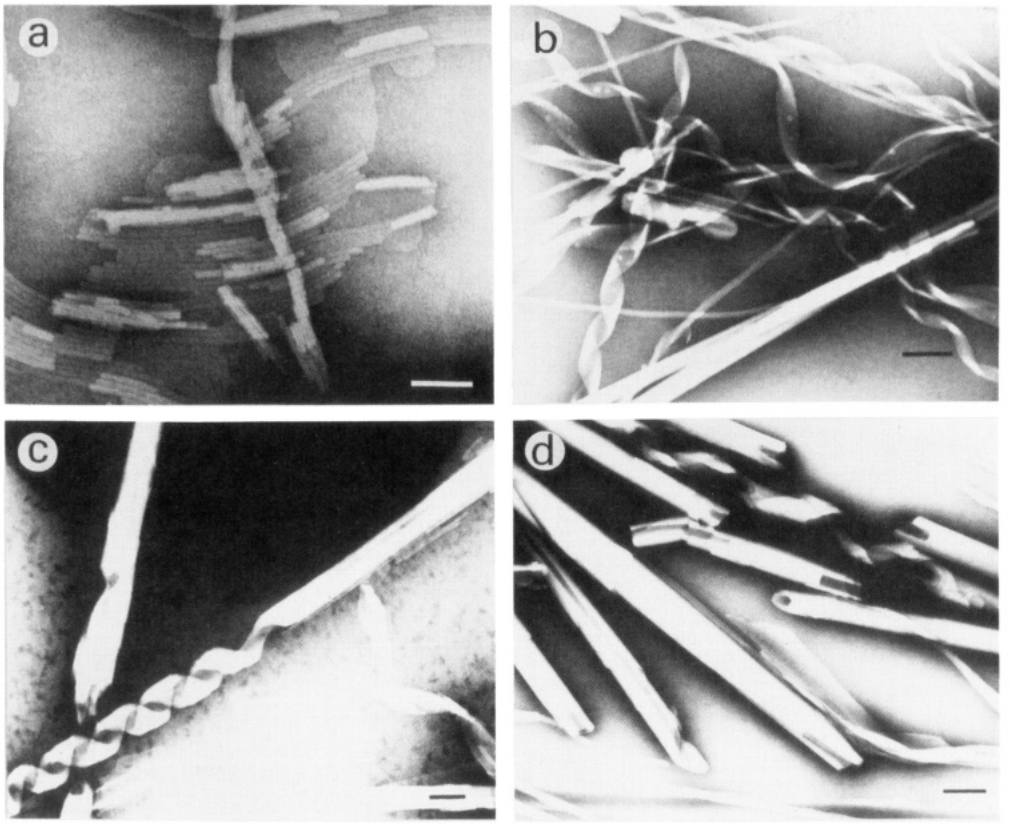

FIGURE 4: Electron micrograph of the formation process of cigar-like scrolls from DMPA in acidic solution (pH 5.0): (a) linear double-helical strand; (b) ribbon structure; (c) incomplete cigar-like scrolls from ribbon structure; (d) complete cigar-like scrolls. All scale bars represent 0.1 μm.

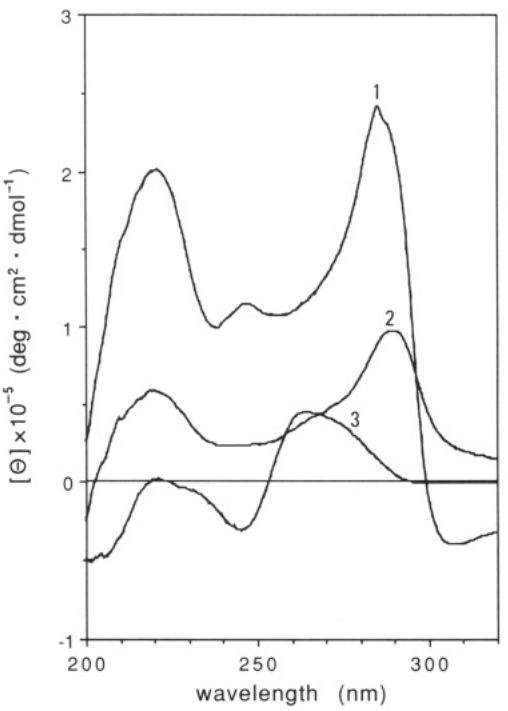

FIGURE 5: CD spectra of DMPA in acidic solution: curve 1, DMPA at pH 5.5; curve 2, DMPA at pH 6.0; curve 3, poly(A) at pH 5.0.

strands would thus appear to be inhibited by the addition of DMPU.

Discussion

Different types of helical structures were constructed from phospholipid–nucleoside conjugates, and the structural elements determining the helical morphology of the phospholipid–nucleoside conjugates were sought. The most essential

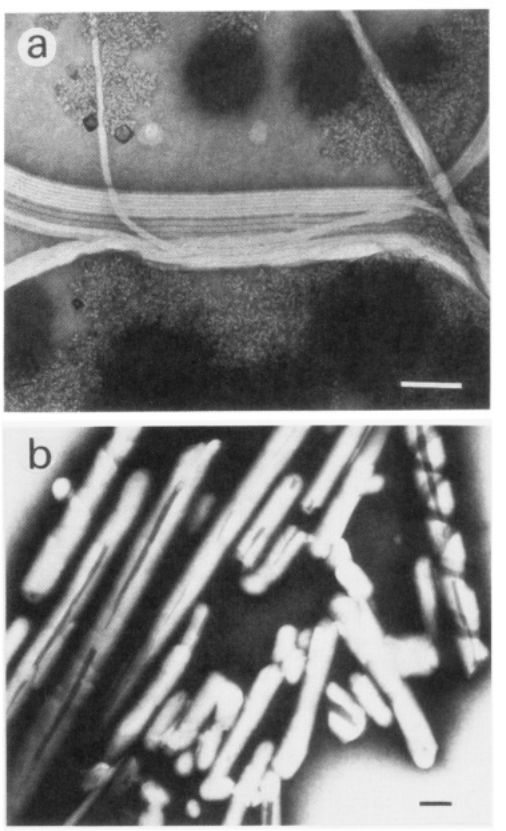

FIGURE 6: Electron micrograph of an extended tape structure (a) and cigar-like scrolls (b) from DLPA. All scale bars represent 0.1 μm.

structural elements for helical structure formation were found to be a nucleic acid base moiety and two alkyl chain moieties. Stable helical structures were not formed when any one of these elements was absent or modified. For instance, di-

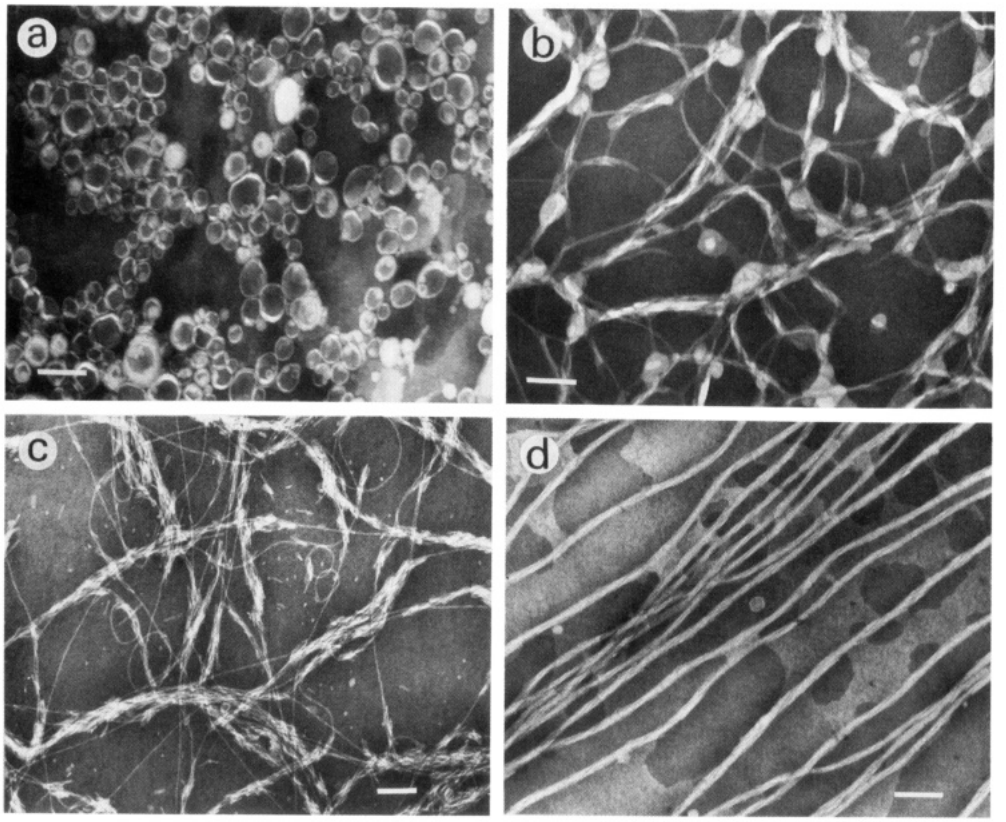

FIGURE 7: Electron micrograph of the formation process of multihelical strands from DMPC in 50 mM Tris-HCl (pH 8.0): (a) vesicles immediately following sonication at 45 °C for 45 min; (b) network structures after aging at 40 °C for 1 day; (c) incomplete multihelical strands after aging at 25 °C; (d) multihelical strands after aging at 25 °C for 1 week. All scale bars represent 0.1 μm.

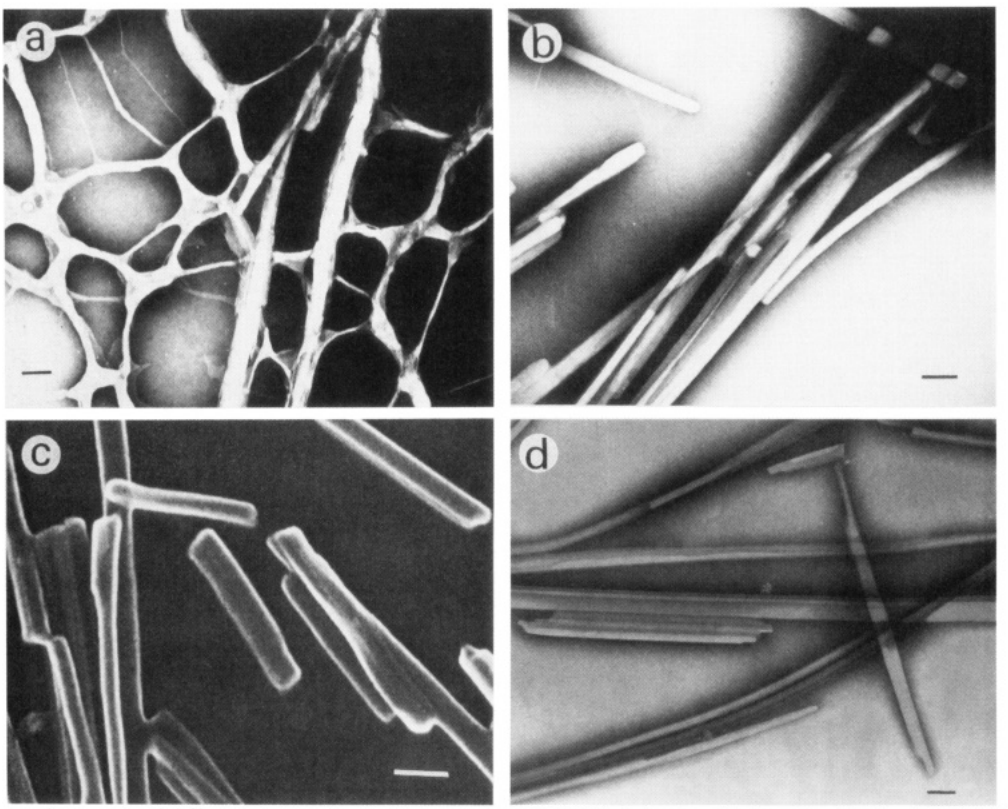

FIGURE 8: Electron micrograph of different types of helical strands from DMPU: (a) network structures in 50 mM Tris-HCl (pH 8.0); (b) platelet structures in 50 mM Tris-HCl (pH 8.0); (c) scanning electron micrograph of platelet structures in 50 mM Tris-HCl (pH 8.0); (d) platelet structures in acidic solution (pH 5.5). All scale bars represent 0.1 μm.

myristoyl-5′-phosphatidyl-D-ribose and dimyristoyl-*sn*-glycero-3-phosphocholine without a nucleic acid base moiety were incapable of helical structure formation under the same condition. In addition, dimyristoyl-5′-phosphatidyl-N^4-ethenoadenosine, in which an amino group at the 4-position and a nitrogen at the 3-position were protected, could not form

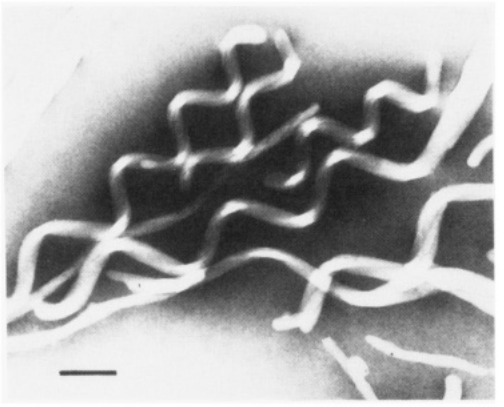

FIGURE 9: Electron micrograph of helical strands from a 1:1 mixture of DMPA and DMPU in 50 mM Tris-HCl (pH 8.0). The scale bar represents 0.1 μm.

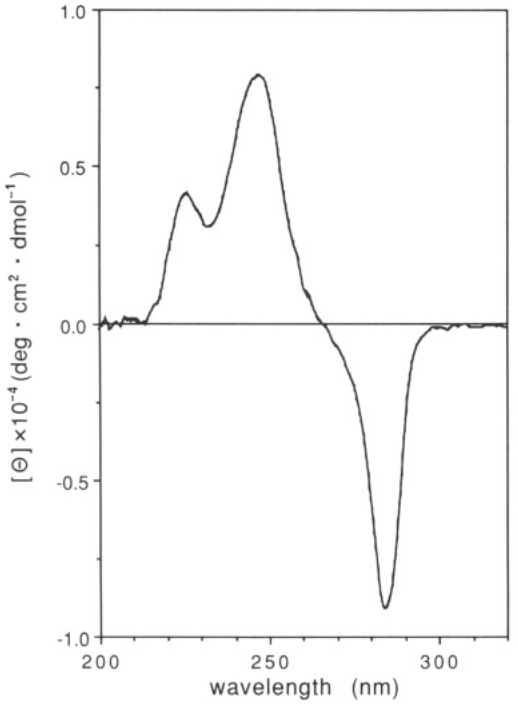

FIGURE 10: CD spectrum of a 1:1 mixture of DMPA and DMPU in 50 mM Tris-HCl (pH 8.0).

any helical strand (Yanagawa et al., 1988).

Alkyl group chain length and the kind of nucleic acid base of a phospholipid–nucleoside conjugate were determining factors of morphology and helical structure formation. Among various diacyl-5′-phosphatidyladenosines, the efficiency of formation was noted to decrease with decrease in the alkyl group chain length. The effect of this length on formation followed the order DMPA (14 carbons) > DLPA (12 carbons) > DDPA (10 carbons) > DOPA (8 carbons). In a previous paper (Yanagawa et al., 1989a), assessment was made of the effects of this chain length: in the case of diacyl-5′-phosphatidylcytidine with longer chain length (18 and 20 carbons), such as dioctadecanoyl and diicosanoyl groups, and with shorter chain length (10 and 8 carbons), such as didecanoyl and dioctanoyl groups, there was no helical structure formation. It would thus follow that a chain length of 14–16 carbons should lead to optimal helical structure formation and a chain length of two diacyl groups would thus appear important.

Dimyristoyl-5′-phosphatidylnucleosides with different nucleic acid bases were shown to produce different types of helical structures in alkaline and acidic solutions. In alkaline solution, DMPA produced thick multihelical structures, DMPC gave thin multihelical strands, and DMPU gave crystalline platelet structures. Morphology varied according to the structure of the nucleic acid base moiety of phospholipid–nucleoside conjugates. That is, the interactions between nucleic acid base moieties by stacking and hydrogen bonding were the factors determining morphology. A strong Cotton effect in CD spectra was evident in many phospholipid–nucleoside conjugates. For DMPA, in particular, this effect was prominent with a peak at 266 nm, thus indicating the possibility of stacking between adenine base moieties in single strands. The CD profile of DMPA resembled that of single-helical poly(A) strands at the same pH and temperature.

In acidic solution, morphology of DMPA changed greatly with the formation of cigar-like scrolls consisting of many double-helical strands. The formation of these double-helical structures was supported by a larger positive peak at 285 nm. The conformation of poly(A) is a single-strand helix in neutral and alkaline solutions and a double-strand helix in acidic solution (Holcomb & Tinoco, 1965). In poly(A), the transition of a single-strand helix to a double-strand helix has been shown to occur at pH 5.0–5.5, but with DMPA, it occurred at a pH the same as that of poly(A). Hydrogen bonds between the adenine base in poly(A) are on a rotation axis. Each adenine residue forms two hydrogen bonds between an adenine amino group, and nitrogen 1 of the adenine ring is protonated (Rich et al., 1961). The double-helical structure formed from DMPA at acidic pH may be similar to that from poly(A) at acidic pH.

DMPC produced a single-helical strand as the minimum structural unit in neutral and alkaline solution. Poly(C) forms a single-helical structure in neutral and alkaline solution and a double-stranded helical structure in acidic solution (Guschlbauen, 1976). X-ray diffraction studies (Langridge & Rich, 1963) have shown the double-helical structure to be a helix similar to that of poly(A), the differences being that only one shared proton is bound by two cytosine residues and this proton forms a third hydrogen bond in acidic solution between pH 3.5 and 5.5. In poly(A), aggregate formation gradually increased with lowering of pH; however, in poly(C), it increased rapidly with decrease in pH (Slegers & Fiers, 1973). Poly(C) is precipitated more easily than poly(A) by protonation at acidic pH. With phospholipid–nucleoside conjugates, DMPA formed double-helical structures at acidic pH, while DMPC did not, producing rather only large lamellar structures at acidic pH. In DMPA, precipitate formation gradually increases with lowering of pH, while DMPC precipitates more rapidly at a pH less than 6. The behavior of DMPA and DMPC at acidic pH is quite close to that of poly(A) and poly(C) at acidic pH.

DMPU produced platelet structures in both alkaline and acidic solution. At an early stage of aging, DMPU formed large vesicles which gradually became network structures and then finally platelet structures. The latter consisted of single-helical strands as the minimum structural unit. DMPU was incapable of forming superhelical structures but could produce platelet structures whose single-helical strands aligned parallel. It is well established that nucleotide bases in aqueous solution have a strong tendency to associate. This is referred to as stacking (Powell et al., 1972). The degree of interaction between the bases of a dinucleotide phosphate varies greatly from one compound to another. The interaction between the bases in ApA or CpC is considerably greater than in UpU (Davis et al., 1968). At neutral or alkaline pH the oligomers possess a single-stranded stacked base helical conformation at low temperature (Brahms et al., 1967). Poly(A) and

poly(C) are believed to take a helical structure as described above. Poly(U), however, has no secondary structure (Simpkins & Richards, 1977). Since the helix–coil transition in single-stranded polynucleotides seems to be essentially noncooperative (Brahms et al., 1966), we expect the base–base interactions in single-stranded polynucleotides to be essentially similar to those in dinucleotides and even mononucleotides. The degree of stacking between base moieties of phospholipid–nucleoside conjugates is probably similar to that in polynucleotides or dinucleotides. Thus, DMPA and DMPC could form multihelical strands, and DMPU was incapable of forming multihelical strands. The capacity for structure formation may depend on an interaction and balance between alkyl and nucleotidyl groups. That is, in DMPU a single-helical strand might be constructed by assistance of the interaction between the hydrophobic alkyl chain moieties, since only stacking between uracil bases is very weak. In addition, the single-helical strand aligned structure formation of DMPU may be due to the greater predominance of interactions between hydrophobic alkyl groups than to stacking between nucleic acid base moieties. DMPA and DMPC could twist around each other to produce multihelical strands since the stacking between nucleic acid bases was stronger than hydrophobic interactions between alkyl chain moieties. The importance of balance between alkyl and nucleotidyl groups was also supported by the following result. DMPC with a chain length of 14 carbons formed a multihelical strand, while dipalmitoyl-5′-phosphatidylcytidine with longer chain length (16 carbons) produced a double-helical strand (Yanagawa et al., 1988a). This indicates that DMPC could produce a multihelical strand since the stacking between cytosine bases was stronger than hydrophobic interactions between alkyl chain moieties, and dipalmitoyl-5′-phosphatidylcytidine formed a double-helical strand since the stacking between cytosine bases was not stronger than hydrophobic interactions between alkyl chain moieties.

In summary, we developed new types of helical strands that assemble spontaneously in aqueous solution. From the standpoint of the origin of life, these helical strands may possibly serve as models of prebiotic templates (Kanaya & Yanagawa, 1986) and prebiotic assembly, which may lead to spontaneous polymerization. Research is presently being conducted for greater clarification of the structures and functions of resulting helical strands from various phospholipid–nucleoside conjugates.

Acknowledgments

We are greatly indebted to Dr. S. Shuto of Toyo Jozo Co., Ltd., for providing the *Streptomyces* phospholipase D. Cordial thanks are also due to Mr. S. Kondo for conducting the scanning electron micrography, Dr. H. Takayanagi and JEOL for measurement of the NMR spectra, and Misses M. Oku and S. Yoshida for measurements of the FAB-MS.

Registry No. DMPA, 94401-40-8; DMPC, 94401-42-0; DMPU, 94401-43-1; DMPG, 139895-05-9; DDPA, 139895-06-0; DOPA, 139895-07-1.

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