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5'-Phosphatidylnucleosides Spontaneously Assemble to Form Circular and Linear Helical Strands

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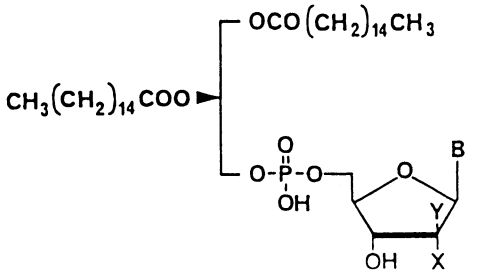
Phospholipid-nucleoside conjugates containing two long alkyl chains and a nucleotidyl residue have been synthesized and their self-organization and morphology have been investigated. 5'-Phosphatidyl-cytidine and 5'-phosphatidyldeoxycytidine spontaneously assembled to form circular and linear strands. Image processing analysis of the electron micrograph of the strands confirmed that they are indeed double helix reminiscent of the double-helical structure of nucleic acids.

Molecular helicity is a fascinating property displayed by biological polymers such as polypeptides and nucleic acids. In particular, well known is the double helix present in DNA,[1] whose formation, structure, and function have been the subject of very extensive studies.

Nucleic acids such as DNA and RNA consist of successive mononucleotide units covalently linked through phosphodiester bridges between the 3' position of one mononucleotide unit and the 5' position of the next. A mononucleotide unit noncovalently linked through certain hydrophobic groups would be a good mimic of a mononucleotide unit covalently linked through phosphodiester bonds. They would be expected to self-assemble in an aqueous solution and form helical strands like DNA and RNA.[2] To date, the formation of helical aggregates of natural amphiphiles such as steroid acid,[3,4] hydroxy fatty acid,[5] and phospholipid,[6] and synthetic amphiphiles[7-9] in aqueous solution has been observed. A great interest in the construction of superstructure similar to DNA and RNA by self-assembly of the mononucleotide unit has stimulated us to provide phospholipid-nucleoside



(1) B = Cytosin-1-yl, X = OH, Y = H
(2) B = X = OH, Y = H
(3) B = N^4-Ethenocytosin-1-yl, X = OH, Y = H
(4) B = Cytosin-1-yl, X = Y = H

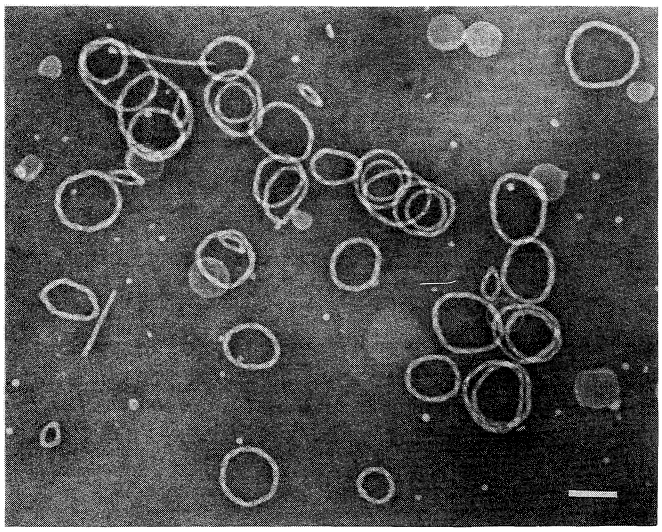


Fig. 1. Electron micrograph of circular helical strands formed from 5'-phosphatidylcytidine in the presence of 0.2 M KCl after aging at 25 °C overnight. Scale bar represents 0.1 μm.

conjugates such as 5'-phosphatidylnucleosides having two long alkyl chains and a nucleotidyl group in a molecule.[10-12]

In this paper, we report the formation of circular and linear helical strands from 5'-phosphatidylnucleosides and their morphology.

5'-Phosphatidylnucleosides were enzymatically synthesized from 1,2-dipalmitoyl-sn-glycero-3-phosphocholine and the corresponding nucleoside using Streptomyces phospholipase D[13] and obtained in 50-80% yields.[12] Synthesis of 5'-phosphatidyl-N^4-ethenocytidine (**3**) was readily accomplished by stirring a solution of 5'-phosphatidylcytidine (**1**) in 40% aqueous chloroacetaldehyde-chloroform (5:1) at 50 °C for 30 h.[14]

5'-Phosphatidylnucleoside (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, 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 (ϕ 5 mm x 110 mm), sonicated (Branson Sonifier B-1200, 30W) at 50 °C for 45 min, and aged at 25 °C overnight.

For negatively stained electron microscopic observation, a drop of the sample was placed on a carbon-coated collodion grid, the excess sample was removed with filter paper, 1% phosphotungustate was applied to the grid, and it was immediately examined under a JEM 1200 EX at 80 kV. Automatic image processing was performed on a microdensitometer (Joyce Loebl, model 6) by the use of an image processing program (JEOL, SEMPER).[15]

After sonication at 50 °C for 45 min aqueous 5'-phosphatidylcytidine (**1**) produced vesicles mainly with diameter of 0.015-0.03 μm. The vesicles were slowly transformed into circular helical strands with diameter of 0.05-0.15 μm in the presence of 0.2 M KCl after aging at 25 °C overnight (Fig. 1). The circular helical strands were present along with vesicles with diameter of 0.05-0.1 μm. Vesicles with a rod-shaped bud or rod-like aggregates found during the aging appear to be metastable intermediates between the vesicles and the circular

strands. The cyclizing process seems to be completed in a week at 25 °C.

Salt concentration and pH affected the morphology of 5'-phosphatidylcytidine. At a lower KCl concentration (0.01-0.05 M), 5'-phosphatidylcytidine produced linear helical strands (Fig. 2a) and at a higher KCl concentration (0.5-1.0 M), it formed only vesicular structures. The optimal KCl concentration and pH for the formation of the circular strand were 0.1-0.2 M and 8.0. No formation of the circular and linear strands was observed in an acidic solution. As shown in Fig. 2b, the linear helical strands had grooves of ≈100 Å in diameter and helical pitch of ≈240 Å. The geometry of the helix was right-handed. The diameter, pitch, and geometry of the helix of the circular strands were much the same as those of the linear strands.

Complete ionization of the phosphate group of 5'-phosphatidylcytidine by addition of NaOH or KOH stimulated the formation of circular helical strands. However, a free acid form of 5'-phosphatidylcytidine did not produce the circular strands in the same buffer solution because of its poor solubility. Analogues of 5'-phosphatidylcytidine such as 5'-phosphatidyl-D-ribose (**2**) and 5'-phosphatidyl-N^4-ethenocytidine (**3**) could not form circular nor linear helical strand. These results indicate that stacking and hydrogen bonding between the bases and complete

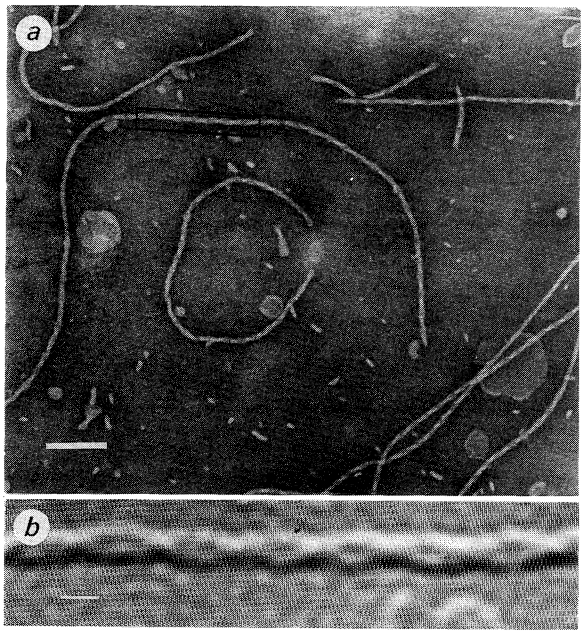


Fig. 2. a. Electron micrograph (scale bar, 0.1 μm) of linear helical strands formed from 5'-phosphatidylcytidine in the presence of 0.01 M KCl after aging at 25 °C overnight. b. Fourier transferred and differentiated image (scale bar, 100 Å) of the part boxed by a rectangle in Fig. 2a.

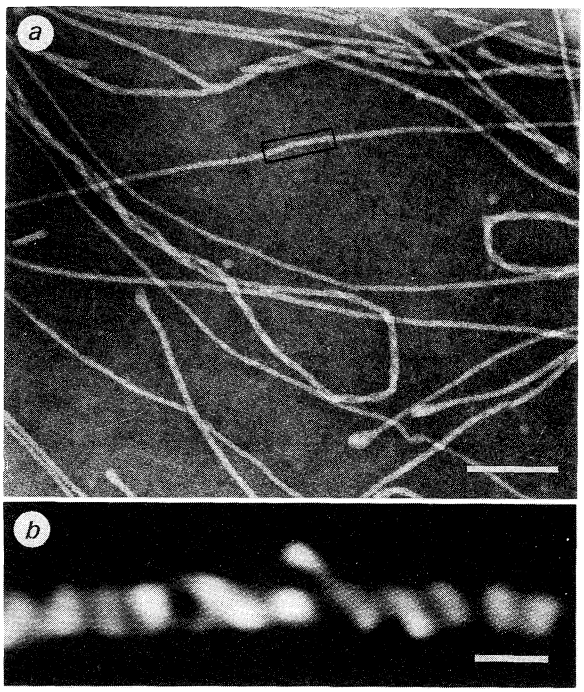


Fig. 3. a. Electron micrograph (scale bar, 0.1 μm) of linear helical strands formed from 5'-phosphatidyldeoxycytidine in the presence of 0.2 M KCl after aging at 25 °C overnight. b. Fourier transferred image (scale bar, 100 Å) of the part boxed by a rectangle in Fig. 3a.

ionization of a phosphate group of 5'-phosphatidylcytidine are necessary for the formation of the circular helical strands.

5'-Phosphatidyldeoxycytidine (**4**) also produced circular helical strands at 0.5 M KCl concentration. It formed linear helical strands at a lower KCl concentration (0.1-0.2 M) (Fig. 3a) and only vesicular structures at a higher KCl concentration (1 M and above). As shown in Fig. 3b, the linear strands formed from 5'-phosphatidyldeoxycytidine had grooves of ≈65 Å in diameter and helical pitch of ≈52 Å. Therefore, the strands were more compactly folded than those of 5'-phosphatidylcytidine. The right-handed double helices existed in the strands.

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 extension 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 result in a change in their morphology and function.

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References

1) J.D. Watson and F.H.C. Crick, Nature (London), 171, 737 (1953).
2) A.L. Lehninger, "Biochemistry," Worth Publishers, New York (1970), pp.241-263.
3) J.F. McCrea and S. Angerer, Biochim. Biophys. Acta, 42, 355 (1960).
4) N. Ramanathan, A.L. Currie, and J.R. Colvin, Nature (London), 190, 779 (1961).
5) T. Tachibana, T. Mori, and K. Hori, Bull. Chem. Soc., Jpn., 54, 73 (1981).
6) K.C. Lin, R.M. Weis, and H.M. McConnell, Nature (London), 296, 164 (1982).
7) H. Hidaka, M. Murata, and T. Onai, J. Chem. Soc. Chem. Commun., 1984, 562.
8) N. Nakashima, S. Asakuma, J.M. Kim, and T. Kunitake, Chem. Lett., 1984, 1709.
9) J-H. Fuhrhop, P. Schnieder, and J. Resenberg, J. Am. Chem. Soc., 109, 3387 (1987).
10) E.K. Ryu, R.J. Ross, T., Matsushita, M. MacCoss, C.I. Hong, and C.R. West, J. Med. Chem., 25, 1322 (1982).
11) J.J. Oltvoort, G.A. van der Marel, C.A.A. van Boeckel, and J.H. van Boom, Recl. Trav. Chim. Pays-Bas, 102, 523 (1983).
12) S. Shuto, S. Ueda, S. Imamura, K. Fukukawa, A. Matsuda, and T. Ueda, Tetrahedron Lett., 28, 199 (1987).
13) S. Imamura, E. Matsumura, H. Misaki, and N. Mutoh, Japan Kokai Tokkyo Koho, JP-58152481 (1982).
14) J.A. Secrist III, J.R. Barrio, N.J. Leonard, and G. Weber, Biochemistry, 11, 3499 (1972).
15) W.O. Saxton and T.L. Koch, J. Microscopy, 127, 69 (1982).

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