CHEMISTRY LETTERS, pp. 1709 - 1712, 1984.

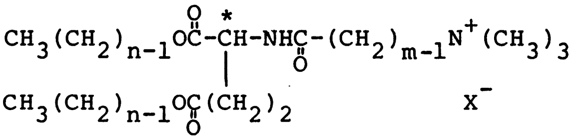
HELICAL SUPERSTRUCTURES ARE FORMED FROM CHIRAL AMMONIUM BILAYERS[1)]

Naotoshi NAKASHIMA, Sumitoshi ASAKUMA, Jong-Mok KIM,
and Toyoki KUNITAKE*

Department of Organic Synthesis, Faculty of Engineering,
Kyushu University, Fukuoka 812

Electron microscopy showed the growth of vesicles of chiral ammonium amphiphiles to helical aggregates. The transformation was determined by the physical state and the chemical structure of the components.

It has been shown by us[2)] and others[3,4)] that amino acid-derived, double-chain ammonium amphiphiles produce well-developed bilayer membranes. Some of the chiral bilayers showed peculiar spectral properties which are sensitively dependent on the physical state of the bilayer and the chemical structure of component amphiphiles.[5-10)] In particular, anionic dyes bound to chiral, ammonium bilayers exhibited marked enhancement of induced circular dichroism.[7)] Absorption spectral data suggested specific arrangements of bound dyes on the bilayer surface.[8,10)] These data imply that the bilayer aggregates assume asymmetrical molecular arrangements. We report in this paper that ordinary chiral bilayer vesicles in fact undergo metamorphoses to large helical aggregates. A related observation was made independently by Yamada and co-workers,[11)] and their finding is arranged for simultaneous publication in this Journal.



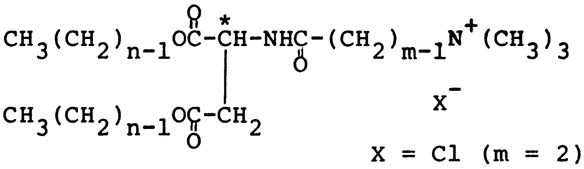
$$\begin{array}{l}\mathrm{CH_3(CH_2)_{n-1}O\overset{O}{\overset{\|}{C}}\text{-}\overset{*}{C}H\text{-}NH\underset{O}{\underset{\|}{C}}\text{-}(CH_2)_{m-1}N^+(CH_3)_3} \\ \qquad\qquad\quad\;\; | \\ \mathrm{CH_3(CH_2)_{n-1}O\underset{O}{\underset{\|}{C}}(CH_2)_2}\end{array}$$

X^-

$X = Cl$ (m = 2)
$= Br$ (m = 11)

1 - **6**

1, $2C_{12}$-L-Glu-C_2N^+ (n = 12, m = 2)
2, $2C_{12}$-L-Glu-$C_{11}N^+$ (n = 12, m = 11)
3, $2C_{12}$-D-Glu-$C_{11}N^+$ (n = 12, m = 11)
4, $2C_{12}$-DL-Glu-$C_{11}N^+$ (n = 12, m = 11)
5, $2C_{14}$-L-Glu-C_2N^+ (n = 14, m = 2)
6, $2C_{14}$-L-Glu-$C_{11}N^+$ (n = 14, m = 11)



$$\begin{array}{l}\mathrm{CH_3(CH_2)_{n-1}O\overset{O}{\overset{\|}{C}}\text{-}\overset{*}{C}H\text{-}NH\underset{O}{\underset{\|}{C}}\text{-}(CH_2)_{m-1}N^+(CH_3)_3} \\ \qquad\qquad\quad\;\; | \\ \mathrm{CH_3(CH_2)_{n-1}O\underset{O}{\underset{\|}{C}}\text{-}CH_2}\end{array}$$

X^-

$X = Cl$ (m = 2)
Br (m = 11)

7 - **9**

7, $2C_{12}$-L-Asp-C_2N^+ (n = 12, m = 2)
8, $2C_{12}$-L-Asp-$C_{11}N^+$ (n = 12, m = 11)
9, $2C_{14}$-L-Asp-C_2N^+ (n = 14, m = 2)

Amphiphiles **1** - **9** were prepared in these laboratories and identified by IR and NMR spectra and by elemental analyses. They gave transparent aqueous dispersions by sonication. Electron microscopy(instrument, Hitachi H-600) was performed as follows: amphiphiles(3 mM in water, M = mol•dm^{-3}) were mixed with 2% aqueous uranyl acetate, sonicated for 1 min(Branson Sonifier 185, 40W), aged at temperatures 10° below the phase transition(T_c)[12] for given periods of time, and placed on carbon-coated Cu meshes. Aging of the bilayers of **1** and **7** was done in an ice bath because of their low T_c.

Figure 1A is an electron micrograph of aqueous $2C_{12}$-L-Glu-$C_{11}N^+$ after aging at 20 °C for 1 h. Highly developed vesicles(single- and double-walled) with diameter of 300-1000 Å are found. The vesicles are slowly transformed into helices, as can be seen in a micrograph obtained after aging for 2 d(Fig. 1B). Helical structures

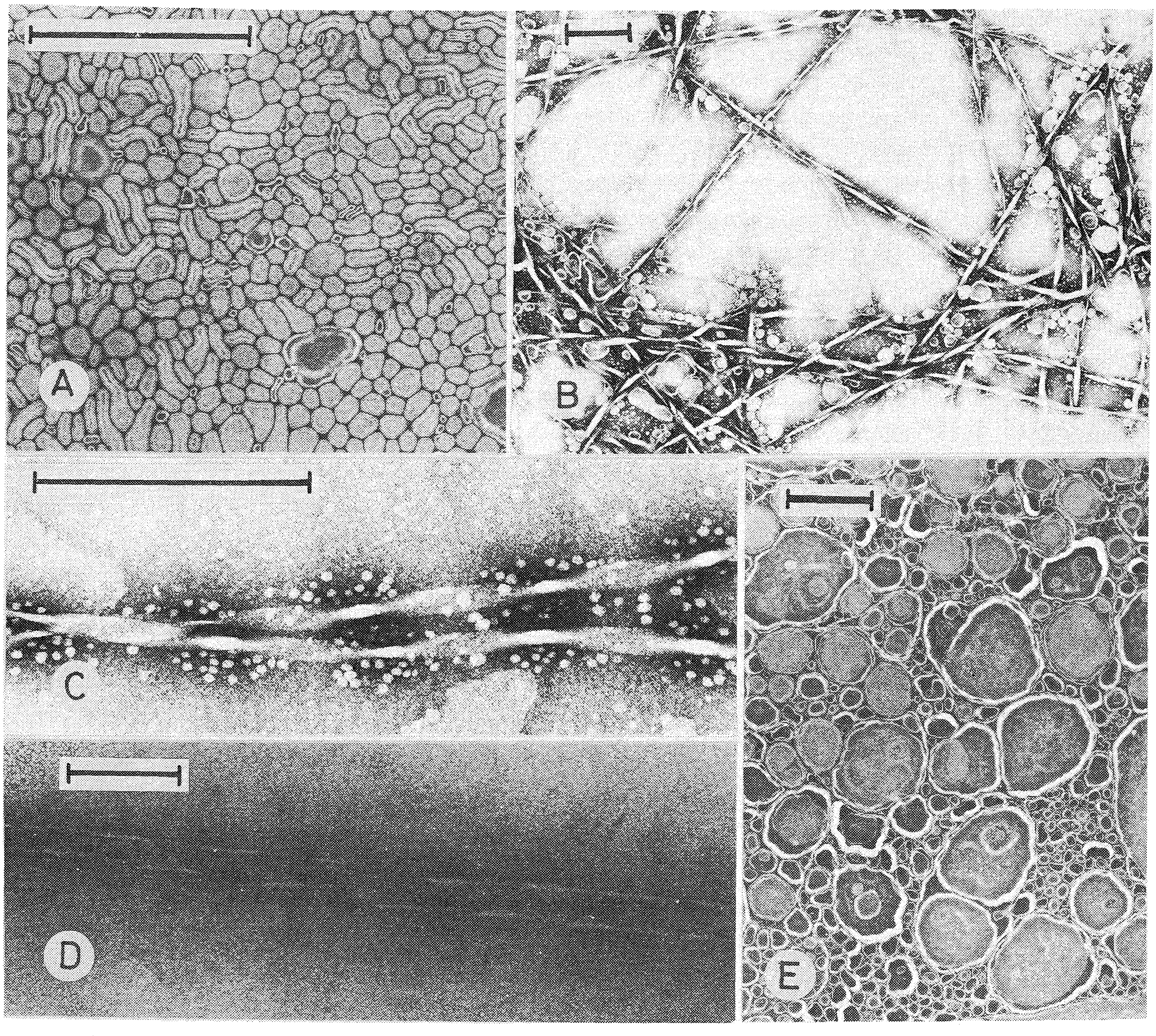


Fig. 1. Electron micrographs of aqueous dispersion of $2C_{12}$-L-Glu-$C_{11}N^+$(3 mM) stained by uranyl acetate. Scale bars, 0.5 μm. Aging condition: A, 20 °C, 1 h; B and C, 20 °C, 2 d; D, 20 °C, 1 week; E, 35 °C, 15 min after D.

(length, ca. 10 µm; diameter, ca. 500 Å; helical pitch, ca. 5000 Å) are present along with vesicles(diameter, 500-3000 Å). The detailed structure of the helix is not yet clear, however, an enlarged micrograph(Fig. 1C) suggests that helices are produced from twisted tapes. The twisting process appears to be complete in ca. a week, as shown by the presence of a large helical tube(length, ca. 10 µm; diameter, ca. 1500 Å; pitch, ca. 5000 Å) of Fig. 1D.

The molecular arrangements in vesicular and helical bilayers seems to be subtly different. The T_c value of a vesicular dispersion of $2C_{12}$-L-Glu-$C_{11}N^+$ (short aging) is 33 °C, while that of the helical aggregate(prolonged aging) is 34 °C. The presence of the two peaks is clearly seen for an aqueous sample of intermediate aging. The helical structures are converted completely to multi-vesicular and lamella structures(Fig. 1E), when a helix-rich aqueous sample(cf. Fig. 1D) is kept at 35 °C(only 1 ° above T_c of the helix) for 15 min.

Enantiomeric amphiphile, $2C_{12}$D-Glu-$C_{11}N^+$, similarly showed a slow growth to helices. The sense of the helix of $2C_{12}$-L-Glu-$C_{11}N^+$ is right-handed, and that of $2C_{12}$-D-Glu-$C_{11}N^+$ is left-handed, as observed by dark field optical microscopy.[13] In the case of racemic $2C_{12}$-DL-Glu-$C_{11}N^+$, initially formed vesicles are converted to rod-like aggregates, but not to helices. It is evident that the chirality of the component amphiphile determines the helical sense .

The influence of the molecular structure on the helix formation was subsequently studied for other types of amphiphile. Figure 2A is an electron micrograph of $2C_{12}$-L-Asp-$C_{11}N^+$ dispersions after 1 h aging. Unlike $2C_{12}$-L-Glu-$C_{11}N^+$(cf. Fig. 1A) helices are already formed. Twisted ropes are made from smaller threads, and edges of vesicles are seemingly tucked into the ropes. Bundles of tubes are found

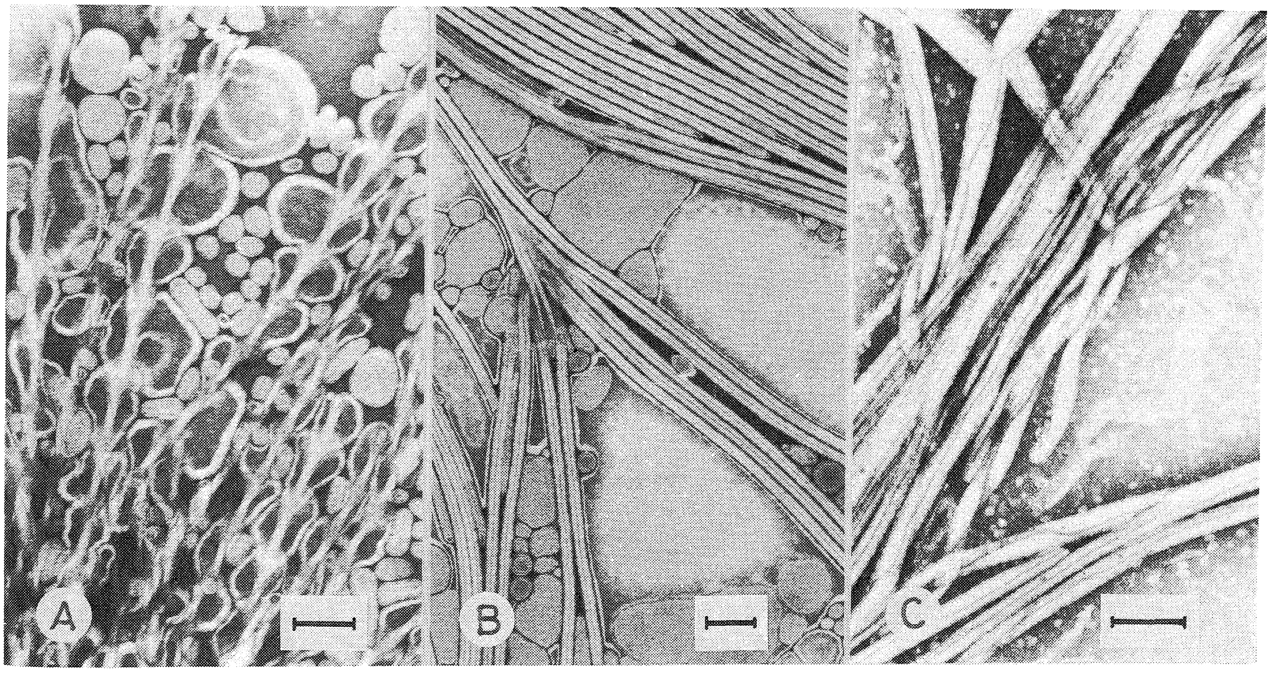


Fig. 2. Electron micrographs of aqueous dispersion of $2C_{12}$-L-Asp-$C_{11}N^+$(3 mM). Aging condition; A and B, 20 °C, 1 h; C, 20 °C, 1 d. Scale bars, 1000 Å.

together with vesicles in other areas of the view(Fig. 2B). After aging one day at 20 °C, vesicles are completely gone and slightly wavy tubes are seen abundantly (Fig. 2C).

Helix formation is not significantly affected by the tail length, and the morphological behavior of $2C_{14}$-L-Glu-$C_{11}N^+$ is analogous to that of $2C_{12}$-L-Glu-$C_{11}N^+$. In contrast, the length of spacer methylene(C_m) is critical. Vesicles are observed for amphiphiles with C_2 spacer(1, 5, 7, and 9) and helices are not formed even after aging for 10 days. Short spacer methylene appears to interfere with effective hydrogen bonding of the amide unit that should be essential for helix formation.

Formation of helical aggregates from natural products has been known for a long time. Phospholipids produce helical liposomes.14-16) Tachibana et al.17,18) conducted detailed investigations on helical aggregates of 12-hydroxystearic acid and their work was extended by Hidaka et al. to 2-hydroxydodecylamino acid.19) McCrea et al.20) and Ramanathan et al.21) observed helical microfibrils of bile acids. The present findings by us and by Yamada et al.11) establish that helical structures are readily formed in water from chiral, synthetic bilayers. It is an advantage of the synthetic system that it is possible to prepare helical aggregates from *designed* component amphiphiles.

This research is supported in part by Special Cordination Funds for Promoting Science and Technology from Science and Technology Agency of Japan.

References

1) Contribution No. 739 from Department of Organic Synthesis.
2) T. Kunitake, N. Nakashima, S. Hayashida, and K. Yonemori, Chem. Lett., 1979, 1413.
3) Y. Murakami, A. Nakano, and K. Fukuya, J. Am. Chem. Soc., 102, 4253 (1980).
4) Y. Murakami, A. Nakano, and H. Ikeda, J. Org. Chem., 47, 2137 (1983).
5) T. Kunitake, N. Nakashima, and M. Shimomura, Y. Okahata, K. Kano, and T. Ogawa, J. Am. Chem. Soc., 102, 6642 (1980).
6) T. Kunitake, N. Nakashima, and K. Morimitsu, Chem. Lett., 1980, 1347.
7) N. Nakashima, H. Fukushima, and T. Kunitake, Chem. Lett., 1981, 1207.
8) N. Nakashima, H. Fukushima, and T. Kunitake, Chem. Lett., 1981, 1555.
9) N. Nakashima and T. Kunitake, J. Am. Chem. Soc., 104, 4261 (1982).
10) N. Nakashima, R. Ando, H. Fukushima, and T. Kunitake, J. Chem. Soc., Chem. Commun., 1982, 707.
11) K. Yamada, H. Ihara, T. Fukumoto and C. Hirayama, Chem. Lett., submitted. We extend our appreciation to these authors for communication of their unpublished data.
12) T_c value(peak-top temperature in the DSC thermogram): 1, <5 °C; 2, 33 °C; 3, 33 °C; 4, 21 °C; 5, 26 °C; 6, 47 °C; 7, 7 °C; 8, 31 and 33 °C; 9, 29 °C.
13) N. Nakashima, S. Asakuma, and T. Kunitake, submitted for publication.
14) R. Virchow, Virchows Archiv, 6, 571 (1854) as cited in H. Kelker, Mol. Cryst. Liq. Cryst. 21, 1 (1973).
15) K. -C. Lin, R. M. Weis, and H. M. McConnel, Nature, 296, 164 (1982).
16) I. Sakurai and Y. Kawamura, personal communication.
17) T. Tachibana, K. Kayama, and H. Takeno, Bull. Chem. Soc. Jpn., 42, 3422 (1969).
18) T. Tachibana, T. Mori, and K. Hori, Bull. Chem. Soc. Jpn., 54, 73 (1981).
19) H. Hidaka, M. Murata, and T. Onai, J. Chem. Soc., Chem. Commun., 1984, 562.
20) J. F. McCrea and S. Angerer, Biochim. Biophys. Acta, 42, 357 (1960).
21) N. Ramanathan, A. L. Currie, and J. R. Colvin, Nature, 190, 779 (1961).

(Received July 12, 1984)