CHEMISTRY LETTERS, pp. 227 - 230, 1984.

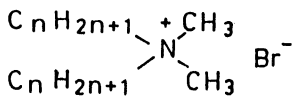
DYNAMIC TRANSFORMATION OF THE MORPHOLOGY OF DIALKYLAMMONIUM BILAYER AGGREGATES[1)]

Naotoshi NAKASHIMA, Sumitoshi ASAKUMA,
Toyoki KUNITAKE,* and Hirokazu HOTANI†
Department of Organic Synthesis, Faculty of Engineering,
Kyushu University, Fukuoka 812
†Department of Biophysics, Faculty of Science,
Kyoto University, Kyoto 606

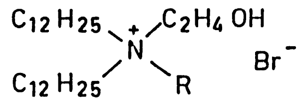
Optical and electron microscopies showed dynamic morphological changes of the aqueous bilayer aggregate of dialkylammonium salts among large vesicles, fibers and small vesicles.

Simple dialkylammonium amphiphiles such as $2C_nN^+2C_1$ and $2C_{12}N^+C_2OH{\cdot}R$ have been shown to produce vesicular(diameter, a few hundred to a few thousand Å) and lamellar aggregates of the bilayer membrane in dilute aqueous solution.[2,3)] Subsequent studies proved that the physicochemical characteristics of these bilayers are intrinsically the same as those of the biolipid bilayer.

Recently, direct observations by optical microscopy were reported for static and dynamic morphological variations of lecithin liposomes.[4-8)] These changes are closely related to the function of biological membranes such as cell fusion and fission. It would be extremely interesting if analogous morphological changes are observable for simple, synthetic bilayers. In this communication we describe morphological transformations of dialkylammonium bilayer aggregates as examined by optical and electron microscopes. The static, light-microscopic observation of lamellar mesophases(vesicle and tubule) was reported by Bacon et al. for a mixture of dodecylammonium chloride and didodecyldimethylammonium chloride.[9)]



$2C_nN^+2C_1$
(n=12, 14, 16)



$2C_{12}N^+C_2OH{\cdot}R$
($R = CH_3$, C_2H_4OH)

$2C_{12}N^+2C_1$(1 mg) was added to 1 ml of distilled water, allowed to swell and then shaken gently by hand. A transparent dispersion thus obtained was placed on a slide glass and observed at room temperature(20 - 25 °C) by a dark-field light microscope (Olympus BH-2). As shown in Fig. 1, the aggregate morphology is remarkably variable. There are flexible fibers extending over a distance of several to several hundred μm(a), large tubules(b), spherical vesicles of diameter of 0.5 - 10 μm(c), vesicles connected to tubules(d), twisted vesicles(e), tubules protruding from vesicles(f), and concave vesicles(g). Smaller particles are seen as dots. The Brownian motion is clearly noticeable for fibers and vesicles. These morphologies do not change significantly at 50°C(the crystal-to-liquid crystal phase transition temperature, T_C, of $2C_{12}N^{+}2C_1$ is below 10°C).[10] A similar observation has been made independently by Kachar et al.[11]

The dynamic aspect was recorded on a video tape by using an ultra-sensitive TV camera(Ikegami CTC-9000).[12] Figure 2 illustrates examples of the dynamic transformation recorded. In the case of a tubule attached to a vesicle(Fig. 2a), the tubule is elongated while the vesicle shrinks. A vesicle with protuberance is converted to a round vesicle via a rugged intermediate(Fig. 2b). A third example(Fig. 2c) includes transformation between a tadpole and a tripod. These conversions occur instantaneously or within a few seconds.

Morphological changes at the finer scale could be elucidated by electron microscopy. Figure 3 shows electron micrographs(instrument, Hitachi H-600) of a freeze fracture replica(Eiko Engineering, freeze specimen processing device FD-2A) of aqueous $2C_{12}N^{+}2C_1$ obtained by gentle shaking. In Fig. 3a, vesicles(diameter, 0.2-1 μm) and fibrous aggregates(length, several tens to hundreds of μm) are found in agreement with the optical microscopic result of Fig. 1. Many of the flexible fibers are attached to vesicles at one end. At the other end the fiber simply terminates or appears to disintegrate into small particles. The particles with diameter of less than 500 Å are scattered all over the view field. Figure 3b is an enlarged micrograph of the sample. A tubule(diameter, 2000 Å) branches into smaller tubules and fibers. Figure 3c is probably the final stage and the mode of disintegration is recorded surprisingly well. Smooth fibers are transformed into trains of particles(diameter, 300 - 400 Å) which gradually separate from each other.

When $2C_{14}N^{+}2C_1$ and $2C_{16}N^{+}2C_1$ were dispersed in water by gentle shaking at temperatures below T_C(T_C = 14 °C for $2C_{14}N^{+}2C_1$ bilayer and 28 °C for $2C_{16}N^{+}2C_1$ bilayer),[10] microcrystalline dispersions(size, several tens of μm) were found by optical microscopy. When the temperature was raised beyond T_C or when the initial dispersion was performed at $T > T_C$, vesicles(diameter, 0.5 - 10 μm) were formed overwhelmingly and fibrous aggregates, if formed, were shorter(several tens of μm). Dialkylammonium amphiphiles with the hydroxyethyl group at the head, $2C_{12}N^{+}C_2OH{\cdot}R$, were dispersed in water(30°C) by gentle shaking. They produced mostly vesicles (diameter, a few μm) at room temperature and these morphologies did not change at 50°C (T_C : below 10°C).

Dynamic morphological changes described in this study possess remarkable similarities to those observed in the phospholipid system. This suggests that these morphological properties are widely observable for bilayer-forming compounds, both natural and synthetic. The present results provide a new vista in the field of the synthetic bilayer membrane.

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

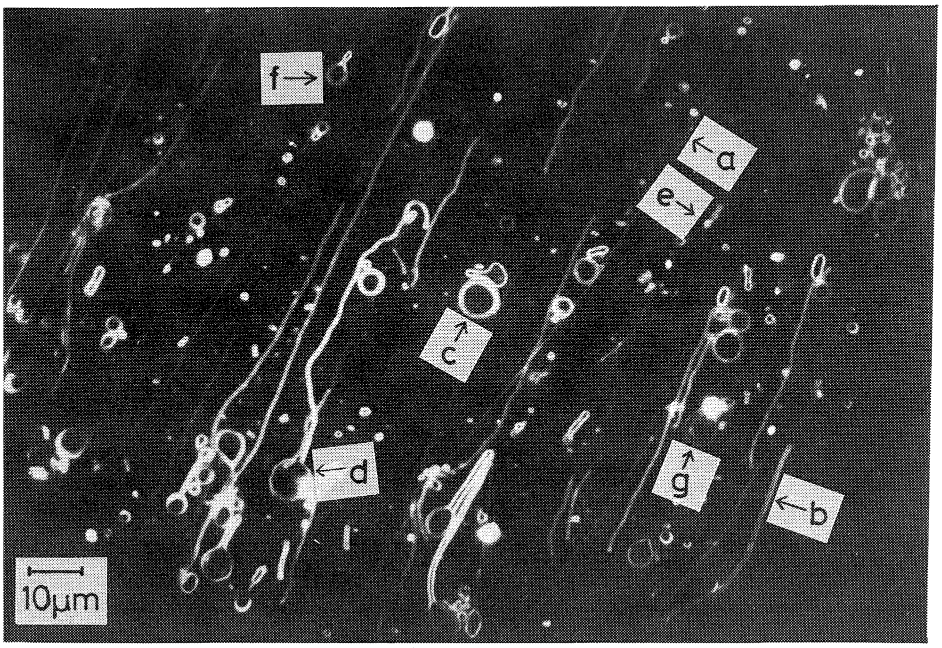


Fig. 1. Dark-field optical micrograph of aqueous dispersion of $2C_{12}N^{+}2C_{1}$.

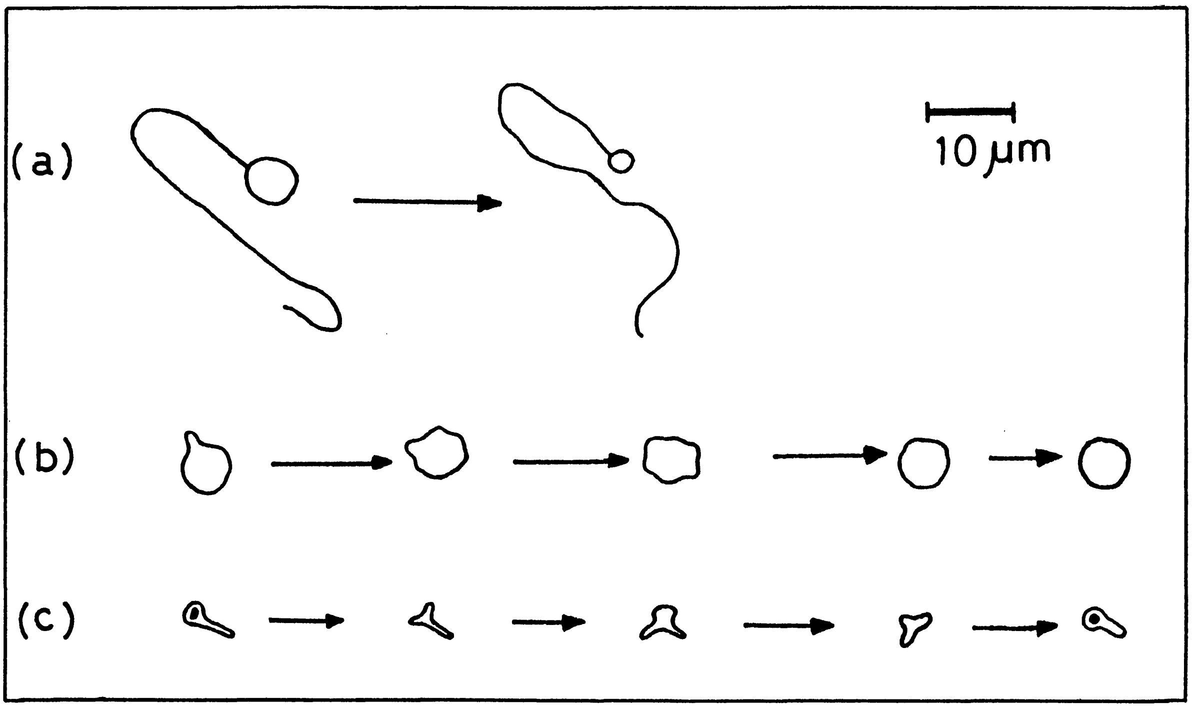


Fig. 2. Schematic illustrations of morphological transformation. The relative sizes are the same as those observed on a video monitor.

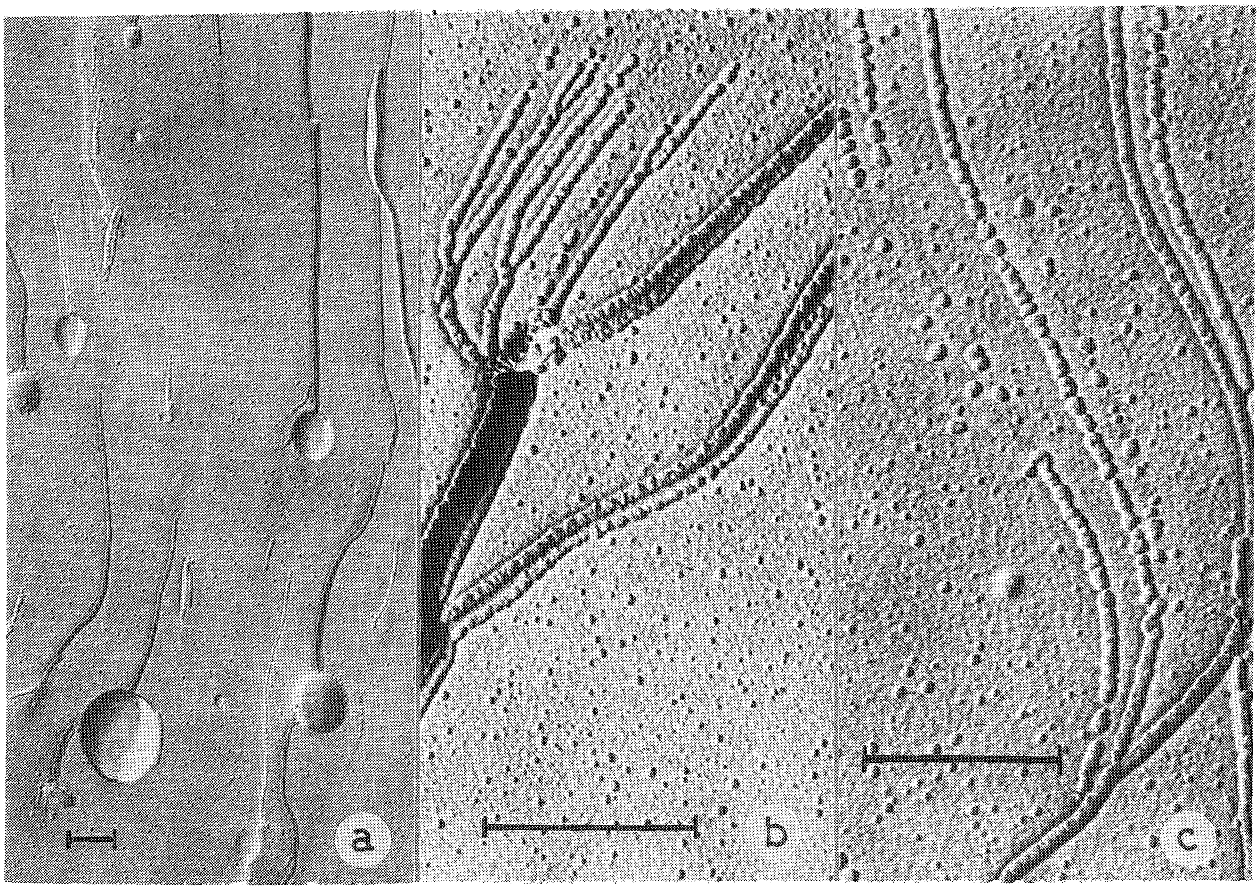


Fig. 3. Electron micrographs of feeze fracture replicas of aqueous dispersion of $2C_{12}N^{+}2C_{1}$. Scale bar, 0.5 μm.

References

1) Contribution No. 718 from Department of Organic Synthesis.
2) T. Kunitake and Y. Okahata, J. Am. Chem. Soc., 99, 3860 (1977); T. Kunitake, Y. Okahata, K. Tamaki, F. Kumamuru, and M. Takayanagi, Chem. Lett., 1977, 387.
3) T. Kunitake and Y. Okahata, Chem. Lett., 1977, 1337.
4) W. Harbich, R. M. Servuss, and W. Helfrich, Phys. Lett., 57A, 294 (1976).
5) E. Boroske, M. Elwenspoek, and W. Helfrich, Biophys. J., 34, 95 (1981).
6) P. Yager, J. P. Sheridan, and W. L. Peticolas, Biochim. Biophys. Acta, 639, 485 (1982).
7) K. L. Lin, R. M. Weis, and H. M. McConnell, Nature, 296, 164 (1982).
8) H. Hotani, and S. Onishi, Seibutsubutsuri(Biophysics), 22, S39 (1982).
9) W. E. Bacon, M. E. Neubert, P. J. Wildman, and D. W. Ott, Mol. Cryst. Liq. Cryst., 90, 307 (1983).
10) Y. Okahata, R. Ando, and T. Kunitake, Ber. Bunsenges. Phys. Chem., 85, 789 (1981).
11) B. Kachar, D. F. Evans, and B. W. Ninham, J. Colloid Interface Sci., in the press.
12) H. Hotani, J. Mol. Biol., 156, 791 (1982).

(Received November 12, 1983)