CHEMISTRY LETTERS, pp. 25-28, 1990.

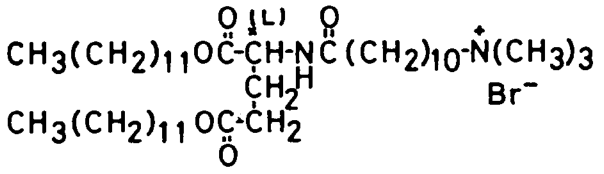
Direct Observation of Helices and Vesicles of Ammonium Bilayers by High-Resolution, Scanning Electron Microscopy[1)]

Yuichi ISHIKAWA, Taisei NISHIMI, and Toyoki KUNITAKE*
Department of Organic Synthesis, Faculty of Engineering,
Kyushu University, Fukuoka 812

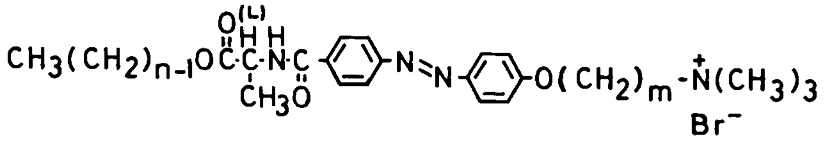
The steric structures of helices and vesicles formed from bilayer membranes of ammonium amphiphiles were observed without metal coating by high-resolution scanning electron microscopy. These observations agreed with those made by transmission electron microscopy.

Bilayer membranes are basically two-dimensional arrays of component molecules, and a remarkable range of higher-order morphologies are created by relatively small realignments of those molecules. The morphological variation is, therefore, an intrinsic part of the bilayer chemistry of both natural lipids and synthetic amphiphiles.

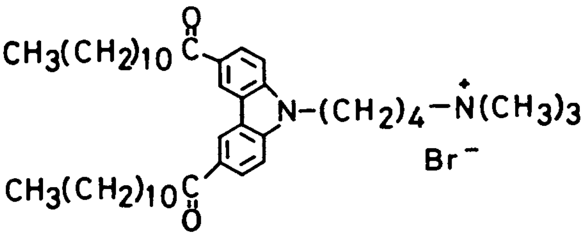
When a TEM observation is made in combination with the negative-staining technique, cross-sectional (two-dimensional) views are obtainable. Steric (three-dimensional) pictures become available through the freeze-fracture replica technique; however, artifacts have been a serious problem at finer resolutions. Although scanning electron microscopy (SEM) readily gives three-dimensional pictures, its resolution is inferior to that of TEM and metal shadowing is usually required for non-conducting specimens in order to avoid charge-up.



: **1**



: **2**



: **3**

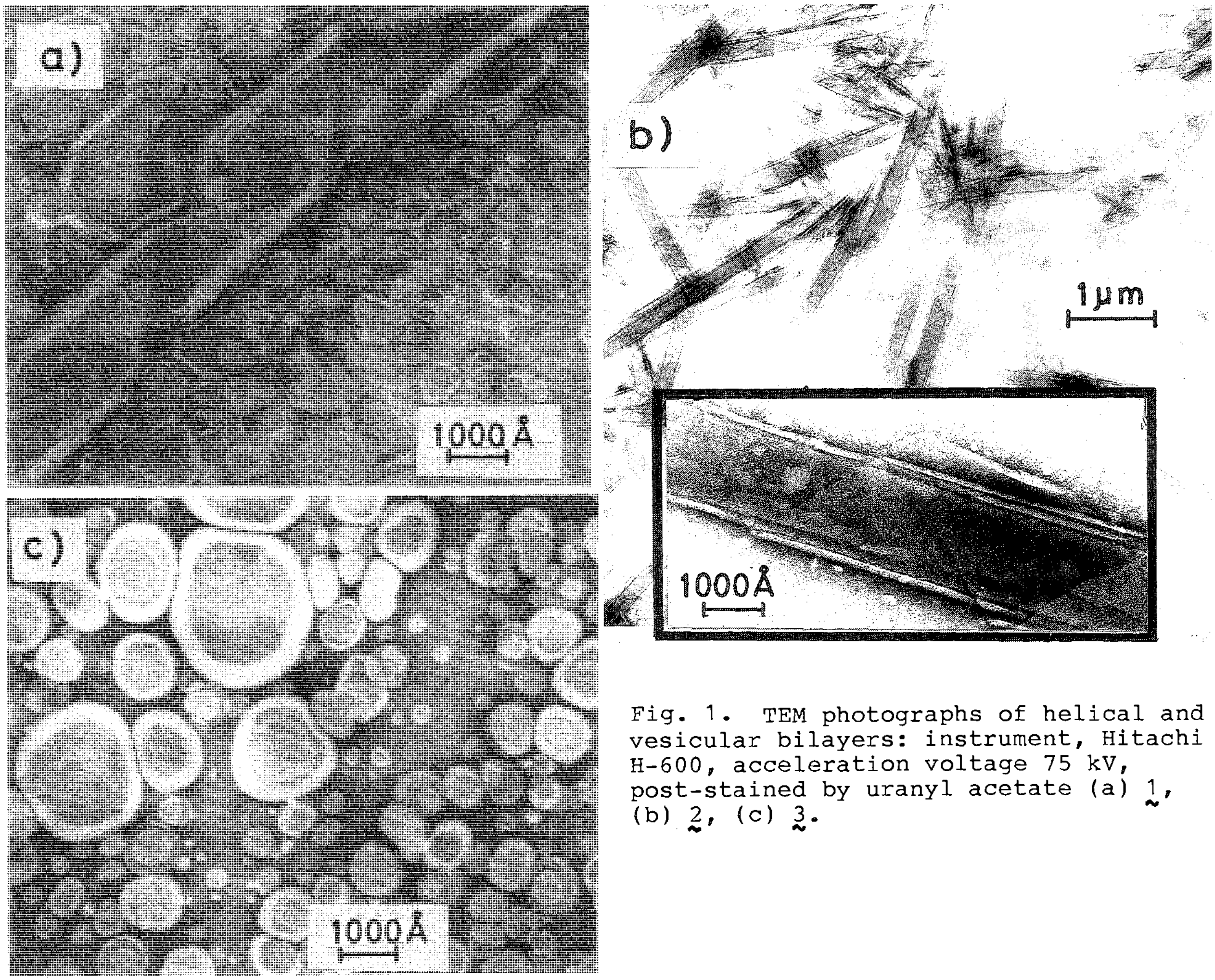


Fig. 1. TEM photographs of helical and vesicular bilayers: instrument, Hitachi H-600, acceleration voltage 75 kV, post-stained by uranyl acetate (a) **1**, (b) **2**, (c) **3**.

A recent advance in SEM technology made it possible to observe objects at low acceleration voltages, thus eliminating the need of metal coating.2) We applied this methodology to observation of the three-dimensional structure of synthetic bilayer aggregates, and compared the results with those of the corresponding TEM pictures. The three amphiphiles (**1**, **2**, **3**) were selected for the SEM examination, because they provide very different bilayer aggregates.3-6)

The preparation of compounds **1**,3,4) **2**,5) and **3**6) have been described. They were sonicated in de-ionized water (Bransonic Cell Disruptor 185, 30 W, 1 min) to give 2 mM transparent dispersions. After aging for one day at room temperature, small drops of the dispersions were placed on carbon-coated Cu grids, allowed to stand for a few min. Extra liquid was removed by filter paper and the samples were dried at 1 mmHg for 1 h. They were used as SEM specimens (Fig. 2, a-d). TEM (Fig. 1, a-c) specimens and some SEM (Fig. 2, e,f) specimens were obtained by additionally applying a saturated aqueous solution of uranyl acetate to the original SEM specimens.

Figure 1 shows TEM photographs. As described before,3) helical superstructures (helical pitch, ca. 3300 Å) develop from vesicular dispersions of

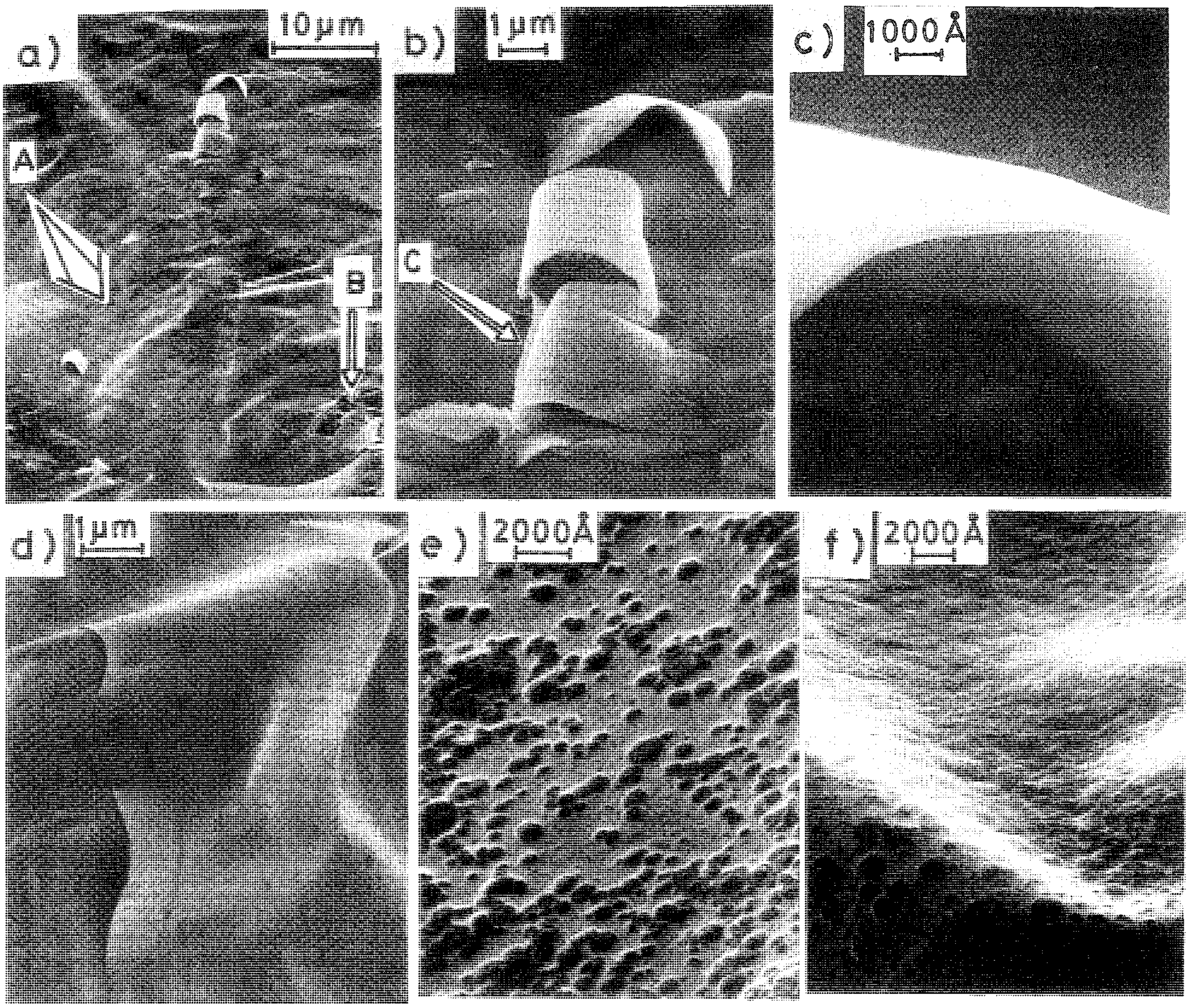


Fig. 2. High-resolution SEM photographs of bilayer aggregates, instrument, Hitachi S-900. The Cu-grids were placed against electron beam at an angle of 50 . (a),(b),(c), sample **1**, acceleration voltage 16 kV; (d) sample **2**, 2 kV; (e),(f), vesicles of **3**, post-stained by uranyl acetate, 16 kV.

1. Figure 1a shows both of helices and vesicles. Chiral, single-chain amphiphile **2**, gave multi-cylindrical helices with the helical pitch of ca. 4000 Å (Fig. 1b). Georger et al.[7)] and Fuhrhop et al.[8,9)] observed very similar giant helices (pitch, ca. 1 - 10 μm) which were produced from lecithin and saccharide surfactants.

In contrast, typical vesicles with diameters of 200-2000 Å are formed from **3**, as shown in Fig. 1c. They are a mixture of single-walled and multi-walled vesicles.

Figure 1 is a collection of the corresponding SEM micrographs. The acceleration voltages were set as low as possible, in order to avoid charge-up and sample degradation by electron beam in the absence of conductive metal coating. The presence of the helical superstructure is clearly seen in Fig. 2a among other apparently fractured structures. The helix could not be found abundantly. Most of the original helices may have been destroyed during the sample preparation (see A in Fig. 2a as a typical example), since the aqueous dispersion is full of the helical superstructure, as reported before.3) Similar destruction phenomena have been reported by Georger et al.7) The film formation by morphological reorganization also proceeds during the same period. The carbon coat appears to be exposed in area B of Fig. 2a, but the surrounding area is covered by a very thin film. The structural detail can be examined by micrographs obtained at greater magnifications (Fig. 2b and 2c). The helix is right-handed, according to the previous optical microscopic observation.3) The thickness of the helical tape is estimated to be 280 Å from Fig. 2c. Since the thickness of the bilayer unit of **1** has been determined to be 59.6 Å in a cast film,10) this helical tape must be composed of 4 - 5 bilayer units. Unwrapping of the tape is seen in C of Fig. 2b. The surface of the helical tape is remarkably smooth.

Figure 2d show a smooth sheet of **2** which covers the matrix carbon film. Its thickness is less than 200 Å. The helical superstructure is found for this compound in a TEM picture (Fig. 1b). The structural transformation must have taken place during the sample preparation. The sheet structure is apparently not detected by TEM.

In the case of typical vesicles of **3**, the SEM figures (Fig. 2e and 2d) similarly indicate the presence of vesicles of diameter of 200-800 Å. These diameters agrees with those in the TEM figure of Fig. 1c, although the vesicles look flattened in the former.

In conclusion, we observed three-dimensional morphologies of some bilayer aggregates by SEM, avoiding possible artifacts due to staining or metal coating. The high-resolution SEM allows direct examination of the steric structures of organic aggregates, compared with the conventional SEM technique.

The authors are grateful to Drs. Michio Goto and Takashi Nagatani of Naka Works, Hitachi Ltd. for their advice and SEM operation.

References

1) Contribution No. 916 from Department of Organic Synthesis.
2) Hitachi technical data > SEM, No.37 (1988).
3) N. Nakashima, S. Asakuma, and T. Kunitake, J. Am. Chem. Soc., 107, 509 (1985).
4) N. Nakashima, S. Asakuma, J.-M. Kim, and T. Kunitake, Chem. Lett., 1984, 1709.
5) for example. M. Nakashima, K. Morimitzu, and T. Kunitake, Bull. Chem. Soc. Jpn., 57, 3253 (1984).
6) T. Kunitake, M. Shimomura, Y. Hashiguchi, and T. Kawanaka, J. Chem. Soc., Chem. Commun., 1985, 833.
7) J.H. Georger, A. Singh, R.R. Price, J.M. Schnur, P. Yager, and P.E. Schoen, J. Am. Chem. Soc., 109, 6169 (1987).
8) J-H. Fuhrhop, P. Schneider, J. Rosenberg, and E. Boekema, J. Am. Chem. Soc., 109, 3387 (1987).
9) J-H. Fuhrhop, P. Schneider, E. Boekema, and W. Helfrich, J. Am. Chem. Soc., 110, 2861 (1988).
10) S. Asakuma and T. Kunitake, Chem. Lett., submitted.

(Received August 30, 1989)