AFM Observation of a Supramolecular Rod-like Structure of Bilayer Membrane Formed from Tripeptide-Containing Amphiphiles

Katsuhiko Ariga, Norihiro Yamada,*† Masanobu Naito,† Emiko Koyama,†† and Yoshio Okahata*
Department of Biomolecular Engineering, Tokyo Institute of Technology, 4259 Nagatsuda, Midori-ku, Yokohama 226-8501
†Faculty of Education, Chiba University, 1-33 Yayoi-Cho, Inage-ku, Chiba 263-8522
††Department of Chemistry, University of Tsukuba, Tsukuba 305-8751

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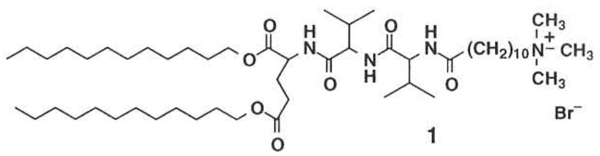
Rod-like supramolecular structure composed of hydrogen-bonded bilayers of tripeptide-containing amphiphiles was observed with an atomic force microscopy (AFM). Presence of peeling-off structure in monolayer thickness suggests that the rods are made from multi-wall of bilayer units.

It has been reported that several kinds of amphiphiles form fiber-like and/or spiral structures, which had been mainly observed by conventional transmission electron microscope (TEM).[1–9] However, three-dimensional images of the supramolecular structure have not been fully investigated. It should be observed by current-developed techniques such as energy-filtered TEM, scanning confocal microscopy, and atomic force microscopy (AFM). Recently, modifications in AFM techniques have been widely proposed on the basis of other measuring forces such as friction force and viscoelasticity. These techniques would allow the structure to be analyzed from various points of view. Therefore, we must open the way to observation of the supramolecular structure by AFM technique. As the first step, we just have started an AFM observation on supramolecular assemblies of a new class of amphiphile having a tripeptide moiety.[10,11]



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Amphiphile 1[12] with a Glu-Val-Val unit (L isomers), two C_{12} chains, and quarternary ammonium head group was used as an example of the tripeptide-containing amphiphile. All the experiments were carried out by use of the air-dried cast film from the aqueous solution of **1**. The aqueous solution was translucent. A definitive evidence of the aggregation was obtained by Fourier transform infrared (FT-IR) spectroscopy, because the aqueous solution (1 mM) gave the amide I and amide II peak at 1637 and 1546 cm^{-1}, respectively, indicating the presence of the parallel-chain β-sheet structure at the tripeptide part. The same spectra were obtained when the water was removed by evaporation (1633 and 1548 cm^{-1}, for amide I and amide II, respectively), and hence the aggregate structure was maintained intact in the air-dried cast film.

We then applied the AFM to the cast film. Amphiphile **1** was cast on freshly cleaved mica from the 1 mM aqueous solution followed by air-drying. AFM images were taken by a Nanoscope IIIa (Digital Instruments, Santa Barbara, CA) with tapping mode in air. According to manufacturer, the normal spring constant was 20–100 Nm^{-1}. Drive frequency was around 300 kHz. Scanning speed was at a line frequency of 1 Hz with 256 pixels per line. Film morphology was observed in 5 μm, 1 μm, and 200 nm scales. Image at 5 μm scale is shown in Figure 1. Rod- or fiber-like structures are visible and they are aligned and assembled. Similar morphological characteristics were also confirmed by TEM observation. Figure 2 shows the image at 1 μm scale where supramolecular rods with diameter of 50–300 nm were stacked. Surprisingly, surface of all the rods was peeled off (see parts indicated by arrows).

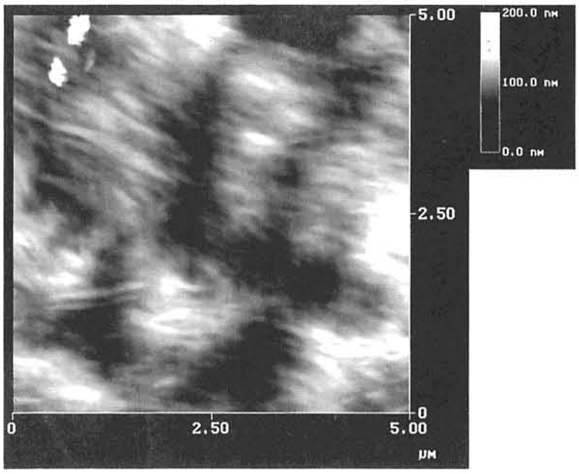

Figure 1. AFM image (5 x 5 μm) of cast film of **1** on mica

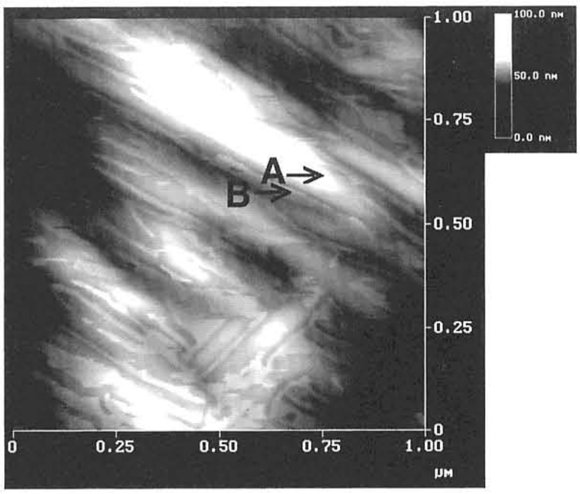

Figure 2. AFM image (1 x 1 μm) of cast film of **1** on mica (A) Peelded part and (B) unpeeled part.

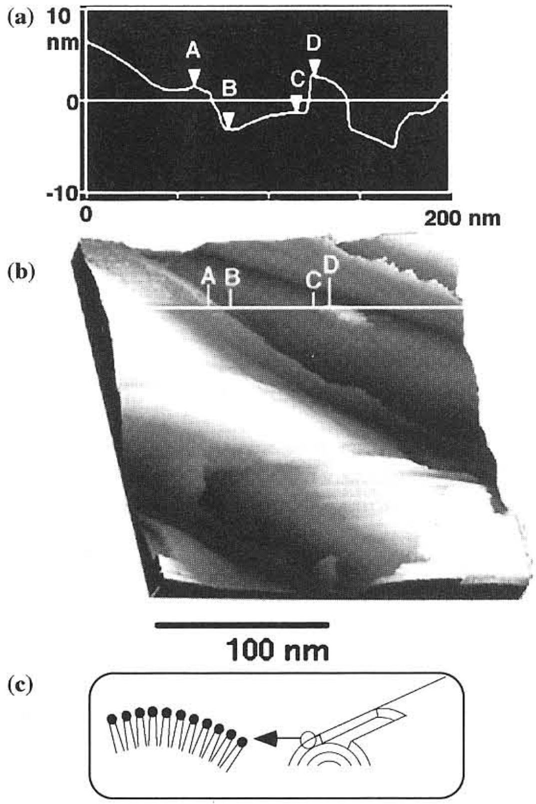

Figure 3. Three-dimensional image of magnified AFM image (200 x 200 nm) of cast film of **1** on mica (a) and its depth profile (b) on the indicated line. In the three-dimensional image, vertical and horizontal scales are equalized. In the depth profile, height difference between A and B is 4.7 nm and that between C and D is 4.0 nm. Plausible model of the supramolecular structure is drawn in (c).

In order to elucidate the obtained structure, we investigated the details of the AFM image. Figure 3b shows three-dimensional representation of the magnified image (200 × 200 nm) where vertical and horizontal scales are equalized. The representation confirms that the supramolecular structure is not flat and has some degree of curvature. Cross-sectional profile of the image was also investigated (Figure 3a). Thicknesses of skin of the rods are measured at two points and they are 4.0 and 4.7 nm as indicated in the figure caption. Although viscoelastic properties and tilt angle of amphiphiles in assembled structure would affect these values, the obtained data were in a good agreement with molecular dimension of amphiphile **1** (ca. 4.5 nm in length from CPK model), *i.e.*, thickness of the skins of the rods is almost equal to the monolayer thickness of **1**. This result suggests the one of the possibilities that the supramolecular rods of **1** cast from the aqueous solution are composed of multi-walls of bilayer units and the outer most monolayers are easily peeled off (Figure 3c). As seen in Figure 2, only surface layer was peeled off in all the rods. Instability of the outer most layers should be further investigated in future.

In this communication, we just have demonstrated that AFM is the one of the very useful tools to investigate submicron-size supramolecular structures. Systematical studies on the present and the related systems would provide important information on the design of supramolecular structures from artificial peptides.

References and Notes

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12 Synthesis of **1** was described breifly in ref 10: Anal. Found: C, 62.40; H, 10.43; N, 5.30%. Calcd for $C_{53}H_{103}N_4O_7Br{\cdot}2H_2O$: C, 62.14; H, 10.53; N, 5.47%.