Formation of Mesoscopic Patterns with Molecular-Level Flatness by Simple Casting of Chloroform Solutions of Tripeptide-Containing Amphiphiles

Katsuhiko Ariga,* Jun-ichi Kikuchi, Kikou Narumi,† Emiko Koyama,†# and Norihiro Yamada†
Graduate School of Materials Science, Nara Institute of Science and Technology (NAIST), 8916-5 Takayama, Ikoma, Nara 630-0101
†*Department of Education, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522*

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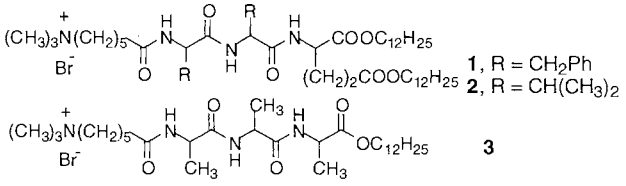
An AFM investigation revealed that air-dried cast films of tripeptide-containing amphiphiles had a flat texture with regular mesoscopic patterns.

The preparation of ultrathin film based on the self-assembling nature of amphiphilic molecules is one of the promising methods to create structures of nanometer-scale and/or mesoscopic size. Although a simple casting method is regarded as a practical technique to obtain thick multi-bilayer structures,[1] Shimomura *et al.* recently reported the formation of micrometer-scale mesoscopic patterns on the cast films of polyion complex amphiphiles.[2] Sano *et al.* reported a formation of mesoscopic tapes upon spontaneous assembly of carboxyazobenzene derivertives on water.[3] It is also known that immobilized bilayers from aqueous phospholipid vesicles give regular patterns.[4] These examples suggest that the spontaneous process of amphiphile assemblies would give a variety of mesoscopic patterns under appropriate control. We have successfully controlled the assembling behaviors of tripeptide-containing amphiphiles in various solvents by the combination of the hydrophobic interaction and hydrogen bonding. In water and CCl_4, the amphiphiles formed well-developed fibrous assemblages which can be fixed in their cast films.[5,6] In contrast, $CHCl_3$ was found as a medium that does not induce aggregation of the amphiphiles probably due to its medium polarity. Therefore, casting from the $CHCl_3$ solution has possibility to induce spontaneous pattern formation upon gradual assembly. In the present study, the morphologies of the cast films of the tripeptide-containing amphiphiles from $CHCl_3$ solution have been investigated using atomic force microscopy (AFM) in order to demonstrate that a subtle change in the molecular structures results in a variety of flat mesoscopic patterns. The patterns have a mesoscopic size in two dimensions and a nanometer-scale thickness.



The amphiphiles **1** and **2** have dialkyl chains with Phe-Phe-Glu and Val-Val-Glu moieties, respectively, and **3** is the monoalkyl amphiphile with an Ala-Ala-Ala unit.[5] Their $CHCl_3$ solutions and the cast films were analyzed by Fourier transform infrared (FT-IR) spectroscopy.[5] FT-IR spectra of $CHCl_3$ solution and cast film of **1** are shown in Figure 1. In the spectrum of the $CHCl_3$ solution at 16 mmol·dm^{-3} (Figure 1a), amide A, amide I, and amide II peaks were found at 3412, 1667, and 1497 cm^{-1}, respectively, indicating that hydrogen bonding was not formed. Such diagnostics was precisely described by Miyazawa *et al.*[7] The peak position of $\nu_{as}(CH_2)$ for the $CHCl_3$

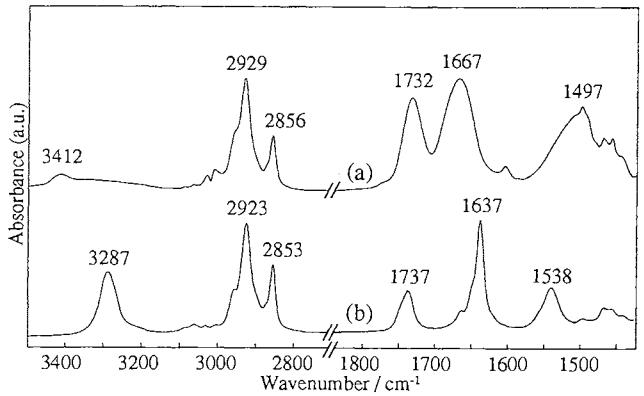


Figure 1. FT-IR spectra of **1** at 25 °C: A, $CHCl_3$ solution; B, cast film.

solution of **1** (2929 cm^{-1}) indicates that the alkyl chains are spatially separated from each other.[8] These spectral aspects mean that the amphiphile molecules are well solvated and molecularly dispersed. Similar spectral features were observed even when the concentration was raised to 200 mmol dm^{-3}. Drastic peak shifts were seen in its cast films (Figure 1b). The cast films were prepared by spontaneous evaporation of the $CHCl_3$ solution on CaF_2 (for FT-IR) and mica (for AFM). The lower shifts of amide A and amide I to 3287 and 1637 cm^{-1}, respectively, and higher shift of amide II to 1538 cm^{-1} indicate the formation of a parallel β-sheet structure, according to previous reports.[5,9,10] Lower shift of $\nu_{as}(CH_2)$ in the spectrum of the cast film of **1** (2923 cm^{-1}) indicates an increase in the trans-conformer in the alkyl chains. Similar spectral characteristics were observed for the other cast films.[11] These amphiphiles were converted from the monomerically-dispersed state to an ordered assembly with a parallel β-sheet of multiple hydrogen bonding upon solvent evaporation.

The morphologies of these cast films were then investigated using AFM.[5] Flat morphologies with characteristic patterns were observed in all the cases. A 500 × 500 nm image of **1** has a footprint-like pattern (Figure 2A). The rectangle units are aligned and are partially intersected in the 1000 × 1000 nm image of the cast film of **2** (Figure 2B). Flat-tape-like patterns can be detected in the 500 × 500 nm images of the cast film of **3** (Figure 2C). Similar images were reproducibly observed everywhere in the corresponding cast films. These images have only a few nanometer steps, *i.e.*, the surface patterns have a thickness of only molecular size (4.5 nm from CPK model). Since we spread enough amount of the amphiphiles to cover the mica surface with the multilayers, the observed steps must not be created between the bare surface and the molecules epitaxially adsorbed on the surface.[12] The formation of well-oriented parallel β-sheet structure indicated by the FT-IR suggests that the monolayer-type

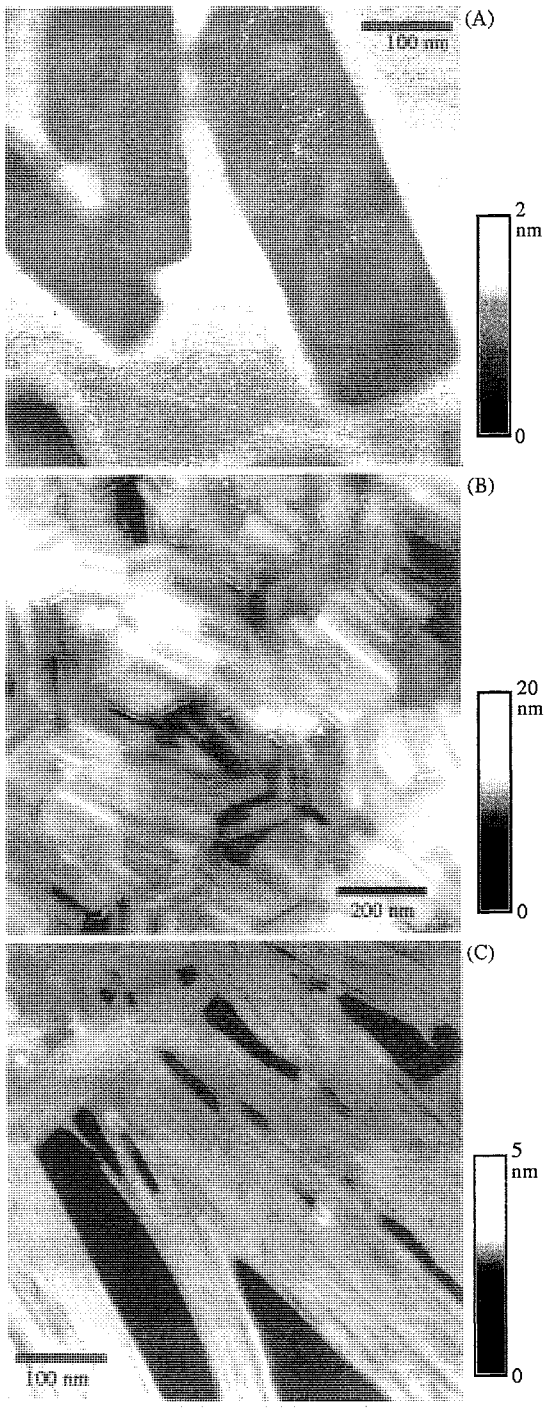


Figure 2. AFM images of the cast films: A, **1**; B, **2**; C, **3**.

structure would exist as a unit. The steps might represent the partial absence of the surface monolayer or dislocation of the lamella layers. Similar layer steps were reported for the mesoscopic domains in the fluid smectic phase of liquid crystals.[13] The mechanism of the pattern formation is still unknown. The formation of mesoscopic regular structures through dewetting and/or solvent evaporation processes have been reported for polymeric materials.[2,14–16] Some of these examples were explained by immobilization of dissipative structures.

In this paper, we demonstrated the formation of the mesoscopic patterns with molecular-level flatness by the simple casting method. The origin of these structures would be gradual assembly through multiple hydrogen bonding upon solvent evaporation. The steric effect between the side chains and/or the alkyl chains affects the assembling process, probably resulting in the mesoscopic patterns specific to the amphiphiles. Therefore, we believe that various patterns can be designed by selecting peptide units and be modified by controlling the evaporation process.

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References and Notes

Current address: Department of Chemistry, University of Tsukuba, Tsukuba 305-8751.

1 Y. Wakayama and T. Kunitake, *Chem. Lett.*, **1993**, 1425.

2 N. Maruyama, O. Karthaus, K. Ijiro, M. Shimomura, T. Koito, S. Nishimura, T. Sawadaishi, N. Nishi, and S. Tokura, *Supramol. Sci.*, **5**, 331 (1998).

3 M. Sano, A. Kamino, and S. Shinkai, *Langmuir*, **15**, 13 (1999).

4 Z. Shao, J. Mou, D. M. Czajkowsky, J. Yang, and J.-Y. Yuan, *Adv. Phys.*, **45**, 1 (1996).

5 N. Yamada, K. Ariga, M. Naito, K. Matsubara, and E. Koyama, *J. Am. Chem. Soc.*, **120**, 12192 (1998).

6 K. Ariga, N. Yamada, M. Naito, E. Koyama, and Y. Okahata, *Chem. Lett.*, **1998**, 493.

7 Y. Masuda, K. Fukushima, T. Fujii-Imae, and T. Miyazawa, *Biopolymers*, **8**, 91 (1969).

8 H. L. Casal and H. H. Mantsch, *Biochim. Biophys. Acta*, **779**, 381 (1984).

9 C. M. Toniolo and M. Palumbo, *Biopolymers*, **16**, 219 (1977).

10 J. Bandekar, *Biochim. Biophys. Acta*, **1120**, 123 (1992).

11 Peak tops (cm^{-1}) of amide A, $\nu_{as}(CH_2)$, amide I, and amide II: 3279, 2923, 1632, and 1546 in cast film of **2**; 3289, 2921, 1631, and 1542 in cast film of **3**.

12 P. Quan, H. Nanjo, T. Yokoyama, and T. M. Suzuki, *Chem. Lett.*, **1998**, 1133.

13 D. R. Link, G. Natale, R. Shao, J. E. Maclennan, N. A. Clark, E. Körblova, and D. M. Walba, *Science*, **278**, 1924 (1997).

14 O. Karthaus, K. Ijiro, and M. Shimomura, *Chem. Lett.*, **1996**, 821.

15 G. Widawski, M. Rawiso, and B. François, *Nature*, **369**, 387 (1994).

16 S. Sheiko, E. Lermann, and M. Möller, *Langmuir*, **12**, 4015 (1996).