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Nano-patterning based on Amphiphilic Peptide to form Ribbon-like Structure at Air/water Interface

Makoto Hattori[1], Shujiro Hayashi[2], Hidenori Yokoi[2], Shuguang Zhang[3], Masayoshi Tanaka[1] and Takatoshi Kinoshita[1]*

[1]Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya, 466-8555, Japan
Fax: 81-052-735-5267, e-mail: kinoshita.takatoshi@nitech.ac.jp
[2]Menicon Co., Ltd. 5-1-10, Takamoridai, Kasugai, Aichi, 487-0032, Japan
[3]Center for Biomedical Engineering NE47-379, Massachusetts Institute of Technology, Cambrige, MA 02139-4307, USA

Peptide having an alternative sequence of hydrophobic and charged amino acid residues, RADA16 [CH_3CO-(Arg-Ala-Asp-Ala)$_4$-$CONH_2$], was synthesized by solid phase method. A monolayer of RADA16 was prepared by spreading a solution of the peptide on deionized water and transferred onto mica substrate by Langmuir-Blodgett (LB) method. The LB film was observed by atomic force microscopy (AFM), showed a stripe pattern. The width of the stripe was corresponded with a length of the β-structure chain of RADA16, suggesting that the stripe pattern was formed by intermolecular hydrogen bonding of RADA16. A new peptide, RFDF16 (CH_3CO-(Arg-Phe-Asp-Phe)$_4$-$CONH_2$), which was substituted phenylalanine (F) as a hydrophobic amino acid residue instead of alanine (A) of RADA16, was also synthesized. RFDF16 formed a stable monolayer at air/water interface. The AFM image of RFDF16 LB film showed orderly arranged stripe pattern.

Key words: amphiphilic peptide / β-sheet / LB film / stripe pattern

1. INTRODUCTION

Much attraction is explored to application of novel functional materials based on molecular self-assembly of which origin is biology. Peptides and proteins are useful as a building blocks to create nano-structured materials, because the character of each amino acid and its sequence promote to form favorable secondary structure such as α-helix, β-sheet and flexible loop. These structural units show inter/intra-molecular interaction, and thus result into specific higher-ordered nano-organizations[1]-[5]. Actually, peptides constructed various nano-architectures by self-assembly such as nanofibers[6][7], nanotubes[8], vesicles[9] and nano-templates[10] etc. Recently, β-sheet aggregation having orderly arranged nano-structure, especially fibrous object owing to its ability of intermolecular hydrogen bonding, is focused[11]-[15]. RADA, which is composed by Arg-Ala-Asp-Ala sequence, is one of the peptide building blocks forming β-sheet nanofibril. RADA16, which is composed by four RADA sequence, forms hydrogel in aqueous medium at quite low concentration (<0.5 wt%)[14]. Such three-dimensional self-assembled structure would be applied as a tissue culture[15].

It was considered that RADA16 formed a ribbon-like bilayer object with the nonpolar residues inside and the charged residues outside in aqueous media, and thus electrostatic interaction would be contributed to the cross-linking of RADA16. It was suggested that design of the alternative sequence of nonpolar residues and charged residues would be essential to construct a unique nano-structure. We have focused on the construction of self-assembled structure of RADA16 at air/water interface, expecting that stable ribbon-like monolayer object having hydrophilic and hydrophobic phases would be formed.

In this study, RADA16 monolayer was prepared by LB method and surface morphology was observed by AFM. Furthermore, another peptide building block, RFDF16, whose hydrophobic amino acid residues are different, was prepared. Construction of monolayer of RFDF16 at air/water interface was also investigated.

2. EXPERIMENTAL

2.1 Materials

The amphiphilic peptides, RADA16 and RFDF16 were prepared by solid phase method using Fmoc strategy (Scheme 1). The obtained peptides were characterized by MALDI-TOF mass spectrometry (Figure 1).

CH_3CO-R-A-D-A-R-A-D-A-R-A-D-A-R-A-D-A-$CONH_2$

RADA16

CH_3CO-R-F-D-F-R-F-D-F-R-F-D-F-R-F-D-F-$CONH_2$

RFDF16

Scheme 1 Amino acid sequences of amphiphilic peptides, RADA16 and RFDF16.

2.2 Preparation of Peptide Monolayer

The surface pressure-area (π-A) isotherms of

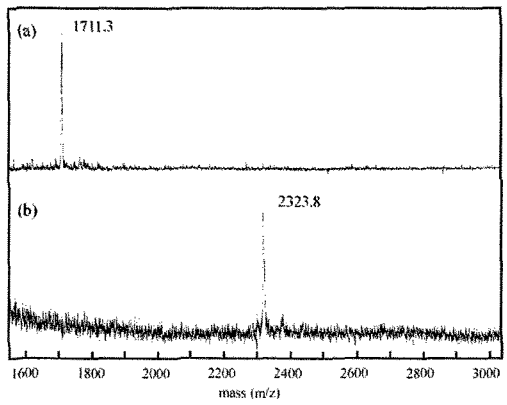

Figure 1 MALDI-TOF mass spectra of peptides: found $[M+H]^+$ (calc. $[M+H]^+$); (a) RADA16: m/z 1711.3 (1713.8) and (b) RFDF16: m/z 2323.8 (2322.6).

RADA16 and RFDF16 were measured using a NL-BIO40-MWCT (Nippon Laser & Electronics Lab.) at 25℃. The monolayer was prepared by spreading a solution of the peptide in benzene/2,2,2-trifluoroethanol (6:4) at a concentration of approximately 0.1 mg/ml. The monolayer was compressed at a speed of 5 mm/min and surface pressure was measured by Wilhelmy plate. The monolayer was transferred onto a freshly cleaved mica substrate by LB method.

2.3 AFM Observation

AFM observation was carried out on Nanoscope IIIa (Digital Instruments) using a silicon cantilever (NCH-10V, Veeco Instruments) operated in tapping mode. All images were obtained in air at room temperature.

3. RESULTS AND DISCUSSION

3.1 RADA16 Monolayer Behavior and Morphology of LB film

Figure 2 shows π-A isotherms of RADA16 at air/water interface. The surface pressure was increased at a certain area by compressing the spread RADA16. The obtained curve was typical as formation of monolayer, thus, it was suggested that RADA16 formed monolayer. From the π-A isotherm of RADA16, a limiting area per molecule was estimated to be 0.3 nm^2, which was much smaller than the expected value, 2.58 nm^2, on the basis of β-sheet formation, suggesting unexpected aggregation or partial dissolution of the peptide into the subphase. The monolayer was successfully transferred onto mica substrate at 1 mN/m by LB technique. The surface morphology of LB film was observed by AFM (Figure 3(a)). The AFM image revealed that RADA16 was orderly arranged in nano-stripe structure whose width was estimated to be ca. 6~8 nm and thickness, difference in height between the surface of nano-stripe and that of mica (Figure 3(b)), was ca. 0.6 nm. Here, we show the schematic representation of peptide RADA16 in the β-sheet structure at air/water interface (Figure 4)[16]. The width and thickness of the nanofibers of β-sheet were supposed to be 5.5 nm and 0.6 nm, respectively. These values were corresponded with the observed stripe

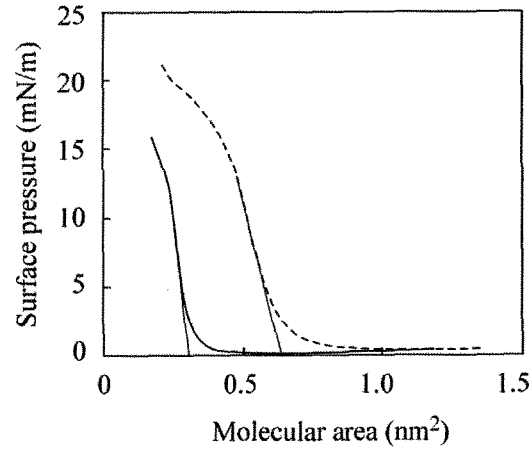

Figure 2 Surface pressure-area (π-A) isotherms for monolayer of RADA16 at air/water interface (solid line: deionized water, dotted line: 0.1 M NaCl solution, gray lines: extrapolations to the x axis from the points of steepest slope on the isotherms).

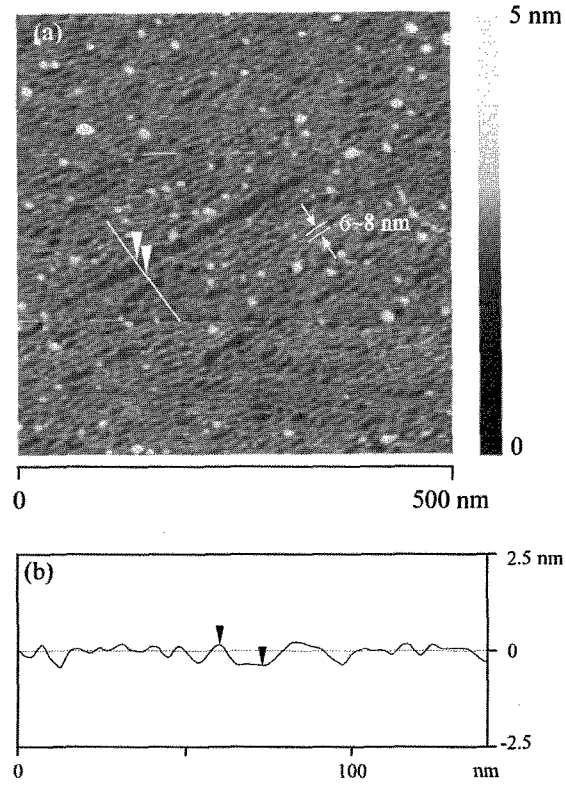

Figure 3 (a) AFM image of RADA16 LB film transferred onto mica substrate at 1 mN/m and (b) cross section of the line in (a). The height difference of the two places is ca. 0.6 nm.

pattern by AFM measurement. Therefore, arranged stripe pattern was composed by assembled β-sheet nanofibers. It was also supported that the observed low value of limiting area per molecule in π-A isotherms was caused by peptide dissolution of RADA16 into subphase. Then, in order to prevent the dissolution of peptide into subphase, π-A isotherm of RADA16 was measured on 0.1 M NaCl solution (Figure 2). The

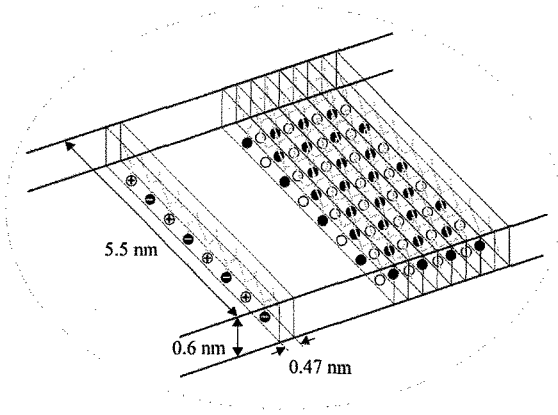

Figure 4 Schematic representation of RADA16 formed β-sheet structure at air/water interface (The length of β-strand is 5.5 nm, the thickness is 0.47 nm, the molecular area is $5.5 \times 0.47 = 2.58$ nm^2).

limiting area was estimated to be 0.63 nm^2, which was larger than the former one, suggesting that the peptide dissolution into subphase was suppressed due to salting out effect. However, this value was still smaller than the theoretical value.

3.2 RFDF16 Monolayer Behavior and Morphology of LB film

We considered that RADA16 monolayer was not stable at air/water interface due to the peptide hydrophobicity was very weak. Then, a newly designed polypeptide, RFDF16, in which the hydrophobic amino acid, alanine of RADA16 was displaced to phenylalanine, was synthesized. By spreading a benzene/TFE(6:4) solution of RFDF16 onto deionized water, the π-A isotherm was obtained (Figure 5). The surface pressure was sharply increased by compression of the spread RFDF16, suggesting the formation of extremely stable monolayer at air/water interface. The π-A isotherm showed a limiting area per molecule of ca. 2.2 nm^2. This value was approximately corresponded with theoretical molecular area of RFDF16 formed β-sheet structure (2.58 nm^2), however, it was slightly small. This result would be attributed that electrostatic interaction makes the aggregation of RFDF16 compact. It was also confirmed by FT-IR measurements that RFDF16 monolayer on CaF_2 forms β-sheet structure.

Figure 6 showed surface morphology of RFDF16 LB film which was transferred onto mica substrate at 2 mN/m. The AFM image showed orderly arranged nano-stripe structure whose width was estimated to be ca. 6~8 nm and thickness was ca. 0.7 nm, suggesting that the stripe pattern was constructed with organization of β-sheet nanofibers. These values were corresponded with the theoretical size of RFDF16 forms β-sheet structure.

4. CONCLUSION

We demonstrated that the LB films of amphiphilic peptide to form β-sheet were produced arranged nano-stripe structure. Peptides RADA16 and RFDF16 having alternate sequence of hydrophobic and charged amino acid residues formed monolayer at air/water

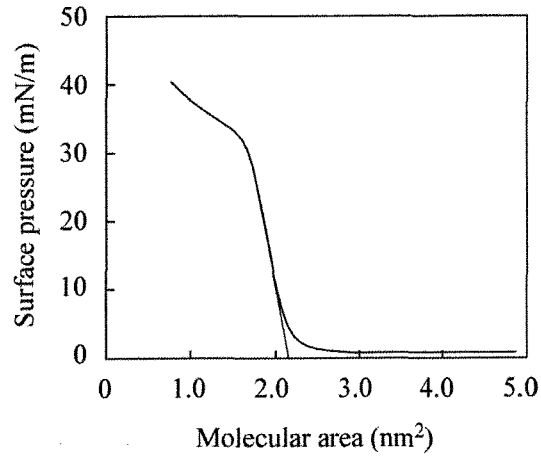

Figure 5 Π-A isotherm for monolayer of RFDF16 at air/water interface (gray line: extrapolation to the x axis from the point of steepest slope on the isotherm).

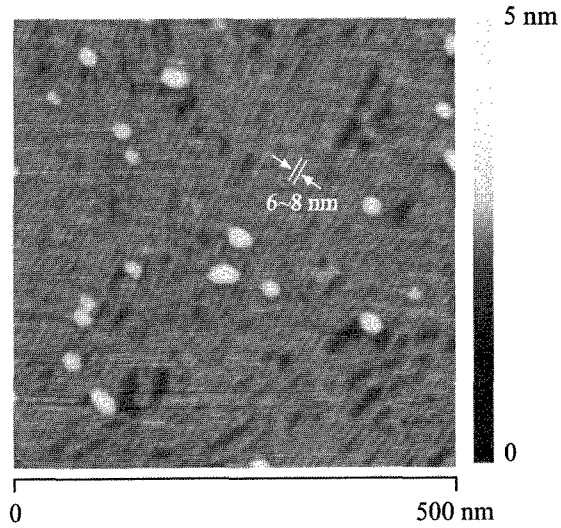

Figure 6 Surface structure of RFDF16 LB film transferred onto mica substrate at 2 mN/m by AFM measurement.

interface. These LB films transferred onto mica substrate were formed orderly arranged stripe pattern composed of β-sheet nanofibers. RFDF16 monolayer was more stable onto deionized water than RADA16 monolayer, due to the strong polarity difference. It was suggested that not only a total design of the alternative sequence but also selection of the hydrophobic residue of the peptide were essential to construct a stable nano-architecture. These results would be applied as a novel surface modification technique.

5. REFERENCES

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