The Construction of the Two-dimensional Array of the Fibrous Objects from the Mixture of RFDF16–PEG and RFDF16 at Air/Water Interface

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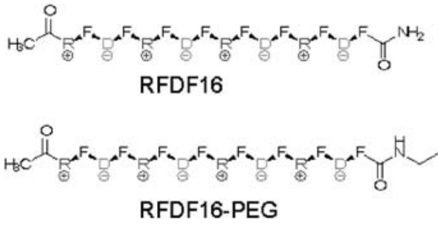
The mixture of β-sheet peptide–PEG diblock copolymer and β-sheet peptide spread at air/water interface formed a two-dimensional array of the fibrous objects. The fibrous objects constructed by intermolecular hydrogen bonding of β-sheet peptides were regulated by compressing of the monolayer. When the spread peptide moieties fully located on the water surface, the distance between the fibers was efficiently modulated by the fraction of PEG segments.

One of the most fascinating properties of block copolymers is their ability to form an ordered nanoarchitecture by self-assembly. Self-assembly of amphiphilic block copolymer composed of rod–coil structure at air/water interface can construct unique two-dimensional patterns.[1–4] Such a bottom-up approach attracts much attention to develop a novel molecular device in nanometer scale. Especially, peptide which adopts β-sheet structure is an attractive building block, because of its ability of intermolecular hydrogen bonding to give an orderly arranged structure of fibrous objects.[5–8]

Recently, modulation of the two-dimensional morphology by addition of metal ions or controlling compressing stress is featured to obtain a highly utilizable nanodevice.[9–11] We have reported that a peptide having a repeating sequence of Arg(R)–Phe(F)–Asp(D)–Phe(F) for four times, RFDF16, formed β-sheet fibrous objects on solid substrate by transferring from air/water interface.[12] Thus, we have focused on the mixing effect of the amphiphilic block copolymer, which is composed of RFDF16 as a rod and poly(ethylene glycol) (PEG) segments as a coil, into the β-sheet fibrous object. In this report, construction of an arranged array of fibrous object was investigated by spreading the mixture of RFDF16 and RFDF16–PEG diblock copolymer at air/water interface. The dependency of the mixing ratio for the two-dimensional morphology will be also discussed.

Monolayer behavior, when RFDF16–PEG and RFDF16 were mixed in several ratios, was carried out at air/water interface including 0.3 M NaCl to avoid unexpected dissolution of RFDF16 unit into water subphase (Scheme 1). The surface pressure–area (π–A) isotherms for the mixtures of RFDF16–PEG and RFDF16 are shown in Figure 1. Isotherm obtained from RFDF16 indicated a sharp increasing of surface pressure, suggesting that a monolayer efficiently formed at air/water interface. On the other hands, isotherms obtained from the mixture of RFDF16–PEG and RFDF16 showed increasing in stepwise. At low surface pressure, gradual increase of the surface pressure suggested a typical liquid-expanded monolayer, which was often observed in monolayer of block copolymer containing PEG segments.[1–3] RFDF16–PEG located at air/water interface anchoring by RFDF16 segments assembled in insoluble aggregates by intermolecular hydrogen bonding, while PEG segments adopt a flattened conformation in contact with the water subphase due to their affinity for water. At a surface pressure of near 9 mN/m, pseudoplateau can be observed in the isotherms of the mixture containing RFDF16–PEG. This behavior could be associated with the dissolution of PEG segment into water subphase. This pseudoplateau agreed with the case observed for polystyrene–poly(ethylene oxide) (PS–PEO) diblock copolymer and PEO homopolymer.[1,9] There is a conformational transition, through which PEG segments leave the surface and go into the water subphase to form a brush conformation.



Scheme 1.

At high surface pressure, the isotherms showed steep increase due to the interaction between RFDF16 units. The area per molecule (A_{obs}), which was estimated by customary extra polation of the steep increase in the compression isotherm to $\pi = 0$, was summarized in Table 1. Each value was closer to the calculated area of RFDF16 ($A_{\mathrm{cal}} = 2.58$), rather than the value of RFDF16–PEG ($A_{\mathrm{cal}} = 9.18$), supporting that the brush conformation was constructed as compressing the monolayer at high surface pressure. The estimated values gradually increased, as the containing ratio of RFDF16–PEG increased, though the studied RFDF16 segments in either of RFDF16 and RFDF16–PEG are monodisperse and same in molecular weight. It was suggested that PEG segments essentially influenced the behavior of the monolayer even it was dissolved into the water subphase.

The two-dimensional array of the fibrous objects was

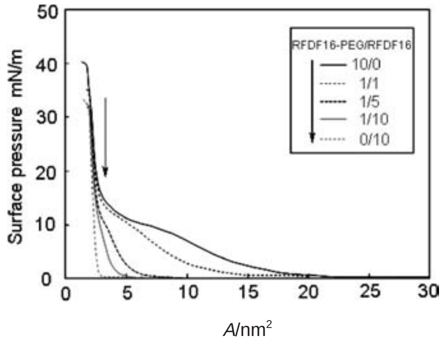


Figure 1. π–A isotherms for the mixtures of RFDF16–PEG and RFDF16 with mixing ratio from 10/0 to 0/10 at the air/water interface.

Table 1. The limiting area per molecule for the mixture of RFDF16–PEG and RFDF16

	RFDF16–PEG /RFDF16	A_{obs} (nm^2/molecule)	A_{cal} (nm^2/molecule)[a]
1	0/10	2.50	2.58
2	1/10	2.64	3.23
3	1/5	2.75	3.76
4	1/1	3.04	6.13
5	10/0	3.20	9.68

[a] As an average value for RFDF16, 2.58 nm^2 and PEG, 7.10 nm^2.

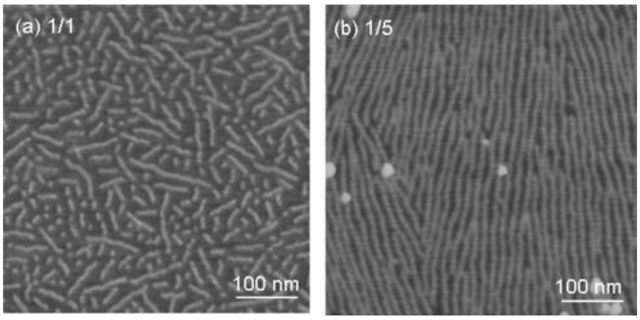


Figure 2. AFM images of the monolayer transferred on mica substrate at 4 mN/m for the mixing ratio of RFDF16–PEG/RFDF16; (a) 1/1, and (b) 1/5.

remarkably influenced by mixing ratio of RFDF16–PEG and RFDF16 at low surface pressure. The monolayer which was obtained by mixing RFDF16–PEG and RFDF16 in several feed ratio at air/water interface was transferred onto a freshly cleaved mica substrate at 4 mN/m by Langmuir–Blodgett (LB) method.[13] FT-IR spectra of the obtained LB films revealed that the peptides formed antiparallel β-sheet structure. AFM images were shown in Figure 2. When the fraction of RFDF16–PEG was large, grains and short fibers were formed (Figure 2a). Such structures were supposed to be constructed by β-sheet structure of RFDF16 as a core and PEG on the periphery of the RFDF16 aggregates. The size of the structures, due to the bulkiness of the PEG periphery, would be determined by the ratio of the RFDF16 segments and PEG segments at air/water interface. Thus, the LB film obtained by the mixture of RFDF16–PEG and RFDF16 shows the fibrous object, when the propagation of β-sheet fiber is allowed through partial relaxation from the steric hindrance of PEG segments. Fibrous objects regulated in two dimensions were observed in the mixing ratio; 1/5 (Figure 2b). The width of the fibrous objects was estimated to be 6–8 nm, which was agreed with the theoretical value of RFDF16 width assuming that if it formed β-sheet structure. It was suggested that the observed fibrous objects were attributed to propagation of the hydrogen bonding between RFDF16 segments. It was noteworthy that fibrous objects were efficiently obtained even at low surface pressure, where RFDF16–PEG fully located at air/water interface.

The construction of the two-dimensional array of the fibrous objects at low surface pressure, where PEG segments are spreading on water surface, could be achieved the readily modulation of the distance between fibers. The distance of the observed fibers in each mixing ratio of RFDF16–PEG and RFDF16 was estimated as an average value counted 100 points in the corresponding AFM images (Table 2). The distance increased as the fraction of RFDF16–PEG was increased. It was suggested that the distance of the fibers was occupied with PEG segments on the periphery of the β-sheet structure, i.e., PEG segments played a decisive role as a spacer, which controlled the distance between the fibrous objects. The detailed investigations for the control of the two-dimensional arrangement of the fibrous objects, which would be dependent on the fraction of PEG segments, are now in progress.

Table 2. Distance between the fibers in the two-dimensional arrays

	RFDF16–PEG /RFDF16	D_{obs}/nm[a]	D_{net}/nm[b]
1	0/10	8.1	2.6
2	1/10	12.0	6.5
3	1/5	14.6	9.1
4	1/1	18.9	13.4
5	10/0	19.0[c]	13.5

[a] Distance of fibers estimated as an average value counted 100 points of the distance from top to top. [b] Deduction of the width of RFDF16 segments (5.5 nm) from D_{obs}. [c] Distance between grains, since just grains were observed from the corresponding AFM image.

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13 The monolayer was prepared by spreading a solution of the peptide in benzene/2,2,2-trifluoroethanol (6:4 v/v) at a concentration of approximately 0.1 mg/mL through a micro-syringe onto the deionized water surface. After evaporation of organic solvents, the monolayer was compressed at a speed of 5 mm/min, and the surface pressure was measured by Wilhelmy plate. The monolayer was transferred onto the mica substrate in vertical method at a speed of 5 mm/min.

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