Nano-Phase Separation in the Monolayer Composed of α-Helical Copolypeptide at Air/Water Interface

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Nanometer-scale stripe pattern in the monolayer composed of α-helical copolypeptide was characterized with atomic force microscope (AFM). This monolayer was formed with the copolypeptide composed of poly(ε-benzyloxycarbonyl L-lysine)$_x$-poly[(γ-methyl L-glutamate)$_{y-z}$/(L-glutamic acid)$_z$] (PLLZ$_x$–P(MLG$_{y-z}$/LGA$_z$)). AFM image of Langmuir–Blodgett (LB) film of this copolypeptide showed a nanometer-scale stripe pattern. This pattern seems to be based on the nanophase-separated structure in the copolypeptide monolayer at air/water interface.

Two-dimensional pattern formation on substrate using functional molecules is important for making a novel molecular device such as high-density data storage device, etc. Much effort[1–6] has already been made to produce two-dimensional pattern in monolayers composed of long alkyl chains with functional terminal. Polypeptides with well-defined secondary structure are expected to be highly functional monolayers[7] whose two-dimensional pattern is systematically and effectively controllable by their molecular weight and side chain modifications, etc.

In this study, we prepared monolayer composed of an α-helical copolypeptide at air/water interface. It was confirmed from the AFM image of the LB film that the copolypeptide formed a well-defined nanometer-scale stripe pattern in the monolayer. This pattern seems to be based on phase-separated structure of the copolypeptide monolayer at air/water interface.

An α-helical copolypeptide composed of poly(ε-benzyloxycarbonyl L-lysine)$_x$-poly[(γ-methyl L-glutamate)$_{y-z}$/(L-glutamic acid)$_z$] (PLLZ$_x$–P(MLG$_{y-z}$/LGA$_z$)) (Figure 1(a)) was prepared as follows: PLLZ$_x$ block was prepared by the polymerization of the N-carboxy anhydride of ε-benzyloxycarbonyl L-lysine (LLZ-NCA) in tetrahydrofuran (THF) with *n*-hexylamine as an initiator. And then, PLLZ$_x$–PMLG$_y$ block copolypeptide was prepared using the *N*-carboxy anhydride of γ-methyl L-glutamate (MLG-NCA) in *N*,*N*-dimethylformamide (DMF) solution with the terminal amino group of PLLZ$_x$ as an initiator. The introduction of hydrophilic gultamic acid residue (PLLZ$_x$–P(MLG$_{y-z}$/LGA$_z$)) was carried out with saponification of PMLG$_y$ block in 2,2,2,-trifluoroethanol (TFE)/water (7:1 vol) which contains potassium hydroxide (KOH) 7 times of MLG residues in molar ratio. The average degree of polymerization (x, y) and saponification degree (z/y) was estimated from ^{1}H-NMR analysis of trifluoroacetic acid (TFA) solution of the polypeptide. As a result, x and y was 25 and 60, respectively, and then z/y was 0.3, that is to say, 18 in 60 MLG residues were saponificated to be LGA residues (PLLZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$)).

Circular dichroism spectrum showed that the polypeptide adopts α-helix conformation in TFE. Diameter of α-helical PLLZ and PMLG is 1.66 nm and 1.20 nm, respectively.[8,9] The

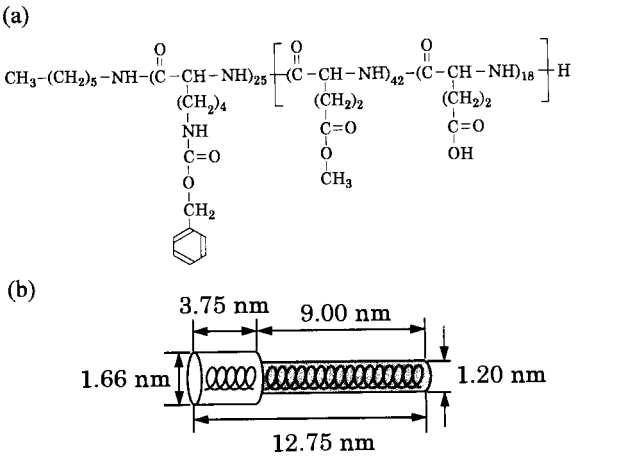

Figure 1. (a) Chemical structure of PLLZ$_{25}$-P(MLG$_{42}$/LGA$_{18}$). (b) Schematic illustration and size of PLLZ$_{25}$-P(MLG$_{42}$/LGA$_{18}$).

pitch of α-helix is 0.54 nm, so the length of PLLZ$_{25}$ and P(MLG$_{42}$/LGA$_{18}$) segments is 3.75 nm and 9.00 nm, respectively (Figure 1(b)).

The monolayer was prepared by spreading a DMF/benzene (1:20 in vol) solution on water at pH 5. The monolayer was compressed up to a surface pressure of 25 mN/m, and then, was transferred onto a freshly cleaved mica substrate by the horizontal drawing-up method at 25 mN/m to obtain the LB film with single layer.

The morphology of the LB film was observed by atomic force microscope (Nano-Scope IIIa, Digital Instruments) with a contact mode (Figure 2(a)). From the depth of the cavity that was made by scratching with a cantilever, the thickness of the LB film was estimated to be ca. 1.2 nm. This value is equivalent to the diameter of α-helix rod of PMLG segment. This means that the PLLZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$) lie on the mica substrate. And then, nanometer-scale image of the LB film was observed by AFM (Figure 2(b)). The AFM image showed the stripe pattern composed of alternate thick and thin domains whose difference in height was ca. 0.3 nm. This value is almost equivalent with the difference between the radius of α-helical PLLZ and that of PMLG, 0.23 nm. It may say, therefore, the thick domain corresponds to the molecular array of the hydrophobic PLLZ$_{25}$ segment and thin domain that of the partial hydrophilic P(MLG$_{42}$/LGA$_{18}$) segment, respectively. And the interval of the stripe was estimated to be ca. 24 nm. This value is equivalent with twice the length of PLLZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$) (12.75 nm). This suggests that the PLLZ$_{25}$–P(MLG$_{42}$/LGA$_{18}$) aggregate by head to head and tail to tail, resulting in the formation of nanophase-separated structure (Figure 3).

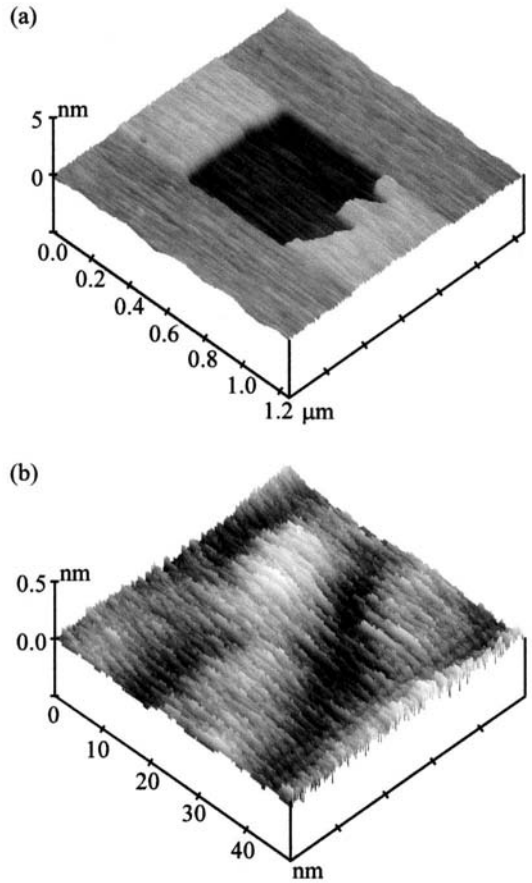

Figure 2. (a) AFM image (1.25 μm×1.25 μm) of $PLLZ_{25}$-$P(MLG_{42}/LGA_{18})$ LB film on mica substrate after scratching with a cantilever. (b) Image (48.9 nm ×48.9 nm) from same LB film.

In conclusion, a nanophase-separated structure could be observed in the $PLLZ_{25}$–$P(MLG_{42}/LGA_{18})$ LB film on the mica obtained by transferring the monolayer at air/water interface.

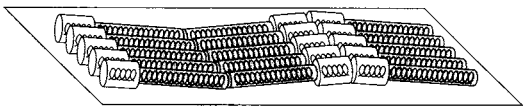

Figure 3. Schematic illustration of the nanophase separated structure of $PLLZ_{25}$-$P(MLG_{42}/LGA_{18})$ LB film on mica substrate.

The structure has well-defined stripe pattern which is based on the secondary structure of polypeptide. It is expected that this nanometer-scale pattern may be regulated by the side chain structure and the degree of polymerization of the component polypeptide segments.

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