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Molecular shape of polypeptides and the nanometer-scale pattern formation in their monolayers

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Abstract: To produce the fine and high performance molecular membrane system, diblock copolypeptide poly(ε-benzyloxycarbonyl-L-lysine)-poly(γ-methyl-L-glutamate/L-glutamic acid) ($PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$) and triblock copolypeptide poly(L-leucine)-poly(L-glutamic acid)-poly(L-leucine) ($PLLeu_{54}$-$PLGA_{80}$–$PLLeu_{54}$) were prepared. The morphology of the Langmuir-Blodgett (LB) films composed of these polypeptides was characterized with atomic force microscope (AFM). The AFM image of $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ LB film showed the nanometer-scale stripe pattern. The branching pattern was seen as the disorder of the stripe. This structure seems to be based on the large difference between the diameter of α-helical $PLLysZ_{25}$ segment and that of $P(MLG_{42}/LGA_{18})$. The AFM image of $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ LB film showed more sophisticated stripe pattern, having no branching. And this nanometer-scale structure was controlled by pH environment. These patterns composed of di- or triblock copolypeptide seem to be based on the nanophase-separated structure constructed in the monolayer at air/water interface.

Key words: amphiphilic block copolypeptide, monolayer at air/water interface, phase separation, nanometer-scale, pattern formation

1. Introduction

Nanoscale two-dimensional patterning on substrate using functional molecules is important for making a novel molecular device such as high-density data storage, etc. Much effort[1-11] has already been made to produce two-dimensional pattern in monolayers of numerous compounds with long alkyl chain(s) and several copolymers with statistical main chain structure. Polypeptides with well-defined secondary structure are expected to be highly functional monolayers, in addition, its two-dimensional regular pattern in the monolayer may be controllable by their molecular weight and side chain modifications, etc. Several studies[12-14] concerning with the self-assembled monolayers (SAMs) on substrate using polypeptide derivatives have been reported. However, the two-dimensional ordering of these rod-like polymers on substrate has not been observed. It was shown, on the other hand, that the adsorption of proteins onto SAM templates could yield the micrometer scale ordered layers on substrate.[15, 16]

In this study, we prepared monolayer composed of di- or triblock copolypeptide at air/water interface. It was confirmed from the AFM image of the LB film that the copolypeptide formed a nanometer-scale stripe pattern in the monolayer. These patterns seem to be based on phase-separated structure of the copolypeptide monolayers at air/water interface.

2. Experimental Section

2.1 Preparation of Block Copolypeptide

2.1.1 Diblock Copolypeptide

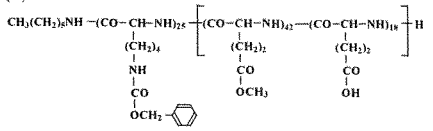
A diblock copolypeptide composed of poly(ε-benzyloxycarbonyl L-lysine)$_x$-poly[(γ-methyl L-glutamate)$_{y-z}$/(L-glutamic acid)$_z$] ($PLLysZ_x$-$P(MLG_{y-z}/LGA_z)$) (Fig. 1(a)) was prepared as follows: $PLLysZ_x$ block was prepared by the polymerization of the N-carboxy anhydride of ε-benzyloxycarbonyl L-lysine (LLysZ-NCA) in tetrahydrofuran with n-hexylamine as an initiator. And then, $PLLysZ_x$-$PMLG_y$ diblock 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 $PLLysZ_x$ as an initiator. The introduction of hydrophilic gultamic acid residue ($PLLysZ_x$-$P(MLG_{y-z}/LGA_z)$) was carried out with saponification of $PMLG_y$ block in water/2,2,2-trifluoroethanol (1:7 in 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 residues in 60 MLG residues were saponificated to be LGA residues

($PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$).

2.1.2 Triblock Copolypeptide

A triblock copolypeptide composed of poly(L-leucine)$_m$-poly(L-glutamic acid)$_n$-poly(L-leucine)$_m$ ($PLLeu_n$-$PLGA_m$-$PLLeu_n$) (Fig. 1(b)) was prepared as follows: poly(γ-benzyl L-glutamate) ($PBLG_m$) block was prepared by the polymerization of the N-carboxy anhydride of γ-benzyl L-glutamate (BLG-NCA) in dioxane/benzene (1:19 in vol.) with hexamethylenediamine as an initiator. And then, $PLLeu_n$-$PBLG_m$-$PLLeu_n$ triblock copolypeptide was prepared using the N-carboxy anhydride of L-leucine (LLeu-NCA) in DMF solution with the terminal amino groups of $PBLG_m$ as an initiator. The saponification of $PBLG_m$ block ($PLLeu_n$-$PLGA_m$-$PLLeu_n$) was carried out with saponification of $PBLG_m$ block in water/methanol/2-propanol (1:2:2 in vol.) which contains equimolar KOH to BLG residues. The ^{1}H-NMR spectra of the polypeptide in TFA solution showed that the average degree of polymerization m and n was 80 and 54, respectively, and then all BLG residues were saponificated to be LGA residues ($PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$).

(a)



(b)

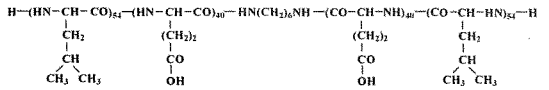


Fig. 1 Chemical structures of $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ (a) and $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ (b).

2.2 The Molecular Size of the Polypeptide

Diameter of α-helical PLLysZ and PMLG was estimated to be 1.66 nm and 1.20 nm, respectively, based on the X-ray data in the literature.[17, 18] The pitch of α-helix is 0.54 nm, so the length of $PLLysZ_{25}$ and $P(MLG_{42}/LGA_{18})$ segments is 3.75 nm and 9.00 nm, respectively (Fig. 2(a)).

And for $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$, diameter of α-helical PLLeu and PLGA was estimated to be 1.3 nm and 1.1 nm by wide-angle X-ray diffraction measurements of their cast films, respectively. The length of $PLLeu_{54}$ and $PLGA_{80}$ segments is 8.10 nm and 12.0 nm, respectively (Fig. 2(b)).

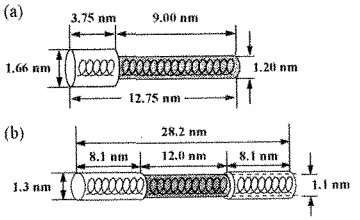


Fig. 2 Schematic illustrations and sizes of $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ (a) and $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ (b).

2.3 Preparation of the LB film

Langmuir-Blodgett (LB) films were prepared with an automatic Langmuir trough (NLE-BIO40-MWCT) (Nippon Laser & Electronics Lab.) interfaced with a NEC PC-9821 personal computer. Surface pressure was monitored by a Wilhelmy-type film balance.

For preparation of $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ LB film, a DMF/benzene (1:20 in vol.) solution of the polymer was spread onto aqueous solution at pH=5. Then, the monolayer was compressed up to a surface pressure of 25 mN/m at a rate of 5 mm/min. For AFM observation, single layer of $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ was transferred onto freshly cleaved mica substrate by the horizontal drawing-up method, and for FT-IR/RAS measurement, single layer was transferred onto gold substrate in the similar manner.

For preparation of $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ LB film, a TFA/chloroform (1:20 in vol.) solution of the polymer was spread onto aqueous solution at pH=4 and 12, respectively. Then, the monolayer was compressed up to a surface pressure of 17 mN/m at a rate of 5 mm/min. The transferring of monolayer to substrate was performed in the similar manner as above.

2.4 AFM observation

Atomic force microscope (AFM) observation was carried out on Nano-Scope Ⅲa (Digital Instruments). A silicon nitride cantilever with a spring constant of 0.06 N/m was used to acquire images in contact mode. All images were recorded in air at room temperature.

3. Results and Discussions

3.1 Monolayer Characteristics of Polypeptides at Air/Water Interface.

Fig. 3 shows the surface pressure-area (π-A) isotherm for monolayer of $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ at the air/water interface. Extrapolations of the steep increase part of the isotherm to π=0 gave the limiting area of $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ molecule, $A_{pH=5}$. The value of $A_{pH=5}$ was shown in Table I together with the calculated value of the area per molecule when it oriented parallel and normal to the monolayer, respectively ($A_\perp$, $A_{//}$). The value of $A_{pH=5}$ was between $A_\perp$ and $A_{//}$. This indicates that $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ is not perfectly parallel or normal to the air/water interface.

Fig. 4 shows the π-A isotherms for monolayer of $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ at the air/water interface. From the isotherms, limiting area $A_{pH=4}$ and $A_{pH=12}$ was obtained, respectively. The values of $A_{pH=4}$ and $A_{pH=12}$ were shown in Table I together with the calculated value. The area/molecule of $PLGA_{80}$ segment with random coil conformation was estimated to be 7.83 nm^2/molecule based on the freely-jointed chain model. The values of $A_{pH=4}$ and $A_{pH=12}$ were far from that of $A_\perp$. This indicates that $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ was nearly parallel to the air/water interface at subphase pH = 4 and 12.

By the way, $A_{pH=12}$ was smaller than $A_{pH=4}$. This may be based on that the $PLGA_{80}$ block segment with random coil conformation sank into the subphase.

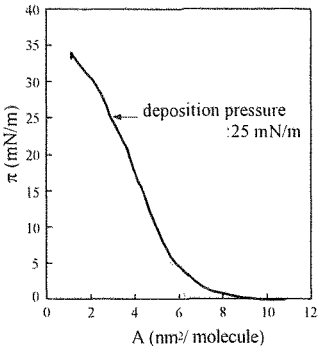


Fig. 3 π-A isotherm for monolayer of $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ with 0.1 mol/l KCl in aqueous solution at subphase pH=5.

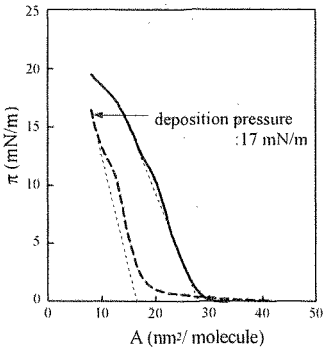


Fig. 4 π-A isotherms for monolayer of $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ with 0.1 mol/l KCl in aqueous solution at subphase pH=4 (▬▬) and 12 (▪▪▪▪▪▪).

Table I. Limiting area estimated from π-A isotherms.

		observed (nm²/molecule)	calculated (nm²/molecule)	
			$A_{//}$	$A_{\perp}$
$PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$	$A_{pH=5}$	6.3	16.99	2.39
$PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$	$A_{pH=4}$	28.0	34.26	1.51
	$A_{pH=12}$	15.5	28.88	7.83

3.2 Morphology of the LB Film

3.2.1 α-Helical Diblock Copolypeptide

The secondary structure of the $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ LB film on gold substrate transferred from aqueous surface whose subphase pH=5 was estimated by FT-IR reflection absorption spectroscopy (FT-IR/RAS) (data is not shown). The peaks based on amide I and II absorptions were observed at 1670 and 1551 cm^{-1}, respectively. It is indicated that the $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ adopts α-helix conformation in the LB film. The morphology of the $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ LB film was observed by AFM (Fig. 5).[19] 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 PLLysZ and that of PMLG, 0.23 nm. It may say, therefore, the thick domain corresponds to the molecular array of the hydrophobic $PLLysZ_{25}$ 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 $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ (12.75 nm). This suggests that the $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ aggregate by head to head and tail to tail, resulting in the formation of nanophase-separated structure. And the branching pattern was also observed. This structure seems to be based on the large difference between the diameter of α-helical $PLLysZ_{25}$ segment and that of $P(MLG_{42}/LGA_{18})$.

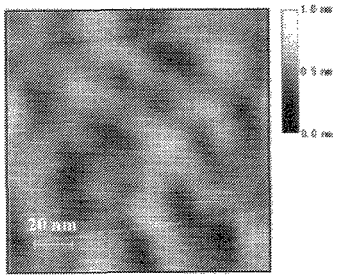


Fig. 5 AFM image (140 nm × 140 nm) of $PLLysZ_{25}$-$P(MLG_{42}/LGA_{18})$ LB film on mica substrate transferred from monolayer on aqueous solution at subphase pH=5.

3.2.2 α-Helical Triblock Copolypeptide

α-helix structure of $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ LB film on gold substrate was also confirmed by FT-IR/RAS measurements (amide I; 1660 cm^{-1}, amide II; 1549 cm^{-1}). The morphology of the LB film transferred from $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ monolayer on aqueous solution at subphase pH = 4 was observed by AFM (Fig. 6). 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 PLL and that of PLGA, 0.1 nm. It may say, therefore, the thick domain corresponds to the molecular array of the hydrophobic $PLLeu_{54}$ segment and thin domain that of the hydrophilic $PLGA_{80}$ segment, respectively. And the interval of the stripe was estimated to be ca. 29 nm. This value is equivalent with the length of $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ (28.2 nm). The triblock copolypeptide LB film constructed more sophisticated stripe, that is, few branching patterns were seen.

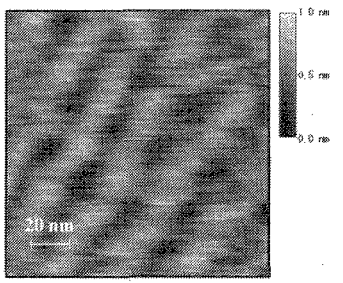


Fig. 6 AFM image (140 nm × 140 nm) of $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$) LB film on mica substrate transferred from monolayer on aqueous solution at subphase pH=4.

3.2.3 Helix-Loop-Helix Copolypeptide

The secondary structure of the $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ LB film transferred from aqueous surface whose subphase pH=12 was estimated by FT-IR/RAS (data is not shown). The shoulder band based on random coil conformation was observed at 1538 cm^{-1} in addition to the peaks based on α-helix conformation (1660 and 1549 cm^{-1}). It seems to be caused by that the $PLGA_{80}$ segment adopts random coil conformation because of the ionization of carboxyl group. α-helix structure of PLLeu segments is known to be independent of pH, therefore, it is indicated that the $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ forms "helix-loop-helix" conformation in the LB film. The morphology of the LB film transferred from $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$ monolayer on aqueous solution at subphase pH = 12 was observed by AFM (Fig. 7). Comparing with Fig. 6, the interval of the stripe was short (ca. 23 nm) and difference in height of thick and thin domains was large (ca. 0.9 nm). It may say, therefore, the thick domain corresponds to the molecular array of the hydrophilic loop segment and thin domain that of the hydrophobic helix segment, respectively. And there were lots of branching patterns. This structure seems to be based on the large difference between the diameter of helix segment and that of loop segment. It was suggested that stripe pattern was able to be controlled by pH stimulus.

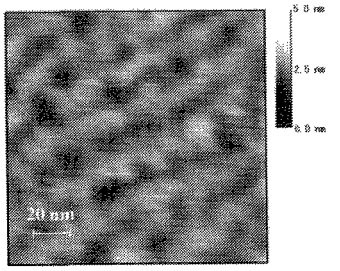


Fig. 7 AFM image (140 nm × 140 nm) of $PLLeu_{54}$-$PLGA_{80}$-$PLLeu_{54}$) LB film on mica substrate transferred from monolayer on aqueous solution at subphase pH=12.

4. Conclusion

In conclusion, to produce the fine and high performance molecular membrane system, the monolayer composed of di- or triblock copolypeptides were formed at air/water interface and transferred on the mica substrate. The AFM images of these copolypeptide LB films showed well-defined nanoscale stripe patterns. These patterns seem to be based on the nanophase-separated structure in the copolypeptide monolayer at air/water interface. It is expected that this nanoscale pattern may be systematically and effectively controllable by the side chain structure, chemical property and the size of the helix or loop segments. Such nanophase-separeted template will be applied to electrical, optical, medical fields and so on, as a novel functional interface.

To produce finer and higher performance molecular membrane system, we are trying pattern formation by using the monodisperse polypeptide which was made by the recombinant DNA method.[20]

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References

[1] G. P. Lopez, H. A. Biebuyck and G. M. Whitesides, *Langmuir*, **9**, 1513 (1993).
[2] S. J. Stranick, A. N. Parikh, Y. -T. Tao, D. L. Allara and P. S. Weiss, *J. Phys. Chem.*, **98**, 7636 (1994).
[3] K. Tamada, M. Hara, H. Sasabe and W. Knoll, *Langmuir*, **13**, 1558 (1997).
[4] Y. Oishi, Y. Torii, T. Kato, M. Kuramori, K. Suehiro, K. Ariga, K. Taguchi, A. Kamino, H. Koyano and T. Kunitake, *Langmuir*, **13**, 519 (1997).
[5] W. Mizutani, T. Ishida, S. -I. Yamamoto, H. Tokumoto, H. Hokari, H. Azehara and M.Fujihira, *Appl. Phys. A*, **66**, S1257 (1998).
[6] P. Qian, H. Nanjo, T. Yokoyama and T. M. Suzuki, *Chem. Lett.*, 1229 (1999).
[7] K. Nishiyama, T. T. Kie and I.Taniguchi, *Chem. Lett.*, 753 (1999).
[8] Y. Liu, W. Zhao, X. Zheng, A. King, A. Singh, M. H. Rafailovich, J. Sokolov, K. H. Dai, E. J. Kramer, S. A. Schwarz, O. Gebizlioglu and S. K. Sinha, *Macromolecules*, **27**, 4000 (1994).
[9] T. L. Morkved, M. Lu, A. M. Urbas, E. E. Ehrichs, H. M. Jaeger, P. Mansky and T. P. Russell, *Science*, **273**, 931 (1996).
[10] I. I. Potemkin, E. Y. Kramarenko, A. R. Khokhlov, R. G. Winkler, P. Reineker, P. Eibeck, J. P. Spatz and M. Möller, *Langmuir*, **15**, 7290 (1999).
[11] S. M. Baker, K. A. Leach, C. E. Devereaux, D. E. Gragson, *Macromolecules*, **33**, 5432 (2000).
[12] K. Fujita, N. Bunjes, K. Nakajima, M. Hara, H. Sasabe and W. Knoll, *Langmuir*, **14**, 6167 (1998).
[13] M. Niwa, M. Morikawa and N. Higashi, *Langmuir*, 15, 5088 (1999).
[14] S. Kimura, Y. Miura, T. Morita and Y. Imanishi, *J. Polym. Sci.*, **38**, 4826 (2000).
[15] G. P. Lopez, H. A. Biebuyck, R. Härter, A. Kumar and G. M. Whitesides, *J. Am. Chem. Soc.*, **115**, 10774 (1993).
[16] R. G. Chapman, E. Ostuni, M. N. Liang, G. Meluleni, K. E. Gloria, L. Yan, G. Pier, H. S. Warren and G. M. Whitesides, *Langmuir*, **17**, 1225 (2001).
[17] R. L. Shuler and W. A. Zisman, *Macromolecules*, **5**, 487 (1972).
[18] H. L. Yakel, Jr., *Acta Cryst.*, **6**, 724 (1953).
[19] H. Yokoi, T. Kinoshita, Y. Tsujita and H. Yoshimizu, *Chem. Lett.*, 1210 (2000).
[20] H. Yokoi, T. Kinoshita and D. A. Tirrell, *Polym. Prepr., Jpn.*, **50**, 628 (2001).

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