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Fabrication of vertically and unidirectionally oriented polypeptide assemblies on self-assembled monolayers by stepwise polymerization

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A polypeptide assembly prepared by stepwise polymerization on a self-assembled monolayer consisting of amino-alkanethiol and dialkyl disulfide oriented vertically and unidirectionally to the surface.

Vertically oriented α-helical polypeptide assemblies in the biological membrane are closely related to the vectorial signal transfer through the membrane. For example, in a photosynthesis system, the specific location of a special pair chlorophylls, two pherophytins, and two quinines bound to the vertically oriented α-helical polypeptide bundle yields the photo-induced vectorial electron transfer through the membrane.[1] Further, the vertically oriented α-helical polypeptide assemblies whose molecular dipole moment aligns unidirectionally provide optical switches based on second-order nonlinear effects.[2] Studies on vertically and unidirectionally oriented polypeptide assembly systems may be important not only to the understanding of a simple and/or essential mechanism for the signal transduction through biological membrane but also may provide the basis for a molecular device capable of transferring information. Recently, the preparation of vertically oriented α-helical polypeptide assemblies such as Langmuir–Blodgett (L–B) films,[3,6] self-assembled monolayers (SAMs),[4] and grafted polypeptide layers prepared by the polymerization of *N*-carboxyanhydride of amino acids (NCA) on the initiator immobilized substrate surfaces[5] has been reported. The monolayers and L–B films are practically insufficient because of the lack of physical stability due to the fact that the individual peptide chain remains unfixed. For SAMs, the antiparallel α-helix packing is significantly preferable to a parallel one because of the dipole–dipole interaction between the α-helices. On the other hand, in the grafted polypeptide layers on the substrate, the individual α-helical rod has unidirectionally alignment.

In this paper, we report a novel approach for the preparation of vertically and unidirectionally oriented α-helical polypeptide assembly on a substrate. The method used in this study involves the stepwise polymerization of amino acids on a mixed SAM composed of amino-alkanethiol and dialkyl disulfide.

A mixed SAM consisting of 11-amino-1-undecanthiol (C11N) and *n*-butyl disulfide (C4) on a gold-deposited glass substrate was prepared by immersing the substrate in a 0.1 mM ethanol solution containing C11N and C4 for 24 h, then the substrate was rinsed with ethanol several times. The molar ratio of C11N and C4 was 1 : 4.5. In this condition, the lateral density of an amino group was calculated to be *ca.* 2.0 nm^2 molecule^{-1}, assuming the ideal mixing of C11N and C4. This value is larger than the cross sectional area of α-helical poly(L-Leu), 1.5 nm^2 molecule^{-1},[6] when they orient perpendicularly to the monolayer. A SAM consisting of only C11N was prepared in a manner similar to that above. The substrates were mounted in the flow cell of a surface plasmon resonance (SPR) apparatus equipped with a high refraction index prism (n = 1.923). Dimethylformamide (DMF) was passed over the surfaces of the SAMs at 10 μL min^{-1} at 28 °C. The DMF flow over the surfaces of the SAMs were replaced with DMF solution of activated ester for Fmoc-L-Leu[7] (1 mM) to attach the Fmoc-L-Leu to the amino group on the surfaces. This coupling reaction between activated Fmoc-L-Leu and the amino group on the SAM surface was monitored by the resonance angle changes, $\Delta\theta$, of SPR obtained after periodically replacing the pure DMF solution. It is well known that $\Delta\theta$ is proportional to the thickness and refraction index of the layer formed on a gold surface.[8] The coupling reaction was run until no more changes of $\Delta\theta$ were detected. The introduction of activated Fmoc-L-Leu into the SAMs surfaces induced the increase of $\Delta\theta$ to the equilibrium value within *ca.* 150 min. This finding implies that the coupling reaction between activated Fmoc-L-Leu and the amino group on the SAM surface reaches completion within 150 min. After the reaction, the flow over the surface was replaced with pure DMF to rinse the surface. DMF solution containing 20 vol% piperidine was passed over the surfaces to remove the amino terminal Fmoc-protecting group. The piperidine solution flow was replaced with pure DMF to rinse the surface for 12 h. This reaction cycle was repeated successively to obtain the poly(L-Leu) layer on the SAMs. Fig. 1 shows the $\Delta\theta$ changes of the C11N/C4 mixed SAM and the C11N SAM by the stepwise polymerization of L-Leu residues on the surface, respectively. On the C11N/C4 mixed SAM surface, the value of $\Delta\theta$ increased in proportion to the number of coupling reaction cycles; that is to say, the successive addition of L-Leu residue to the mixed SAM surface proceeded quantitatively. On the other hand, the changes of $\Delta\theta$ on the C11N SAM were very small, and the value of $\Delta\theta$ remained approximately constant after the second reaction. This result implies that sufficient intermolecular spacing among amino groups on the surface is required for the peptide chain-growing reaction. Whitesell *et al.*[5] used a bulky aminotrithiol as an

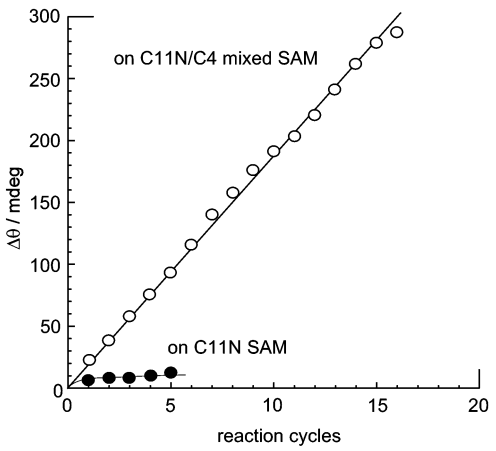

Fig. 1 Relationship between shift of resonance angle of SPR and number of peptide synthesis cycle on C11N/C4 mixed SAM and C11N SAM.

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immobilized initiator on the surface for preparation of grafted polypeptide layers by NCA polymerization. In our method, suitable amino group spacing on the surface for the peptide chain-growing reaction can be easily secured by formation of the mixed SAM consisting of amino-alkanethiol and dialkyl disulfide.

We obtained a layer of L-Leu 16mer (poly(L-Leu)$_{16}$) on the surface of the mixed SAM composed of C11N and C4 by stepwise polymerization. The thickness of the poly(L-Leu)$_{16}$ layer on the mixed SAM was determined by the multi-solvent SPR method.[9] By this method, the thickness of the poly(L-Leu)$_{16}$ layer was 2.22 nm.

The structure and orientation of the poly(L-Leu)$_{16}$ layer was investigated by FTIR-RAS measurements. Fig. 2 shows the FTIR-RAS of the poly(L-Leu)$_{16}$ layer on the C11N/C4 mixed SAM. The spectrum of the polypeptide layer showed typical amide I ($\nu_{C=O}$) and amide II (δ_{N-H}) absorption near 1654 cm^{-1} and 1545 cm^{-1}, respectively, indicating a α-helical conformation.[10] The helicity of poly(L-Leu)$_{16}$ was estimated to be 77 % from the ratio of peak intensity at 1654 cm^{-1} assigned to a α-helical conformation to that of β-sheet (1635 cm^{-1}) and random (1679 cm^{-1}) conformation,[10] which were obtained by peak deconvolution of the amide I band. The tilt angle of the α-helical axis of the poly(L-Leu)$_{16}$ from the surface normal was estimated to be 25.3° from the ratio of the individual intensities of amide I to amide II absorption bands, $D = A_I/A_{II}$.[11] The molecular length of poly(L-Leu)$_{16}$ can be evaluated to be 2.4 nm by assuming that the polypeptide takes α-helical structure with a helical pitch of 0.15 nm per amino acid residue. Considering these values, the layer thickness of poly(L-Leu)$_{16}$ was calculated to be 2.17 nm. This value was similar to that obtained by SPR measurement. In Fig. 3, the structural model of the polypeptide layer prepared by stepwise polymerization on the mixed SAM surface is proposed.

In conclusion, the vertically and unidirectionally oriented polypeptide assembly can be easily obtained by the stepwise polymerization of amino acids using a conventional solid-phase peptide synthesis method on a mixed SAM surface consisting of amino-alkanethiol and dialkyl disulfide. This method has the advantage of permitting simple preparation of oriented polypeptide assemblies consisting of sequential polypeptide having functional groups such as electron donors and acceptors at the specific positions. This system may be useful for signal transduction devices. Further studies involving the vectorial electron transfer through these polypeptide assemblies are underway.

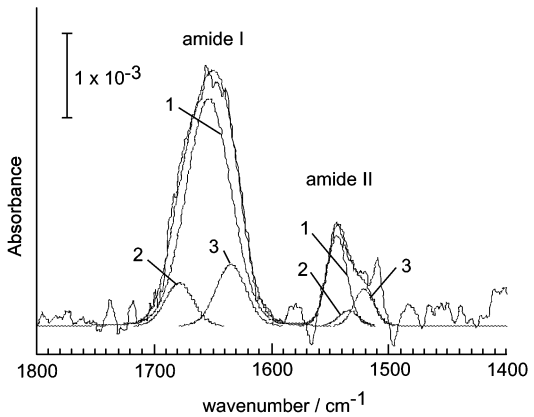

Fig. 2 FTIR-RAS of poly(L-Leu)$_{16}$ on C11N/C4 mixed SAM, and peak deconvolution of amide I and amide II band to 1: α-helical (1654 cm^{-1} and 1545 cm^{-1}), 2: random coil (1679 cm^{-1} and 1535 cm^{-1}), and 3: β-sheet (1635 cm^{-1} and 1522 cm^{-1}) conformation.

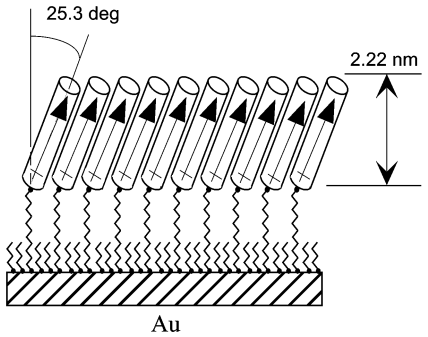

Fig. 3 Structural model of poly(L-Leu)$_{16}$ layer on C11N/C4 mixed SAM.

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7 Activation of Fmoc-L-Leu was carried out as follows. Fmoc-L-Leu was dissolved in DMF (1 mM) with benzotriazole-1-yloxytris(dimethylamino)phophonium hexafluorophosphate (BOP), *N*-methylmorpholine (NMM), and *N*-hydroxybenzotriazole (HOBt). The molar ratio of Fmoc-L-Leu, BOP, NMM, and HOBt was 1 : 1 : 1.5 : 1. This solution was stirred for 5 min at room temperature.

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$$D = A_I / A_{II} = K \frac{0.5(\sin<\theta>\sin 39)^2 + (\cos<\theta>\sin 39)^2}{0.5(\sin<\theta>\sin 75)^2 + (\cos<\theta>\sin 75)^2} \quad (1)$$

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