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Preparation of Vertically and Unidirectionally Oriented α-Helical Sequential Polypeptide Assemblies on Self-Assembled Monolayers

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A novel approach to the preparation of vertically and unidirectionally oriented α-helical polypeptide assemblies on a substrate was investigated. It involved the stepwise addition of amino acids to a growing polypeptide chain, which was bound by a mixed self-assembled monolayer (SAM) composed of amino-alkanethiol and dialkyl disulfide on a gold-deposited glass plate. The each reaction step on the SAM surface was monitored by resonance angle changes of the surface plasmon for the plate. The thickness, the conformation, and the orientation of the polypeptide layer were investigated by the surface plasmon resonance and the FTIR reflection-absorption spectroscopy, respectively. The α-helical polypeptide bound on the SAM surface oriented vertically and unidirectionally to the substrate. The advantage of our method was simplicity preparation for such oriented molecular assembled layer consisted of sequential polypeptide.

Key words: sequential polypeptide assembly, vertically and unidirectionally orientation, stepwise peptide synthesis, self-assembled monolayer, surface plasmon resonance

1. INTRODUCTION

Vertically oriented α-helical polypeptide assemblies in the biological membrane are closely related to the vectorial signal transfer through the membrane. For example, a photosynthesis system contains two reaction center chlorophylls, and two another chlorophylls, two pherophytins, two quinines, and one nonhem iron atom. These molecules are bound to the vertically oriented α-helical polypeptide bundle. The specific location of these molecules in the bundle yields the photo-induced vectorial electron transfer through the membrane.[1,2] Furthermore, the vertically oriented α-helical polypeptide assemblies whose molecular dipole moment aligns unidirectionally provide optical switches based on second-order nonlinear effects.[3] 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 reception and transduction through biological membrane but also may provide the basis for a molecular device capable of receiving and transferring of information. Recently, the preparation of vertically oriented α-helical polypeptide assemblies such as monolayers[4-6], Langmuir-Blodgett (L-B) films[7,8], SAMs[9-11], and grafted polypeptide layers prepared by the polymerization of *N*-carboxyanhydride of amino acids (NCA) on the initiator immobilized substrate surfaces[12-15] has been reported. However, the monolayers and L-B films are insufficient and lack adequate practical physical stability due to fact that the individual peptide chain does not remain fixed. For SAMs, the antiparallel α-helix packing is significantly preferable to a parallel one because of the dipole interaction between the α-helical polypeptides in the absorbed solution. On the other hand, in the grafted polypeptide layers on the substrate, the individual α-helical rod has unidirectional alignment, through the sequential polypeptide whose functional groups locate specifically in the rod cannot be obtained by NCA polymerization on the substrate.

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, to which the conventional solid-phase peptide synthesis method is applied.

This method has the advantage that it permits easily preparation for such oriented molecular assemblies consisted of sequential polypeptide having functional groups at the designed positions, compared with NCA polymerization on the surface.

2. EXPERIMENTAL

2.1 Substrate Preparation

Gold-deposited glass plate (refraction index: n = 1.923, Nippon Laser & Electronics Lab) was used for substrate. A mixed SAM consisting of 11-amino-1-undecanthiol (C11N) and n-butyl disulfide (C4) on the gold surface 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 fixed to 1:4.5. A SAM consisting of only C11N was prepared in a manner similar to that above.

2.2 Stepwise Polymerization of Amino Acids on SAM Surface

The substrates were mounted in the flow cell of a surface plasmon resonance apparatus (SPR, Nippon Laser & Electronics Lab, SPR670B) equipped with a high refraction index prism (n=1.923). Dimethylformamide (DMF) was passed over the surfaces of the SAMs at 10 μL / min at 28°C. Activation of Fmoc-L-Leu was carried out as follows. Fmoc-L-Leu was dissolved in DMF (1 mM) with benzotriazole-1-yloxytris-(dimethylamino)-phophonim hexafluorophophate (BOP), *N*-methylmorpholine (NMM), and *N*-hydroxy-benzotriazole (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. The DMF flow over the surfaces of the SAMs was periodically replaced with DMF solution of activated Fmoc-L-Leu to attach the Fmoc-L-Leu to the amino group on the surfaces. This coupling reaction between activated Fomc-L-Leu and the amino group on the SAM surface was monitored by the resonance angle changes, Δθ, of SPR. It is well known that the Δθ is proportional to the thickness and refraction index of the layer formed on a gold surface.[16] The coupling reaction was run until no more changes of Δθ were detected. 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 surface to remove the amino terminal Fmoc-protecting group. The piperidine solution flow was replaced with pure DMF to rinse the surface for 12 h, after which replacement the Δθ did not change. This reaction cycle was repeated successively to obtain the poly(L-Leu) layer on the SAM.

2.3 FTIR-RAS Measurements

Fourier transfer reflection-absorption spectra (FTIR-RAS) of polypeptide layer on the gold surface were measured with a Perkin Elmer, Spectrum 2000 equipped with a PIKE, 80Spec reflectance accessory. Incident angle was set at 80°. The 1800 cm^{-1} – 1400 cm^{-1} regions of spectra were analyzed as a sum of Gaussian / Lorentzian (9:1) composition of individual bands. Molecular orientation of the polypeptide layer on the SAM surface was assessed according to the ratio method using FTIR-RAS.[17] By comparison of the theoretical values of the ratio of amide I and amide II absorbencies with the experimental values of FTIR-RAS, the tilt angle, γ, of the α-helix axis from the surface normal was determined from eq. 1,

$$D_{obs} = A_I / A_{II} = C\frac{2[0.5(3\cos^2\gamma - 1)][0.5(3\cos^2\theta_I - 1)] + 1}{2[0.5(3\cos^2\gamma - 1)][0.5(3\cos^2\theta_{II} - 1)] + 1} \quad (1)$$

where, D_{obs}, θ_I, θ_{II}, and C represent the observed ratio of amide I (A_I) and amide II (A_{II}) absorbencies, the angle between the transition moment (amide I and amide II) and the helix axis, and the scaling constant, respectively. The angle of the transition moment from the helix axis was 39° for θ_I and 75° for θ_{II}, respectively.[18] The scaling factor, C = 1.49, was determined from the ratio of amide I and amide II absorbencies for a poly(L-Leu) in a KBr pellet taking as a random orientation.

2.4 SPR Measurements

The layer thicknesses, d, of organic thin films on the gold surface were determined by multi-solvent SPR method.[19] The thickness of the layers were calculated from the resonance angle changes in DMF and iso-propanol (iso-ProOH) using eq. 2,

$$d = \frac{\lambda(\sin\theta_s - \sin\theta_r)}{2n_p\pi\sin^2\theta_r}\frac{-\varepsilon_m + n_s^2}{\sqrt{-\varepsilon_m n_s^2}} \Big/ \sqrt{1 - n_p^2\sin^2\theta_r\left(\frac{1}{n^2} + \frac{n^2}{\varepsilon_m n_s^2}\right)} \quad (2)$$

where, n_p, n_s, n, and ε_m represent the refraction index of prism, flow solution, organic layer on gold surface, and dielectric constant of gold, respectively, and θ_s and θ_r represent the resonance angle of SPR for the organic layer on gold surface and pure gold surface, respectively, and λ represent a wavelength of incident laser beam.

3. RESULTS AND DISCUSSION

3.1 Stepwise Polypeptide Synthesis on SAM surface

Figure 1 shows the shifts in the resonance angle of the SPR, Δθ, for the C11N/C4 mixed SAM and the C11N SAM on the gold-deposited glass plate, respectively, during first peptide attachment reaction cycle. The introduction of activated Fmoc-L-Leu into the SAMs surfaces induced the increase of Δθ 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. Furthermore, the equilibrium value of

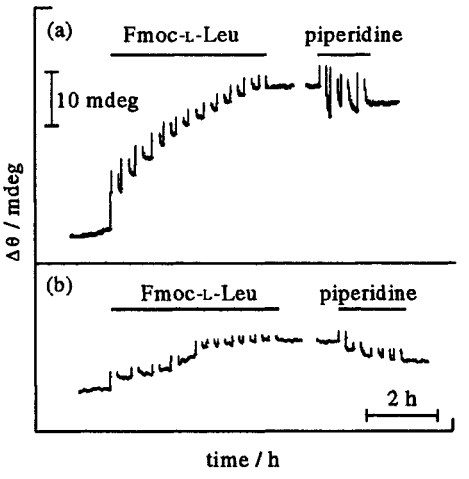

Fig. 1. Shift of resonance angle of SPR for (a) C11N/C4 mixed SAM and (b) C11N SAM during one peptide attachment reaction cycle, respectively.

$\Delta\theta$ after one reaction cycle on the C11N SAM was smaller than that on the C11N/C4 SAM.

Figure 2 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.

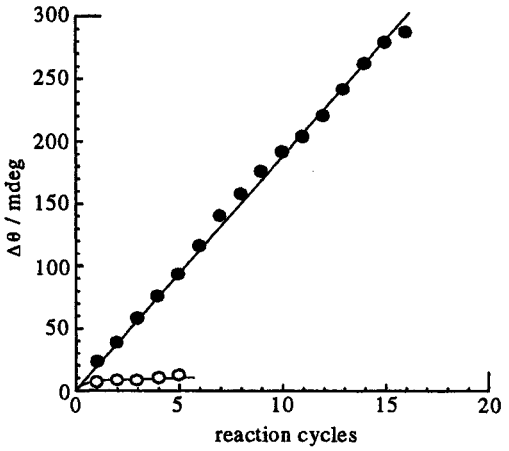

Fig. 2. Relationship between shift of resonance angle of SPR and number of peptide synthesis cycles on (●) C11N/C4 mixed SAM and (○) C11N SAM.

These results imply that sufficient intermolecular spacing among amino groups on the surface is required for the peptide chain-growing reaction. Whitesell et al.[12,13] used a bulky aminotrithiol as an immobilized initiator on the surface for preparation of grafted polypeptide layers by NCA polymerization. In our method, the 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.

3.2 Structure of the Polypeptide Layer on the SAM Surface

We obtained the 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 structure of poly(L-Leu)$_{16}$ layer was investigated by FTIR-RAS and SPR measurements. Figure 3 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. The peak deconvolution indicated that the poly(L-Leu)$_{16}$ on the SAM surface was in mainly α-helix conformation[20] more than 77% in the assembly.

The orientation of the poly(L-Leu)$_{16}$ layer on the C11N/C4 mixed SAM was determined by the FTIR-RAS, in which the transition moment oriented vertically to the surface shows intensive adsorption. In the case of α-helical polypeptide, the transition moment

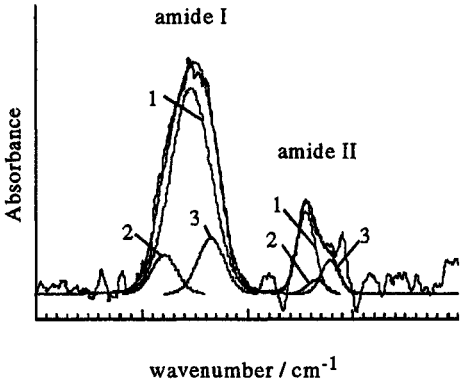

Fig. 3. FTIR-RAS of poly(L-Leu)$_{16}$ on C11N/C4 mixed SAM, and peak deconvolution of amide I and amide II band to 1: α-helical, 2: random coil and 3: β-sheet conformation.

of amide I absorption orients nearly parallel to the helix axis whereas that of amide II absorption perpendicular to the axis. The ratio of the individual intensities of amide I to amide II band of α-helix, therefore, reflects orientation of the α-helical axis on the substrate. The tilt angle of the α-helical axis from the surface normal was estimated to be 25.3° from the eq. 1. Thus, poly(L-Leu)$_{16}$ prepared by stepwise polymerization exists as α-helical structure and nearly vertical orientation on the mixed SAM surface.

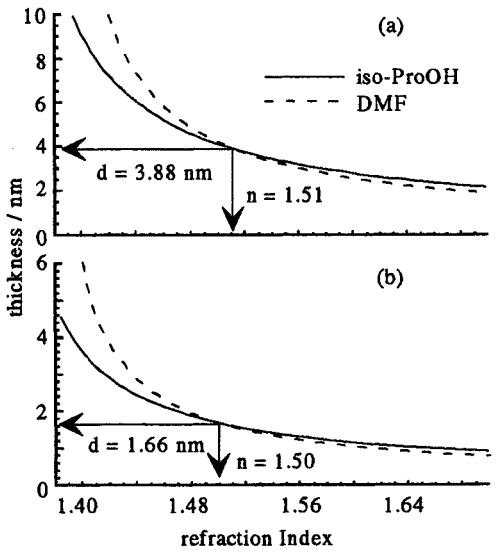

Fig. 4. Calculation of film thickness and refraction index of (a) poly(L-Leu)$_{16}$ -C11N/C4 mixed SAM and (b) C11N/C4 mixed SAM from SPR data in DMF and iso-ProOH.

The thickness of poly(L-Leu)$_{16}$ layer on the mixed

SAM was determined by the multi-solvent SPR method. The resonance angle, θ, of SPR is strongly dependent on thickness, d, and refraction index, n, of organic thin layer on the gold surface. Figure 4 shows the thickness versus refraction index curves calculated from the SPR data of the poly(L-Leu)$_{16}$ – C11N/C4 mixed SAM layer and the C11N/C4 mixed SAM layer on the gold surface, respectively, using eq. 2. The solid and dashed lines correspond to the SPR data for the layers in iso-ProOH and DMF, respectively. The thickness of the layers are obtained to be 3.88 nm for the poly(L-Leu)$_{16}$ – C11N/C4 mixed SAM layer and 1.66 nm for the C11N/C4 mixed SAM layer, respectively, from the intersection point of the iso-ProOH and DMF curves. From these results, the thickness of the poly(L-Leu)$_{16}$ layer on the SAM surface was estimated to 2.22 nm. This value indicated that the tilt angle of the α-helical poly(L-Leu)$_{16}$ (molecular length of the α-helical rod is 2.4 nm) is 22.3°, which value was similar to that obtained by FTIR-RAS measurement. In Figure 5, The structural model of polypeptide layer prepared by stepwise polymerization on the mixed SAM surface is proposed.

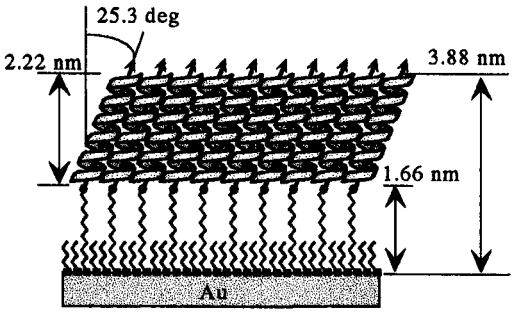

Fig. 5. Structural model of poly(L-Leu)$_{16}$ layer on C11N/C4 mixed SAM.

In conclusion, the vertically and unidirectionally oriented polypeptide assembly can be easily obtained by the stepwise polymerization of amino acids using the conventional solid-phase peptide synthesis method on the 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 polypeptides having functional groups such as electron donors and receptors at the designed positions. This system may be employed in signal transduction devices. Further studies involving the vectorial electron transfer through these polypeptide assemblies are underway.

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