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Photo-induced Vectorial Electron Transfer through Oriented Metal Coordinated Peptide Assembly on a Self-Assembled Monolayer

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We prepared vertically and unidirectionally oriented metal coordinated $Leu_2His(Co(II))Leu_6His(Co(II))Leu_6$ assemblies containing C_{60} at the terminal by condensation reaction on a SAM surface having ferrocene moieties. The peptide obtained by the stepwise polymerization oriented vertically and unidirectionally to the mixed SAM surface and the His moieties were formed complexes with Co(II) in the assembly. We have demonstrated that vectorial electron transfer through the peptide assemblies on the mixed SAM. The Co(II)-His complexes in the peptide assembly on the SAM accelerated the electron transfer coupled with the macro-dipole moment of the peptides through the assembly. Furthermore, upon photoexcitation of ferrocenyl group on the SAM surface, electron transfer occurred from the excited ferrocenyl group to the electron acceptor C_{60}. This method has the advantage of permitting simple preparation of oriented peptide assemblies consisting of sequential peptide having functional groups such as electron donors and acceptors at the desired positions. This system may be useful for signal transduction devices.

Key words: Vertically and Unidirectionally Orientation, Metal Coordinated Polypeptide Assembly, Stepwise Polymerization, Photocurrent, Electrochemical Properties

1. INTRODUCTION

The spatially specific arrangement of functional groups in membrane proteins is closely related to the vectorial signal transfer through a biological membrane. For example, in a photosynthesis system, the specific location of a special pair chlorophylls, two pheophytins, and two quinines bound to an α-helical peptide bundle in a lipid membrane yields the photo-induced vectorial electron transfer through the membrane.[1,2] Further, a vertically oriented α-helical peptide assemblies whose molecular dipole moment aligns unidirectionally provide optical switches based on second-order nonlinear effects.[3,4] Studies on vertically and unidirectionally oriented peptide 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. The fabrication of vertically oriented α-helical peptide assemblies such as Langmuir-Blodgett (LB) films[5-7] and self-assembled monolayers (SAMs)[8-11] 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 LB 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 unidirectional alignment, through the sequential polypeptide whose functional groups locate specifically in the rod cannot be obtained by NCA polymerization on the substrate. Recently, we have reported that fabrication of vertically and unidirectionally oriented peptide assemblies on self-assembled monolayers by the stepwise polymerization.[16] This method has the advantage of permitting simple preparation of oriented peptide assemblies consisting of sequential peptide having functional groups such as electron donors and acceptors at the specific positions.

In this paper, we have reported that the fabrication of vertically and unidirectionally oriented metal coordinated peptide assembly by the stepwise polymerization on a substrate. We demonstrated the vectorial electron transfer through the peptide assembly coupled with macro-dipole moment of the peptides. Furthermore, we demonstrated photo-induced vectorial electron transfer through the metal coordinated peptide assembly having electron acceptor, C_{60}, at the *N*-terminal from ferrocenyl groups, which were fixed at the SAM surface by UV light irradiation. This system may be useful for organic signal transduction devices such as supra-integrated photo-diode.

2. EXPERIMENTAL

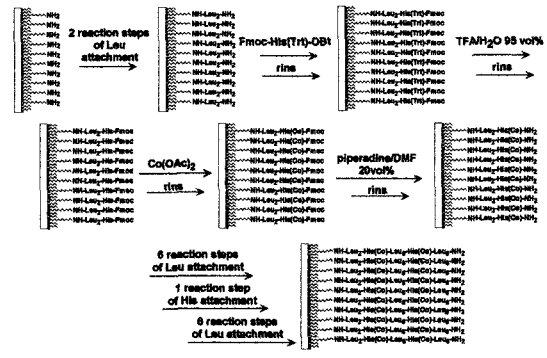
2-1. Substrate Preparation

Gold-deposited glass plate (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, and then the substrate was rinsed with ethanol several times.

The molar ratio of C11N and C4 was fixed to 1:4.5. Furthermore, a mixed SAM composed of C11N, C4, and 6-ferrocenyl-1-hexanethiol (C6Ferro) was prepared in a manner similar to the above. The molar ratio of C11N, C4 and C6Ferro was fixed to 1:4:1.

2-2. Stepwise Polymerization of Amino Acids on SAM Surfaces

The metal coordinated peptide having $Leu_2His(Co)Leu_6His(Co)Leu_6$ sequence on the mixed SAM surface was obtained by the modified method of conventional solid-phase peptide synthesis method[17] according to the literature.[16] Activation of Fmoc-amino acid was carried out as follows. Fmoc-amino acid was dissolved in DMF (1 mM) with benzotriazol–1–yloxytris(dimethylamino)phophonium hexafluorophosphate (BOP), *N*-methylmorpholine (NMM), and *N*-hydroxy-benzotriazole (HOBt). The molar ratio of Fmoc-amino acid, BOP, NMM, and HOBt was 1:1:1.5:1. This solution was stirred for 5 min at room temperature. First, the substrate was immersed in dimethylformamide (DMF) solution of activated ester for Fmoc-L-Leu for 2 hours to attach the Fmoc-L-Leu to the amino group on the surfaces. After the reaction, the surface of the substrate was rinsed by pure DMF and then the substrate was immersed in DMF solution containing 20 vol% piperidine to remove the amino terminal Fmoc-protecting group for 1 hour. After the reaction, the surface of the substrate was rinsed by pure DMF until the pH of the immersed DMF solution was neutral. This reaction cycle was repeated two times to obtain the Leu-Leu layer on the SAM. Next, the Fmoc-L-His(Tri) was coupled to the Leu-Leu layer by the same protocol and then the substrate was immersed in aqueous solution containing 95 vol% trifluoroacetic acid to remove the Tri-protecting group of the His moiety for 1 hour. After the reaction, the surface was rinsed by pure water until the pH of the aqueous solution was neutral and the substrate immersed in a 0.1 M aqueous solution containing Cobalt(II) acetate for 1day to form complex between Co(II) and His residues in the peptide layer on the SAM. After the complexation between the His residues and Co(II), the substrate was rinsed with deionized water and pure DMF several times to remove free Co ion and to exchange the medium from water to DMF. Then the terminal Fmoc protecting group was removed by the same protocol described above. These reaction cycle were repeated successively to obtain the $Leu_2His(Co)Leu_6His(Co)Leu_6$ layer on the SAM (Scheme 1). The introduction of C_{60} moiety at the *N*-terminal of the peptide was carried out as follows. 1,2-dihydro-1,2-methanofullerene[60]-61-carboxylic acid (C_{60}-COOH) was synthesized according to the literature.[18] C_{60}-COOH was dissolved in bromobenzene (1 mM) with dicyclohexylcarbodiimide (DCC) and HOBt. The molar ratio of C_{60}-COOH, DCC, and HOBt was 1:1:1. The substrate having the peptide assembly was immersed in the solution of activated ester for C_{60}-COOH for 1 day to attach the C_{60} to the amino terminal of the peptide. After the reaction, the surface of the substrate was rinsed by pure bromobenzene and then the substrate was dried in vacuo. Peptide assembly consisted in Leu 16 mer, Leu_{16}, on the mixed SAM surface was fabricated by the same protocol.



Scheme 1. Fabrication of Co(II)-coordinated peptide assembly on the SAM.

2-3. Spectroscopic Measurements

The Fourier transform infrared reflection-absorption spectra (FTIR-RAS) of the peptide assemblies on the mixed SAM 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 the spectra were analyzed as a sum of the Gaussian / Lorentzian composition of individual bands. When the ratio of the Gaussian / Lorentzian was 9/1, the sum of the calculated individual bands was best fit to the experimental spectra. The molecular orientation of the peptides on the SAM surface was assessed according to the ratio method using FTIR-RAS. By comparison of the theoretical values of the ratio of the amide I and II absorbencies with the experimental values of FTIR-RAS, the tilt angle of the helix axis from the surface normal was determined.[19,20] The UV-Vis reflection adsorption spectra of the Co(II) coordinated peptide assemblies on the mixed SAM surface were measured with spectrophotometer (Jasco, V-550) equipped with ARV-474 reflectance accessory. Incident angle was fixed at 45°.

2-4. Electrochemical Measurements

The electrochemical measurements of the peptide assemblies on the mixed SAM were performed using a potentiostat (Niko Keisoku Co. Ltd., NPGA-2501) connected to a personal computer (AD Instruments Co. Ltd., PowerLab 2/25) at room temperature. A conventional three-electrode setup was used with the peptide assemblies coated gold substrate as the working electrode, Ag/AgCl as the reference electrode, and a platinum wire as the counter electrode in a teflon cell. The electric potential was measured with respect to the reference electrode. The supporting electrolyte was KCl (0.2 M). The area of the working electrode exposed to the electrolyte solution was 1.13 cm^2.

3. RESULTS AND DISCUSSION

3.1. Structural Study of Peptide Layers on the Mixed SAM Surface

The structure of the $Leu_2His(Co)Leu_6His(Co)Leu_6$ layer was investigated by FTIR-RAS and UV-Vis reflection absorption measurements. Figure 1 shows the FTIR-RAS of the peptide layer on the C11N/C4

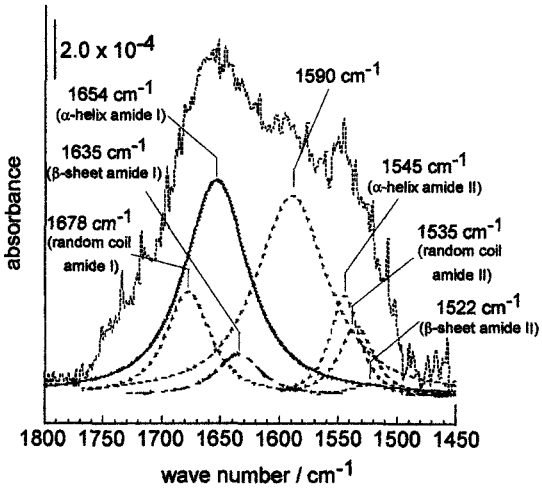


Figure 1. FTIR-RAS of Co(II)-coordinated peptide assembly on the SAM.

mixed SAM. The spectrum of the peptide 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 peptides on the SAM surface were in mainly α-helix conformation[21,22] more than 68 % in the assembly. The orientation of the peptide 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 peptide, the transition moment 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 29°.[19]

In the spectra, a carbonyl-stretching band of CH_3COO^- group was observed strongly at 1590 cm^{-1}. This result suggested that the C=O bond of the CH_3COO^- oriented perpendicular to the surface in the peptide assembly, because the RAS emphasized vibrational modes perpendicular to the surface normal. Further, in the UV-Vis reflection adsorption spectrum of

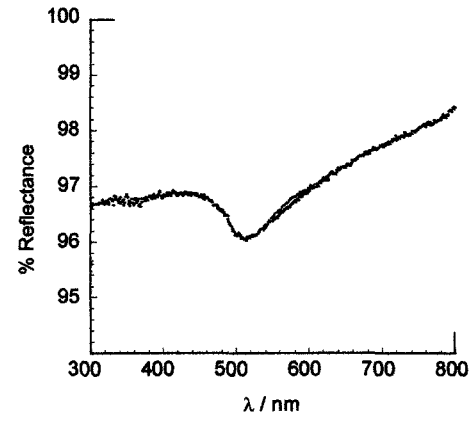


Figure 2. UV-Vis reflection adsorption spectrum of Co(II)-coordinated peptide assembly on the SAM.

the $Leu_2His(Co)Leu_6His(Co)Leu_6$ layer (Figure 2), the absorption band was observed at 514 nm, which was assigned to the octahedral coordination geometry around Co(II) atom. These results suggested that the Co(II) formed complex with four imidazole groups of His residues in square-planner geometry and two apical CH_3COO^- groups capped the square-planner face in the vertically and unidirectionally oriented peptide assembly.

3.2. Electrochemical Properties of Peptide Layers on the Mixed SAM

We demonstrated the vectorial electron transfer through the Co(II)-coordinated peptide assemblies on the mixed SAM. The electrochemical measurements of the Co(II)-coordinated peptide assemblies on the SAM were carried out at room temperature. A conventional three-electrode setup was used with a Co(II)-coordinated peptide assemblies coated gold substrate as the working electrode, Ag/AgCl as the reference electrode, and a platinum wire as the counter electrode in a teflon cell. The electric potential was measured with respect to the reference electrode. The supporting electrolyte was KCl (0.2 M). The area of the working electrode exposed to the electrolyte solution

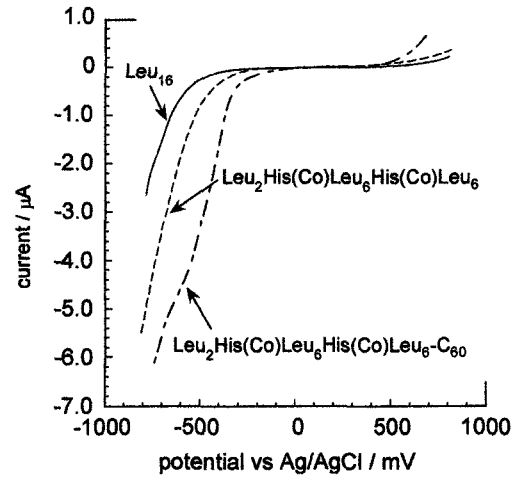


Figure 3. Current (I)–voltage (V) plots for peptide assemblies on the gold-deposited glass plate.

was 1.13 cm^2. Figure 3 shows the current (I)-voltage (V) curves of the Co(II)-coordinated peptide assemblies, with and without electron donor, ferrocenyl group, at the SAM surface and electron acceptor, C_{60}, at the peptide terminal. In this figure, the I-V curve of vertically and unidirectionally oriented α-helical peptide assembly composed of Leu 16 mer, Leu_{16},[16] was shown to compare with the electrochemical properties of Co(II)-coordinated peptide assemblies, $Leu_2His(Co)Leu_6His(Co)Leu_6$. The shape of the I-V curves determined for the peptide assemblies composed Leu_{16} or $Leu_2His(Co)Leu_6His(Co)Leu_6$ are very similar. A significant current increase was observed at negative potentials. Presumably, electron flow through the peptide assembly along the peptide macro-dipole moment is accelerated. Furthermore, the current through the Co(II)-coordinated peptide assembly was

larger than that through the Leu_{16} assembly. In addition, introduction of electron donor and acceptor pair to the Co(II)-coordinated peptide assembly shows large non-linear effect of electron flow through the peptide assembly. This result suggested that the Co(II)-His complexes, electron donor, ferrocene and electron acceptor, C_{60}, in the peptide assembly prompted the vectorial electron transfer coupled with the peptide macro-dipole moment through the peptide assembly on the SAM.

3-3. Photo-induced Vectorial Electron Transfer through the Peptide Assembly

Photo-induced vectorial electron transfer through the Co(II)-coordinated peptide assembly, which had electron donor, ferrocene, at the substrate surface and electron acceptor, C_{60}, at the *N*-terminal of the peptide, was investigated using the three-electrode setup described above. Co(II)-coordinated peptide assembly modified electrode was photoirradiated with a 500-w Xe lamp (SANEI Denki Co. Ltd., XEF-501S) using a fiber bundle array. The measured photocurrents generated by photoirradiation of the Co(II)-coordinated peptide assembly is shown in Figure 4 with repeating on-off switching of the photoirradiation. The remarkable photocurrent was observed Co(II)-coordinated peptide

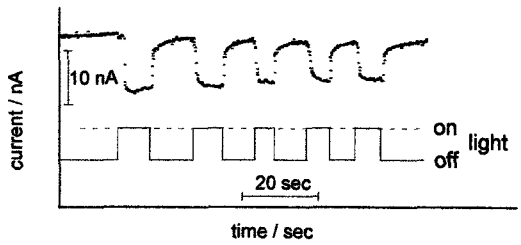


Figure 4. Time course of the photocurrents of Ferro / $Leu_2His(Co(II))Leu_6His(Co(II))Leu_6$ array in 200 mM KCl solution at 0 mV upon photo-irradiation by 500-w Xe lamp.

assembly containing ferrocene and C_{60}. The mechanism of the photo-induced vectorial electron transfer through the peptide assembly is proposed as follows. Upon photoexcitation of ferrocenyl group on the SAM surface, electron transfer occurred from the excited ferrocenyl group to the electron acceptor C_{60} at the *N*-terminal of the peptide. The Co(II)-His complexes in the peptide assembly and macro-dipole moment of the peptides accelerated the vectorial electron transfer from the gold surface to the aqueous phase through the Co(II)-coordinated peptide assembly.

In summary, the vertically and unidirectionally oriented metal-coordinated peptide assembly can be easily obtained by the stepwise polymerization of amino acids using the modified conventional solid-phase peptide synthesis method on the mixed SAM surface consisting of amino-alkanethiol and dialkyl disulfide. We have demonstrated that vectorial electron transfer through the peptide assemblies on the mixed SAM. The Co(II)-His complexes in the peptide assembly on the SAM accelerated the electron transfer coupled with the macro-dipole moment of the peptides through the assembly. This vectorial electron transfer could be regulated by the introduction of photo-sensitive electron donor and acceptor to the peptide assembly. This system may be useful for signal transduction devices.

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