

The adsorption of guest molecule onto self-assembled monolayer of α -helical polypeptide.

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For the purpose of preparation of α -helix standing monolayer on Au substrate, poly (γ -methyl L-glutamate) with -SH moiety at the terminal (PMLG₂₄-SH) was prepared and adsorbed on Au surface in 2,2,2-trifluoroethanol (TFE). Adsorption isotherm of PMLG₂₄-SH obtained from QCM measurements was typical Langmuir type, which indicates the formation of PMLG₂₄-SH monolayer on the substrate. The adsorption area and amount estimated from the isotherm supported that the α -helix axis is almost normal to the monolayer plane. FT-IR RAS measurement confirmed that PMLG₂₄-SH formed α -helix conformation in the monolayer on the substrate. And then diphenylacetylene was introduced into the monolayer as a guest molecule. The adsorption isotherm of diphenylacetylene was also Langmuir type, indicating that the guest molecule could be fixed at the adsorption site in the PMLG₂₄-SH monolayer. Furthermore, the adsorption amount and FI-IR spectrum supported that the orientation of the guest diphenylacetylene in the monolayer seems to be different from that in its cast film.

Key words: guest molecule, self-assembled monolayer, polypeptide, and Au substrate

1. INTRODUCTION

It is considered that the orientation changes of α -helix unit of membrane protein plays an important role of functional regulation of biological membranes such as the transport of specific ions or the signal transductions [1]. The α -helical polypeptide, therefore, may be useful for various applications to novel intelligent materials on the basis of their molecular orientation control.

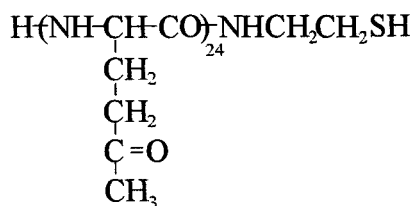
In order to analyze and mimic these functions, it has been studied to control the orientation of α -helix molecules in artificial membrane systems. Recently, it is reported that the α -helix standing monolayer could be formed at air-water and oil-water interface [2-4], respectively. It is important that the monolayer formed at air-water interface can be transferred on various of substrates. However, LB film seems to be unstable because of a weak interaction between monolayer and substrate. On the other hand, it is reported that a stable self-assembled monolayer (SAM) was obtained by adsorption of α -helical polypeptide with -SH group on a gold surface [5-7]. In this case, SAM has a strong interaction of Au-S bond, and is expected to have sufficient physical stability for practical use [6-9]. We noticed the possibility that the anisotropic structure of the monolayer based on the vertical orientation of helix rod effects to the orientation of the guest molecule adsorbed on SAM.

In this study, we prepared the SAM with vertical orientation of α -helical polypeptide and aimed for the construction of novel functional

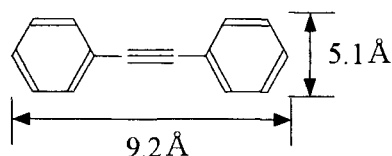
interface based on adsorption of a liquid crystalline guest molecule on this SAM template. Especially, we focused the influence of anisotropic structure of the SAM on the orientation of the adsorbed guest molecule.

2. EXPERIMENTAL

Poly (γ -methyl L-glutamate) with -SH moiety at the terminal was prepared by polymerization of N-carboxyanhydride of γ -methyl L-glutamate (MLG-NCA) with S-triphenylmethylsisteamine as initiator in N,N-dimethylformamide (DMF) solution. And then removal of triphenylmethyl group was carried out in trifluoroacetic acid (TFA) solution of the polypeptide. The average degree of polymerization of PMLG moiety was 24 by ¹H-NMR analysis (Varian XL-200 spectrometer) in TFA solution of the sample (Scheme 1, PMLG₂₄-SH). The secondary structure of PMLG₂₄-SH was confirmed to α -helix conformation in 2,2,2-trifluoroethanol (TFE) by circular dichroism (CD) measurement (Jasco J-820K spectropolarimeter).



Scheme 1 Molecular structure of PMLG₂₄-SH.



Scheme 2 Molecular structure and size of diphenylacetylene.

In order to obtain the adsorption isotherms of PMLG₂₄-SH, gold-coated quartz crystal microbalance (QCM) resonators (9 MHz) were immersed in TFE solutions containing various amounts of peptide for 24 hours and washed with TFE, and frequency shifts were monitored in air after drying. The frequency changes were followed to a universal frequency counter (model 53131A) connected to a personal computer (IBM Thinkpad 600), and transformed into mass changes by using the Sauerbrey equation [10]. The SAM of PMLG₂₄-SH, which was prepared by immersing gold substrate in TFE solution of the polypeptide (0.2 mM) for 24 hours, was used for guest molecule adsorption and FT-IR measurements.

And we used diphenylacetylene as a guest molecule of SAM for adsorption experiments (Scheme 2). The amounts of adsorption of the guest molecule on the SAM were determined by QCM technique (9 MHz) under both *in situ* (methanol) and dry state. In order to obtain the adsorption isotherms of the guest molecule, the SAM was immersed in methanol solutions of various diphenylacetylene concentration for 3 hours and washed with methanol, and frequency shifts were monitored in air after drying. We used Well-type cell for *in situ* measurements of the adsorption behavior of the guest molecule in methanol.

The spectrum of FT-IR was recorded on a Perkin Elmer Spectrum 2000 equipped with MCT (Mercury-Cadmium-Telluride) detector with a resolution of 4 cm⁻¹. For RAS measurements, FT 80 Specular Reflectance attachment was used. The tilt angle of α -helical axis from the surface

$$\frac{A^I_{\text{obs}}}{A^II_{\text{obs}}} = K \frac{A^I_{\text{cal}}}{A^II_{\text{cal}}} \quad (1)$$

$$\frac{A^I_{\text{cal}}}{A^II_{\text{cal}}} = \frac{(\sin\theta \sin 39^\circ)^2/2 + (\cos\theta \cos 39^\circ)^2}{(\sin\theta \sin 74^\circ)^2/2 + (\cos\theta \cos 74^\circ)^2} \quad (2)$$

normal was calculated by the method proposed by Samulsky et al. [5]

Here, θ is an angle between the helix axis and the axis normal to plane of the monolayer A^I_{obs} and A^II_{obs} are the absorbance of amide I and amide II, respectively, observed by FT-IR measurements. $A^I_{\text{cal}} / A^II_{\text{cal}}$ is the calculated ratio of absorbance.

3. RESULTS AND DISCUSSION

The adsorption isotherm of PMLG₂₄-SH was Langmuir type, which supports that PMLG₂₄-SH formed SAM on Au. The saturated adsorption of the peptide was obtained in the solution whose peptide concentration above 0.1 mM and the saturated amount was estimated to be 1.62×10^{-6} mol/m². The SAM, which was obtained in 0.2 mM solution of the peptide, was used for the guest adsorption and FT-IR measurements. The area per molecule of PMLG₂₄-SH, A_{sam} , was shown in Table 1. The value of A_{sam} was obtained from the experimental value of the saturated amount of the peptide. The calculated values of A_{\parallel} and A_{\perp} were the area per molecule of PMLG₂₄-SH whose degree of polymerization is 24, when they orientated parallel (A_{\parallel}) and normal (A_{\perp}) to the monolayer. The values of A_{sam} was very close to that of A_{\perp} . It is clear that α -helix axis is almost normal to the monolayer plane and the SAM contains very small defects. FT-IR RAS measurement of the SAM was carried out. It exhibited two bands at 1662 cm⁻¹ and 1552 cm⁻¹ assigned Amide I and Amide II of the typical α -helix, respectively, indicating that PMLG₂₄-SH formed α -helix conformation in the monolayer on the substrate. And the tilt angle of α -helical axis from the surface normal was calculated to be 37°. In this way, we prepared the well-packing SAM in which α -helical polypeptide tilted to almost normal direction of the monolayer.

Figure 1 shows frequency changes with time obtained with *in situ* measurements for the

Table 1 Molecular occupied area of PMLG₂₄-SH from QCM measurements.

(nm ² /molecule)		
observed	calculated	
A_{sam}	$A_{\alpha \perp}$	$A_{\alpha \parallel}$
1.02	1.25	4.32

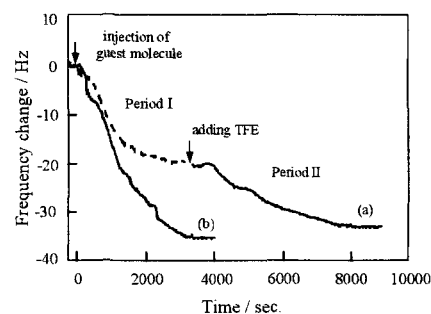


Figure 1 QCM frequency changes versus time for the adsorption of diphenylacetylene onto SAM at methanol solution ((a), Period I), the subsequent additional of TFE ((a), Period II), and methanol solution containing TFE (3 vol%) (b).

subsequent adsorption of guest diphenylacetylene on PMLG_{2,4}-SH SAM/gold QCM electrode in methanol. The frequency was decreased after injection of the guest molecule (Figure 1 (a), period I), which supported that the guest diphenylacetylene was adsorbed onto SAM. In addition, adsorption amount of diphenylacetylene was further increased by the addition of small amount of TFE which is poor solvent for the guest molecule (Figure 1 (a), period II). In this case the concentration of TFE in methanol was 3 % in volume. Figure 1 (b) shows the frequency changes when the guest molecule was injected in the mixed solvent (3 % TFE / 97 % methanol) prepared previously. It is clear that the frequency decrease of (a) and (b) is almost the same. The adsorption amount of diphenylacetylene was found to be dependent on the TFE concentration and showed the maximum at 3 % TFE. This implies that the guest molecule is rather stable in the monolayer phase than in the TFE added solution. And then the reason why the adsorption amount of the guest molecule was decreased over 3 % of TFE is the precipitation of the guest molecule in the solution. The adsorption isotherms of the guest both in methanol (Figure 2 (a)) and 3 % of TFE (Figure 2 (b)) were Langmuir type. This shows that the guest molecule could be fixed at the adsorption site in the SAM. Moreover, the amount of saturated adsorption was estimated to be $0.96 \times 10^{-5} \text{ mol/m}^2$ (Figure 2 (a)) and $1.36 \times 10^{-5} \text{ mol/m}^2$ (Figure 2 (b)), respectively. So we prepared the SAM on which diphenylacetylene was adsorbed at 1.0 mM of methanol solution (Sample I) and mixed solution (Sample II) for FT-IR measurements, respectively. We estimated the molecular occupied area of diphenylacetylene, A_{guest} , adsorbed on the SAM (Table 2). The value of A_{guest} was obtained from the experimental saturated amount of adsorption of the guest molecule. Both the values of A_{guest} of Sample I (0.17 nm^2) and Sample II (0.12 nm^2) were smaller than the calculated area of the guest molecule ($0.47 \text{ nm}^2 = 0.92 \text{ nm} \times 0.51 \text{ nm}$ in Scheme 2). Thus the orientation of the guest in the SAM seems to be different from that in its cast film due to the anisotropic structure of SAM, i.e., α -helix regularly standing template.

We measured FT-IR RAS spectrum of diphenylacetylene adsorbed onto the SAM to confirm the presence of the guest in the SAM. Figure 3 shows the FT-IR RAS spectrum of the Sample I (a) and the FT-IR spectrum of the guest molecule in KBr pellet (b). The presence of the guest in the SAM was confirmed by appearance of the peak based on diphenylacetylene (1606 cm^{-1} , 1516 cm^{-1} and 1460 cm^{-1} , respectively). However, these peaks were observed at higher wavenumbers than those observed in the KBr pellet (1601 cm^{-1} , 1500 cm^{-1} and 1442 cm^{-1} , respectively). In addition, the absorbance of these three peaks was also different from those obtained by KBr method. It is clear

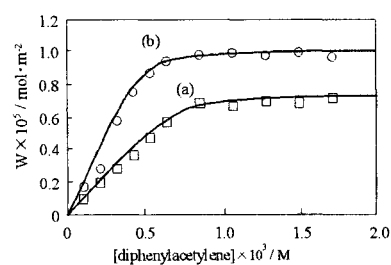


Figure 2 Adsorption isotherm of diphenylacetylene on SAM at methanol solution (a) and methanol solution containing TFE (3 vol%) (b).

Table 2 Molecular occupied area of diphenylacetylene adsorbed on SAM from QCM measurements.

	(nm ² /molecule)
	A_{guest}
Sample I	0.17
Sample II	0.12

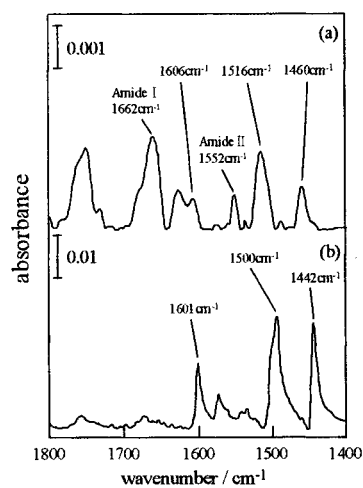


Figure 3 FT-IR reflection absorption spectrum of Sample I (a) and FT-IR spectrum of diphenylacetylene in KBr pellet (b).

Table 3 Tilt angle (θ) of α -helix from normal to the monolayer.

	θ
SAM	37°
Sample I	29°
Sample II	25°

that the guest molecule had a unique orientation in the SAM somewhat different from the random one. The values of tilt angle of PMLG₂₄-SH in the SAM, Sample I and Sample II were shown in Table 3. Interestingly, as adsorption amount of the guest was increased, the tilt angle of the peptide from the surface normal became smaller, i.e., α -helix rod in the SAM approach to the perfect standing. This fact may be explained in terms of space filling effect of guest molecule, i.e., the guest molecules could be fixed at the adsorption site between the standing α -helices, which resulted in the decrease in the tilt angle of the helix rods in the SAM.

4. CONCLUSIONS

We prepared the SAM with helical peptide standing and well-packing on gold surface. Diphenylacetylene used as a guest molecule of the SAM showed Langmuir type adsorption on the SAM. In addition, the molecular orientation of the guest molecule seems to differ from that in its cast film due to the anisotropic structure of the SAM. SAMs of α -helical peptide are interesting materials for the template for the orientation control of a guest molecule adsorbed and may be applicable to a part of the nanoscale electronic molecular devices.

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