Photoresponsive Change of the Surface Potential Generated by Helical Peptide Self-Assembled Monolayers

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(Received March 28, 2005; Accepted May 16, 2005; Published August 15, 2005)

ABSTRACT: Photoresponsive helical peptides, which are composed of two helical peptide segments with an intervening azobenzene unit, were synthesized, and the self-assembled monolayers (SAMs) were formed on gold. The helical peptides were shown to take vertical orientation on gold by Fourier transform infrared reflection-absorption spectroscopy, and to change the molecular orientation reversibly upon UV and visible light irradiation due to photoisomerization of the azobenzene unit. The structural changes upon photoirradiation resulted in alterations of the surface potential of the peptide SAMs. The helical peptides in the SAM are suggested to change the molecular orientation in a cooperative manner upon photoirradiation. [DOI 10.1295/polymj.37.599]

KEY WORDS Helix Peptide / Surface Potential / Photoresponse / Self-assembled Monolayer / Azobenzene /

Self-assembled monolayers (SAMs) of organic molecules have attracted much attention due to the regular structure on the nanometer scale,¹ and organic molecules may replace the silicon-base devices in future when the miniaturization by the top-down method will face the physical limit and the change in the principle of operation of the electronic devices.² Since proteins elicit various functions in the living system such as photoenergy harvest, electron transfer, and energy conversion, polypeptides as a model of proteins are convincing starting compounds for the molecular devices.³

Helical peptides have been extensively studied as a model of protein secondary structure. Their regular structure is formed by intramolecular hydrogen bonds between the amide proton at the 5th residue and the carbonyl oxygen at the 1st residue. The molecular alignment of peptide bonds along the helix generates a large dipole moment.⁴ Notably, helices tend to self-assemble into a bundle structure, which is often crucial for the protein functions such as ion-channel formation. Using this property of self-assembling of helices, self-assembled monolayers (SAMs) of the helical peptides on gold have been reported.⁵ The peptide SAMs have been investigated mainly from two aspects: mediator for electron transfer at a long distance⁵ and generation of surface potential.⁶ Both aspects are strongly related with the large dipole moment along the helix.

When the helical peptides are immobilized *via* the C terminal and the N terminals are exposed to the surface, positive surface potential is generated due to the positive partial charge on the N terminal.⁶ Since the intensity of the surface potential is a function of the dipole moment, change of helix orientation on gold should result in change of surface potential of the helix SAM. In the present study, we have synthesized novel helical peptides having two α -helical segments with an intervening azobenzene unit (Figure 1, Nunchaku peptide and Nunchaku Type-L). The azobenzene unit is well known to isomerize reversibly upon UV and visible light irradiation. The azobenzene-containing helix peptide SAM should therefore show photoresponsive change of the surface potential due to the molecular orientation change. The two helix segments of the Nunchaku peptide are designed to take the opposite helix sense. Layer-by-layer structure, where the helix layer at the top of the SAM is formed above the helix layer at the bottom with a intervening layer of azobenzene, may be promoted in the Nunchaku peptide SAM by using the phase separation property according to the difference of the helical sense.

EXPERIMENTAL

Materials

DL-6,8-Thioctic amide (DL-6,8-lipoamide) was pur-

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Scheme 1.

and the residual solid was treated with *n*-hexane. AzL16SS(D-Ala)

A DMF solution of HL16SS, BaAzCOOH (32 mg), HATU (32 mg), and DIEA (0.026 mL) was stirred for 3 h at 0 °C under N₂ atmosphere and for 21 h at room temperature. The solution was evaporated, and the residue was dissolved in chloroform and washed with NaHCO₃aq (3 times), NaCl_{aq}, KHSO₄aq (2 times), and NaCl_{aq}. After drying the chloroform layer with MgSO₄, the solution was evaporated, and the residue was purified by an LH20 column using chloroform/methanol (1/1 v/v) as eluant. Yield: 22 mg (42%) TLC: R_f(A) = 0.44, R_f(E) = 0.64.

¹H NMR (400 MHz, methanol-*d*₄): δ (ppm) 0.80–0.93 (48H, LeuC^δH₃), 1.40–1.90 (92H, (CH₃)₃C, Leu-C^βH₂, LeuC^γH, AibCH₃, NHCH₂(CH₂)₄, AlaC^βH₃), 1.86, 2.42 (2H, m, CHCH₂CH₂SS), 2.51, 2.71 (4H, m, CH₂CH₂), 3.08–3.19 (4H, brm, NHCH₂(CH₂)₄, CHCH₂CH₂SS), 3.53 (1H, m, CHCH₂CH₂SS), 3.87,

4.01, 4.21 (9H, brm, LeuC^αH, AlaC^αH), 7.25 (1H, d, AlaNH), 7.75–7.89 (8H, brm, C₆H₄), 8.17–8.43 (19H, LeuNH, AibNH, NHCH₂(CH₂)₄).

MS (FAB, matrix; nitrobenzyl alcohol): *m/z* 2241 (calcd for C₁₁₂H₁₈₉N₂₂O₂₁S₂ [M + H]⁺ 2242) *m/z* 2264 (calcd for C₁₁₂H₁₈₈N₂₂O₂₁S₂Na [M + Na]⁺ 2264)

AzL16SS(L-Ala). AzL16SS(L-Ala) was synthesized by the method similarly to AzL16SS(D-Ala). Yield: 18 mg (30%) TLC: R_f(D) = 0.62.

¹H NMR (400 MHz, methanol-*d*₄): δ (ppm) 0.80–1.00 (48H, LeuC^δH₃), 1.40–1.90 (92H, (CH₃)₃C, Leu-C^βH₂, LeuC^γH, AibCH₃, NHCH₂(CH₂)₄, AlaC^βH₃), 1.86, 2.42 (2H, m, CHCH₂CH₂SS), 2.48, 2.70 (4H, m, CH₂CH₂), 3.07–3.17 (4H, brm, NHCH₂(CH₂)₄, CHCH₂CH₂SS), 3.52 (1H, m, CHCH₂CH₂SS), 3.87, 3.99, 4.23 (9H, brm, LeuC^αH, AlaC^αH), 7.19 (1H, d, AlaNH), 7.74–7.87 (8H, brm, C₆H₄), 8.12–8.23 (19H, LeuNH, AibNH, NHCH₂(CH₂)₄).

MS (FAB, matrix; nitrobenzyl alcohol): m/z 2241 (calcd for $C_{112}H_{189}N_{22}O_{21}S_2$ $[M + H]^+$ 2242) m/z 2264 (calcd for $C_{112}H_{188}N_{22}O_{21}S_2Na$ $[M + Na]^+$ 2264)

TFA·HAAzL16SS. TFA·HAAzL16SS was synthesized by the method similarly to TFA·HL16SS.

Nunchaku peptide. A DMF/chloroform (1/1 v/v) solution of TFA·HAAzL16SS, B16OH (57 mg), HATU (15 mg), and DIEA (0.023 mL) was stirred for 3 h at 0 °C under N_2 atmosphere and for 31 h at room temperature. The solution was evaporated, and the residue was dissolved in chloroform. The chloroform solution was washed with water, and was dried with $MgSO_4$. The solution was evaporated, and the residue was purified by an LH20 column using chloroform/methanol (1/1 v/v) as eluant. The product was further purified by an LH60 column using chloroform/methanol (1/1 v/v) as eluant. Yield: 64 mg (75%) TLC: $R_f(D) = 0.38$.

1H NMR (400 MHz, methanol- d_4): δ (ppm) 0.64–0.93 (48H, $LeuC^{\delta}H_3$), 1.32–1.72 (161H, $(CH_3)_3C$, $LeuC^{\beta}H_2$, $LeuC^{\gamma}H$, $AibCH_3$, $NHCH_2(CH_2)_4$, $AlaC^{\beta}H_3$), 1.86, 2.39 (2H, m, $CHCH_2CH_2SS$), 2.48, 2.68 (4H, m, CH_2CH_2), 3.04–3.12 (4H, brm, $NHCH_2(CH_2)_4$, $CHCH_2CH_2SS$), 3.64 (1H, m, $CHCH_2CH_2SS$), 3.83, 3.97, 4.18, 4.28 (17H, brm, $LeuC^{\alpha}H$, $AlaC^{\alpha}H$), 7.22 (1H, d, $LeuNH$), 7.72–7.84 (8H, brm, C_6H_4), 8.03–8.23 (34H, $LeuNH$, $AibNH$, $AlaNH$, $NHCH_2(CH_2)_4$).

MS (FAB, matrix; nitrobenzyl alcohol): m/z 3853 (calcd for $C_{192}H_{322}N_{38}NaO_{37}S_2$ $[M + Na]^+$ m/z 3850)

Nunchaku Type-L. Nunchaku Type-L was synthesized by the method similarly to Nunchaku peptide. Yield: 16 mg (23%) TLC: $R_f(D) = 0.38$.

1H NMR (400 MHz, chloroform- d): δ (ppm) 0.80–1.00 (72H, $LeuC^{\delta}H_3$), 1.45–1.80 (125H, $(CH_3)_3C$, $LeuC^{\beta}H_2$, $LeuC^{\gamma}H$, $AibCH_3$, $NHCH_2(CH_2)_4$), 1.85, 2.42 (2H, m, $CHCH_2CH_2SS$), 2.52, 2.75 (4H, m, CH_2CH_2), 3.02–3.20 (4H, brm, $NHCH_2(CH_2)_4$, $CHCH_2CH_2SS$), 3.55 (1H, m, $CHCH_2CH_2SS$), 3.90, 4.00, 4.20, 4.35 (13H, brm, $LeuC^{\alpha}H$, $AlaC^{\alpha}H$), 7.78–7.90 (8H, brm, C_6H_4), 7.25, 7.43, 7.97–8.28 (27H, $LeuNH$, $AibNH$, $AlaNH$, $NHCH_2(CH_2)_4$).

MS (FAB, matrix; nitrobenzyl alcohol): m/z 3059 (calcd for $C_{152}H_{260}N_{30}O_{29}S_2Na$ $[M + Na]^+$ m/z 3057)

Preparation of Self-Assembled Monolayers

Gold substrates for reflection-absorption spectroscopy and surface potential measurements were prepared by vapor deposition of chromium (30 nm) as adhesion layer and then gold (99.99%, 200 nm) on a slide glass (76 × 26 mm). In the case of gold substrates for UV measurements, chromium (1 nm) and

gold (8 nm) were successively deposited on quartz substrate (38 × 12 mm).

SAMs were prepared by incubation of each gold substrate in an ethanol solution of the peptide (0.1 mM) for 24 h. The substrates were then washed by ethanol and dried under N_2 gas. The bicomponent SAMs, Nunchaku/SSL16B (1/1 mol/mol) and Nunchaku Type-L/SSL16B (1/1 mol/mol), were prepared similarly.

UV-vis Measurement

UV-vis measurements were carried out on a Shimadzu UV-2450PC. Filters were used for UV irradiation, Toshiba UV-35 or UV-33, or visible light irradiation, Toshiba Vis-430, in combination with a Xe lamp (500 W, JASCO Co.).

Fourier Transformed Infrared Reflection-absorption Spectroscopy (FTIR-RAS)

FTIR-RAS measurements were performed on a Nicolet Magna 850 Fourier transformed infrared spectrophotometer with a Harrick Model RMA-1DG/VRA reflection attachment. The incident light angle was set at 85°, and the number of interferogram accumulation was 500.

The molecular orientation of the peptide monolayer on gold was determined on the basis of the amide I/amide II absorbance ratio in the FTIR-RAS spectrum according to eq (1) under an assumption of a uniform distribution of the helical peptides along the surface normal.^{5,6}

$$\frac{I_1}{I_2} = C \frac{2 \left[\frac{1}{2}(3 \cos^2 \gamma - 1) \right] \left[\frac{1}{2}(3 \cos^2 \theta_1 - 1) \right] + 1}{2 \left[\frac{1}{2}(3 \cos^2 \gamma - 1) \right] \left[\frac{1}{2}(3 \cos^2 \theta_2 - 1) \right] + 1} \quad (1)$$

where I_i , C , γ , and θ_i (subscripts of 1 and 2 represent respectively amide I and II) represent the observed absorbance, the scaling constant ($C = 1.5$ for the peptides), the tilt angle of the helix axis from the surface normal, and the angle between the transition moment of amide vibration mode and the helix axis, respectively. The values of θ_1 and θ_2 were taken to be 39° and 75°, respectively.

Surface Potential

Surface potential of the peptide SAMs were measured by the Kelvin Probe technique with using a home-made apparatus under a high vacuum of *ca.* 10^{-6} torr.⁸ Hg lamp (200 W, Hamamatsu LC5) with a filter (Sigma Koki, UTVAF-50S-33F) was used for UV irradiation on the SAMs, and Xe lamp (500 W,

Figure 2. UV-vis absorption spectral change of Nunchaku peptide (1.3×10^{-5} M) in ethanol upon; a) UV irradiation at 360 nm. b) Vis irradiation for 10 min at >430 nm after UV irradiation.

Ushio UI-501C) with a filter (Toshiba, Vis-430) was for visible light irradiation.

Polarization-modulation IR-RAS (PMIR-RAS)

PMIR-RAS measurements were performed on a Nicolet Magna 850 Fourier transform infrared spectrophotometer equipped with a Hinds Instruments PEM-90 photoelastic modulator system with a II/ZS50 ZnSe head. The modulation frequency of this system was $2 \times 50 = 100$ kHz. A GWC instruments SSD-100 synchronous sampling demodulator and Nicolet SST (simultaneous synchronous technique) module and software were used for signal processing. The incident light angle was set at 80° , and the accumulation number was 500. Filters were used for UV irradiation, Toshiba UV-35 or UV-33, and for visible light irradiation, Toshiba Vis-430, in combination with Xe lamp (350 W, Hamamatsu L2480).

RESULTS AND DISCUSSION

Photoisomerization of the Peptides in Ethanol

Photoisomerization of the Nunchaku peptide in an ethanol solution was analyzed by UV measurement (Figure 2). The absorption around 365 nm is assigned to the trans azobenzene unit ($\epsilon = 2.3 \times 10^4$ M $^{-1}$ cm $^{-1}$),⁹ and the intensity decreased significantly upon UV light irradiation. On the other hand, the small peak at 460 nm, which appeared upon UV light irradiation, is assigned to the cis azobenzene unit. Since the UV spectrum after the visible light irradiation was the same as that before UV light irradiation, the azobenzene unit in the peptides photoisomerizes reversibly by UV and visible light irradiation. Nunchaku Type-L and AzL16SS also showed similar photoisomerization. Content of cis isomer after UV irradiation and isomerization rate were similar among

these peptides in ethanol.

Photoisomerization on Gold Substrate

Photoisomerization of the peptide monolayer on gold was examined by UV measurement.¹⁰ The absorption around 370 nm due to the trans form of the azobenzene unit in the SAMs of Nunchaku peptide, Nunchaku Type-L, and AzL16SS decreased upon UV irradiation, and restored the intensity reversibly upon visible light irradiation (Figures 3a–c). The reversible photoisomerization was also observed with the bicomponent SAMs of Nunchaku peptide/SSL16B and Nunchaku Type-L/SSL16B (Figures 3d and e).

However, the cis contents after UV irradiation on the peptide SAMs were lower than those in ethanol, and decreased in the order of Nunchaku/SSL16B (57%) > Nunchaku Type-L/SSL16B (50%) > AzL16SS (42%) > Nunchaku Type-L (35%) > Nunchaku (32%). This order indicates that the photoisomerization from trans to cis form occurs easily in the bicomponent SAMs, where the Nunchaku peptide or the Nunchaku Type-L are spaced out by the hexadecapeptide, SSL16B. The differences in the cis contents upon UV irradiation are thus explained in terms of the steric congestion around the azobenzene unit. The photoisomerization from trans to cis form should occur easily in the mixed SAMs, because the peptide segments in the top layer, which is located above the bottom hexadecapeptide layer connected just adjacent to gold, can move in the free space generated by the spacing of the inserted SSL16B in the bottom layer.

Molecular Orientation of the Peptide SAMs

The amide I and II regions in the FTIR-RAS spectrum of the Nunchaku peptide SAM are shown in Figure 4. The average tilt angle of the helices was

Figure 3. UV-vis absorption spectral change of the peptide SAMs; a) Nunchaku peptide SAM, b) Nunchaku Type-L SAM, c) AzL16SS SAM, d) Nunchaku peptide/SSL16B SAM, and e) Nunchaku Type-L/SSL16B SAM; before UV irradiation (thick lines), after UV irradiation (intermediate), and after Vis irradiation (thin lines).

estimated from the intensity ratio of amide I and II, and the result is summarized in Table I together with the tilt angles of the other SAMs.

Notably, the helices in the peptide SAMs take a vertical orientation on gold. Especially, the mixed SAMs showed the small tilt angles among these SAMs. In the mixed SAMs, the direction of the dipole moment of SSL16B is opposite to that of Nunchaku

peptide or Nunchaku Type-L. The helices in the mixed SAM therefore can align tightly without suffering electrostatic repulsion due to the dipole moment, when SSL16B and Nunchaku peptide or Nunchaku Type-L molecules stand side by side on gold. This result is in good agreement with the previous report by Fujita *et al.*¹¹

Figure 4. FTIR-RAS spectrum of the Nunchaku peptide SAM.

Table I. The ratio amide I/amide II absorbance and the tilt angle (γ , degree) of the peptide SAMs

SAM	D_{obs}	γ
Nunchaku	2.38	44
Nuchaku type-L	3.25	37
AzL16SS	2.14	49
Nunchaku/SSL16B	4.68	29
Nunchaku type-L/SSL16B	6.42	23

Surface Potentials of the SAMs

True surface potentials of the peptide SAMs are usually difficult to obtain because the surface is contaminated by various adsorbates due to the electrostatic interaction. A baking process under high vacuum usually is effective to remove these adsorbates from the surface. In the present case, the SAMs were heated at 70 °C for three hours under high vacuum, then were subjected to UV and visible light irradiation repeatedly. After this treatment on the SAMs, the photoresponse of the surface potential of the peptide SAM was recorded.

In either peptide SAM, positive surface potentials of 200–300 mV were observed. The positive potentials can be explained from the fact that Nunchaku peptide, Nunchaku Type-L, and AzL16SS are immobilized on gold through the C terminal, and the positively charged N terminal is exposed to the surface in these peptide SAMs. The positive surface potentials of these peptide SAMs are also consistent with the previous report.^{12,13} However, the potential differences between the monocomponent SAMs and the mixed SAMs are relatively small, though SSL16B, which is connected to gold through N terminal, should partly cancel out the positively charged surface in the mixed SAMs. There are several reports which suggest that electrons are injected from gold to thin polyimide

Figure 5. Change of surface potential upon photoirradiation: ○; Nunchaku peptide SAM, ●; Nunchaku Type-L SAM.

film or peptide layer deposited on gold. This effect may be the reason for the similar positive surface potentials between these peptide SAMs.^{12,13}

In the case of the Nunchaku peptide SAM, UV irradiation decreased the surface potential by 30–60 mV, and the visible light irradiation increased the surface potential (Figure 5). Similarly, the surface potential of the Nunchaku Type-L SAM decreased by 20–30 mV upon UV irradiation, and increased upon visible light irradiation (Figure 5). On the other hand, the AzL16SS SAM, where the top helical peptide layer lacks, showed a constant surface potential irrespective of UV and visible light irradiation. These peptide SAMs were indicated to isomerize the azobenzene unit reversibly depending on the UV or visible light irradiation as described before. Therefore, the surface potential change of the Nunchaku peptide and the Nunchaku Type-L SAMs upon light irradiation should be due to the molecular orientation change of the helices in the top layer but not due to the helices in the bottom layer adjacent to gold nor the azobenzene unit.

This explanation is evidenced spectroscopically by PM-IRRAS measurements about the orientation change upon light irradiation on the SAMs. When the Nunchaku peptide SAM was irradiated by UV and visible light, the intensity ratio of amide I and II bands decreased upon UV irradiation and reversibly increased to the original level upon visible light irradiation (Figure 6). The same result was obtained with the Nunchaku Type-L SAM. The effects of the light irradiation on the intensity ratio of amide I and II bands and the tilt angles are summarized in Table II. The absorbance (D_{obs}) changes reversibly upon photoirradiation in the cases of the Nunchaku peptide SAM and the Nunchaku Type-L SAM (Tables IIa, b).

On the other hand, in the cases of the mixed SAMs, the photoresponsive behavior was not apparently

Figure 6. The Change of PM-IRRAS spectrum of the Nunchaku peptide SAM upon photoirradiation; a) before UV irradiation b) after UV irradiation c) after Vis irradiation.

Table II. The spectral change of amide I/amide II absorbance and the tilt angle (γ , degree) upon photoirradiation;

a) Nunchaku peptide SAM; b) Nunchaku Type-L SAM; c) Nunchaku/SSL16B SAM d) Nunchaku Type-L/SSL16B SAM

a)	Nunchaku peptide SAM	D_{obs}	γ
	before UV irradiation	2.1	47
	after UV irradiation	1.6	53
	after Vis irradiation	2.0	48

b)	Nunchaku Type-L SAM	D_{obs}	γ
	before UV irradiation	3.2	37
	after UV irradiation	2.7	40
	after Vis irradiation	3.1	38

c)	Nunchaku/SSL16B SAM	D_{obs}	γ
	before UV irradiation	3.7	34
	after UV irradiation	3.4	36
	after Vis irradiation	3.5	35

d)	Nunchaku Type-L/SSL16B SAM	D_{obs}	γ
	before UV irradiation	5.2	27
	after UV irradiation	5.4	26
	after Vis irradiation	5.8	25

observed. The Nunchaku/SSL16B SAM increased the surface potential upon UV irradiation, and decreased upon visible light irradiation in some cases. But, this response was lost after repeating cycles of UV and visible light irradiation. The Nunchaku Type-L/SSL16B SAM did not show a regular photoresponse neither. In the cases of the mixed SAMs, the helices in the top layer are loosely packed due to the presence of the spacing SSL16B in the bottom layer. These helices therefore may adopt various orientations upon photoisomerization of the azobenzene unit. Indeed, the average tilt angles of the helices did not show photoresponsive changes (Tables IIc and d), although the photoisomerization of the azobenzene unit occurred (Figure 3).

The helices are difficult to change the molecular orientation in the Nunchaku peptide SAM and the Nunchaku Type-L SAM as shown by the suppressed cis contents upon UV irradiation (Figure 3). The tight molecular packing in these peptide SAMs should hinder the alterations of the molecular orientation. However, when the helices in the monocomponent SAMs change the molecular orientation upon photoirradiation, the helices move in a cooperative manner to change the average tilt angles and the surface potentials. This cooperativity was lost in the mixed SAMs due to the free space generated by the presence of the spacing peptides without the azobenzene unit.

CONCLUSIONS

Helix-azobenzene-helix peptides were immobilized on gold with a vertical orientation. Upon photoirradiation of UV and visible light, the surface potential of the peptide SAMs reversibly changed due to the orientation change of the helices. The helices are tightly packed in the SAM, which should allow the cooperative movement of the helices upon photoirradiation. Cooperativity should be one of the characteristics of the peptide SAM.

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