Azobenzene–Helical Peptide Conjugate: Electronic Structure and Photoisomerization in Solution and on Surface

Mika Wakabayashi, Shigekatsu Fujii, Tomoyuki Morita, and Shunsaku Kimura*
Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Kyoto-Daigaku-Katsura, Nishikyo-ku, Kyoto 615-8510

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A novel helical peptide derivative with an intervening azobenzene unit was synthesized, and the effects of the peptide segments on the electronic properties of the azobenzene unit, immobilization on solid surfaces, and photoisomerization in solution and on surface were studied.

Construction of a well-defined structure from molecules and its control are essential for understanding of biological systems at a molecular level and development of molecular-based nanotechnology.[1] We have focused our attention on helical peptides as a component for functional molecular systems because helical peptides have a rigid and periodic structure, show unique electric properties, and form regular self-assemblies. We have prepared self-assembled monolayers on a solid substrate from helical peptides carrying functional groups at the terminal or at the side chains, and demonstrated that the helical peptides therein exhibit various functions such as molecular wire,[2] photodiode,[3] rectifier,[4] and switch.[5,6]

In this study, as another type of photoresponsible molecular switch, a novel helical peptide derivative with an intervening azobenzene unit was synthesized (**9-Azo-8**, Figure 1). **9-Azo-8** is a *p*-aminophenylazobenzoic acid derivative carrying [L-leucine (Leu)–α-aminoisobutyric acid (Aib)]$_4$–L-alanine (Ala) at the N-terminal and (Leu-Aib)$_4$ at the C-terminal. Control derivatives, **B-Azo** having one Ala residue at the N-terminal, **B-Azo-8** with one Ala residue at the N-terminal and one helical peptide segment at the C-terminal were also prepared (Figure 1). The electronic structure of the azobenzene unit and its photoisomerization were here studied in solution. Furthermore, the N-terminal of **9-Azo-8** was modified with a disulfide group (**SS9-Azo-8**), which allowed the peptide being chemically immobilized on gold via an Au–S linkage. Accordingly, photoisomerization on surface was studied as well. Operations on surface are important for practical device application.

All the azobenzene derivatives were synthesized by the liquid-phase method according to the literature[5] and identified by ^{1}H NMR spectroscopy and mass spectrometry. Figure 2 shows the absorption spectra of **B-Azo**, **B-Azo-8**, and **9-Azo-8** in the form of trans isomer in methanol. The absorption maxima (π–π^*) are 406 and 412 nm for **B-Azo** and **B-Azo-8**, respectively, whereas **9-Azo-8** shows the maximum at 357 nm, significantly blue-shifted by ca. 50 nm. A similar blue shift from the control derivatives was observed in DMF ($\lambda_{max} = 368$ nm, blue-shifted by 58 nm from **B-Azo-8**) and dichloromethane ($\lambda_{max} = 367$ nm, blue-shifted by 33 nm from **B-Azo-8**). The blue shift may be explained by two possible reasons. One is that the negatively charged C-terminal of the helix affects the electronic structure of the azobenzene unit. The other is distortion from the coplanar structure of the trans azobenzene unit due to the electrostatic attraction between the N- and C-terminals of the helices in **9-Azo-8**. In contradiction to our expectation, the absorption of **9-Azo-8** did not change with heating up to 80 °C nor with mixing trifluoroacetic acid up to 50% in DMF. These perturbations usually unfold the helical structure by disrupting the hydrogen bonds if it exists. It is though hard to speculate at this point, but the helices in **9-Azo-8** may be stabilized specifically to retain the helicity partially even under the rigorous conditions.

Photoisomerization was studied in methanol. The methanol solution of each azobenzene derivative was irradiated with a UV light for a certain period and the absorption spectra were recorded. The absorption spectra of the **B-Azo** and **B-Azo-8** solutions did not change by UV irradiation (410 nm) even after 3 h irradiation. It is reported that the trans isomer is strongly favored for *p*-aminophenylazobenzoic acid and isomerization from the cis form to trans form is very fast.[7] It is thus considered that the azobenzene unit of **B-Azo** and **B-Azo-8** isomerized into the cis form by UV irradiation but quickly recovered to the trans form before the absorption measurement. In contrast, **9-Azo-8** isomerized from trans form to cis form by UV irradiation (350 nm). π–π^* Absorption of the trans form decreased while n–π^* absorption of the cis form centered at 260 nm increased (Figure 3, left). Irradiation for about 30 min led to the photostationary state. After that, upon irradiation by a visible light (460 nm, Figure 3, center) or keeping in the dark (Figure 3, right), the spectrum

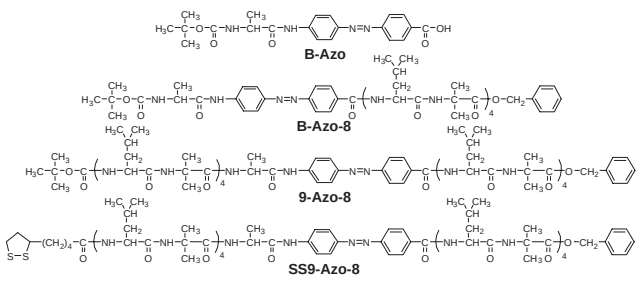

Figure 1. Chemical structures of the azobenzene derivatives.

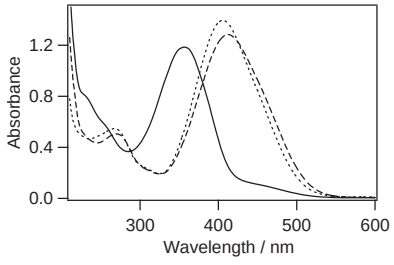

Figure 2. Absorption spectra of **B-Azo** (dotted line), **B-Azo-8** (dashed line), and **9-Azo-8** (solid line) in methanol at a concentration of 4×10^{-5} M.

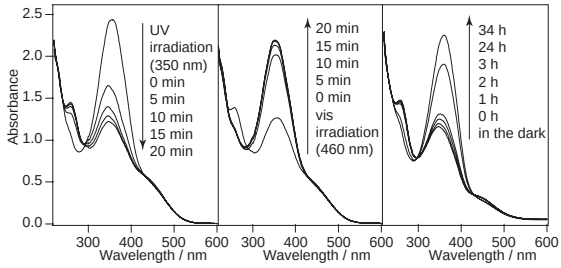

Figure 3. Changes of the absorption of **9-Azo-8** in methanol upon irradiation with a UV light (350 nm, left) and visible light (460 nm, center), and keeping the sample in the dark (right).

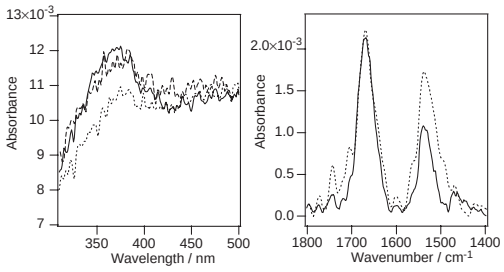

Figure 4. Absorption spectra (left) and IRRAS spectra (right) of **SS9-Azo-8** on gold before UV irradiation (solid line), after UV irradiation (dotted line), and after keeping in the dark for 24 h (dashed line, only for absorption).

gradually recovered to the original shape. It took 20 min with a visible light and 34 h in the dark. We consider that, in **9-Azo-8** in the trans form, the azobenzene unit is partially distorted as suggested by the blue shift in the absorption and its energy level is raised, while the electrostatic attraction between the terminals of the two helices should stabilize the cis form because the terminals are brought close to each other in the cis form. These effects should slow down the isomerization from the cis form to trans form compared to **B-Azo** and **B-Azo-8**.

Conformation of the peptide segment was studied by circular dichroism spectroscopy in methanol. Both **B-Azo-8** and **9-Azo-8** showed a negative peak at 202 nm and a shoulder at 222 nm which are characteristic for 3_{10}-helical conformation. But the molar ellipticities (deg cm^2 dmol^{-1} residue) were quite different, ca. -5000 for **B-Azo-8** carrying one helix whereas ca. -10000 for **9-Azo-8** carrying two helices. This is quite interesting because this result suggests that there is cooperativity between the two helices in **9-Azo-8** despite separation by the azobenzene unit in the trans form. Hydrogen-bond formation between the terminals of the two helices is not plausible, but electrostatic interaction is possible. An electric field generated by the dipole moment of either helix can orient the amide groups of the other helix. With UV irradiation, the molar ellipticity of **9-Azo-8** decreased to about the half value, which is close to the value of **B-Azo-8**. This finding also supports the electrostatic interaction working in the trans form of the azobenzene unit.

To confirm the photoswitching operation on surface, **SS9-Azo-8** was immobilized on a gold substrate via an Au–S linkage by immersion of the substrate into an ethanol solution of the peptide for 24 h. **SS9-Azo-8** showed an absorption maximum at 370 nm which is close to that in solution, showing the electronic structure of the azobenzene unit remains intact. The surface density was determined from the absorbance at 370 nm and molar extinction coefficient of the azobenzene unit to be 0.26 molecules/nm^2. This density is about 30% coverage of vertical and hexagonal packing of the helices estimated by computational geometry optimization by the AM1 method in MOPAC 2002 package (CAChe, Fujitsu). Reversible photoisomerization was observed. UV irradiation induced isomerization from the trans form to cis form and then the absorption spectrum fully recovered by keeping the substrate in the dark for 24 h (Figure 4, left).

To study the effect of photoisomerization on molecular orientation, infrared reflection–absorption spectroscopy (IRRAS) was carried out. The IRRAS spectra before and after UV irradiation are shown in Figure 4, right. Amide I and II were observed at 1670 and 1540 cm^{-1}. The tilt angle of the N-terminal helix from the surface normal was calculated from the absorbance ratio of the amide I and II according to the literature[8] with a modification for two helices. The helix at the C-terminal of the azobenzene unit was assumed to be uniformly tilted from the axis of the N-terminal helix. The angles of the axes of the two helices were assumed to be 0° for the trans form and 53° for the cis form, respectively, estimated by the AM1 calculation. The tilt angles of the N-terminal 9 mer helix on gold were determined to be 48° for the trans form and 60° for the cis form. Notably, photoisomerization of the intervening azobenzene unit induced the change in the molecular orientation.

In conclusion, a helical peptide derivative with an intervening azobenzene unit was synthesized. The azobenzene unit showed significant blue shift of absorption band and underwent reversible photoisomerization. The molecule immobilized on gold was found to reversibly photoisomerize with change of molecular orientation. The effects by the peptide chain on the electronic structure and photoisomerization of the azobenzene unit needs to be clarified by the future work.

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