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ORIGINAL CONTRIBUTION

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Photo-responsive behavior of a monolayer composed of an azobenzene containing polypeptide in the main chain

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Abstract A photoresponsive polypeptide, two α-helical poly(γ-methyl L-glutamate)s joined with an azobenzene (MAzoM, $Mn = 11000$), have been prepared. Monolayers of the polypeptide were formed at air–water interface and the photo-responsive behavior of the monolayer obtained was investigated. A trans to cis photo-isomerization of the azobenzene moiety in the main chain of MAzoM induced by UV light irradiation resulted in a bending structure formation in the main chain of the polypeptide via photo-induced changes in the geometry of the azobenzene chromophore. As a result, the limiting area per molecule of the MAzoM monolayer was decreased. Based on the degree of the decrease in the limiting area per molecule, it was estimated that the bending angle between the two α-helical rods of MAzoM molecule under UV light irradiation was ca. 140°. The photo-responsive behavior of the MAzoM monolayer was reversible and consisted along with the photo-isomerization of azobenzene moiety.

Key words Photo-responsive polypeptide – azobenzene – poly(γ-methyl L-glutamate) – monolayer – photo-induced structural changes

Introduction

It has been recognized that structural changes of a receptor induced by external signals are an essential process of a signal transmission in a biological system. In a vertebrate photo-sensory system, a photon was accepted by a retinal in a photo-receptor, rhodopsin, and a photo-isomerization of the retinal-induced structural changes of the receptor [1]. The photo-induced structural changes of the rhodopsin finally regulate an open/closed state of a sodium channel [2, 3].

Investigations on stimulus-responsive behavior in artificial systems may be important to understand a mechanism of a signal reception and transmission in a molecular level. Synthetic polypeptides containing photo-chromic moieties have been prepared as models of a photo-receptor protein and their photo-induced changes in the structure and function were investigated [4–22]. Ciardelli et al. [11] have shown the photo-induced α-helix $\rightleftharpoons$ coil and β-structure $\rightharpoonup$ coil transition of azobenzene modified poly(L-glutamic acid) in aqueous solution. Moreover, Kinoshita et al. [20–22] have shown the photo-induced α-helix $\rightleftharpoons$ coil transition of poly(L-glutamic acid) membrane containing pararosaniline groups in the side chains and they demonstrated the photo-control of the mass transport across the membrane. In a previous study, we reported photo-induced high-order structural changes of a photo-responsive amphiphilic sequential polypeptide, consisting of two amphiphilic α-helical segments joined by an azobenzene moiety [23, 24]. The polypeptide formed a micellar aggregate in an aqueous solution and a trans-membrane bundle in a lipid bilayer membrane by self-association in the dark. Photo-irradiation induced a bending of the polypeptide by a trans-cis photoisomerization of the azobenzene moiety. The photo-induced structural changes of the polypeptide resulted in a disaggregation of the micelle in aqueous solution and a destabilization of the transmembrane bundle, respectively.

On the other hand, monolayer systems of photo-reponsive polypeptides directly provide information on the photo-induced structural changes of the individual molecules, i.e., a simplification of the photo-responsive phenomena as a two-dimensional event. In this case the photo-induced changes either in surface pressure or in surface area of the monolayers have been investigated [25–27]. The surface pressure changes upon light irradiation of poly(L-lysine) monolayer containing azobenzene in the side chains have been reported by Malcolm et al. [28].

We report here on photo-induced structural changes of a polypeptide monolayer composed of two α-helical poly(γ-methyl L-glutamate) rods joined with an azobenzene as a photo-induced geometry controllable site in the main chain. That is, in this monolayer system, we attempt to directly demonstrate the photo-induced bending structure formation of the polypeptide molecule owing to the photo-isomerization of the azobenzene moiety in the main chain.

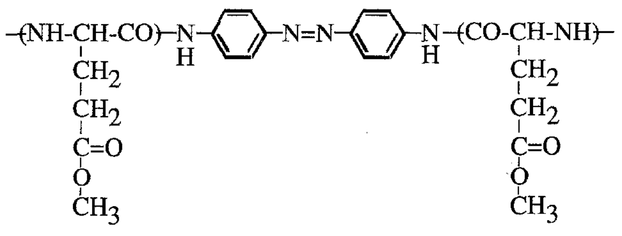
Experimental

Materials

A photo-responsive polypeptide, poly(γ-methyl L-glutamate) having an azobenzene moiety in the main chain (MAzoM) (Scheme 1), was obtained by a polymerization of N-carboxyanhydride of L-glutamic acid γ-methyl ester [29] in dimethylformamide (DMF) solution with p,p'-diaminoazobenzene as an initiator. The molar ratio of the anhydride to the initiator was 60. The polymerization occurred at room temperature over 24 h. The DMF solution was poured into water. Then the residue obtained was dissolved in DMF again and MAzoM was precipitated with water. This procedure was repeated until unreacted diaminoazobenzene was not detected spectroscopically. A number average molecular weight of 11 000 was estimated from the molar ratio of the azobenzene moiety to the γ-methyl L-glutamate residue of MAzoM. The ratio was determined by the absorbance at 375 nm on the basis of the molar extinction coefficient of azobenzene in DMF solution of MAzoM.

MAzoM in trimethylphosphate solution is in the α-helical conformation which was confirmed by the molar ellipticity at 222 nm, $[\theta]_{222}$, in the circular dichroism (CD) spectra. The helical content estimated was 81% from the value of $[\theta]_{222}$. A photo-isomerization of the azobenzene moiety did not affect the α-helical conformation of MAzoM, as confirmed by CD spectra.

Scheme 1



$\overline{\text{Mn}} = 11000$

Methods

Surface pressure–area measurements

A surface pressure–area (π-A) isotherm of MAzoM monolayer was measured with a Langmuir film balance (Nippon Laser & Electronics Lab., NL-LB-240-MWC). A Teflon trough was filled with doubly distilled and Milli-Q treated water, and spectroscopic grade 1,2-dichloroethane was used as a spreading solvent. About 5 mg of MAzoM was weighed and dissolved in 10 mL 1,2-dichloroethane. A measured small amount of the solution was delivered to the water surface from a Termo micro syringe. The temperature of the subphase was controlled at 25 °C. Compression of the monolayer was carried out successively.

Photo-induced surface area changes of MAzoM monolayer at a constant surface pressure were measured as follows. MAzoM monolayer was compressed to the desired surface pressure and then kept a constant pressure while the photo-induced surface area changes were recorded continuously.

Spectroscopic measurements

Absorption spectra of MAzoM monolayer at air–water interface were directly measured with a spectro multi channel photo detector (Otsuka Electronics Co. Ltd., MCPD-1000) to elucidate the photo-isomerization of the azobenzene moiety of MAzoM.

A secondary structure of MAzoM monolayer was estimated from the CD spectra (Jasco J-600 spectropolarimeter) of a deposited MAzoM film on a quartz plate. The deposition of MAzoM monolayer was carried out at 21 mN/m.

Microscopic measurement

Microscopic observation of MAzoM monolayers at air–water interface was done with an ellipsometric microscope (Nippon Laser & Electronics Lab., NL-EMM633) without adding any marker such as a fluorescence probe. This microscopic technique is essentially an ellipsometry [30, 31], and hence a monolayer image arises from a refractive index difference between the monolayer and the subphase.

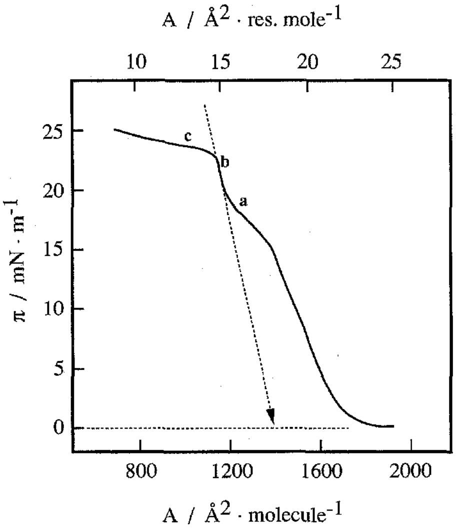
Irradiation

A light source used for UV light (250 nm $< \lambda <$ 380 nm) irradiation was a 500-w super-high-pressure mercury lamp (Ushio, USH-500D) equipped with a Toshiba UV-D33S glass filter.

Results and discussion

Figure 1 shows a surface pressure(π)–area(A) isotherm for MAzoM at 25 °C. The abscissa indicates the area per MAzoM molecule. In addition, the surface area of the monolayer is also plotted as the area per monomer residue of MAzoM in this figure. The isotherm has a shoulder at $A = 1250$ Å^2/molecule (point a in Fig. 1). The structure of MAzoM monolayer at this area was directly observed with an ellipsometric microscope and compared with those at two different regions (b and c in Fig. 1) below $A = 1250$ Å^2/molecule. Figure 2 shows micrographs of

Fig. 1 Surface pressure–area isotherm for MAzoM at 25 °C



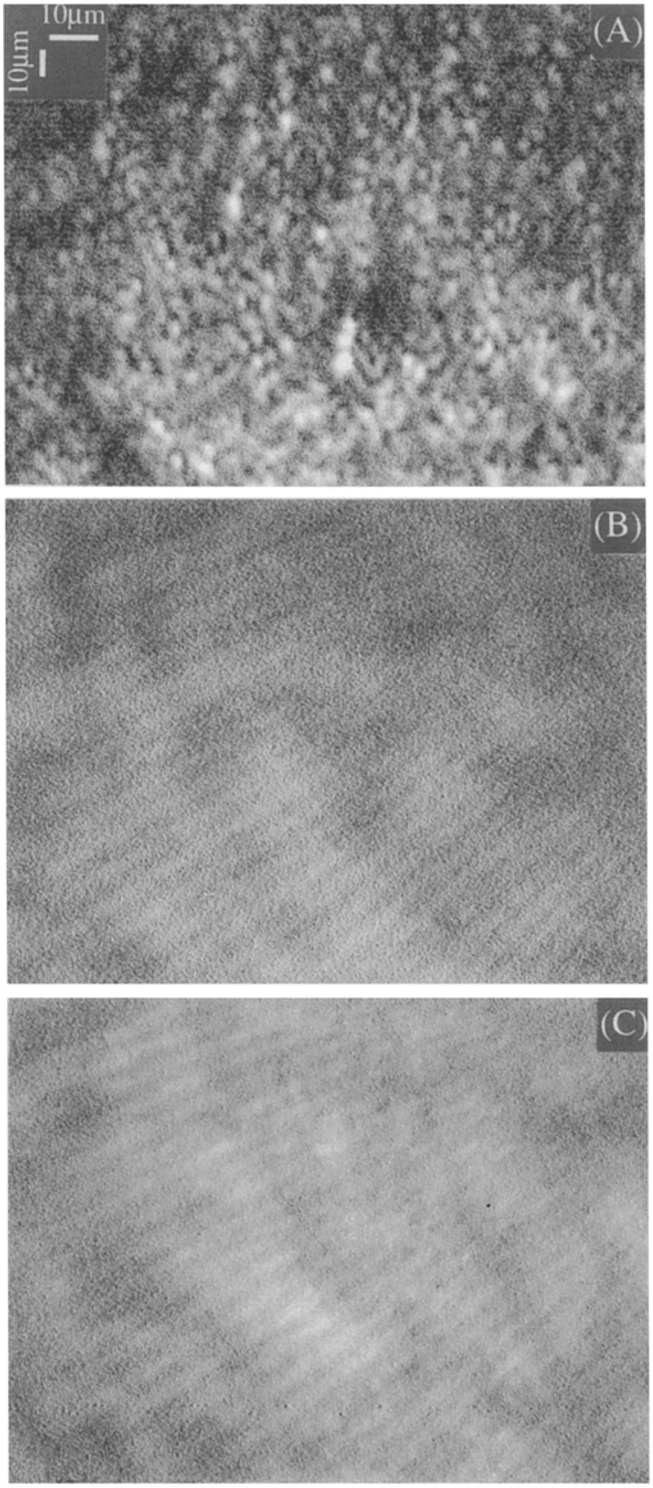


Fig. 2 Ellipsometric microscopes of MAzoM monolayer at 25 °C. (A): the shoulder region of the π-A curve at $A = 1250$ Å^2/molecule indicated point a in Fig. 1. (B): $A = 1150$ Å^2/molecule in the linear portion of the π-A curve (b in Fig. 1). (C): $A = 1000$ Å^2/molecule in the plateau portion of the π-A curve (c in Fig. 1)

MAzoM monolayer. The small domains were clearly seen in the monolayer at $A = 1250\ \text{Å}^2$/molecule (Fig. 2A). Upon further compression (b and c in Fig. 1), the domains were not observed and a uniform image appeared (Figs. 2B and C). These results suggested that MAzoM molecules were reoriented to form a closed-packing arrangement in the monolayer when the monolayer was compressed to the surface area below the shoulder a of the π-A isotherm. Furthermore, the conformation of MAzoM monolayer at air–water interface was estimated from the CD spectra with a deposited film of MAzoM monolayer (Fig. 3). The deposition of MAzoM monolayer was carried out at $\pi = 21$ mN/m. The CD spectra of deposited MAzoM film exhibited the two negative bands at 208 nm and 222 nm typical of stable right-handed α-helix. Moreover, the limiting area per monomer residue of MAzoM which was given by extrapolation of the linear portion through point b of the π-A isotherm to zero pressure (dashed line in Fig. 1) was 18.1 Å^2/res.mole. The value calculated from x-ray diffraction measurements of α-helical poly(γ-methyl L-glutamate) in the solid film in which the molecules are packed hexagonally as shown to 17.9 Å^2/res. mole [32]. It was confirmed that MAzoM molecules formed an orderly array of α-helices lying flat at the air–water interface in the solid condensed monolayer. Similar results for monolayers of α-helical polypeptides have been reported by Malcolm et al. [33]. The photo-induced behavior of MAzoM monolayer, such as photo-isomerization and surface area changes upon UV light irradiation or dark adaptation, was investigated under the solid condensed state of the monolayer.

An azobenzene is known to undergo configurational changes from trans to cis form by UV light irradiation involving changes in two important characteristics, geometry and polarity of the photo-chrome, that is, the overall geometry of the azobenzene changes from planner to nonplaner form [34, 35] and the dipole moment increase from 0.5 to 3.1 D [36]. In our case, the changes in geometry of the azobenzene moiety in MAzoM main chain would directly lead to a bending of MAzoM molecule. We have attempted to estimate the bending structure formation of MAzoM molecules from the photo-induced surface area changes of MAzoM monolayer. Figure 4 shows the photo-induced changes in the absorption spectra of MAzoM monolayer at air–water interface. The surface pressure of the monolayer was controlled at 23 ± 0.3 mN/m. The absorption spectrum of dark-adapted MAzoM monolayer exhibited the main absorption band at 370 nm assigned to the trans π-π^* transition of the azobenzene moiety. The UV light irradiation created a metastable nonplaner cis isomer of the azobenzene revealed by a strong decrease of the main absorption band. The value of a photo-conversion of the trans-cis isomerization of the azobenzene moiety was estimated from the decrease of the absorbance at 370 nm. The photo-conversion of this isomerization obtained was ca. 75%. Furthermore, the photo-isomerization of the azobenzene moiety in MAzoM monolayer was reversible. Figure 5 shows the changes in relative absorbance of the main absorption band of MAzoM monolayer, $\text{Abs}_{370}/\text{Abs}_{370,\,\text{dark}}$, upon UV light irradiation and dark adaptation. The $\text{Abs}_{370,\,\text{dark}}$ is the adsorption intensity at 370 nm of the MAzoM monolayer in the dark before UV irradiation. The relative absorbance at 370 nm could be recovered by keeping the monolayer in the dark after UV light irradiation. These results implied that the azobenzene moiety could reversibly change by the photo-stimulation from trans to cis isomer, even in the monolayer state.

To elucidate the effect of the photo-induced configurational changes of the azobenzene moiety on the molecular

Fig. 3 CD spectrum of deposited MAzoM film on a quartz plate. The deposition was carried out at 21 mN/m

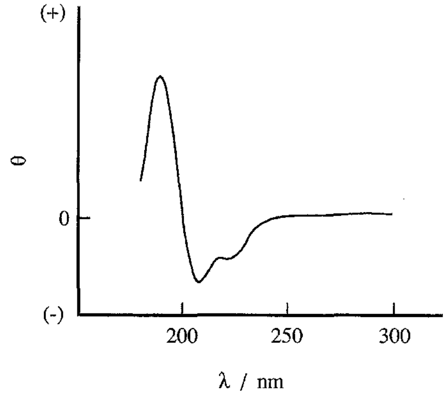
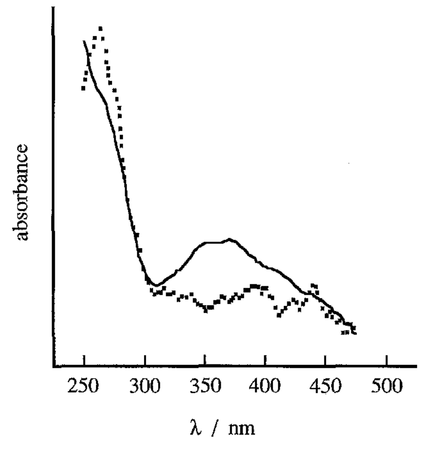


Fig. 4 Absorption spectra of MAzoM monolayer at air–water interface at 25 °C. (——): in the dark adaptation, (··· ··· ···): upon UV irradiation. Surface pressure was controlled at 23 mN/m



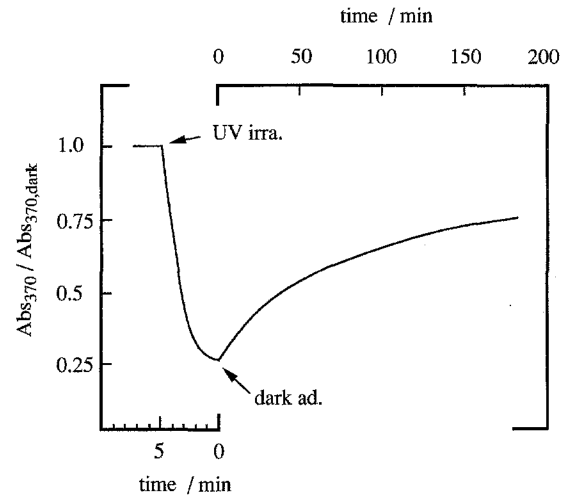


Fig. 5 Changes in the relative absorbance at 370 nm of MAzoM monolayer, $Abs_{370}/Abs_{370,\mathrm{dark}}$, by UV light irradiation and dark adaptation at 25 °C. Surface pressure was controlled at 23 mN/m

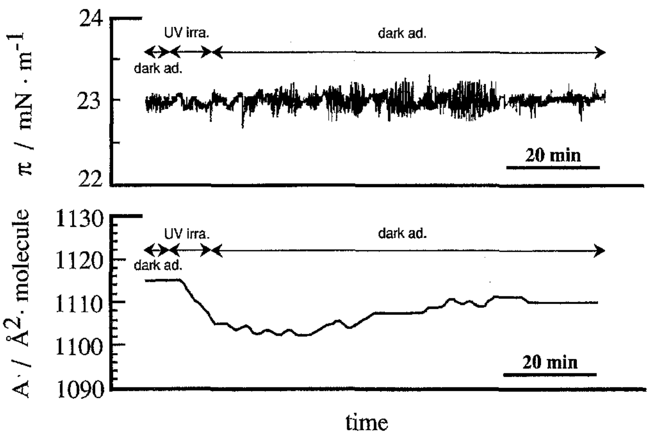


Fig. 6 Changes in the molecular area of MAzoM at air–water interface by UV light irradiation and dark adaptation at 25 °C. Surface pressure was controlled at 23 mN/m

structure of MAzoM monolayer, photo-induced surface area changes of the monolayer were investigated under the constant surface pressure 23 ± 0.3 mN/m. Figure 6 shows the surface area changes of MAzoM monolayer upon UV light irradiation and dark adaptation at 25 °C. The monolayer shrunk by the UV light irradiation and expanded upon dark adaptation, and the photo-induced surface area changes were reversible. Moreover, the photo-induced changes in the surface area of MAzoM monolayer occurred concomitantly with the absorbance changes at 370 nm (Fig. 5). On the other hand, solution studies showed that the UV light irradiation did not affect the main chain α-helical structure of MAzoM, as confirmed by CD spectra. From these results, the photo-induced surface area changes of MAzoM monolayer could be explained as follows. The UV light irradiation induced a bending of the α-helical MAzoM rod via a photo-isomerization of the azobenzene moiety in the main chain from trans to cis configuration. The photo-induced bending structure of MAzoM molecule resulted in a reduction of the molecular area of MAzoM at air–water interface owing to a decrease in the distance between terminals of the molecule. An additional important finding was that the photo-induced changes in the area of MAzoM monolayer responded more slowly than the spectral changes. The photo-induced surface area changes may arise from the re-arrangement of the bending MAzoM molecules induced by the photo-isomerization of the azobenzene moiety in the main chain. The inter-molecular interaction of MAzoM molecules in the solid condensed monolayer may serve to slow down the rate of their re-arrangement process.

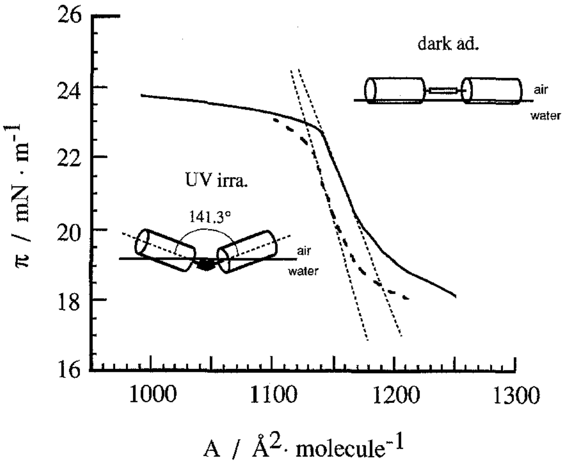


Fig. 7 Surface pressure–area isotherms for MAzoM under the UV light irradiation (– – – – –), and dark adaptation (——) at 25 °C

Figure 7 indicates π-A isotherms for solid condensed MAzoM monolayer below A = 1250 Å^2/molecule at 25 °C before and after UV light irradiation. The isotherm upon UV light irradiation was obtained by plotting the surface area per MAzoM molecule at photo-stationary state against the controlled surface pressure under the UV light irradiation. It can be seen, from the changes in shape of the π-A isotherm, that MAzoM monolayer became contracted owing to the bending of MAzoM molecule upon the UV light irradiation. That is to say, the UV light irradiation induced a decrease in the limiting area per MAzoM molecule from 1395 to 1316 Å^2/molecule which was obtained by extrapolation of the linear portion of the π-A isotherm to zero pressure. From the limiting area changes, we estimated a bending angle, θ, between the two α-helical rods of MAzoM molecule according to

$$A_{\mathrm{UV}} = A_{\mathrm{dark}} \cdot \sin(\theta/2) , \tag{1}$$

where A_{dark} and A_{UV} are the limiting area per MAzoM molecule before and after UV irradiation, respectively. The value of θ obtained from Eq. (1) was 141.3 degrees. It should be noted that the θ obtained from Eq. (1) is an average value of the bending angles of all MAzoM molecules in the monolayer, since the photo-conversion of the azobenzene moiety did not be considered.

In conclusion, the photo-stimulated geometrical changes of the azobenzene moiety of MAzoM can be amplified into macroscopic structural changes of the monolayer, expansion and contraction. Furthermore, it is suggested that the monolayer system for stimuli-responsive molecules provide major important information concerning the stimuli-induced structural changes of the individual molecule.

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