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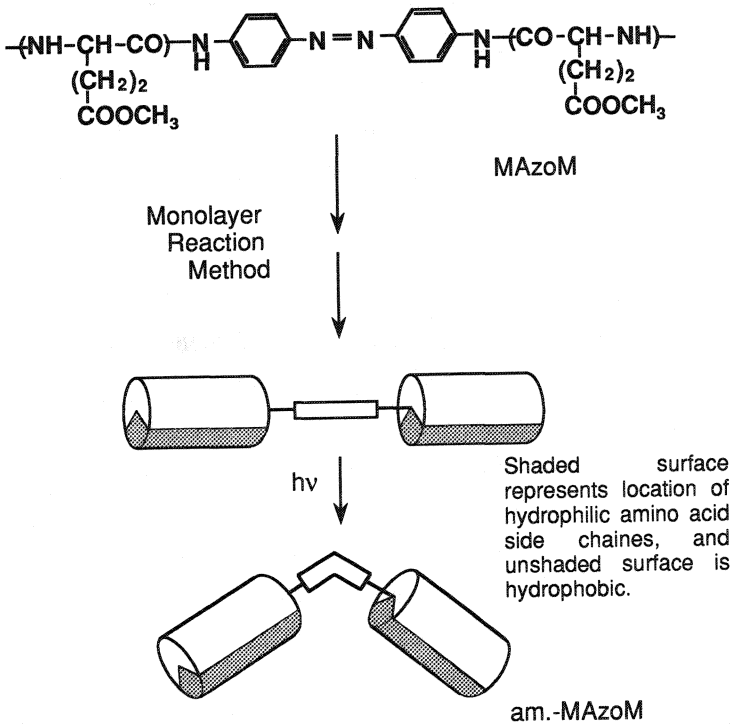
Photocontrol of Micellar Structure of an Azobenzene Containing Amphiphilic Sequential Polypeptide

Masahiro HIGUCHI,* Norihiko MINOURA, and Takatoshi KINOSHITA†
Department of Organic Materials, National Institute of Materials and Chemical Research, Tsukuba, Ibaraki 305
†Department of Materials Science & Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466

Photo-induced structural changes were observed for an aggregate composed of amphiphilic sequential polypeptides with an azobenzene moiety in the main chain. The polypeptides associated with each other and formed micelle in aqueous solution in the dark. Photo-isomerization of the azobenzene moiety of the polypeptides induced a deaggregation of the micelle, via the bending structure formation of the polypeptide at the azobenzene moiety.

Functions of biological membranes such as signal transduction, are closely related to high-order structural changes of membrane proteins induced by external stimulation.[1] During the past several years, photocontrol of structure and functions of membranes,[2] gels, [3] and micelle[4] containing azobenzene derivatives have been reported as a model of photoreceptor. More recently, amphiphilic α-helical polypeptides have been synthesized as an artificial protein and employed to build up a functional structure.[5]

We have already reported a bundle arrangement of amphiphilic α-helixes, which are prepared by a "Monolayer Reaction Method".[6] We report here on photo-induced structural changes of a bundle consisted of photoresponsive amphiphilic sequential polypeptides having an azobenzene moiety in the main chain



Scheme 1. Preparation of am.-MAzoM

prepared by the monolayer reaction method. Photoisomerization of theazobenzene moiety induced remarkable structural changes of the bundle consisted of amphiphilic sequential polypeptides.

The starting material, two poly(γ-methyl L-glutamate)s jointed with an azobenzene (MAzoM) was obtained by the polymerization of the N-carboxyanhydride of L-glutamic acid γ-methyl ester with p,p'-diaminoazobenzene as an initiator in dimethylformamide. The molar ratio of anhydride to initiator was 60. A number average molecular weight of 11000 was estimated from the molar ratio of the azobenzene moiety to the γ-methyl L-glutamate residues of MAzoM. The ratio was determined by the absorbance at 375 nm on the basis of the molar extinction coefficient of the azobenzene in MAzoM dimethylformamide solution. The photoresponsive amphiphilic sequential polypeptide (am.-MAzoM) was prepared by the selective saponification of MAzoM side chains according to the monolayer reaction method, which was described in detail elsewhere.[6a] A known amount of MAzoM dissolved in 1,2-dichloroethane was placed at the air-water interface in a hydrophobic vessel with a syringe. The area occupied by the monolayer was decreased by moving a hydrophobic barrier to form the solid condensed state of the monolayer. When the area per the monomer residue reached 15 Å, aqueous solution of potassium hydroxide was injected into the aqueous phase beneath the solid condensed monolayer to selectively saponify MAzoM side chains oriented into the aqueous phase (Scheme 1). The L-glutamic acid content of the am.-MAzoM was estimated to be 34 mol% from an NMR analysis.

Figure 1 shows the circular dichroism (CD) spectra of am.-MAzoM in trimethyl phosphate (TMP) and aqueous solution containing 0.1 mol dm^{-3} KCl at pH 6.9, respectively. The CD spectrum of am.-MAzoM in TMP exhibited the two negative bands at 208 nm and 222 nm typical of stable right-handed α-helix. On the other hand, the CD spectrum of am.-MAzoM in aqueous solution showed a remarkable variation, i.e.,

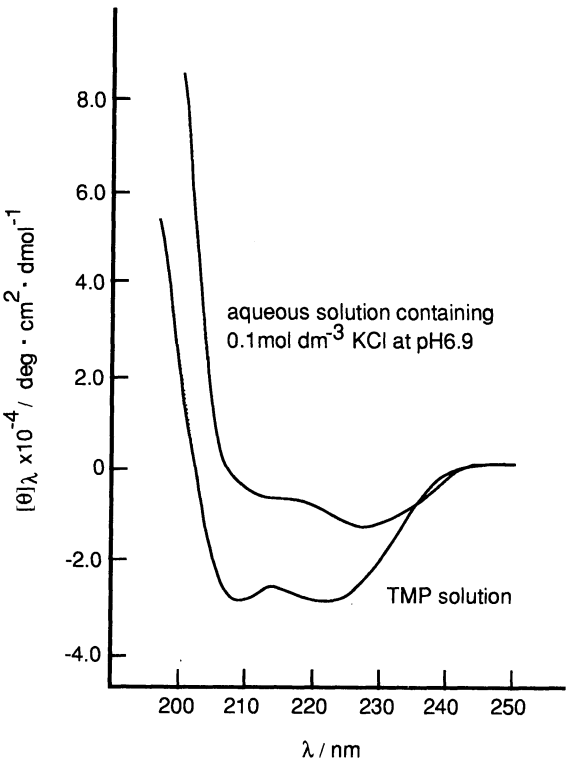


Fig. 1. CD spectra of am.-MAzoM in TMP and aqueous solution containing 0.1 mol dm^{-3} KCl at pH 6.9.

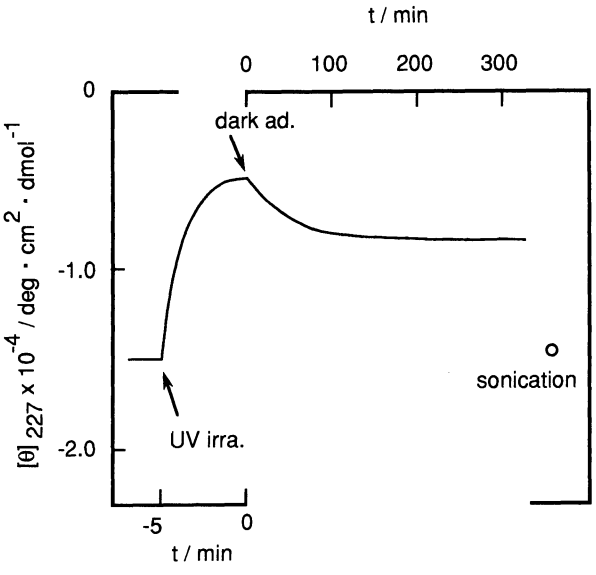


Fig. 2. Photo-induced changes in the minimum ellipticity,$[\theta]_{227}$ of am.-MAzoM in aqueous solution containing 0.1 mol dm^{-3} at pH 6.9.

the distortion of the spectrum with red shifting of 222 nm band toward 227 nm and decreases of 208 nm band. The same distortions of the spectra have been shown with aggregated α-helical polypeptides.[7] Thus, the am.-MAzoM has an α-helical conformation even in aqueous solution at pH 6.9 and the several amphiphilic α–helical rods are considered to form the micellar aggregate, which has hydrophilic exterior (glutamic acid side chains) and hydrophobic inner pore surrounded by methyl glutamate side chains, to dissolve in aqueous solution. It is also noted here, that a random copolypeptide containing the same amount of L-glutamic acid residues was insoluble in aqueous solution.

The trans to cis photoisomerization in azobenzene moiety of am.-MAzoM in aqueous solution containing 0.1 mol dm^{-3} KCl at pH 6.9 was induced by UV irradiation. On the base of the changes in absorbance at 374 nm, it was estimated that 60% of the trans form was converted into the cis isomers. Figure 2 shows the changes in $[\theta]_{227}$ of am.-MAzoM in aqueous solution containing 0.1 mol dm^{-3} KCl at pH6.9 upon UV irradiation and dark adaptation. During the UV irradiation, the $[\theta]_{227}$ value decreased accompanying a deaggregation of the micelle composed of am.-MAzoMs. After removal of the light, it increased and attained an equilibrium value within *ca.* 100 min, however, the original $[\theta]_{227}$ value could not be recovered. By the sonication of the am.-MAzoM aqueous solution in the dark the original $[\theta]_{227}$ value could be returned. These results suggested that the UV irradiation induced the denaturation of the amphiphilic character of am.-MAzoM owing to the photoisomerization of azobenzene moiety from trans to cis form resulting in the deaggregation of the micelle.

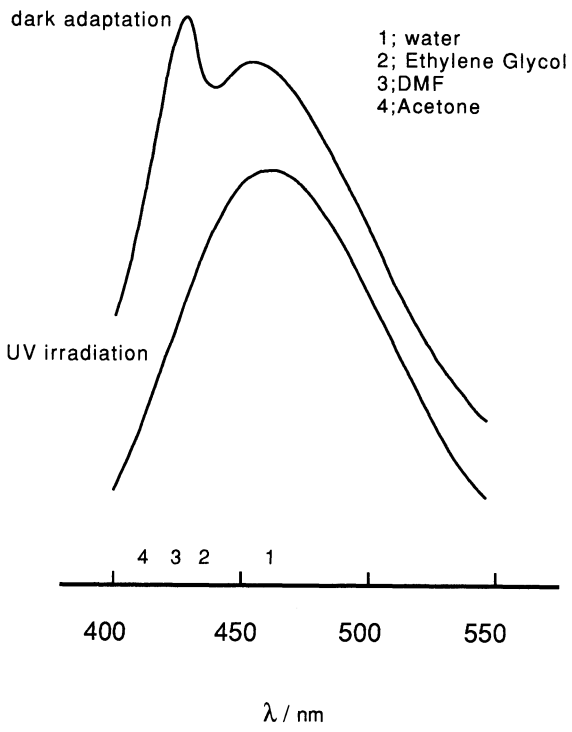


Fig. 3. Changes in fluorescence spectra of AN in am.-MAzoM aqueous solution containing 0.1 mol dm^{-3} at pH 6.9.

We elucidated the photoinduced micellar structure changes of am.-MAzoM by means of fluorescence spectroscopic measurements. It is well-known that fluorescence characteristics, such as emission maxima and fluorescence intensity, of anilinonaphthalene (AN) are very sensitive to the environmental polarity around AN.[8] The emission maxima of AN shifts to higher wavelength with increase solvent polarity. Figure 3 shows fluorescence spectra of AN in am.-MAzoM aqueous solution containing 0.1 mol dm^{-3} KCl at pH 6.9 before and after UV irradiation. The emission maxima of AN in various solvents are shown in this figure. The excitation wavelength of AN was 348 nm. Two emission maxima were observed at 429 nm and 455 nm. Environmental polarity of AN for the emission at 429 nm and 455 nm corresponded to that between acetone and dimethylformamide, and that of water, respectively. The former was assigned to the emission of AN in the hydrophobic internal pore of am.-MAzoMs micellar aggregate, and the latter in the external aqueous solution. On the other hand, the UV irradiation induced a noticeable difference in the fluorescence spectra, i.e., the

emission band at 429 nm vanished. This implies that the AN was released from the micelle consisted of am.-MAzoMs to the external aqueous solution phase. These results could be explained as follows. The denaturation of amphiphilic character of am.-MAzoM owing to the photoisomerization of the azobenzene moiety induced the deaggregation of the micelle, resulted in the distraction of hydrophobic inner pore.

In conclusion, the micellar structure of photoresponsive amphiphilic sequential polypeptides could be controlled by the photo-stimulation. This system may be employed in a photoresponsive protein model. Further studies, the photo-control of am.-MAzoMs structure in a lipid bilayer membrane are under investigation.

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