# Synthesis and Circular Dichroic and Photoresponsive Properties of a Graft Copolymer Containing an Azoaromatic Polypeptide Branch and Its Membrane

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ABSTRACT: Graft copolymers composed of a photoresponsive copolypeptide branch from β-benzyl L-aspartate and β-*p*-phenylazobenzyl L-aspartate ($C_1$Azo), attached to a poly(2-hydroxyethyl methacrylate) (PHEMA) backbone, were newly synthesized. The graft copolymers exhibited different circular dichroic properties in the solution in a mixture of 1,2-dichloroethane (DCE) and methanol from the corresponding branches in DCE. The effect of the addition of trimethyl phosphate to the solution upon the circular dichroisms was also different between the graft copolymers and the corresponding branches. Membranes immersed in DCE showed the same CD curves as those in the solution in a mixture of DCE and methanol. In the case of the graft copolymers containing $C_1$Azo moieties, a photoinduced conformational change of the polypeptide chains was observed in the membrane immersed in DCE as well as in the solution. The effect of trimethyl phosphate on the photoinduced conformational change was also observed both in the solution and in the membrane.

## Introduction

The photoresponsive conformational change of a polypeptide is of much interest in relation to the mechanism of vision and in view of the possible application to reversible photoresponsive materials. But most reports are concerned with the photoinduced conformational change of the polypeptide in the solution, and only a few investigations on the membrane have been performed. A membrane from cross-linked poly(L-glutamic acid) containing pararosaniline groups in the polymer side chain was prepared.[1] The membrane showed photoinduced changes in the secondary structure of the polypeptide in aqueous media at weak alkaline pH. Another photoresponsive porous membrane with adsorbed poly(L-glutamic acid) containing azobenzenesulfonate moieties on the pore wall was reported.[2] Photoirradiation induced the irreversible helix to coil transition of the adsorbed poly(L-glutamic acid) with the photoisomerization of azobenzenesulfonate moieties.

In a solid membrane the occurrence of an efficient conformational change by photoirradiation is considered to be difficult. However, a conformational change in polymer chains of the membrane is expected in a system in which the polymer chains form a domain structure by microphase separation in the membrane. In an appropriate solvent, the polymer chains in the domain can be mobile as in solution, in the solid, insoluble membrane as a whole.

Recently, we have prepared a new polymer membrane containing a polypeptide microdomain from polyvinyl/polypeptide graft copolymers. A microdomain from a hydrophilic polypeptide such as poly(L-glutamic acid) forms penetrated permeating pathways across the membrane supported by a hydrophobic vinyl polymer such as poly(butyl methacrylate) in water. When the membrane is immersed in water, a good solvent for the polypeptide but not for the vinyl polymer, the conformational states of the polypeptide in the membrane could be controlled by the effect of pH,[3a-c] divalent ion,[3d] urea,[3e] and ammonium salt.[3f] The permeability of the substrates such as phenylethane-1,2-diol, glucose, and raffinose across the membrane was able to be regulated by these external stimuli.

In the present study new polyvinyl/polypeptide graft copolymers were synthesized, carrying as the branch the polymer of aspartic ester of an azobenzene derivative, which is known to undergo a photoresponsive conformational change in an organic solution.[4] The circular dichroic properties of the graft copolymers in the solution and in the membrane and the conformational change of the polypeptide chain on photoirradiation were investigated. As the photoresponsive polypeptide, the copolypeptide consisting of *β-phenylazobenzyl* ($C_1$Azo) L-aspartate and β-benzyl L-aspartate (BLA) was used. Poly(2-hydroxyethyl methacrylate) (PHEMA) was employed as the backbone because 1,2-dichloroethane (DCE), in which the copolypeptides are known to undergo a photoinduced conformational change, is a poor solvent for PHEMA, so that the membrane was stable in DCE. This paper describes the first example of the photoinduced conformational change of polypeptide chains in the membrane from the novel polyvinyl/polypeptide graft copolymers containing pendant azobenzene moieties.

## Experimental Section

**Materials**: The synthetic route of the graft copolymers is summarized in Scheme I. Synthesis of *N*-methyl-*N*-(4-vinylphenethyl)ethylenediamine (1) was reported previously.[5] 2-Hydroxyethyl methacrylate (HEMA) was distilled under reduced pressure. β-Benzyl L-aspartate *N*-carboxylic anhydride (BLA-NCA, **2**),[6] β-(4-phenylazobenzyl) L-aspartate *N*-carboxylic anhydride ($C_1$Azo-NCA, **3**)[7,8] were synthesized according to the literature. *N*,*N*-Dimethylformamide (DMF) was distilled over potassium hydroxide under reduced pressure. The other chemicals such as DCE, methanol, and trimethyl phosphate (TMP) of reagent grade were used without further purification.

**Copolymerization of 1 and HEMA. Synthesis of the Backbone Copolymer 4.** Radical copolymerization of 1 and HEMA was carried out in a sealed glass tube at 45 °C with 2,2′-azobis(2,4-dimethylvaleronitrile) (V-65) as the initiator and ethanol as the solvent (30 mL). After a definite period of time (1.5 h), the tube was opened and the contents were poured into a large excess of ether. The precipitated white, powdery polymer was dried under reduced pressure. The obtained polymers are summarized in Table I. The content of the unit of 1 in copolymer 4 was determined from the area ratio of the signal of $-COOCH_2-$ of HEMA to the signal of phenylene protons of 1 in the $^1H$

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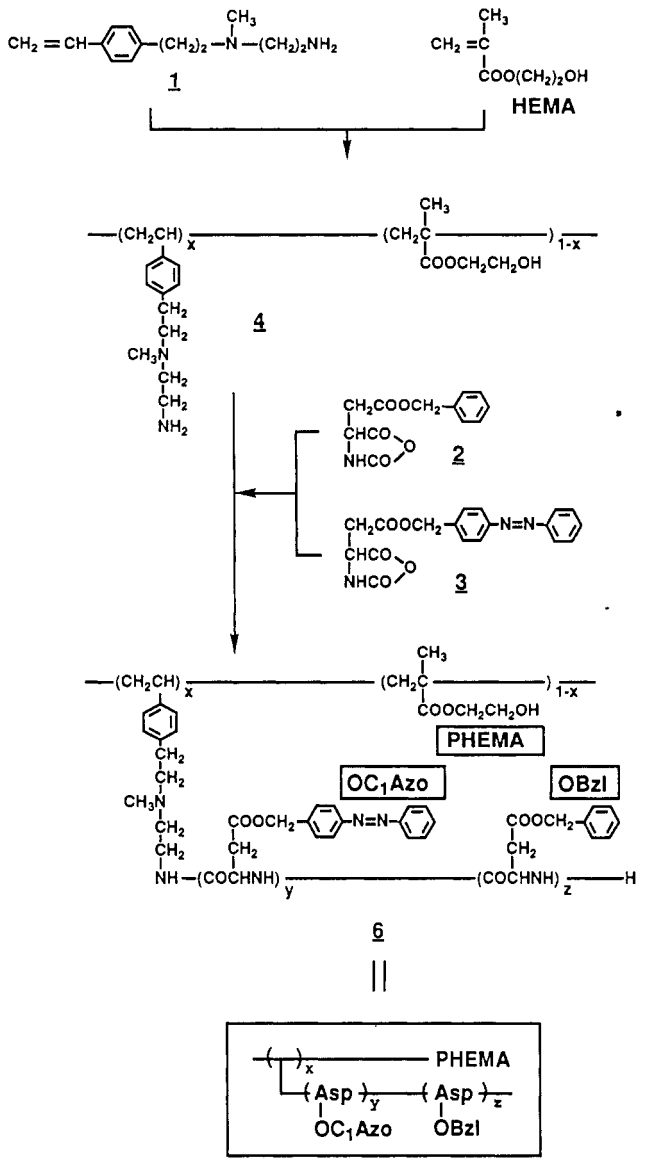
**Scheme I**




NMR spectrum in DMSO-$d_6$. The intrinsic viscosity ([$\eta$]) of **4** was about 0.5 dL·g$^{-1}$ (methanol, 25 °C). The synthesis of the backbone copolymer **5** (content of **1** = 0.008) composed of **1** and butyl methacrylate was reported previously.[3b]

**Grafting Reaction: Attachment of the Polypeptide Branch.** The synthesis of graft copolymers **6** and **7** was carried out by the copolymerization of BLA-NCA (**2**) and $C_1$Azo-NCA (**3**) initiated by the primary amino group of the backbone copolymer **4**. To a solution of **4** (1 g, content of primary amino group is $8.4 \times 10^{-3}$ to $3.2 \times 10^{-2}$ mmol) in DMF (10 mL) was added DMF solutions of **2** and **3** (2–4 mL, total NCA concentration = 0.2 M), and the mixture was stirred at 35 °C until the disappearance of absorptions of the anhydride due to NCA in the IR spectrum, for about 5 days. The reaction mixture was homogeneous throughout the reaction. Then the reaction mixture was poured into a large excess of ether, and the precipitated yellow, powdery polymer was filtered off and dried under reduced pressure. The average degree of polymerization of the poly(amino acid) chains and the composition of the units from **2** and **3** were determined by the $^1$H NMR spectrum in $CF_3$-COOD; since the content of **1** in **4** was known, the average number of amino acid residues in a branch of the graft copolymers was estimated by using the area ratio of the signal of $-COOCH_2-$ of HEMA in the backbone, that of $-COOCH_2-$ in the aspartate, and that of total aromatic protons. The estimated contents of the azobenzene moieties from $^1$H NMR were identical with those calculated from the molar extinction coefficients ($\epsilon$) of the *trans*-azobenzene moieties[4] in the dark adapted product.

A copolypeptide corresponding to the branch **8** was prepared from **2** and **3** initiated by **1** under similar conditions described above. The graft copolymers **9** and **10** with poly(butyl methacrylate) (PBMA) as the backbone were synthesized by using **5** instead of **4** under similar conditions described above.

**Turbidimetry.** The polymer was dissolved in a mixture of DCE and methanol (1:1 (v/v)) in 2 wt %. The obtained transparent solution (0.2 mL) was added to a mixture (3.8 mL) of DCE and methanol of various compositions. Turbidity was determined by the transmittance at 600 nm of the freshly obtained solution (light path length = 10 mm).

**Preparation of Membrane.** A yellow and transparent membrane was obtained by casting the DMF solution of the graft copolymer on a flat quartz plate (thickness = 3 mm) followed by evaporation in vacuo.

**Photoirradiation.** A quartz plate attaching the membrane was placed in a quartz cell (light path length = 10 mm) filled with DCE, and irradiation was carried out with a 300-W Xe lamp (ILC Cermax LX-300F): for light of 330–350 nm a Corning 7-37 filter was used and for light of 430–450 nm Corning 3-74 and 7-59 filters were used.

**Measurements.** $^1$H NMR spectra were recorded on JEOL GX-400 and GSX-270 instruments. IR spectra were recorded on a Hitachi 260-30 spectrophotometer. UV/vis spectra and transmittance were recorded on JASCO UVDEC-1 and U-best 50 spectrophotometers. Circular dichroism (CD) spectra were obtained by using a JASCO J-500 spectropolarimeter. The concentrations of the polymer solutions were 1.2–1.6 g·L$^{-1}$. On the irradiated solutions and membranes, special care was taken in order to accomplish the spectral measurements quickly. No structural and conformational changes are considered to occur during the measurements since the UV/vis spectra just before and after the measurement of the CD spectrum were identical.

## Results and Discussion

**Synthesis of Graft Copolymers.** Graft copolymers and the compounds corresponding to the branches synthesized are listed in Chart I. The graft copolymers with PHEMA as the backbone (**6** and **7**) were insoluble in 1,2-dichloroethane (DCE) but soluble in a mixture of DCE and methanol and could form membranes stable in DCE. In the graft copolymerization, the hydroxyl group of the backbone copolymer **4** is not considered to initiate reaction because no reaction was found to occur between HEMA and BLA-NCA (**2**) under the same conditions. The formation of a graft copolymer, but not the mixture of **4** and copoly(aspartate), was confirmed by turbidimetry. Turbidity of the product in the mixture of chloroform and methanol was quite different from the blend of the corresponding homopolymers, as exemplified in Figure 1 (**6c**). In the case of the graft copolymer **6c** composed of PHEMA and poly(aspartate) (degree of branching ($x$) = 0.011, $\overline{DP}$ ($y + z$) = 70, $C_1$Azo content ($y/(y + z)$) = 0.81), turbidity appeared only in the high content of chloroform or methanol in the mixed solvent. On the other hand, the blend of polymers **4a** and **8** (composition in weight and chain length of copoly(aspartate) were identical between the blended polymer and the graft copolymer) showed turbidity also in a 3:7 (v/v) mixture of methanol and chloroform. All the products obtained by reacting **2**, **3**, and **4** showed similar turbidimetry curves to Figure 1a. Thus, these products were confirmed to be the graft copolymers with the polypeptide branch linked at the primary amino group of **4**.

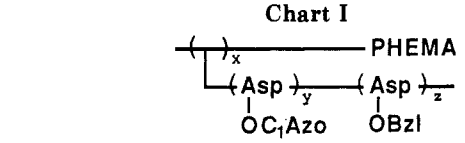
**Circular Dichroism of a Solution of Graft Copolymers.** In order to examine the conformation of the polypeptide branch of the graft copolymers, circular dichroism (CD) spectra of the solution were measured. The graft copolymers with PHEMA as the backbone (**6**) exhibited different CD associated with the amide n–$\pi^*$ tran-

**Table I**
**Radical Copolymerization of 1 and HEMA**

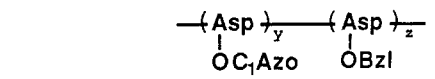
| | monomers | | initiator | | | degree of branching, | |
|---|---|---|---|---|---|---|---|
| | 1, mg | HEMA, $cm^3$ | V-65, mg | product, g | yield, % | $x$,[a] % | $[\eta]$,[b] $dL \cdot g^{-1}$ |
| **4a** | 78 | 9.9 | 18 | 1.5 | 15 | 1.1 | 0.57 |
| **4b** | 126 | 3.9 | 37 | 1.2 | 29 | 4.4 | 0.51 |
| **4c** | 61 | 3.7 | 37 | 0.76 | 19 | 2.3 | *c* |
| **4d** | 39 | 5.0 | 16 | 0.80 | 16 | 0.5 | *c* |
| **4e** | 54 | 7.0 | 17 | 1.7 | 24 | 1.3 | *c* |

[a] Estimated by $^1H$ NMR. [b] Methanol, 25 °C. [c] Not measured.

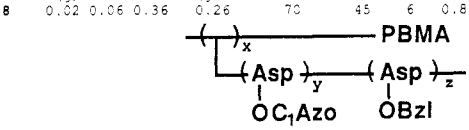
**Chart I**




| | backbone | | NCA (g) | | product | yield | x | y | z | y/(y+z) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | (g) | 2 | 3 | (g) | (%) | | | | |
| 6a | 4c | 0.50 | 0.15 | 0.77 | 1.07 | 82 | 0.023 | 19 | 6 | 0.76 |
| 6b | 4b | 0.13 | 0.07 | 0.47 | 0.45 | 75 | 0.044 | 31 | 9 | 0.79 |
| 6c | 4a | 0.27 | 0.09 | 0.51 | 0.65 | 82 | 0.011 | 57 | 13 | 0.81 |
| 6d | 4d | 0.66 | 0.07 | 0.31 | 0.75 | 75 | 0.005 | 28 | 14 | 0.67 |
| 7 | 4e | 1.76 | 1.74 | - | 2.62 | 75 | 0.013 | - | 40 | - |




| | 1 (g) | NCA (g) 2 | 3 | product (g) | yield (%) | y | z | y/(y+z) |
|---|---|---|---|---|---|---|---|---|
| 8 | 0.02 | 0.06 | 0.36 | 0.26 | 70 | 45 | 6 | 0.88 |




| | backbone | | NCA (g) | | product | yield | x | y | z | y/(y+z) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | (g) | 2 | 3 | (g) | (%) | | | | |
| 9 | 5 | 0.33 | 0.03 | 0.13 | 0.32 | 70 | 0.008 | 26 | 13 | 0.68 |
| 10 | 5 | 1.30 | 0.70 | - | 1.46 | 73 | 0.008 | - | 40 | - |

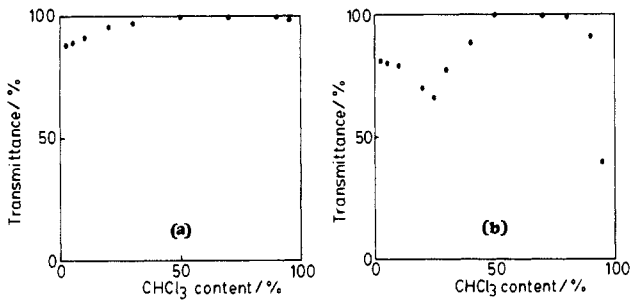



**Figure 1.** Turbidimetric curves of (a) graft copolymer **6c** and (b) a mixture of **4a** and **8**, in a mixture of chloroform and methanol.

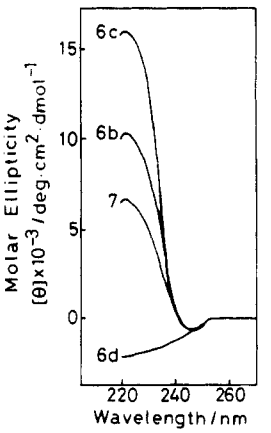



**Figure 2.** CD spectra of graft copolymers **6** and **7** in a mixture of DCE and methanol (9:1 (v/v)).

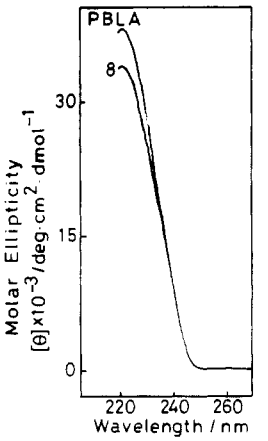



**Figure 3.** CD spectra of polypeptides **8** and PBLA, corresponding to the branches of the graft copolymers, in DCE.

sition from the corresponding branches.

The CD spectra of the solution of the graft copolymers in a mixture of DCE and methanol (9:1 (v/v)) and the corresponding branches in DCE are shown in Figures 2 and 3, respectively.

The copolypeptides **8** corresponding to the branches of **6b** exhibited circular dichroism characteristic of a left-handed $\alpha$-helix (molar ellipticity at 222 nm ($[\Theta]_{222}$) = $3.4 \times 10^4$). Poly($\beta$-benzyl L-aspartate) (PBLA) was found to show a circular dichroism of a left-handed $\alpha$-helix in DCE similar to that in chloroform (Figure 3). The graft copolymer **9** with PBMA as the backbone also exhibited a typical circular dichroism of a left-handed $\alpha$-helix ($[\Theta]_{222} = 3.0 \times 10^4$).

In contrast, the graft copolymers with PHEMA as the backbone (**6b** and **6c**) exhibited a weak positive band at 222 nm and a very weak negative band at 245 nm (Figure 2). The graft copolymer **7** composed of PBLA as the branch and PHEMA as the backbone in a mixture of DCE and methanol showed a CD curve (Figure 2) similar to that of the other graft copolymers with PHEMA as the backbone, while the graft copolymer **10** with PBMA as the backbone exhibited a circular dichroism of a left-handed $\alpha$-helix in DCE. Methanol in the amount of 10%, which is required to dissolve the graft copolymers with PHEMA as the backbone, is not considered to influence the conformation of the polypeptide chain, since the addition of 10% methanol to the solution of the corresponding branches in DCE did not change the CD spectra. Furthermore, no spectral changes were observed when the methanol solution of PHEMA was added to the DCE solution of the branch **8** in an amount corresponding to the composition, in weight, of the graft copolymer **6b**.

These results indicate an interaction between the hydroxyl group of PHEMA as the backbone and the polypeptide chain as the branch which lie closely together due to the covalently linked structure. The decrease in the ellipticities of the graft copolymers from those of the

Table II
Molar Ellipticities of Polymers in a DCE–Methanol Mixture (9:1 (v/v))

| | polymer (branch) | $10^3[\theta]_{222}$ TMP content, % 0 | 12.5 | 75 |
|---|---|---|---|---|
| graft copolymers with PHEMA as the backbone | **6b** (79% $C_1$Azo)[a] | +11 | +11 | +1.1 |
| | **6c** (81% $C_1$Azo) | +16 | +16 | +6.8 |
| | **7** (PBLA branch) | +6.6 | +7.3 | 0.0 |
| corresponding branches | **8** (88% $C_1$Azo) | +34 | +26 | +14[c] |
| | PBLA[b] | +38 | +31 | -41 |
| graft copolymers with PBMA as the backbone | **9** (68% $C_1$Azo) | +30 | +26 | +17[c] |
| | **10** (PBLA branch) | +25 | +21 | -29 |

[a] $C_1$Azo = phenylazobenzyl aspartate. [b] $\overline{DP}$ = 40. [c] $[\theta]_{215}$.

corresponding branches indicates that a part of the branch of the graft copolymer takes a random conformation because of the interaction with PHEMA. The other part of the branch that is not interacting with PHEMA is considered to take a specific secondary structure such as the α-helix. Since the graft copolymer **6d** with a low degree of branching and a relatively short branch did not show any peak around 220–270 nm, all of the polypeptide chain in **6d** is considered to interact with PHEMA.

**Solvent Effect on CD Spectra.** It is well-known that the conformation of polypeptide is much dependent of the nature of solvents. We investigated the effect of trimethyl phosphate (TMP) which is known to induce the right-handed α-helix for PBLA[9] or the azo-modified PBLA.[10,11]

The effect of solvent on circular dichroism was also different between the graft copolymers with PHEMA as the backbone (**6**) and the corresponding branches (**8**). The changes in the molar ellipticities at 222 nm ($[\theta]_{222}$) by the addition of TMP in 12.5% and 75% are summarized in Table II.

The effect of the addition of 12.5% TMP to the solutions of **6b** and **6c** in a mixture of DCE and methanol (9:1 (v/v)) was different from that on the solution of the corresponding branch **8** in DCE. On the addition of 12.5% TMP to the solution of the branch **8**, a decrease in $[\theta]_{222}$ by 23% was observed similar to that of PBLA. A similar decrease in $[\theta]_{222}$ was observed for the graft copolymers **9** and **10** with PBMA as the backbone in DCE. On the other hand, the addition of 12.5% TMP to the solutions of **6b** and **6c** in a mixture of DCE and methanol (9:1 (v/v)) did not change the ellipticities at 222 nm. In the case of the graft copolymer **7** with PBLA as the branch, the addition of 12.5% TMP caused an 10% increase in $[\theta]_{222}$. The small amount of TMP added to the solution of the graft copolymer with PHEMA as the backbone is considered to influence the interaction between the polypeptide branch and PHEMA and to interact more strongly with PHEMA than the polypeptide chain.

In contrast to the case in PBLA, the addition of 75% TMP to the DCE solution of the $C_1$Azo branch (**8**) (88 mol % azo) brought about a further decrease in $[\theta]_{222}$ but did not induce the inversion of the helix sense and caused the blue shift of the amide n–π* transition by 7 nm. Similar behaviors were also observed in the graft copolymer **9** with PBMA as the backbone. Since the helix sense of the copolyaspartate composed of 8 mol % of $C_1$Azo and 92 mol % of BLA is known to be reversed on the addition of more than 35% of TMP,[11] TMP is considered to interact with the $C_1$Azo residue rich in **8** (88 mol %) rather than with the polypeptide, so that the sign of $[\theta]_{222}$ of **8** was not reversed on the addition of 75% TMP.

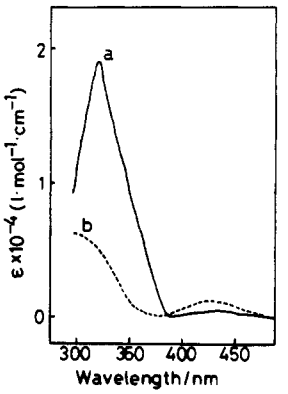



**Figure 4.** Effect of irradiation on absorption spectrum of graft copolymer **6c** in a mixture of DCE and methanol (9:1 (v/v)).

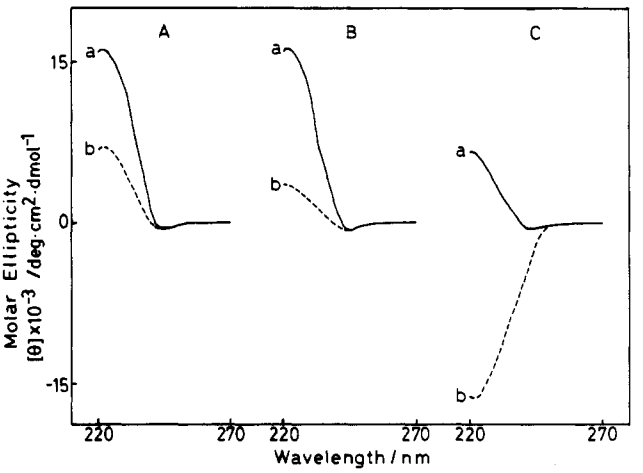



**Figure 5.** Effect of irradiation on CD spectrum of graft copolymer **6c** in a mixture of DCE and methanol (9:1 (v/v)), in the presence of TMP: (a) before UV irradiation and after visible light irradiation; (b) after UV irradiation. TMP content: (A) 0%; (B) 12.5%; (C) 75%.

**Photoirradiation of a Solution of Graft Copolymers.** UV irradiation of the solution (mixture of DCE and methanol (9:1 (v/v)) of the graft copolymers with the copolyaspartate branch containing about 80 mol % of the $C_1$Azo group (**6b** (degree of branching = 0.044, $\overline{DP}$ = 40) and **6c** (degree of branching = 0.011, $\overline{DP}$ = 70)) caused a decrease in the absorption at 323 nm due to the *trans*-azobenzene moieties from $\epsilon = 1.9 \times 10^4$ to $6.0 \times 10^3$ within 5 min and an increase in the absorption at 430 nm due to the cis structure (Figure 4, **6c**).

At the same time, the molar ellipticity at 222 nm was reduced from $1.6 \times 10^4$ to $6.7 \times 10^3$ (Figure 5A, **6c**). The original value in the absorption and CD spectra were reproduced by irradiation with visible light for about 2 h, and thus these changes were fully reversible. On the other hand, the graft copolymers with a shorter branch (**6a**, degree of branching = 0.023, $\overline{DP}$ = 25, azo content = 76 mol %) or a lower degree of branching (**6d**, degree of branching = 0.011, $\overline{DP}$ = 40, azo content = 67 mol %) exhibited the photoisomerization of the azobenzene moieties, but no change in CD spectra were observed with the isomerization. Taking into account the changes in the absorbance in **6a–d** around 222 nm between *trans*- and *cis*-azobenzene moieties, the changes in CD spectra in **6b** and **6c** around 222 nm are considered to be due to the conformational change of the polypeptide chain. Detectable induced CD associated with π–π* transitions of the azo aromatic side chain was observed only in **6b** and **6c** ($\overline{DP}$ = 40 and 70, respectively), and the maxi-

mum ellipticities were $2.1 \times 10^3$ at 340 nm. On UV irradiation, the induced CD decreased accompanying the decrease in the absorption at 323 nm. In all cases examined, no specific informations about the conformational variations of the polypeptide chain were obtained from the side-chain CD bands.

On the basis of the considerations about the influence of TMP on the interaction between the polypeptide and PHEMA chains, the CD spectral change of the solution containing TMP on photoirradiation is expected to be different from that of the solution without TMP.

Copolypeptides composed of BLA and β-*m*-phenylazobenzyl L-aspartate (azo content = 9–92 mol %)[10] or of BLA and β-*p*-phenylazobenzyl L-aspartate (azo content = 8 mol %)[11] are known to undergo conformational changes on UV irradiation in a mixture of DCE and TMP, while no or little conformational changes occur without TMP. Figure 5 shows the change in the CD spectra of the solution (DCE + methanol + TMP) of the graft copolymer **6c** before and after photoirradiation at various contents of TMP. The photoisomerization of the azobenzene moieties on irradiation occurred concurrently with the changes in the CD spectra similar to those in a mixture of DCE and methanol.

An increasing TMP content caused the increase in the magnitude of the variation in $[\theta]_{222}$ on irradiation, and an inversion of the sign of $[\theta]_{222}$ on irradiation was eventually achieved at the addition of 75% TMP, in the graft copolymers **6b** ($\overline{DP}$ = 40) and **6c** ($\overline{DP}$ = 70) as well as the corresponding branch 8 ($\overline{DP}$ = 51).[12] In the case of **6c**, the isomerization of the azobenzene moieties from trans to cis and a decrease in $[\theta]_{222}$ on UV irradiation were fast at the content of 75% TMP as well as without TMP and reached the photostationary state within 5 min. The full recovery of the *trans*-azobenzene structure and of the original value of $[\theta]_{222}$ on the visible light irradiation was completed within 5 min at a TMP content of 75%, in contrast to the requirement of 2 h for the solution without TMP.

In the case of the graft copolymer **6b** ($\overline{DP}$ = 40), the photoisomerization (trans → cis and cis → trans) of the azobenzene moieties and the simultaneous conformational changes were fast enough and were completed within 15 min with full recovery at any content of TMP.

In the corresponding branch (8, $\overline{DP}$ = 51), the changes in CD spectra upon irradiation completed within 10 min, however, were not fully reversible in a mixture of DCE and TMP (TMP content = 12.5–75%) as shown in Figure 6. On the addition of TMP, the irradiation of visible light caused about 90% recovery of the trans structure of the azobenzene moieties within 10 min, and a longer irradiation did not cause the further change. Since the isomerization process is reported to be completely reversible in the copolyaspartate composed of 8 mol % of $C_1$-Azo and 92 mol % of BLA in the mixture of DCE and TMP,[11] the irreversible process observed in 8 rich in $C_1$-Azo residues (88 mol %) can be interpreted in terms of the interaction between the $C_1$Azo residue and TMP as discussed for the effect of TMP on the conformation of 8.

In this respect, it is of interest that full recovery of absorption and CD spectra could be achieved for the graft copolymer **6c** (degree of branching = 0.011, $\overline{DP}$ = 70) in the presence of TMP. TMP is considered to interact more strongly with PHEMA than with the $C_1$Azo group to keep the polypeptide chains mobile for the reversible conformational changes.

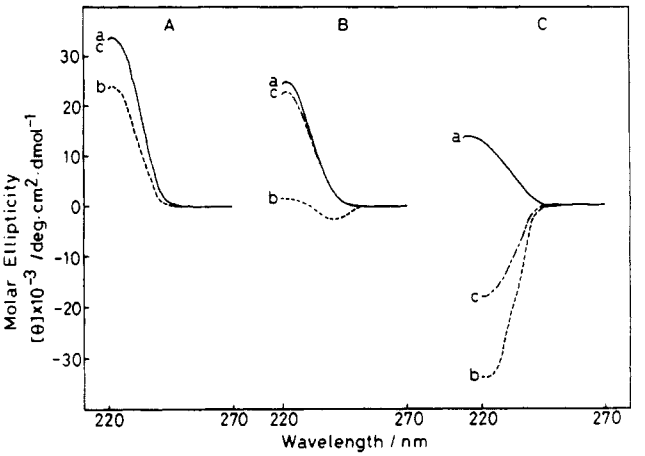



**Figure 6.** Effect of irradiation on CD spectrum of polypeptide 8, corresponding to branch of **6c**, in DCE in the presence of TMP: (a) before UV irradiation, (b) after UV irradiation, (c) after visible light irradiation. TMP content: (A) 0%; (B) 12.5%; (C) 75%.

Since the addition of any amount of TMP to the solution of the graft copolymer **6b** (degree of branching = 0.044, $\overline{DP}$ = 40) did not influence the rate of the photoisomerization and the simultaneous conformational change in contrast to **6c**, the polypeptide chains of **6c** is considered to be more strongly interacted with PHEMA than **6b** because of the lower degree of branching than **6b** and/or of the higher degree of polymerization of the polypeptide than **6b**.

**Photoirradiation of the Membrane of a Graft Copolymer.** The CD profiles of the membrane immersed in DCE of the graft copolymers **6b** and **6c** immersed in DCE were the same as those in the solution in a mixture of DCE and methanol (9:1 (v/v)). Since no change in CD spectra was observed by the rotation of the quartz plate with the membrane on the CD measurement, no linear dichroisms are considered to affect the CD spectra.

UV and visible light irradiation of the membrane of the graft copolymers **6b** and **6c** exhibited similar conformational changes with the photoisomerization of the azobenzene moieties as observed in the solution.

Upon UV irradiation of the membrane of the graft copolymers **6b** and **6c** with the polyaspartate branch containing about 80 mol % of the $C_1$Azo group, immersed in DCE, the absorption at 323 nm was reduced to 33% in strength with an increase in the absorption at 430 nm within 5 min, indicating photoisomerization of the azobenzene moieties (Figure 7, **6c**). At the same time, the ellipticity at 222 nm in the CD spectra was reduced to 30% of the original value (Figure 8A, **6c**). The original forms of the absorption and the CD spectra were reproduced by irradiation with visible light for about 2 h; thus these changes in the absorption and the CD spectra were fully reversible also in the membrane.

On the addition of 12.5–75% TMP to DCE in which the membrane of **6b** or **6c** was immersed, the photoisomerization and the simultaneous conformational change of the polypeptide chains were observed similarly as in the solution (Figure 8, **6c**). The change in CD spectra on the photoirradiation in the presence of TMP was fully reversible as observed similarly in the solution.

On the other hand, the graft copolymers with a shorter branch (**6a**) or a lower degree of branching (**6d**) exhibited no change in the CD spectra with isomerization in the solution or in the membrane under the conditions described above.

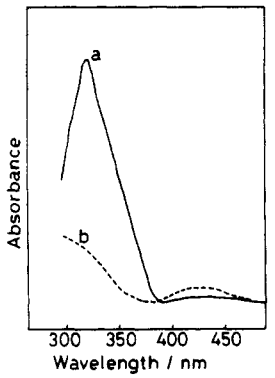



**Figure 7.** Effect of irradiation on absorption spectrum of membrane of graft copolymer **6c** immersed in DCE: (a) before UV irradiation and after visible light irradiation; (b) after UV irradiation.

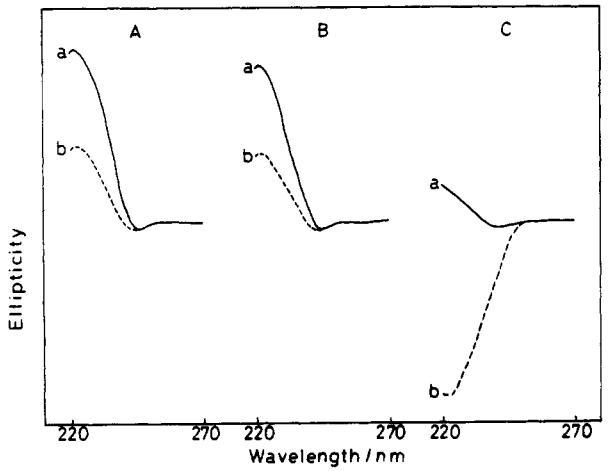



**Figure 8.** Effect of irradiation on CD spectrum of membrane of graft copolymer **6c** immersed in DCE in the presence of TMP: (a) before UV irradiation and after visible light irradiation; (b) after UV irradiation. TMP content: (A) 0%; (B) 12.5%; (C) 75%.

## Conclusion

In the membrane from the graft copolymers **6b** and **6c** with the $C_1$Azo polyaspartate branch immersed in DCE or in a mixture of DCE and TMP, the fully reversible conformational change of the polypeptide chain accompanied with the trans–cis photoisomerization of the attached azobenzene moieties was observed. This is the first example of the reversible conformational change of the polypeptide in the membrane stable in organic media from a graft copolymer, brought about by the photoisomerization of the pendant azobenzene moiety. The polypeptide chain in the membrane exhibited a similar conformational change as observed in the solution, though the membrane was stable in DCE or in a mixture of DCE and TMP. Thus, the graft copolymer structure was demonstrated to be effective in inducing an efficient conformational change in a solid membrane by photoirradiation.

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## References and Notes

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(8) The melting point of **3** did not agree with that in the literature.[7] By the recrystallization of the crude product four times from tetrahydrofuran (distilled from sodium–benzophenone ketyl just before use) and hexane (distilled from sodium–benzophenone ketyl), the melting point was 186–187 °C (lit. mp 240–240.5 °C from ethyl acetate–hexane.) Anal. Calcd for $C_{16}H_{15}N_3O_5$ (**3**): C, 61.19; H, 4.28; N, 11.89. Found: C, 61.16; H, 4.31; N, 11.82. Recrystallization from ethyl acetate (distilled over phosphorus pentoxide) and hexane gave the product with the same melting point as that from tetrahydrofuran–hexane. Since further recrystallization caused no change in the melting point and the IR spectral data agreed with those in the literature, the obtained product was used for the subsequent reactions.
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(13) In the literature,[4] a copolypeptide composed of β-benzyl L-aspartate and β-*p*-phenylazobenzyl L-aspartate ($\overline{DP}$ = 40, azo content = 81 mol %) was reported to show the inversion of the helix sense ($[\Theta]_{222} = 2.9 \times 10^4 \rightarrow -1.9 \times 10^4$) on UV irradiation in DCE. The copolypeptide was reported to be almost insoluble in DCE, and the soluble part was used for the CD measurements. In our experiment, the copolypeptide **8** ($\overline{DP}$ = 51, azo content = 88 mol %) was fully soluble in DCE, and the inversion of the helix sense on UV irradiation was not observed ($[\Theta]_{222} = 3.4 \times 10^4 \rightarrow 2.3 \times 10^4$ on UV irradiation).