Photoregulation of Permeability across a Membrane from a Graft Copolymer Containing a Photoresponsive Polypeptide Branch

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Abstract: The first example of the photoregulation of the permeability across a membrane containing a polypeptide by photoinduced conformational change of the polypeptide chains without any concomitant change in electrostatic repulsion along the polypeptide chain was achieved. A new polyvinyl/polypeptide graft copolymer composed of a photoresponsive copolypeptide branch from β-*p*-phenylazobenzyl L-aspartate and β-benzyl L-aspartate attached to a poly(butyl methacrylate) backbone was synthesized and the membrane was prepared. The permeation rate of mandelic acid across the membrane immersed in trimethyl phosphate (TMP) was raised by about six times with UV irradiation and was suppressed on irradiation with visible light. The permeation rates of other polar and nonpolar substrates such as *N*-((benzyloxy)carbonyl)-D,L-alanine, acetone, and biphenyl were also raised with UV irradiation and were suppressed on irradiation with visible light. The photoinduced permeability change thus observed is correlated to the conformational change of the polypeptide chains in the membrane. In the CD spectrum of the membrane immersed in TMP, the UV irradiation changed the sign of the CD band associated with the peptide n–π* transition from positive at 215 nm to negative at 222 nm within 15 min, and the original CD profile was recovered by irradiation with visible light within 5 min or by standing in the dark for about 5 days, indicating the inversion of the helix sense of the polypeptide chains in the membrane from left-handed to right-handed with UV irradiation and the reversion on irradiation with visible light or dark adaption.

Conformational change of a polypeptide controlled by external stimuli is known to govern biological functions such as transport properties across a membrane[1] or the mechanism of vision.[2,3] The chromophore of rhodopsin, the photoreceptor protein in the vertebrate eye, is 11-*cis*-retinal. The primary event in visual excitation is the isomerization of 11-*cis*-retinal to the *all-trans* form. Photoexcited rhodopsin triggers an enzymatic cascade leading to the hydrolysis of guanosine 3′,5′-phosphate and the consequent closure of cation-specific channels in the plasma membrane. The blockage of the influx of more than 10^6 Na^+ ions by a single photon results in the hyperpolarization of the plasma membrane, which is passively communicated to the synapse.[3] Thus, it is of much interest to construct a system in which a change in permeability across a membrane is achieved by a conformational change of the polypeptide in the membrane induced by the photoisomerization of the chromophore attached to the polypeptide. However, studies on photoresponsive conformational change of the polypeptide have been concerned with the conformational change in the solution, and investigations for a membrane have been performed much less than those for solutions. There have been only a few reports[4-6] concerning the photoregulation of permeability across a membrane by the regulation of the conformational state of the polypeptide in the membrane with irradiation. Permeability of water through a membrane composed of poly(L-glutamic acid) containing the pendant azobenzenesulfonate group[4] was reported to be irreversibly changed and was accompanied by photoinduced conformational change of the polypeptide. Permeability of potassium chloride[5] and phenylethane-1,2-diol[6] in water through a membrane composed of poly(L-glutamic acid) containing the pendant pararosaniline group was reported to be reversibly regulated by the photoinduced conformational change of the polypeptide. In these systems, isomerization of azobenzenesulfonate from trans to cis or ionic dissociation of the rosaniline groups on the UV irradiation causes an increase in electron density in the neighborhood of the polypeptide chain, resulting in electrostatic repulsion along the polypeptide chain. This electrostatic repulsion is considered to induce the conformational change of the polypeptide. Thus, it is impossible in these systems to exclude the contribution of the photoinduced change in the electron density along the polypeptide chain to the change in the permeability. There has been no example of photoinduced permeability change across a membrane *without* a concomitant change in electrostatic repulsion.

We have reported that the permeability of a substrate such as phenylethane-1,2-diol across a membrane containing the polypeptide microdomain (permeating pathway) from a graft copolymer composed of a poly(L-aspartic acid) or poly(L-glutamic acid) branch attached to the poly(butyl methacrylate) (PBMA) backbone could be regulated by external stimuli such as pH,[7-9] divalent ion,[10] urea,[11] and ammonium salt.[12] We have also investigated[13] the circular dichroic and photoresponsive properties of a graft copolymer composed of the polyasparate branch with the pendant azobenzene group attached to the poly(2-hydroxyethyl methacrylate) backbone in solution and membrane. Herein, we report the first example of the photoregulation of the permeability across a membrane containing the polypeptide *without* any contribution of electrostatic repulsion along the polypeptide chain. As the membrane, a novel polyvinyl/polypeptide graft copolymer (**1**) composed of the photoresponsive copolypeptide[14] branch from β-(4-phenylazo)benzyl L-aspartate and β-benzyl L-aspartate attached to the PBMA backbone was used.

(1) For example: Alberts, B.; Bray, D.; Lewis, J.; Baff, M.; Roberts, K.; Watson, J. D. *Molecular Biology of the Cell*, 2nd ed.; Garland Publishing: New York, 1989; pp 300-323.
(2) Wald, G. *Nature (London)* **1968**, *219*, 800.
(3) Stryer, L. *Biochemistry*, 3rd ed.; W. H. Freeman and Company: New York, 1988; pp 1027-1040.
(4) Sato, M.; Kinoshita, T.; Takizawa, A.; Tsujita, Y.; Ito, R. *Polym. J.* **1988**, *20*, 761.
(5) Sato, M.; Kinoshita, T.; Takizawa, A.; Tsujita, Y. *Polym. J.* **1989**, *21*, 369.
(6) Sato, M.; Kinoshita, T.; Takizawa, A.; Tsujita, Y. *Polym. J.* **1988**, *20*, 729.
(7) Maeda, M.; Kimura, M.; Hareyama, Y.; Inoue, S. *J. Am. Chem. Soc.* **1984**, *106*, 250.
(8) Higuchi, S.; Mozawa, T.; Maeda, M.; Inoue, S. *Macromolecules* **1986**, *19*, 2263.
(9) Chung, D.-w.; Higuchi, S.; Maeda, M.; Inoue, S. *J. Am. Chem. Soc.* **1986**, *108*, 5823.
(10) Maeda, M.; Aoyama, M.; Inoue, S. *Makromol. Chem.* **1986**, *187*, 2137.
(11) Chung, D.-w.; Maeda, M.; Inoue, S. *Makromol. Chem.* **1988**, *189*, 1635.
(12) Chung, D.-w.; Kurosawa, K.; Maeda, M.; Inoue, S. *Makromol. Chem.* **1988**, *189*, 2731. Chung, D.-w.; Tanaka, K.; Maeda, M.; Inoue, S. *Polym. J.* **1988**, *20*, 933.
(13) Aoyama, M.; Youda, A.; Watanabe, J.; Inoue, S. *Macromolecules* **1990**, *23*, 1458.
(14) Ueno, A.; Anzai, J.; Osa, T.; Kodama, Y. *J. Polym. Sci., Polym. Lett. Ed.* **1977**, *15*, 407.

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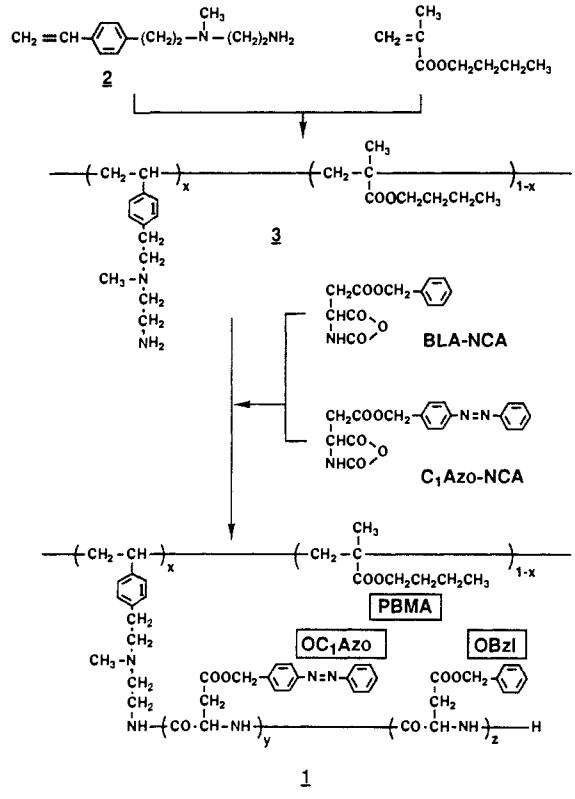
Scheme I



x = 0.006, y = 28, z = 13

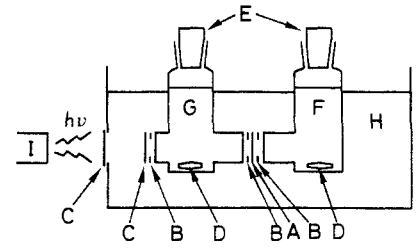


Figure 1. Experimental setup for permeation: (A) membrane; (B) silicone rubber; (C) quartz plate; (D) stirring bar; (E) stopper; (F) feed side (substrate solution); (G) receipt side (trimethyl phosphate); (H) water bath (thermostated); (I) light source.

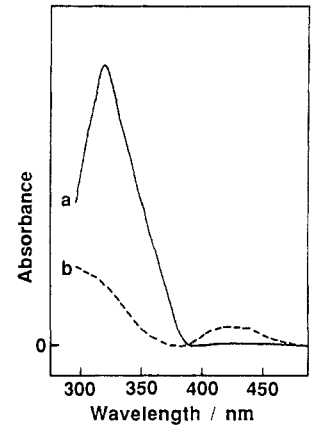


Figure 2. Effect of photoirradiation on the absorption spectrum of the membrane from **1** immersed in trimethyl phosphate: (a) before UV irradiation and after visible light irradiation; (b) after UV irradiation.

Experimental Section

Materials. Preparation of **1** was carried out as shown in Scheme I. Synthesis of *N*-methyl-*N*-(4-vinylphenethyl)ethylenediamine (**2**)[15] was reported previously. β-Benzyl L-aspartate *N*-carboxylic anhydride (BLA-NCA)[16] and β-(4-phenylazo)benzyl L-aspartate *N*-carboxylic anhydride (C_1Azo-NCA)[17] were prepared according to the literatures. Dichloromethane was distilled over calcium hydride. Butyl methacrylate was distilled over calcium hydride under reduced pressure. The other chemicals such as 1,2-dichloroethane (DCE) and trimethyl phosphate (TMP) of reagent grade were used without further purification.

Polymerization. Preparation of backbone copolymer **3** by radical copolymerization of **2** and butyl methacrylate was reported previously.[8] The amount of **2** in copolymer **3** (x) was estimated as 6.0×10^{-3} from the ^{1}H NMR spectrum in $CDCl_3$. The average molecular weight of **3** was 3.0–4.0 × 10^4 as estimated by gel permeation chromatography (polystyrenes as standard, eluted by chloroform). Synthesis of graft copolymer **1** was carried out by copolymerization of BLA-NCA and C_1 Azo-NCA initiated by the primary amino group of backbone copolymer **3**. To a solution of **3** (326 mg) in dichloromethane (20 mL) was added dichloromethane solutions of BLA-NCA (30.5 mg in 5 mL) and C_1Azo-NCA (130 mg in 50 mL), and the mixture was stirred at room temperature until the disappearance of absorptions of the anhydride group due to NCA in the IR spectrum. The reaction mixture was homogeneous throughout the reaction. Then the reaction mixture was poured into a large excess of hexane at 0 °C, and the precipitated yellow powdery polymer was filtered off and dried under reduced pressure. The average degree of polymerization of the poly(amino acids) chain ($y + z$) and the content of the residue containing the azo group ($y/(y + z)$) were determined from the ^{1}H NMR spectrum in CF_3COOD. The estimated content of the azobenzene moieties from ^{1}H NMR was identical with that estimated from the molar extinction coefficient of the *trans*-azobenzene group in the dark-adapted product.

Preparation of Membrane. A yellow and transparent membrane for the permeation was obtained by casting the DCE solution (2 mL) of **1** (60 mg) on a flat plate (diameter 3.1 cm) made of poly(tetrafluoroethylene), followed by evaporation under reduced pressure. The thickness of the membrane was about 30 μm. The membrane thus obtained was stable in TMP. A membrane for the circular dichroism (CD) measurement was prepared by casting a few drops of the DCE solution of **1** (0.3 wt %) on a flat quartz plate followed by evaporation in vacuo. The quartz plate attaching the membrane was placed in a quartz cell (light-path length = 10 mm) that was filled with TMP and irradiated by a 300 W Xe lamp (ILC Cermax LX-300F): for light of 330–350 nm with a Corning 7-37 filter was used and for that of 430–450 nm Corning 3-74 and 7-59 filters were used.

Permeation. Permeation of a substrate across the membrane immersed in TMP was carried out at 30 °C as shown in Figure 1. As the substrate, biphenyl, 1,3,5-triphenylbenzene, *N*-((benzyloxy)carbonyl)-DL-alanine, *N*-((benzyloxy)carbonyl)-D,L-valine, D,L-mandelic acid, benzamide, 2-hydroxyacetophenone, phenylethane-1,2-diol, hydantoin, and acetone were used. The initial concentration of the substrate was 1.0×10^{-1} M for biphenyl, 1.0×10^{-2} M for 1,3,5-triphenylbenzene, and 7.0×10^{-2} M for the other substrates. A TMP solution of the substrate was introduced into one side (feed side) of the cell and pure TMP was introduced into the other side (receipt side) of the cell. The membrane was irradiated from the receipt side of the cell with use of the same light source and filters as for CD measurements. The amount of permeated substrate was determined by the absorption at 254 nm for substrates with aromatic groups such as biphenyl and mandelic acid and that at 270 nm for nonaromatic substrates, i.e., hydantoin and acetone.

Measurements. UV-vis spectra and transmittance were recorded on a JASCO U-best 50 spectrophotometer. Circular dichroism spectra were obtained with a JASCO J-500 spectropolarimeter.

Results and Discussion

Circular Dichroism of a Membrane. In order to examine the conformation of the polypeptide chain in the membrane from **1** immersed in TMP, CD spectra were measured. In the dark-

(15) Maeda, M.; Nitadori, Y.; Tsuruta, T. *Makromol. Chem.* **1980**, *181*, 2251.

(16) Fuller, W. D.; Verlander, M. S.; Goodman, M. *Biopolymers* **1976**, *15*, 1869.

(17) Ueno, A.; Anzai, J.; Osa, T.; Kodama, Y. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 549. The melting point of C_1Azo-NCA did not agree with that in the literature. 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. 240–240.5 °C from ethyl acetate–hexane). Anal. Calcd for $C_{18}H_{15}N_3O_5$: 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.

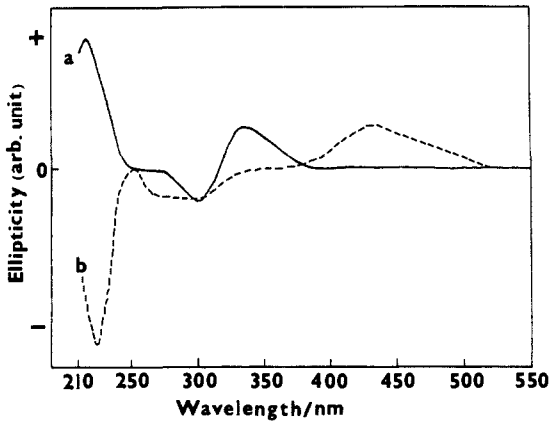


Figure 3. Effect of photoirradiation on the CD spectrum of the membrane from **1** immersed in trimethyl phosphate: (a) before UV irradiation and after visible light irradiation; (b) after UV irradiation.

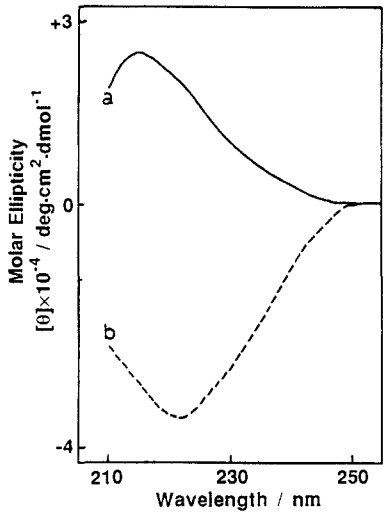


Figure 4. Effect of photoirradiation on the CD spectrum of the solution from **1** in the mixture of trimethyl phosphate and 1,2-dichloroethane (9:1 (v/v)): (a) before UV irradiation and after visible light irradiation; (b) after UV irradiation.

adapted membrane, the absorption spectrum showed that the azobenzene moieties had the trans structure (Figure 2), and a positive CD band associated with the peptide n–π^* transition at 215 nm indicating a left-handed α-helix was observed (Figure 3). Linear dichroism is not considered to affect the CD spectra because no change in CD spectra was observed with the rotation of the quartz plate with the membrane in the CD measurement. The UV irradiation caused a decrease in the absorption at 323 nm due to the *trans*-azobenzene moieties within 15 min and an increase in the absorption at 430 nm due to the cis structure. At the same time, the CD band was changed to negative at 222 nm indicating a right-handed α-helix. The original absorption and CD profiles were recovered completely by irradiating with visible light within 5 min or by standing in the dark for about 5 days. An induced CD band with positive sign around 340 nm associated with the π–π^* transition of the azoaromatic side chain was observed in the dark and after the irradiation with visible light. A new induced CD band with positive sign around 450 nm associated with the n–π^* transition appeared after the UV irradiation. Thus the reversible inversion of the helix sense of the polypeptide chains accompanied by the photoisomerization of the azobenzene moieties in the membrane was confirmed. The CD profiles of the membrane immersed in TMP before and after the irradiation were very similar to those of the solution in the mixture of TMP and DCE (9:1 (v/v)), and the molar ellipticities of the solution at 215 nm before the UV irradiation and at 222 nm after the UV irradiation were $+2.5 \times 10^4$ and -3.5×10^4, respectively (Figure 4). If the molar ellipticities observed are assumed to reflect the helix contents of the polypeptide chains in **1**, the helix contents before and after

Table I. Effect of Light on Permeation Rate across a Membrane from **1**, Immersed in Trimethyl Phosphate, at 30 °C

substrate[a]	permeation rate, %·h^{-1} dark	UV irradiation	visible light irradiation	ratio of UV/dark
D,L-mandelic acid	2.1×10^{-2}	1.2×10^{-1}	2.7×10^{-2}	5.7
Z-D,L-Ala[b]	3.0×10^{-2}	1.1×10^{-1}	3.0×10^{-2}	3.7
Z-D,L-Val[c]	4.4×10^{-2}	1.0×10^{-1}	4.7×10^{-2}[d]	2.3
benzamide	1.5×10^{-1}	3.1×10^{-1}	1.7×10^{-1}	2.1
2-hydroxy-acetophenone	3.4×10^{-1}	5.0×10^{-1}	3.4×10^{-1}	1.5
phenylethane-1,2-diol	2.3×10^{-1}	3.5×10^{-1}	2.6×10^{-1}	1.5
hydantoin	1.4×10^{-1}	2.1×10^{-1}	1.2×10^{-1}	1.5
acetone	6.7×10^{-1}	1.0	7.0×10^{-1}	1.5
biphenyl[e]	3.5×10^{-1}	5.1×10^{-1}	3.0×10^{-1}	1.5
1,3,5-triphenyl-benzene[f]	2.4×10^{-2}	4.2×10^{-2}	2.5×10^{-2}	1.8

[a] 7.0×10^{-2} M. [b] *N*-((Benzyloxy)carbonyl)-D,L-alanine. [c] *N*-((Benzyloxy)carbonyl)-D,L-valine. [d] Adapted to dark for 7 days after UV irradiation. [e] 1.0×10^{-1} M. [f] 1.0×10^{-2} M.

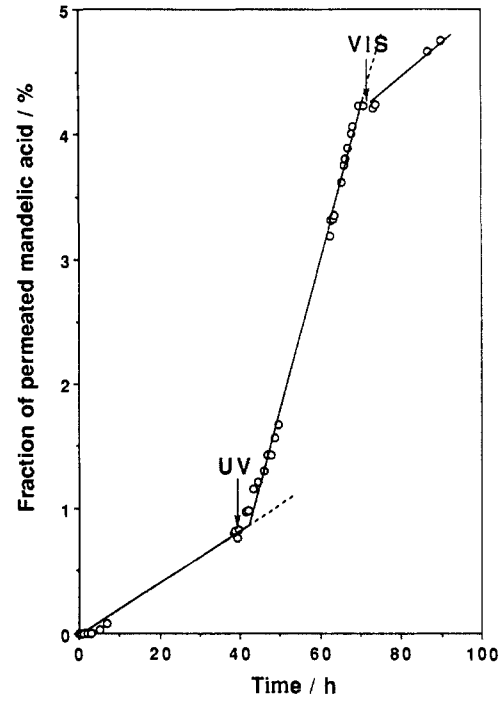


Figure 5. Permeation profile of D,L-mandelic acid (0.07 M) across the membrane from **1** immersed in trimethyl phosphate on photoirradiation.

the UV irradiation are estimated to be 63% and 88%, respectively.[18,19] A similar increase in the helix content on the UV irradiation is considered to take place also in the membrane judging from the CD profiles.

Photoregulation of Permeability across a Membrane. The effects of irradiation with UV and visible light on the permeation

(18) The helix contents were calculated on the basis of the molar ellipticity of 4.0×10^4 at 222 nm for fully α-helical polypeptide such as poly(L-glutamic acid) in water. For poly(β-benzyl L-aspartate), the molar ellipticites in chloroform and trimethyl phosphate are reported to be $+4.3 \times 10^4$ at 219 nm and -3.1×10^4 at 223 nm, respectively: Giancotti, V.; Quadrifoglio, F.; Crescenzi, V. *J. Am. Chem. Soc.* **1972**, *94*, 297. For poly[β-(4-phenylazo)-benzyl L-aspartate-*co*-β-benzyl L-aspartate] (content of the residue containing azo group = 81 mol %) in 1,2-dichloroethane, it is reported that the molar ellipticities before and after UV irradiation are $+2.9 \times 10^4$ at 215 nm and -1.9×10^4 at 222 nm, respectively: Ueno, A.; Anzai, J.; Osa, T.; Kadoma, Y. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 549.

(19) The ellipticity associated with n–π^* transitions is known to be especially sensitive to variations in the dihedral angles along the peptide bond: Woody, R. W. *J. Polym. Sci., Macromolecular Rev.* **1977**, *12*, 181. It is known that architectural dissymmetry, for instance the handedness of helices, has a major effect on the optical rotatory power of a polypeptide molecule: Bodanszky, M. *Peptide Chemistry*; Springer-Verlag: New York, 1988; pp 45–48.

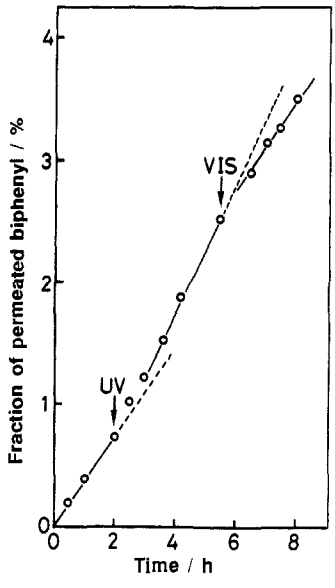


Figure 6. Permeation profile of biphenyl (0.1 M) across the membrane from **1** immersed in trimethyl phosphate on photoirradiation.

rates of various substrates are summarized in Table I. Figure 5 shows the permeation profile of D,L-mandelic acid (concentration 7.0×10^{-2} M), a polar substrate, across the membrane. In the dark, the permeation rate was 2.1×10^{-2} %·h^{-1}. The UV irradiation remarkably raised the permeation rate to 1.2×10^{-1} %·h^{-1}, about six times higher than that in the dark. The subsequent visible light irradiation suppressed the permeation rate to 2.7×10^{-2} %·h^{-2}. In the cases of other polar substrates, such as *N*-((benzyloxy)carbonyl)-D,L-alanine, *N*-((benzyloxy)carbonyl)-D,L-valine (Z-D,L-Val), and benzamide, the UV irradiation caused the permeation rate to increase by more than 2-fold. A similar but a bit smaller increase in the permeation rate on the UV irradiation took place also for 2-hydroxyacetophenone, phenylethane-1,2-diol, hydantoin, and acetone.

The effects of lights were observed also for nonpolar substrates. When biphenyl (1.0×10^{-1} M) was used as a nonpolar substrate (Figure 6), the substrate permeated by 3.5×10^{-1} %·h^{-1} in the dark, and the permeation rate increased to 5.1×10^{-1} %·h^{-1} on UV irradiation. The subsequent irradiation of visible light suppressed the permeation rate to 3.9×10^{-1} %·h^{-1}. 1,3,5-Triphenylbenzene (1.0×10^{-2} M) permeated by 2.4×10^{-2} %·h^{-1} in the dark, and the permeation rate increased to 4.2×10^{-2} %·h^{-2} with UV irradiation. The subsequent irradiation of visible light suppressed the permeation rate to 2.5×10^{-2} %·h^{-1}, and this photoregulation of the permeation rate could be repeated as shown in Figure 7.

Thus, the UV irradiation raised the permeation rate of polar and nonpolar substrates across the membrane from **1**, and the subsequent irradiation of visible light suppressed it to almost the original state, although not completely. On irradiation with both UV and visible light, the permeation rate became constant within an hour, as exemplified in Figure 5, for all substrates examined. In the permeation of Z-D,L-Val, for example, after the permeation rate was raised from 4.4×10^{-2} to 1.0×10^{-1} %·h^{-1} with UV irradiation, the membrane was adapted to the dark. The permeation rate gradually decreased and eventually reached 4.7×10^{-2} %·h^{-1} after about 7 days. Times required for the change in the permeation rates were not only on the irradiation of UV and visible lights but also in the adaption to dark, comparable with

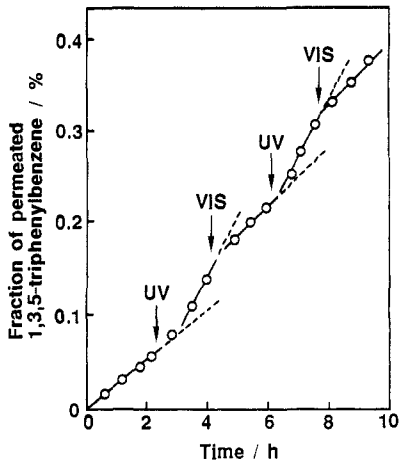


Figure 7. Permeation profile of 1,3,5-triphenylbenzene (0.01 M) across the membrane from **1** immersed in trimethyl phosphate on photoirradiation.

those required for the change in the conformation of the polypeptide chains in the membrane. No change in the appearance of the membrane was observed during the permeation experiment.

In the graft copolymer composed of the poly(butyl methacrylate) backbone and the poly(β-benzyl L-aspartate) branch, which corresponds to the structure of **1** without phenylazo substituents, the microphase separation of poly(butyl methacrylate) and poly(β-benzyl L-aspartate) domains was observed.[8] The polypeptide domains have been considered to form a permeating pathway penetrating the membrane after the hydrolysis of poly(β-benzyl L-aspartate) to poly(L-aspartic acid). On the other hand, the rates of permeation of biphenyl and 1,3,5-triphenylbenzene across the membrane prepared from the homopolymer of butyl methacrylate (PBMA) were about one-fourth (1.4×10^{-1} %·h^{-1}) and about one-tenth (2.7×10^{-3} %·h^{-1}), respectively, of those across the membrane from graft copolymer **1**. The result indicates that the substrates permeate predominantly through the permeating pathway composed of the polypeptide chains of **1**.

The change in permeability on photoirradiation is, thus, considered to be correlated with the photoinduced inversion of the helix sense of the polypeptide chains in the membrane. The substrates are considered to hardly permeate the inside of the rigid helix of the polypeptide chain but are more likely to permeate through the space between the polypeptide chains. In the inversion of the helix sense of the polypeptide chains in the membrane from **1**, the increase in the helix content of the polypeptide chains and/or possibly the change in the helix size is considered to induce the decrease in the volume occupied by the polypeptide chains and to cause the looseness of the space between the polypeptide chains, resulting in the expansion of the permeating pathway. The change in the polarity of the azobenzene moiety by the isomerization is considered to affect, at least partly, the permeability of polar substrates. However, such an effect is excluded for nonpolar substrates. Although there is the possibility that the inversion of the helix sense of the polypeptide chains leads to the change in the overall morphology of the membrane, no detectable change was observed in the X-ray diffraction of the membrane, being amorphous before and after UV irradiation.

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