PHOTORESPONSIVE PEPTIDE AND POLYPEPTIDE SYSTEMS IV: LIGHT-INDUCED CONFORMATIONAL CHANGES IN ϵ-POLY(L-LYSINE) WITH PHOTOCHROMIC SIDE CHAINS†

HIROYUKI YAMAMOTO‡, YASUKO MIYAGI and AYAKO NISHIDA

Institute of High Polymer Research, Faculty of Textile Science and Technology, Shinshu University, Ueda 386 (Japan)

TORU TAKAGISHI

Department of Applied Chemistry, College of Engineering, University of Osaka Prefecture, Sakai 591 (Japan)

SHOJI SHIMA

Department of Agricultural Chemistry, College of Agriculture, University of Osaka Prefecture, Sakai 591 (Japan)

(Received December 12, 1986; in revised form February 4, 1987)

Summary

ϵ-Poly(L-lysine) containing a high mole percentage of azo aromatic side chains was synthesized by the active ester method. The photochemical properties of the azo polypeptide, ϵ-poly(N^{α}-phenylazobenzoyl-L-lysine) (ϵ-PPABLL), were investigated by absorption and circular dichroism (CD) spectroscopy in hexafluoro-2-propanol (HFIP). The photochromism of the absorption band in the near UV and visible wavelength region was found to be mostly reversible as a function of irradiation time at different wavelengths (360 nm and 460 nm) owing to the trans–cis photoisomerization of the azo aromatic moieties. The CD spectrum exhibited multistage photochromism on irradiation by light. The whole CD spectrum, especially at the dichroic bands at 300 nm (positive), 340 nm (negative) and 365 nm (trans, positive), showed a peculiar reversible photochromism on irradiation by 360 nm and 460 nm light, except the first irradiation time course. The position and magnitude of the negative dichroic band at 225 nm suggested that the backbone conformational change of ϵ-PPABLL was a light-induced partial reversible transition from a β-form-rich structure to a random-coil-rich structure.

†Part III is ref. 1.

‡Author to whom correspondence should be addressed.

0047-2670/87/$3.50

1. Introduction

Photochromic polypeptides are interesting systems because of the relevance to the molecular mechanism of photoregulation in biological materials and processes [2 - 4]. Neutral polypeptides containing azo aromatic chromophores were first reported by Goodman and coworkers with optical rotatory dispersion [5, 6] and circular dichroism (CD) techniques [7 - 9]. However, their polypeptides were too stable to cause photochromism in solvents such as water, trimethyl phosphate and trifluoroacetic acid [7]. More recently, two groups have studied the light-induced reversible conformational properties of anionic photochromic polyaspartates and polyglutamates containing azobenzene residues [10 - 15]. The results clarified that the number of methylene groups and the content of azo aromatic moieties in the side chain are the critical factors in causing a light-induced inversion of the helical sense of the backbone. On cationic polypeptides, we have already reported the synthesis and reversible photochromic properties of α-poly(N^{ϵ}-phenylazobenzoyl-L-lysine) (α-PPABLL) with a high mole percentage of azo aromatic side chains [16, 17].

Cationic ε-poly(L-lysine) (ε-PLL) with the shortest side chains is a structural isomer of α-poly(L-lysine) and the conformational aspects of ε- and α-polylysines differ greatly [18, 19]. Cationic ε-PLL has been investigated from the standpoints of synthesis and conformation [20], microbial production and bacteriophage interactions [21 - 24], and binding ability of small molecules such as dyes and metal ions [25, 26]. In this paper, we describe the synthesis and photoresponsive properties of ε-PPABLL with azo aromatic side chains. The photochromic results are discussed in comparison with those for α-PPABLL.

2. Experimental procedure

2.1. Materials

ε-PLL hydrochloride hydrate (ε-PLL HCl H_2O) was produced from the culture broth of *Streptomyces albulus*. The microbiological method for the preparation was described in detail in earlier articles [21 - 24]. The molecular weight of ε-PLL HCl H_2O was determined to be about 4000 (degree of polymerization, 25 - 30) from chromatographic and spectrophotometric methods [21 - 24]. Azo dye, *p*-(phenylazo)benzoic acid (a guaranteed reagent from the Tokyo Chemical Industries Company Limited) was converted to *p*-nitrophenyl ester as described previously [17].

ε-PPABLL was synthesized as follows. ε-PLL HCl H_2O (91.5 mg, 0.5 mmol) was dissolved in 1 cm^3 of water. After diluting the solution with 2 cm^3 dimethylformamide (DMF), triethylamine (0.6 mmol) was added and then *p*-(phenylazo)benzoic acid *p*-nitrophenyl ester (1.5 mmol) in 7.5 cm^3 DMF was added. The reaction mixture was stirred at room temperature for 60 h. Ether was added to the resulting mixture and the precipitate was

centrifuged, washed with dioxane and ethanol, and finally refluxed in ethanol for 3 h, and dried. The yield was 126 mg (75%). The calculated analysis for $(C_{19}H_{20}N_4O_2)_n$ is C, 67.84%; H, 5.99%; N, 16.66% (as 100% azo moieties). The percentages found were C, 67.49%; H, 6.09%; N, 16.82%. ϵ-PPABLL is only soluble in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and dichloroacetic acid.

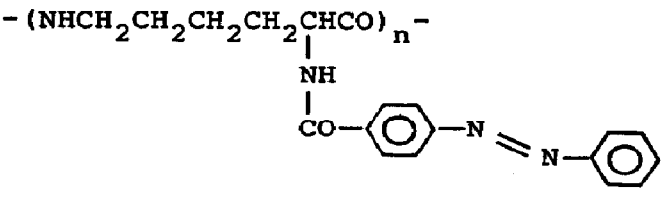
2.2. Methods

ϵ-PPABLL solution was prepared and kept in the dark for a few days to ensure the trans conformation at the beginning of the measurements. The absorption spectra and CD spectra were measured with a Jasco UVIDEC-1 spectrometer and a CD J-40A spectrometer respectively. The concentrations of the sample were (2×10^{-5}) - (2×10^{-4}) mol dm^{-3} in HFIP. The spectral data were expressed in terms of mean residue ellipticity $[\theta]$ (deg cm^2 dmol^{-1}) and molar extinction coefficient ϵ (dm^3 mol^{-1} cm^{-1}). No observable photoisomerization occurs during recording of the CD spectra.

Irradiations of the sample solutions were carried out at 25 °C with a mercury lamp (400 W) filtered with narrow-band interference filters from Toshiba Limited. The light intensity was determined by chemical actinometry using potassium ferrioxalate [27] to be 1.8×10^{19} photons cm^{-2} s^{-1} at 360 nm and 1.3×10^{19} photons cm^{-2} s^{-1} at 460 nm.

3. Results and discussion

The chemical structure of *trans*-ϵ-PPABLL is



ϵ-PPABLL, containing a quantitative amount of azo aromatic groups in the side chains, was synthesized by a procedure similar to that described previously [17]. Normally azobenzene and its derivatives exist in the thermodynamically more stable trans ground state [28, 29]. The effect of solvent on the photoisomerization of azo dyes is well known [30 - 32]. The photochromism of *p*-(phenylazo)benzoic acid in HFIP as a function of irradiation time at different wavelengths and the course of the dark adaptation has been examined [32]. The trans conformer of the azo dye in HFIP isomerized to the cis conformer on irradiation at 360 nm for 5 min, whereas the cis conformer isomerized to the photostationary state on irradiation at 460 nm for 5 min. The absorption spectrum of ϵ-PPABLL in HFIP exhibits four main trans absorption bands at 197 nm (ϵ = 30 000 dm^3 mol^{-1} cm^{-1}), 228 nm (ϵ = 12 000 dm^3 mol^{-1} cm^{-1}), 326 nm (ϵ = 23 300 dm^3 mol^{-1} cm^{-1})

and 430 nm (ϵ = 1250 dm^3 mol^{-1} cm^{-1}). The absorption bands at 326 nm and 430 nm are associated with the π–π^* and n–π^* transitions of the side-chain azobenzene moieties respectively. These bands exhibit photochromism on irradiation at 360 nm as depicted in Fig. 1. On irradiation at 360 nm for 5 min, the absorption band at 228 nm shifts to 256 nm (ϵ = 14 000 dm^3 mol^{-1} cm^{-1}) and the ϵ value of the band at 326 nm decreases to 6750 dm^3 mol^{-1} cm^{-1} (the peak at 300 nm, ϵ = 9700 dm^3 mol^{-1} cm^{-1}), whereas the weak absorption band at 430 nm becomes more intense (ϵ = 2750 dm^3 mol^{-1} cm^{-1}). The photochromism of ϵ-PPABLL from cis to the photostationary state is 95% reversible, as depicted in Fig. 2.

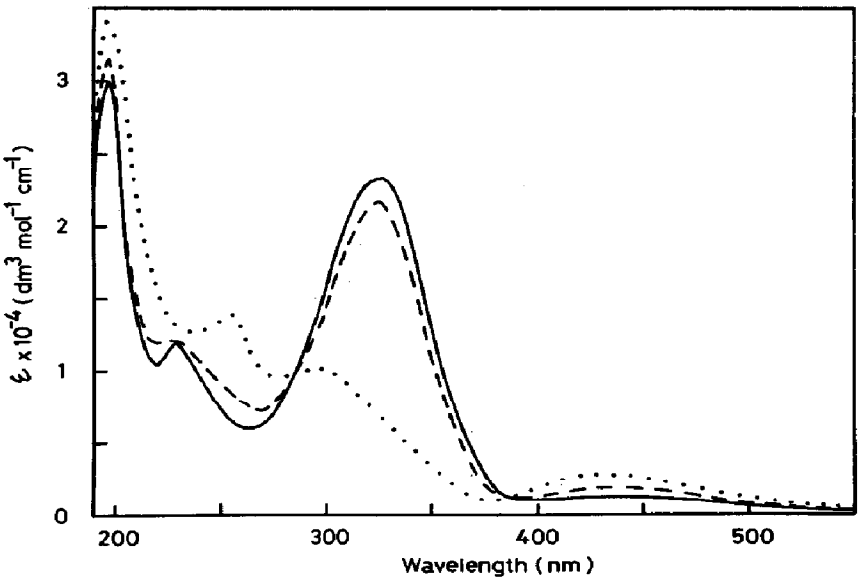


Fig. 1. Absorption spectra of ϵ-PPABLL in HFIP at 25 °C: ——, trans, before irradiation; ....., cis, after irradiation at 360 nm for 5 min; – – –, photostationary state, after re-irradiation at 460 nm for 5 min.

Figure 3 shows the CD spectrum of ϵ-PPABLL in HFIP in the 205 - 520 nm wavelength region. Before irradiation, seven dichroic bands were observed at 520 nm (positive), 420 nm (negative), 365 nm (positive), 340 nm (negative), 300 nm and 265 nm (positive), and 225 nm (negative). Light produces a photochromism in the CD spectrum of ϵ-PPABLL in HFIP. On irradiation at 360 nm for 10 min, the positive band at 520 nm became more intense, the negative band at 420 nm decreased, the dichroic bands at 365 nm and 340 nm disappeared, the positive band at 300 nm became negative at 290 nm, the positive band at 265 nm became more intense at 250 nm, and finally the peptide transition band at 225 nm became weak. On re-irradiation at 460 nm for 10 min, the spectrum before irradiation (dark) regenerated again in the 400 - 520 nm wavelength region, a negative band at 345 nm newly appeared, the band at 300 nm became positive again, and the peptide band at 205 nm became intense again.

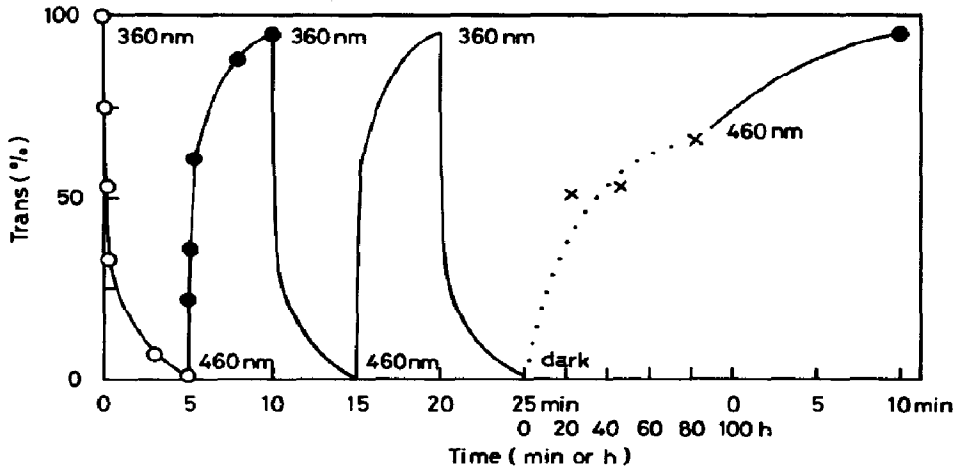


Fig. 2. Kinetics of the photochemical isomerization at 325 nm absorption band of azo aromatic chromophore in ϵ-PPABLL in HFIP at 25 °C: ○, irradiation at 360 nm; ●, irradiation at 460 nm; ×, dark adaptation.

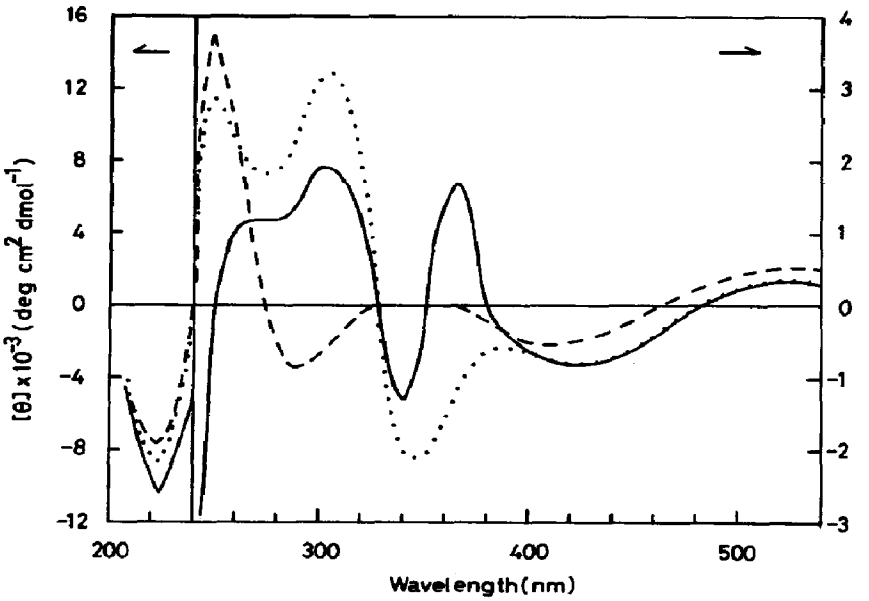


Fig. 3. Circular dichroism spectra of ϵ-PPABLL in HFIP at 25 °C: ——, before irradiation; – – –, after irradiation at 360 nm for 10 min; ·····, after re-irradiation at 460 nm for 10 min.

Figure 4 shows the change of the dichroic bands at 345 - 365 nm of ϵ-PPABLL on irradiation at 360 nm and 460 nm. Firstly, before irradiation in the dark, the dichroic band at 365 nm exhibited positive ellipticity with $[\theta]_{365} = 1700$. On irradiation at 360 nm the dichroic band disappeared after 5 min but no change was observed on irradiation at 460 nm. This is the first stage of a photochromism of ϵ-PPABLL. Secondly, after irradiation at 360 nm, the disappeared dichroic band appeared again, showing negative ellip-

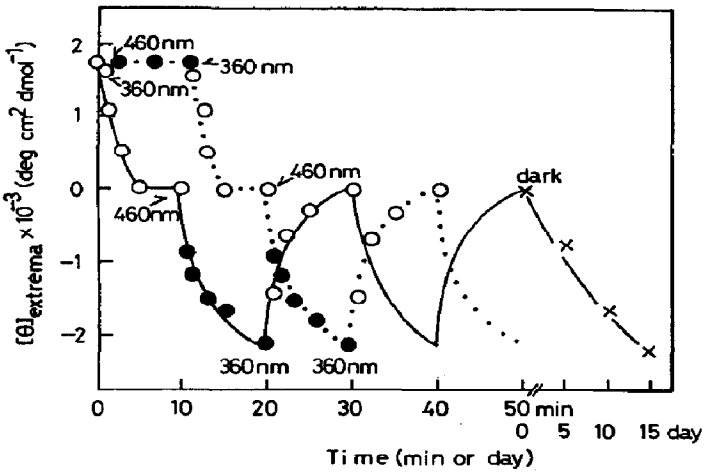


Fig. 4. Change in the ellipticities at extrema (positive at 365 nm, negative at 345 nm) of ϵ-PPABLL in HFIP as a function of irradiation time at different wavelengths and of the dark adaptation time: ○, irradiation at 360 nm; ●, irradiation at 460 nm; ×, dark adaptation; ———, first irradiated at 360 nm; ·····, first irradiated at 460 nm.

ticity of $[\theta]_{345} = -2100$ (after 10 min) on re-irradiation at 460 nm. The ellipticity of the negative dichroic band at 345 nm decreased to zero on irradiation again at 360 nm. Alternatively, after irradiation at 460 nm, the unchanged positive dichroic band at 365 nm disappeared on re-irradiation at 360 nm for 5 min. On irradiation again at 460 nm, the disappeared band appeared with a negative ellipticity of $[\theta]_{345} = -2100$ (after 10 min) and the negative ellipticity at 345 nm disappeared again to zero on irradiation at 360 nm. These are the second stages of the photochromism. The third stage is a repetition of the second photo-reversible stages, that is, from zero to negative ellipticity at 345 nm. When the disappeared ellipticity (after irradiation at 360 nm) was kept in the dark, the negative ellipticity appeared gradually and the ellipticity increased to $[\theta]_{345} = -2100$ after two weeks. Strangely, the dark adaptation did not regenerate the original positive ellipticity at 365 nm. Although the reasons for this peculiar photochromic behaviour are unclear at present, the possibility of a superimposed photochemical reaction between the chiral environments of the backbone and the symmetrical azo aromatic dyes may be an important consideration.

The negative dichroic band at 225 nm is because of either the peptide amide transitions or the coupling of the transition dipole moments of dyes with the transition moments of the peptides [17, 33, 34]. Assuming that the band with $[\theta]_{225} = -10\,000$ mainly arises from the peptide transitions, the backbone conformation of ϵ-PPABLL in HFIP could be assigned to be the mixed 60%(β-form)–40%(random-coil) structure before irradiation (dark, freshly prepared). On irradiation at 360 nm for 10 min, the ellipticity of the transition of ϵ-PPABLL in HFIP decreases from −10 000 to −7600 (in magnitude) at 225 nm, suggesting a decreased β-form content. On re-irradiation at 460 nm for 10 min, the ellipticity increases to −8600, suggesting a partial regeneration of the β-form structure.

The photoresponsive properties of ε-PPABLL are compared with those of the structural isomeric polypeptide α-PPABLL. Light-induced absorption properties of both ε-PPABLL and α-PPABLL in HFIP are essentially similar and reversible. However, the light-induced conformational aspects of the backbone and of the azo aromatic moieties in the side chains exhibited significant differences. The conformational change of ε-PPABLL in Fig. 3 may be because of the partial transition of a β-form-rich tertiary structure by the *trans–cis* photoconversion, whereas the light-induced conformational change of α-PPABLL was from the highly helical (before irradiation, $[\theta]_{221} = -27\,600$ and $[\theta]_{207} = -29\,000$) to the less helical structure (after irradiation, $[\theta]_{221} = -18\,500$ and $[\theta]_{207} = -22\,400$) [17]. As for the dichroic bands associated with the azo aromatic chromophores under chiral environments above 250 nm wavelength, the behaviour of ε-PPABLL with seven dichroic bands in Figs. 3 and 4 is more complicated than that of α-PPABLL, which exhibits only two dichroic bands at 340 nm and 435 nm, although both ε-PPABLL and α-PPABLL exhibit a typical photochromism at selected wavelengths on irradiation with visible light of wavelength 360 nm and 460 nm.

Acknowledgments

The authors wish to thank Professor T. Hayakawa for his encouragement. The present work was supported by Grant-in-Aid for Scientific Research 61490014 from the Ministry of Education, Science and Culture.

References

1 H. Yamamoto, A. Nishida, Y. Miyagi and T. Takagishi, *Polym. Prepr. Jpn.*, *35* (1986) 2470.
2 R. Lovrien, P. Pesheck and W. Tisel, *J. Am. Chem. Soc.*, *96* (1974) 244.
3 R. Van der Veen, R. Houguest and W. Prins, *Photochem. Photobiol.*, *19* (1974) 197.
4 J. C. Lagarias and H. Rapoport, *J. Am. Chem. Soc.*, *102* (1980) 4821.
5 M. Goodman and A. Kossoy, *J. Am. Chem. Soc.*, *88* (1966) 5010.
6 M. Goodman and M. Falxa, *J. Am. Chem. Soc.*, *89* (1967) 3863.
7 M. Goodman and E. Benedetti, *Biochemistry*, *7* (1968) 4226.
8 E. Benedetti, A. Kossoy, M. Falxa and M. Goodman, *Biochemistry*, *7* (1968) 4234.
9 E. Benedetti and M. Goodman, *Biochemistry*, *7* (1968) 4242.
10 A. Ueno, J. Anzai, T. Osa and Y. Kodama, *J. Polym. Sci., Polym. Lett. Ed.*, *15* (1977) 407.
11 A. Ueno, K. Takahashi, J. Anzai and T. Osa, *Macromolecules*, *13 (1980)* 459.
12 A. Ueno, K. Takahashi, J. Anzai and T. Osa, *J. Am. Chem. Soc.*, *103* (1981) 6410.
13 J. L. Houben, O. Pierono, A. Fissi and F. Ciardelli, *Biopolymers*, *17* (1978) 799.
14 O. Pieroni, J. L. Houben, A. Fissi, P. Constantino and F. Ciardelli, *J. Am. Chem. Soc.*, *102* (1980) 5913.
15 F. Ciardelli, O. Pieroni, A. Fissi and J. L. Houben, *Biopolymers*, *23* (1984) 1423.
16 H. Yamamoto and A. Nishida, *Macromolecules*, *19* (1986) 943.
17 H. Yamamoto, *Macromolecules*, *19* (1986) 2472.
18 D. Balasubramanian, C. C. Kalita and J. Kovacs, *Biopolymers*, *12* (1973) 1089.

19 D. Balasubramanian, *Biopolymers, 13* (1974) 407.
20 D. R. S. Kushwara, K. B. Mathur and D. Balasubramanian, *Biopolymers, 19* (1980) 219.
21 S. Shima and H. Sakai, *Agric. Biol. Chem., 41* (1977) 1807.
22 S. Shima and H. Sakai, *Agric. Biol. Chem., 45* (1981) 2497.
23 S. Shima and H. Sakai, *Agric. Biol. Chem., 45* (1981) 2503.
24 S. Shima, Y. Fukuhara and H. Sakai, *Agric. Biol. Chem., 46* (1982) 1917.
25 T. Takagishi, T. Ueno, N. Kuroki, S. Shima and H. Sakai, *J. Polym. Sci., Polym. Chem. Ed., 22* (1984) 1281.
26 T. Takagishi, N. Kuroki, S. Shima, H. Sakai, H. Yamamoto and A. Nakazawa, *J. Polym. Sci., Polym. Lett. Ed., 23* (1985) 329.
27 J. G. Calvert and J. N. Pitts, Jr., *Photochemistry*, Wiley, New York, 1966, p. 783.
28 G. S. Hartley, *J. Chem. Soc.* (1938) 633.
29 J. W. Le Févre and J. Northcott, *J. Chem. Soc.* (1953) 867.
30 G. M. Wyman, *Chem. Rev., 55* (1955) 625.
31 H. Kalinowski and H. Kessler, *Top. Stereochem., 7* (1973) 295.
32 H. Yamamoto and A. Nishida, *J. Chem. Soc. Jpn.* (1985) 2338.
33 R. E. Ballard, A. J. McCaffery and S. F. Mason, *Biopolymers, 4* (1966) 97.
34 H. Yamamoto and A. Nakazawa, *Biopolymers, 23* (1984) 1367.