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Light-induced Conformational Changes of Polypeptides. Photoisomerization of Azoaromatic Polypeptides

Akihiko Ueno,* Jun-ichi Anzai, Tetsuo Osa, and Yoshinori Kadoma[†]
Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980
[†]*Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, 2-Chome, Surugadai, Kanda, Chiyoda-ku, Tokyo 101*

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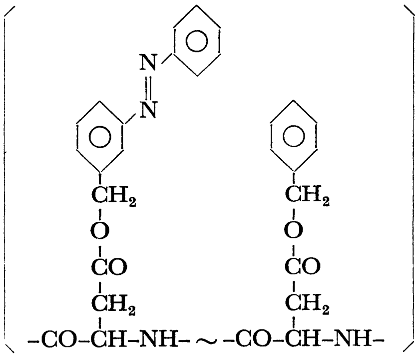
Copolymers of β-benzyl L-aspartate with β-*m*- or *p*-(phenylazo)benzyl L-aspartate were prepared in order to investigate the effect of side-chain photoisomerization on their conformations. These polymers in 1,2-dichloroethane show a CD band at 222 nm characteristic of left-handed α-helices. After irradiation, remarkable changes of the 222 nm band were observed for both *meta* and *para* copolymers with more than 50% azo residues. The decrease in ellipticity observed for the *meta* copolymers indicates that photoisomerization induces a significant instability in the left-handed α-helical conformation. On the other hand, the *para* copolymers change their sign from positive to negative by irradiation, confirming the reversal in the helix sense. In the course of subsequent relaxation of the irradiated *para* copolymer with 59% azo residues, an abrupt change in the CD spectra was found above 50% *cis* followed by a gradual change on decreasing the content of *cis* isomers. Concerning the polymers with azoaromatic side chains including copolymers with γ-benzyl L-glutamate, the helical sense seems to affect appreciably the sign of the side-chain CD bands.

The conformation of substituted benzyl esters of poly(aspartic acid) is determined by the nature and position of the substituent. Revesal in the helix sense from left- to right-handed α-helix is induced by the *para* substitution of the benzene ring of poly(β-benzyl L-aspartate) (PBLA) with a methyl, cyano, or nitro group.[1] Furthermore, the chlorine substituted PBLAs assume different handed helices depending on the position of the chlorine atom attached to the benzene ring.[2] From these observations a geometrical change such as *cis-trans* isomerization of the appropriate side chains might be expected to reverse the helix sense. Such a system, if realized, can be compared with visual purple, rhodopsin, since its polypeptide part (opsin) undergoes a conformational change induced by *cis-trans* photoisomerization of its chromophoric part (retinal). In nature, this light-induced conformational change is a trigger which excites the nerve cells of the retinal rods. From this view point, we prepared some copolymer series containing azobenzene moieties in their side chains. Photoisomerization of a polypeptide side chain itself was reported for the copolymers of L-*p*-(phenylazo)phenylalanine[3] and also for poly(γ-cinnamyl L-glutamate).[4] However, no conformational change could be induced by irradiation probably because of their conformational stability. In a previous paper,[5] we reported the results of the copolymer series of γ-benzyl L-glutamate with β-*m*- or *p*-(phenylazo)benzyl L-aspartate, and showed evidence supporting the following processes which occur independently.

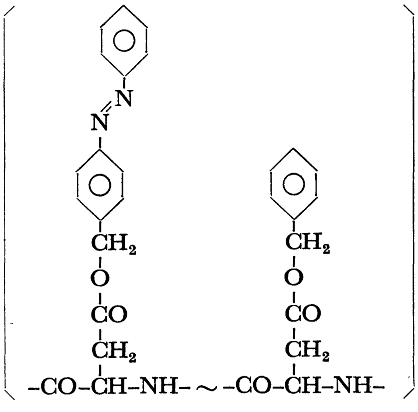
$$\text{random coil} \underset{\Delta}{\overset{h\nu}{\rightleftarrows}} \text{right-handed helix} \tag{1}$$

$$\text{left-handed helix} \underset{\Delta}{\overset{h\nu}{\rightleftarrows}} \text{random coil} \tag{2}$$

Reversal in helix sense could be attained for these copolymer series. In this paper, the results of copolymer series of β-benzyl L-aspartate with β-*m*- or *p*-(phenylazo)benzyl L-aspartate (*meta* or *para* copolymer series) are described. The following process which involves reversal in the helix sense has been confirmed to occur for some of these polymers.



meta copolymer series



para copolymer series

$$\text{left-handed helix} \underset{\Delta}{\overset{h\nu}{\rightleftarrows}} \text{right-handed helix} \tag{3}$$

The side-chain *cis* isomer, formed by irradiation, undergoes subsequent relaxation in the dark back to the original *trans* form. The light-induced conformational changes are, therefore, reversible, and provide a unique system which responds to light signals in an on-off fashion.

Results and Discussion

The ultraviolet and visible spectrum of poly[β-*m*-

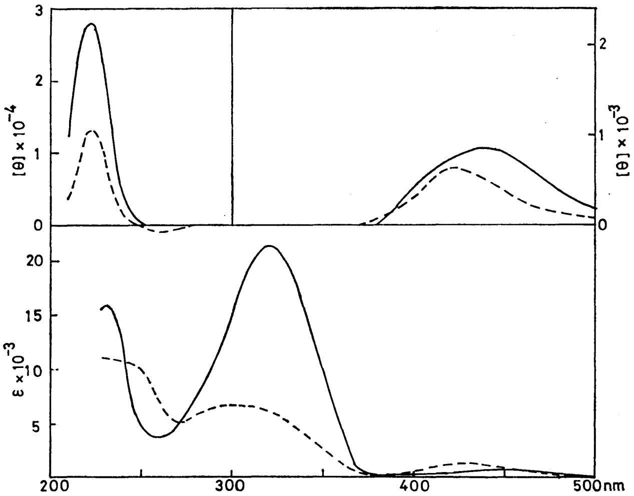


Fig. 1. CD and ultraviolet-visible spectra of mPALA in 1,2-dichloroethane before (——) and after (----) irradiation.

(phenylazo)benzyl L-aspartate] (mPALA) is essentially that of azobenzene itself (Fig. 1).⁷⁾ The band at 320 nm is attributed to π-π^* transition ($\varepsilon=22000$) and that at 450 nm to n-π^* transition ($\varepsilon=700$).⁸⁾ The irradiated polymer shows a spectrum resembling that of *cis*-azobenzene reported by Gerson *et al.*⁷⁾ The important aspect of the spectrum after irradiation is the decrease in absorption intensity in the π-π^* transition region. All of the *meta* and *para* copolymer series show similar spectra with various intensities corresponding to their content of azo residues.

The circular dichroism (CD) spectra of *meta* copolymer series before and after irradiation show dichroic bands associated with the n-π^* transitions of the main-chain amide and side-chain azo chromophores at 222 nm and 420—450 nm, respectively. No dichroic band was observed in the region of the π-π^* transition of the chromophore. The values of $[\theta]$ at the extrema are plotted against the content of azo residues (mol % of (phenylao)benzyl L-aspartate in copolymers) (Fig. 2). Molar ellipticities of the side-chain band were calculated with use of the molar concentration of azo residues. The spectra around 222 nm before irradiation are almost independent of the copolymer composition, and are very similar to those of poly(β-methyl L-aspartate) which assumes a left-handed α-helical conformation.⁹⁾ These observations suggest that overlapping of allowed transitions of side-chain chromophores with those from the peptide backbone is either small or absent in the polymers.¹⁰⁾ The values of $[\theta]$ at 222 nm do not seem to be affected by irradiation for the copolymers with azo residues of smaller than 50%. However, the values decrease for the copolymers of 67 and 92% azo contents and mPALA (Figs. 2 and 7). The value of $[\theta]$ at 222 nm is generally used to characterize the helicity, the decreased values indicating the increase in instability for the left-handed α-helices of the polymers. The value of $[\theta]_{222}=28000$ for mPALA is reduced to 13000 by irradiation (Figs. 1 and 2), confirming the formation of an appreciable amount of random-coil form. Its reduced specific viscosity, η_{sp}/c ($c=0.1$ in 1,2-dichloroethane), measured at 25 °C changes from 0.23 to 0.49 by photoirradiation. This suggests the increased volume induced by collapse of the helical form.

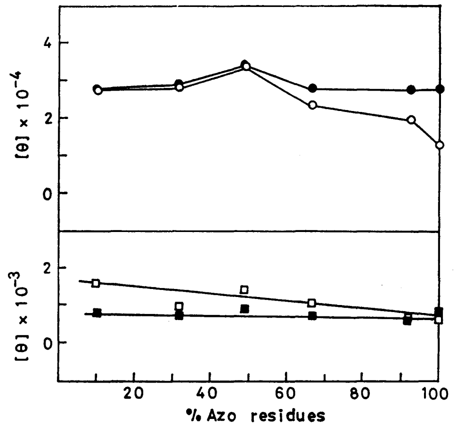


Fig. 2. Variation of maximum ellipticities associated with the side-chain n-π^* (■,□) and the amide n-π^* (●,○) transitions before (■,●) and after (□,○) iradiation for *meta* copolymer series as a function of the azo content.

Proton NMR spectra at 100 MHz were measured for mPALA (Fig. 3) in order to obtain further evidence for the partial helix-coil transition. The spectrum of the fully helical mPALA in 0.3% v/v trifluoroacetic acid/deuteriochloroform (TFA/$CDCl_3$) solution shows a peak at 4.37 ppm from α-CH proton and a broad doublet at 3.28 and 2.76 ppm from the β-CH_2 protons. The spectrum of the random-coil form of mPALA in 3% v/v TFA/$CDCl_3$ solution shows a peak at 4.86 ppm from the α-CH proton (shoulder of the peak from the benzyl CH_2

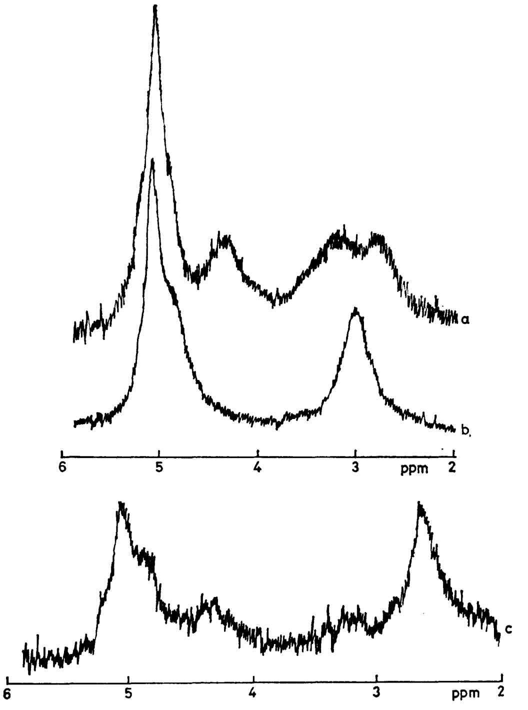


Fig. 3. 100-MHz NMR Spectra of mPALA. a; α-Helix (0.3% v/v TFA/$CDCl_3$), b; random coil (3% v/v TFA/$CDCl_3$), c; mPALA after irradiation ($CDCl_3$).

protons) and a single peak at 3.01 ppm from the β-CH_2 protons. The chemical shifts are close to those reported for the L-aspartate polymers.[11] The spectrum of mPALA after irradiation in $CDCl_3$ shows a complex pattern. The two α-CH chemical shifts observed at 4.40 and 4.90 ppm correspond to the α-helix and the random-coil peaks, respectively. Another change to be noted is that for the β-CH_2 resonance, which shows a sharp peak at 2.66 ppm probably from the random-coil form overlapped by the high field component of the β-CH_2 doublet of the helix. Compared with the chemical shift of the random coil caused by TFA addition, it is shifted upfield. The cause of the shift is unknown, but it might be due to the absense of TFA or a ring current effect of formed *cis* isomers. The results of NMR spectra might be in line with those of CD spectra.

The signs of the n-π^* CD band of the side-chain azo chromophores are always positive, not changing on photoirradiation (Fig. 2). The values of $[\theta]$ before and after irradiation are independent of the content of azo residues, which confirms side chain-main chain interactions as the origin of the dichroism band.

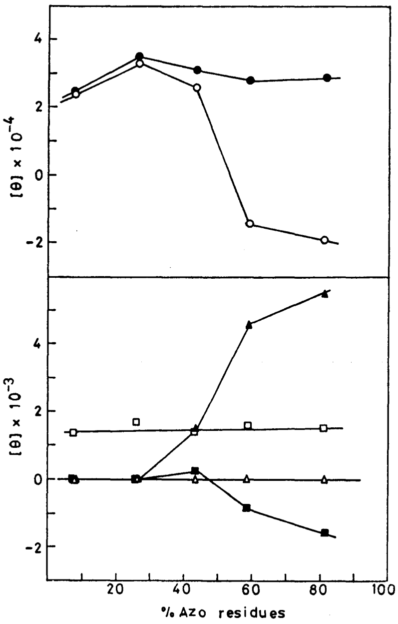


Fig. 4. Variation of maximum or minimum ellipticities associated with the side-chain n-π^* (■,□) π-π^* (▲,△) and the amide n-π^* (●,○) transitions before (■,▲,●) and after (□,△,○) irradiation for *para* copolymer series as a function of the azo content.

The CD spectra of *para* copolymer series show dichroic bands associated with the side-chain π-π^* transition as well as the n-π^* transitions of the main chain amide and side-chain azo chromophores. The values of $[\theta]$ at the extrema before and after irradiation are plotted against the content of azo residues (Fig. 4). All the spectra around 222 nm before irradiation are essentially those for the left-handed α-helices. The spectra after irradiation differ remarkably from those before irradiation for the copolymers of 59 and 81% azo contents (Fig. 7). These copolymers before irradiation display maxima with the values of $[\theta]_{222}=28000$ for 59% azo copolymer and $[\theta]_{215}=29000$ for 81% azo copolymer,[12] after irradiation minima with the values of $[\theta]_{224}=-14000$ for the former and $[\theta]_{222}=-19000$ for the latter. These observations demonstrate the reversal in the helix sense of these copolymers. Some indication of double trough is also consistent with helix reversal. Since the absolute values of $[\theta]$ are appreciably smaller than the value of ≈ 40000 reported for right-handed α-helices,[13] the copolymers after irradiation cannot be entirely right-handed helices. In contrast to the above polymers, the effect of irradiation on conformation is negligible for the polymers with smaller amount of azo residues. Certain amount of azo residues is necessary to cause the pronounced conformational changes. A critical azo content seems to exist at about 50%.

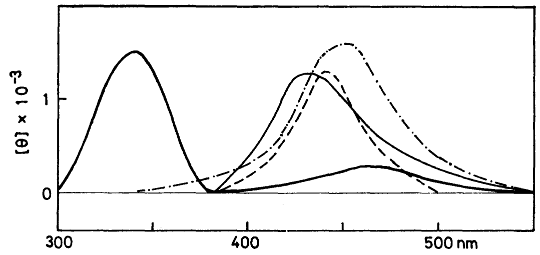


Fig. 5. CD spectra associated with the side-chain transitions of *para* copolymer series in 1,2-dichloroethane. Before irradiation: azo content 43% (——). After irradiation: azo content 7.4% (– – –), 26% (-·-·-), 43% (——).

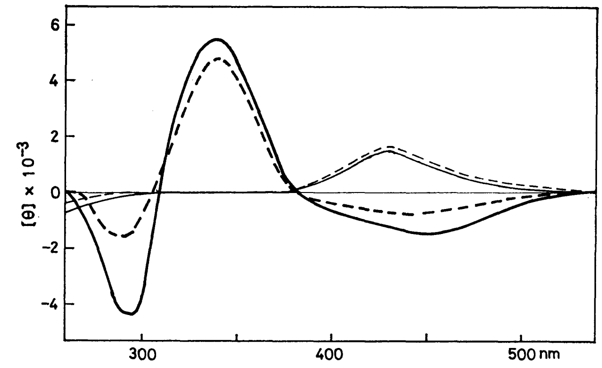


Fig. 6. CD spectra associated with the side-chain transitions of *para* copolymer series in 1,2-dichloroethane. Before irradiation: azo content 59% (----), 81% (——). After irradiation: azo content 59% (– – –), 81% (——).

The CD spectra associated with the side-chain n-π^* and π-π^* transitions of *para* copolymer series before and after irradiation are shown in Figs. 5 and 6.[14] Before irradiation, the CD spectra of the two copolymers with azo residues of 7.4 and 26% exhibit no dichroism in the region of the side-chain π-π^* transition, whereas the spectra of the copolymers with more azo residues exhibit distinct dichroism bands in the region. A distinct difference in the observed π-π^* CD bands exists between the copolymer with 43% azo residues and the copolymers with more azo residues. A simple positive band is observed for the former while the spectra of the latter are rather complicated, showing a peak in the 340-nm region and a trough in the 295-nm region with a cross-over near 310 nm. The separation of these bands is small, confirming the assignment of the band to exciton resonance coupling[15] of spatially adjacent azoaromatic chromophores.[16] The side chains of these polymers are allowed to arrange themselves in close packing in favor of the delocalization of the electronic excitation. Such exciton resonance coupling was also observed in the π-π* transition region of azo chromophores by Goodman *et al.*[10] for some azoaromatic polypeptides composed of (phenylazo)phenylalanine or its analogues. In the case of (phenylazo)benzyl L-aspartate polymers, the exciton resonance coupling seems to be very difficult because of the allowed movement of the side chains and the relatively large distances between the side chains. In fact, it does not appear for other copolymer series of (phenylazo)benzyl L-aspartate involving the copolymer series with γ-benzyl L-glutamate. Thus it might be possible only when some steric requirements are satisfied concerning the side-chain conformation.

In the region of the side-chain n-π* transition, circular dichroism is absent or small before irradiation for the copolymers with azo residues smaller than 40%. On further increase in azo residues, there appears a positive band and subsequently a negative band. It is difficult to explain the complicated spectral behavior. Nevertheless, we believe that the origin of the band is side chain-main chain interaction since there must be a profound difficulty for the electronic interactions between the adjacent side chains due to a small value for the transition moment of the n-π* transition and the large distance between the side chains.

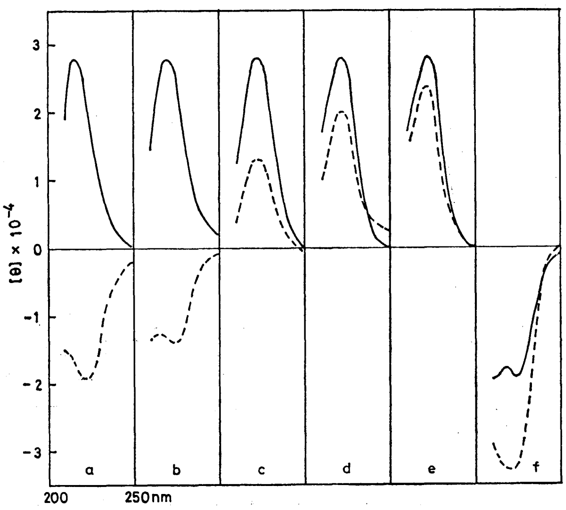


Fig. 7. CD spectra associated with the amide n-π* transition before (——), and after (----) irradiation in 1,2-dichloroethane for the polymers which undergo light-induced conformational changes. a, b; *para* Copolymers with azo residues of 81 and 59% respectively. c, d, e; *meta* Copolymers with azo residues of 100, 92, and 67% respectively. f; Copolymer of β-*m*-(phenylazo)benzyl L-aspartate and γ-benzyl L-glutamate (89: 11).

Photoirradiation causes significant changes in the side-chain CD bands of the *para* copolymers. The dichroic band associated with the π-π* transition vanishes. On the other hand, a positive band appears in the region of the n-π* transition. The complete disappearance of the π-π* CD band, in spite of the presence of about 20% *trans* isomers in the photostationary state, indicates the importance of *trans-trans* side-chain interactions for this CD band. The intensity of the azo n-π* transition after irradiation does not depend on the content of azo residues in the copolymers (Fig. 4), which assumes side chain-main chain interactions as the origin of this band.

The CD spectra associated with the peptide n-π* transition of the polymers which undergo light-induced conformational changes are summarized in Fig. 7. The spectral changes induced by irradiation can be classified into three types; left-handed to right-handed helix (*para* copolymers with azo residues of 59 and 81%), left-handed helix to random coil (*meta* copolymers with azo residues of 100, 92, and 67%) and random coil to right-handed helix (copolymer of β-*m*-(phenylazo)benzyl L-aspartate and γ-benzyl L-glutamate; 89: 11). We cannot obtain the spectra of pure *cis* azoaromatic polypeptides since 100% conversion could not be attained in our experimental conditions (about 80% *cis* in the photostationary state). Thus the conformational changes cannot be regarded as those occurring between pure *trans* and pure *cis* polypeptides.

TABLE 1. CD SIGNS OF THE SIDE-CHAIN n-π* AND π-π* BANDS[a)]

Copolymer series[b)]	Helix sense	Before irradiation		After irradiation
		n-π*	π-π*	n-π*
meta	right	−		−
meta	left	+		+
para	right	−	−	+
para	left	+	+	+

a) Signs for the copolymers showing exciton resonance coupling are not included. b) Copolymer series of γ-benzyl L-glutamate with β-*m*- and *p*-(phenylazo)-benzyl L-aspartate are also designated as *meta* and *para* copolymer series.

The signs of the side-chain CD bands are summarized in relation to the helical senses in Table 1. Konishi and Hatano showed that the CD signs of the α-band on aromatic side-chain chromophores in many ester derivatives of poly(glutamic acid) and poly(aspartic acid) are determined by the helical sense of polymers, positive for left-handed helices and negative for right-handed helices.[17] Concerning the (phenylazo)benzyl L-aspartate copolymers including copolymers with γ-benzyl L-glutamate, we also find that the CD signs of the side-chain n-π* and π-π* bands are determined by the backbone conformation of the polymers, positive for left-handed helices and negative for right-handed helices though some exceptions are present. Exceptional behavior is encountered for the *para* copolymers which show exciton resonance coupling and the right-handed *para* copoly-

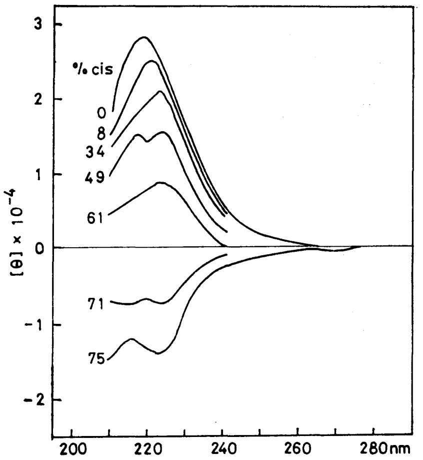


Fig. 8. CD Spectra of *para* copolymer with 59% azo residues at different *cis* contents in the course of relaxation.

mers after irradiation. So far there is no explanation for these exceptions.

The relaxation process back to the original state was studied for the *para* copolymer with 59% azo residues by CD spectra.[18] The CD spectra associated with the amide n-π^* transition change in a complicated manner in the course of relaxation (Fig. 8).[19] The behavior of the CD change can be attributed to the mixing of different conformations. After complete decay of the side-chain chromophores into the *trans* isomers the original CD spectrum was again obtained. The values of $[\theta]$ at 222 nm in the course of relaxation are plotted against the *cis* content (Fig. 9). It shows an abrupt change of $[\theta]_{222}$ at more than 50% *cis*. It should be noted that the transition is caused by variation in the ratio of different configurations of the side chains. The usual conformational transitions involving reversal in helix sense have been shown on copolymers composed of different amino acids such as β-benzyl L-aspartate and substituted benzyl L-aspartate by variation in the monomer ratios.[1] The present result demonstrates that the energy required for the conformational change comes from *cis* forms in the side chain, or light energy.

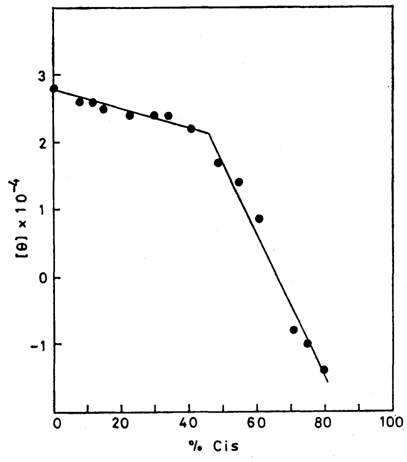


Fig. 9. Variation of $[\theta]$ values around 222 nm for *para* copolymer with 59% azo residues as a function of *cis* content.

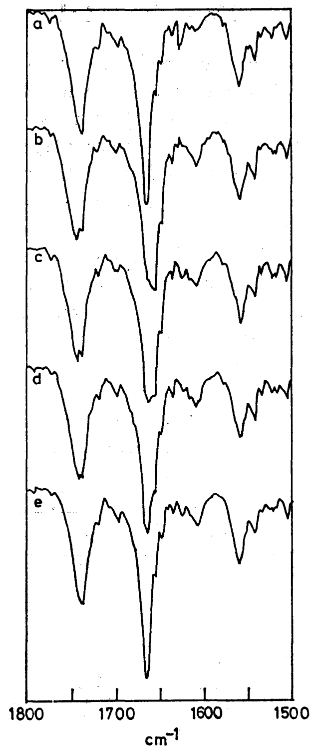


Fig. 10. Infrared spectra of the *para* copolymer with 59% azo residues before (a) and after (b; 0 min, c; 20 min, d; 50 min, e; 20 h) irradiation.

The infrared spectra of the *para* copolymer with 59% azo residues in the course of relaxation are shown in Fig. 10. Before irradiation, the spectrum of the copolymer shows the amide I at 1665 cm^{-1}, the amide II at 1560 cm^{-1} and the ester carbonyl at 1738 cm^{-1} characteristic of left-handed α-helices.[1c] On the other hand, the spectrum after irradiation shows shifted bands, the amide I at 1655 cm^{-1}, the amide II at 1558 cm^{-1} and the ester carbonyl at 1744 cm^{-1}, characteristic of right-handed α-helices. In the spectrum, absorptions attributable to the left-handed helix remain as shoulders. Thus the polymer after irradiation is not purely right-handed helical but contains left-handed conformation. The spectrum after 20 min shows comparable absorption intensities of both helices. The spectrum is finally restored to the original one.

Conclusion

The light-induced reversal in polypeptide helix sense was observed for the *para* copolymers which contain more than 50% azo residues. The conformational change occurs abruptly at more than 50% *cis*. Thus, the light-induced conformational change enables the polypeptides to act as elements which response to light in an on-off fashion. The system as well as systems such as left-handed helix to random coil and random coil to right-handed helix can be compared with visual purple, rhodopsin. Return to the original state is promoted by an isomerase for rhodopsin, but occurs spontaneously in these systems.

Experimental

All polymers were prepared according to the procedure described previously[5] with various ratios of β-benzyl L-aspartate *N*-carboxy anhydride (NCA) and β-*m*- or *p*-(phenylazo)benzyl L-aspartate NCA. Mole percentage of azo residues in the copolymers was estimated from their nitrogen contents obtained by elemental analyses. The reduced specific viscosity, η_{sp}/c, of each polymer was measured for a 0.1% 1,2-dichloroethane solution at 25 °C. Viscosity measurement could not be carried out for poly[β-*p*-(phenylazo)benzyl L-aspartate] and the *para* copolymer with 81% azo residues due to their poor solubility in 1,2-dichloroethane. The data on these polymers are summarized in Table 2.

TABLE 2. YIELDS AND VISCOSITIES OF AZOAROMATIC POLYPEPTIDES

Copolymer series	mol % azo NCA[a]	mol % azo residues[b]	Yield mg	Yield %	η_{sp}/c[c]
meta	10	9.7	235	54	1.13
meta	30	32	125	77	0.66
meta	50	49	65	63	0.44
meta	70	67	41	52	0.79
meta	90	92	28	42	0.23
meta	100	100	218	75	0.23
para	10	7.4	216	69	0.55
para	30	26	79	65	0.71
para	50	43	103	62	0.59
para	70	59	80	29	0.66
para	90	81	66	47	d)
para	100	100	63	61	e)

a) mol % of β-*m*- or *p*-(phenylazo) benzyl L-aspartate NCA reacted with β-benzyl L-aspartate NCA. b) Determined from the nitrogen content of elemental analysis. c) Measured for 0.1% 1,2-dichloroethane solutions at 25 °C. d) Almost insoluble. e) Insoluble.

Photoirradiation was carried out with a 500-W xenon lamp. A Corning 7-37 filter was used to pass the light of 320—380 nm. The *cis* content was calculated from the absorbance at 320 nm assuming that the absorbance is essentially proportional to the concentration of the *trans* isomer in view of the low extinction of the *cis* isomer for the wavelength.[20] Ultraviolet and visible spectra were measured with a JASCO UVIDEC-1 spectrophotometer using 3×10^{-5} M (azo residues) solutions. NMR spectra were recorded on a JEOL PS-100 spectrometer. Solutions of *ca.* 10 mg/ml were prepared in $CDCl_3$ containing tetramethylsilane as an internal reference. CD spectra were recorded on a JASCO J-20 circular dichrograph apparatus at 25 °C with cell thickness 0.1 and 0.01 cm using 0.3—1 mg/ml solutions. Due to the absorption of solvent, no spectra could be obtained below 210 nm. Molar ellipticities were calculated for the band around 222 nm by use of molar concentration of the amide group, while the molar concentration of the azo residues was used for the calculation of the ellipticities associated with the extrinsic bands. No CD spectrum of poly[β-*p*-(phenylazo)benzyl L-aspartate] could be measured due to its insolubility in 1,2-dichloroethane. As for the *para* copolymer with 81% azo residues, soluble part was used. On the irradiated solutions, special care was taken in order to accomplish the spectral measurements quickly.

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14) The [θ] values of the side-chain CD bands of the *para* copolymer with 59% azo residues reported[7] differ from those given here since the values in this paper are calculated based on the molar concentrations of the azo residues.

15) (a) W. Moffitt, *Proc. Natl. Acad. Sci. U. S. A.*, **42**, 736 (1956); (b) M. Kasha, *Radiat. Res.* **20**, 55 (1963); (c) W. Rhodes, *J. Am. Chem. Soc.*, **83**, 3609 (1961).

16) The precipitous appearance of the exciton resonance coupling on increasing azo residues might indicate that the azo residues are not distributed at random in the copolymers with high azo residues.

17) Y. Konishi and M. Hatano, *J. Polym. Sci., Polym. Lett. Ed.*, **14**, 351 (1976).

18) The half-life of this copolymer is 730 min at 40 °C starting from the photostationary state.

19) No time parameter is given in Fig. 8 since the solution is occasionally warmed for the convenience of measurements covering wide *cis* percentages.

20) C. S. Paik and H. Morawetz, *Macromolecules*, **5**, 171 (1972).