Light-induced Conformational Changes of Polypeptides. Random Copolymers of γ-Benzyl-L-glutamate with *m*- and *p*-Phenylazobenzyl-L-aspartates

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Copolymers of γ-benzyl-L-glutamate with *m*- and *p*-phenylazobenzyl-L-aspartates were prepared for investigating the effect of side-chain photoisomerization on their conformations. A study was made of the circular dichroism associated with the n,π^* transition of the amide group, as well as the extrinsic bands associated with the azo chromophore. The intensity of the 222-nm amide band seems unaffected by the interaction with the side-chain chromophore. The circular dichroism spectra before irradiation indicate that poly[β-(*m*-phenylazobenzyl)-L-aspartate] (mPALA) adopts a left-handed α-helical form and the other copolymers a right-handed form in 1,2-dichloroethane. After irradiation, remarkable changes of the 222-nm band were observed for the meta copolymer with 89.3% azo residues and mPALA. The increased absolute ellipticity for the former indicates that photoisomerization enhances the helicity of the polymer. Decrease in ellipticity for the latter indicates that photoisomerization induces significant instability of the left-handed α-helical conformation. In the course of subsequent relaxation of mPALA, an abrupt change in the CD spectra was found above 50% of cis followed by a gradual change with decreasing cis%.

The conformation of ester derivatives of poly(L-aspartic acid) has been shown to depend on the nature of the side chain. Left- and right-handed α-helical conformations are usually identified by the circular dichroism (CD), IR or NMR spectra. Poly(β-benzyl-L-aspartate) (PBLA) was shown to adopt a left-handed α-helical conformation,[1,2] but the para substitution of the benzene ring with a methyl, chloro, cyano, or nitro group induces a reversal of the helical sense to a right-handed α-helical conformation.[3–11] The chloro substituted PBLAs assume different handed helices depending on the position of the chlorine atom attached to the benzene ring.[12] These observations indicate that the balance of forces is so delicate that a small change in the side-chain conformation could reverse the helix sense. This prompted us to realize conformational changes of polyaspartates by inducing a configurational change of their side chains. From this view point we prepared some copolymer series containing azobenzene groups in their side chains. We report here the results of copolymers of γ-benzyl-L-glutamate with *m*- and *p*-phenylazobenzyl-L-aspartate. The side-chain azo chromophore undergoes trans-to-cis photoisomerization and subsequent relaxation back to the original trans form. The energy balance of the polymers might be perturbed by the configurational change, and the conformational changes as exemplified below might be possible.

$$\text{left-handed helix} \underset{\Delta}{\overset{h\nu}{\rightleftharpoons}} \text{right-handed helix} \tag{1}$$

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

$$\text{random coil} \underset{\Delta}{\overset{h\nu}{\rightleftharpoons}} \text{right-handed helix} \tag{3}$$

Process 1 has been confirmed to occur for a copolymer of β-benzyl-L-aspartate and β-(*p*-phenylazobenzyl)-L-aspartate (41 : 59).[13] In this report, Processes 2 and 3 are discussed. Photoisomerization of polypeptide side chains itself has been reported for the copolymers of L-*p*-(phenylazo)phenylalanine[14] and also for poly(γ-cinnamyl-L-glutamate).[15] However, no conformational change could be induced by irradiation probably due to their conformational stability.

Experimental

Materials. The solvents used for recrystallization of *N*-carboxy anhydries (NCA) and for polymerization were purified shortly before use, dioxane and hexane by distillation over sodium, ethyl acetate by distillation over CaH_2, and triethylamine by drying and distillation over KOH. For the measurements of optical properties in solution, "Dotite Spectrosol" grade 1,2-dichloroethane was used without further purification.

*p-Aminobenzyl Alcohol (**1**).* A mixture of *p*-nitrobenzyl alcohol (30 g), dry methanol (50 ml) and palladium carbon (5 weight%) (1.5 g) was agitated in an autoclave under an atmosphere of hydrogen (initial pressure was 100 kg/cm^2). After the catalyst had been removed by suction filtration, the filtrate was evaporated to dryness under reduced pressure. The crude product was recrystallized from ethanol. Yield, 18.5 g (77%); mp 58—60 °C (lit,[16] 63—64 °C). Found: C, 67.71; H, 7.22; N, 11.30%. Calcd for C_7H_9NO: C, 68.27; H, 7.37; N, 11.37%.

*m-Aminobenzyl Alcohol (**2**).* *m*-Nitrobenzyl alcohol (30 g) was reduced, as described above, to *m*-aminobenzyl alcohol. Yield, 15.9 g (66%); mp 91—93 °C (lit,[17] 95—96 °C). Found: C, 68.29; H, 7.47; N, 11.15%. Calcd for C_7H_9NO: C, 68.27; H, 7.37; N, 11.37%.

*p-Phenylazobenzyl Alcohol (**3**).* A mixture of alcohol **1** (12 g) and ethanol (40 ml) was added with stirring to a mixture of nitrosobenzene (12 g), ethanol (120 ml) and glacial acetic acid (12 ml). After the mixture had been stirred for 2 h, the product was crystallized upon cooling. The crude solid was recrystallized from ethanol. Yield, 9.9 g (48%); mp 142—143.5 °C (lit,[18] 142.5—143 °C). Found: C, 72.78; H, 5.72; N, 13.16%. Calcd for $C_{13}H_{12}N_2O$: C, 73.56; H, 5.70; N, 13.20%.

*m-Phenylazobenzyl Alcohol (**4**).* This alcohol was prepared from **2** (12 g) and nitrosobenzene (12 g) in the same way as for **3**. The crude product was purified by distillation (177 °C/3 mmHg). Yield, 18.8 g (91%); mp 36 °C (lit,[19] 34.5—36 °C). Found: C, 73.50; H, 5.68; N, 13.10%. Calcd for $C_{13}H_{12}N_2O$: C, 73.56; H, 5.70; N, 13.20%.

*p-Phenylazobenzyl-L-aspartate (**5**).* A mixture of L-

TABLE 1. YIELDS, VISCOSITIES, AND CIS PERCENTAGE IN THE PHOTOSTATIONARY STATE

Copolymer series$^{a)}$	Mol% azo NCA$^{b)}$	Mol% azo content$^{c)}$	Yield		$\eta_{sp}/c^{d)}$	Cis%
			mg	%		
p	50	46.5	19	18	0.04	69
p	70	74.0	22	22	—	74
p	90	85.9	27	26	0.16	68
p	100	100	63	61	—	70
m	30	26.2	62	63	0.51	88
m	50	45.9	46	44	0.37	75
m	70	78.0	5	4	—	68
m	90	89.3	64	53	0.28	62
m	100	100	218	75	0.23	68

a) p: para-copolymer. m; meta-copolymer. b) Mol% of *m*- or *p*-phenylazobenzyl-L-aspartate NCA reacted with γ-benzyl-L-glutamate NCA. c) Determined from the nitrogen content of elemental analysis. d) 0.1% 1,2-dichloroethane solution.

aspartic acid (5.8 g), alcohol **3** (10 g), *p*-toluenesulfonic acid (9.2 g), and dioxane (60 ml) was heated with stirring at 80 °C for 24 h. The reaction mixture was evaporated to dryness, and the residue was neutralized with a sodium hydrogencarbonate solution. The precipitated crystalline product was filtered, and washed with acetone. This was recrystallized from a mixed solution of dioxane and water (1 : 1 by volume). Yield, 1.3 g (8.4%); mp 229—229.5 °C; $[\alpha]_D=+0.63$ ($c=3.1$, dichloroacetic acid). IR: 1735, 1585 cm^{-1}. Found: C, 62.26; H, 5.38; N, 12.33%. Calcd for $C_{17}H_{17}N_3O_4$: C, 62.38; H, 5.23; N, 12.84%.

m-Phenylazobenzyl-L-aspartate (6). Ester **6** was prepared from alcohol **4** (10 g) and L-aspartic acid (5.8 g) in the same way as for **5**. Yield, 0.86 g (5.8%); mp 218—218.5 °C; $[\alpha]_D=+0.93°$ ($c=1.5$, acetic acid). IR: 1720, 1585 cm^{-1}. Found: C, 62.41; H, 5.45; N, 12.38%. Calcd for $C_{18}H_{15}N_3O_5$: C, 62.38; H, 5.23; N, 12.84%.

p-Phenylazobenzyl-L-aspartate NCA (7). This NCA was prepared from amino acid **5** (2.5 g) and phosgene using the reported procedure.[20] Yield, 1.3 g (48%); mp 240—240.5 °C. IR: 1860, 1788, 1725 cm^{-1}. Found: C, 61.41; H, 4.42; N, 12.02%. Calcd for $C_{18}H_{15}N_3O_5$: C, 61.19; H, 4.28; N, 11.89%.

m-Phenylazobenzyl-L-aspartate NCA (8). This NCA was prepared from amino acid **6** (2 g) and phosgene using the reported procedure.[20] Yield, 1.2 g (56%), mp 133.5—134.5 °C. IR: 1850, 1800, 1735 cm^{-1}. Found: C, 61.27; H, 4.27; N, 11.86%. Calcd for $C_{18}H_{15}N_3O_5$: C, 61.19; H, 4.28; N, 11.89%.

Poly[β-(p-phenylazobenzyl)-L-aspartate] (pPALA). NCA **7** was dissolved in dioxane (0.1 M), and a 0.004-M solution of triethylamine in dioxane (monomer/initiator=40) was added. Polymerizatiom was carried out at room temperature for 5 days. The reaction mixture was added to vigorously stirred ethanol. The precipitate was collected on a filter, washed with several portions of ethanol and dried *in vacuo* at 40 °C. Found: C, 65.79; H, 4.83; N, 13.08%. Calcd for $(C_{17}H_{15}N_3O_3)_n$: C, 66.01; H, 4.89; N, 13.58%.

Poly[β-(m-phenylazobenzyl)-L-aspartate] (mPALA). mPALA was prepared from NCA **8** in the same way as for pPALA. Found: C, 66.24; H, 4.91; N, 13.73%. Calcd for $(C_{17}H_{15}N_3O_3)_n$: C, 66.01; H, 4.89; 13.58%.

Polymers. Copolymers were prepared in an analogous way to that for homopolymers by using varing ratios of γ-benzyl-L-glutamate NCA and *m*- or *p*-phenylazobenzyl-L-aspartate NCA. Mole percentage of azo residue 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 pPALA because of its insoluble character, or for the copolymers containing 74.0% *p*-phenylazobenzyl residue and 78.0% *m*-phenylazobenzyl residue because of low yields of the samples. Data on the homopolymers and copolymers are summerized in Table 1.

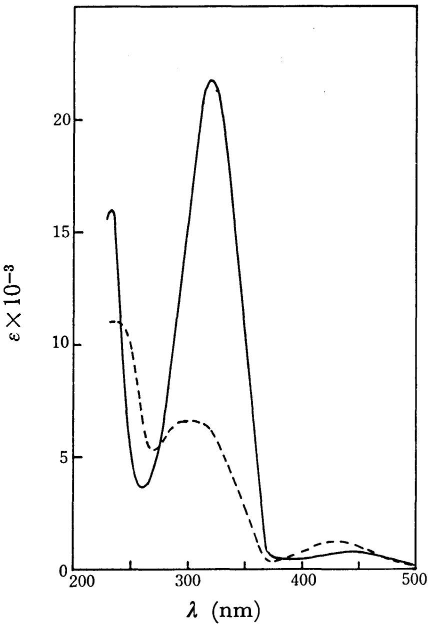

Fig. 1. Ultraviolet spectra of mPALA before (——) and after (---) irradiation.

Photoisomerization. Photoirradiation was carried out on solutions with concentration of 1-mg/ml using a Corning 7—37 filter which permits the light of 310—380 nm to pass. The cis percentage was calculated from the absorbance at 320 nm assuming that the absorbance is substantially proportional to the concentration of the trans isomer in view of the low extinction of the cis isomer at the wavelength.[21]

Measurements. CD spectra were recorded on a JASCO J-20 circular dichrograph apparatus with cell thickness of 0.1 and 0.01 cm. Due to the absorption of the solvent no spectra could be obtained below 210 nm. Molar ellipticities were calculated for the supposedly intrinsic band around 222 nm using the molar concentration of amide groups, while the molar concentration of the azo groups was used for the calculation of the ellipticities associated with the extrinsic bands. Ultraviolet (UV) spectra were measured with a JASCO UVIDEC-1 spectrophotometer.

Results and Discussion

Photoisomerization. The UV spectra of mPALA before and after irradiation are shown in Fig. 1. The major electronic transitions of this polymer are essentially those of azobenzene.[22] The band for mPALA before irradiation in the 320 nm region ($\varepsilon=22000$) is attributed to π,π^* transition and the band in the 430 nm region ($\varepsilon=700$) to n,π^* transition.[23] The spectrum after irradiation resembles the spectrum of *cis*-azobenzene reported by Gerson and co-workers.[22] The spectra

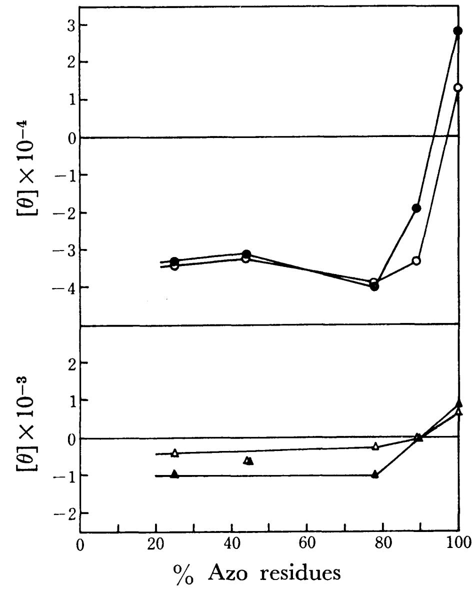

Fig. 2. Variation of the maximum or minimum ellipticities associated with the side-chain n,π* (▲,△) and the amide n,π* (●,○) transitions before (▲,●) and after (△,○) irradiation for the meta-copolymers as a functions of the azo content.

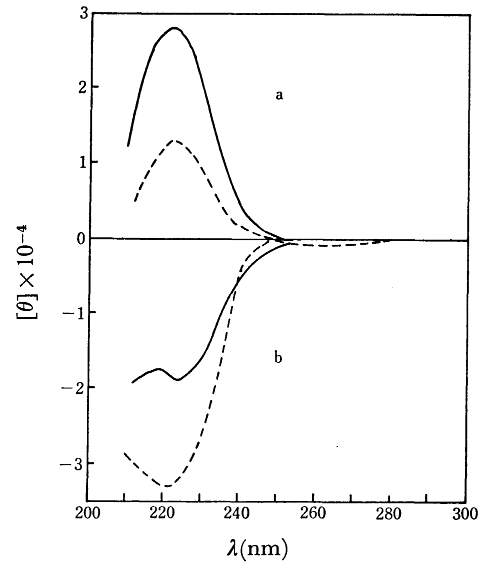

Fig. 3. CD spectra of mPALA (a) and the meta-copolymer with the azo content of 89.3% (b) before (——) and after (---) irradiation.

of the other polymers before and after irradiation are similar to those of mPALA in the regions of the azo π,π* and n,π* transitions.

The cis percentages of all the polymers at the photostationary state are given in Table 1. The values of the para copolymer series are almost the same and close to 70%. On the other hand, a considerable decrease in cis percentage is observed for the meta copolymer series on proceeding from the copolymer with the least azo residue to the copolymer which contains 89.3% azo residue. The decreased cis conversion for the meta-copolymers may result from steric restrictions between the side-chain meta-azobenzene groups.

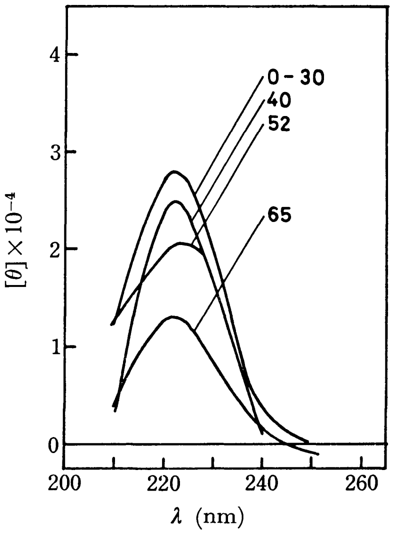

Fig. 4. CD spectra of mPALA at different cis per cents in the course of relaxation.

The cis percentage of mPALA seems slightly higher as a polymer with side-chains of maximal steric restrictions. This might be due to the conformational difference.

CD Spectra of meta-Copolymers. The maximum or minimum ellipticities associated with the amide n,π* transition at 222 nm before and after irradiation are plotted against the mole percentage of azo residue (Fig. 2). The values are almost independent of the copolymer composition for the copolymers with less than 80% azo residue. These observations suggest that the overlapping of allowed transitions of the side-chain chromophore with those from the peptide main chain is either small or absent in the polymers. This is in contrast to that for other azoaromatic copolypeptides which contain *p*-(*p*-hydroxyphenylazo)phenylalanine residue.[24] For these polymers, decreasing intensity of the peptide main-chain band was observed with increase in mole percentage of the azo residue. If one takes $[\theta]_{222}=-40000\pm10\%$ for 100% right-handed α-helix,[25] the meta-copolymers are all confirmed to exist as right-handed α-helices. On the other hand, the CD spectrum of mPALA before irradiation shows a maximum with the value of $[\theta]_{222}=28000$ (Fig. 3), confirming the conclusion together with the results of its IR spectrum that the helix of mPALA is left-handed in 1,2-dichloroethane. The ellipticities at 222 nm seem unaffected by irradiation for the copolymers with less than 80% azo residue. However, irradiation causes significant changes in the ellipticity values of the copolymers with more azo residue. The absolute ellipticity of 89.3% azo copolymer increases from 19000 to 33000 by irradiation, confirming the increased percentage of helicity. This enhanced helix content might be due to the release of steric restrictions between the trans side chains by the formation of cis isomers. The ellipticity of mPALA decreases from 28000 to 13000 by irradiation, confirming the induced instability for its left-handed α-helical conformation. This observation could be due to the partial transition from the left-handed α-helix to a random coil or a right-handed

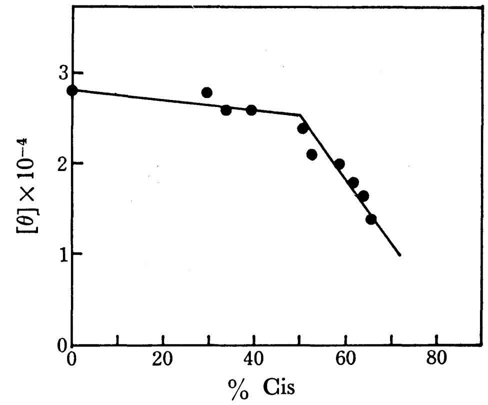

Fig. 5. Variation of the maximum ellipticities around 222 nm for mPALA as a function of the cis per cent.

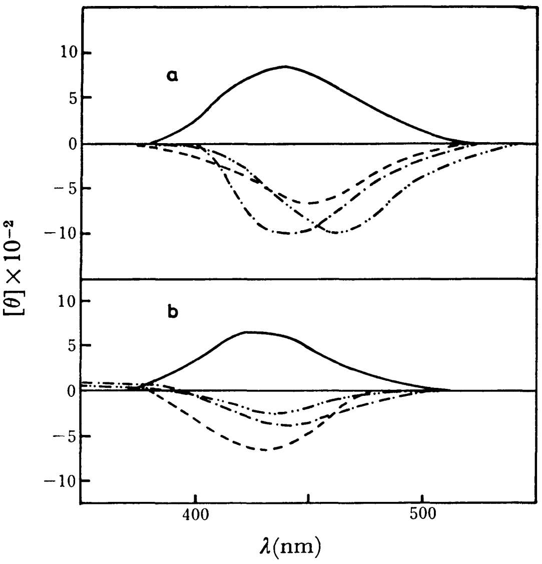

Fig. 6. CD spectra associated with the side-chain n,π* transition before (a) and after (b) irradiation for the meta-copolymers. Azo%: 100(——); 78.0(—··—); 45.9 (---); 26.2 (—·—).

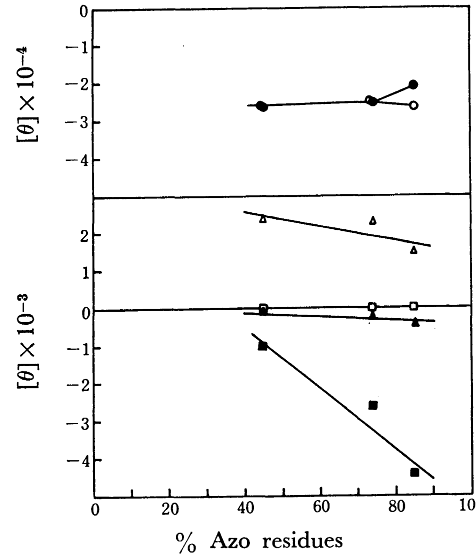

Fig. 7. Variation of the maximum or minimum ellipticities associated with the side-chain n,π* (▲,△), π,π* (■,□) and the amide n,π* (●,○) transitions before (▲,■,●) and after (△,□,○) irradiation for the para-copolymers as a function of the azo content.

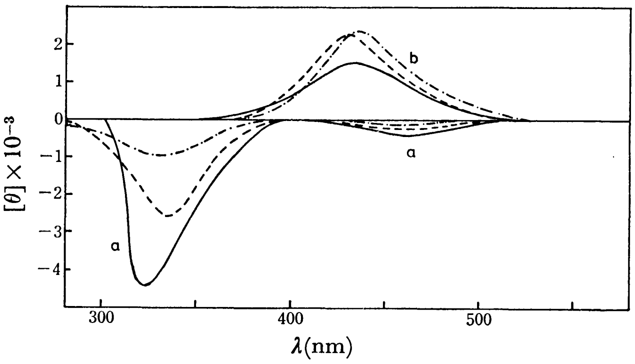

Fig. 8. CD spectra associated with the side-chain n,π* and π,π* transitions before (a) and after (b) irradiation for the para-copolymers. Azo%: 85.9 (——); 74.0 (---); 46.5 (—·—).

α-helix. We reported that the copolymer of *p*-phenylazobenzyl-L-aspartate and β-benzyl-L-aspartate (59 : 41) undergoes a light-induced conformational change from left- to right-handed helix.[13] The CD band associated with the peptide n,π* transition of this copolymer changes its sign from positive to negative by irradiation, giving clear evidence for inversion of its helix sense. In the case of mPALA, however, it is difficult only by the CD spectrum to determine which of the conformational transformations is responsible for the observation. NMR spectra could, in principle, give some information.

Subsequent relaxation of mPALA back to the original conformation was followed by its CD spectra (Fig. 4). Maximum ellipticities at 222 nm are plotted as a function of cis percentage (Fig. 5). It shows an abrupt change of $[\theta]_{222}$ at over 50% followed by a gradual change with decrease. The result demonstrates that the energy required for the conformational change comes from cis forms in the side chain, *viz.*, ultimately from light energy.

The CD spectra associated with the n,π* transition of the side-chain chromophore before and after irradiation are given in Fig. 6, maximum or minimum ellipticities being plotted against mole percentage of azo residue (Fig. 2). The copolymers show no dichroic band in the region of the π,π* transition of the side-chain chromophore. One important aspect is the relation between the sign of the side-chain CD band and the backbone helix sense. There is a possibility that the sign of a side-chain band is opposite corresponding to the left- and right-handed helices.[20] The CD spectra of the meta copolymer series provide one example of the corresponding behavior since mPALA shows a positive band while the right-handed copolymers show negative bands. Usually the origin of induced dichroic bands are discussed in the terms of side chain-side chain and side chain-main chain interactions. In the case of the former interactions, the induced circular dichroism is expected to be dependent on the concentration of the side-chain chromophores in the copolymers. No such dependency was observed for the polymers we studied. The ellipticities of the side-chain CD bands before and after irradiation are almost independent of mole percentage of azo residue in the copolymers unless conformational changes are induced. Thus the side-chain n,π^* CD band should be attributed to the coupling between the backbone and the side chain.

CD Spectra of para-Copolymers. The minimum ellipticities associated with the amide n,π^* transition are plotted against mole percentage of azo residue in Fig. 7. The CD spectrum of pPALA could not be measured since it is insoluble in 1,2-dichloroethane. The spectra of the copolymers show that they assume a right-handed α-helical conformation. Photoirradiation did not change the minimum ellipticities of the copolymers in the region of the amide n,π^* transition except for the azo 85.9% copolymer. This copolymer shows increase in absolute ellipticity after irradiation, which indicates an increase of helicity though not so remarkable as observed for the 89.3% meta-azo copolymer.

The CD spectra before and after irradiation associated with the side-chain n,π^* and π,π^* transitions are given in Fig. 8. Their maximum or minimum ellipticities are plotted against the mole percentage of azo residue in Fig. 7. The side-chain transitions are affected remarkably by irradiation. The ellipticity for the n,π^* transition increases a great deal with inversion of its sign, whereas the circular dichroism for the π,π^* transition vanishes. Similarly to the case for the meta-copolymers, the ellipticities of the side-chain n,π^* CD bands before and after irradiation seem to be almost independent of the mole percentage of azo residue. This confirms the fact that the origin of the CD bands resides in side chain-main chain interactions. However, the absolute ellipticity of the side-chain π,π^* band observed only before irradiation increases with increasing azo residue, which suggests side chain-side chain interactions as the origin of the CD band.

Conclusion

The effect of irradiation on the backbone conformation is remarkable for the two polymers of the meta copolymer series, the 89.3% azo copolymer and mPALA For the former, stability of the right-handed α-helical conformation is promoted. On the other hand, significant instability is induced for the left-handed α-helical conformation of the latter. The conformational change of mPALA occurs abruptly at greater than 50% cis. Thus, the light-induced conformational change can be compared with visual purple, rhodopsin, where its retinal component undergoes photoisomerization accompanied by a change in conformation of opsin.

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