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Solvent-induced Conformational Changes of Arylazo-substituted Polyaspartates

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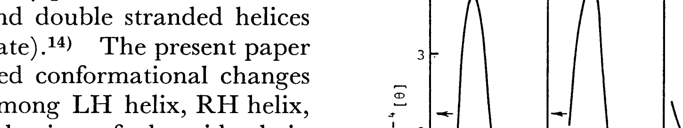
Copolymers of β-benzyl L-aspartate and β-[*p*-(phenylazo)phenethyl] L-aspartate were prepared and their conformations studied by circular dichroism (CD) spectroscopy. The copolymers with less than 40% azo amino acid residues were found to be left-handed (LH) helical, those with more azo amino acid residues right-handed (RH) helical in 1,2-dichloroethane (DCE) solution. The former undergo LH helix→random coil transition on addition of hexafluoro-2-propanol (HFIP) to their DCE solutions, the latter RH helix→LH helix→random coil transition on addition of HFIP. All polymers in DCE exhibit a negative CD band in the side-chain n-π* transition region. The side-chain π-π* CD band was observed only for the copolymers with the highest azo amino acid residues (67%). Its absolute ellipticity decreases with increasing HFIP content, finally disappearing at 10% HFIP. The sign of the n-π* CD band of the copolymer remains negative irrespective of the helix reversion, its absolute ellipticity being unchanged below 10% HFIP and decreasing on further increase in HFIP content. The results suggest solvent-induced changes in orientation or mobility of the side-chain phenylazo moieties.

Circular dichroism (CD) measurements have been extensively used to evaluate the conformation of polypeptides in solution, the evaluation being based on the peptide CD bands. However, it is very difficult to assign the conformation of polypeptides with aromatic side chains since the usually very useful indications are made useless by the presence of additional side-chain CD bands in the same spectral region.[1] In spite of this unfavorable effect, the side-chain CD might provide information of main-chain conformation as well as orientation, mobility and arrangement of side chains themselves. As examples of polypeptides with aromatic side chains, some arylazo polypeptides have been prepared, and their chiroptical properties investigated.[1–10] Goodman *et al.* prepared polypeptides composed of (phenylazo)phenylalanine and its analogues,[2–6] and observed that the peptide CD bands of their α-helices are significantly perturbed by the presence of the aromatic side chains. There is no such complication for the arylazo polyaspartates[7–9] and azo-modified poly(L-glutamic acid).[1,10] The use of these polypeptides would establish a relationship between side-chain CD and main-chain conformation. Various conformations are needed in order to gain insight into the relationship. Arylazo polyaspartates seem to be suitable since polyaspartates often exhibit conformational versatility.[11] Light-induced conformational changes, such as left-handed (LH) helix→right-handed (RH) helix and LH helix→random coil transitions have been observed on some arylazo polyaspartates.[7–9] We have prepared a new series of arylazo polyaspartates composed of β-[*p*-(phenylazo)phenethyl] L-aspartate and β-benzyl L-aspartate,



and found that their conformations are very sensitive to solvent. Several reports have appeared on solvent-induced conformational changes of polypeptides: helix reversion for polyaspartates[11d,12] and poly[L-β-[3,4-bis(benzyloxycarbonyloxy)phenyl]-α-alanine],[13] transformations between single and double stranded helices for poly(γ-benzyl D-L-glutamate).[14] The present paper deals with the solvent-induced conformational changes of the arylazo copolymers among LH helix, RH helix, and random coil. The behavior of the side-chain CD during the course of conformational change was investigated in order to elucidate the relationship between the side-chain CD and the main-chain conformation.

Results and Discussion

The CD spectra in the region below 260 nm measured for the copolymers dissolved in 1,2-dichloroethane (DCE) are shown in Fig. 1. The spectra of copolymers with less phenylazo residues (azo amino acid residues< 40%) exhibit a positive CD band around 222 nm,

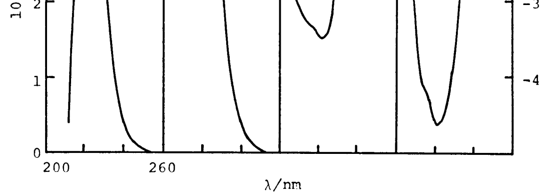


Fig. 1. CD spectra of the copolymers with different contents of phenylazo residues in DCE solution. Phenylazo content: 11% (a); 26% (b); 46% (c); 67% (d).

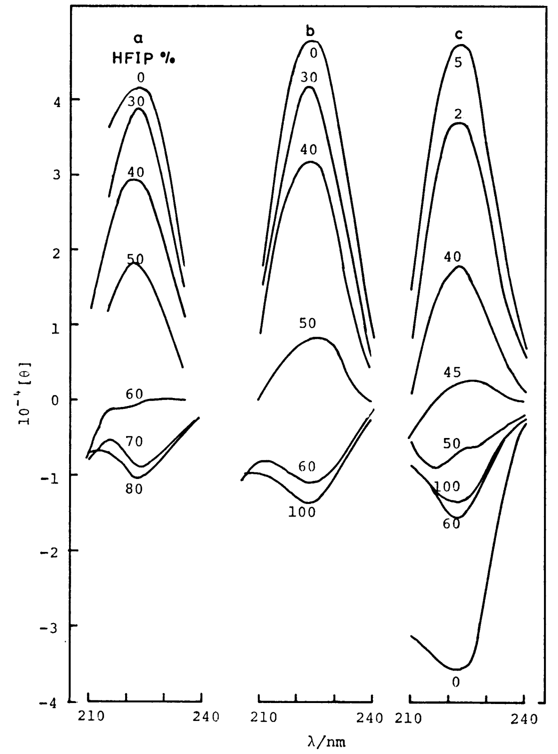


Fig. 2. CD spectra of the copolymers with phenylazo residues of 11% (a), 26% (b), and 46% (c) in mixed solutions of DCE and HFIP.

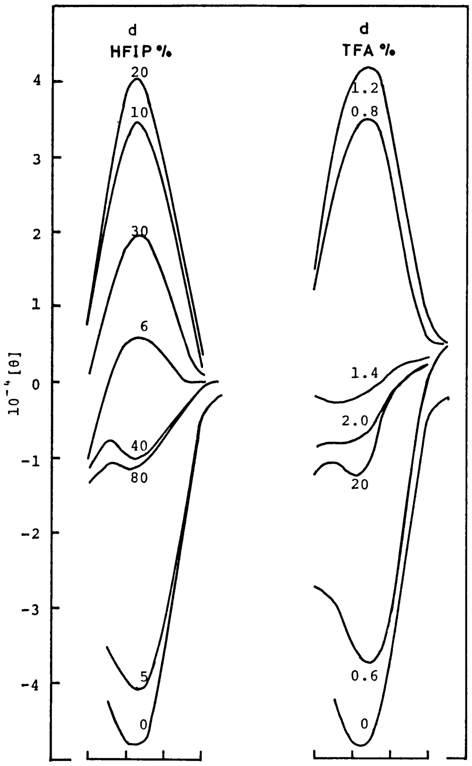


Fig. 3. CD spectra of the copolymer with 67% phenylazo residues in DCE–HFIP and DCE–TFA solvent systems.

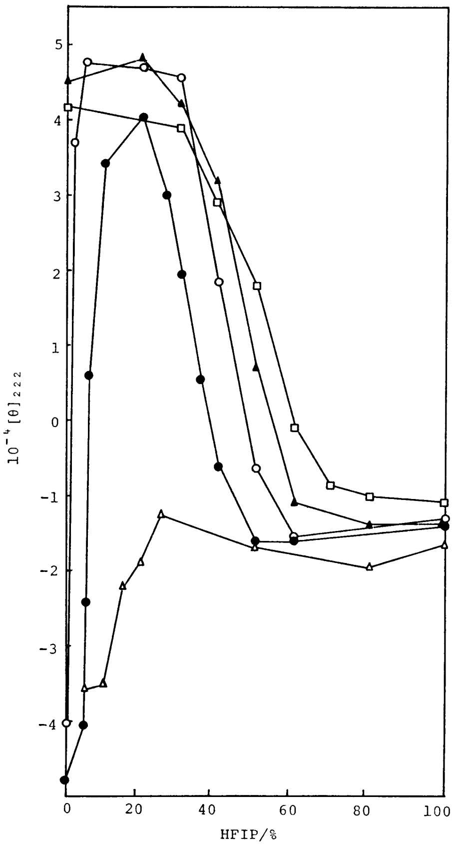


Fig. 4. Dependence of the ellipticity at 222 nm on HFIP content.
Phenylazo content: 11% (□); 26% (▲); 46% (○); 67% (●); 100% (△).

characteristic of the LH helix, those with more phenylazo residues (>40%) a negative CD band around 222 nm, characteristic of the RH helix. The homopolymer with 100% azo residues being insoluble in DCE, no spectral measurement was carried out in this solvent.

The CD spectra of the polymers were found to change on addition of hexafluoro-2-propanol (HFIP) to their DCE solutions (Figs. 2, 3, and 4). The molecular ellipticities $[\theta]$ for the copolymers with more than 40% phenylazo residues change from negative to positive on increasing HFIP content (up to 20%), confirming the conformational transition from RH helix to LH helix. Further increase in HFIP content causes another change in the $[\theta]$ values from positive to negative, which may correspond to the conformational transition from LH helix to random coil; increase in HFIP content above 50% causes no significant change. The following transition process is thus established.

$$\text{RH helix} \xrightarrow{\text{HFIP}} \text{LH helix} \xrightarrow{\text{HFIP}} \text{random coil} \quad (1)$$

The anomalous $[\theta]$ values for the random coil suggest that the conformation of these copolymers is not entirely random, possibly due to side chain-side chain steric interactions. Bradbury *et al.* reported a similar phenomenon, the conformational transitions in their case being induced by addition of trifluoroacetic acid

TABLE 1. ELEMENTAL ANALYSES AND VISCOSITIES OF COPOLYMERS OF β-BENZYL L-ASPARTATE AND β-[p-(PHENYLAZO)PHENETHYL] L-ASPARTATE

Mol % phenylazo residues	Found (%)			Calcd (%)			η_{sp}/c
	C	H	N	C	H	N	
10.53	64.24	5.41	7.63	64.69	5.40	7.63	1.41
25.92	64.87	5.42	9.02	65.25	5.38	9.02	3.97
45.79	65.20	5.35	10.35	65.79	5.35	10.35	2.36
67.24	65.72	5.42	11.54	66.26	5.33	11.54	2.50
100	65.81	5.51	12.47	66.85	5.31	13.00	—

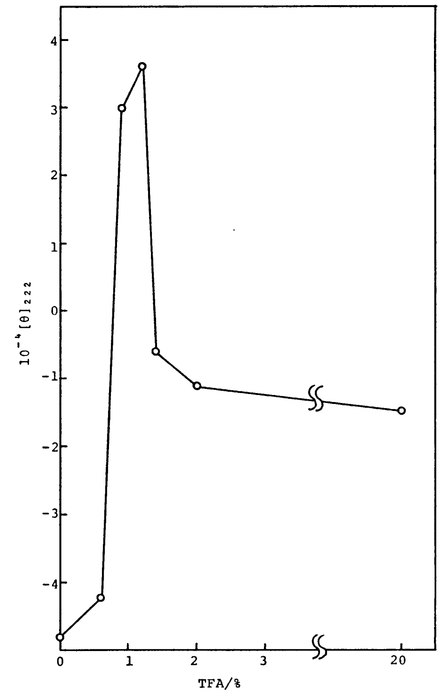


Fig. 5. Dependence of the ellipticity at 222 nm on TFA content for the copolymer with 67% phenylazo residues.

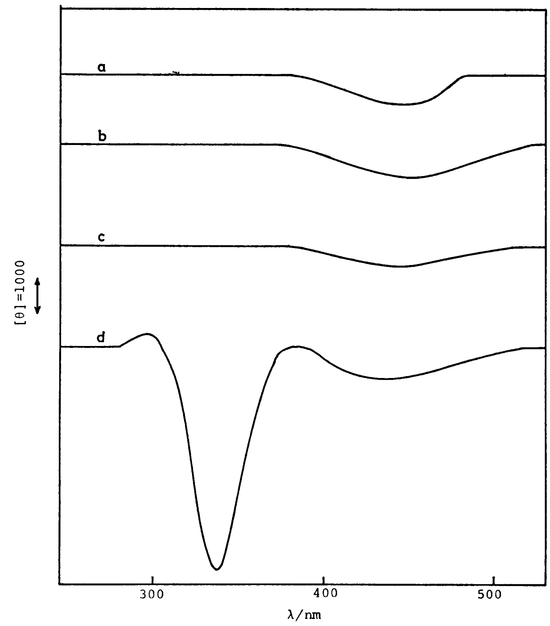


Fig. 6. CD spectra associated with the side-chain n-π* and π-π* transitions in DCE.
Phenylazo content: 11% (a); 26% (b); 46% (c); 67% (d).

(TFA) or dichloroacetic acid to chloroform solutions of copolymers of β-ethyl L-aspartate and β-benzyl L-aspartate.[12a] RH Helix→LH helix transition for the arylazo polyaspartates we studied was also induced by TFA addition, in agreement with their results (Fig. 5). The helix reversal for arylazo polyaspartates occurs on addition of a very small amount of TFA (<2%). The resulting LH helix readily turns into the random form on further addition of TFA, in line with the behavior of TFA as a random-coil promoting solvent.

In contrast to the copolymers with more than 40% phenylazo residues, the copolymers with less phenylazo residues are LH helical in pure DCE, undergoing only LH helix→random coil transition on addition of HFIP.

$$\text{LH helix} \xrightarrow{\text{HFIP}} \text{random coil} \qquad (2)$$

The homopolymer with 100% phenylazo residues is insoluble in DCE but soluble in HFIP. Its CD spectra at different HFIP contents were therefore measured on solutions prepared by adding DCE to the HFIP solution. The spectrum of the polymer in 100% HFIP exhibits a negative band around 222 nm attributable to the random-coil form. No positive band appears on increasing the DCE content, the spectrum attributable to a RH helix being finally attained (Fig. 4). Thus, the following process should occur if HFIP is added to the DCE solution.

$$\text{RH helix} \xrightarrow{\text{HFIP}} \text{random coil} \qquad (3)$$

Processes (1), (2), and (3) might arise from an alteration of the energy balance as a consequence of hydrogen bonding of HFIP to the side-chain ester groups, or to the main-chain amide groups.

The CD spectra associated with the side-chain n-π* and π-π* transitions were obtained for the polymers in DCE solution (Fig. 6). Molar ellipticities of the side-chain CD bands were calculated using the molar concentration of phenylazo residues. Among the four copolymers used, only one with 67% phenylazo residues exhibits the π-π* CD band, suggesting that the π-π* CD arises from electronic interactions between the side-chain azobenzene moieties. On the other hand, the n-π* CD band appears for all copolymers, the sign being the same regardless of the conformational differences. The minimum ellipticities around 440 nm are similar irrespective of copolymer composition. The results suggest that side chain-main

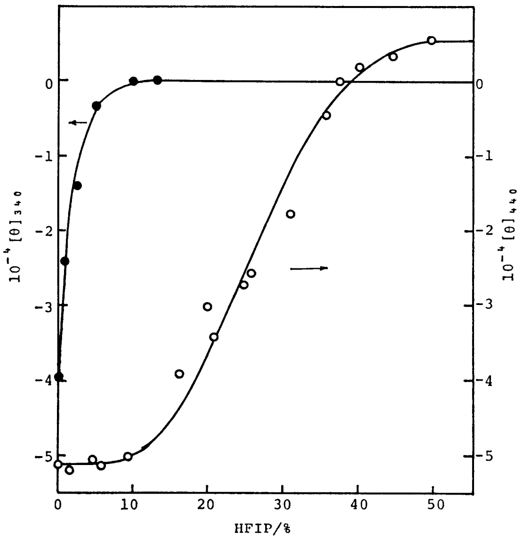


Fig. 7. Dependence of the ellipticities of the side-chain n-π^* and π-π^* CD bands on HFIP content for the copolymer with 67% phenylazo residues.
○: n-π^* CD band around 440 nm, ●: π-π^* CD band around 340 nm.

chain electronic interactions give rise to the band. Addition of HFIP to a DCE solution of the copolymer with 67% phenylazo residues alters both side-chain CD bands. The π-π^* CD band decreases in intensity with increasing HFIP content, finally disappearing at 10% HFIP where the conformation of the polymer is LH helical (Fig. 7). This indicates that the side chain - side chain electronic interactions, which are origin of the π-π^* CD band, become impossible, probably due to the disordered arrangement of the side-chain chromophores in the HFIP-solvated LH helix. The n-π^* CD band of the copolymer with 67% phenylazo residues shows no change up to 10% HFIP in spite of the observed RH helix→LH helix transition in this solvent range, confirming the insensitivity of this band to helix nature. Further increase in the HFIP content causes a decrease in the absolute ellipticity, even in the solvent range where the main-chain conformation remains LH helical. The absolute ellipticity continues to decrease over the HFIP concentration range in which LH helix→random coil transition occurs, the $[\theta]$ value becoming constant with a positive sign for the random-coil form. This behavior of the n-π^* CD band indicates that helical structures are necessary for its appearance. In the helical structures the side chains might be limited in mobility, adopting certain geometric positions in relation to the asymmetric centers of the main-chain backbone.

Experimental

CD spectra were recorded on a JASCO J-20 circular dichrograph apparatus at 25 °C using 0.3—1 g/l solutions. "Dotite Spectrosol" grade DCE and "Merck Urasol" grade HFIP were used without further purification. Due to the absorption of DCE, no spectra could be obtained below 210 nm. Molecular ellipticities for the band around 222 nm were calculated by means of the molar concentration of the amide group. Ellipticities for the side-chain CD bands were calculated by means of the molar concentration of the phenylazo residues. Polymers used for the CD measurements were synthesized as described below.

*p-(Phenylazo)phenethyl Alcohol (**1**).* The alcohol was prepared from *p*-aminophenethyl alcohol (25 g) and nitrosobenzene (19.5 g) by the method reported previously.[8] Yield, 30.5 g (74%); mp 90—91 °C. Found: C, 74.04; H, 6.22; N, 12.33%. Calcd for $C_{14}H_{14}N_2O$: C, 74.30; H, 6.25; N, 12.38%.

β-[p-(Phenylazo)phenethyl] L-*Aspartate (**2**).* Amino acid **2** was prepared from alcohol **1** (13.3 g) and L-aspartic acid (7.83 g) by the method reported previously.[15] Yield, 15.8 g (79%); mp 199—200 °C. IR (KBr): 1740, 1600, 1240 cm^{-1}. Found: C, 63.09; H, 5.56; N, 12.23%. Calcd for $C_{18}H_{19}N_3O_4$: C, 63.32; H, 5.62; N, 12.31%.

β-[p-(Phenylazo)phenethyl] L-*Aspartate N-Carboxy Anhydride (**3**).* *N*-Carboxy anhydride (NCA) was prepared from **2** (4 g) and trichloromethyl chloroformate (2.36 g).[16] The crude product was recrystallized twice from ethyl acetate and hexane. Yield, 0.93 g (22%); mp 160—161 °C. IR (KBr): 1858, 1800, 1766, 1740, 1180 cm^{-1}. Found: C, 62.33; H, 4.80; N, 11.64%. Calcd for $C_{19}H_{17}N_3O_5$: C, 62.11; H, 4.67; N, 11.44%.

Polymers. Polymers with different azo amino acid contents were prepared from **3** and *β*-benzyl L-aspartate NCA by varying the ratio of the two NCAs. Polymerization was carried out in dioxane at room temperature for 5 d using triethylamine as an initiator (monomer/initiator ratio 50:1). The reaction mixture was added to ethanol stirred vigorously. The precipitate was collected by filtration, washed with several portions of ethanol and dried *in vacuo* at 40 °C. Mole percentage of azo amino acid residues in the copolymers was estimated from the nitrogen content obtained by elemental analysis. The reduced specific viscosity, η_{sp}/c, for each polymer was measured for a 0.1% DCE solution at 25 °C. Viscosity measurement for the polymer composed of 100% azo amino acid residues could not be carried out due to its insolubility in DCE. Data for the polymers are sammarized in Table 1.

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