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Conformational Aspects of Polypeptide Structure. XXVI. Azoaromatic Side-Chain Effect from Poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine*

Murray Goodman and Ettore Benedetti

ABSTRACT: We have undertaken conformational analyses of polymers of L-*p*-(*p'*-hydroxyphenylazo)phenylalanine and copolymers of this amino acid with *N*-(3-hydroxypropyl)-L-glutamine. We compared the ultraviolet, optical rotatory dispersion, and circular dichroism spectra of these materials under neutral, acidic, and basic conditions with results obtained from the model compound, *N*-acetyl-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine methyl ester. In trimethyl phosphate the homopolymer and copolymers exist as right-handed α helices. We have evidence for exciton resonance coupling of spatially adjacent azoaromatic chromophores. In aqueous solutions above pH 10 but below pH 11.9, the homopolymer also exists as a right-handed α helix. Above pH 12 we observe a major change in the circular dichroism spectra. At high salt concentration the magnitude of the Cotton effect is reduced to the values assumed by the model compound under the same conditions. Lastly, in trifluoroacetic acid the azoaromatic residues are protonated. The circular dichroism spectrum provides evidence for an ordered polymer structure, perhaps of the polyelectrolyte type.

It is known that polypeptides containing large chromophores such as the indole, imidazole, and substituted aromatic groups exhibit side-chain in addition to main-chain (peptide) Cotton effects. These side-chain Cotton effects arise because the normally symmetric chromophores are in a dissymmetric environment. The sources of such environments include the asymmetric α-carbon of the peptide group, ordering of the main chain and/or with respect to each other, and intermolecular interactions between adjacent aggregated chains.

It is not completely clear what contribution the side chains make to the optical rotatory dispersion or circular dichroism spectra of polypeptides because side-chain Cotton effects often overlap those arising from the peptide groups. Several aromatic amino acids with auxochromic substituents on the aromatic ring have been investigated in our laboratories such as L-*p*-nitrophenylalanine (M. Goodman, unpublished results), L-*p*-aminophenylalanine (Goodman and Peggion, 1967), and L-*p*-(phenylazo)phenylalanine (Goodman and Kossoy, 1966). In our present report we extend our work on azoaromatic polypeptides and describe the synthesis and the conformational characterization of poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine and copolymers of

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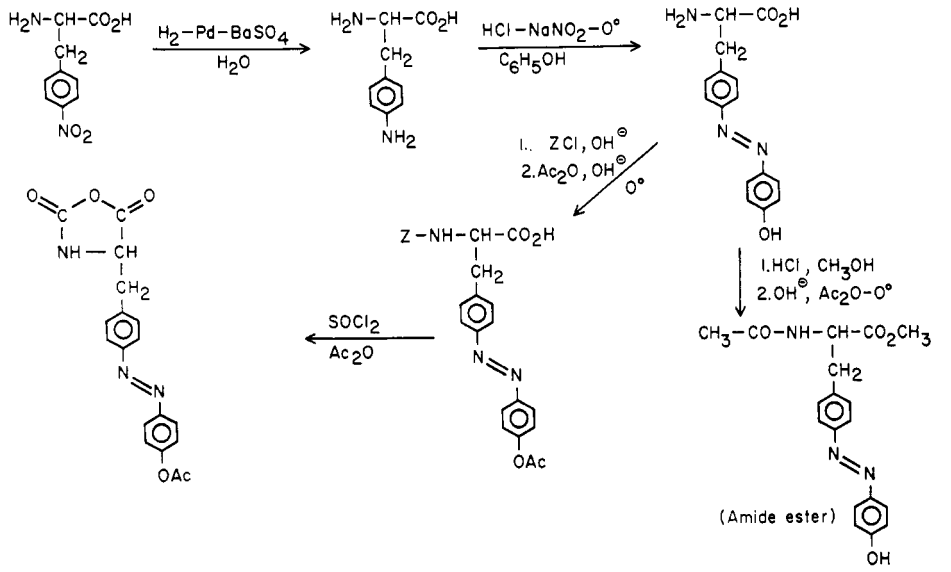
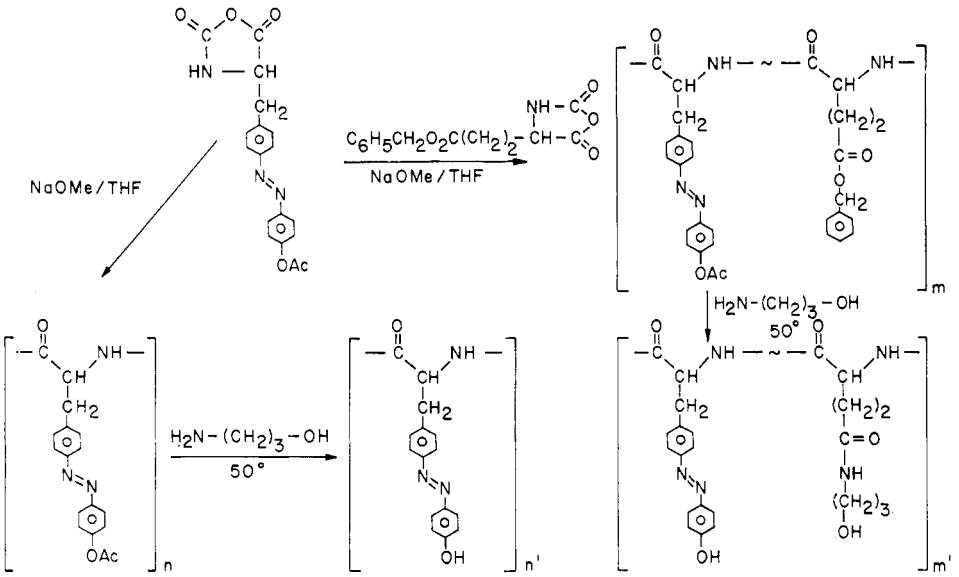
SCHEME I



SCHEME II



L-*p*-(*p'*-hydroxyphenylazo)phenylalanine with *N*-(3-hydroxypropyl)-L-glutamine in neutral, acidic, and basic systems. We also compare results from polymers with findings from the monomeric model compound, the *N*-acetyl-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine methyl ester.

Results and Discussion

The synthesis of the amino acid and the monomeric model compound is outlined in Scheme I. The starting material for the synthesis, L-*p*-nitrophenylalanine, was catalytically reduced to L-*p*-aminophenylalanine by the method of Bergmann (1952). The latter compound was diazotized and coupled with phenol to yield L-*p*-(*p'*-hydroxyphenylazo)phenylalanine (Bar-Eli and Katchalski 1963). This azoaromatic amino acid was treated successively with methanolic HCl and acetic anhydride–base to obtain the amide ester model compound. *N*-Carbobenzoxylation, followed by acetylation, gave the *N*-carbobenzoxy-*O*-acetyl derivative of the amino acid which was then treated with thionyl chloride to yield the corresponding α-amino acid *N*-carboxyanhydride. The *N*-carboxyanhydride was polymerized in dry tetrahydrofuran using freshly prepared sodium methoxide as an initiator to obtain poly-L-*p*-(*p'*-acetoxyphenylazo)-phenylalanine. Subsequent removal of the *O*-acetyl group was accomplished *via trans*-amidolysis with 3-amino-1-propanol (Lupu-Lotan *et al.*, 1965). Copolymers of L-*p*-(*p'*-acetoxyphenylazo)phenylalanine with

TABLE I: Ultraviolet and Visible Absorption Spectra (λ_{max} (mμ)(log ϵ)) in Trimethyl Phosphate and Trifluoroacetic Acid.

Compound	Solvent				
	Trimethyl Phosphate			Trifluoroacetic Acid	
HO—⟨⟩—N=N—⟨⟩	430 (3.20)	345 (4.33)	240 (3.13)	465 (4.60)	249 (3.59)
Amide ester	430 (3.20)	352 (4.50)	238 (3.13)	468 (4.61)	250 (3.60)
Poly-L-*p*-(*p'*-hydroxyphenylazo)-phenylalanine	430 (3.30)	354 (4.47)	243 (3.12)	468 (4.62)	250 (3.66)

γ-benzyl-L-glutamate were prepared and converted into the copolymers containing free phenolic hydroxyls by the amidolysis procedure noted above. Simultaneously the benzyl ester groups were converted into hydroxypropylamide side chains. These procedures are illustrated in Scheme II. We measured the optical rotatory dispersion and circular dichroism of poly-L-*p*-(*p'*-acetoxyphenylazo)phenylalanine in dioxane and observed simple Cotton effects in the azoaromatic absorption region essentially identical with those we reported for poly-L-*p*-(phenylazo)phenylalanine in the same solvent. Conformational analysis of the polymer and copolymers was carried out by means of ultraviolet, optical rotatory dispersion, and circular dichroism spectroscopy in neutral (trimethyl phosphate), basic (aqueous solution at pH >10), and acidic (trifluoroacetic acid) conditions.

Studies in Trimethyl Phosphate. The results of ultraviolet and visible spectral absorption from *trans-p*-hydroxyazobenzene, the monomeric model compound (amide ester), and the azopolypeptides are tabulated in Table I.

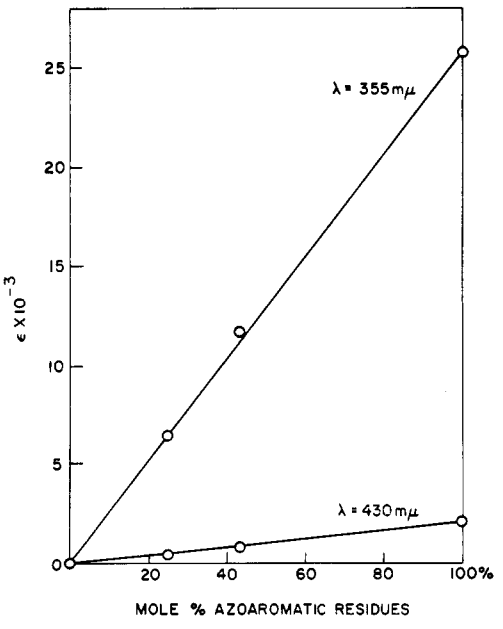


FIGURE 1: Absorbances at 430 and 345 mμ of the polymer and copolymer solutions in trimethyl phosphate as function of the mole per cent of azoaromatic *N*-carboxyanhydride n the starting monomer solution.

In trimethyl phosphate the major bands are attributed to an n–π^* (430 mμ), a π–π^* (345 mμ), and a ϕ–ϕ^* ($\sim$250 mμ) electronic transition (Jaffe *et al.*, 1958). As previously reported (Goodman and Kossoy, 1966) we use the visible spectra of the polymer in trimethyl phosphate to determine the copolymer composition. The absorbances were measured at 430 and 345 mμ and plotted against the mole per cent of L-*p*-(*p'*-acetoxyphenylazo)-phenylalanine-*N*-carboxyanhydride in the starting monomer solutions (Figure 1). As previously pointed out, this technique is useful in determining the composition because of the difficulties in the standard microchemical analysis for carbon and hydrogen percentages when azoaromatic compounds are involved. On the basis of related copolymerization studies, we assume random sequential distribution for these polymers.

Optical rotatory dispersion and circular dichroism studies of the polymer and copolymers show that such materials in trimethyl phosphate exist as right-handed α helices. All the polymers exhibit an azoaromatic Cotton effect centered in the 350-mμ region in addition to the Cotton effects arising from the peptide main-chain chromophores below 230 mμ. In particular the optical rotatory dispersion spectra (Figure 2) of the poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine display a large trough centered at 363 mμ flanked by two smaller peaks

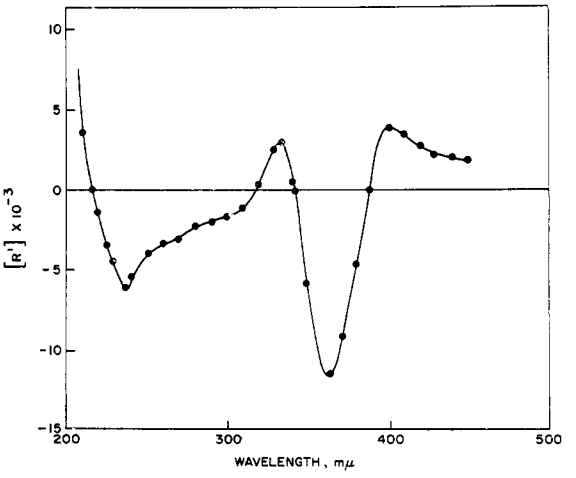


FIGURE 2: Optical rotatory dispersion spectrum of poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in trimethyl phosphate.

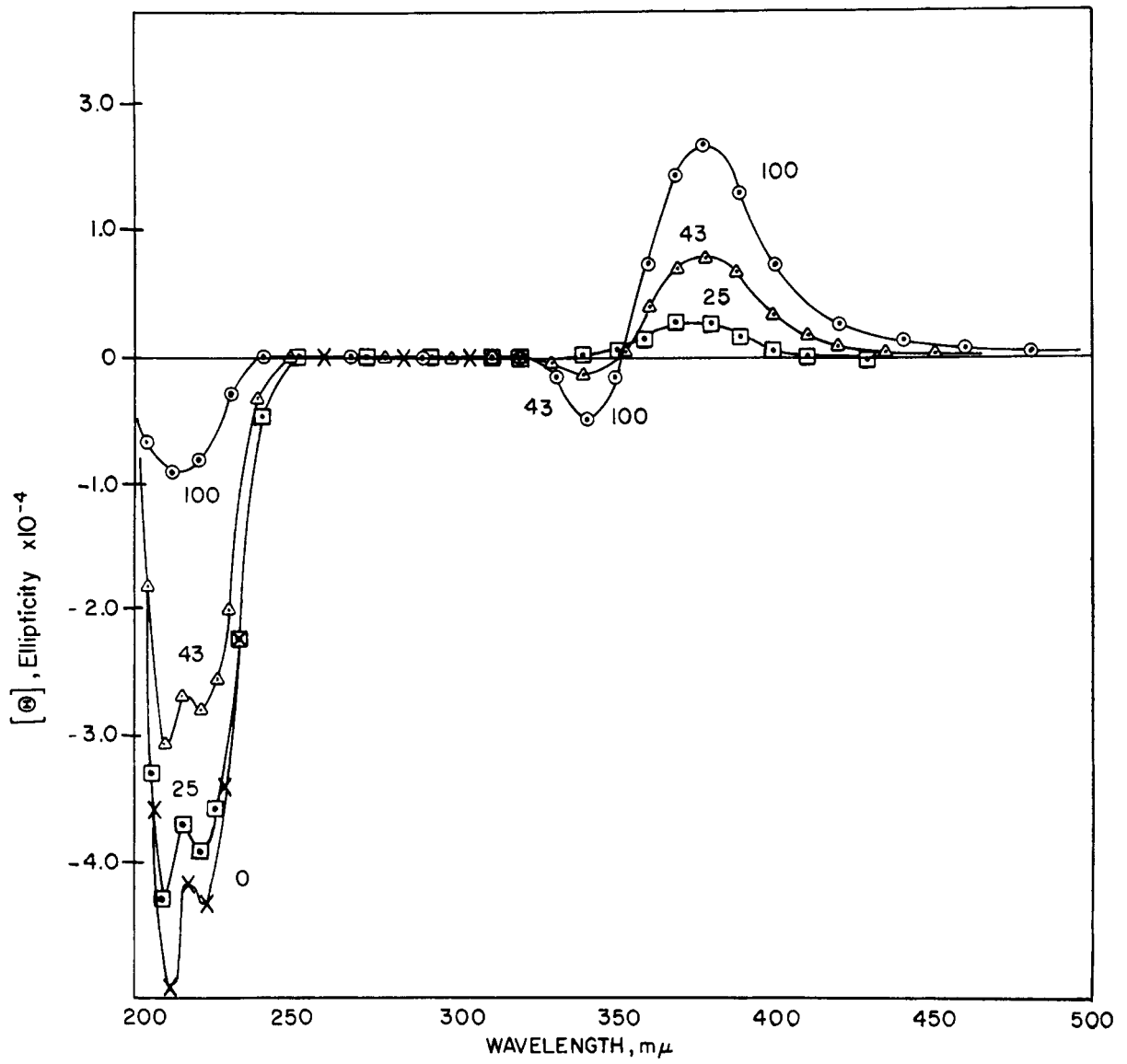


FIGURE 3: Circular dichroism spectra of poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine, poly-*N*-(3-hydroxypropyl)-L-glutamine, and copolymers of L-*p*-(*p'*-hydroxyphenylazo)phenylalanine with *N*-(3-hydroxypropyl)-L-glutamine in trimethyl phosphate. Numbers of the curve denote the mole per cent of azoaromatic residues in the polymer.

centered at 398 and 334 mμ which we assign to exciton resonance coupling of spatially adjacent azoaromatic chromophores. The large values of the transition moment for the $\pi-\pi^*$ transition and the geometry of the α helix which allows the side chains to arrange themselves in close packing are all in favor of the delocalization of the electronic excitation (Moffitt, 1956; Kasha, 1963). This results in a splitting of the simple $\pi-\pi^*$ band of the azoaromatic chromophore into a parallel and perpendicular component with respect to the α-helix axis. No other possible assignment seems available at the moment for such bands because the next allowed transition for the azoaromatic chromophore is far down in the ultraviolet region (about 300 mμ).

The circular dichroism spectrum (Figure 3) shows clearly that the Cotton effect is of complex origin. This Cotton effect for the side-chain chromophore involves a large peak in the 385-mμ region and a small trough in the 340-mμ region with a cross-over at 352 mμ. Again the separation of these bands is small. These results confirm our assignment of the band to a $\pi-\pi^*$ transition split by exciton resonance coupling of the spatially adjacent side-chain azoaromatic chromophores. The effect becomes smaller as the concentration of azoaromatic residues in the copolymers decreases, which demonstrates that the local concentration of azoaromatic side chains plays an important role in the origin of this particular Cotton effect.

In the region below 240 mμ the circular dichroism spectrum of the two copolymers and the poly-*N*-(3-hydroxypropyl)-L-glutamine (Figure 3) exhibit the well-established patterns of the right-handed α helix, *i.e.*, troughs at 222 and 212 mμ. Poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine does not exhibit two resolved bands in this spectral region. In Figure 4 we report the molar residue rotation for the 233-mμ band of the optical ro-

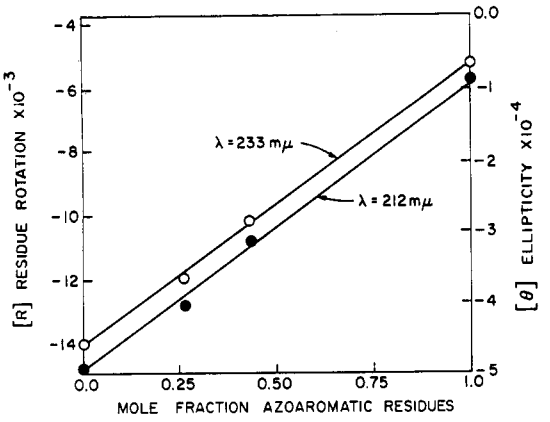


FIGURE 4: Molar residue rotation for the 233-mμ trough and molar ellipticity of the 212-mμ band as functions of the mole fraction of azoaromatic residues in the polymers.

tatory dispersion *vs.* the mole fraction of azoaromatic residues in the copolymers. In this same figure, we also include findings based on the 212-mμ circular dichroism band. The linearity of such plots supports our thesis that these polymers and copolymers exist in this solvent as right-handed α helices. On the other hand, the decreasing intensity of the peptide main-chain ellipticity bands in the region below 230 mμ must be ascribed to overlapping of allowed transitions of the side-chain chromophore with those from the peptide main chain. As a matter of fact the monomeric model compound, the amide ester, exhibits at least two bands of positive ellipticity in the region 230–200 mμ in trimethyl phosphate while the polymers present negative bands in this region. In addition, for the azoaromatic polypeptides the magnitudes of the Cotton effect in the 350-mμ region is enhanced relative to the amide ester. The azoaromatic Cotton effect in the polymers arises probably from both interactions among the side-chain chromophores and between side chains and the asymmetric centers of the main chain.

Studies in Aqueous Solutions. A conformational study of poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in aqueous solution at pH values above 10 was carried out by means of ultraviolet and circular dichroism spectroscopy.

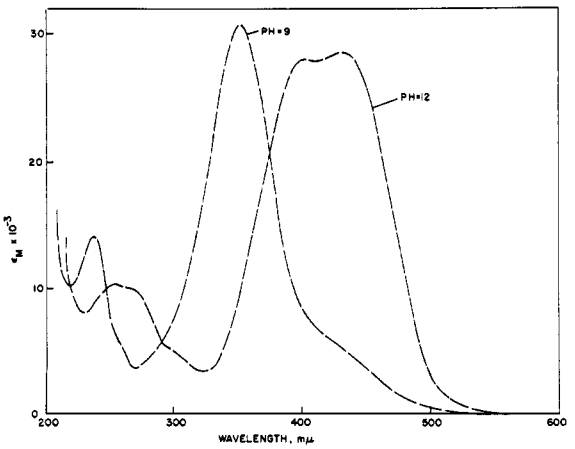


FIGURE 5: Ultraviolet and visible spectra of the *N*-acetyl-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine (amide ester) in aqueous solutions at pH 9.7 and 12.0.

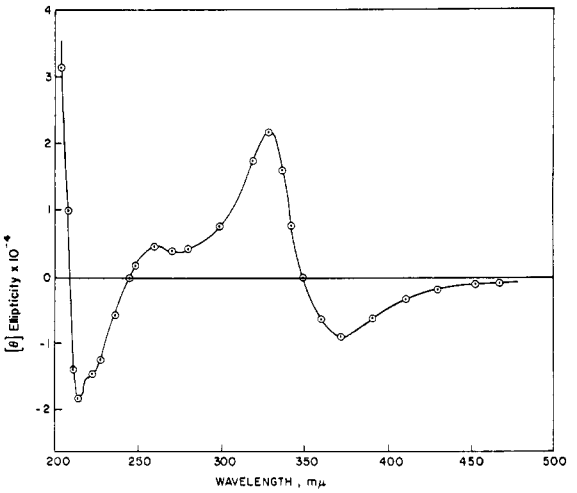


FIGURE 6: Circular dichroism spectrum of poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in aqueous solution at pH 10.2–11.9 with 0.2 M NaCl added.

Yeh and Jaffe (1959) reported on the acid dissociation constant of the phenolic function (pK_2) of the *p*-hydroxyazobenzene in 20% ethanol. They found that pK_2 is equal to 8.38. The thermodynamic data for the acid dissociation of the phenolic hydroxyl for phenol (Cohn and Edsall, 1943), tyrosine (Tanford and Roberts, 1952), and poly-L-tyrosine (Katchalski and Sela, 1953) are 9.78, 9.66, and 9.5, respectively. On the other hand, in Figure 5 we report the ultraviolet and visible spectra of the amide ester at pH 9.7 and 12.0. At the lower pH the model compound exhibits the classical bands associated with the azo compound such as *p*-hydroxyazobenzene (Izmailski and Milliaresi, 1961). A band appears at 350 mμ with a pronounced shoulder at about 430 mμ and another band is evident at 238 mμ with a shoulder at 260 mμ. At this pH we believe that very little ionization occurs. At the higher pH, the spectrum is completely different. It shows two bands at 432 and 401 mμ, a shoulder at about 300 mμ, and an additional two bands at 270 and 258 mμ. We consider that at this pH essentially 100% of the phenolic hydroxyls are ionized. From these results we assume that the pK_2 of the monomeric model compound has a value near 10.

By stirring at room temperature poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine dissolves in aqueous solution at pH 10.2 with 0.2 M NaCl added. The circular dichroism spectrum (Figure 6) displays a trough at 370 mμ, two peaks at 330 and 260 mμ, and two troughs at 222 and 214 mμ. The first three bands arise from the side-chain azoaromatic chromophores, whereas the last two bands arise from the peptide main-chain chromophore. The shape and the position of the latter two bands enable us to assign the conformation of the polymer as a right-handed α helix. The intensities of the two bands associated with the peptide chromophore are somewhat lower than that reported in the literature (Holzwarth and Doty, 1965) for well-formed right-handed α helices. However

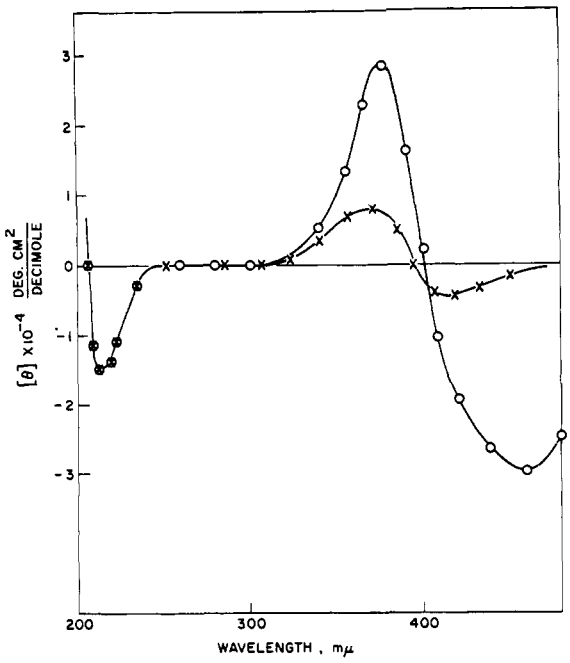


FIGURE 7: Circular dichroism spectrum of poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in aqueous solution at pH 12.3: (O) 0.2 M NaCl and (×) 0.9 M NaCl.

in the same spectral region, the amide ester model compound exhibits two positive bands which can contribute ellipticity of opposite sign to the bands arising from the peptide main chain. The three bands above 250 mμ are assigned to identical transitions as above for solutions of the polymer in trimethyl phosphate.

From the results on the acid dissociation constant (pK_2), we believe that when the polymer is un-ionized it behaves in a similar manner to that observed in neutral solvent (trimethyl phosphate). The trough at 370 mμ and the peak at 330 mμ arise from splitting of the same π–π^* transition by an exciton resonance coupling mechanism of spatially close packed azoaromatic residue along the polymer chain. The band at 260 mμ can be tentatively assigned to a pure aromatic transition such as Φ–Φ* involving orbitals completely localized on the benzene rings (Jaffe *et al.*, 1958). Only small changes are observed in the circular dichroism spectrum when the pH is increased from 10.2 to 11.9. In this range a large proportion of the phenolic hydroxyls are successively ionized. However, their presence does not affect the overall conformation of the main chain. Upon further ionization a drastic alteration occurs in the circular dichroism spectrum (Figure 7), which affords a broad trough at 480 mμ and a very large peak at 380 mμ. The small band in the aromatic region at about 260 mμ vanishes. The intensities of these bands decrease to low values upon addition to the solutions of large amounts of neutral electrolytes such as NaCl. This electrostatic effect is shown in Figure 8, where we report the ellipticity of the peak at 380 mμ for solutions at pH 12.3 *vs.* the concentration of neutral electrolyte added. The highly charged polyelectrolyte chain at low ionic strength tends to approach a fully extended, rodlike shape, so that the solution becomes highly ordered because of interactions

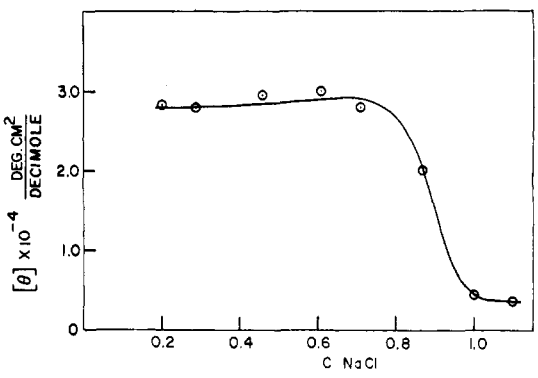
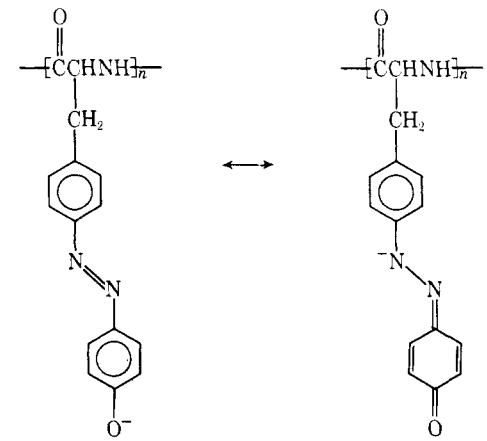


FIGURE 8: Molar ellipticity of the 380-mμ circular dichroism band of poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in aqueous solutions at pH 12.3 as function of neutral electrolyte added.

between charges. This can explain the enhancement in ellipticity observed. Part of the contribution to the ordered structure at these high pH values may come from quinonoid forms of the type



Interactions among anionic and quinonoid forms of the side chains can lead to enhanced stability for the specific conformation. This could explain the high concentration of salt required to denature the ordered form. When however large concentrations of added salt are present, the Cotton effects decrease to levels comparable with that observed for the monomeric model compound (amide ester) since the polymer must assume a random coil structure. This contention is supported by the fact that only a broad negative band centered at about 208 mμ is present in the peptide chromophoric region. A helix–coil transition occurs between pH 11.9 and 12.3 in the presence of salt. It is interesting to note that addition of a neutral electrolyte to a solution of the polypeptide at pH values lower than 11.9 does not lead to any substantial change in helical content. Thus, we believe that the helix formed by these polypeptides is quite stable.

Studies in Trifluoroacetic Acid. In this solvent, the azoaromatic residues are fully protonated and the chromophores exhibit electronic transitions of different energy. The values of λ_{max} and the extinction coefficient for the π–π^* transition of *p*-hydroxyazobenzene, the

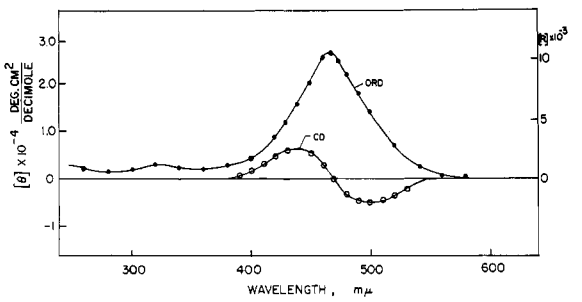


FIGURE 9: Optical rotatory dispersion and circular dichroism spectrum of poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in trifluoroacetic acid.

amide ester, and the polymer are tabulated in Table I. The optical rotatory dispersion and circular dichroism spectra are represented in Figure 9. It is clear that the conjugated acid of the azoaromatic chromophore also exhibits induced asymmetry. Further, the appearance of the Cotton effect in trifluoroacetic acid is strong evidence for a high state of ordering in this hydrogen-bonding solvent. This Cotton effect is very similar to that recently reported for poly-L-*p*(phenylazo)phenylalanine (Goodman and Kossoy, 1966). The shapes of the optical rotatory dispersion and circular dichroism spectra clearly suggest that the interactions of the side-chain azoaromatic residues produce an exciton band splitting phenomenon. We believe that the structure is ordered and must probably be of a polyelectrolyte extended chain type.

We investigated photoisomerization of the polymer either in trimethyl phosphate, H_2O, or trifluoroacetic acid solutions and contrary to our findings with poly-L-*p*-(phenylazo)phenylalanine (Goodman and Falxa, 1967) we observed no *trans–cis* photoisomerization.

Experimental Section

L-*p*-Nitrophenylalanine and phenol were obtained, respectively, from the Cyclo Chemical Corp. and Allied Chemical Co. and were used without further purification. All solvents used were of reagent grade. Melting points were taken using a Kofler hot stage and are uncorrected.

Optical Rotatory Dispersion and Circular Dichroism. Measurements of optical rotatory dispersion and circular dichroism were carried out with a Cary 60 spectropolarimeter equipped with a circular dichroism attachment. The measurements were taken in 0.1-, 0.5-, and 1.0-mm cylindrical cells from Optical Cell Co., Brentwood, Md. Corrected residue rotations [*R'*] were calculated from the equation, $[R'] = 3MRW \cdot [\alpha]_\lambda^T/10^2(n^2 + 2)$, where *MRW* = mean residue weight and *n* = refractive index of solvent (uncorrected for wavelength).

Ultraviolet Absorbance. The ultraviolet spectra were recorded using a Cary 14 double-beam spectrophotometer. The concentration used was 0.05% or less.

Preparation of Polymer Aqueous Solution for Optical Rotatory Dispersion, Circular Dichroism, and Ultraviolet Measurements. The polypeptide was weighed out in a 10-ml volumetric flask, approximately 5 ml of 0.2 M NaCl solution was added, and the solutions were titrated with 0.1 N NaOH solution. The pH was measured simultaneously with a Beckman Research pH meter, Model 1019. Finally the volume was built up to the 10-ml mark with distilled water.

Compounds. Elemental analyses for the compound reported in this section are tabulated in Table II. They were executed at A. Bernhard Laboratories, West Germany.

L-*p-Aminophenylalanine.* This material was prepared according to the procedure of Bergmann (1952). The L-*p*-nitrophenylalanine was reduced by catalytic hydrogenation in water. The material was recrystallized four times from water, mp 225–227° (Bergmann reported mp 254°).

L-*p*-(*p'-Hydroxyphenylazo)phenylalanine.* The precursor (diazonium salt of L-*p*-aminophenylalanine) of this compound was prepared according to the procedure of Bar-Eli and Katchalski (1963). L-*p*-Aminophenylalanine (4.0 mmoles) in 1 N HCl (4 ml) was diazotized with 0.5 N sodium nitrite solution (6 ml) at 0°. The mixture was stirred for 30 min in an ice bath, neutralized with 2 N potassium bicarbonate (20 ml), and diluted with 10% sodium acetate solution (40 ml) in order to keep the mixture at pH 5. Phenol (4.3 g) in 70 ml of water was then added in one batch with vigorous stirring. After 1 hr a yellow precipitate was filtered and washed several times with water, then acetone and ether. The crude material was crystallized from water (with traces of acid), mp 285° dec. The final yield was 90%.

*N-Carbobenzoxy-*L-*p-(p'-hydroxyphenylazo)phenylalanine.* The procedure of Katchalski and Sela (1953) for the preparation of *N,O*-dicarbobenzoxy-L-tyrosine was followed. L-*p*-(*p'*-Hydroxyphenylazo)phenylalanine (10 mmoles) was dissolved in 4 N NaOH (5.2 ml) and treated with carbobenzoxy chloride (3.1 ml) and 4 N NaOH (6.0 ml) at 0° with vigorous stirring. The addition was carried out over a period of 25 min. The pH of the reaction mixture was maintained above 10 by adjusting the flow rate of the carbobenzoxy chloride and sodium hydroxide; the *N,O*-dicarbobenzoxy derivative precipitated during the addition. After 1 hr, 200 ml of 1% solution of dioxane in water was added and the mixture was stirred at room temperature for 3 hr. During this time the precipitate dissolved. The mixture then was acidified to pH 4 with a 6 N HCl solution. The yellow precipitate was filtered, washed with water, and then dissolved in ether. The solution was extracted with 1 N HCl and then with saturated solution of NaCl; finally it was dried over $MgSO_4$. The solvent was removed *in vacuo* and the resulting yellow solid (4.0 g, mp 177–181°, yield 95%) was washed with *n*-hexane, dried, and used in the next step without further purification.

*N-Carbobenzoxy-*L-*p-(p'-acetoxyphenylazo)phenylalanine.* *N*-Carbobenzoxy-L-*p*-(*p'*-hydroxyphenylazo)-phenylalanine (9.5 mmoles) was dissolved with vigorous stirring at 0° in 1 N NaOH solution (9.5 ml) and 10 ml of pyridine. The ice-cold solution was treated with acetic anhydride (0.84 ml) and 1 N NaOH solution (9.5 ml). The addition was carried out over a period of 20 min, with vigorous stirring. The pH of the reaction mixture

TABLE II: Elemental Analyses.

Compound	Calcd (%)			Found (%)			%[a] Residue of the Sample
	C	H	N	C	H	N	
L-Amino acid	63.15	5.25	14.73	62.95	5.25	14.61	
Amide ester	63.33	5.61	12.31	63.19	5.55	12.33	
O-Acetyl-poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine	66.01	4.89	13.58	65.58	4.75	13.40	0.85
Poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine	67.40	4.90	15.72	66.15	5.13	15.66	1.13
Copolymer I[b]	58.39	6.43	15.33	58.27	6.31	15.25	
Copolymer II[c]	55.55	6.91	14.21	55.47	6.97	14.29	

[a] The residues were reported as white and granular powders with no traces of carbon withheld. [b] 25.0% L-*p*-(*p'*-hydroxyphenylazo)phenylalanine–75% γ-benzyl-L-glutamate copolymer. [c] 43.7% L-*p*-(*p'*-hydroxyphenylazo)phenylalanine–56.3% γ-benzyl-L-glutamate copolymer.

was maintained between 9 and 11, adjusting the flow rate of the above reagent. The mixture was then acidified with 6 N HCl solution. The precipitate was then extracted twice with 100 ml of ethyl acetate. The combined organic layers were washed with 1 N HCl solution and saturated solution of NaCl. Finally, the organic solution was dried over $MgSO_4$. The solvent was removed *in vacuo*. The resulting oil was dissolved in chloroform and by addition of *n*-hexane a yellow precipitate was obtained with mp 126–130° (yield 89%).

L-p-(p'-Acetoxyphenylazo)phenylalanine N-Carboxyanhydride. The procedure reported in the literature was followed. *N*-Carbobenzoxy-L-*p*-(*p'*-acetoxyphenylazo)-phenylalanine (6.0 mmoles) was dissolved in 10 ml of acetic anhydride and heated with thionyl chloride (4.5 ml). The round-bottom flask (50 ml) was equipped with a condenser and a drying tube. The mixture was heated at 40° for 10 min. The precipitate was filtered in a drybox and washed with *n*-hexane. Recrystallization four times from tetrahydrofuran–*n*-hexane afforded 1.8 g of a yellow solid, mp 185° dec (yield 83%).

Poly-L-p-(p'-acetoxyphenylazo)phenylalanine. L-*p*-(*p'*-Acetoxyphenylazo)phenylalanine *N*-carboxyanhydride (0.58 g, 1.6 mmoles) was dissolved in 30 ml of dry tetrahydrofuran. The polymerization was initiated with sodium methoxide (0.16 ml of 0.1 N; monomer/initiator 100) and allowed to proceed at room temperature. After 48 hr the precipitated polymer was filtered and washed with tetrahydrofuran, and subsequent extraction with dioxane in Soxhlet for 48 hr afforded 0.4 g (80% yield) of yellow polymer.

γ-Benzyl-L-glutamate N-Carboxyanhydride. The procedure reported in the literature (Blout and Karlson, 1956) was followed.

Copolymers of γ-Benzyl-L-glutamate with L-p-(p'-Acetoxyphenylazo)phenylalanine. γ-Benzyl-L-glutamate *N*-carboxyanhydride and L-*p*-(*p'*-acetoxyphenylazo)phenylalanine *N*-carboxyanhydride were weighed out in different molar ratios and polymerized according to the procedure reported above for the homopolymer. The monomer/initiator ratio was always 100; the reactions were carried out in 2–3% tetrahydrofuran solutions, using as initiator a 0.1 N sodium methoxide solution. The polymerization was allowed to proceed at room temperature for 6 days. The viscous reaction mixtures were decanted into vigorously stirred *n*-hexane. The fibrous yellow solids were reprecipitated from tetrahydrofuran–*n*-hexane and dried (yield 75–85%).

Poly-L-p-(p'-hydroxyphenylazo)phenylalanine. This polymer was prepared *via trans*-amidolysis with 3-amino-1-propanol according to a slightly modified procedure reported in the literature (Lupu-Lotan *et al.*, 1965). Poly-L-*p*-(*p'*-acetoxyphenylazo)phenylalanine (0.30 g, 9.7 mmoles) was mixed with 3-amino-1-propanol (1.45 g, 19.4 mmoles, 3-amino-1-propanol/polymer 20) in a 2-in. test tube. The tube was corked and placed in a bath at 55 ± 2°. It was left for 70 hr with occasional stirring. The resulting red gel was taken up in deionized water and dialyzed *vs.* water repeatedly. Following this the free hydroxy polymer (0.25 g, yield 95%) was obtained by lyophilization.

Copolymers of L-p-(p'-Hydroxyphenylazo)phenylalanine with N-(3-Hydroxypropyl)-L-glutamine. These materials were prepared *via trans*-amidolysis with 3-amino-1-propanol according to the procedure reported above starting from the copolymers of L-*p*-(*p'*-acetoxyphenylazo)phenylalanine with γ-benzyl-L-glutamate. The ratio 3-amino-1-propanol/copolymer was always 20 and the reaction was carried out in an oil bath at 45 ± 2° for 72 hr. After dialysis and following lyophilization the yields were in the range of 80–90%.

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Conformational Aspects of Polypeptide Structure. XXVII. Solvent Effects on Azoaromatic Polypeptides*

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ABSTRACT: Two azoaromatic polypeptide systems have been studied in mixed solvents. Poly-L-*p*-(phenylazo)-phenylalanine and its copolymer with γ-benzyl-L-glutamate exist as a right-handed α helix in dioxane, as a random coil in approximately 70% trifluoroacetic acid–30% dioxane mixed solvent, and as an extended ordered polyelectrolyte in nearly pure trifluoroacetic acid. With the poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine and its copolymers with *N*-(3-hydroxypropyl)-L-glutamine we find a right-handed α helix and a conformation with only side-chain order depending upon the nature of the mixed solvent systems $(CH_3)_3PO_4$–CF_3COOH.

At high trifluoroacetic acid concentration the structure of the *p*-hydroxyazoaromatic polymer assumes an extended polyelectrolyte conformation.

The electronic and steric interactions of component amino acids determine allowed polypeptide conformation in solution. In order to understand better the influence of these forces we undertook to study the interactions between side-chain chromophores and the optically active centers of the main chain and between spatially adjacent side-chain chromophores by optical rotatory dispersion, circular dichroism, and ultraviolet absorption spectroscopy. Polypeptides containing aromatic side chains with auxochromic substituents are well suited for such studies. Indeed aromatic side-chain effects have been noted for polymers derived from L-tyrosine (Fasman *et al.*, 1964; Beychok and Fasman, 1964; Pao *et al.*, 1965), L-histidine (Norland *et al.*, 1963; Beychok *et al.*, 1965), L-tryptophan (Fasman *et al.*, 1965), L-phenylalanine (Sage and Fasman, 1966; Auer and Doty, 1966), and derivatives such as L-*p*-aminophenylalanine (Goodman and Peggion, 1967), L-*p*-nitrophenylalanine (M. Goodman, unpublished results), and L-*p*-(phenylazo)phenylalanine (Goodman and Kossoy, 1966). A survey of aromatic side-chain contributions to polypeptide structure will appear in an article from our laboratories (Goodman *et al.*, 1968). The helical porphyrin *d*-urobilin (Moscowitz, 1964) and naturally occurring proteins (Goodman and Toniolo, 1968) have shown analogous effects.

We reported in previous studies (Goodman and Kossoy, 1966) that L-*p*-(phenylazo)phenylalanine polypeptides form right-handed helices in dioxane solutions and extended structures in trifluoroacetic acid. In dioxane these macromolecules exhibit Cotton effects associated with the 320-mμ azoaromatic absorption maximum, while in trifluoroacetic acid they produce Cotton effects in the 425-mμ absorption region of the conjugated acid of the azoaromatic group. Conformational characterization of polypeptides derived from L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in trimethyl phosphate, trifluoroacetic acid, and aqueous solutions has been reported (Goodman and Benedetti, 1968). Exciton resonance coupling of the spatially adjacent side-chain azoaromatic chromophores produces splitting of the π–π^* transitions in the systems studied.

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