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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and derivatives such as L-*p*-aminophenylalanine (Goodman and Peggion, 1967), L-*p*-nitrophenylalanine (M. Goodman, unpublished results), and L-*p*-(phenylazo)phenylalanine (Goodman and Kossoy, 1966).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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In this paper we describe solvent-induced conformational changes of these azoaromatic polypeptides. We demonstrate conformational changes in the case of poly-L-*p*-(phenylazo)phenylalanine from a helix in dioxane to a random coil in certain dioxane–trifluoroacetic acid mixtures, to an extended conformation in pure trifluoroacetic acid. The polymers derived from L-*p*-(*p'*-hydroxyphenylazo)phenylalanine form right-handed α helices in trimethyl phosphate and aqueous solutions (between pH 10 and 11.9), whereas in trifluoroacetic acid a different ordered regular structure exists; however, we believe that the azoaromatic side chains are not extremely well ordered with respect to each other in all of the above solvents.

The extent of exciton resonance interactions between the azoaromatic chromophores which produce splitting of the π–π^* transition in the pure solvents is extremely enhanced in mixtures of trimethyl phosphate–trifluoroacetic acid. Depending upon the method of preparation of these solutions, we find that poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine assumes different conformations. In the case where we dissolve the polymer in trimethyl phosphate–trifluoroacetic acid mixtures (method A) we find an extremely well-ordered right-handed α helix. In the case where we dissolve the polymer in either component and then add the other (method B) we find no secondary structure in the peptide chromophoric region, but observe splitting of the π–π^* band in the azoaromatic region with an opposite sign of ellipticity to that noted for the same transition when method A is used to prepare the solution.

TABLE I: Ultraviolet and Visible Spectra in Dioxane–Trifluoroacetic Acid Mixtures.

Solvent Mixtures % Acid	49.7% L-*p*-(Phenylazo)phenylalanine–50.3% γ-Benzyl-L-glutamate Copolymer		Poly-L-*p*-(phenylazo)-phenylalanine	Model Compd[b]
	$\lambda_{320-323}$	$\lambda_{420-423}$	$\lambda_{420-423}$	λ_{420}
0	4.07	2.14[a]	ins[c]	2.38[a]
20	4.06	2.25[a]	ins	2.66[a]
40	4.05	2.36[a]	ins	2.98[a]
60	4.04	2.45[a]	ins	3.31[a]
70	3.98	3.33	ins	4.07
80	3.38	3.73	4.26	4.31
90	3.17	4.01	4.38	4.39
100	2.25	4.07	4.42	4.42

[a] Not a minimum or a maximum. [b] *N*-Acetyl-L-*p*-(phenylazo)phenylalanine. [c] Insoluble.

Results and Discussion

A. Poly-L-p-(phenylazo)phenylalanine. The transformation of the azoaromatic chromophore to its conjugate acid was followed by recording both the optical rotatory dispersion and the absorption spectra of a copolymer composed of 49.7 mole % L-*p*-(phenylazo)-phenylalanine–50.3 mole % γ-benzyl-L-glutamate and the homopolymer poly-L-*p*-(phenylazo)phenylalanine in dioxane–trifluoroacetic acid solvent mixtures. Extreme care was taken to avoid *cis–trans* photoisomerization of the azoaromatic group by storing the sample in the dark except during the measurement.

Previous "optical titration" studies have been conducted to ascertain the helix–coil transitions for a number of polypeptide systems by following values of the derived constant, b_0, as a function of solvent composition (Karlson *et al.*, 1960). We examined the conformational changes by studying the values of the Cotton effect arising from the main-chain and side-chain chromophores.

ABSORPTION SPECTRA. The transformation of the azoaromatic residue to its conjugate acid was measured by the changes in the molar extinction coefficient at 425 and 320 mμ in dioxane–trifluoroacetic acid mixtures (Table I). The strong absorption maximum near 320 mμ has been assigned to a π–π^* azoaromatic transition for azobenzene and related compounds in the *trans* configuration (Jaffe *et al.*, 1958). In the conjugate acids of these *trans* compounds, the π—π^* transition is shifted to the 420-mμ spectral region.

The sigmoidal nature of the ϵ_{425} solvent composition relationship for the monomeric model compound, *N*-acetyl-L-*p*-(phenylazo)phenylalanine methyl ester, the 49.7% azo copolymer, and the azo homopolymer indicate that protonation of the azo group occurs over a narrow range of dioxane–trifluoroacetic acid mixtures. Unfortunately, the azo homopolymer is insoluble in dioxane–trifluoroacetic acid containing less than 80% acid.

The azoaromatic residues are unprotonated in solvent mixtures containing less than 60% trifluoroacetic acid as can be seen by the absence of an absorption band at 425 mμ and the large absorption band at 320 mμ. The transition from unprotonated to protonated azo structure for the model compound occurs when the solvent contains roughly between 65 and 75% trifluoroacetic acid. For the copolymer the azo groups are protonated when the solvent contains between 75 and 85% trifluoroacetic acid.

OPTICAL ROTATORY DISPERSION. The location and magnitude of the Cotton effects of the azoaromatic copolymer in dioxane–trifluoroacetic acid solvent mixtures are shown in Figure 1. The curve obtained for this azoaromatic copolymer in dioxane solution exhibits two Cotton effects in the range studied which are attributed to the unprotonated azoaromatic group and to the helical peptide group. The magnitudes of these Cotton effects decrease as the percentage of trifluoroacetic acid increases in the solvent mixture. No Cotton effects are observed in 80% trifluoroacetic acid solutions in the regions 500–240 mμ. Cotton effects attributed to the protonated azoaromatic groups are complete in solvent mixtures containing more than 90% trifluoroacetic acid. It is not possible to penetrate through the peptide Cotton effect region with solvents containing 80% or more tri-

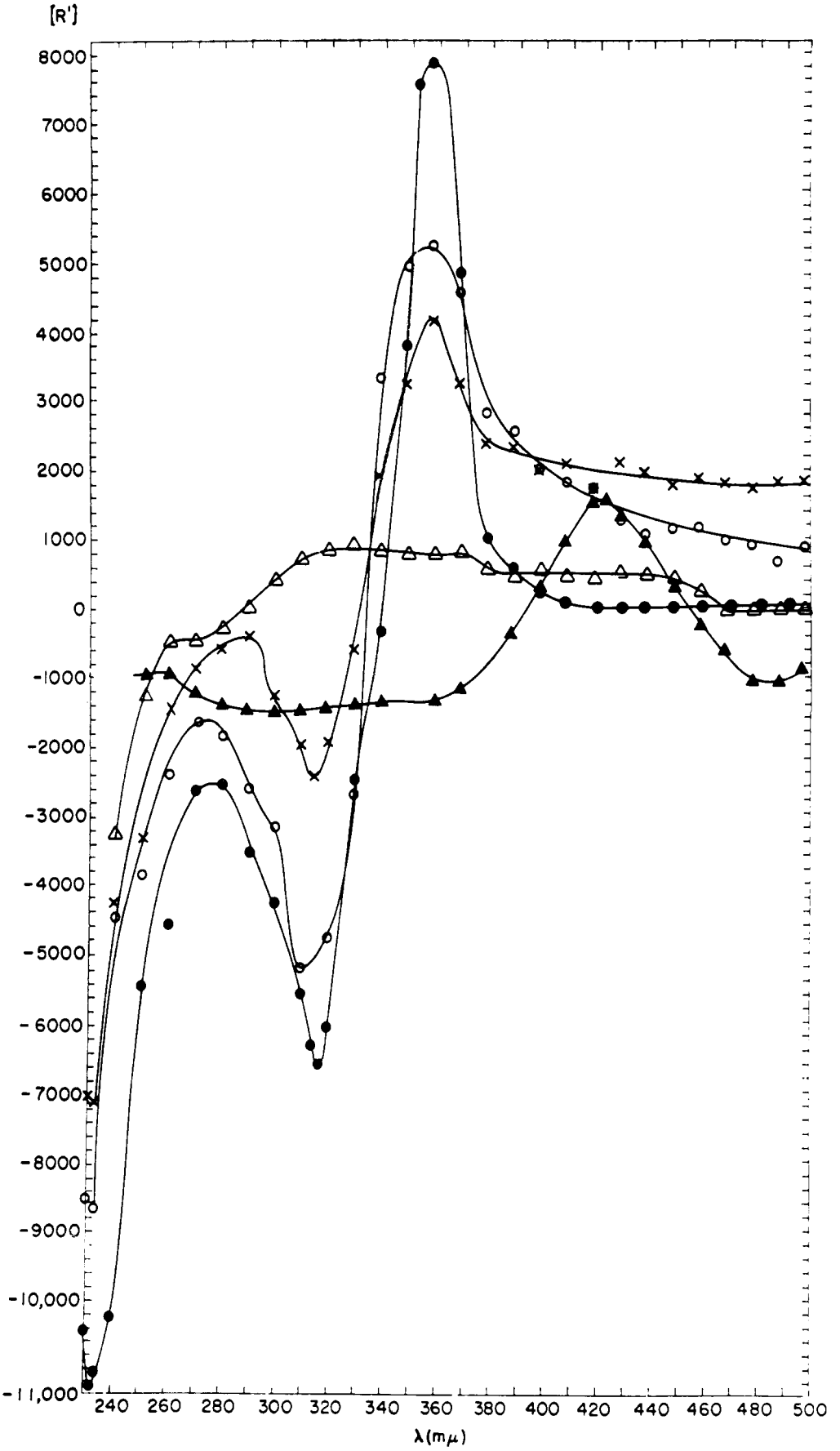

FIGURE 1: Optical rotatory dispersion spectrum of poly-L-*p*-(phenylazo)phenylalanine (49.7%) and γ-benzyl-L-glutamate copolymer (50.3%) in trifluoroacetic acid–dioxane mixtures. Solvent: (▲) trifluoroacetic acid; (△) 80% trifluoroacetic acid–20% dioxane; (×) 70% trifluoroacetic acid–30% dioxane; (○) 60% trifluoroacetic acid–40% dioxane; (●) dioxane.

fluoroacetic acid. These results indicate that a helix exists in solvent mixtures containing low percentages of trifluoroacetic acid, that a disordered structure obtains in solvent mixtures containing approximately 80% (v/v) trifluoroacetic acid, and that a fully extended polymer is encountered in solvent mixtures which are nearly pure trifluoroacetic acid. We have reported (Goodman and Kossoy, 1966) that the residue rotations in dioxane at 233 mμ for the copolymers containing between 49 and 80 mole % azoaromatic residues remain near −11,000 deg (cm²/dm). This finding allowed us to conclude that the copolymer conformation is predominantly helical in dioxane.

The intensity of the Cotton effect arising from the azoaromatic group is directly proportional to the mole fraction of azoaromatic residues in the copolymers. The magnitude of this Cotton effect in the homopolymer is substantially larger than that encountered with the model compound. This enhancement of rotational strength arises in part from interactions between azoaromatic residues and the chromophores in the dissymmetric helical main chain.

The azoaromatic side chains probably do not interact with each other in dioxane since the intensities of the 320-mμ Cotton effect are directly proportional to the mole per cent azo residue content (Bradley *et al.*, 1966). The residue rotation for the copolymer at 233 mμ gradually decreases in magnitude as the per cent of trifluoroacetic acid in the solvent mixture increases to 70%. These results indicate a partial denaturation of the helices in the acid-containing solutions. No azoaromatic Cotton effects are observed for this copolymer in solvent mixtures containing 80% trifluoroacetic acid. Also the residue rotation at 240 mμ (furthest wavelength of penetration) in this solvent shows a value of −3000 deg (cm²/dm). These findings demonstrate that the copolymer is largely disordered in this solvent mixture.

Evidence for the extended structure is obtained from the dependence of apparent weight-average molecular weights upon the polymer concentration in dichloroacetic acid. The apparent molecular weight for the 49.7% azo copolymer is 2.0×10^4 when C_0 is 0.6 and 7.5×10^4 when C_0 is 0.1. The increase in apparent molecular weight as concentration decreases is indicative of a polyelectrolyte effect. By contrast, the apparent weight-average molecular weight of a sample of poly-γ-benzyl-L-glutamate in dichloroacetic acid solutions is 3.0×10^4 when C_0 is 0.1. The glutamate polypeptide is not a polyelectrolyte, and the molecular weight plot does not exhibit the large increase in apparent molecular weight upon dilution.

In trifluoroacetic acid solutions the azoaromatic Cotton effect is in the region of the π–π^* protonated azoaromatic transition. This 425-mμ Cotton effect peak is flanked by two shallow troughs near 310 and 490 mμ. This relationship of a large peak flanked by smaller troughs has been attributed to exciton resonance coupling of chromophores (Tinoco, 1964). The intensities of the residue rotation of the azo polypeptides at 425 mμ show a dependence upon the square of the percentage of the azoaromatic residues in the copolymers in trifluoroacetic acid. This can be indicative of side-chain–side-chain interactions. In addition, the enhanced rotation of the 425-mμ Cotton effect for the polymers as compared with the monomeric model compound can be ascribed to electronic interactions of the ordered side chains. The conformational transitions discussed above occur in about the same acid content as the helix–coil transition of poly-L-alanine and require a much greater strong acid concentration than is needed to denature helical poly-γ-benzyl-L-glutamate. We have demonstrated that this azoaromatic series possesses an additional conformational change when the azoaromatic residues are protonated. It appears that the main chain becomes extended and the protonated azoaromatic groups remain ordered.

B. Poly-L-p-(p'-hydroxyphenylazo)phenylalanine. The change from the azoaromatic chromophore to its conjugated acid was followed by recording the absorption and the circular dichroism spectra of the polymer in trimethyl phosphate–trifluoroacetic acid mixtures prepared in different ways. Since we observe no photoisomerization of the *p*-hydroxyazoaromatic groups in either of the two pure solvents or the mixed solvent we took no special care to store the samples in the dark.

ABSORPTION SPECTRA. The results of the ultraviolet and visible spectra of the polymer and the monomeric model compound, *N*-acetyl-L-*p*-(*p'*-hydroxyphenylazo)-phenylalanine methyl ester, are tabulated in Table II.

TABLE II: Ultraviolet Spectral Results of π–π^* Transitions in Trimethyl Phosphate–Trifluoroacetic Acid Mixtures.

Solvent Mixture % Acid	Poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine		Model Compound
	Method A $\lambda_{340-345}$	Method B $\lambda_{340-345}$	λ_{352}
0	4.41	4.41	4.50
2	4.28	4.36	4.52
5	4.27	4.35	4.45
10	4.26	4.32	4.45
20	4.23	4.32	4.53
100	3.34[a]	3.34[a]	3.19[a]

[a] Not a minimum or a maximum.

The detectable peaks corresponding to the major electronic transitions of these substances have been assigned (Jaffe *et al.*, 1958). In nonacidic solvents the band in the 430-mμ region is attributed to an n–π^* transition while the band in the 345-mμ region is due to a π–π^* transition. The third major peak in the absorption spectrum has been tentatively assigned to a Φ–Φ* transition involving ϕ orbitals localized in the 2(2′) and 3(3′) atoms of the benzene rings. In strongly acidic solvents the protonated azoaromatic chromophore shows the π–π^* transition shifted toward the red (468 mμ) from that encountered for protonated azoaromatic compounds with-

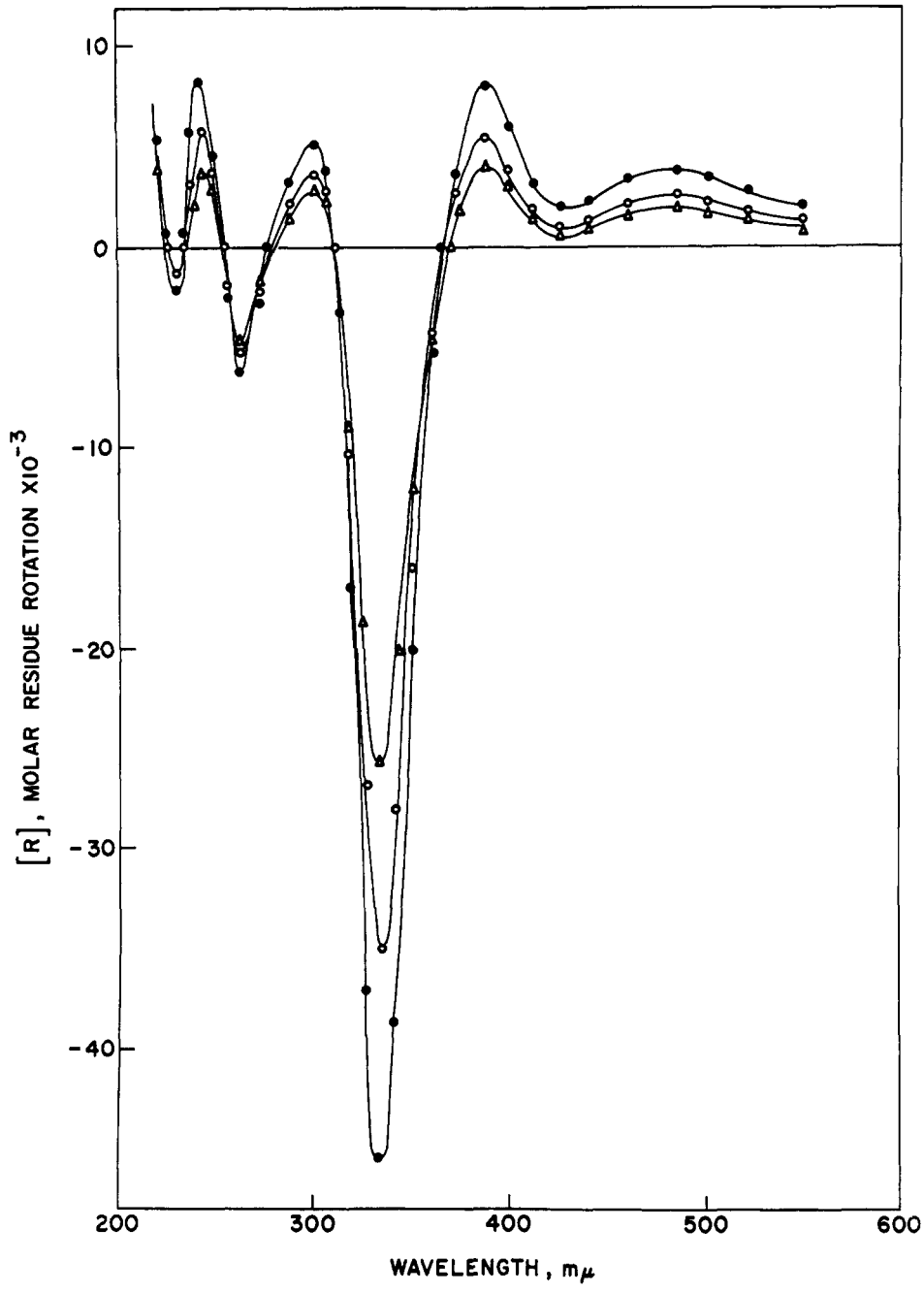

FIGURE 2: Optical rotatory dispersion spectrum of poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in trifluoroacetic acid–trimethyl phosphate mixtures prepared by method A (*c* 0.02%). Solvent: (△) 0.1% trifluoroacetic acid–99.9% trimethyl phosphate; (○) 2.0% trifluoroacetic acid–98.0% trimethyl phosphate; (●) 5.0% trifluoroacetic acid–95.0% trimethyl phosphate.

out auxochromes (425 mμ). Transition from the azo to the protonated chromophore was found to occur between 35 and 45% trifluoroacetic acid in trimethyl phosphate both for the polymer and the model compound.

Generally an enhancement of the extinction coefficient for the π–π^* transition of azoaromatic compounds upon addition of small concentrations of acidic solvents insufficient to convert a measurable fraction of the free base into conjugate acid has been observed (Jaffe and Gardner, 1958). We obtained the same result for our model compound. However in the case of the polymer we observe a hypochromism of the π–π^* band. In pure trimethyl phosphate the polymer exhibits an ultraviolet absorption band of intensity some 20% less than that of the monomeric model compound in the same solvent. For trimethyl phosphate–trifluoroacetic acid mixtures the results depend upon the two procedures employed for the preparation of the polymer solutions as noted above (methods A and B). In mixtures of trimethyl phosphate–trifluoroacetic acid containing 5% trifluoroacetic acid the intensity of the absorption band for the polymer is reduced by an approximate factor of 0.4 as compared with the results from the model compound. These hypochromic effects in absorption bands of the

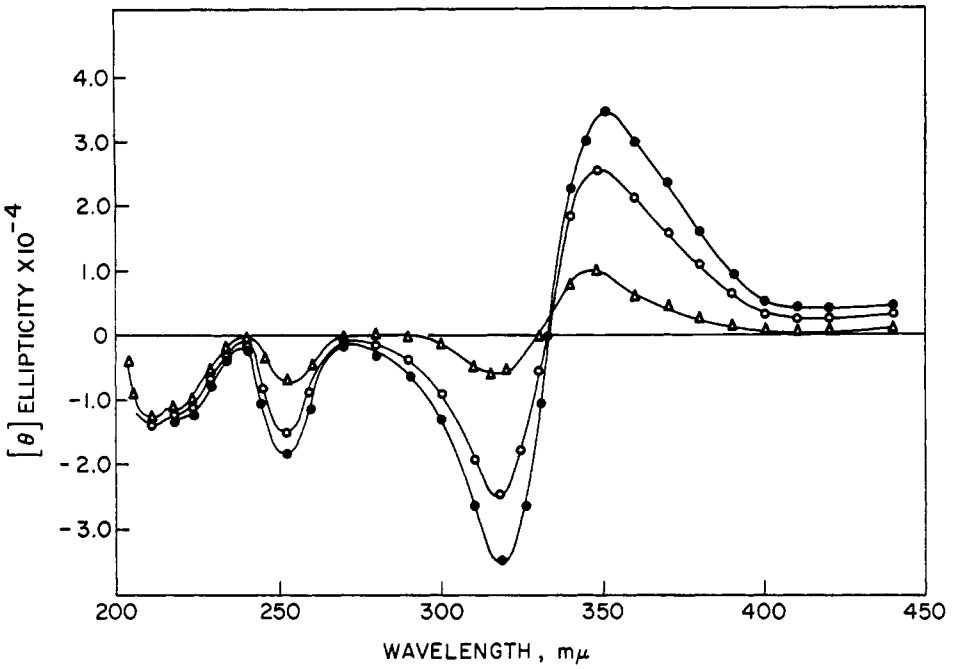

FIGURE 3: Circular dichroism spectrum of poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in trimethyl phosphate–trifluoroacetic acid mixtures prepared by method A. (△) 0.1% trifluoroacetic acid–99.9% trimethyl phosphate; (○) 1.0% trifluoroacetic acid–99.0% trimethyl phosphate; (●) 5.0% trifluoroacetic acid–95.0% trimethyl phosphate.

side-chain azoaromatic chromophore substantiate an extremely ordered side-chain–side-chain structure around the main helix (Gratzer, 1967).

For acidic solvent content higher than 15–20% and smaller than 60–70% the polymer is insoluble when prepared according to method A, or precipitates out after a short time if the solution is prepared according to method B. We were able to establish that protonation of the azo group occurs for 40–45% trifluoroacetic acid in the mixture prepared according to method B. The fully protonated chromophore exhibits its π–π^* transition at 468 mμ with an intensity essentially identical with that of the model compound (no hypochromicity).

Optical Rotatory Dispersion. We have carried out measurements of the optical rotatory dispersion for poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in mixed solvent trimethyl phosphate–trifluoroacetic acid (method A) with concentration of trifluoroacetic acid insufficient to protonate the azoaromatic chromophore. The spectrum (Figure 2) is complex, showing a weak broad band in the 480-mμ region which may arise from an n–π^* transition from the azoaromatic grouping, a typical exciton split Cotton effect composed of two small positive lobes at 388 and 300 mμ, and a huge trough ($[R]$ = −47,000) at 333 mμ. In addition a simple Cotton effect occurs in the 250-mμ region (trough at 262 mμ, peak at 242 mμ, crossover at 252 mμ). It is not possible to deduce the structure of the main chain from optical rotatory dispersion results because of overlapping of this 250-mμ Cotton effect with the Cotton effect arising from the peptide chromophore which exhibits a trough at 233 mμ. Consequently, we decided to investigate circular dichroism where we hoped to avoid overlapping of the side-chain and main-chain bands.

Circular Dichroism. The circular dichroism spectra of the polymer solutions in trimethyl phosphate–trifluoroacetic acid mixtures, prepared according to methods A and B, are shown in Figures 3 and 4. The curve obtained for the polymer in pure trimethyl phosphate exhibits two Cotton effects in the region of the π–π^* transition of the azoaromatic chromophore, a very weak negative band at 252 mμ and a broad band in the peptide chromophoric region below 235 mμ (Goodman and Benedetti, 1968). A previous study on polymers and copolymers of L-*p*-(*p'*-hydroxyphenylazo)phenylalanine and *N*-(3-hydroxypropyl)-L-glutamine has shown that in pure TMP such materials exist as right-handed α helices. We base these assignments on the linearity of the intensity of the molar residue rotation in either the peptide (233 mμ) or the azoaromatic chromophoric regions with the mole fraction of azoaromatic residues in the series of polymers and copolymers.

Examination of the circular dichroism spectrum of trimethyl phosphate–trifluoroacetic acid mixtures prepared according to method A indicates that the polymer is still a right-handed α helix and now the side chains arrange themselves in a tightly packed manner around the main chain probably aligned side to side with respect to each other. The two bands in the peptide chromophoric region shed light on the conformation of the main chain. Their position, shape, and ratio of intensity are all consistent with a right-handed α-helical conformation (Holzwarth and Doty, 1965). Their magnitude is lower than that found for α-helical polypeptides with no chromophores in the side chain. However, one must recall that other azoaromatic transitions with positive ellipticity are allowed in the same region and must certainly overlap with the peptide transitions, leading to lowered intensities for these bands. In mixtures of tri-

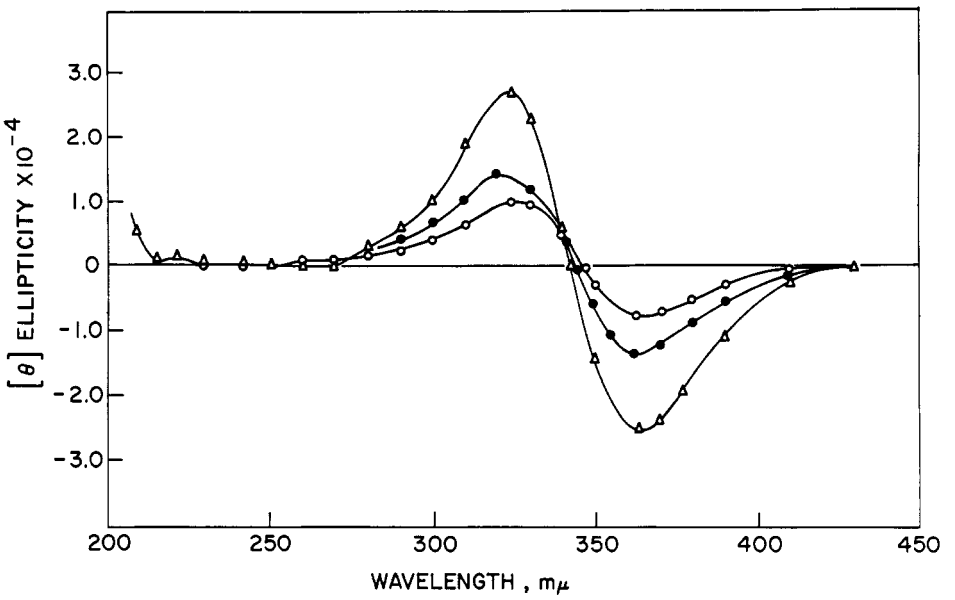

FIGURE 4: Circular dichroism spectrum of poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in trimethyl phosphate–trifluoroacetic acid mixtures prepared by method B (*c* 0.02%). (△) 2.0% trifluoroacetic acid–98.0% trimethyl phosphate; (●) 5.0% trifluoroacetic acid–95.0% trimethyl phosphate; (○) 20.0% trifluoroacetic acid–80.0% trimethyl phosphate.

methyl phosphate–trifluoroacetic acid prepared according to method B and containing insufficient trifluoroacetic acid to produce protonation of the chromophore, no α-helical structure is detectable in the peptide chromophoric region. We believe that the observed exciton resonance coupling splitting of the azoaromatic bands (above 250 mμ) is indicative of side-chain order for these polymers. The solution of the polymer in trimethyl phosphate–trifluoroacetic acid mixture containing approximately 5% of trifluoroacetic acid and prepared according to method A exhibits three bands in the circular dichroism spectrum above 240 mμ, a peak centered at 348 and two troughs centered at 319 and 252 mμ arising from induced optically active electronic transitions of the azoaromatic chromophore. The intensities of the first two bands are nearly the same but of different sign; their separation is less than 30 mμ and the crossover point is located at 333 mμ. These findings suggest that the bands arise from the π–π^* electronic transition of the azo group split by exciton resonance coupling of the spatially adjacent chromophores. The symmetry of the *p*-hydroxyazobenzene side chain (C_{2h} for the *trans* conformation) ensures a large value for the transition moment of the π–π^* transition. On the other hand, the geometry of the α helix is such that the side chains are spatially close to each other so that the conditions for the delocalization of the electronic excitation are established. As a result a large and effectively continuous series of levels produces two components, for which the transition moments do not vanish, one polarized roughly parallel to the axis of the side chain and nearly perpendicular to the helix axis and the other polarized perpendicularly to the axis of the azoaromatic groups and parallel to the α helix. A possible alternate explanation of the cause of the band at short wavelength could be the Φ–π_1^* transition which should have opposite polarization as compared to the π–π^* transition (Jaffe *et al.*, 1958). However, the magnitude of the transition moment for this band is very small and its energy should be greater than that found. We assign the band centered at 252 mμ to a pure aromatic transition, the Φ–Φ^*, which involves orbitals localized on the benzene rings. Jaffe and Gardner (1958) calculated for azobenzene that there must be actually four such transitions possible, of which two are allowed and may be degenerated. Our findings for the position of this band are in good agreement with Jaffe's calculation and with the experimental value reported in the literature for *p*-hydroxyazobenzene (Jaffe *et al.*, 1958; Baba, 1958).

For the solution prepared according to method B the circular dichroism spectrum exhibits two bands: a trough at 365 mμ and a peak at 323 mμ with crossover at 345 mμ. We believe that these bands arise from a splitting of the π–π^* transition of the azoaromatic group for reasons analogous to that noted above. No bands are detectable from the aromatic groups at 250 mμ and in the peptide chromophoric regions below 230 mμ. We ascribe these results to the lack of structure in the main chain. We found a splitting of the protonated azo grouping at 468 mμ when trimethyl phosphate–trifluoroacetic acid mixed solvent is used with trifluoroacetic acid concentration 60% or greater. The conformation of the polypeptide is independent of the method of preparation of the solutions. For high percentages of trifluoroacetic acid no other Cotton effects are observed down to 240 mμ. These results suggest the idea that an ordered side-chain structure exists for these solutions which may be analogous to the extended polyelectrolyte found for poly-L-*p*-(phenylazo)phenylalanine at high trifluoroacetic acid composition in the mixed solvent.

Finally we wish to point out that both the solutions, prepared according to the two methods, are stable over a long time and a large temperature range (−60 to +150°). A concentration dependence of the circular

dichroism spectrum of solutions prepared by method B shows that for trimethyl phosphate/polymer molar ratio greater than 10^6, the curve becomes identical with that obtained with solutions prepared by method A. This result demonstrates that trifluoroacetic acid interacts with the polymer chain with much greater affinity than trimethyl phosphate. When mixtures of trimethyl phosphate and trifluoroacetic acid are employed the trifluoroacetic acid acidity is substantially leveled. The conformation of the polymer is determined primarily by the large excess of trimethyl phosphate in the solvent mixture. If on the other hand trifluoroacetic acid is used initially to dissolve the polymer followed by trimethyl phosphate the conformation is primarily determined by the interactions of the trifluoroacetic acid with the polymer chains which might involve specific hydrogen bonding with the peptide main chain or with the phenolic group of the side chain. Subsequent addition of trimethyl phosphate does not materially alter this polymer–solvent interaction until the ratio of trimethyl phosphate to polymer is extremely high (10^6 or greater). If trimethyl phosphate is used to dissolve the polymer and the concentration of the polypeptide is relatively large (trimethyl phosphate/polymer 10^4 or less) there are sufficient sites on the polymer chain so that the trifluoroacetic acid added can frequently encounter peptide or side-chain groupings. Thus, for all practical purposes the trifluoroacetic acid acidity is not leveled and is the dominant factor in determining the polypeptide conformation. When the trimethyl phosphate/polymer ratio is large (10^6 or greater) the trifluoroacetic acid subsequently added encounters only trimethyl phosphate solvent molecules and is thus leveled in acidity. The polymer conformation then is determined primarily by the trimethyl phosphate solvent molecules.

Conclusions

We have examined two azoaromatic polypeptide systems in mixed solvents. We have shown that poly-L-*p*-(phenylazo)phenylalanine goes from right-handed α helix in dioxane to a random coil in 75–80% trifluoroacetic acid in dioxane to an extended ordered polyelectrolyte in solvent where trifluoroacetic acid concentration is greater than 90%.

We have also uncovered conformational changes for poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in trimethyl phosphate–trifluoroacetic acid solvent systems. We found evidence for right-handed α helices when premixed trimethyl phosphate–trifluoroacetic acid solvent is used, when the solvent contains no more than 10% trifluoroacetic acid. We found a different order for the azoaromatic side chains and an apparent absence of main-chain order if trifluoroacetic acid is allowed to interact extensively with the polypeptide solute. At this time we are unable to assign a specific structure that the polypeptide assumes under these conditions. When the trifluoroacetic acid composition of the solvent exceeds 45% we observe only protonated azoaromatic structure which probably leads to an extended ordered polyelectrolyte structure.

Experimental Section

Materials. Trimethyl phosphate and dioxane (spectroquality from Matheson Coleman and Bell) were redistilled and dioxane was passed through a column of neutral alumina just prior to use in order to remove peroxide. Trifluoroacetic acid was obtained from Aldrich and was used without further purification. Preparations of the polymers and copolymers used for all the measurements have been described previously (Goodman and Kossoy, 1966; Goodman and Benedetti, 1968).

Apparatus. The optical rotatory dispersion and circular dichroism measurements were recorded on the Cary 60 automatic recording spectropolarimeter equipped with a circular dichroism attachment. A special water-cooled cell holder was used to ensure constant temperature solutions as well as to obtain other temperature ranges. All ultraviolet and visible spectra were recorded either on the Cary 14 or on a Perkin-Elmer Model 350 spectrophotometer using 0.1-, 0.2-, or 0.5-mm fused-quartz (Suprasil) cylindrical cells from Optical Cell Co., Brentwood, Md. Extreme care was used in cleaning the cells and in the placement of the cells in the cell holder to avoid shifts in the base line. Molecular weight determinations were carried out using a Beckman Model E analytical centrifuge.

Preparation of Solutions. It has been reported (Goodman and Falxa, 1967) that poly-L-*p*-(phenylazo)phenylalanine photoisomerizes from *trans* to *cis* in the presence of light. Special caution was taken to exclude all light in the preparation of solutions of these polymers. They were irradiated only during a brief period in the spectrophotometer or spectropolarimeter. The samples were initially dissolved in the dark with magnetic stirring in trifluoroacetic acid. Sufficient dioxane was added to the 5-ml volumetric flask to obtain the desired dioxane–trifluoroacetic acid mixture. The stoppered flasks were sealed with a paraffin film and then covered with several layers of masking or electrical tape. The samples were kept in the dark at least 24 hr before scanning. The solutions of poly-L-*p*-(*p'*-hydroxyphenylazo)phenylalanine in trifluoroacetic acid–trimethyl phosphate mixtures were prepared without such special care since no photoisomerization has been observed. Addition of either component to the other was carried out slowly in a thermostated bath in order to avoid heating of the solutions upon mixing. All measurements were carried out on the solutions as soon after preparation as was possible. A second series of measurements was accomplished after a long delay (more than 7 days). During this period no change was noted in the ultraviolet, optical rotatory dispersion, or circular dichroism spectra.

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Conformational Aspects of Polypeptide Structure. XXVIII. Side-Chain Cotton Effect from Poly-L-*p*-(2′-hydroxy-5′-methylphenylazo)phenylalanine*

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ABSTRACT: Quantitative diazotization of poly-L-*p*-aminophenylalanine, followed by coupling of this unisolated intermediate with *p*-cresol, leads to poly-L-*p*-(5′-hydroxy-2′-methylphenylazo)phenylalanine. Conformational analysis of this azoaromatic polypeptide was carried out in hexafluoro-2-propanol, dimethylacetamide, and trifluoroacetic acid. In the first two solvents, which we generally considered helix supporting, side-chain Cotton effects are detected, while for the third solvent no ellipticity band is observed. We point out that macromolecules with bulky side chains may not be able to assume a completely random structure because steric interactions lead to specific preferred conformations. The resulting level of order or occasional periodicity in the main chain or in the side chains or in both produces the observed Cotton effects.

For most polymers derived from α-amino acids containing strongly absorbing side-chain chromophores such as imidazole, indole, or aromatic groups, unique assignment of secondary structure in solution is difficult (Goodman *et al.*, 1968). The difficulties arise mainly from overlapping of the optically active electronic transitions of the side chain with the transitions from the main-chain amide chromophores. On the other hand, amino acid aromatic side chains substantially affect the conformation of poly-α-amino acids and proteins in solution (Goodman and Toniolo, 1968). Their structure and conformation are in part determined by electronic and steric interactions among side-chain chromophores and between the side chains and the optically active centers in the polypeptide main chain. Optical methods such as optical rotatory dispersion or circular dichroism are powerful tools to yield information on side-chain structure and over-all conformational relationship in poly-α-amino acids and naturally occurring materials.

Several aromatic amino acids containing auxochromic substituents in the side chains have been investigated in our laboratories. We recently reported on the synthesis and conformational characterization of two azoaromatic polypeptides: poly-L-*p*-(phenylazo)phenylalanine (Goodman and Kossoy, 1966) and poly-L-*p*-(*p*′-hydroxyphenylazo)phenylalanine (Goodman and Benedetti, 1968). In the present paper we report on the synthesis and conformational analysis of a new azoaromatic poly-

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