Conformational Studies of Poly-α-amino Acids with Aromatic-Side-Chain Effects

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In recent years scientists have achieved a revolution in the understanding of the structure of fibrous and globular proteins by means of X-ray diffraction analysis. Pauling's α helix,[1] Perutz's[2] isomorphous replacement, and the derivation of expressions of X-ray scattering by helices by Cochran, Crick, and Vand[3] were all established during this period. Complete structural determinations have been presented for myoglobin,[4] hemoglobin,[5] lysozyme,[6] and ribonuclease.[7] It is expected that X-ray diffraction analysis of these proteins in the presence of substrates under conditions of complete biological activity will lead to elucidation of structure–function relationships for these biological macromolecules.[8]

Many biological substances cannot be crystallized and thus cannot be investigated by X-ray diffraction analysis. Even when biopolymers can be crystallized, it is a hit or miss proposition to find multiple isomorphous heavy atom derivatives.

It has been demonstrated by many workers that polypeptides can form α helices and that they maintain their structure in appropriate solutions.[9] Therefore many workers have concentrated their efforts on the solution properties of proteins and polypeptides.

Optical rotatory dispersion (ORD) has been most successful in estimating the presence of secondary structure in solution for proteins and polypeptides.[9] In proteins and poly-α-amino acids the peptide chromophores present two electronic transitions (n–π* and π–π*) in the accessible isotropic absorption region and produce characteristic Cotton effects which can be related to secondary structure. Side-chain chromophores such as aromatic groups also produce Cotton effects. Each Cotton effect is set upon the background provided by the tails of all the other Cotton effects. The background rotation may be sufficiently large or change sufficiently rapidly that small Cotton effects are obscured.

Circular dichroism (CD), which measures asymmetric absorption phenomena directly, exhibits rotation values only over the frequency where absorption occurs. Thus, no background optical activity exists to complicate interpretations. Optical rotatory dispersion and circular dichroism are intimately related by the Kronig–Kramer transform.[10]

Holzwarth and Doty,[11] by means of circular dichroism, demonstrated the presence of three bands, two negative, one positive, in the peptide chromophoric region (225–185 mμ). These bands were carefully assigned with the aid of polarized absorption spectra to the n–π* transition and the two allowed π–π* exciton bands predicted by Moffitt.[12–14]

Amino acid aromatic side chains substantially affect the conformation of poly-α-amino acids and proteins in solution.[15] Their structure and conformation are in part determined by electronic and steric interactions between side-chain chromophores and the optically active centers in the polypeptide main chain. Aromatic-side-chain effects have been investigated by optical rotatory dispersion, circular dichroism, and ultraviolet absorption techniques. It is the expressed purpose of this article critically to summarize data accumulated from conformational studies of poly-α-amino acids with aromatic side chains.

Aromatic-Side-Chain Effects from Poly-α-amino Acids

A. Poly-L-tyrosine. Aromatic-side-chain effects have been confirmed by optical rotatory dispersion, circular dichroism, and ultraviolet absorption studies of poly-L-tyrosine.[16–18] Although poly-L-tyrosine in dimethylformamide and in pyridine has been found[19] to possess a positive b_0 value of the Moffitt equation, it has been demonstrated[16–18,20] that this poly-α-amino acid exists as a right-handed α helix, instead of the expected[12–14] left-handed α-helical conformation (*cf.* ref 9, 21, 22).

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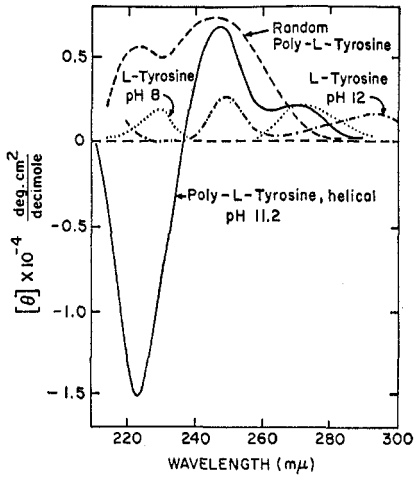

Figure 1. Circular dichroism of poly-L-tyrosine and L-tyrosine in aqueous solutions.[17]

This anomalous behavior has been attributed to the electronic interactions between tyrosyl aromatic-side-chain chromophores which presumably have such large contributions to the optical rotatory dispersion spectra that the peptide contribution is obscured in the tyrosyl absorption region.[16,23,24] These effects are easily discernible in the optical rotatory dispersion spectra of helical poly-L-tyrosine.[16]

Similar results were demonstrated[17] by circular dichroism spectra (Figure 1), which reveal three dichroic bands for the helical conformation, two positive at 270 and 248 mμ and a negative one at 224 mμ, whereas the random-coil form exhibits only two positive bands at 245 and 225mμ. An unusual behavior characteristic of helical poly-L-tyrosine is that the rotational strength of the n–π^* peptide transition at 224 mμ is lower than expected;[11] presumably a side-chain chromophore transition of opposite sign rotational strength is responsible for this apparently low value of helix content.

Quantum mechanical treatment[18] of the existing optical rotation data obtained from helical poly-L-tyrosine in solution suggests that, although there are aromatic-side-chain interactions, there is no evidence to support the existence of a stable helical arrangement of the tyrosyl side-chain groups.

Energy calculations have been carried out[25] for several isolated homopolymers of α-amino acids in order to find the most stable regular conformations. In the case of poly-L-tyrosine a lower energy in the right-handed than in the left-handed α-helical form has been found.

However, with poly-L-tyrosine there has been disagreement over the screw sense of the α helix. A recent study[26] by means of dipole moments, rotational relaxation times, and other parameters expressing polydispersity of poly-L-tyrosine in dilute solutions of quinoline show that in such a system the polymer can exist as a left-handed α helix. The screw sense of the helix was determined by observing the change in the z component (the z axis lies along the helix axis) of the dipole moment, $\langle\mu_z\rangle$, of 1.0 ± 0.1 D per residue on going from poly-L-tyrosine to poly-L-3-bromotyrosine.

We believe that poly-L-tyrosine under appropriate conditions can be left handed or right handed. Calculations of the minimum potential energy of poly-L-tyrosine, copolymer studies of L-tyrosine with γ-benzyl-L-glutamate, and optical rotatory dispersion and circular dichroism measurements of poly-L-tyrosine in aqueous base and also in dimethyl sulfoxide all support a right-handed structure. On the other hand, dipole moment measurements and related experiments in quinoline indicate that the poly-L-tyrosine helix is left handed. We find no contradiction in these assignments and suggest that solvent–polymer interactions may indeed convert the polymer from one conformation to another. It should be recalled that the energy difference between right- and left-handed poly-L-tyrosine is only 1.8 kcal/mol of residue. Molecular models indicate that the aromatic rings in the side chain of the poly-L-tyrosine are more easily accessible in the left-handed helical structure as compared with the right-handed one. The stability gained from the solvent–side-chain interactions (quinoline–phenol group) can be greater than the difference in energy between the left- and right-handed helical forms. We have other evidence from poly-L-aspartate esters (see section on poly-L-aspartate esters) that solvent can alter polymer conformation substantially.

B. Poly-L-phenylalanine. Weak Cotton effects have been detected[25–27] in the wavelength region where the phenyl-side-chain chromophore exhibits a number of absorption maxima (300–225 mμ) for copolymers containing L-phenylalanine. Since poly-L-phenylalanine is water insoluble, these results were obtained[27–29] by studying the optical rotatory dispersion and circular dichroism spectra of block copolymers of L-phenylalanine with DL-glutamate. The three small aromatic Cotton effects in the 258–246-mμ region of the optical rotatory dispersion curve of block copoly DL-Glu-L-Phe-DL-Glu (40:20:20) may be attributed to aromatic-side-chain chromophores existing in a dissymmetric environment. The weak negative trough at 237 mμ further suggests a right-handed α-helical conformation. These effects disappear when the helical conformation is disrupted.

The question of whether the ^1L_b transition in the Platt notation[30] in the 255–275-mμ region corresponding to a symmetry-forbidden π–π^* transition is opti-

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cally active has been answered.[31] The extreme weakness of the Cotton effects in poly-L-phenylalanine with respect to those in poly-L-tyrosine corresponding to this transition can be understood to a first approximation, considering that the benzene ring has an effective C_{2v} local symmetry. Then the dissymmetric molecular environment provided for the aromatic chromophore by the amino acid substituent is ineffective in generating $\pi-\pi^*$ Cotton effects if the π-molecular orbitals are constructed as a linear combination of $2p_z$ atomic orbitals. In this case the $\pi-\pi^*$ transitions have the electric dipole transition moments in the plane of the ring and the magnetic dipole transition moments perpendicular to that plane.[32] No amount of mixing of the $\pi-\pi^*$ transitions can lead to a nonvanishing scalar product of them. Therefore no optically active $\pi-\pi^*$ transition can be observed. Charge transfer or mixing of transitions between aromatic and amide chromophores or simply mixing of the $\pi-\pi^*$ transitions with some perpendicular transitions of the aromatic, such as $\sigma-\pi^*$, $n-\pi^*$, or transitions involving 3d orbitals must be considered in order to explain the aromatic Cotton effects.[31] In the case of tyrosine the oxygen atom of the aromatic chromophore is a ready source of nonbonding orbitals for $n-\pi^*$ transitions. As a matter of fact, poly-L-tyrosine presents a detectable long-wavelength aromatic Cotton effect. In poly-L-phenylalanine, which does not have n orbitals available, a less effective mechanism of mixing of $\sigma-\pi^*$ transitions or transitions involving 3d orbitals, ignoring charge transfer or mixing with the amide chromophore, must be invoked. This can be a reasonable explanation for the exceptional weakness of these aromatic Cotton effects. Generally, monosubstituted benzene rings exhibit two additional transitions in the accessible isotropic absorption region, near 210 and 185 mμ, stronger than the transitions in the 255–275-mμ region. The 210-mμ band, classified ^1L_a, is also forbidden but probably involves a contribution from the first allowed $\pi-\pi^*$ transition which overlaps it at shorter wavelengths.[33] Upon appropriate ring substitution both of these latter bands may shift to longer wavelengths. Cotton effects in the 215–225-mμ region of several benzene derivatives have been reported,[34] such as α-amino and α-hydroxy aromatic acids. Rosenberg[35] concluded on the basis of ORD comparisons of phenylalaninol and tyrosinol with the corresponding amino acids that the ^1L_a transition of the phenyl ring is optically active. Verbit and Inouye[36] arrived at the same conclusion for a series of phenylcyclopropane derivatives.

All these facts support the concept that poly-α-amino acids with aromatic side chains cannot be given unique assignments of screw sense because of overlapping absorption bands resulting from aromatic transitions and main-chain amide chromophores.

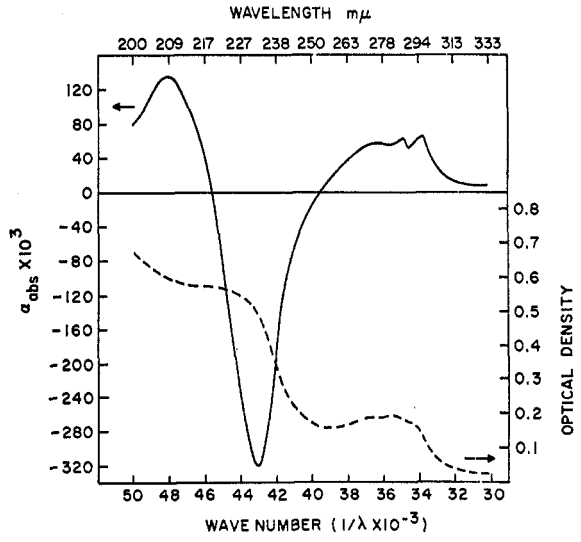

Figure 2. Optical rotatory dispersion and ultraviolet absorption spectra of poly-L-tryptophan film.[37]

C. Poly-L-tryptophan. Optical rotatory dispersion studies of poly-L-tryptophan have also shown that side-chain chromophores can affect the b_0 of the Moffitt equation. It has been demonstrated[37] that poly-L-tryptophan, despite its positive b_0 value, is a right-handed α helix. This conclusion is based on the linear b_0 relationship of copolymers with γ-benzyl-L-glutamate and the observation of the negative 233-mμ Cotton effect (Figure 2) usually found in right-handed α-helical poly-α-L-amino acids. The two positive Cotton effects in the 290–270-mμ region are assigned to a conformation-dependent interaction between indole residues, which probably stack in a helical array or interact with the backbone peptide bonds. The hypochromicity found for the tryptophanyl residues in the helical polypeptide affords supporting evidence for this aromatic-side-chain effect.

A recent study on the optical rotatory properties of a block copolymer of γ-ethyl-DL-glutamate (160 residues) and L-tryptophan (30 residues) in trifluoroethanol has been described.[38,39] Peaks at 280, 233, and 200 mμ and a trough at 218 mμ were observed. In contrast to the previous study,[37] the latter results would appear to cast doubts on the correct choice of helical sense of the conformation of poly-L-tryptophan, since strong overlapping of Cotton effects of peptide main-chain chromophores and indole side-chain chromophores are evident.

A recent investigation[40] of L-tryptophan has shown

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that the CD spectrum in the 250–300-mμ region consists of at least three positive dichroic bands centered at 291, 281, and 272 mμ. The positions of these bands are approximately the same as those of the absorption peaks of the indole chromophore, thus suggesting their origin. Below 250 mμ there are four ellipticity bands. Only the shoulder at 218 mμ reflects its counterpart in the exceedingly strong ultraviolet absorption band of indole.

Circular dichroism studies[39] of the block copolymer noted above show a rather complex spectrum in the region 260–300 mμ with a negative band at 290 mμ, a positive band at 272 mμ, and two shoulders at 280 mμ and at 286 mμ. Below 250 mμ two positive bands and a single negative one are present respectively at 226, 210, and 190 mμ. The maximum of the band at 226 mμ is shifted 8 mμ toward the red with respect to the corresponding uv indole band probably from the partial overlapping of this band with the 210-mμ peptide band. The two dichroic bands at 210 and 190 mμ are of the same sign and position as the CD bands observed and calculated for polypeptides of a right-handed α-helical conformation. However, because of the possible presence of optically active electronic transitions of the indole chromophore that overlap the peptide chromophoric bands, a definite conclusion about the type of helical conformation exhibited by poly-L-tryptophan remains difficult.

D. Poly-L-histidine and Derivatives. The imidazole-side-chain group of poly-L-histidine has absorption bands much lower than those of the aromatic groups, but enhanced imidazole chromophore contributions have been detected. The helical sense of poly-L-histidine was originally assigned[41] a left-handed α-helical conformation in aqueous solution. A more recent conformational study[42] of poly-L-histidine suggests a right-handed α helix as indicated by the marked change in the optical rotatory dispersion spectrum between pH 4.97 and 5.78.

The circular dichroism spectra of poly-L-histidine indicate that between pH 3 and 6 this poly-α-amino acid undergoes a random coil to right-handed α helix transition. However, the magnitude of the characteristic negative $n-\pi^*$ ellipticity band near 220 mμ is much lower than expected,[11] suggesting that the polypeptide possesses a low helical content or the imidazole side chain possesses a positive circular dichroism band in the region where the peptide bands in the α-helical conformation exhibit negative bands.

Optical rotatory dispersion and infrared studies[41] of poly-1-benzyl-L-histidine in the solvent system chloroform–dichloroacetic acid indicate that this poly-α-amino acid exists in three different reversibly interconvertible forms depending upon the solvent composition. It is suggested that form I (100% chloroform) is an un-ionized random chain, form II (chloroform containing one residue equivalent of dichloroacetic acid) is an ionized left-handed α helix, and form III (chloroform with a large excess of dichloroacetic acid) is an ionized random chain.

E. Poly-β-benzyl-L-aspartate and Derivatives. Certain poly-α-amino acids derived from L-aspartic acid such as poly-β-benzyl-L-aspartate appear to be in the left-handed α-helical conformation.[43,44] An interesting reversal from the left- to the right-handed helical sense of the polypeptide was found[45–49] upon the introduction of a nitro group in the *para* position of the aromatic ring in the side chain of poly-β-benzyl-L-aspartate. It was demonstrated that this reversal occurs when 26–32 mole % of β-(*p*-nitrobenzyl)-L-aspartate is present in the copolymer. Also, unusually large negative b_0 values are obtained at nitro residue contents greater than 50 mole %, indicating the formation of a very stable helix.

A 330-mμ circular dichroism band is observed which is attributed to long-wavelength electronic transition resulting from dissymmetric array of nitroaromatic groups. Statistical analysis[49] of the dependence of this Cotton effect on the nitroaromatic residue mole fraction of a copolymer in chloroform leads to a conclusion that pairwise interactions between two nitrobenzyl groups separated by four residues can be responsible for the experimental observations.

A recent optical rotatory dispersion study[50] in chloroform and trifluoroethanol comparing β-methyl with β-benzyl-L-aspartate supports our contention that pairwise interactions between nitroaromatic groups are important to the stability of the right-handed α helix (Figure 3).

Circular dichroism studies[51] on copolymers β-(*p*-nitrobenzyl)-L-aspartate–β-benzyl-L-aspartate have once again established the transition from the left-handed to the right-handed α helix at approximately 20% of nitroaromatic residues in chloroform, while in trimethyl phosphate such a transition is not observed. In this solvent copolymers are in the random-coil conformation for a content of nitroaromatic residues less than 40% and in the right-handed α-helix for higher content of nitroaromatic residues.

We have examined the CD spectra of the β-(*p*-nitrobenzyl)-L-aspartate polymers and copolymers and found that the long-wavelength 320–330-mμ transition observed in chloroform is absent. Another Cotton effect at approximately 300 mμ is observed in trimethyl

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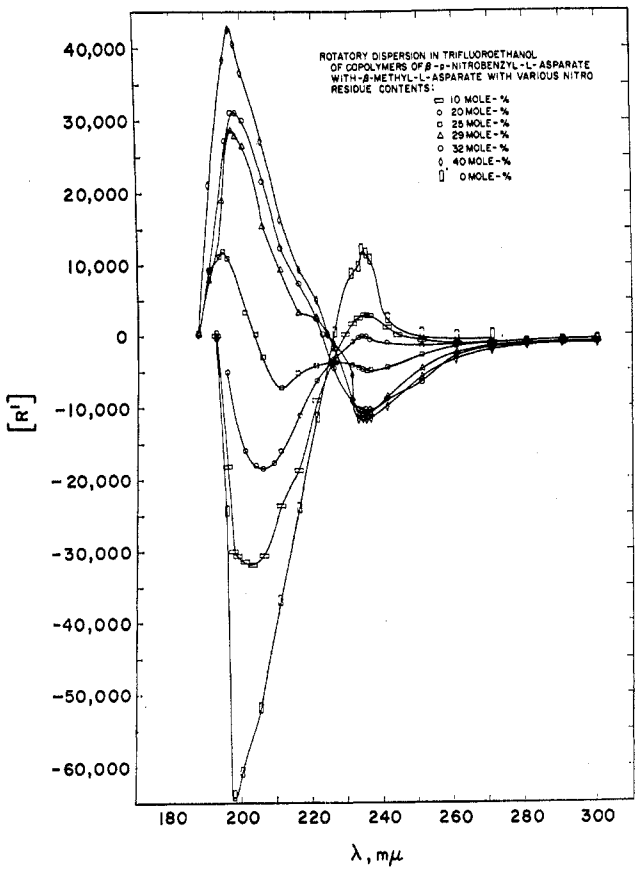

Figure 3. Optical rotatory dispersion of copolymers of β-(*p*-nitrobenzyl)-L-aspartate with β-methyl-L-aspartate in trifluoroethanol.[50]

phosphate. Similar results for nitroaromatic chromophores were observed for poly-L-nitrophenylalanine in dimethylacetamide (see later section). Rae[52] reported on the nature of the solvent effect on the nuclear magnetic resonance of nitroaromatic groups. He studied a series of trisubstituted benzenes in deuteriochloroform and dimethyl sulfoxide. He found that the protons *ortho* to the nitro group are substantially shifted upfield by change of solvent from deuteriochloroform to dimethyl sulfoxide. He interprets these results in terms of rotation of the nitro group about the bond joining it to the benzene ring. This, of course, decreases the electronic interactions between the aromatic and the nitro groups. The polar nitro group would be expected to interact with polar solvent more extensively in this uncoupled conformation. This would lead to an upfield shift of an adjacent proton because the nitro orbitals are no longer in a plane where they shield adjacent protons.

Our evidence indicates that a right-handed α helix exists in chloroform and trimethyl phosphate by virtue of the CD spectra through the peptide Cotton effect region (below 230 mμ). However, in the former, side chains are organized as indicated by the 330-mμ CD band, but in the latter, the side chains are disorganized since the absorption of the nitrobenzyl homopolymer at 300 mμ is identical with that found in the model compound.

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Analogous aromatic effects have been reported[53,54] for polymers containing poly-(β-*p*-methyl-, -chloro-, -cyano-, and -nitrobenzyl)-L-aspartate. The introduction of a methyl, chloro, or cyano group into the *para* position of the aromatic ring in the side chain of poly-β-benzyl-L-aspartate also causes a reversal from the left- to the right-handed in the helical sense of the poly-α-amino acid in a chloroform solution, as is the case of the nitro group.

In similar studies[46,47] of copolymers containing poly-γ-(*p*-nitrobenzyl)-L-glutamate this nitroaromatic effect was not observed since the side-chain aromatic group is much more flexible and therefore cannot be dissymmetrically arrayed about a main-chain helix.

A suggestion for the anomalous sense of the helix has been made[55] by Scheraga and his group by means of energy calculations carried out for isolated (single-stranded) homopolymers. The existence of the poly-α-amino acids, derived from L-aspartate esters, in the left-handed α helix is primarily due to a balance of forces. The electrostatic energy contribution can overcome the stabilization of the right-handed helix by the nonbonded energy interactions.

The results on the poly-L-aspartate esters show that these copolymers can take up right-handed or left-handed helices, depending on the temperature of the chloroform solutions. Reversal of the helix sense was also observed by changing the solvent.[54,56]

The change in conformation from right handed to left handed or *vice versa* must be accompanied by an over-all gain in entropy of the system polymer–solvent. This gain can be obtained either by mixing previously ordered solvent molecules with the bulk solvent or from a change in order of the side chain. If differences in the side-chain–main-chain or side-chain–side-chain interactions can stabilize the right-handed helix more than the left-handed helix, the change in helix sense will be accompanied by a gain in entropy because of a loss of order of the side chains. We have shown that such effects can be produced by substitution of different ester groups which can change the polymer–solvent interactions.

F. Poly-L-*p*-nitrophenylalanine. Nitroaromatic chromophore effects have been detected by optical rotatory dispersion and circular dichroism in the aromatic absorption region of poly-L-*p*-nitrophenylalanine and several copolymers containing L-*p*-nitrophenylalanine.[57] In dimethylacetamide and dimethylformamide some copolymers exhibit positive Cotton effects in the 400–300-mμ region; these Cotton effects are attributed to the existence of the nitroaromatic chromophore in a dissymmetric environment. The nitrophenyl Cotton effect is observed when as little as 9.5%

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L-*p*-nitrophenylalanine was incorporated into the copolymer. Negative Cotton effects with troughs near 238 mμ further suggest that the copolymers exist as right-handed α helices. In addition, a statistical analysis of the circular dichroism curves of the copolymers indicates the existence of side-chain–main-chain and first and second neighbor interactions between nitroaromatic groups.

However, in contrast to the copolymers, the homopolymer, poly-L-*p*-nitrophenylalanine, exhibits a negative Cotton effect near 310 mμ. This Cotton effect corresponds to a long-wavelength electronic transition arising from a dissymmetric array of the nitroaromatic chromophore. Contrary to expectations, the dispersions of the copolymers in trifluoroacetic acid and dichloroacetic acid are complex. The nature of these discrepancies cannot be explained on the basis of existing data.

G. Poly-L-*p*-aminophenylalanine and Derivatives. Strong conformational aromatic-side-chain effects have been observed[58] for amino analogs of poly-L-tyrosine. In tetrahydrofuran the optical rotatory dispersion and circular dichroism of poly-N^{ω}-carbobenzoxy-L-*p*-aminophenylalanine reveals a positive Cotton effect centered at 245 mμ which can be assigned to the π–π^* electronic transitions of *para*-substituted benzene rings existing in a dissymmetric environment; this band disappears in trifluoroacetic acid, a helix-breaking solvent.

Conformational effects from poly-L-*p*-aminophenylalanine[58] in the acidic pH range are comparable to those found for poly-L-tyrosine[16,17] in the basic pH range (Figure 4). Poly-L-*p*-aminophenylalanine exists as a random coil between pH 1.08 and 2.56. However, when the pH is raised from 2.56 to 2.78, a sharp change in optical rotatory dispersion, circular dichroism, and ultraviolet absorption is observed. Positive Cotton effects at 290 and 245 mμ corresponding to π–π^* electronic interactions of aromatic-side chain amino groups appear. This is attributed to a random-coil to α-helix transition over this pH region, even though only half the amino groups are deprotonated. The negative Cotton effect with a trough at 230 mμ further suggests a right-handed α helix at pH 2.78 or higher.

H. Poly-L-*p*-(phenylazo)phenylalanine. Azoaromatic effects have been observed in a series of azopolypeptides derived from L-*p*-(phenylazo)phenylalanine and γ-benzyl-L-glutamate.[59] The optical rotatory dispersion spectra of the azopolypeptides in dioxane (Figure 5) reveal an intense positive Cotton effect at 360 mμ which arises from interactions between the side-chain azoaromatic chromophores and the asymmetric centers in the ordered peptide main chain. In trifluoroacetic acid the appearance of another positive Cotton effect with a peak at 425 mμ is attributed to the interactions among the highly ordered protonated azoaromatic side chains arrayed about an extended peptide main chain.

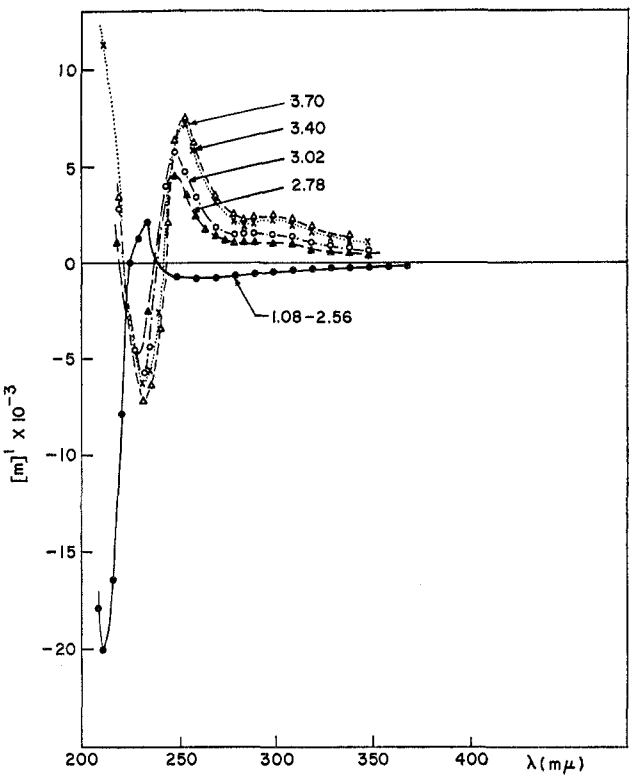

Figure 4. Optical rotatory dispersion of poly-L-*p*-aminophenylalanine in aqueous solutions.[58]

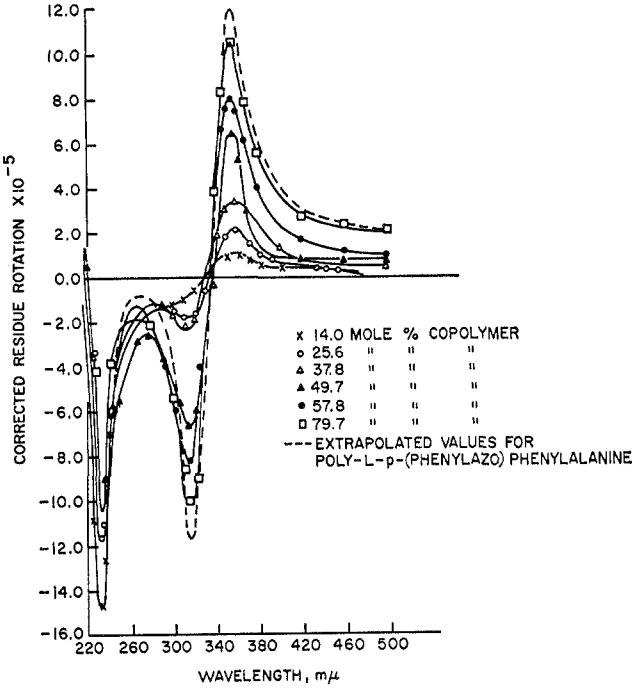

Figure 5. Optical rotatory dispersion of azoaromatic polypeptides in dioxane.[59]

Photoisomerizations (*cis–trans*) of these poly-α-amino acids with azoaromatic side chains have been characterized by optical rotatory dispersion.[60] Upon irradiation in dioxane at 320 mμ, the λ_{max} of the azoaromatic π–π^* transition, the half-time for the partial conversion of the *trans* (unirradiated) form of the azopolypeptides to the less stable *cis* form is about 180 min, while the

(58) M. Goodman and E. Peggion, *Biochemistry*, **6**, 1533 (1967).
(59) M. Goodman and A. Kossoy, *J. Am. Chem. Soc.*, **88**, 5010 (1966).
(60) M. Goodman and M. L. Falxa, *ibid.*, **89**, 3863 (1967).

half-time for the reverse reaction (relaxation) in the dark is about 90 min.

The optical rotatory dispersion Cotton effect trough at 233 mμ remains essentially unchanged, while the side-chain Cotton effect near 335 mμ decreases. These results indicate that the helical conformation of the polypeptide backbone is unaffected by the *cis–trans* photoisomerization.

In contrast, upon irradiation in trifluoroacetic acid at 425 mμ, the λ_{max} of the conjugate acid, the half-time for the partial conversion of the *trans* protonated azo-aromatic side chains to photoequilibrium form containing *cis*-protonated configuration is only about 160 min. Unexpectedly, the half-time for the relaxation in the dark is much longer, 210 min. Also, the optical rotatory dispersion Cotton effect at 425 mμ for the *trans* form is replaced at somewhat lower wavelengths by Cotton effects that are of opposite sign and greater magnitudes. These observations are attributed to side-chain–side-chain interactions which tend to stabilize the extended *cis* form, once it is formed by irradiation.

Conclusion

There is, today, much interest in the aspects of protein structure–function relationship. Specifically scientists are concerned with possible conformational alterations which accompany interactions of biological macromolecules with other cellular constituents. Optical methods such as optical rotatory dispersion and circular dichroism spectra shed light on structural changes in biopolymers which are not primarily due to over-all change in helix content but rather to local conformations at particular sites. These may not affect any periodic or nonperiodic arrangement of peptide main chain bonds. Poly-α-amino acids with aromatic side chains provide an important model to study such delicate and subtle changes in conformation. Optical activity and circular dichroism are extremely sensitive to the environment and interactions of the aromatic side chains. We have discussed the difficulties in interpreting data for poly-α-amino acids where aromatic chromophoric effects overlap peptide group electronic transitions. Because of this, much remains uncertain for the specific analysis of poly-α-amino acid and protein conformations. However, these findings can be used as a powerful tool to yield information on side-chain structure–function relationships. Perhaps in the future we can obtain fascinating results on aromatic-side-chain effects in biopolymer structure by use of high-frequency nuclear magnetic resonance. Coupling patterns for *ortho* and *meta* protons should reveal specific interactions and orientations of the aromatic group with respect to the main chain.

The Chemistry of the Polyhedral Species Derived from Transition Metals and Carboranes

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The first definitive research in boron hydride chemistry was conducted by Stock and his students who succeeded in preparing small quantities of several boron hydrides (B_2H_6, B_4H_{10}, B_5H_9, B_5H_{11}, B_6H_{10}, and $B_{10}H_{14}$). This early work was necessarily carried out using the then newly developed vacuum-line technique. Later work by Schlesinger and his students at the University of Chicago led to improved preparative methods for the boron hydrides and uncovered many new reactions of these novel species. Throughout these early days little was known with regard to the structures of the boron hydrides. Consequently, the boron hydrides were regarded as unstable and highly reactive chemical oddities of unknown importance until X-ray and electron diffraction techniques elucidated their unusual structures and the unconventional multicenter bonding associated with these structures.

Following World War II, a high-energy fuel program was established under military auspices with the intent of producing large quantities of liquid boron hydride derivatives. The impetus which this program gave to boron hydride research culminated in the discovery of the extraordinarily stable polyhedral borane ions[1] ($B_nH_n^{2-}$, n = 10 and 12) and the icosahedral $B_{10}C_2H_{12}$ carboranes.[1] These highly symmetrical species are stabilized by three-dimensional electron delocalization, and they may be considered to be the aromatic members of the boron hydride series. These discoveries have since led to the characterization of the entire polyhedral ion and the corresponding isoelectronic carborane series (n = 6–12 in $B_nH_n^{2-}$ and $B_{n-2}C_2H_n$).

While these developments are significant in themselves, more recent work[2] has opened up a new field of research which combines polyhedral carborane and transition metal chemistries in much the same way as the first metallocenes were fashioned from aromatic organic species and transition metal derivatives. Several families of polyhedral species are now known in

(1) See M. F. Hawthorne in "The Chemistry of Boron and its Compounds," E. L. Muetterties, Ed., John Wiley & Sons, Inc., New York, N. Y., 1967, p 223, or E. L. Muetterties and W. H. Knoth," Polyhedral Boranes," Dekker Publishing Co., New York, N. Y., 1968, for recent reviews of this subject.

(2) M. F. Hawthorne, D. C. Young, T. D. Andrews, D. V. Howe, R. L. Pilling, A. D. Pitts, M. Reintjes, L. F. Warren, Jr., and P. A. Wegner, *J. Am. Chem. Soc.*, **90**, 879 (1968).