# The Synthesis, Structure, and Optical Properties of Some Copolypeptides Containing Nonpolar Amino Acid Residues*

Henry E. Auer† and Paul Doty

ABSTRACT: *In situ* block copolymerizations are described which were used to prepare block copoly-DL-glutamate–poly-L-(nonpolar residue)–poly-DL-glutamates. The nonpolar residues incorporated were L-leucine in one case, and L-phenylalanine in another. Mixed-batch copolymerizations using the same constituents were also carried out. Proper conditions for the syntheses were worked out from the kinetics of copolymerization. The resulting copolypeptides were characterized by determining their molecular sizes and chemical compositions. Measurements of their optical rotatory dispersions, circular dichroism, absorption spectra, and fluorescence emission spectra in aqueous solution were performed. On the basis of these results, it is demonstrated that the block copolymerizations yielded true block copolymers, and that the central blocks containing the nonpolar residues are $\alpha$-helical.

The optical rotatory dispersion of L-phenylalanine residues is anomalous and strongly conformation dependent. There is no evidence for hypochromism or exciton splitting at 260 m$\mu$ in block poly-L-phenylalanine; the formation of excited dimers, however, is apparent from the emission spectra.

This paper and the succeeding one describe studies on the physical and thermodynamic properties of polypeptides containing L-leucine and L-phenylalanine. In the present work we shall first discuss the preparation and characterization of some block and mixed-batch copolypeptides containing these amino acid residues, and then present a study of their spectroscopic properties. In the following paper (Auer and Doty, 1966), an investigation of the conformational stability of the copolypeptides is considered.

The block copolypeptides employed in these studies consisted of three blocks arranged in the sequence poly-DL-glutamate–poly-L-(nonpolar amino acid residue)–poly-DL-glutamate. This system was designed to render the poly-L-leucine or poly-L-phenylalanine block soluble while still permitting the conformation of the central block to be determined from its optical rotatory power (Gratzer and Doty, 1963). The first objective in this paper, then, is to describe the synthesis of these block copolypeptides, and to demonstrate that the desired product is in fact provided. Closely allied to this is the second problem of showing that the natural conformation of the L-leucine residues and of the L-phenylalanine residues in the central blocks is the $\alpha$-helix. Without this knowledge, an investigation of conformational stability has no meaning. Finally, the optical properties of the nonpolar residues in these copolymers were examined since this was the first time that such peptide chains could be studied in transparent aqueous medium.

## Experimental Section

*N-Carboxyanhydrides.* Amino acid *N*-carboxyanhydrides (NCA's)[1] were obtained from Pilot Chemicals, Inc., Watertown, Mass. One portion of L-leucine NCA was purified by sublimation at 50 $\mu$ of Hg at 65°. All other samples of NCA's were dissolved in dry ethyl acetate, shaken with $Ag_2O$ and anhydrous $MgSO_4$, and then twice recrystallized at −20° from dry ethyl acetate by addition of dry petroleum ether (bp 38–49°) to opalescence. The final crystals were repeatedly washed with petroleum ether and finally dried *in vacuo* at 50°. They were stored until use at −20°.

*Block Copolymerizations.* The technique of *in situ* block copolymerization described by Gratzer and Doty (1963) was adopted for use in the present work. This procedure is preferred to that of preparing each intermediate in view of the finding that chain termination may occur during the isolation of polypeptides from polymerizing media (Mitchell *et al.*, 1957; Lundberg and Doty, 1957). A careful study of the kinetics of the copolymerizations was therefore conducted.

The copolymerizations were all carried out in dry benzene, at a monomer concentration of 2 g/dl. The initiator was *n*-hexylamine. The method of following and treating the kinetics was that of Lundberg and

* From the Department of Chemistry, Harvard University, Cambridge, Massachusetts. *Received January 10, 1966.* Portions of this work constituted part of a dissertation submitted by H. E. A. in partial fulfillment of the requirements for the degree of Doctor of Philosophy. It was supported by a research grant from the National Science Foundation (GB-1328).

† Predoctoral Fellow of the National Science Foundation (1961–1962) and the National Institutes of Health (1962–1964). Present address: Polymer Department, The Weizmann Institute of Science, Rehovoth, Israel.

[1] Abbreviations used in this paper: NCA, L-amino acid *N*-carboxyanhydride; ORD, optical rotatory dispersion.

Doty (1957). The polymerization of each block was allowed to proceed until about 95% of the monomer was exhausted, as determined from preliminary homopolymerizations. At this point the monomer for the succeeding block was added. The last block was allowed to polymerize overnight.

The kinetics of the block copolymerizations observed in this way revealed that the growth of the polymer proceeded in three stages, with approximately exponential decay of each of the first two stages. The discontinuities corresponding to the second and third stages occurred precisely at the times at which each of the NCA's intended for the second and third blocks, respectively, were added. By comparison, the kinetics of mixed-batch copolymerizations incorporating the same NCA's, in about the same proportions, revealed only a single, continuous polymerization reaction. These results are fully consistent with the expectation that such block copolymerizations yield block copolypeptides with essentially complete segregation of amino acid residues into blocks.

*Preparative Polymerizations.* Larger polymerization reactions were then carried out. The γ-benzyl ester forms of the products were precipitated by pouring the reaction mixture into a 10- to 20-fold excess of isopropyl ether. They were completely soluble in all the organic solvents used in this work. To obtain water-soluble derivatives, the products were debenzylated with gaseous hydrogen bromide (Blout and Idelson, 1956). The free acid form was collected, the benzyl bromide was removed by Soxhlet extraction with acetone, and the product was dissolved in dilute $NaHCO_3$. Whereas the random copolypeptides yielded clear solutions after this treatment, the solutions of the block copolymers were turbid. The latter preparations were clarified either by fractional precipitation at low pH values, or by ultracentrifugation of a solution brought to moderately low pH and high ionic strength in order to promote aggregation. The resulting preparations yielded clear solutions after dialysis *vs.* distilled water. They were then lyophilized for storage. Spectra of the water-soluble leucine copolymers showed that removal of the benzyl protecting groups had been complete.

*Characterization of the Products.* The molecular weights of the copolymers were obtained by means of the calibration of the intrinsic viscosity of poly-γ-benzyl-L-glutamate against its molecular weight given by Mitchell *et al.* (1957). Viscosities of solutions of the copolymers in trifluoroacetic acid or dichloroacetic acid were determined using Ubbelohde viscometers with solvent flow times of about 90 sec, thermostated at 25.33 ± 0.02°.

Consistent and reproducible determinations of amino acid composition were more readily obtained by means of acid hydrolysis of the polypeptides than by a combined determination of carboxyl groups and total nitrogen using potentiometric titration and micro-Kjeldahl analysis, respectively. The hydrolyses were carried out in sealed tubes at 106–110°, using 12 M HCl. The yields of amino acid, calibrated *vs.* norleucine added as an internal standard, were determined on a Spinco Model 120 amino acid analyzer. The results for the block copolymers were reproducible after 3–4 days of hydrolysis. The hydrolysates of the mixed-batch copolymers gave an unusual peak in the elution patterns, about 0.1 the size of the glutamic acid peaks. This peak was conclusively identified as diglutamic acid (Auer, 1965).

*Instrumental.* Ultraviolet absorption spectra were recorded on a Beckman DK-2A spectrophotometer. Samples were contained in cells of high-transmission fused silica obtained from Quaracell Products, Inc.

Fluorescence emission spectra were for the most part taken on an Amino-Bowman spectrophotofluorometer, using 1 cm × 1 cm fluorescence cuvets of fused silica. The light source was a 500-w xenon arc. The output was recorded on a Moseley Model 2D2 X-Y recorder. A few determinations were made on a Zeiss spectrofluorometer, Model ZFM 4C.[2] Calibration of the excitation and emission monochromators was carried out using phenylalanine as a standard by comparing the spectra obtained to the results of Teale and Weber (1957).

Measurements of optical rotatory dispersion (ORD) were taken with a Rudolph Model 200S photoelectric spectropolarimeter. Details are given in Auer and Doty (1966). Values of $[m']$ are always referred to content of nonpolar residues.

Spectra of circular dichroism were obtained on an apparatus constructed by Holzwarth (1965). This device uses the optical system and recorder of a Beckman DK-2A spectrophotometer, and generates circularly polarized light by means of an electrooptic quarter-wave plate. A lock-in amplifier selectively amplifies the dichroism signal, which is then recorded directly as a function of wavelength by the pen drive of the spectrophotometer.

## Results

*Physical Characterization of the Copolypeptides.* A description of the copolypeptides used in these studies is provided in Table I. It has been assumed, where necessary, that the value of $DP_w$, the weight-average degree of polymerization, found from the intrinsic viscosity of the γ-benzyl derivatives, holds also for the sodium salt forms. In every case, the composition and the value of $DP_w$ of the polymerization products were close to that expected from the conditions of polymerization. The number of nonpolar amino acid residues in the block copolymers was generally about 50; the low value given for 6LG is probably a consequence of having determined its intrinsic viscosity in dichloroacetic acid rather than in trifluoroacetic acid. For in the former, the helical sequences (see Discussion) are probably not unfolded (Fasman, 1962), so that the frictional drag of the polymer is smaller than it would be if the central portion were unfolded. Furthermore, it

[2] We wish to thank Professor G. D. Fasman and Dr. S. S. Lehrer of Brandeis University for making this instrument available to us.

TABLE I: Molecular Size and Chemical Composition of the Copolypeptides.

| Polymer[a] | Type | Intrinsic Viscosity (dl/g[b]) | Amino Acid Compn of L-Leu(L-Phe)–DL-Glu | Residues/Molecule (wt av)[c] γ-Benzyl Form L-Leu (L-Phe) | γ-Benzyl Form DL-Glu | Na Salt Form L-Leu (L-Phe) | Na Salt Form DL-Glu | $M_w$ |
|---|---|---|---|---|---|---|---|---|
| 4LG | Block | 0.324[d] | 24.6:75.4[e] | | | 51 | 157 | 29,500 |
| A4LG[f] | Block | | 23.4:76.6 | | | 46[g] | 162[g] | |
| 5LG | Mixed batch | 0.210 | 29.1:70.9 | | | 38 | 92 | 19,600 |
| 6LG | Block | 0.219 | 23.0:77.0 | 30 | 99 | | | 24,900 |
| 2PG | Block | | 23.0:77.0 | | | | | |
| 3PG | Mixed batch | 0.23 | 32.4:67.6 | | | 46 | 97 | 21,400 |
| 5PG | Block | 0.322 | 22.6:77.4 | 54 | 184 | | | 48,200 |
| 5PG, fraction 2[h] | Block | | 25.6:74.4 | | | 61[i] | 177[i] | 35,700 |

[a] L, P, and G indicate that the copolymer contains, respectively, L-leucine residues, L-phenylalanine residues, and DL-glutamate residues. [b] Determined on the γ-benzyl ester derivative in dichloroacetic acid. [c] The listings under this heading refer to the final form of the polymer studied. The numbers were obtained from the value of $DP_w$ derived from the intrinsic viscosity using the calibration of Mitchell *et al.* (1957) and the amino acid composition. It was assumed where necessary that the value of $DP_w$ found on the γ-benzyl ester derivative holds also for the sodium salt derivative. [d] Determined on the sodium salt derivative in trifluoroacetic acid. [e] Determined by combined carboxyl titration and micro-Kjeldahl nitrogen analysis. [f] Fraction of polymer 4LG obtained by precipitation between pH 4.4 and 4.0. [g] Determined on the assumption that $DP_w$ for polymer 4LG is valid for this derivative. [h] Fraction of polymer 5PG obtained from the supernatant after two ultracentrifugations, at lowered pH and raised ionic strength, at 100,000*g* for 20 min. [i] Determined on the assumption that the $DP_w$ obtained with polymer 5PG is valid for this derivative.

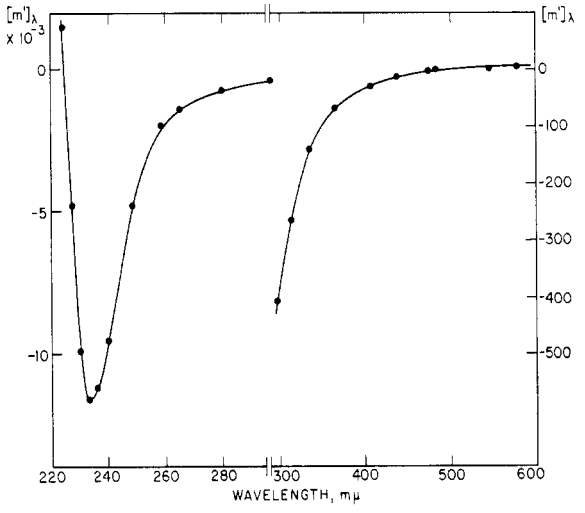


FIGURE 1: Optical rotatory dispersion of block poly-L-leucine in 0.1 M $Na_2HPO_4$. $[m']_\lambda$ is based on content of L-leucine residues.

is to be expected that the actual content of leucine in 4LG and 6LG should be similar, since the extent of incorporation of leucine residues in each, based on the amount of initiator used, was the same. For sample 2PG, the mole ratio of phenylalanine NCA to initiator was 30, while for sample 5PG this ratio was 40. In Table I it is seen that 5PG contains about 54 phenylalanine residues/polymer molecule. From the difference in the initiation ratios, we may expect that 2PG contains about 0.75 this number, or about 42 phenylalanine residues/molecule.

*Optical Rotatory Power.* The ORD values of the copolypeptides were examined from the related viewpoints of using the results both to help establish natural conformation, and to permit the tracing of changes from ordered to disordered conformations. The ORD curve of the supposed block of poly-L-leucine in 0.1 M $Na_2HPO_4$ is given in Figure 1. The trough of the Cotton effect in the far ultraviolet falls at 233 mμ, with a value for $[m']_{233}$ of about −11,500°. There is a crossover to positive rotations at 225 mμ. Analysis of the ORD curve by means of the Moffitt–Yang equation,

$$[m']_\lambda = \frac{a_0\lambda_0^2}{\lambda^2 - \lambda_0^2} + \frac{b_0\lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} \qquad (1)$$

eq 1 (Moffitt and Yang, 1956), yields a value for $b_0$ of $-467 \pm 7°$ when $\lambda_0$ is set at 212 mμ, for the wavelength range 578–296.7 mμ. These characteristics are very similar to those found for polypeptides known to be α-helical (Simmons *et al.*, 1961).

Curves of the ORD of the supposed block of poly-L-phenylalanine in water and in trifluoroacetic acid and of the mixed-batch copolymer of L-phenylalanine and DL-sodium glutamate in 0.1 M $Na_2HPO_4$ are given in Figure 2. It is seen that the curves of the supposed block polypeptide in water and in trifluoroacetic acid are

drastically different. These dispersions were analyzed according to the Moffitt–Yang equation (eq 1) with a value for $\lambda_0$ of 212 m$\mu$, and also by means of the one-term Drude equation (eq 2). The parameters $\lambda_c$ and $A$ in this equation are characteristic constants of the dispersion, and were determined by adopting the Yang–

$$[m']_\lambda = \frac{A}{\lambda^2 - \lambda_c^2} \qquad (2)$$

Doty plotting technique (Yang and Doty, 1957). The resulting values of the rotatory parameters are given in Table II.

TABLE II: Optical Rotatory Parameters of Block Poly-L-phenylalanine.

| Solvent | Moffitt–Yang Eq $b_0$ ($\lambda_0$ 212 m$\mu$) | One-Term Drude Eq $\lambda_c$ (m$\mu$) | One-Term Drude Eq $A \times 10^{-8}$ |
|---|---|---|---|
| Water | −120° | 202 ± 1 | 1.79 |
| Trifluoroacetic Acid | −380° | 243 ± 5 | −0.157 |

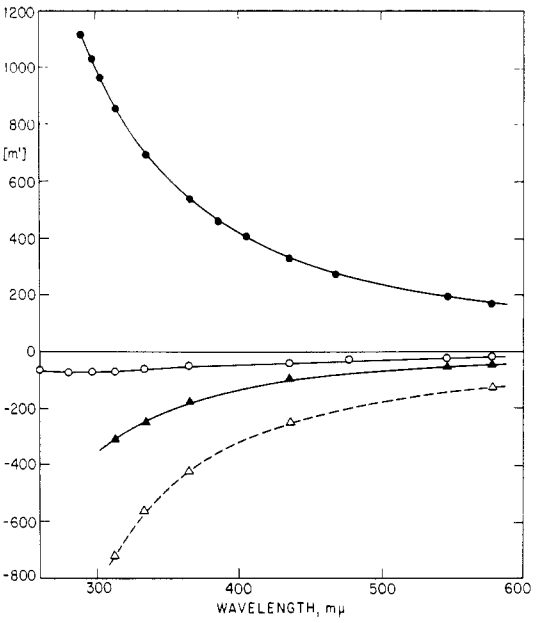


FIGURE 2: Optical rotatory dispersions of block and mixed-batch copoly-L-phenylalanine–DL-sodium glutamates. Solid curves: ●, block in water; ▲, block in trifluoroacetic acid; ○, random in 0.1 M $Na_2HPO_4$. Dashed curve: mixed-batch copoly-L-leucine–DL-sodium glutamate in trifluoroacetic acid. $[m']_\lambda$ is based on content of L-nonpolar amino acid residues.

It is concluded in the Discussion that the block synthesis yielded block copolymers. If the block of poly-L-phenylalanine is $\alpha$-helical in water and disordered in trifluoroacetic acid (see Discussion), the values of $b_0$ obtained in these two reference states are anomalous when compared to the usual values of −450 to −650° for the $\alpha$-helix and about 0° for the disordered chain (Urnes and Doty, 1961). These differences in value of $b_0$ are accompanied by marked changes in $\lambda_c$ and $A$ in the one-term Drude equation, and are clearly due to the presence of a chromophoric side chain in this polymer. Similar anomalies have been found with homopolypeptides of the other aromatic residues (see Urnes and Doty, 1961).

The circular dichroism spectra of the block polypeptides in water were obtained as a further aid in determining conformation. Ellipticity spectra could not be measured below 210 m$\mu$ for the leucine copolymer, and below 230 m$\mu$ for the phenylalanine copolymer, due to the absorption of light by the DL-sodium glutamate residues, and in the case of phenylalanine by the additional absorption of the phenyl chromophores. These factors decrease the amount of signal reaching the detector. The block of poly-L-leucine exhibits negative dichroism from 245 m$\mu$ to the limit of the observations. There is an extremum at 223 m$\mu$, with the ellipticity $[\theta]_{223}$ attaining −4.2 (±0.4) × $10^4$ deg cm$^2$ dm$^{-1}$. There is a notch at 217 m$\mu$, and then a further increase in value of $-[\theta]$, with the magnitudes of negative dichroism becoming greater than that at the extremum at 223 m$\mu$. These features are similar to those found for $\alpha$-helical polypeptides and sperm whale myoglobin by Holzwarth and Doty (1965).

The observable ellipticity spectrum of the block of poly-L-phenylalanine shows negative dichroism beginning at 245 m$\mu$ and increasing in magnitude monotonically with decreasing wavelength, to the limit of resolution. However, the magnitudes of the ellipticity in this wavelength range are smaller than for block poly-L-leucine and other nonchromophoric materials (Holzwarth and Doty, 1965). The value of $[\theta]_{240}$ is −0.13 × $10^4$ deg cm$^2$ dm$^{-1}$, and that of $[\theta]_{235}$ is −0.38 × $10^4$ deg cm$^2$ dm$^{-1}$. Although the magnitudes of $-[\theta]$ are small, the over-all shape of the observable spectrum is similar to that of nonchromophoric $\alpha$-helical polypeptides (Holzwarth and Doty, 1965).

*Absorption and Fluorescence Spectra.* Further spectroscopic studies were made on the water-soluble products of the block and mixed-batch copolymerizations incorporating L-phenylalanine. These measurements were designed to establish a structural difference between the two copolymers. Then, after the conformational basis of this distinction would be made clear by independent considerations, the dependence of the optical phenomena on conformation could be examined.

The absorption spectrum of the block of poly-L-phenylalanine was compared with that of the mixed-batch copolymer in the region of the symmetry-forbidden $\pi \rightarrow \pi^*$ transition at 260 m$\mu$. There was a

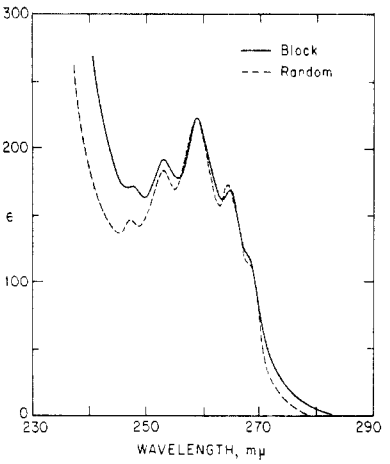


FIGURE 3: Absorption spectrum of block and mixed-batch copoly-L-phenylalanine–DL-sodium glutamates in water.———, block copolymer after correction for scattering background; – – –, mixed-batch copolymer.

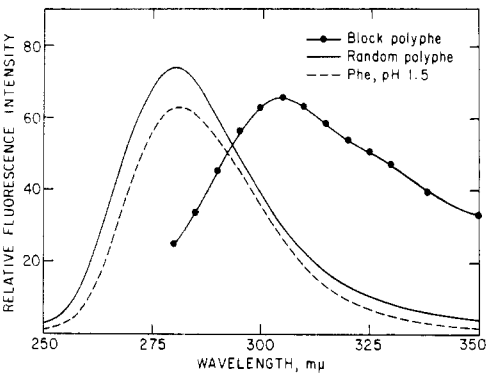


FIGURE 4: Fluorescence emission spectra of L-phenylalanine (——), and of block (—●—) and mixed-batch (———) copoly-L-phenylalanine–DL-sodium glutamates in water. The block copolymer was excited at 265 mμ, the others at 261 mμ The intensities are arbitrary and are not meant to refer one curve to another.

fairly pronounced scattering background in the spectrum of the block copolymer, amounting to about 20% of the absorption at 259 mμ. This was corrected most readily by a simple extrapolation of the scattering base line of the recorded spectrum itself, using a large French curve. The resulting spectrum, and that of the mixed-batch L-phenylalanine copolymer, which required no scattering correction, are shown in Figure 3. The curve for the block of poly-L-phenylalanine is the mean of three different spectra and their associated extrapolations. The molar extinction coefficient at the 259 mμ peak is 223 l. mole$^{-1}$ cm$^{-1}$ ±0.57%, and that for the mixed-batch L-phenylalanine copolymer, the mean of two recorded spectra, is 224 l. mole$^{-1}$ cm$^{-1}$ ±1.0%. These values, and the close over-all similarity between the two curves, show that there is no observable distinction between the absorption properties of the two polymers in this region.

The fluorescence emission spectra of L-phenylalanine at pH 1.5 and of aqueous solutions of the mixed-batch and block copolymers containing L-phenylalanine are presented in Figure 4. The relative fluorescence intensity is arbitrary in each spectrum, and is not meant to relate one spectrum to another. The excitation wavelengths were 261 mμ for the curves of L-phenylalanine and the mixed-batch copolymer, and 265 mμ for the curve of the block copolymer.

The emission spectra of phenylalanine and the mixed-batch copolymer are essentially identical. The wavelength of maximum emission, $\lambda_{max}$, is 282 mμ for the former and 280 mμ for the latter. The wavelength of half the maximum emission intensity on the long-wave side, $\lambda_+$, is 303 mμ for L-phenylalanine and 301 mμ for the mixed-batch copolymer, both indicative of a fairly narrow emission band. The slight broadness of the band for the mixed-batch copolymer on the short-wave side is due to the concealed scatter peak from the excitation at 261 mμ. The emission spectrum of the block copolymer, however, differs considerably from the two spectra just considered. The emission is red shifted and considerably broadened, with $\lambda_{max}$ at about 305 mμ and $\lambda_+$ at 350 mμ. The emission is still finite at 450 mμ.

These features are characteristic of emission which has recently been found with a number of low molecular weight substances at very high concentrations, in contrast to their emission spectra in dilute solution. Since the absorption spectra in these cases remain unchanged, it has been postulated that an excited dimer, or excimer, comprising one excited chromophore and one ground-state chromophore, is formed (Stevens and Hutton, 1960). Fluorescence emission characteristic of excimer formation has also been found in isotactic polystyrene (Yanari *et al.*, 1963) and in α-helical poly-L-tyrosine and (presumably helical) poly-L-tryptophan (Lehrer and Fasman, 1964). The present results suggest that in the product of the block polymerization, the side chains have distinct orientational correlations of sufficient extent and duration to permit excimer formation to occur. On the other hand, the side chains in the mixed-batch copolymer are relatively disoriented such that the fluorescence emission is virtually identical with that of a dilute solution of L-phenylalanine.

## Discussion

*Block Copolypeptides.* In order to show that the products of block copolymerization are indeed block copolypeptides, we note first that these preparations are completely soluble in both organic solvents and in water. This indicates that there was essentially no homopolymerization of L-leucine NCA or L-phenylalanine NCA, because such homopolymers are insoluble in both organic and aqueous media. Therefore, the nonpolar amino acids were incorporated into copolymers with DL-glutamate.

Evidence for formation of true block copolymers is drawn first from a study of optical rotatory power. A comparison of the ORD curves of the products of the block and mixed-batch copolymerizations for each of the nonpolar amino acids reveals marked differences between the two. In the case of the leucine copolypeptides, treatment of the ORD curves by means of the Moffitt–Yang equation reveals that the leucine residues in the supposed block copolymer were fully helical in 0.1 M $Na_2HPO_4$. In the mixed-batch copolymer, on the other hand, they were 50% disordered in the same medium (Auer and Doty, 1966). The phenylalanine copolymers also exhibited markedly different ORD curves, which however are not amenable to conventional analysis by means of the Moffitt–Yang equation, as we have seen. In water, the supposed block copolymer has a strongly positive ORD curve, whereas the mixed-batch copolymer has a shallow negative dispersion with a minimum in the region of 300 mμ. From these considerations, it is seen that the technique of block copolymerization yields copolymers whose structures, as reflected by their optical rotatory properties, are quite different from those of copolymers which are intentionally of mixed sequence. These observations are thus consistent with the formation of true blocks of nonpolar amino acids in the products of block copolymerization.

Second, the supposed block copolymer of L-phenylalanine displays a fluorescence emission spectrum indicative of excimer formation, a phenomenon which is characteristic of systems containing ordered chromophores. The mixed-batch copolymer, however, yields a fluorescence spectrum which is virtually identical with that of free L-phenylalanine. This evidence indicates that the phenylalanine side chains in the supposed block copolymer, but not in the random copolymer, are ordered sufficiently to permit electronic interactions between them. The distinction may be attributed to the presence of true blocks in the former.

Finally, from the kinetics of block *vs.* random copolymerization, it was shown in the Experimental Section that essentially blocklike addition of monomer proceeds in the former synthesis, but not in the latter. The three items just reviewed clearly suggest that block copolymerization is successfully accomplished. We may, therefore, proceed safely in the knowledge that the blocks intended actually do exist. The possibility of imperfect junctions between blocks is not obviated, however.

*α-Helical Conformation.* The next step is to show that the conformation assumed by the nonpolar L-amino acid residues in the central blocks of the copolymers is the α-helix. We begin with block poly-L-leucine, by noting that high molecular weight preparations of this material are α-helical both in the solid state and in mild organic solvents (Bamford *et al.*, 1956; Blout *et al.*, 1960; Brandt and Budrys, 1964; Downie *et al.*, 1957; Fasman, 1962). The optical rotatory power of block poly-L-leucine indicates that it too is α-helical. First, we found that the trough of the Cotton effect and the value of $b_0$ are both similar to those of known α-helical polypeptides. [The values of both $-[m']_{233}$ and $-b_0$ are slightly lower in magnitude than are those of other polypeptides (Simmons *et al.*, 1961; Blout *et al.*, 1962; Urnes and Doty, 1961). The reason for this is unclear.]

Second, further similarities between the optical rotatory properties of block poly-L-leucine and those of known helical polypeptides are revealed by comparing the best values of $\lambda_0$ in the Moffitt–Yang equation for a given wavelength range as determined by the statistical method of Sogami *et al.* (1963). These are presented in Table III. It is seen that there is close agreement between the values of $\lambda_0$ for block poly-L-leucine and in the other polypeptides for each wavelength range. This is a good indication that the optical rotatory properties of all these polypeptides have a common origin in the helical disposition of the polypeptide backbone.

Third, it was noted above that the circular dichroism spectrum of block poly-L-leucine contains features closely resembling those found for α-helical polypeptides. Finally, the infrared spectra of gelled preparations of the block copolymer in a $D_2O$–dioxane solvent contains a band, at 1542 $cm^{-1}$, assigned to the poly-L-leucine block (Auer and Doty, 1966). The position of this band is characteristic of the amide II transition when the polypeptide backbone is in the α-helical conformation (Bamford *et al.*, 1956; Blout *et al.*, 1960).

In summary, the evidence from the ORD and its analysis by the Moffitt–Yang equation, the circular dichroism, and the infrared spectra clearly demonstrate that block poly-L-leucine is α-helical in aqueous medium (and in nonpolar organic solvents (Auer and Doty, 1966). It is clear that L-leucine residues tend to fold into the α-helix spontaneously. Therefore, in the mixed-batch copolymer they may be expected to partition themselves between helical and disordered states.

We now turn to the proposition that the conformation of block poly-L-phenylalanine is α-helical. To begin with, there is evidence that high molecular weight poly-DL-phenylalanine contains a significant proportion of α-helical residues in films and in chloroform containing 0.5% dichloroacetic acid or formamide (Horn *et al.*, 1959; Lapp and Marchal, 1958). This would arise from the apparent presence in racemic copolypeptides of long sequences of high optical purity (Tsuboi *et al.*, 1963). Block poly-L-phenylalanine in 1:1 $D_2O$–dioxane yields an infrared spectrum with an amide II band at 1540 $cm^{-1}$ (Auer and Doty, 1965). The intensity of this band corresponds well with the proportion of L-phenylalanine residues in the block copolypeptide and its position, which is similar to that found with block poly-L-leucine, agrees well with that expected for the protonated amide group when hydrogen bonded in the α-helix.

The circular dichroism spectrum of block poly-L-phenylalanine, in the limited range observed, is similar to that for α-helical polypeptides, but has somewhat lower magnitudes. This situation has also been observed in poly-L-tyrosine, another aromatic polypeptide, in the region 250–220 mμ (Beychok and Fas-

TABLE III: Best Values of $\lambda_0$ for Some $\alpha$-Helical Polypeptides.

| Polymer[a] | Region I | | Region II | | Regions I + II | |
|---|---|---|---|---|---|---|
| | Wavelength Range (mμ) | $\lambda_0$ (mμ) | Wavelength Range (mμ) | $\lambda_0$ (mμ) | Wavelength Range (mμ) | $\lambda_0$ (mμ) |
| PALGA[b] | 242–313 | 220 | 300–589 | 212 | 242–589 | 218 |
| PGA[b] | 244–320 | 220 | 300–589 | 214 | 244–589 | 219 |
| PTGA[b] | 240–320 | 219 | 300–620 | 210 | 240–620 | 218 |
| PLy[c] | | | 296.7–546 | 212 | 240–546 | 218 |
| PGA[c] | | | | | 240–546 | 218 |
| PBLG[c,d] | | | 296.7–546 | 212 | 248–546 | 219 |
| PBLG[c,e] | | | 296.7–546 | 210 | 248–546 | 216 |
| PLu | 240–296.7 | 219 | 296.7–578 | 215 | 240–578 | 218 |

[a] PALGA, mixed-batch copoly-L-alanine–L-lysine–L-glutamic acid (30:28:42); PGA, poly-L-glutamic acid; PTGA, mixed-batch copoly-L-tyrosine–L-glutamic acid (4:96), PLy, poly-L-lysine; PBLG, poly-$\gamma$-benzyl-L-glutamate; PLu block poly-L-leucine. [b] Urnes (1963). [c] Leonard and Foster (1963). [d] Ethylene chloride solution. [e] Dioxane solution.

man, 1964). The negative dichroism found in this wavelength range was assigned to the $n \rightarrow \pi^*$ transition of the helical amide groups. The single band observed with poly-L-tyrosine has smaller magnitudes of ellipticity, and an extremum at a higher wavelength, than have nonchromophoric helical polypeptides. These deviations were attributed to optically active transitions of the phenolic side chain. In view of the anomalous circular dichroism displayed by poly-L-tyrosine, it appears that the circular dichroism of block poly-L-phenylalanine may be consistent with the existence of the $\alpha$-helical conformation.

Thus from the apparent helical conformation in poly-DL-phenylalanine, and the present evidence from infrared and circular dichroic spectra, it may be concluded that block poly-L-phenylalanine is most probably $\alpha$-helical in aqueous solution and in favorable organic solvents.

*Optical Properties.* Electronic interactions between chromophores held in fixed array are known to occur in many polymer systems. They are frequently dependent on conformation. A comparison of the optical properties of phenylalanine residues in the helical and disordered states can be made from this point of view. First we consider the optical rotatory power of these materials. The ORD of block poly-L-phenylalanine in water is drastically different from that which it displays in trifluoroacetic acid (Figure 2, Table II). It is found (Auer and Doty, 1966) that save for a solvent effect on the optical rotation, the change from one form of rotation to the other is sudden, and may be taken to represent a helix–random coil transition. Thus, in trifluoroacetic acid, block poly-L-phenylalanine is disordered. The pronounced changes in the rotatory power of block poly-L-phenylalanine in the two solvents considered are due to the disruption of the helical conformation by trifluoroacetic acid. They are quite drastic and clearly reflect the effects of changes in the juxtaposition of the side chain chromophore, over and above the changes in rotatory power due to the amide chromophores in the polypeptide backbone. There are apparently strong interactions between the phenyl chromophores in the $\alpha$-helix which are absent when this conformation is disrupted.

It is seen in Figure 2 that the ORD of mixed-batch copoly-L-phenylalanine–DL-sodium glutamate in water lies close to that of the block polypeptide in trifluoroacetic acid, in which the block is disordered. Therefore the phenylalanine residues in the mixed-batch coplymer are also largely disordered. A comparison of the absorption spectrum of the block polypeptide with that of the mixed-batch copolymer revealed that both the position and intensity of the $\pi \rightarrow \pi^*$ transition at about 260 mμ were identical for the two copolymers; that is, neither hypochromicity nor exciton splitting is observed in the helical polypeptide. Thus one must conclude that both the energy and dipole strength remain unchanged when the helical conformation is disrupted.

It is of interest to inquire at this point whether there is interaction of the magnetic moments of this lowest $\pi \rightarrow \pi^*$ transition when the chromophores are arrayed along the $\alpha$-helix. The pronounced changes in the optical rotation at higher wavelengths indicate that there are such interactions involving the transitions at higher energies. The circular dichroism spectrum, it should be noted, revealed no observable ellipticity bands between 280 and 250 mμ. Preliminary recordings of the optical rotatory dispersion of block poly-L-phenylalanine, however, have revealed the presence of small Cotton effects in this region, of the order of 100° from peak to trough on a scale of reduced molar rotation. (The optical rotatory dispersion of the random copolymer in this region was not examined.) These preliminary results show that the magnetic transition moments of the phenyl chromophores are capable of mutual interaction.

It may be expected that in proteins also this magnetic moment may, under appropriate conditions, inter-

act with its environment to produce a nonvanishing rotatory strength, and to undergo changes in magnitude if the environment should change. The Cotton effects in this region may consequently be sensitive to local changes of conformation in proteins.

Also, the fluorescence emission spectrum of block poly-L-phenylalanine was found to be characteristic of excimer interaction between the chromophores in this polymer. Thus it is possible that aromatic chromophores in proteins may be suitably arrayed for excimer formation to occur. In this respect, the emission characteristics of these chromophores would probably be altered making available new avenues for energy transfer in such biological systems.

In conclusion, we have shown that the proper control of conditions, the technique of *in situ* copolymerizations of NCA's initiated by a primary amine yields products with a high degree of segregation of amino acid residues into blocks. In the present investigation, block polymers were employed to obtain the information that blocks of poly-L-leucine and poly-L-phenylalanine are $\alpha$-helical in solution. This knowledge is a prerequisite to the study of the conformational stability of these materials. An investigation of selected optical properties of L-phenylalanine residues in block and random copolypeptides revealed that certain phenomena, such as the fluorescence emission and the ORD, are strongly dependent on conformation. It has already been found that this is true for other aromatic amino acid residues both in polypeptides and in proteins. The observation of a similar dependence on structure with L-phenylalanine extends the usefulness of this technique to a broader spectrum of local environments in protein molecules.

## Note Added in Proof

The optical properties of a block copolymer of L-phenylalanine are discussed by Sage and Fasman (1966) and are in essential agreement with those reported here.

## References


Auer, H. E. (1965), Ph.D. Thesis, Harvard University, Cambridge, Mass.

Auer, H. E., and Doty, P. (1966), *Biochemistry 5*, 1716 (following article).

Bamford, C. H., Elliott, A., and Hanby, W. E. (1956), Synthetic Polypeptides, New York, N. Y., Academic, pp 155, 157.

Beychok, S., and Fasman, G. D. (1964), *Biochemistry 3*, 1675.

Blout, E. R., de Lozé, C., Bloom, S. M., and Fasman, G. D. (1960), *J. Am. Chem. Soc. 82*, 3787.

Blout, E. R., and Idelson, M. (1956), *J. Am. Chem. Soc. 78*, 497.

Blout, E. R., Schmier, I., and Simmons, N. S. (1962), *J. Am. Chem. Soc. 84*, 3193.

Brandt, W. W., and Budrys, R. S. (1964), *J. Biol. Chem. 239*, 1442.

Downie, A. R., Elliott, A., Hanby, W. E., and Malcolm, B. R. (1957), *Proc. Roy. Soc. (London) A242*, 325.

Fasman, G. (1962), Proceedings of the International Symposium, Polyamino Acids, Polypeptides, and Proteins, Madison, Wis., 1961, p 221.

Gratzer, W. B., and Doty, P. (1963), *J. Am. Chem. Soc. 85*, 1193.

Holzwarth, G. (1965), *Rev. Sci. Instr. 36*, 59.

Holzwarth, G., and Doty, P. (1965), *J. Am. Chem. Soc. 87*, 218.

Horn, P., Marchal, J., and Lapp, C. (1959), *Compt. Rend. 248*, 233.

Lapp, C., and Marchal, J. (1958), *Compt. Rend. 247*, 86.

Lehrer, S. S., and Fasman, G. D. (1964), *Biopolymers 2*, 199.

Leonard, W. J., Jr., and Foster, J. F. (1963), *J. Mol. Biol. 7*, 590.

Lundberg, R. D., and Doty, P. (1957), *J. Am. Chem. Soc. 79*, 3961.

Mitchell, J. C., Woodward, A. E., and Doty, P. (1957), *J. Am. Chem. Soc. 79*, 3955.

Moffitt, W., and Yang, J. T. (1956), *Proc. Natl. Acad. Sci. U. S. 42*, 596.

Sage, H. J., and Fasman, G. D., (1966), *Biochemistry 5*, 286.

Simmons, N. S., Cohen, C., Szent-Gyorgyi, A. G., Wetlaufer, D. B., and Blout, E. R. (1961), *J. Am. Chem. Soc. 83*, 4766.

Sogami, M., Leonard, W. J., Jr., and Foster, J. F. (1963), *Arch. Biochem. Biophys. 100*, 260.

Stevens, B., and Hutton, E. (1960), *Nature 186*, 1045.

Teale, F. W. J., and Weber, G. (1957), *Biochem. J. 65*, 476.

Tsuboi, M., Mitsui, Y., Wada, A., Miyazawa, T., and Nagashima, N. (1963), *Biopolymers 1*, 297.

Urnes, P. J. (1963), Ph.D. Thesis, Harvard University, Cambridge, Mass.

Urnes, P., and Doty, P. (1961), *Advan. Protein Chem. 16*, 401.

Yanari, S. S., Bovey, F. A., and Lumry, R. (1963), *Nature 200*, 242.

Yang, J. T., and Doty, P. (1957), *J. Am. Chem. Soc. 79*, 761.