

Helix-Coil Transition of Poly-L-ornithine in Solution*

Satya R. Chaudhuri and Jen Tsi Yang

ABSTRACT: Optical rotatory dispersion and circular dichroism of poly-L-ornithine (PO) in aqueous solution revealed a low helical content of about 20% even at pH above 11, when the side groups of the polymer were un-ionized. The corresponding intrinsic viscosity decreased gradually with increasing pH, suggesting that the polymer contracted with deprotonation. The striking contrast between PO and poly-L-lysine,

which has one more methylene group in each residue and is completely helical at pH above 11, is attributed largely to hydrophobic interactions among the side chains. Addition of methanol or dioxane to the aqueous solution increased the helical content. In 40% methanol (v/v) a sharp helix-coil transition occurred near pH 9.5 (apparent), and above 40% methanol at pH 11.7 (apparent) the polypeptide was essentially helical.

The helix-coil transition of synthetic polypeptides in solution has been extensively investigated (for reviews, see Urnes and Doty, 1961; Katchalski *et al.*, 1964; Schellman and Schellman, 1964). However, the effect of side chains on the stability of helical structures is not yet well understood. The side chains have been found to play a significant role in determining the conformation of poly- α -amino acids in solution. For example, PGA¹ is more helical than poly-L-aspartic acid when conditions are comparable (Brahms and Spach, 1963; Jacobson, 1965). Again, poly-L-alanine, which has a CH₃ group as side chain, can form the α helix, whereas polyglycine, which has no side chain, cannot form an α -helical structure (Gratzer and Doty, 1963; Bixon *et al.*, 1963; Schellman, 1955). The helical structure of the copolymers of L-glutamic acid-L-leucine and L-glutamic acid-L-phenylalanine is more stable against temperature than that of the corresponding homopolymer, PGA (Fasman *et al.*, 1964; Sage and Fasman, 1966). We have tried to determine the influence of the side chains on the helix-coil transition of PO as compared to that of PL, which has one more methylene group per residue in the side chain.

Experimental Procedures

Materials. The polypeptides, PO-HBr (lots 0-8 and 0-10) and PL-HBr (lot L-62) were purchased from Pilot Chemicals, Watertown, Mass. Lots 0-10 and L-62 were dissolved in water and dialyzed first against 0.01 N HCl (to convert the hydrobromide into the hydrochloride) and then against water ex-

haustively to remove excess chloride ions. Lot 0-8 was dialyzed only against water. Our experiments were done with lot 0-10 unless stated otherwise. The concentrations of the polypeptide solutions were determined by the micro-Kjeldahl nitrogen analysis. Methanol and *p*-dioxane were of spectroquality; all other chemicals were reagent grade.

Methods. Viscosities were measured at 25.0° in a Ubbelohde-type viscometer with a flow time of more than 100 sec for water. Sedimentation velocities were determined with a Spinco Model E ultracentrifuge at 59,780 rpm, using the schlieren optics.

The molecular weight (*M*) of PO was estimated from the Flory equation (Mandelkern and Flory, 1952), $M^{2/3} = Ns^0[\eta]^{1/3}\eta_0/\Phi^{1/3}p^{-1}(1 - \bar{v}\rho)$, where *N* is Avogadro's number, *s*⁰ sedimentation coefficient, $[\eta]$ the intrinsic viscosity, \bar{v} the partial specific volume of the polymer, ρ the solvent density, and $\Phi^{1/3}p^{-1}$ a constant whose experimental value is 2.5×10^6 . The $[\eta]$ of PO in 0.2 M NaCl was 2.31 dl/g for lot 0-8 and 2.46 dl/g for lot 0-10, and the corresponding *s*⁰ values were 3.57 and 2.50 S. Taking $\bar{v} = 0.781$ ml/g (Appelquist and Doty, 1962), we found the molecular weights of the two PO samples to be 335,000 (lot 0-8) and 202,000 (lot 0-10).

Optical rotatory dispersion was measured with a Cary 60 spectropolarimeter at 25°, using cells of path lengths 0.1, 1, and 100 mm. The concentration of each polypeptide was about 0.2%. The rotations were expressed in terms of mean residue rotation, $[m]$, or reduced mean residue rotation, $[m']$, in deg cm²/dmole. $[m']$ equals $[m]$ multiplied by $3/(n^2 + 2)$. We assumed the refractive index, *n*, of the methanol-water mixture to be the same as that of pure water. The visible rotatory dispersion between 300 and 600 m μ was fitted with the Moffitt equation with λ_0 preset at 212 m μ (Moffitt and Yang, 1956).

Circular dichroism was measured with a Jasco ORD-CD-UV-5 at 25°, using cells of path lengths of 0.1, 0.5, and 10 mm. The concentration of the polymers was again about 0.2%. The data were expressed in

* From the Cardiovascular Research Institute and the Department of Biochemistry, University of California San Francisco Medical Center, San Francisco, California 94122. Received June 1, 1967. This work was aided by grants from the U. S. Public Health Service (HE-06285, GM-10880, and K3-GM-3441).

¹ Abbreviations used that are not listed in *Biochemistry* 5, 1445 (1966) are: PO, poly-L-ornithine; PL, poly-L-lysine; PGA, poly-L-glutamic acid.

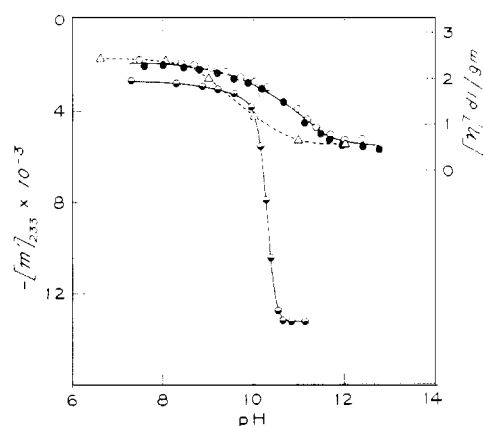


FIGURE 1: Intrinsic viscosity of poly-L-ornithine in 0.2 M NaCl and optical rotation of poly-L-ornithine and poly-L-lysine in 0.2 M KCl. $[\eta]$: Δ , PO (lot 0-8). $[m]_{233}$: \bullet , PL; \circ , PO (lot 0-8); \bullet , PO (lot 0-10).

terms of mean residue ellipticity, $[\theta]$, in $\text{deg cm}^2/\text{dmole}$. $[\theta]$ is related to the difference in molar absorptivity of the left and right circularly polarized components $\Delta\epsilon$ by $[\theta] = 3300\Delta\epsilon$.

The pH of the solutions was measured with a Radiometer pH meter 25 equipped with a scale expander, using a G-222 B glass electrode and a K-130 calomel electrode. Solutions of PO and PL in 0.2 M KCl were titrated in a glass vessel in a water jacket, which was attached to a constant-temperature bath at $25.0 \pm 0.1^\circ$, under nitrogen flush, which had been saturated with the solvent vapor. Standard KOH was added from a Gilmont ultramicroburet. The activity coefficient of the hydroxyl ions, which was required for the calculation of the degree of neutralization, was determined by measuring the pH of the solvent containing a known concentration of KOH.

Results

pH-Induced Transition. A helix-coil transition can be brought about in some polypeptides, such as PGA and PL, by changing the degree of ionization of the side groups. Such a change of conformation will change the hydrodynamic and the optical properties of the polymers. Figure 1 shows the variation of intrinsic viscosity and optical rotation of two PO samples with pH; the rotation of one PL sample was included for comparison. (The appearance of a trough at $233 \text{ m}\mu$ indicates the presence of the helical structure; its magnitude increases with the helical content; cf. Figure 5a.) PO seemed to be predominantly in the coiled form and its conformation changed very little even at pH 12.8. In contrast, PL underwent a sharp coil-to-helix transition near pH 10.5. Similarly, the $-b_0$ of the Moffitt equation (Moffitt and Yang, 1956) for PO (not shown here) increased from close to zero at neutral pH to about 120 above pH 12. We used the empirical equations (Yang, 1967): fraction of helix $= -([m]_{233} + 2000)/13,000$ and fraction of helix $= -b_0/630$, and found that the helical content of PO was only about 25% even at pH 12, where most of the

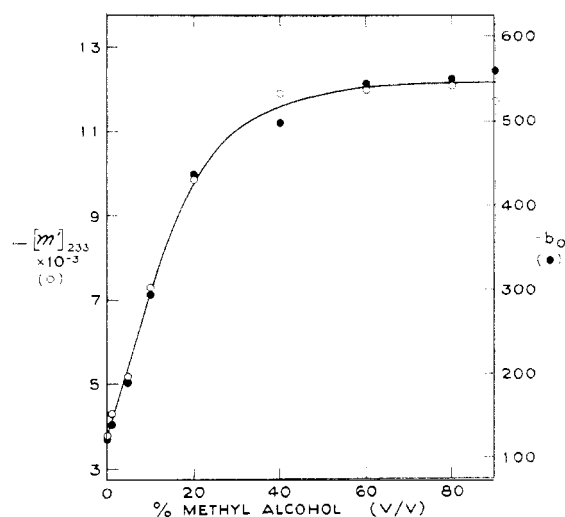


FIGURE 2: Helix-coil transition of poly-L-ornithine in methanol-water mixtures at pH 11.7 (apparent).

NH_3^+ groups in the side chains become un-ionized (see Titration of PO, below), whereas PL was almost completely helical at pH 10.5. Our estimate of the helical content of PO agrees well with that reported by Blauer and Alfassi (1967), who based their estimate on the b_0 parameter only. Those authors used a PO sample having a degree of polymerization of 100, whereas the degree of polymerization of our samples was more than 1000. The viscosity data in Figure 1 seem to support the contention that only partial helices are present in PO at high pH, since either the coiled form or the perfect helix would have a higher intrinsic viscosity than a partial helix (Doty *et al.*, 1957).

Solvent-Induced Transition. Addition of nonaqueous solvents such as methanol and dioxane to the aqueous solution of PO should stabilize the helical conformation. Figure 2 shows the helix-coil transition of PO in methanol-water mixture at pH 11.7 (apparent). The transition was complete at about 40% methanol (v/v) and further addition of methanol did not change the optical parameters very much. The b_0 and $[m]_{233}$ of PO reached plateaus at about -550 and $-12,000$, respectively; corresponding values in water alone (pH 11.7) were -120 and -3800 . The amount of maximum helical content of PO in methanol-water was estimated to be 80-90%.

Figure 3 illustrates the variation of $[m]_{233}$ and $[\theta]_{222.5}$ (the circular dichroism minimum characteristic of a helix) with pH for PO in 40% methanol. A sharp transition occurred near pH 9.5 (apparent), in marked contrast to the behavior in aqueous solution (Figure 1). Using the empirical equation (Yang, 1967) fraction of helix $= (4000 - [\theta]_{222.5})/42,000$, we estimated from the circular dichroism a maximum helical content of about 90% for PO in methanol-water. This value is in good accord with that estimated from the $233\text{-m}\mu$ trough of optical rotatory dispersion.

We also studied the effect of dioxane on the helix-coil transition of PO in solution. Again there was a sharp transition near pH 9.5 (apparent) (Figure 4) for PO in 50% dioxane (v/v). The magnitude of $[m]_{233}$

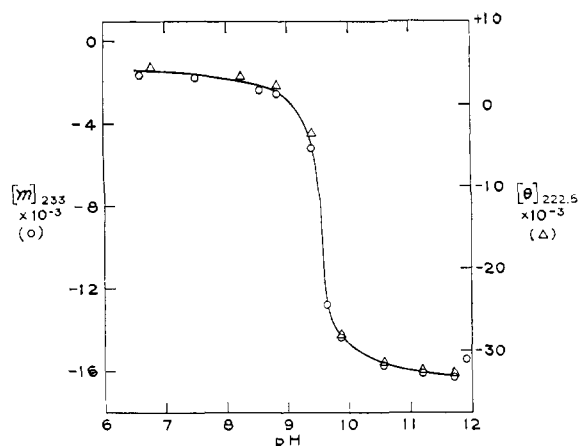


FIGURE 3: Helix-coil transition of poly-L-ornithine in 40% methanol as a function of pH.

and $[\theta]_{222.5}$ at high pH suggests the formation of the same amount of helical content in dioxane-water and methanol-water (40:60, v/v) mixtures (*cf.* Figure 3). One notable difference between the two mixtures was that PO (0.2%) in 50% dioxane began to precipitate at pH 9.8 (apparent) but redissolved on further addition of alkali (above pH 11.5) (see the dashed line in Figure 4).

The profiles of ultraviolet rotatory dispersion and circular dichroism of PO in the different solvents and at different pH (Figure 5a,b) values substantiate the occurrence of a helix-coil transition. As is evident from Figure 5a, the aqueous solution of PO at neutral pH (curve 1) exhibited a negative trough at 205 $m\mu$ characteristic of a random coil. Raising the pH to 11.7 decreased the magnitude of the trough accompanied by a red shift, and a very shallow trough around 233 $m\mu$ appeared (curve 2). The optical rotatory dispersion at pH 11.7 resembles that of a mixture of coiled and helical conformations. In 40% methanol at pH 11.7 (apparent) (curve 3), however, the polymer exhibited the distinctive 233- $m\mu$ trough and 198- $m\mu$ peak typical of a helical structure (Blout *et al.*, 1962; Yang, 1967). The Cotton effects of PO in 50% dioxane at pH 11.7 (apparent) (curve 4) nearly coincided with those in methanol-water mixture (curve 3), suggesting that PO has nearly the same amount of helical content in both mixtures.

The circular dichroism profiles in Figure 5b reflect the same conformational changes as those observed in Figure 5a. The coiled form (curve 1) had a large negative circular dichroic band below 210 $m\mu$, a small positive one around 215 $m\mu$ (Holzwarth and Doty, 1965; Yang, 1967), and another much smaller negative one near 243–244 $m\mu$. The magnitude of this last band was so small (see the insert) that it could only be detected by increasing the concentration of the polypeptide, by using cells of longer path length, or both. This band has recently been reported to be present in the coiled form of poly-L-glutamic acid with a minimum at 238 $m\mu$ (Carver *et al.*, 1966; Yang, 1967). Raising the pH of the solution depressed the positive 215- $m\mu$ band and at pH 11.7 (curve 2) a small

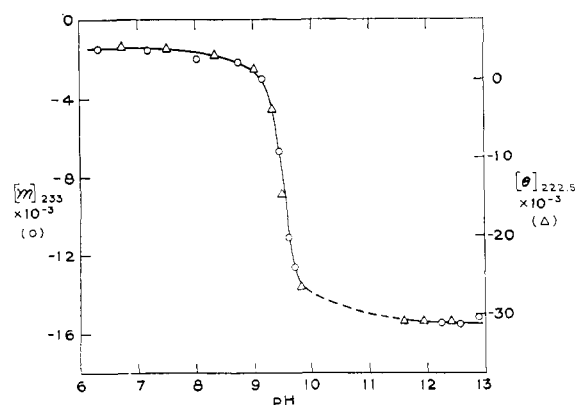


FIGURE 4: Helix-coil transition of poly-L-ornithine in 50% dioxane as a function of pH.

negative band appeared at 222.5 $m\mu$, as would be expected of a mixture of coiled and helical forms. In 40% methanol at pH 11.7 the circular dichroism clearly revealed a double minimum at 222.5 and 209 $m\mu$, both of which are typical of a helical conformation (Holzwarth and Doty, 1965). The same was true for PO in 50% dioxane at pH 11.7 (curve 4). These findings are in close agreement with the conclusions drawn from Figure 5a.

Figure 6 summarizes the visible rotatory dispersion of PO in solution (between 300 and 600 $m\mu$). The spectra are featureless. The coiled form (curve 1) was the more levorotatory and the levorotation decreased as helical content increased. In 50% dioxane at pH 11.7 (curve 4), helical content was so high that PO actually became dextrorotatory above 500 $m\mu$.

Titration of PO. The intrinsic dissociation constants pK_i of the NH_3^+ group in PO and in PL are expected to be similar. However, to assure that our PO sample was not highly charged at pH 12 and thereby unable to complete the coil-to-helix transition at that pH, we performed titration experiments for our PO and PL samples in 0.2 M KCl. (We acknowledge the referees' suggestion for this experiment.) Figure 7 shows a plot of pK_{app} ($=pH - \log(\alpha/(1 - \alpha))$) *vs.* the degree of dissociation (α) (Wada, 1960; Zimm and Rice, 1960;

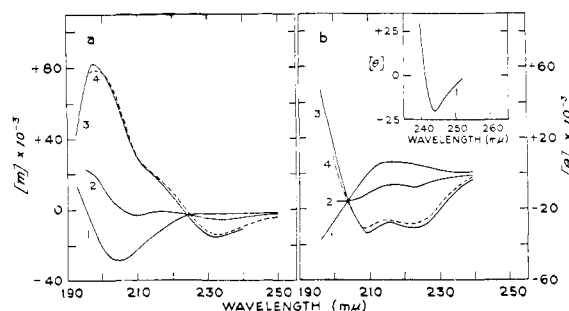


FIGURE 5: Poly-L-ornithine studies. (a) Ultraviolet rotatory dispersion of poly-L-ornithine in different solvents and at different pH values. (b) Circular dichroism of poly-L-ornithine in different solvents and at different pH values. Curve 1: in water (pH 6.6); curve 2: in water (pH 11.7); curve 3: in 40% methanol (pH_{app} 11.7); curve 4: in 50% dioxane (pH_{app} 11.7).

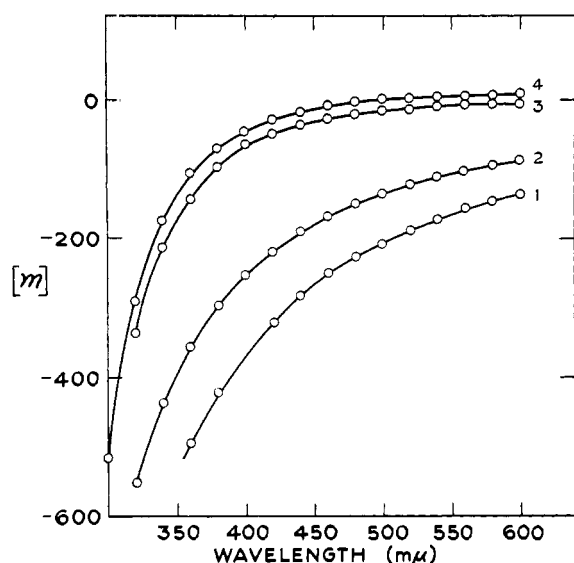


FIGURE 6: Visible rotatory dispersion of poly-L-ornithine in different solvents and at different pH values. Curves 1-4: same as in Figure 5.

Nagasawa and Holtzer, 1964). In the insert we show the pH vs. α curves. The two PO samples have almost identical curves and the curves between PO and PL show very small differences; at any given value of α , the pH of the PL solution is slightly higher than that of the PO solution, or, to put it in another way, at any given pH PO is slightly less charged than PL. Following Wada's suggestion (Wada, 1960), we can divide the titration curve into three regions: I represents the coiled form and III the helical form, whereas II is the helix-coil transition zone. For PL the transition occurs in the region of $\alpha = 0.5-0.8$, but for PO it starts at about $\alpha = 0.7$. Region III, characteristic of a complete helix, is absent in PO even at an α as high as 0.9. Extrapolation of the data for both polypeptides gave a pK_i of 10.4 for PL and 10.1 for PO (the latter value might be slightly underestimated because of the difficulties in extrapolation).

Discussion

The free energy of formation associated with coil-to-helix transition of polypeptides in aqueous solution is slightly negative (Kauzmann, 1959) and therefore the α -helix should have only a marginal stability. Changes such as introduction of charges of the same sign to the side groups can easily break up the helices. Such is the case for PGA and PL at neutral pH. Elimination of the electrostatic repulsion, however, is not sufficient to stabilize the helix of PO, *e.g.*, at pH 12, suggesting that the standard free-energy change for the formation of PO helix must be less negative than that of PL helix.

In mixed solvents, however, PO undergoes a sharp transition when the electrostatic repulsion is minimized (Figure 3). The use of mixtures of water and organic solvents can influence the helical stability in two ways. First, in a less polar medium the pK of the ionizable side

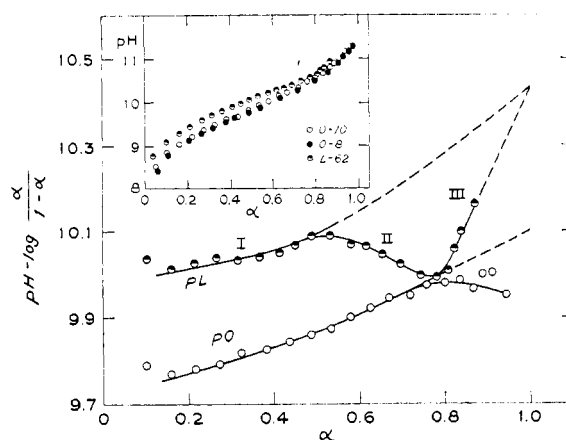


FIGURE 7: Titration of poly-L-ornithine and poly-L-lysine in 0.2 M KCl.

groups changes and the electrostatic repulsion among side groups is reduced. For instance, the pK of the carboxyl group in PGA rises from 4.5 to more than 8 in 50% dioxane; thus, this polymer became helical even at an apparent pH of 7.3 (Iizuka and Yang, 1965). Likewise, the NH_3^+ group in PO and PL tend to dissociate into the NH_2 form in the presence of, say, methanol or dioxane. But this factor alone is insufficient to stabilize the helices (see Figure 3). A second and perhaps more important factor is the lowering of the water activity in the mixed solvents. Water competes with the amide linkages for the formation of hydrogen bonds. Thus, a decrease in its activity tends to stabilize the intrachain hydrogen bonds of the helices. On the other hand, hydrophobic interactions (discussed later) are weaker in the mixed solvents so that stabilization of the helices by side chains becomes less important.

The contrast in physical properties between PO and PL is striking. As has been stated earlier, in aqueous solution under conditions when PL is almost fully helical, PO is only about 25% helical. Again, Rosenheck and Doty (1961) observed that when a dilute solution of PL at high pH (10.8) was heated, a helix-to- β transition occurred. Sarkar and Doty (1966) showed that even at neutral pH PL can be converted into the β form in the presence of sodium dodecyl sulfate. Surprisingly enough, PO does not undergo such a transition to the β structure under any of the conditions cited. It is interesting that if an aqueous solution of PL (0.01%) at pH 10.0, where it is about 80% helical ($[\theta]_{222} = -29,450$), is heated it cannot be converted into the β form (S. R. Chaudhuri and J. T. Yang, unpublished results). This explains, at least in part, why PO in aqueous solution (with low helical content) cannot form a β structure. Again, a solution of PL in methanol-water mixture (40:60, v/v) when heated at pH above 11 does not form the β structure, though it is fully helical. This suggests that hydrophobic interactions must also be involved in the formation of a β structure of PL. Moderately concentrated solutions of PL (about 0.1%) began to precipitate at pH above 11, but with PO no such precipitate could be detected even at pH

12.9. Blauer and Alfassi (1967), however, found that their PO having a degree of polymerization of 100 became turbid at pH above 11. This difference in solubility is probably related to the different helical content of the two polymers, PL and PO, at high pH, since Cassim and Yang (1967) showed that a helical polypeptide such as PGA tends to form ordered aggregates, and it is possible that random coils would not entangle in dilute solutions enough to precipitate.

We have shown in Figure 7 that the pK_i 's of PO and PL are almost identical; if anything, PO is slightly more easily deprotonated at high pH than is PL. Therefore, electrostatic interactions cannot be responsible for the instability of the helical conformation of PO. Since the submission of our paper, Grouke and Gibbs (1967) have reached the same conclusion that "in the higher pH range the poly-L-ornithine is not significantly charged and yet water soluble and unable to form a complete α helix at room temperature."

The differences in properties of PO and PL as discussed above may thus be ascribed to the difference in the aliphatic side chains of the respective polypeptides. (There are cases, however, where nature of the end groups may be more important in stabilizing the helix. For instance, PGA actually has one less methylene group in the side chains than does PO, but PGA forms perfect helices when the carboxyl groups are unionized.) In recent years the idea of hydrophobic interactions among side chains has been invoked to explain helical stability of many polypeptides and proteins (Kauzmann, 1959; Tanford *et al.*, 1960; Hermans and Scheraga, 1961; Wetlaufer, 1962; Tanford, 1962). Some earlier observations made on similar systems deserve consideration. Under comparable conditions, poly-L-aspartic acid in aqueous solution is lower in helical content than is PGA, just as PO is lower than PL. Again, Lotan *et al.* (1966) recently demonstrated that the amount of helical content of polymers of the N^5 -(ω -hydroxyalkyl)-L-glutamines in water depends on the number of methylene groups in the side chain $(CH_2)_2CONH(CH_2)_mOH$; for instance, the polymer was nonhelical with $m = 2$, about 20% helical with $m = 3$, and 65% helical with $m = 4$. It appears, therefore, that in a homologous series the fewer the number of CH_2 groups in the side chain, the less stable will be the helical conformation; for instance, PO has three CH_2 groups as compared with four in PL, and poly-L-aspartic acid has one CH_2 group as compared with two in PGA. Blauer and Alfassi (1967) also pointed out that PO has a smaller maximal area of contact between adjacent side chains than PL and therefore hydrophobic interactions must be less in PO than in PL. These findings support the contention that hydrophobic side-chain interactions stabilize the helical conformation in aqueous solution.

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