Helix–Coil Transition of Poly-δ-benzyl-L-α-aminoadipate and Poly-L-α-aminoadipic Acid in Solution[1]

Shigeo Kubota, Felicia Gaskin, and Jen Tsi Yang*

Contribution from the Cardiovascular Research Institute and Department of Biochemistry and Biophysics, University of California, San Francisco, California 94122. *Received July 14, 1971*

Abstract: The helix–coil transition of poly-δ-benzyl-L-α-aminoadipate (PBLAA) in chloroform–dichloroacetic acid mixtures was followed by optical rotation. The thermodynamic parameters for helix formation were determined according to the Zimm–Bragg theory. The enthalpy change, ΔH, for adding an amide residue to a helical region was 900 cal/mole (after correction for sample polydispersity). The initiation factor, σ, was 2.5×10^{-5}, suggesting that the transition of PBLAA was more cooperative than that of its homolog, poly-γ-benzyl-L-glutamate ($\sigma = 1\text{–}2 \times 10^{-4}$). The charge- and temperature-induced helix–coil transition of poly-L-α-aminoadipic acid (PLAAA) and poly-L-glutamic acid (PLGA) in 0.1 *M* NaCl was followed with potentiometric titration and optical rotation. The data were analyzed by the theories of Zimm and Bragg and of Zimm and Rice. For uncharged coil-to-helix transition the enthalpy and entropy changes were $\Delta H = -880$ cal/mole and $\Delta S = -2.3$ eu for PLAAA and $\Delta H = -990$ cal/mole and $\Delta S = -2.8$ eu for PLGA. The absolute values of ΔH dropped markedly in the region of the charge-induced transition, whereas ΔS remained essentially unchanged. The initiation factor σ' was about $8\text{–}9 \times 10^{-4}$ for both PLAAA and PLGA, which was virtually independent of temperature and pH of the solution.

As part of a program to assess the influence of side chains on the stability of polypeptide helices, we studied the helix–coil transition of synthetic polymers, the monomeric units of which are homologs of the naturally occurring amino acids. Previously, we reported that poly-γ-*N*-carbobenzoxy-L-α,γ-diaminobutyrate undergoes a "normal" temperature-induced helix–coil transition in mixed solvents of chloroform and dichloroacetic acid (DCA); that is, high temperature favors the coiled form as in the denaturation of proteins.[2] This direction of transition is opposite to that of poly-δ-*N*-carbobenzoxy-L-ornithine and poly-ε-*N*-carbobenzoxy-L-lysine, which undergoes an "inverse" transition; that is, high temperature favors the helical form. The only difference between the three polypeptides is the number of methylene groups (m) in their side chains (m = 2, 3, and 4, respectively). Similarly, poly-L-ornithine in aqueous solution has a low helical content of about 20% even when the polymer is completely un-ionized at high pH.[3] This is in striking contrast to poly-L-lysine, which is fully helical at pH above 11. It is also known that poly-γ-benzyl-L-glutamate (PBLG) and other helix-forming polypeptides have a right-handed sense of helix,[4a] whereas poly-β-benzyl-L-aspartate, whose side chain is one methylene group less than that of PBLG, has a left-handed sense of twist.[4b] Similarly, un-ionized poly-L-glutamic acid (PLGA) at pH below 5 is completely helical,[4a] but un-ionized poly-L-aspartic acid is only about 20% helical in aqueous solution.[4c] Here again, the difference lies in one methylene group of the side chains. Thus, the size of the nonpolar group in the side chains seems to play an important role in the stability of helical conformation. The question that arises is whether increasing the methylene groups would further increase the hydrophobic interactions, thereby augmenting the stability of polypeptide helices, or whether there is a limit in each series of homologs beyond which the effect of additional methylene groups would cause little improvement in helical stability. Toward this end we have now extended our investigations to the homologs of PBLG and PLGA. In this paper we report the syntheses and conformational studies of poly-δ-benzyl-L-α-aminoadipate (PBLAA) and poly-L-α-aminoadipic acid (PLAAA), both of which have one more methylene group in the side chain (m = 3) than the corresponding PBLG and PLGA (m = 2).

The helix–coil transition of polypeptides in solution can be induced by changes in solvent composition and temperature, and for polypeptides having ionizable side groups, by changes in the pH of the solution. We followed the conformational changes with optical rotation and, when applicable, potentiometric titration. The data were then analyzed according to the theories of Zimm and Bragg[5] and Zimm and Rice,[6] which define two parameters, s and σ. Here s is the equilibrium constant for adding an amide residue to a helical segment and σ characterizes the initiation of a helical segment and also the sharpness of the transition. For aqueous solutions Snipp, *et al.*,[7] have recently derived the equation for equilibrium constant s' as a function of pH or the average degree of dissociation $\bar{\alpha}$, which reduces to s when $\bar{\alpha}$ approaches zero.

Results and Discussion

Poly-δ-benzyl-L-α-aminoadipate. Conformation. Optical rotatory dispersion (ORD) in the visible region (above 300 nm) for high molecular weight PBLAA (n = 1000) in chloroform (a helix-promoting solvent) at 25° can be fitted with the Moffitt equation,[8] which gave

(1) This work was aided by grants from the U. S. Public Health Service (HE-06285, GM-10880, and GM-K3-3441).

(2) G. Gaskin, S. Kubota, and J. T. Yang, *J. Amer. Chem. Soc.*, 91, 6525 (1969).

(3) S. R. Chaudhuri and J. T. Yang, *Biochemistry*, 7, 1379 (1968).

(4) (a) See, for example, G. D. Fasman in "Poly-α-Amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, Chapter 11; (b) E. R. Blout and R. H. Karlson, *J. Amer. Chem. Soc.*, 80, 1259 (1958); (c) J. Brahms and J. Spach, *Nature (London)*, 200, 72 (1963).

(5) (a) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, 28, 1246 (1958); 31, 526 (1959); (b) B. H. Zimm, P. Doty, and K. Iso, *Proc. Nat. Acad. Sci. U. S.*, 45, 160 (1959).

(6) B. H. Zimm and S. A. Rice, *Mol. Phys.*, 3, 391 (1960).

(7) R. L. Snipp, W. G. Miller, and R. E. Nylund, *J. Amer. Chem. Soc.*, 87, 3547 (1965).

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

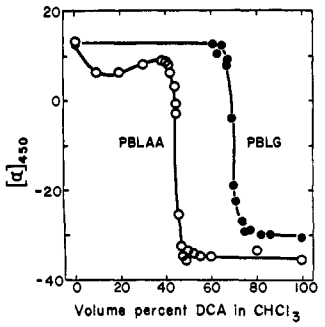


Figure 1. Helix–coil transition of poly-δ-benzyl-L-α-aminoadipate (PBLAA) and poly-γ-benzyl-L-glutamate (PBLG) in chloroform–dichloroacetic acid mixtures at 25°.

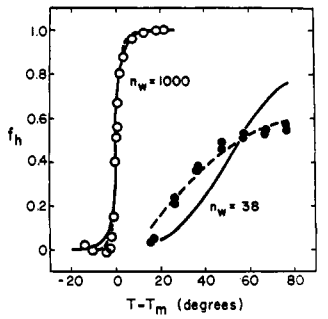


Figure 2. Fraction of helix (f_h) of poly-δ-benzyl-L-α-aminoadipate as a function of temperature, T, minus midpoint temperature, T_m, in chloroform–dichloroacetic acid (DCA) mixtures. Sample n_w = 1000 (○) was studied in 64:36 ($CHCl_3$–DCA (v/v) and T_m set at −0.7°; sample n_w = 38 (●) in 81:19 $CHCl_3$–DCA with T_m = −42°. Lines are theoretical curves: solid, assuming monodispersity; broken, assuming a distribution of molecular weights. See text for details.

$b_0 = -620$ and $a_0 = 260$ deg cm²/dmole. In the ultraviolet region the ORD displayed a 233-nm minimum characteristic of an α-helix with $[m']_{233} = -12{,}000$ deg cm²/dmole. In DCA (a coil-promoting solvent), the corresponding b_0 and a_0 were −45 and −190 deg cm²/dmole, respectively. These results are consistent with a right-handed α-helix in chloroform and a random coil in DCA.[9]

Solvent-Induced Transition. Figure 1 shows the helix–coil transition of PBLAA in chloroform–DCA mixed solvents at 25°. The midpoint of the transition occurred at 55 volume % of chloroform. For comparison we included the solvent titration for PBLG, which has one less methylene group in the side chain than PBLAA. Our results with PBLG gave a midpoint of the transition at 31 volume % of chloroform, which agreed with that reported by Karlson, *et al.*[10] (Bradbury, *et al.*,[11] previously reported the transition at 20 volume % of chloroform.) Thus, at 25°, less DCA is required to destroy the helical conformation of PBLAA than of PBLG. Both polypeptides undergo a sharp transition, suggesting a high degree of cooperativity (see analysis of data below).

Temperature-Induced Transition. As in the case of PBLG, temperature induced an "inverse" helix–coil transition of PBLAA in chloroform–DCA mixed solvents; that is, high temperature favored the helical form and low temperature the coiled form. Figure 2 (left half) shows the fraction of helix, f_h, of PBLAA (degree of polymerization, n = 1000) in 64% chloroform as a function of $(T - T_m)$, where T_m is the temperature at the midpoint of the temperature-induced transition. The fractions of helix were calculated from $[\alpha]_{450}$ of PBLAA in the chloroform–DCA mixture, where the upper and lower limits were taken as 100% helix and 100% coil. We have measured the temperature-induced transition in 55, 60, 64, and 68% chloroform and the corresponding transition temperatures were 21.5, 7.7, −0.7, and −10.0°, respectively. In spite of large variations in T_m with solvent composition, the curves of f_h *vs.* $(T - T_m)$ for PBLAA in various solvent mixtures could be superimposed on each other (data not shown).

In order to obtain measurable changes in conformation the temperature-induced helix–coil transition for low molecular weight PBLAA (n = 38) was studied in 81% chloroform. To determine its T_m at this solvent composition it was necessary to use the per cent chloroform *vs.* T_m data for the high molecular weight sample (see above), which extrapolated to a $T_m = -42°$ at 81% chloroform. The plot of f_h *vs.* $(T - T_m)$ for the low molecular weight sample is included in Figure 2 (right half). Here, the f_h's were calculated from the b_0's of the Moffitt equation.[8] This calculation was used because the low molecular weight sample could easily aggregate, which would change significantly the rotation at any wavelength and also a_0, but not b_0. For instance, the b_0 of sample n = 38 in chloroform was −434 at −20° and −420 at 25°, but the corresponding a_0 was 230 and 170 at the two temperatures. Therefore, we used the limits of b_0 found in the temperature-induced transition of high molecular weight sample in 60% chloroform, which were −550 for 100% helix and zero for 100% coil.

Analysis of Data. We treated the experimental data according to the Zimm–Bragg theory, which has been described previously.[5,12] The theory defines two parameters: the initiation factor σ and the equilibrium constant s (for adding an amide residue to a helical segment). For very long polypeptide chains, f_h is given as

$$f_h = \mathrm{d} \ln \lambda / \mathrm{d} \ln s \qquad (1)$$

with

$$\lambda = (1/2)\{1 + s + [(1 - s)^2 + 4\sigma s]^{1/2}\} \qquad (2)$$

where λ is the larger of the two eigenvalues of the Zimm–Bragg matrix. From eq 1 and 2 we obtain

$$f_h = (1/2)\{1 + (s - 1)/[(1 - s)^2 + 4\sigma s]^{1/2}\} \qquad (3)$$

For very short chains f_h is a function of n, s, and σ

$$f_h = \{n(s - 1) - 2 - s^{-n+2}[3(n - 2)s^2 - (7n - 16)s + 4(n - 3)]\}/n(s - 1)\{1 + \sigma^{-1}(s - 1)^2 s^{-n+1} - [(n - 3)(s - 1) + s]s^{-n+2}\} \qquad (4)$$

Furthermore, s is related to temperature by the relationship

$$\ln s = (\Delta H/R)(T - T_m)/TT_m \qquad (5)$$

(9) J. T. Yang in "Poly-α-Amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, Chapter 6.
(10) R. H. Karlson, K. S. Norland, G. D. Fasman, and E. R. Blout, *J. Amer. Chem. Soc.*, 82, 2268 (1960).
(11) E. M. Bradbury, A. R. Downie, A. Elliot, and W. E. Hanby, *Proc. Roy. Soc., Ser. A*, 259, 110 (1960).
(12) F. Gaskin and J. T. Yang, *Biopolymers*, 10, 631 (1971).

where ΔH is the enthalpy for adding an amide residue to a helical segment. Thus, experimental data of f_h's as a function of $(T - T_m)$ for both high and low molecular weight samples allow the determination of σ and ΔH (or s) in eq 1 to 5. This is done by varying σ and $\Delta H/R$ with the aid of a computer program until a best fit with the experimental data is obtained. The solid lines in Figure 2 represent the best least-square fits to the experimental curves. The corresponding values of the thermodynamic parameters were $\Delta H = 690$ cal/mole and $\sigma = 3.8 \times 10^{-5}$.

The agreement between the experimental and calculated results was good for the high molecular weight sample and poor for the low molecular weight one. This was not unexpected since eq 1 is independent of the degree of polymerization, whereas variation in n in eq 4 will influence the shape of the theoretical curve for the low molecular weight sample. As in a previous study,[12] we can employ the Schaefgen–Flory's discrete molecular weight distribution[13]

$$w_n = [(1 - p)^{b+1}/(bp + 1 - p)] \times (p^{n-1})n(n + b - 2)!/(b - 1)!(n - 1)! \quad (6)$$

where w_n is the weight fraction of the n-mer and p and b are two adjustable parameters. The width of the distribution depends on b; the larger the b, the narrower the distribution. The mole fraction size distribution is given by Schaefgen and Flory

$$\chi_{n,b} = (1 - p)^b p^{n-1} \times (n + b - 2)!/(b - 1)!(n - 1)! \quad (7)$$

By introducing a moment-generating function, it can be shown[12] that the weight- and Z-average degrees of polymerization are

$$n_w = [(b - 1)^2 p^2 + (3b - 2)p + 1]/(bp + 1 - p)(1 - p) \quad (8)$$

and

$$n_z = [(b^3 - 3b^2 + 3b - 1)p^3 + (6b^2 - 8b + 3)p^2 + (7p - 3)p + 1]/(1 - p) \times [(b - 1)^2 p^2 + (3b - 2)p + 1] \quad (9)$$

The parameters b and p are so chosen as to give an n_w equal to that determined experimentally (in our case, $n_w = 38$).

The weight fraction, w_n, between $n = 4$ and 150 was calculated with a computer program based on eq 6 for a given set of b and p (p was so set that $n_w = 38$). The fraction of helix was calculated in turn from $f_h = \Sigma w_n f_{hn}$, where f_{hn} was calculated from eq 4. This equation and eq 1 and 5 were combined and the thermodynamic parameters σ and $\Delta H/R$ were varied with a computer program until they best represented the experimental data. Table I lists the results of such calculations. The broken lines in Figure 2 represent the calculated curves with $b = 2$. Clearly, the fit with the experimental points is satisfactory for the low as well as the high molecular weight samples. Note also that at $b = 2$, $M_z/M_w = 1.33$, which is exactly the same as that determined experimentally (see the Experimental Section). Although the decrease in b from 3 to 2 improved the fit, the resultant changes in thermodynamic parameters are insignificant (Table I). If $b = 1$, we would have the broad most probable distribution, but the number of states (degree of polymerization) needed for the summation $\Sigma w_n f_{hn}$ would be too numerous to handle; thus, we stopped our analysis at $b = 2$. The thermodynamic parameters $\Delta H = 900$ cal/mole and $\sigma = 2.5 \times 10^{-5}$ differ from those calculated on the assumption of a monodisperse system (see Table I).

Table I. Effect of Polydispersity on the Thermodynamic Parameters of Poly-δ-benzyl-L-α-aminoadipate in Chloroform–Dichloroacetic Acid

M_z/M_w	b; p	$\sigma \times 10^5$	ΔH, cal/mole	Least-squares errors
1		3.82	690	0.334
1.16	5; 0.857	2.46	850	0.061
1.23	3; 0.900	2.47	880	0.049
1.33	2; 0.924	2.47	900	0.047

Comparison of our results on PBLAA in chloroform–DCA with those obtained for PBLG in ethylene dichloride–DCA[5,14] indicated that the helix–coil transition of PBLAA is significantly more cooperative than that of PBLG ($\sigma = 1.0 \times 10^{-4}$ to 2.0×10^{-4}), even though admittedly different helix-promoting solvents were used in these experiments. But the enthalpies for adding an amide residue to a helical region are similar for the two polypeptides ($\Delta H = 650$–950 cal/mole for PBLG).[14] (The thermodynamic parameters for PBLG as listed here do not include the earlier reported values calculated from calorimetric measurements which were done with moderately concentrated solutions without extrapolation to zero concentration.)

Other poly-α-amino acids studied so far in the PBLG series also undergo inverse temperature-induced helix–coil transitions in chloroform–DCA solvent mixtures: poly-β-benzyl-L-aspartate (PBLA),[15,16] poly-β-(*p*-methylbenzyl)-L-aspartate (PMBLA),[16] and poly-ε-benzyl-D-α-aminopimelate (PBDAP).[16] The thermodynamic parameters for PBLA ($\sigma = 0.6 \times 10^{-4}$ and $\Delta H = 260$ cal/mole[15] and our own finding that $\sigma = 0.5 \times 10^{-4}$ and $\Delta H = 330$ cal/mole) suggest that the degree of cooperativity of this coil → left-handed helix formation is similar to that for the right-handed PBLG ($\sigma = 1$–2×10^{-4}) but the ΔH required is less.

Since we had no low molecular weight samples of PMBLA and PBDAP, we were unable to calculate the thermodynamic parameters. The difficulty of analyzing the f_h *vs.* $T - T_m$ curve of a low molecular weight sample PMBLA is due to the following reason. Since the high molecular weight sample underwent a $CHCl_3$–DCA solvent transition at 25° at a low percentage of DCA (4% by volume) and the solvent and temperature transitions are much broader for a low molecular weight sample than for a high molecular weight sample, the temperature-induced transition in the mixed solvent would probably be small in the experimentally accessible temperature region. Indeed, this was also the case for

(13) J. R. Schaefgen and P. J. Flory, *J. Amer. Chem. Soc.*, 70, 2709 (1948).

(14) (a) T. Ackerman and E. Neumann, *Biopolymers*, 5, 649 (1967); (b) T. V. Barskaya, I. A. Bolotina, and O. B. Ptitsyn, *Mol. Biol.*, 2, 700 (1968); (c) G. Giacometti, A. Turolla, and R. Boni, *Biopolymers*, 6, 441 (1968).

(15) Y. Hayashi, A. Teramoto, K. Kawahara, and H. Fujita, *Biopolymers*, 8, 403 (1969).

(16) F. Gaskin, Ph.D. dissertation, University of California, San Francisco, Calif., 1969.

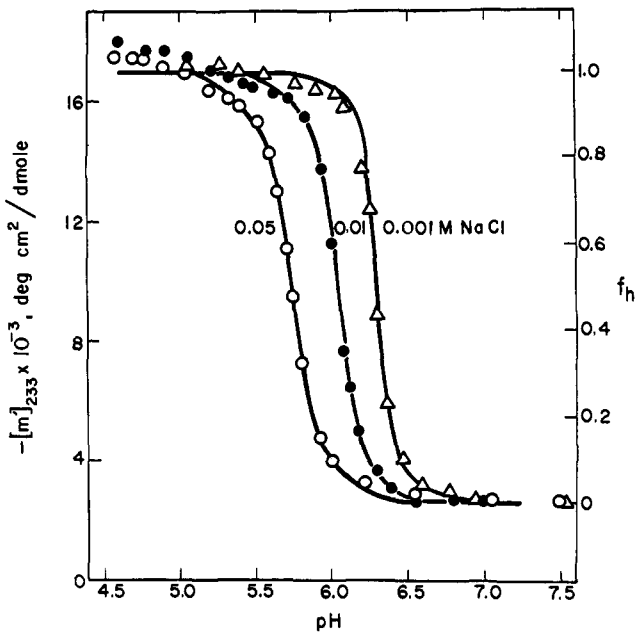


Figure 3. Charge-induced helix–coil transition of poly-L-α-aminoadipic acid in salt solutions at 25°. NaCl concentrations: △, 0.001 *M*; ●, 0.01 *M*; ○, 0.05 *M*. Solid lines are calculated curves based on potentiometric titration.

PBLA (solvent midpoint at 92% $CHCl_3$ for the high molecular weight samples at 25°) when we prepared a sample $n = 55$.

Poly-L-α-aminoadipic Acid. Conformation. PLAAA is highly aggregated at low pH values, more so than PLGA. For instance, in 0.2 *M* NaCl at 25° PLAAA began to precipitate at about pH 4.7, whereas PLGA did not. Thus, all experiments were done in 0.1 *M* NaCl or less. The ORD in the visible region of PLAAA in 0.1 *M* NaCl at 25° gave the following Moffitt parameters[8] in deg cm^2 dmole^{-1}: $b_0 = -580$ and $a_0 = -400$ at pH 4.80 and $b_0 \cong 0$ and $a_0 = -825$ at pH 7.00. The ORD in the ultraviolet region showed at pH 4.80 a minimum at 233 nm and a maximum at 198 nm with $[m']_{233} = -17{,}500$ and $[m']_{198} = 80{,}000 \pm 500$ deg cm^2 dmole^{-1}, values characteristic of an α-helical conformation. The corresponding spectrum in neutral solution showed a minimum at 205 nm with $[m']_{205} = -19{,}000 \pm 200$ deg cm^2 dmole^{-1}, which again is typical of a coiled conformation. Similarly, the CD spectrum for the helical form (pH 4.80) gave a double minimum at 222 and 210 nm with $[\theta]_{222} = -35{,}000$ and $[\theta]_{210} = -33{,}500$ and a maximum at 191 nm with $[\theta]_{191} = 67{,}000$ deg cm^2 dmole^{-1}. The corresponding spectrum for the coiled conformation (pH 7.00) gave a minimum at 197 nm with $[\theta]_{197} = -26{,}000$ deg cm^2 dmole^{-1}, together with a small positive maximum at 218 nm and a small negative minimum at 232 nm. These results support the contention that PLAAA is helical in the uncharged state and coiled in the charged state, as is true of PLGA. The optical activity of the uncharged polypeptide at pH 4.40 remained unchanged on mild heating, indicating the absence of the heat-induced α-to-β transition unlike the uncharged poly-L-lysine.[17]

Charge-Induced Transition; Optical and Potentiometric Titrations. Figure 3 shows the variation in optical rotation at 233 nm with pH at three salt concentrations at 25° (the curve in 0.1 *M* NaCl is included in Figure 5). In all cases the curves for the coiled conformation at high pH values approach a plateau of $[m']_{233} = -2700$ deg cm^2 dmole^{-1}. The helix–coil transition is sharp, a fact indicative of a cooperative process. The levorotations at low pH values, however, do not level off but increase gradually with decreasing pH. This increase most probably results from aggregation of the uncharged polypeptide molecules. For instance, the aggregated PLGA molecules also had a larger levorotation at 233 nm than the unaggregated ones.[18] The results of potentiometric titration (see below) also support this explanation. The solid lines in Figure 3 represent the fraction of α-helix as calculated from the titration curves to be discussed below.

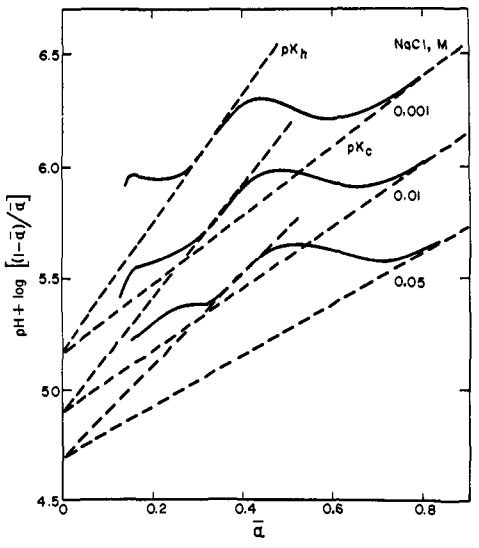


Figure 4. Titration curves of poly-L-α-aminoadipic acid in salt solutions at 25°. NaCl concentrations (from top to bottom): 0.001, 0.01, 0.05, and 0.1 *M*. Solid lines are experimental curves; broken lines represent the titration curves for pure helix and coil.

The apparent dissociation constant, *K*, of a weak polymeric acid can be represented by

$$pK \equiv \text{pH} + \log[(1 - \bar{\alpha})/\bar{\alpha}] = pK_0 + (0.434/RT)(\partial G/\partial Z) \quad (10)$$

where pK_0 is the negative logarithm of the intrinsic dissociation constant of the carboxyl side groups of the polypeptide, $\bar{\alpha}$ the average degree of dissociation, and the last term on the right-hand side the electrostatic free energy accompanying the titration process. Figure 4 represents typical titration curves (solid lines) of PLAAA at three salt concentrations at 25° (the curve in 0.1 *M* NaCl is included in Figure 6). The region above $\bar{\alpha} \cong 0.8$ is the titration of the pure coil, that between $\bar{\alpha} \cong 0.35$ and 0.8 the helix–coil transition region, and that below $\bar{\alpha} \cong 0.35$ the titration of pure helix (the deviations from the broken lines at low α's were attributed to the aggregation of the helical molecules). Following Wada's procedure,[19] we drew two straight lines tangentially to each experimental curve in Figure 4. The two broken lines for each curve then represent the titration curves for pure helix and coil. The extrapolation

(17) P. K. Sarkar and P. Doty, *Proc. Nat. Acad. Sci. U. S.*, **55**, 981 (1966); T. Townend, T. F. Kumosinski, S. N. Timasheff, G. D. Fasman, and B. Davidson, *Biochem. Biophys. Res. Commun.*, **23**, 163 (1966).

(18) J. Y. Cassim and J. T. Yang, *Biochem. Biophys. Res. Commun.*, **26**, 58 (1967).

(19) A. Wada, *Mol. Phys.*, **3**, 409 (1960).

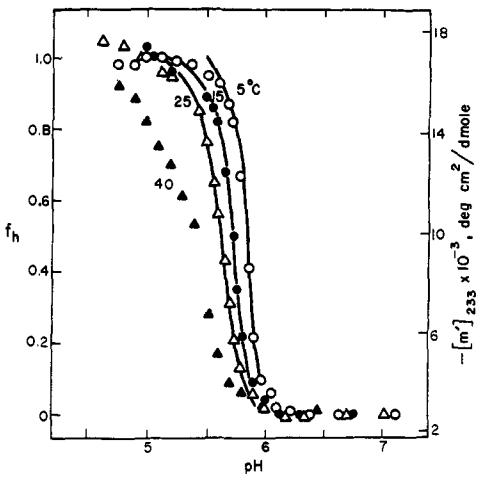


Figure 5. Charge-induced helix–coil transition of poly-L-α-aminoadipic acid in 0.1 M NaCl at several temperatures. Temperatures: ○, 5°; ●, 15°; △, 25°; ▲, 40°. Solid lines are calculated curves based on potentiometric titration.

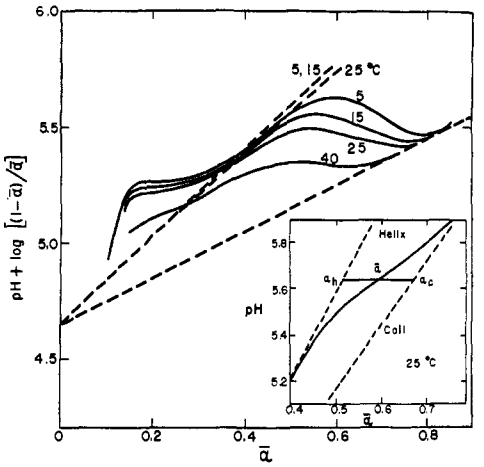


Figure 6. Titration curves of poly-L-α-aminoadipic acid in 0.1 M NaCl at several temperatures. Solid lines are experimental curves; broken lines represent the titration curves for pure helix and coil. Insert: pH *vs.* $\bar{\alpha}$ plot in the helix–coil transition region.

for the pure coil presents no problem, but that for the pure helix (unaggregated) covers only a narrow range of pH or $\bar{\alpha}$. The latter is so drawn that it is tangential to the portion of the curve above the precipitation point of $\bar{\alpha}$, *i.e.*, $\bar{\alpha}_p = 0.32 \pm 0.03$. The two straight lines of each titration curve reach a common intercept, pK_0, within ±0.02 unit. The degree of dissociation for the pure helix and coil at any pH of the solution can thus be read from the relationship

$$pH + \log[(1 - \alpha_h)/\alpha_h] = pK_0 + 0.868 w_h \alpha_h \quad (11a)$$

and

$$pH + \log[(1 - \alpha_c)/\alpha_c] = pK_0 + 0.868 w_c \alpha_c \quad (11b)$$

where w_h and w_c are constants for each titration curve. As can be seen from Figure 4 the pK_0's of PLAAA were 5.17, 4.90, and 4.70 in 0.001, 0.01, and 0.05 M NaCl at 25°, respectively. We also measured the titration curves of PLAAA and PLGA in 0.1 M NaCl at 25° and found that PLGA had a $pK_0 = 4.55$, which was lower by 0.1 unit than that of PLAAA under the same experimental conditions. Our extrapolation procedure differs from that of Nagasawa and Holtzer,[20] in which slightly downward curves were so drawn that all the curves for pure helix and coil at various ionic strengths could be brought to a common intercept. Thus, $pK_0 = 4.55$ for PLGA in both 0.005 to 0.200 M NaCl according to Nagasawa and Holtzer. That the pK_0 of a polyion varies with the ionic strength of the solution is not too surprising, since a simple weak acid such as *N*-acetylglycine also has different pK_0's in various salt solutions.[21] Wada's procedure was also adopted by other laboratories.[22]

The fraction of helix, f_h, at various pH values can be estimated from optical rotation or potentiometric titration. The curves in Figure 4 indicate that PLAAA began to precipitate below $\bar{\alpha}_p = 0.32$. At this average degree of dissociation $[m']_{233} = -17{,}000$, which is independent of the salt concentrations used (see Figure 3). In neutral solutions $[m']_{233} = -2700$. Thus, these two limits can be used to represent pure helix and coil. Below $\bar{\alpha} = 0.32$, however, the levorotation of PLAAA increased gradually with decreasing pH as a result of aggregation of the polypeptide molecules. The estimation of f_h from titration curves has been described in detail by Nagasawa and Holtzer.[20] Briefly, from the results in Figure 4 we can draw the plots of pH *vs.* $\bar{\alpha}$ for the actual experimental data and also for the pure helix and coil (read from the broken lines) (see Figure 6 below). At any chosen pH, we have the degrees of dissociation α, α_h, and α_c. Thus, we have $f_h = (\alpha_c - \bar{\alpha})/(\alpha_c - \alpha_h)$. The solid lines in Figure 3 represent such calculated values for PLAAA. Clearly, the calculated f_h's from titration curves are in good agreement with those obtained from optical rotation.

Effect of Temperature. Figure 5 shows the helix–coil transition of PLAAA at four temperatures. The agreement between f_h's based on optical rotation (points) and potentiometric titration (lines) is again good. We did not calculate the f_h's at 40° because of the complication of aggregation which obscures the true titration curve (see below). The transition at 40° is also less sharp than that at lower temperatures. For comparison, we also measured the optical rotations of PLGA at several temperatures (data not shown) and reached similar conclusions.

Figure 6 shows the titration curves of PLAAA at four temperatures. Again we used linear extrapolations to obtain a pK_0 of 4.65 ± 0.02 at 5, 15, and 25°. The $\bar{\alpha}_p$ values are 0.40 at 5° and 0.38 at 15 and 25°. At these $\bar{\alpha}_p$'s, $[m']_{233}$ (helix) = −17,000 ± 200 deg cm^2 dmole^{-1} (see Figure 5), which is the same as that for $f_h = 1$ without the complication of aggregation as shown in Figure 3. In neutral solutions $[m']_{233}$ (coil) = −2700. Thus, these two limits were again chosen to represent the pure helix and coil (Figure 5). For the titration curve at 40° the unaggregated and aggregated helix regions could not be clearly identified. Linear extrapolation of the pure helix curve would intercept the ordinate at 4.85, which does not coincide with that of the pure coil at 4.65. For this reason we did not calculate the f_h's from the titration curve at 40°.

(20) M. Nagasawa and A. Holtzer, *J. Amer. Chem. Soc.*, **86**, 538 (1964).
(21) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides, Reinhold, New York, N. Y., 1943, p 114.
(22) A. Ciferri, D. Puell, L. Rajagh, and J. Hermans, Jr., *Biopolymers*, **6**, 1019 (1968); G. Conio and E. Patrone, *ibid.*, 8, 57 (1969); G. Conio, E. Patrone, and S. Brighetti, *J. Biol. Chem.*, **245**, 3335 (1970).

For comparison we also measured the titration curves of PLGA at several temperatures (data not shown). The $\bar{\alpha}_p$ values were 0.30 ± 0.02 at 5 and 15°, 0.25 ± 0.02 at 25° and 0.20 ± 0.02 at 40°. At these $\bar{\alpha}_p$'s $[m']_{233}$ (helix) = −14,400 ± 200 deg cm² dmole⁻¹. At neutral pH $[m']_{233}$ (coil) = −2000 deg cm² dmole⁻¹. By choosing these two limits for the pure helix and coil we again found good agreement between the calculated f_h's from optical rotations and titration curves below 30° for $0.1 < f_h < 0.9$. At 40°, however, for reasons still unknown, the helix–coil transition curve based on optical rotation is much less sharp than that calculated from the titration curve (not shown).

Analysis of Data. We analyzed the experimental data at constant temperature by the theories of Zimm and Bragg[5] and Zimm and Rice,[6] as simplified by Snipp, *et al.*[7] Equation 3 for high molecular weight polypeptides can be rewritten as

$$f_h = (1/2)\{1 + (s' - 1)/[(1 - s')^2 + 4\sigma' s']^{1/2}\} \quad (3a)$$

where s' is an equilibrium constant at any chosen pH and temperature, which reduces to s (*cf.* eq 1) when polypeptide is uncharged, *i.e.*, $\alpha = 0$. The parameter σ' is the same initiation factor as σ in eq 3; the prime implies that the polypeptide is ionizable, such as PLAAA; σ' is further assumed to be independent of pH or $\bar{\alpha}$.[6] Equation 3a can be rearranged into[23]

$$(1 - 2f_h)^2/f_h(1 - f_h) = (1 - s')^2/\sigma' s' \quad (12)$$

Thus, a plot of the left side of eq 12 *vs.* $(1 - s')^2/s'$ yields a straight line with a slope of $1/\sigma'$. Applequist[24] has also shown, assuming that σ' is independent of T, that eq 3a can be reduced to

$$df_h/d(1/T) = -\Delta H'/4R\sigma'^{1/2} \quad (13)$$

at $s' = 1$. Thus, a plot of f_h *vs.* $(1/T)$ yields at $s' = 1$ a tangent of $-\Delta H'/4R\sigma'^{1/2}$. The quantities s and s' are given as[7]

$$d \ln s'/d(pH) = 2.3(\alpha_h - \alpha_c) \quad (14)$$

$$\ln s' = \ln s + 2.3 \int_{pH\ (\alpha_c = \alpha_h = 0)}^{pH} (\alpha_h - \alpha_c) d(pH) \quad (15)$$

$$\ln s = -2.3 \int_{pH\ (\alpha_c = \alpha_h = 0)}^{pH\ (f_h = 1/2)} (\alpha_h - \alpha_c) d(pH) \quad (16)[20]$$

Thus

$$\ln s' = 2.3 \int_{pH}^{pH\ (f_h = 1/2)} (\alpha_c - \alpha_h) d(pH) \quad (17)$$

The integrals in eq 16 and 17 can be evaluated graphically from plots of pH *vs.* $\bar{\alpha}$ for the experimental curve (Figure 6, insert) and curves for pure helix and coil based on eq 11. The $\bar{\alpha}$ for the midpoint of the helix–coil transition is read from the plot of f_h *vs.* $\bar{\alpha}$. Alternatively, ln s can be determined graphically from the titration curve such as in Figure 4, since ln s can also be written as[6,20]

$$\ln s = 2.3 \int_0^1 (pK - pK_c)\, d\bar{\alpha} \quad (18)$$

The results, of course, are identical with those based on eq 16.

(23) L. Peller, private communication.
(24) J. Applequist, *J. Chem. Phys.*, 38, 934 (1961).

Since s and s' are the equilibrium constants, we can write the thermodynamic quantities as follows

$$\ln s \equiv -\Delta G/RT = \Delta S/R - \Delta H/RT \quad (19)$$

and

$$\ln s' \equiv -\Delta G'/RT = \Delta S'/R - \Delta H'/RT \quad (20)$$

Here, the unprimed symbols represent the transition from the uncharged coil to the uncharged helix and the primed symbols the transition for the partially or fully charged polypeptides. The enthalpy changes, ΔH and $\Delta H'$, and entropy changes, ΔS and $\Delta S'$, can, of course, be determined from the titration curves at several temperatures by evaluating the s or s' graphically and using a van't Hoff plot of ln s (or ln s') *vs.* $1/T$. In principle, the temperature-induced transition of uncharged polypeptides can also be analyzed by the same procedure as that described for PBLAA using one high molecular weight and one low molecular weight sample. However, the melting temperatures, T_m, at $f_h = 0.5$ for uncharged PLAAA and PLGA were too high to be practical for such analyses.

Uncharged Coil-to-Helix Transition. For uncharged coil-to-helix transition of PLAAA, graphical integration of eq 16 or eq 18 for the data in Figure 4 together with eq 19 gave a free energy change, ΔG, of −240, −230, −230, and −210 cal/mole in 0.001, 0.01, 0.05, and 0.1 *M* NaCl at 25°, respectively. Table II lists the

Table II. Free Energy Changes, ΔG, of the Uncharged Coil-to-Helix Transition of Poly-L-α-aminoadipic (PLAAA) and Poly-L-glutamic Acids (PLGA) in 0.1 *M* NaCl Solutions

Temperature, °C	PLAAA, cal/mole	PLGA, cal/mole
5	−250	−220
15	−230	−200
25	−210	−170
40	(−160)	(−130)

ΔG for the uncharged coil-to-helix transition of PLAAA at several temperatures in 0.1 *M* NaCl. Also included are the data for PLGA for comparison. By plotting ΔG against temperature (not shown), we obtained two straight lines which were essentially parallel. We did not calculate the ΔG at 40° for PLAAA because of the difficulty of extrapolating the curve for pure helix to zero α (see Figure 6). From the $\Delta G - T$ plot, however, we estimated a ΔG of about −160 and −130 cal/mole for PLAAA and PLGA, respectively, at 40°. With the data in Table II we could calculate the enthalpy and entropy changes of the uncharged coil-to-helix transition using a van't Hoff plot (eq 19). The results were $\Delta H = -880$ cal/mole and $\Delta S = -2.3$ eu for PLAAA and $\Delta H = -990$ and $\Delta S = -2.8$ eu for PLGA in 0.1 *M* NaCl.

Partially Charged Polypeptides. For the uncharged helix and the fully charged coil the degrees of dissociation are zero and unity, respectively, and they are not affected by changes in the temperature of the solution. A problem arises, however, when we deal with partially charged polypeptides. Raising or lowering the temperature of the solution will change the average degree of dissociation, $\bar{\alpha}$, of the polymer and pH of the solution. Customarily, however, the thermodynamic quan-

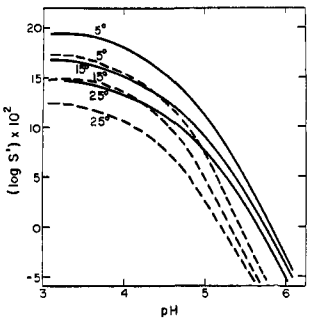


Figure 7. The equilibrium constant, s', as a function of pH at 5, 15, and 25°. Lines: solid, poly-L-α-aminoadipic acid; broken, poly-L-glutamic acid (both in 0.1 M NaCl).

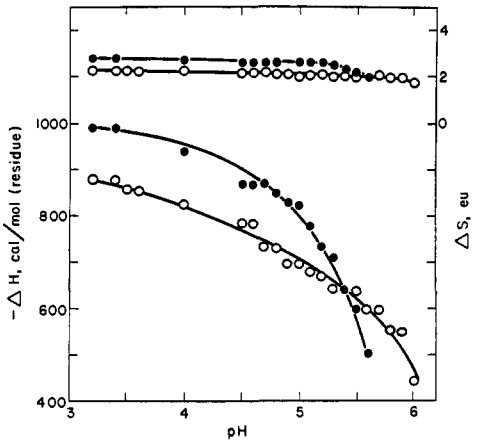


Figure 8. The thermodynamic quantities and initiation factor at various pH's. Lines: solid, poly-L-α-aminoadipic acid; broken, poly-L-glutamic acid (both in 0.1 M NaCl).

tities are determined at constant pH. Thus, we chose constant pH in our analyses. Figure 7 shows the plots of ln s' *vs.* pH in 0.1 M NaCl at several temperatures for PLAAA and PLGA. Here, s' was again determined graphically according to eq 17. Next, plotting ln s' against $1/T$ at constant pH gave a series of straight lines from which we could calculate the ΔH and ΔS values at any chosen pH. The results are summarized in Figure 8. For both PLAAA and PLGA ΔS is virtually constant with respect to pH. In the helix–coil transition region the absolute values of ΔS decrease only slightly with increasing pH, whereas the absolute magnitudes of ΔH for both polymeric acids decrease markedly with increasing pH.

Quantitatively, we can account for the variation of thermodynamic quantities with pH by the following derivations.[23] By differentiating eq 11a and 11b with respect to α_h and α_c, respectively, and combining them with eq 15, we obtain

$$\Delta G' \equiv -RT \ln s' = \Delta G - RT(w_h\alpha_h^2 - w_c\alpha_c^2) + 2RT(w_h\alpha_h - w_c\alpha_c) + RT \ln (\alpha_h/\alpha_c) \quad (21)$$

By differentiating eq 11a, 11b, and 15 with respect to temperature at constant pH, combining them and utilizing the Gibbs–Helmholtz equation, we obtain

$$\Delta H' = \Delta H + R[\alpha_h^2 dw_h/d(1/T) - \alpha_c^2 dw_c/d(1/T)] \quad (22)$$

(There should be another term of $-R[(\alpha_h - \alpha_c)d \ln K_0/d(1/T)]$ on the right side of eq 22. It has been omitted

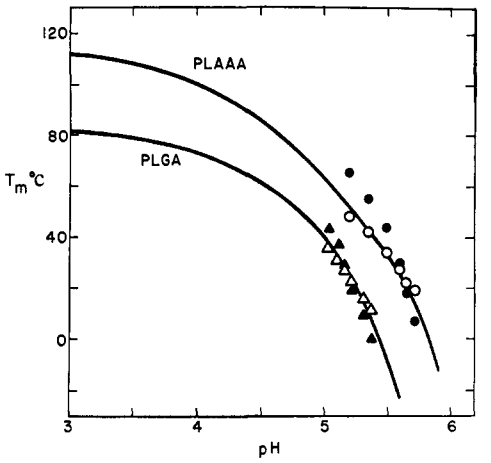


Figure 9. The melting temperatures of poly-L-α-aminoadipic and poly-L-glutamic acids in 0.1 M NaCl at various pH's. Lines, calculated from ΔH and ΔS in Figure 8; points based on optical rotation data (open, at constant pH; closed, the pH subject to minute change with temperature). See text for details.

in the present case, since pK_0 remains essentially constant over the temperature range of 15–25° studied.) By combining eq 21 and 22 with eq 19 and 20, we have

$$\Delta S' = \Delta S + R(w_h\alpha_h^2 - w_c\alpha_c^2) - 2R(w_h\alpha_h - w_c\alpha_c) - RT(\alpha_h^2 dw_h/dT - \alpha_c^2 dw_c/dT) - R \ln (\alpha_h/\alpha_c) \quad (23)$$

In the limiting case of $\alpha_h = \alpha_c = 1$, that is, the polymeric acid is completely ionized, we have

$$\Delta G' = \Delta G + RT(w_h - w_c) \quad (24a)$$

$$\Delta H' = \Delta H + Rd(w_h - w_c)/d(1/T) \quad (24b)$$

and

$$\Delta S' = \Delta S - R[(w_h - w_c) + Td(w_h - w_c)/dT] \quad (24c)$$

From Figure 6 we see that w_h is about 2 and w_c about 1. Thus, the maximum change in ΔG with respect to pH will be about 600 cal according to eq 24a. Also from Figure 6 we conclude that w_c is essentially independent of temperature, that is, $dw_c/d(1/T) = 0$, and further, $dw_h/d(1/T)$ is positive, since w_h decreases with increasing temperature (our experimental data were not precise enough to detect any difference between 5 and 15°). Since the enthalpy changes are negative, $\Delta H'$ becomes less negative with increasing α_h^2 or pH (see Table III below). We estimated that for PLAAA $dw_h/d(1/T)$ was about 300 or $dw_h/dT \cong -0.004$. Thus, in the limiting case where $\alpha_h = \alpha_c = 1$, $\Delta H'$ will be less negative than ΔH by about 600 cal, according to eq 24b. In eq 24c $(w_h - w_c)$ was about 1 and $Td(w_h - w_c)/dT$ about -1. This accounts for the fact that $\Delta S'$ is essentially independent of pH of the solution.

Figure 9 shows the melting temperature, T_m, of PLAAA and PLGA at various pH values. The lines represent the calculated values from the relationship: T_m (°K) $= \Delta H/\Delta S$. The open circles and triangles were obtained from the optical rotation data. From the plots of f_h *vs.* pH at various temperatures (Figure 5), one can read off the f_h's as a function of temperature at constant pH and designate the temperature as T_m at $f_h = 0.5$. The agreement between the two methods of calculation is good. The closed circles and triangles were obtained as follows. By adjusting the pH of the

Table III. Enthalpy Changes and Initiator Factors of Poly-L-α-aminoadipic and Poly-L-glutamic Acids at Various pH

PLAAA				PLGA			
pH	$-\Delta H'/\sigma'^{1/2}$, kcal	$-\Delta H'$, kcal	$\sigma' \times 10^4$	pH	$-\Delta H'/\sigma'^{1/2}$, kcal	$-\Delta H'$, kcal	$\sigma' \times 10^4$
5.20	23.2	0.680	8.6	5.04	29.0	0.795	7.5
5.35	23.2	0.655	8.0	5.11	25.5	0.775	8.8
5.50	22.5	0.625	7.7	5.16	24.6	0.755	9.4
5.60	20.9	0.605	8.4	5.22	24.2	0.735	9.2
5.66	20.5	0.590	8.3	5.31	23.0	0.690	9.0
5.72	20.2	0.580	8.2	5.38	22.8	0.655	8.2

solution to a certain value, say, 5.5, we then varied the temperature of the solution by heating or cooling and measuring its optical rotation at various temperatures, from which we could determine the corresponding f_h's and thereby T_m at $f_h = 0.5$. This method is simple and is used widely when the helix–coil transition of a polymer is studied in a given solvent medium. However, complications arise when the transition is accomplished by the changes in pH of the solution. Heating or cooling such a solution would alter the pH and also the degree of dissociation of the solution. Since the charge-induced transition is sharp, even a slight variation in pH could significantly affect the calculated f_h. This complication perhaps explains the disagreement in Figure 9 between this method and the other two methods mentioned earlier.

Initiation Factor. According to eq 12 we can estimate the initiation factor, σ', by plotting $(1 - 2f_h)^2/f_h(1 - f_h)$ against $(1 - s')^2/s'$ at constant temperature. In the temperature range employed (5–25°), $\sigma' = (5.5 \pm 5) \times 10^{-4}$ for PLAAA and $(15 \pm 5) \times 10^{-4}$ for PLGA, both of which were virtually temperature independent. Our value for PLGA agreed with that reported by Snipp, *et al.* ($\sigma' = (3 \pm 2) \times 10^{-3}$).[7]

To further check whether σ' is independent of pH, we plotted $\ln f_h$ (based on optical rotation) against the reciprocal of absolute temperature and determined the slope $-\Delta H'/4R\sigma'^{1/2}$ at $f_h = 0.5$ (*i.e.*, at $s' = 1$) according to eq 13. With ΔH's known (Figure 8), we can immediately estimate the σ' values at various pH values. The results are summarized in Table III. For all practical purposes we may assume that σ' is virtually independent of pH. The σ' values based on temperature-induced transition are close to those determined with eq 12 from charge-induced transition.

Our studies indicate that the two polymers, PLAAA and PLGA, have essentially the same free energy changes, ΔG (Table II). Furthermore, they appear to have the same degrees of cooperativity, as reflected by their σ' values and enthalpy changes, ΔH (Table III). Thermodynamically, these two polymers show little difference in their helix–coil transition in aqueous solutions. Thus, in the homologous series of PLGA, two methylene groups in the side chains seem to be the critical length for the stability of helical conformation. We have mentioned that un-ionized poly-L-aspartic acid has little helicity,[4c] whereas PLGA is completely helical at pH below 5.[4a] We conclude that a further increase in the number of methylene groups, such as in PLAAA, does not cause additional enhancement of helical stability.

Finally, poly-L-alanine in a block copolymer with poly-DL-glutamic acid is known to be completely helical in aqueous solution.[4a] Replacing one of the hydrogen atoms of the CH_3 group in alanine by a carboxylate group (as in PLGA) or an amino group (as in poly-L-α,β-diaminopropionic acid, unpublished work) immediately destabilizes the helical conformation. This instability can be overcome, however, by lengthening the CH_2 groups in the side chain—to two in the case of the PLGA series and four in the case of the poly-L-lysine series. For the PLGA series the side-chain carboxylate groups presumably are capable of forming hydrogen bonds among themselves, thereby stabilizing the helical conformation. The only exception is poly-L-aspartic acid with only one methylene group where the side-chain COOH group may interfere with the formation of the backbone amide hydrogen bonds of the helical conformation. With the poly-L-lysine series, the side-chain amino groups can only form weak hydrogen bonds; however, the hydrophobic interactions among the long methylene groups must play an important role in the helical stability. These findings are only deduced from studies of polypeptides in aqueous solutions whose side chains are exposed to the solvent medium. Indeed, lowering the water activity, as by the addition of methanol, will transform the partial helices of, say, poly-L-ornithine into perfect helices.[3] At present attempts are being made to classify the 20 amino acids of proteins into helix-forming and non-helix-forming classes. We wish to point out that a non-helix-forming amino acid residue could become a helix-forming one if it were buried inside the protein molecule instead of being exposed on the surface.

Experimental Section

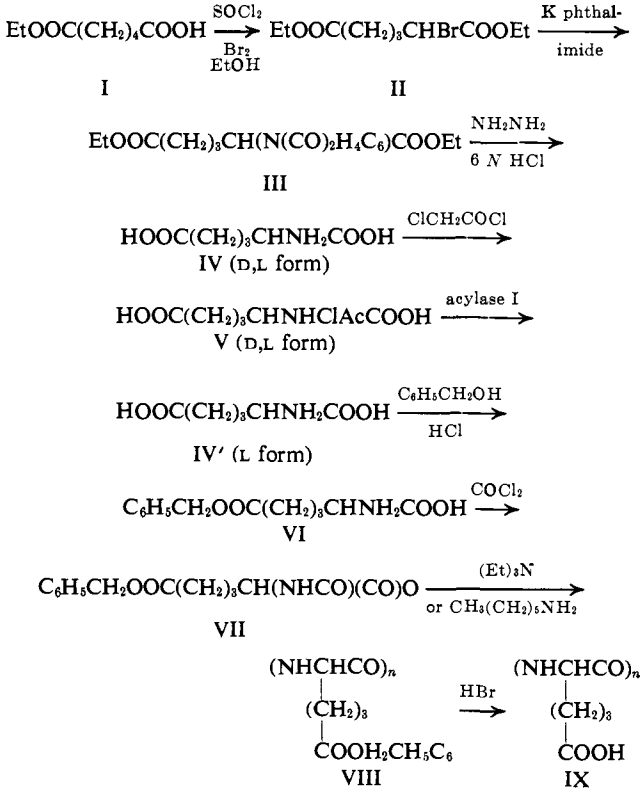
Polypeptides. PBLG (lot G-71; M_w = 340,000) and PLGA (lot G-106; $M_w = 9 \times 10^4$) were purchased from Pilot Chemicals. PBLAA and PLAAA were synthesized according to Scheme I.

Phthalimide Diethyl Adipate (III). The method of preparing III from monoethyl adipate (I) (Eastman Organic Chemicals; mp 28–29°) was essentially the same as the procedure of Schwenk and Papa[25] and Sheenhan and Bolhofer.[26] The product recrystallized from ether–petroleum ether gave an 87.4% yield; mp 50°. *Anal.* Calcd for $C_{18}H_{21}O_6N$: C, 62.2; H, 6.1; N, 4.0. Found: C, 62.3; H, 6.3; N, 4.2.

DL-α-Aminoadipic Acid (IV). III (139 g; 0.40 *M*) was refluxed for 1 hr in 500 ml of ethanol containing 14.1 g of 95% hydrazine (0.42 *M*). The solvent was then evaporated on a rotary evaporator. The crystalline residue was refluxed again in 500 ml of 6 *N* HCl for 1 hr and the mixture allowed to stand overnight in a refrigerator. The crystals were filtered and the filtrate was evaporated to near dryness. Crystalline residues were dissolved in 50 ml of water, 300 ml of ethanol was added, and the solution was adjusted to pH 3.5 with triethylamine. White crystals of IV after filtration were washed with ethanol and dried *in vacuo*. Recrystallization from water–ethanol[27] gave 55.4 g of crystals; yield, 86%. To check for possible contamination in α,δ-diaminoadipic acid, the product

(25) E. Schwenk and D. Papa, *J. Amer. Chem. Soc.*, 70, 3626 (1948).
(26) J. C. Sheehan and W. A. Bolhofer, *ibid.*, 72, 2786 (1950).
(27) J. P. Greenstein, S. M. Birnbaum, and M. C. Otey, *ibid.*, 75, 1994 (1953).

Scheme I



was subjected to thin-layer chromatography on an Eastman chromatogram sheet with a solvent mixture of 40:2:10 isopropyl alcohol–formic acid–water (by volume). Only a single spot was observed (R_f 0.53). *Anal.* Calcd for $C_6H_{11}O_4N$: C, 44.7; H, 6.9; N, 8.7. Found: C, 44.6; H, 7.0; N, 8.5.

Optical Resolution of *N*-Acetyl-DL-α-aminoadipic Acid (V). V was prepared by acetylation of IV according to standard procedure. Optical resolution of V was carried out with hog kidney acylase I by the method of Greenstein, *et al.*[27] The yield was about 80% for the L form ($[\alpha]^{25}D$ 25.0 for 1% in 6 *N* HCl) and 51% for the D form ($[\alpha]^{25}D$ −24.7 for 1% in 6 *N* HCl).

δ-Benzyl-L-α-aminoadipate (VI). VI was prepared by heating IV′ in benzyl alcohol–12 *N* HCl for 30 min,[28] and recrystallized from hot water; yield 40% mp 188°. *Anal.* Calcd for $C_{13}H_{17}O_4N$: C, 62.2; H, 6.8; N, 5.6. Found: C, 62.5; H, 6.9; N, 5.8.

δ-Benzyl-L-α-aminoadipate *N*-Carboxyanhydride (NCA) (VII). The NCA was prepared according to standard procedure using phosgene.[29] After recrystallization (three times) from ethyl acetate–petroleum ether, the product gave an 89–95% yield; mp 67.5°.

PBLAA (VIII). High molecular weight VIII was prepared by polymerizing the NCA in dioxane for 6 hr at room temperature using triethylamine as an initiator. The molar ratio of the NCA to initiator (A/I) was 100 and the NCA concentration 1%. When polymerization was complete, the solution was poured into excess ethanol, causing the polymer to precipitate. The yield was 80%. The polymer had an intrinsic viscosity of 4.40 dl/g in *N*,*N*-dimethylformamide (DMF). The molecular weight was estimated to be 240,000 or the degree of polymerization, n, 1000 from the log $[\eta]$ − log M plot for poly-γ-benzyl-L-glutamate.[30] The sedimentation velocity experiments in DMF showed one symmetric peak with an $S°$ of 3.46 S.

Low molecular weight VIII was prepared in DMF with *n*-hexylamine as an initiator (A/I = 35). The polypeptide was precipitated slowly with ether and the first fraction discarded. The next large fraction (69% yield) was dried, redissolved in DMF, and reprecipitated with water. The sample was dried over P_2O_5 in a vacuum desiccator. This polymer again sedimented as a single peak. By low-speed sedimentation equilibrium studies (c_0 = 0.29–0.57%) (coupled with a synthetic boundary study), the apparent weight-average molecular weight, M_w, was 8,850 (n_w = 38) and the Z-average molecular weight, M_z, 11,800 (n_z = 51). The ratio of M_z/M_w = 1.33. This polypeptide was designated as n = 38. (The partial specific volume, $\bar{v}$, used in calculating the molecular weight was estimated from the relationship of $\bar{v}$ and the number, m, of methylene groups in chemically similar side chains. Since poly-β-benzyl-L-aspartate (m = 1) has a $\bar{v}$ of 0.763 and poly-γ-benzyl-L-glutamate (m = 2) 0.784 ml/g in DMF,[31] PBLAA (m = 3) was assumed to have a $\bar{v}$ of 0.80 ml/g.)

PLAAA (IX). IX was prepared by debenzylation of the high molecular weight VIII sample (n = 1000) in dioxane–chloroform (1:1 by volume) with hydrogen bromide gas.[32] The precipitate was filtered, washed with chloroform, and dried over $CaCl_2$ *in vacuo*. The polymer was then dissolved in water containing equivalent residue moles of NaOH, dialyzed against water, and finally lyophilized to dryness. The yield was about 80%. The polymer had an intrinsic viscosity of 2.04 dl/g in 0.2 *M* NaCl at pH 7 and at 25°. Its molecular weight was estimated to be 100,000 or n = 700, assuming it obeyed the same log $[\eta]$ − log M plot as that of PLGA.[19] Complete removal of the benzyl groups in VIII was confirmed by the absence of any absorption band within the 250–280-nm range.

Chemicals. Dichloroacetic acid (DCA) (Eastman) was freshly distilled under reduced pressure before use. DMF and chloroform were of spectroquality grade (Matheson, Coleman and Bell). All other chemicals were of reagent grade. Water was double distilled.

Preparation of Polypeptide Solutions. PBLAA was dissolved in DMF or in DCA with constant stirring overnight. For transition studies with mixed solvents the polypeptide was first dissolved in DCA; an appropriate volume of chloroform was then added. The polypeptide concentration used was about 0.3%.

PLAAA or PLGA was dissolved in double-distilled water containing equivalent residue moles of NaOH. The solution was then passed through a column of Amberlite MB-1 (Fisher Scientific Co.). Its ionic strength was adjusted by adding an appropriate amount of 1.0 or 1.5 *M* NaCl to the aqueous solution. The concentration of the polypeptide was determined by micro-Kjeldahl analysis. For potentiometric titrations 0.00536 residue mole/l. for PLAAA and 0.0174 residue mole/l. for PLGA were used. For optical rotation measurements they were 0.0167% and 0.0112%, respectively.

Optical Rotation. All optical rotations were measured with a Cary 60 spectropolarimeter. An insulated, channeled aluminum block serving as cell jacket and holder was installed in the cell compartment of the instrument. For measurements at 0° and above, constant temperature was maintained by circulating water (or ice water) through the channels from a Haake constant-temperature bath. For studies below 0° a heat exchanger containing Dry Ice and ethanol, a Haake thermoregulator, and a pump circulating methanol were used. The temperature of the solution in the cell was monitored with a Cu-Constantan thermocouple inserted in the cell holder and read on a millivolt potentiometer (Leeds & Northrup Co.). Fused silica cylindrical cells of various path lengths (Pyrocell S-18-260) were used in all studies. Specially designed cells with heavy walls were used for low-temperature studies and for temperature-induced transition studies. All cells were calibrated with fresh standard sucrose solution (standard sucrose Sample 17, National Bureau of Standards) or with *d*-10-camphorsulfonic acid, which has a circular dichroism, $\epsilon_L - \epsilon_R$, of 2.20 at 290.5 nm.[33] The optical rotation data were expressed in terms of specific rotation, $[\alpha]$, reduced mean residue rotation, $[m']$, or b_0 of the Moffitt equation.[8]

Potentiometric Titration. All titrations were performed in a jacketed glass vessel, which was attached to a constant-temperature bath (±0.05°), under nitrogen flush which had been saturated with water vapor. Standard 0.1 *N* NaOH solution was delivered from a Gilmont ultramicroburet having a capacity of 1 ml. The pH values of aqueous solutions were measured with a Radiometer type pH meter 25 with a scale expander using a GK 2302B type combined electrode. The pH meter was standardized against Beckman buffer solutions for pH values 4, 7, and 10 at different temperatures. The deviation of pH readings from one standard to another was within 0.01 unit over the entire range of pH values, except at 40°, when it was within 0.02 and 0.04 unit at pH values 7 and 10, respectively, after calibration against the pH 4 buffer. Readings of the titrations

(28) N. Izumiya, H. Uchida, and T. Yamashita, *Nippon Kagaku Zasshi*, 79, 420 (1958).
(29) E. R. Blout and R. H. Karlson, *J. Amer. Chem. Soc.*, 78, 941 (1956).
(30) P. Doty, J. H. Bradbury, and A. M. Holtzer, *ibid.*, 78, 947 (1956).
(31) C. DeLoze, P. Saludjian, and A. J. Kovacs, *Biopolymers*, 2, 43 (1964).
(32) D. Ben-Ishai, *J. Org. Chem.*, 17, 1564 (1952).
(33) J. Y. Cassim and J. T. Yang, *Biochemistry*, 8, 1947 (1969).

were taken after each increment of 0.01 or 0.02 ml to a solution of 10 or 15 ml, which was stirred constantly with a Teflon-coated magnetic bar. Below 15° about 1–2 min were required to reach a constant pH reading after each addition of the NaOH solution.

Sedimentation. All sedimentation experiments were performed with a Beckman-Spinco Model E analytical ultracentrifuge. Sedimentation velocity runs were done in single sector cells with Kel F centerpiece and quartz windows and with Schlieren optics. For low-speed sedimentation equilibrium runs, Rayleigh optics were used and the solution and solvent were filled in double sector cells with Kel F centerpieces and sapphire windows. Double-sector capillary centerpieces were used in synthetic boundary measurements. Sedimentation plates were analyzed with a Nikon microcomparator. The number and positions of the fringes for the sedimentation equilibrium runs were read and used to calculate the apparent weight- and Z-average molecular weights, M_w^{app} and M_z^{app}, from a computer program by Dr. D. C. Teller.[34] The reciprocals of M_w and M_z were determined by extrapolating to zero concentration the straight lines of $1/M_w^{app}$ *vs.* the mean concentration (the average of the concentrations at the meniscus and bottom) and of $1/M_z^{app}$ *vs.* twice the mean concentration.

Acknowledgment. We thank Professor L. Peller for several valuable discussions and Professor H. Martinez for providing the computer program for the solution of the Zimm–Bragg equation.

(34) D. C. Teller, Doctoral Dissertation, University of California, Berkeley, Calif., 1965.

A Computer Investigation into the Origin of the Code

Marc S. Rendell

Contribution from the Department of Medicine and Program in Biophysics, State University of New York Downstate Medical Center, Brooklyn, New York 11203. *Received September 28, 1971*

Abstract: Quantum mechanical methods are used to simulate interactions between glycine and polynucleotide base sequences. High-magnitude, configurationally specific interactions with features similar to Watson–Crick base pairing are obtained. Glycine manifests preferential affinities in complexing with the stacked base pairs. The feasibility of selective interaction between polar amino acids and polynucleotide base sequences is considered. The implications of such interactions for the origin of the genetic code are discussed.

1. Introduction

The genetic code has two functions. The first is to preserve information existing in polynucleotide sequences by strict base complementarity upon replication. The other is to permit usage of available information through the relation of a specific amino acid to a given trinucleotide sequence during translation.

The forces subserving the first function are those of Watson–Crick base pairing and are well understood. The specificity of the physical association of adenine to thymine and uracil and of guanine to cytosine has been confirmed both theoretically[1-3] and experimentally.[4-6] Specificity in this case has been demonstrated to be due to the varying feasibility of hydrogen bonding between the charged groups of the bases.

Unlike base pair complementarity, the physical basis for the translation of codon into amino acid remains unclarified. It is clear that distinctive, selective physical interactions between polynucleotide bases and amino acids, either singly or as the side chains of polypeptides, must be involved. In the contemporary translation mechanism, these physical interactions must take place in a complex system consisting of a given tRNA and its complementary aminoacyl synthetase. The crucial interactions involved here most likely reflect the high degree of structure in this system.

The process of translation in the primitive coding system, unlike the contemporary mechanism, could not have taken place in a highly structured environment. Indeed, the primordial coding system arose as a consequence of physical interactions occurring among the relatively unstructured polymers and polymeric subunits in the primitive "soup."[7-10] Here, base pairing ensured conservation of informational sequences. There is far less certainty as to the type of interactions responsible for the primitive translational process. Experimental evidence on this point has only recently begun to appear with the demonstration that polyarginine and polylysine exhibit preferential binding to polynucleotides of varying base composition.[11,12]

In this regard, theoretical investigation indicates that selective interactions between glycine and the individual nucleotide bases are quite feasible and are due to selective component interactions between the carboxyl and amino groups of glycine and oppositely charged moieties of the bases.[13,14] In this paper, it is shown that glycine is capable of selective, configurationally

(1) B. Pullman, P. Claverie, and J. Caillet, *Proc. Nat. Acad. Sci. U. S.*, **55**, 904 (1966).
(2) R. Rein, P. Claverie, and M. Pollak, *Int. J. Quantum Chem.*, **2**, 129 (1968).
(3) H. A. Nash and D. F. Bradley, *Biopolymers*, **3**, 261 (1965).
(4) J. S. Binford, Jr., and D. M. Holloway, *J. Mol. Biol.*, **31**, 91 (1968).
(5) R. A. Newmark and C. R. Cantor, *J. Amer. Chem. Soc.*, **90**, 5010 (1968).
(6) Y. Kyogoku, R. C. Lord, and A. Rich, *ibid.*, **89**, 497 (1967).
(7) F. H. C. Crick, *J. Mol. Biol.*, **38**, 367 (1968).
(8) L. E. Orgel, *ibid.*, **38**, 381 (1968).
(9) C. R. Woese, *ibid.*, **43**, 235 (1969).
(10) A. M. Lesk, *J. Theor. Biol.*, **27**, 171 (1970).
(11) J. C. Lacey and K. M. Pruitt, *Nature (London)*, **223**, 799 (1969).
(12) S. W. Fox, J. C. Lacey, and A. Yuki at the Surface and Colloid Chemistry Symposium, Toronto, Canada, 1970.
(13) R. Rein, M. S. Rendell, and J. P. Harlos in "Molecular Orbital Studies in Chemical Pharmacology," by L. B. Kier, Ed., Springer Verlag, New York, N. Y., 1971.
(14) M. S. Rendell, J. P. Harlos, and R. Rein, *Biopolymers*, **10**, 2083 (1971).