

inant factor in the seemingly anomalous titration curves of the helix and is unimportant in polyglutamic acid under conditions in which the polymer is soluble.

Hill,<sup>23</sup> using a linearized Poisson–Boltzmann equation, and Nagasawa and Holtzer,<sup>24</sup> using numerical integration of the nonlinearized Poisson–Boltzmann equation, have calculated the electrostatic interaction for charges smeared over the surface of a cylinder. These calculations cannot explain the increased electrostatic interaction upon increase in leucine content unless the dielectric constant of the solvent is treated arbitrarily as an adjustable parameter. However, the side-chain carboxyls carry discrete charges and discrete charge state models are more realistic. The equations for the electrostatic potential of discrete charges on a cylinder have not been solved in closed form. Tanford and Kirkwood<sup>25</sup> have obtained solutions for discrete charges attached to spheres of low dielectric medium immersed in a high dielectric solvent. As the discrete charges are moved from the interface and embedded further and further in the low dielectric medium, the electrostatic interaction was found to increase enormously. Embedding the charges causes more of the electric lines of force to act through the low dielectric medium and thus give rise to an increased electrostatic interaction. Molecular models of the  $\alpha$ -helix with both glutamate and leucine side chains attached show the leucine side chain to protrude slightly further than the glutamate side chain. It is thus possible that the increased electrostatic interaction is a result of the increased hydrocarbon content in the vicinity of the carboxyls. Alternatively, the leucine could be looked upon as lowering the effective dielectric constant of the medium through which the charges interact.<sup>26</sup> Changes in water structure near the hydrocarbon side chains may also contribute to the

(23) T. Hill, *Arch. Biochem. Biophys.*, **57**, 229 (1955).

(24) M. Nagasawa and A. Holtzer, *J. Am. Chem. Soc.*, **86**, 531 (1964).

(25) C. Tanford and J. Kirkwood, *ibid.*, **79**, 5333 (1957).

(26) C. Tanford, *ibid.*, **79**, 5348 (1957).

apparent alteration of the effective dielectric constant. If eq. 3 is graphically integrated from a charge density of 0 to 0.2, the electrostatic free energy per unit charge density in the helix may be obtained. Results of such integration are given in Table III. The observed increase in electrostatic free energy is not unreasonable when compared to the results of Tanford and Kirkwood.

Table III. Electrostatic Free Energy in the Helix

Copolymer (% leucine)	$\Delta G_{el}$ , <sup>a</sup> cal./mole/unit charge density
0	250
5.7	200
12.4	485
16.2	570
24.6	960
29.1	1000

<sup>a</sup> At  $\bar{Z}/DP = 0.2$ , a charge density at which all the polymers are still predominantly helical.

Even though we propose that the increased electrostatic interaction in the copolymers results from a lowering of the effective dielectric constant in the region between interacting carboxylates, it does not follow that a corresponding change in  $pK_0$  should occur. The carboxylates may be looked upon as constrained to positions on the surface of a rough cylinder of low dielectric material. Interacting carboxylates have many electric lines of force acting through the low dielectric cylinder. Changes in “roughness” of the surface of the cylinder can produce measurable changes in the electrostatic interaction. In order to shift  $pK_0$ , changes must occur in the electrostatic interaction between a carboxylate and a proton in the solvent. However, in this case the electric lines of force run predominantly through the solvent. Changes in the roughness of the low dielectric cylinder will have far less effect on the interaction.

## The Stability of the Helical Conformation of Random L-Leucine–L-Glutamic Acid Copolymers in Aqueous Solution<sup>1</sup>

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Contribution from the Department of Chemistry, University of Iowa, Iowa City, Iowa. Received February 8, 1965

*The stability in aqueous solution of the helical conformation of random copolymers of L-leucine and L-glutamic acid containing 0 to 30% leucine has been investigated. The standard free-energy change for the transition (coil to helix) was resolved into two components independent of copolymer composition, one characteristic of glutamic acid and the other of leucine. For uncharged glutamic acid residues in 0.200 M NaCl at 25°  $\Delta G^\circ = -130$  cal. mole<sup>-1</sup>,  $\Delta H^\circ \approx -1.0$  kcal. mole<sup>-1</sup>,*

*and  $\Delta S^\circ \approx -2.8$  cal. deg.<sup>-1</sup> mole<sup>-1</sup>. The helix–coil transition temperature for uncharged polyglutamic acid is predicted to be above 90°. For leucine  $\Delta G^\circ = -840$  cal. mole<sup>-1</sup>, indicating the helix with leucine side chains is more stable than the uncharged polyglutamic acid helix relative to their respective random coil conformations.*

(1) Taken in part from the Ph.D. thesis of R. E. Nylund, University of Iowa, 1964.

In the preceding paper<sup>2</sup> the synthesis and titration curves of random L-leucine-L-glutamic acid copolymers were presented. Electrostatic interaction of side-chain carboxyls in the helix was found to be profoundly affected by the presence of leucine. The helical stability of the copolymers is of much interest, but changes in electrostatic interaction must be separated from other factors affecting the stability of copolymers in a helical conformation.

Optical rotation and ultraviolet absorption are frequently used to estimate the helical content of polypeptides. From the work of Tinoco, Halpern, and Simpson,<sup>3</sup> it would appear that the substitution of a leucine for a glutamic acid side chain should have little effect on the ultraviolet absorption of the amide in either the helical or random coil conformations. The optical rotatory dispersion, however, may depend somewhat on the nature of solvent and side chains.<sup>4</sup> For this reason ultraviolet absorption was chosen as the means to follow the helix-coil transition.

### Experimental

The synthesis of copolymers having a random monomer distribution along the chain has been described.<sup>2</sup> The copolymers used in this study are described in Table II of the preceding paper.

Ultraviolet absorption was measured with a Cary 14 spectrophotometer using Beckman far-ultraviolet short-path-length cells with Teflon spacers. The path length of the cells was 0.038 cm., determined by the fringe technique. Aqueous solutions of the polymer ( $\leq 0.1$  wt. %) and of specified NaCl concentration were used in all determinations. Chloride ion begins to absorb significantly in the wave length region of interest. A NaCl solution of the same concentration as in the polymer solution was the reference solution. At wave lengths of 200 m $\mu$  and above matching errors were small. Molar absorptivity  $\epsilon$  ( $M^{-1}$  cm.<sup>-1</sup>) is reported with concentration as moles of amino acid residues per liter. A typical absorbance as a function of pH experiment was performed identical with a titration curve determination except that at each change in pH the absorbance was measured by introducing an aliquot of the solution into the spectrophotometer cell by means of a syringe. Measurements were at 25°, extending from pH  $\sim 8$  down to the precipitation points of the polymers (in 0.2 M NaCl, pH 3.6 for PGA to pH 4.6 for the 29.1 copolymer). Absorbance measurements on a given solution were reproducible to  $\pm 0.01$  and pH to  $\pm 0.03$ .

### Results

It is well-documented that there is a hypochromic effect upon aligning the chromophores of the amide bond in a helical conformation.<sup>3,5-7</sup> The absorption of polyglutamic acid in 0.200 M NaCl as a function of pH was measured at several wave lengths. These data

(2) R. E. Nylund and W. G. Miller, *J. Am. Chem. Soc.*, **87**, 3537 (1965).

(3) I. Tinoco, A. Halpern, and W. Simpson in "Polyamino Acids, Polypeptides, and Proteins," M. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p. 147.

(4) G. D. Fasman, C. Lindblow, and E. Bodenheimer, *Biochemistry*, **3**, 155 (1964).

(5) K. Imahori and J. Tanaka, *J. Mol. Biol.*, **1**, 359 (1959).

(6) P. Doty, *J. Polymer Sci.*, **49**, 129 (1961).

(7) J. Applequist and J. L. Breslow, *J. Am. Chem. Soc.*, **85**, 2869 (1963).

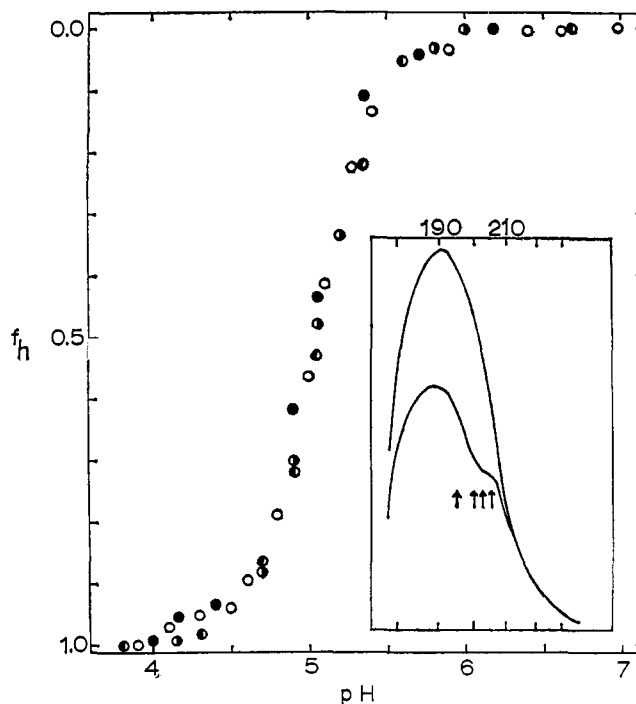


Figure 1. Fraction hypochromicity of polyglutamic acid as a function of pH at 195 (●), 200 (○), 202.5 (◐), and 205 (◑) m $\mu$ . Inset shows the dispersion curves for helix and for random coil.<sup>3</sup>

are shown in Figure 1, where the absorption is reported as the fraction of the total hypochromism observed at that wave length. In order of increasing wave length, the high pH values of  $\epsilon$  ( $\times 10^3$ ) were 7.1, 5.6, 4.0, and 3.0, while the low pH limits were 4.6, 3.1, 2.6, and 2.1. Higher molecular weight samples gave results in agreement with those reported here. When normalized to per cent of total hypochromism, different wave lengths are seen to give superimposable curves within experimental error. A wave length of 200 m $\mu$  was chosen for all other measurements. It is low enough so that there is still an appreciable difference in absorption between the helical and random coil conformation but one at which carboxylate absorption is of minor importance. The absorption at 200 m $\mu$  in 0.200 M NaCl as a function of pH for the various copolymers is shown in Figure 2. The molar absorptivity at high pH is  $5.40 \pm 0.14 \times 10^3$  and is independent of leucine content. This was expected and  $\epsilon_{200}$  is in reasonable agreement with previously published data.<sup>3,5-7</sup> At low pH, the absorptivity levels off shortly before the precipitation point is reached. The minimum absorptivity,  $3.05 \pm 0.1 \times 10^3$ , is essentially the same for each polymer. Beer's law is followed in the concentration range investigated.

As the leucine content increases, the helix-coil transition is shifted toward higher pH, except for the anomalous 5.7 copolymer. Fasman, *et al.*,<sup>4</sup> using optical rotatory dispersion to determine helical content, observed the same effect. They interpret this as indicating that a higher degree of ionization ( $\alpha$ ) is required to bring about the transition as the leucine content is increased. This interpretation assumes the titration curve is independent of leucine content. This assumption has been shown to be invalid.<sup>2</sup> To bring out the effect of the change in the titration curve, the

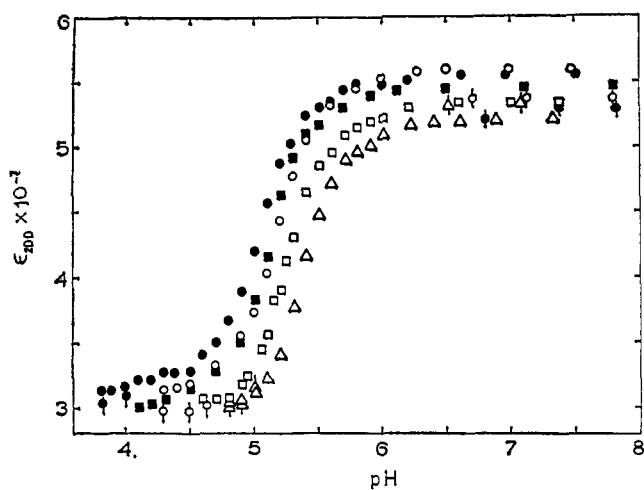


Figure 2. Molar (residue) absorptivity at 200  $m\mu$  of leucine-glutamic acid copolymers. Mole per cent leucine:  $\bullet$ , 0.0;  $\blacksquare$ , 5.7;  $\circ$ , 12.4;  $\square$ , 22.9;  $\triangle$ , 29.1. Precision of determination of maximum and minimum absorptivity is indicated by results of duplicate experiments ( $\circ$ ,  $\phi$ ;  $\bullet$ ,  $\phi$ ;  $\triangle$ ,  $\hat{\triangle}$ ).

absorption data are plotted in Figure 3a vs. the degree of dissociation. The regular trend with leucine content has now disappeared. In Figure 3b the absorption data are shown as a function of the average charge per side chain ( $\bar{p}_z$ ). A regular trend with leucine content is again evident but it is just the reverse of Figure 2. In fact, a lower charge density will bring about the helix-coil transition as the leucine content increases. This, however, is no indication that the inherent stability of the helix is reduced by incorporation of leucine since the titration curves indicate the electrostatic interaction in the helix also increases. In order to determine the effect of introducing leucine residues into the polymer on the inherent stability of the helical conformation in aqueous solution, the effect of the charge on the  $\gamma$ -carboxyls must be eliminated.

What is needed, then, is a parameter which characterizes the transfer of a leucine residue from an uncharged helix to an uncharged random coil. Lack of solubility of pure poly-L-leucine in water precludes actually performing this experiment. Fortunately, it is possible to obtain such information indirectly using experimental data already presented. Zimm and Rice,<sup>8</sup> using a statistical mechanical approach to the helix-coil transition in charged polypeptides, define a parameter  $s$ , the equilibrium constant for the transfer of an ionizable residue to a section of *uncharged helix* from the adjacent section of *uncharged random coil*. The standard free energy change for this process is

$$\Delta G^\circ = -RT \ln s \quad (1)$$

If the polymer molecule assumes the pure helical conformation at  $\alpha = 0$  and completely disordered conformations at  $\alpha = 1$ , Zimm and Rice further show that

$$\ln s = 2.3 \int_{\text{pH}(\alpha=0)}^{\text{pH}(\alpha=1)} (\alpha_c - \alpha) d(\text{pH}) \quad (2)$$

where  $\alpha_c$  is the degree of dissociation of the  $\gamma$ -carboxyls in the random coil and  $\alpha$  is the actual degree of

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

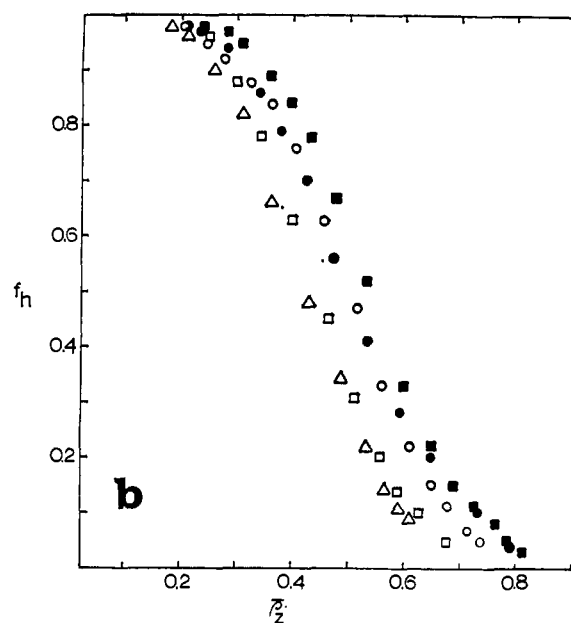
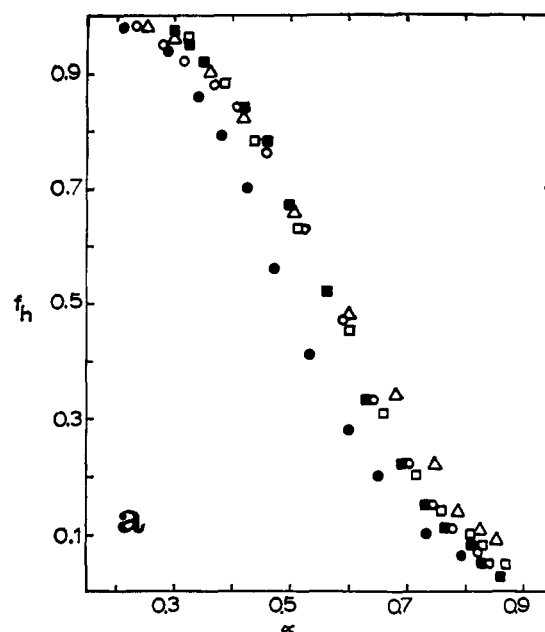


Figure 3. Fraction hypochromicity at 200  $m\mu$  as a function of (a) degree of dissociation; (b) average charge per side chain. Symbols have the same meaning as in Figure 2.

dissociation of the  $\gamma$ -carboxyls in the polymer molecule. The experimental titration curve of the polymer yields  $\alpha$ , and  $\alpha_c$  over the entire pH range may be obtained with reasonable validity by extrapolation from the random-coil region of the titration curve.<sup>2,8,9</sup> Since  $\alpha_c$  and  $\alpha$  can thus be obtained from the titration curve of the polymer,  $\ln s$  and subsequently  $\Delta G^\circ$  may be evaluated by graphical integration of eq. 2.

An alternate procedure to obtain the same information is the method of Nagasawa and Holtzer.<sup>9</sup> At any point in the helix-coil transition it is assumed that molecules will either be completely helical or completely random coil, a somewhat unrealistic though inconsequential<sup>9</sup> assumption. The concentration ( $C_{h \rightarrow}$ ) of helical molecules with a specified number of

(9) M. Nagasawa and A. Holtzer, *J. Am. Chem. Soc.*, **86**, 538 (1964).

charges ( $z$ ) is written in terms of the concentration ( $C_{h^0}$ ) of helical molecules with zero charge, using a simple, multiple equilibria expression. Thus

$$C_{h^-} = C_{h^0}(K_0/a_{H^+})^Z(1/\bar{\gamma}_z) \quad (3)$$

where  $K_0$  is the intrinsic dissociation constant of the  $\gamma$ -carboxyl (4.45),<sup>2,9</sup>  $\bar{\gamma}_z$  the mean activity coefficient of species  $C_{h^-}$ , and  $a_{H^+}$  is the hydrogen ion activity. Analogous expressions can be written for random-coil molecules of varying charge state. In this manner the total concentration of helical (or random-coil) molecules may be written as a function of the uncharged helical (or random-coil) molecules. These equations may be manipulated<sup>9</sup> to give an expression for the standard free-energy change ( $\Delta G^\circ_{c-h}$ ) for taking an uncharged random coil molecule having  $n$   $\gamma$ -carboxyls to an uncharged helical molecule. If graphical integration is carried to the midpoint of the helix-coil transition (h-c midpt.),

$$\Delta G^\circ = \Delta G^\circ_{c-h}/n = -RT \ln s = -2.3RT \int_{pH(\alpha=0)}^{pH(h-c \text{ midpt})} (\alpha_c - \alpha_h) d(pH) \quad (4)$$

where  $\alpha_h$  is the degree of dissociation of the  $\gamma$ -carboxyls in the helix. Nagasawa and Holtzer have shown that evaluation of  $\ln s$  by either eq. 2 or eq. 4 is equivalent.

The free-energy change ( $\Delta G^\circ$ ) for polyglutamic acid and the various copolymers was determined, using both methods, by measuring appropriate areas with a planimeter. The intrinsic dissociation constant of the  $\gamma$ -carboxyls was assumed to be independent of the leucine content of the polymer<sup>2</sup> and  $pK_0$  was taken as 4.45.<sup>9</sup> In the region extending from the precipitation point of a polymer to  $\alpha = 0$ ,  $pH - \log [\alpha/(1 - \alpha)]$  was extrapolated from the observed values just above the precipitation point to 4.45 at  $\alpha = 0$ . A slightly curved extrapolation was used similar to the extrapolation in Figure 7 of the preceding paper. From the extrapolated curve,  $\alpha$ , equal to  $\alpha_h$  in this region, as a function of pH was obtained for the low pH region. To obtain the titration curve for the pure random coil,  $pH - \log [(\alpha/(1 - \alpha))]$  was extrapolated from the pure random coil region of the titration curve to a value of 4.45 at  $\alpha = 0$ , using slightly curved extrapolations similar to those in ref. 2 and 9. Although there was some arbitrariness in making the extrapolation, in general the subsequent areas which had to be measured were large compared to the uncertainty in the areas introduced by the not so precise extrapolations. The helix-coil midpoint was taken as the pH at which the hypochromism in the ultraviolet absorption was one-half of its maximum value. Extrapolation of  $\alpha_h$  past the pure helix part of the experimental titration curve was aided by assuming that

$$\alpha - \alpha_h = \alpha_c - \alpha \quad (5)$$

at the pH of the midpoint of the helix-coil transition. The results of the free-energy determinations are given in Figure 4 for the polymers in 0.2 M NaCl. The precision of the determinations is estimated to be better than 10%. Again, the 5.7 copolymer is anomalous. The value of polyglutamic acid is in good agreement with that found by Nagasawa and Holtzer. The method of Zimm and Rice tends to

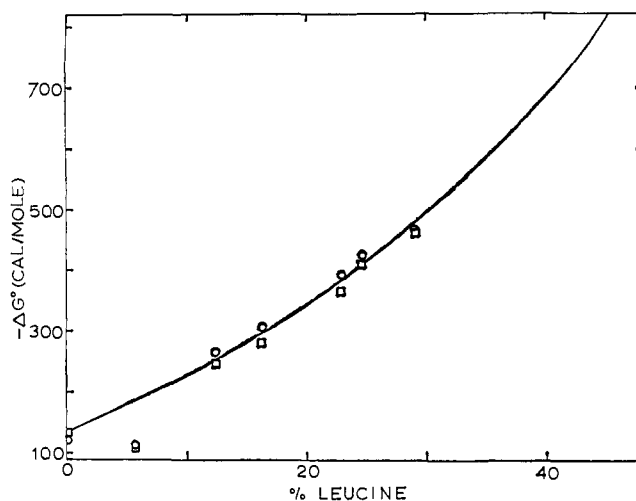


Figure 4. Standard free-energy change for taking uncharged residues from random coil to helix as a function of leucine composition, determined by the methods of Nagasawa and Holtzer (O) and Zimm and Rice (□). Solid line calculated from eq. 6.

give lower values than that of Nagasawa and Holtzer though the differences are not statistically significant.

### Discussion

The helix-coil transition brought about by pH change at constant temperature is the charge-induced transition. The standard free-energy change  $\Delta G^\circ$  will correspond to transferring an uncharged carboxyl from the random coil to an adjacent helical section. When either procedure for obtaining  $\Delta G^\circ$  from the experimental titration curve is applied to the leucine-glutamic acid copolymers,  $\Delta G^\circ$  will still refer to the transfer of an uncharged carboxyl from coil to helix. Since the leucine is randomly distributed and the helix-coil transition is fairly cooperative,<sup>10</sup> the transfer of a carboxyl (glutamic acid residue) must also involve transfer of leucine residues from coil to helix. This must be reflected in the experimental value for  $\Delta G^\circ$ . For every glutamic acid (carboxyl) transferred at any point in the transition,  $f_{leu}/f_{glu}$  leucine residues must on the average be transferred, where  $f_{leu}$  and  $f_{glu}$  refer to the mole fraction of leucine and glutamic acid, respectively, in a copolymer. If it is naively assumed that the free-energy change for transfer of a glutamic acid or of a leucine residue is independent of copolymer composition, the observed  $\Delta G^\circ$  will be given by

$$\Delta G^\circ = \Delta G^\circ_{glu} + (f_{leu}/f_{glu})\Delta G^\circ_{leu} \quad (6)$$

where  $\Delta G^\circ_{leu}$  is the standard free-energy change for transfer of a leucine residue in the leucine-glutamic acid polymers from random coil to helix and  $\Delta G^\circ_{glu}$  is the analogous quantity for an uncharged glutamic acid residue. A least-squares analysis of the data, excluding the 5.7 copolymer, using eq. 6 gives  $\Delta G^\circ_{glu} = -130 \pm 8$  and  $\Delta G^\circ_{leu} = -840 \pm 30$  cal. mole<sup>-1</sup>. Using these values the theoretical curve for  $\Delta G^\circ$  as a function of leucine content of the polymer is shown as the solid curve in Figure 4. The quantitative validity of the theoretical curve beyond the experimental points is obviously unknown. Qualitatively, however, the general shape of the curve must be correct so long

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

as  $\Delta G^\circ$  is defined as in eq. 1. As the percentage of carboxyls decreases, those which remain must carry along more and more leucine residues in undergoing the transition; in the limit where  $f_{\text{glu}} \rightarrow 0$ ,  $\Delta G^\circ \rightarrow -\infty$ . Experimentally this situation could not be approached even if there were no problems with solubility. As the leucine composition is continuously increased, at some point the electrostatic repulsion in the remaining glutamates will be insufficient to bring about the transition to the random coil.

The data show that in 0.2 M NaCl the leucine contribution to the copolymer helix is about 700 cal. mole<sup>-1</sup> more stable than the glutamic acid contribution, relative to the random coil conformations. The conformation offering the least contact with water should be most favored the more hydrocarbon the side chain. It therefore seems reasonable that the helical conformation is more favorable for leucine than for glutamic acid, assuming backbone contributions are independent of side chain. The success in separating the observed  $\Delta G^\circ$  into two parts independent of copolymer composition is much more surprising. If side-chain-side-chain interactions are important, the interactions will, in general, depend on the nature of the side chains. In the  $\alpha$ -helix, a side chain might interact with another side chain three or four units further along the chain as well as with adjacent side chains. Assuming that each side chain can interact with four neighboring side chains, the probability that a leucine will be in a position to interact with at least one other leucine is 0.18 for the 5.7 copolymer and increases to 0.75 for the 29.1 copolymer. If hydrophobic interactions in the context of Nemethy and Scheraga<sup>11</sup> were important here,  $\Delta G^\circ_{\text{glu}}$  and  $\Delta G^\circ_{\text{leu}}$  would be a function of the number of leucine-leucine contacts. Although it is possible that the simple separation into composition-independent factors is fortuitous, no compelling argument is evident to force a more elaborate analysis. In the helical conformation, only the outermost part of the side chain is available to the solvent. If only the innermost portion of the side chains are in hydrophobic contact, a leucine-glutamic acid contact may be little different from a leucine-leucine contact. In this case separation into composition-independent factors might be expected. This forces us to predict that  $\Delta G^\circ_{\text{leu}}$  will be the same in all copolymers containing leucine and in which the inner portion of the other side chains,  $\beta$ - and perhaps  $\gamma$ -positions, resemble leucine.

The titration curves<sup>2</sup> obtained in 0.020 M NaCl were analyzed analogously to the 0.200 M NaCl data. Titration curves for only two copolymers are available and the analysis is much less precise. In 0.020 M NaCl  $\Delta G^\circ_{\text{glu}} = -225$  cal./mole,<sup>9</sup> an approximately 100 cal./mole decrease over  $\Delta G^\circ_{\text{glu}}$  in 0.200 M NaCl. The copolymer data indicate  $\Delta G^\circ_{\text{leu}}$  in 0.020 M NaCl is decreased at least 100 cal./mole over the 0.200 M NaCl value. The significance of this finding is not known.

**Temperature Dependence.** In addition to the stability of the helical conformation at 25° in 0.2 M NaCl, the temperature dependence of the stability is of interest. Titration can be carried out at fixed temperatures and  $\Delta G^\circ$  determined as in the previous section at each temperature. In the case of polyglutamic acid, the

standard free-energy change for taking an uncharged glutamic acid residue from coil to adjacent helical section is obtained as a function of temperature. The standard enthalpy and entropy changes for this process are immediately determinable. With a copolymer,  $\Delta G^\circ$  at each temperature must first be separated into factors characterizing each monomer, using eq. 6. Polyglutamic acid and the 29.1 copolymer were titrated at 10, 25, and 40°, equipment being unavailable to extend the titration over a wider range. For polyglutamic acid  $\Delta H^\circ_{\text{glu}} \approx -1.0$  kcal. mole<sup>-1</sup> and  $\Delta S^\circ_{\text{glu}} \approx -2.8$  cal. deg.<sup>-1</sup> mole<sup>-1</sup>. These values may stand adjusting somewhat when the analysis is extended over a wider temperature range.

In contrast to other estimates of  $\Delta H^\circ$  and  $\Delta S^\circ$  for polyglutamic acid<sup>12,13</sup> which have been obtained, the values determined here do not depend on a knowledge of the initiation parameter associated with the helix-coil transition. Moreover, previous estimates refer to a slightly different process, the transfer of a residue from coil to helix at essentially constant pH, the side chains having a charge in each conformation characteristic of that pH. Therefore,  $\Delta H^\circ$  and  $\Delta S^\circ$  so determined will consist of a charge-dependent and a charge-independent part. Our evaluation will contain only the charge-independent part. Applequist reports  $\Delta H^\circ = -290$  cal. mole<sup>-1</sup> and  $\Delta S^\circ = -0.95$  cal. deg.<sup>-1</sup> mole<sup>-1</sup> in dioxane-water<sup>12</sup> (using a corrected value of the initiation parameter<sup>10</sup>) and  $\Delta H^\circ = -630$  cal. mole<sup>-1</sup> and  $\Delta S^\circ = -2.0$  cal. deg.<sup>-1</sup> mole<sup>-1</sup> in water.<sup>13</sup> The difference between Applequist's and our values may represent the charge contribution or possibly experimental error. From  $\Delta H^\circ$  and  $\Delta S^\circ$  the helix-coil transition temperature for uncharged polyglutamic acid in 0.200 M NaCl may be determined. The transition temperature is approximately the boiling point of water. Once the effect of charge is removed polyglutamic acid is seen to be quite stable, not unlike polyalanine in aqueous solution.<sup>14</sup>

A similar analysis of the 29.1 copolymer indicates  $\Delta H^\circ_{\text{leu}}$  is about half as negative as  $\Delta H^\circ_{\text{glu}}$  and  $\Delta S^\circ_{\text{leu}}$  is much less negative than  $\Delta S^\circ_{\text{glu}}$  and may even be slightly positive. The data are too uncertain to quote numerical values. The direction of the change is compatible with partial removal of a hydrocarbon from contact with water. The helical conformation involving leucine residues will be stable to even higher temperatures than was estimated for uncharged polyglutamic acid.

## Summary

Our study of random copoly- $\alpha$ -L-leucine-L-glutamic acid may be summarized as follows.

(1) The 5.7% leucine copolymer is anomalous in all of its properties. It seems likely that this polymer is contaminated or has not been analyzed correctly, but we have no evidence to support this conjecture.

(2) Before any statements can be made concerning the effect of leucine on helical stability, charge effects must be eliminated.

(3) The observed standard free-energy change for the

(12) J. Applequist, *J. Chem. Phys.*, **38**, 934 (1963).

(13) J. Rifkind and J. Applequist, *J. Am. Chem. Soc.*, **86**, 4207 (1964).

(14) W. Gratzer and P. Doty, *ibid.*, **85**, 1193 (1963).

(11) G. Némethy and H. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).

helix-random coil transition may be described by values characteristic of glutamic acid residues and of leucine residues. The values are independent of the copolymer composition.

(4) The thermodynamic parameters for taking an uncharged glutamic acid residue from random coil to helix in 0.200 *M* NaCl are  $\Delta G^\circ = -130$  cal. mole<sup>-1</sup>,  $\Delta H^\circ = -1.0$  kcal. mole<sup>-1</sup>, and  $\Delta S^\circ = -2.8$  cal. deg.<sup>-1</sup> mole<sup>-1</sup>. The helix-coil transition temperature is greater than 90°. For leucine in leucine-glutamic acid copolymers,  $\Delta G^\circ = -840$  cal. mole<sup>-1</sup>;  $\Delta H^\circ$  and  $\Delta S^\circ$  are more positive than the corresponding values

for glutamic acid residues. The free-energy changes refer to the transition at 25°.

(5) The effect of ionic strength on  $\Delta G^\circ$  is approximately the same for glutamic acid residues as for leucine residues.

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## The Charge-Induced Helix-Random Coil Transition in Aqueous Solution<sup>1</sup>

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*Contribution from the Department of Chemistry, University of Iowa, Iowa City, Iowa. Received February 8, 1965*

*The parameters describing the helix-random coil transition in polypeptides have been determined from experimental data. The initiation parameter ( $\sigma$ ) is  $3 \pm 2 \times 10^{-3}$  for unfractionated, high molecular weight polyglutamic acid and leucine-glutamic acid copolymers in aqueous solution. It is independent of NaCl concentration. In monodisperse oligomers and fractionated polyglutamic acid,  $\sigma$  must vary with chain length if the data are forced to fit a two-parameter equation. At very short chain lengths  $\sigma$  increases. The calculated shape of the transition agrees well with hypochromism measurements.*

### Introduction

The helix to random coil transition in synthetic polypeptides is an example of a one-dimensional cooperative process and has been of considerable interest from the time of its initial observation.<sup>2</sup> The transition has received numerous theoretical treatments<sup>3</sup> each based on an Ising-type model.<sup>4</sup> At least two parameters are needed to describe the transition; (1) a parameter ( $\sigma$ ) characterizing the initiation of a helical section and (2) a parameter ( $s$ ) characterizing the growth of previously formed helical sections. It is the initiation parameter which controls the cooperativeness of the transition such that the transition becomes more cooperative as  $\sigma$  becomes smaller. The transition may be induced by changes in temperature,

solvent, or side-chain charge. Generally it has been assumed that the difficulty in initiating a helical section was entirely entropic. For this reason Zimm and Bragg<sup>3c</sup> suggested that  $\sigma$  may be independent of both solvent and side chain. Recent evidence indicates that  $\sigma$  is not constant<sup>5</sup> and that there may be enthalpic contributions as well.<sup>6</sup>

There are few quantitative analyses of experimental data in terms of the theoretical parameters. Zimm and Bragg estimate  $\sigma$  to be  $2 \times 10^{-4}$  for poly- $\gamma$ -benzyl- $\alpha$ ,L-glutamate in dichloroacetic acid-dichloroethane. Calorimetric data<sup>7</sup> for the same system indicate a somewhat smaller value. In poly- $\epsilon$ -carboboxy-L-lysine  $\sigma$  may be up to an order of magnitude smaller.<sup>8</sup> Rifkind and Applequist<sup>5</sup> have recently estimated  $\sigma$  to be  $5 \times 10^{-3}$  for polyglutamic acid in aqueous solution. In each case the transition was induced thermally.

The charge-induced transition in aqueous solution has received even less attention. Synthetic polypeptides composed of naturally occurring amino acids are generally insoluble in aqueous solution unless the polymer contains some ionic side chain. By ionizing the side chain the helix-random coil transition is induced. In fact it is far more difficult to find the solvent and temperature necessary to bring about the pure thermal transition in nonionic polypeptides than to find conditions under which the charge-induced transition can be observed. Zimm and Rice,<sup>3e</sup> in developing the theory for the charge-induced transition, analyze the titration data of Wada.<sup>9</sup> Although they find  $\sigma$  to be  $1.4 \times 10^{-3}$  for polyglutamic acid (PGA) in 0.0133 *N* NaCl, a value of  $10^{-4}$  was used in all their calculations.

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