

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.

*Acknowledgments.* This investigation was supported in part by Public Health Service Research Grant GM-08409. W. G. M. wishes to thank the John Simon Guggenheim Foundation for a fellowship and Professor P. J. Flory and the Chemistry Department of Stanford University for their kind hospitality during the writing of the manuscript.

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

Robert L. Snipp, Wilmer G. Miller, and Robert E. Nylund

*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-glutamyl 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.

(1) Taken in part from the Ph.D. thesis of R. L. Snipp, University of Iowa, 1965.

(2) P. Doty, A. Holtzer, J. Bradbury, and E. Blout, *J. Am. Chem. Soc.*, **76**, 4493 (1954).

(3) (a) L. Peller, *J. Phys. Chem.*, **63**, 1194, 1199 (1959); (b) J. Gibbs and E. Di Marzio, *J. Chem. Phys.*, **30**, 271 (1959); (c) B. Zimm and J. Bragg, *ibid.*, **31**, 526 (1959); (d) K. Nagai, *J. Phys. Soc. Japan*, **15**, 407 (1960); *J. Chem. Phys.*, **34**, 887 (1961); (e) B. Zimm and S. Rice, *J. Mol. Phys.*, **3**, 391 (1960); (f) S. Lifson and A. Roig, *J. Chem. Phys.*, **34**, 1963 (1961); (g) M. Baur and L. Nosanow, *ibid.*, **37**, 153 (1962); (h) J. Applequist, *ibid.*, **38**, 934 (1963).

(4) E. Ising, *Z. Physik*, **31**, 253 (1925).

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

(6) D. A. Brant and P. J. Flory, *ibid.*, **87**, 663 (1965).

(7) F. Karasy, J. O'Reilly, and H. Bair, *Nature*, **202**, 693 (1964).

(8) F. Karasy, J. O'Reilly, and H. Bair, Sixth International Congress of Biochemistry, New York, N. Y., 1964, Abstracts, p. 158.

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

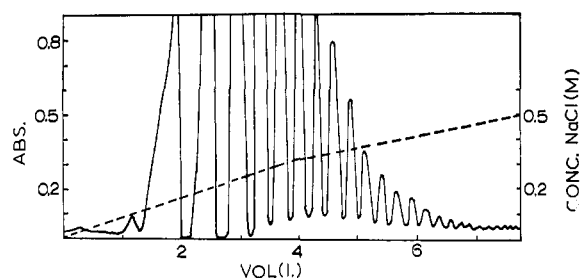


Figure 1a. Fractionation of PGA by gradient elution from DEAE cellulose column. Dashed line indicates NaCl gradient. Effluent monitored by 570-m $\mu$  absorption (ABS) after reaction with ninhydrin.

In this paper an attempt is made to describe quantitatively the charge-induced transition and to investigate effects of chain length, salt concentration, and side-chain composition.

### Experimental

**Polymers (Unfractionated).** Poly- $\alpha$ ,L-glutamic acid having an  $\bar{M}_n$  of 12,000 was prepared by polymerization of benzylglutamate N-carboxyanhydride.<sup>10</sup> A higher molecular weight sample ( $\bar{M}_n = 25,000$ ) was obtained from Pilot Chemical Co. (Lot G-43). Random L-leucine-L-glutamic acid copolymers ( $\bar{M}_n = 7,000$ –12,000) were prepared by copolymerization of the appropriate anhydrides. Their description is given elsewhere.<sup>10</sup> The molecular weight distribution in these polymers is thought to be broad, approaching the random or most probable distribution.<sup>10</sup>

**Monodisperse Oligomers of PGA.** The general procedure to obtain monodisperse oligomers involved enzymic degradation of high molecular weight PGA followed by separation of the small oligomers on DEAE cellulose.<sup>11</sup> In a typical degradation 3 g. of sodium polyglutamate was dissolved in 200 ml. of water and adjusted to pH 4.4. Papain (Worthington Biochemical Corp.) was activated with cysteine and 2 mg. of enzyme was added to the polymer solution. In 4–5 hr. the pH was raised to 6 and the solution stored for chromatographic separation. Approximately 300 mg. of partially degraded polymer was placed in a 4.5  $\times$  50 cm. DEAE cellulose column. For good resolution it was important that the column be repacked after each run. The oligomers were eluted selectively in order of increasing chain length by a linear sodium chloride gradient. Five per cent of the effluent stream was monitored by ninhydrin analysis (Technicon Auto-Analyzer). The remaining effluent was collected by means of a fraction collector. Fractions containing the same oligomer were pooled as were corresponding fractions from several columns.

Desalting the solutions proved to be very difficult as the nonvolatile material was well over 99% NaCl. Numerous methods were tried and two were adopted for routine use. For a solution containing an oligomer of six or fewer residues, the solution was diluted two-fold and reintroduced to the DEAE cellulose (acid form) column. The column was washed with water until the effluent gave a negative chloride test. The

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

(11) W. G. Miller, *ibid.*, **83**, 259 (1961).

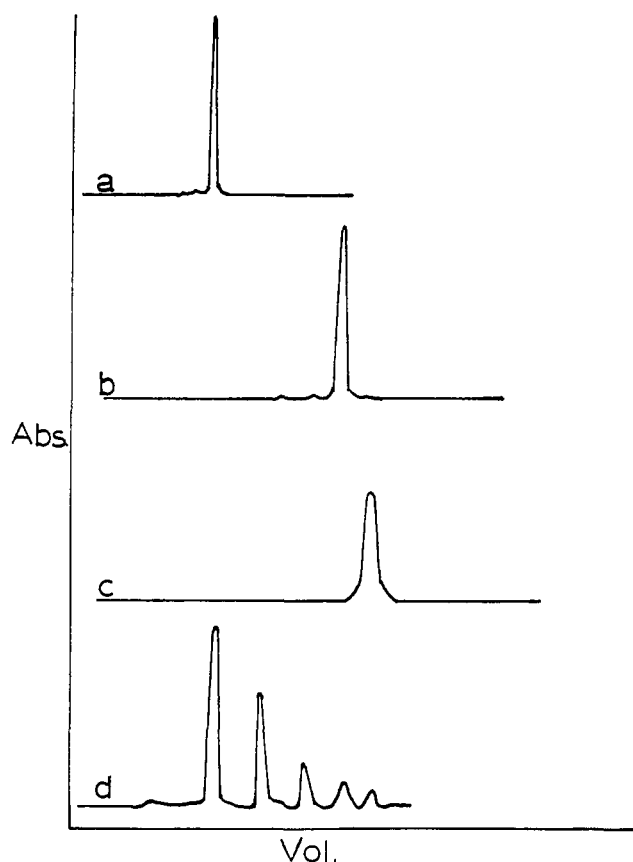


Figure 1b. Purity check on isolated oligomers: (a) dimer, (b) pentamer, (c) hexamer, (d) di-, tri-, penta-, and hexamer.

oligomer was stripped from the column with a dilute HCl solution (pH 2.3) and subsequently lyophilized. Solutions containing an oligomer of seven or more residues were acidified with HCl and allowed to stand for 3 days at 3–5°. The peptide precipitated slowly from solution. The solution was centrifuged; the precipitate was washed and eventually dried in a vacuum desiccator. Oligomers from the tripeptide to the heptadecapeptide were isolated in usable quantities. It becomes increasingly difficult to separate oligomers differing by only one residue as the chain length increases. A typical separation is shown in Figure 1a. Isolated oligomers were rechromatographed to check for purity as illustrated in Figure 1b. In all cases contamination by other oligomers amounted to less than 1%. The oligomers were identified as in ref. 11.

**Fractionated PGA (Narrow Molecular Weight Distribution).** Ten grams of PGA (G-43) was fractionated on a 7  $\times$  60 cm. (500 g.) DEAE cellulose column by gradient elution into approximately 150 fractions. Fractionated polymer was isolated by dialysis followed with lyophilization. Details of the fractionation will be published elsewhere.<sup>12</sup> The polymers isolated from the fractionation are quite narrow in molecular weight distribution.

**Determination of Physical Properties.** Number-average molecular weights of the polymers (acid form) were determined in dimethylformamide using a Mechrolab membrane osmometer. In general, the molecular weights were high enough to eliminate diffusion through

(12) R. L. Snipp and W. G. Miller, in preparation.

the membrane. In instances where diffusion occurred, the data were treated as with the corresponding data for the leucine-glutamic acid copolymers.<sup>10</sup>

In cases where the polymer was isolated as a partial salt, peptide bond concentration of subsequent solutions was determined by absorption at 220 m $\mu$  using a Beckman DU spectrophotometer.

Titration of side-chain carboxyls was carried out as described previously,<sup>10</sup> using either a Beckman GS pH meter or a Cary vibrating reed electrometer.

The helix-coil transition was followed by ultraviolet absorption on a Cary 14 spectrophotometer using Beckman far-ultraviolet short-path-length cells (0.038 cm.). In the fractionated and the monodisperse polymers, all measurements were made at 200 m $\mu$ .

All measurements were made at 25° and in 0.200 M NaCl unless stated otherwise.

### Analysis of Data

The analysis of experimental data to evaluate the variables describing the charge-induced helix-random coil transition at constant temperature will depend on the theoretical framework employed. We follow a simplified and somewhat approximate version of the elegant treatments of Zimm and Rice<sup>3e</sup> and of Zimm and Bragg.<sup>3c</sup> Consider the configuration partition function  $Q$  of a polypeptide homopolymer with  $n$  ionizable side-chain carboxyls per molecule. Statistical weights will be assigned assuming an Ising model with first-neighbor interactions. A statistical weight of unity is assigned to a chain configuration in which all residues are in random-coil conformations. When the chain configuration is such that there are  $k$  helical residues located in  $l$  unbroken helical sequences and the degree of dissociation  $\alpha$  is zero, the weight  $\sigma^l s^k$  is assigned; if  $\alpha > 0$ , the weight  $\sigma^l (s')^k$  is assigned. The initiation parameter describes the extra difficulty in starting a helical sequence compared to the statistical weight  $s'$  assigned to extending it, the molecule being allowed to ionize as it will. Since intramolecular electrostatic interactions vary with  $\alpha$ ,  $s'$  is also a function of  $\alpha$ . In general, electrostatic interactions tend to decrease the stability of the helix more than the random coil; consequently  $s'$  will always be less than  $s$  and approach it only as  $\alpha \rightarrow 0$ . The fraction of residues in the helical conformation  $f_H$  is given by

$$f_H = \frac{1}{n} \frac{d \ln Q}{d \ln s'} \quad (1)$$

or

$$= \frac{1}{n-3} \frac{d \ln Q}{d \ln s'} \quad (2)$$

if the fraction of helix is taken as synonymous with the fraction of the carbonyl oxygens hydrogen bonded. For large  $n$  the difference between the two definitions of  $f_H$  is trivial. Taking the latter definition in conjunction with the statistical weights as assigned, the partition function is given by

$$Q = 1 + \sum_{l=1}^{m/2} \sigma^{-l} \sum_{k=l}^{n-l-2} \frac{(k-l)!(n-k-2)!(s')^k}{l!(l-1)!(n-k-l-2)!} \quad (3)$$

Where  $n$  is large,  $f_H$  becomes<sup>3c,e,h</sup>

$$f_H = 0.5 \{ 1 + (s' - 1) / [(1 - s')^2 + 4\sigma s']^{1/2} \} \quad (4)$$

Equation 4 was applied to all chains having 100 or more residues; eq. 2 and 3 were applied to those with less than 50. In the region of 50 to 100 residues, both equations were utilized.

The quantities  $s'$ ,  $s$ , and  $\sigma$  must be evaluated from experimental data. The titration curves for high molecular weight polymers will be used to evaluate  $s'$  and  $s$ . Consider a polypeptide molecule of fixed conformation  $i$  with variable (0 to  $n$ ) charge on its side chains, characterized by a partition function  $Z_i$  and degree of dissociation  $\alpha_i$ . If the conformation is that of the pure random coil ( $i \equiv c$ )

$$\alpha_c = \frac{1}{2.3n} \frac{d \ln Z_c}{d(\text{pH})} \quad (5)$$

If the fixed conformation is the pure helix ( $i \equiv h$ )

$$\alpha_h = \frac{1}{2.3n} \frac{d \ln Z_h}{d(\text{pH})} \quad (6)$$

Subtracting eq. 5 from eq. 6 gives

$$\frac{d \ln Z_h}{d(\text{pH})} - \frac{d \ln Z_c}{d(\text{pH})} = 2.3n(\alpha_h - \alpha_c)$$

or

$$\ln (Z_h/Z_c)/d(\text{pH}) = 2.3n(\alpha_h - \alpha_c) \quad (7)$$

If the same statistical weights are used as in the configuration partition function

$$Z_h/Z_c = \sigma (s')^n$$

Since  $\sigma$  has been assumed to be independent of pH, eq. 7 can be written as

$$d \ln s' / d(\text{pH}) = 2.3(\alpha_h - \alpha_c)$$

In the limit of low pH,  $\alpha_h = \alpha_c = 0$  and  $s' = s$ . Therefore  $\ln s'$  at any pH is given by

$$\ln s' = \ln s + 2.3 \int_{\text{pH}(\alpha_h = \alpha_c = 0)}^{\text{pH}} (\alpha_h - \alpha_c) d(\text{pH}) \quad (8)$$

Nagasawa and Holtzer<sup>13</sup> have shown that

$$\ln s = -2.3 \int_{\text{pH}(\alpha_h = \alpha_c = 0)}^{\text{pH}(h-c \text{ midpt})} (\alpha_h - \alpha_c) d(\text{pH}) \quad (9)$$

where the upper limit of integration is the pH at the midpoint of the helix-coil transition. Combining eq. 8 and 9 gives

$$\ln s' = 2.3 \int_{\text{pH}}^{\text{pH}(h-c \text{ midpt})} (\alpha_c - \alpha_h) d(\text{pH}) \quad (10)$$

The titration curves for pure helix and pure random coil may be obtained by suitable extrapolation of the experimental titration curves and have been discussed elsewhere.<sup>3e,10,13,14</sup> Thus  $s'$  at any pH as well as  $s$  may be obtained from experimental data.

With high molecular weight polymers to which eq. 4 may be applied,  $\sigma$  may be evaluated from experimental data. On rearranging eq. 4 and taking logarithms

$$\ln \left( \frac{1}{f_H} - 1 \right) = \ln \frac{[(1 - s')^2 + 4\sigma s']^{1/2} + (1 - s')}{[(1 - s')^2 + 4\sigma s']^{1/2} - (1 - s')}$$

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

(14) W. G. Miller and R. E. Nylund, *ibid.*, **87**, 3542 (1965).

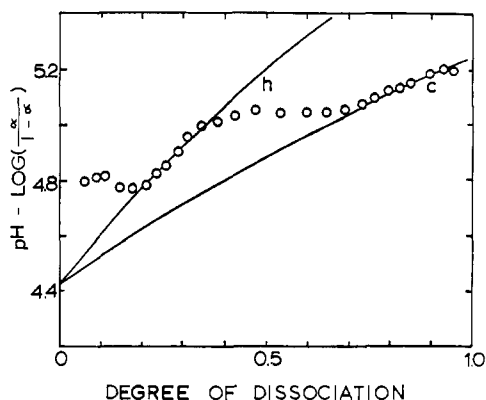


Figure 2a. Typical titration curve showing extrapolation of titration curve for "pure" helix (h) and "pure" coil (c).

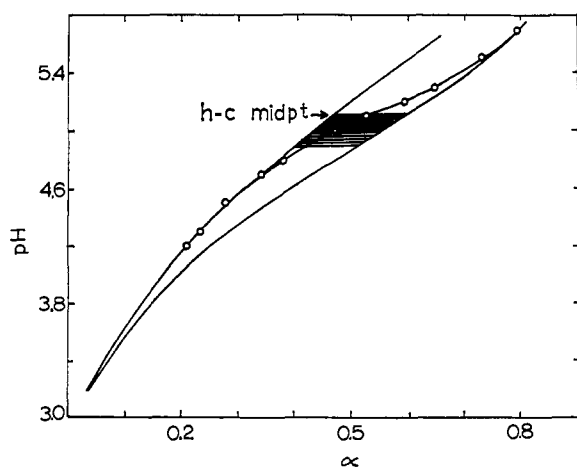


Figure 2b. Determination of  $s'$  from titration curve using eq. 10.  $\ln s'$  is given by shaded area.

A Taylor series expansion gives

$$\ln \left( \frac{I}{f_H} - 1 \right) = \frac{I}{\sigma^{1/2}} \left[ (1 - s') + \frac{1}{2} (1 - s')^2 + \frac{1}{24} (9 - 1/\sigma) (1 - s')^3 + \dots \right] \quad (11)$$

In the region of the helix-coil transition ( $0.9 < s' < 1.1$ ) only the first term is important. Thus  $\sigma$  may be evaluated graphically by plotting the left member against  $(1 - s')$ . Optical measurements may be used to determine  $f_H$  or, alternatively,<sup>3e,13</sup> the titration curve may be utilized assuming

$$\frac{\alpha_c - \alpha}{\alpha - \alpha_h} = \frac{\text{residues in helical conformation}}{\text{residues in random-coil conformation}} \quad (12)$$

If eq. 12 is employed in determining  $f_H$ , the parameters characterizing the charge-induced transition can be completely specified from the titration curve. In practice  $f_H$  is more satisfactorily determined from optical measurements. Equation 11 cannot be used to assess  $\sigma$  for short chains, as eq. 4 is not applicable to them. With such chains  $\sigma$  was treated as an adjustable parameter in eq. 2 and 3 to give the best fit of  $f_H$  to the experimental data.

The preceding treatment is not vigorously applicable to the leucine-glutamic acid copolymers. The electrostatic interaction of the side-chain carboxyls has been

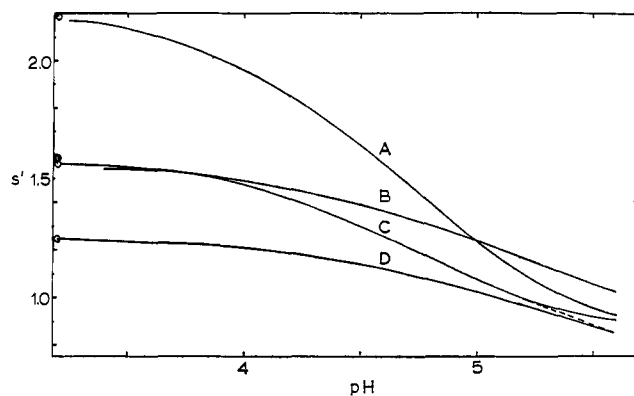


Figure 3. Representative  $s'$ -pH curves at 25° evaluated as in Figure 2: (A) glu-29.1 leu, 0.2 M NaCl; (B) PGA, 0.005 M NaCl (evaluated from data in ref. 13); (C) glu-12.4 leu, 0.2 M NaCl; (D) PGA, 0.2 M NaCl; (O) maximum value of  $s'$  ( $=s$ ) at low pH limit. Dashed line is explained in text.

shown<sup>10</sup> to depend on the leucine content of the copolymer. In a copolymer of a given average composition, the distribution of glutamic acid residues within a molecule will vary from molecule to molecule. Considering for a moment molecules with the same number of glutamic acid residues, the statistical weight at a particular pH will depend upon the distribution of the glutamic acid residues. Thus each actual chain will have, in general, a different titration curve as well as a different helix-coil transition curve. The application of a statistical mechanical treatment would not lead to eq. 3 or 4. For a rigorous treatment the partition function for each actual chain must be given and an average taken over all chains. Since we cannot at present describe theoretically the alteration in electrostatic interaction when leucine residues interpose two carboxylates, it would be impossible to assign statistical weights in the partition functions. Instead we assess an average  $s'$  from the experimental data and use eq. 4 (or eq. 2 and 3) as a phenomenological equation to describe the helix-coil transition. Although each actual chain will have a different titration curve, the average value of  $\alpha_h$  and  $\alpha_c$  may be determined from the experimental titration curves. The difference in the averaged curves,  $\bar{\alpha}_h - \bar{\alpha}_c$ , will be related to the average difference in stability of the pure helical and pure random-coil conformations and thus give an averaged  $s'$  for the system of molecules at each pH. By application of eq. 10, the average  $s'$  as a function of pH may be determined for each copolymer.

## Results

**Polymers (Unfractionated).** In Figure 2a an example of a titration curve is shown along with the extrapolation of the pure helical and pure random-coil regions. In Figure 2b the data are replotted, and the graphical integration of eq. 10 to give  $s'$  is indicated. In Figure 3 representative  $s'$ -pH curves are shown. At low pH,  $s'$  asymptotically approaches  $s$ . Although the titration of charged groups on the surface of a rod has been treated theoretically,<sup>15</sup> lengthy extrapolation of  $\alpha_h$  past the helix-random coil midpoint seems tenuous. The high pH side of the  $s'$  curve is therefore unattainable from our data.

(15) (a) T. Hill, *Arch. Biochem. Biophys.*, **57**, 229 (1955); (b) M. Nagasawa and A. Holtzer, *J. Am. Chem. Soc.*, **86**, 531 (1964).

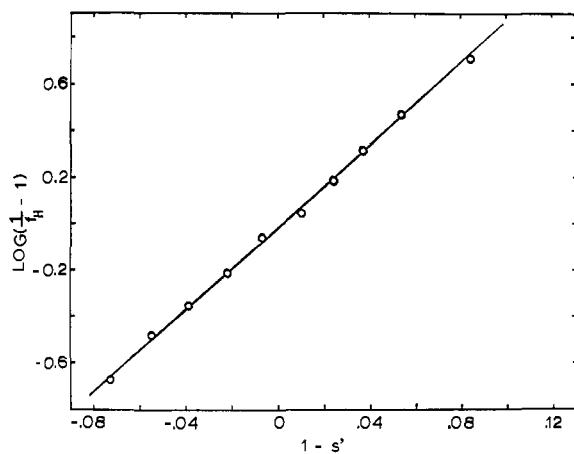


Figure 4. Determination of initiation parameter ( $\sigma$ ) using eq. 11. Data shown are for PGA with no added electrolyte; ultraviolet absorption ( $\epsilon_{200}$ ) used to determine  $f_H$ .

Determination of  $\sigma$  using eq. 11 is shown in Figure 4. A tabulation for several polymer-solvent systems is given in Table I. Titration and absorption data in the preceding papers<sup>10,14</sup> were utilized in determining  $\sigma$  for the copolymers. No effect of electrolyte concentration, molecular weight, or leucine content of the copolymers is apparent. A value for  $\sigma$  equal to  $3 \pm 2 \times 10^{-3}$  best describes the data. This is in agreement with that found in the part charge-part thermal induced transition studied by Rifkind and Applequist. Values of  $s'$  from Figure 3 and a value of  $\sigma = 3 \times 10^{-3}$  were then used in eq. 4 to calculate the helical content as a function of pH. The calculated values can be compared to the transition as measured by optical absorption, as shown in Figure 5.

Table I. Initiation Parameter for Charge-Induced Transition in Unfractionated Polypeptides

Polymer	Mol. wt. ( $\bar{M}_n$ )	NaCl, M	Method <sup>a</sup>	$\sigma \times 10^3$
PGA	12,000	0.200	$\epsilon_{195}$	2.6-2.9
			$\epsilon_{200}$	3.1-3.4
			$\epsilon_{202.5}$	3.1-3.8
			T	2.6
PGA	25,000	0.200	$\epsilon_{200}$	2.0
		0.000	$\epsilon_{190}$	2.9-3.0
			$\epsilon_{200}$	2.3-2.6
PGA <sup>3b</sup>		0.0133	$\epsilon_{200}$	1.4
Glu-5.7 leu <sup>b,c</sup>	7,300	0.200	$\epsilon_{200}$	1.0-1.5
Glu-12.4 leu <sup>b</sup>	7,400	0.200	$\epsilon_{200}$	3.2
Glu-22.9 leu <sup>b</sup>		0.200	$\epsilon_{200}$	3.0-3.6
Glu-29.1 leu <sup>b</sup>	8,800	0.200	$\epsilon_{200}$	3.4-4.6

<sup>a</sup>  $\epsilon_z$ , molar absorptivity at  $x$  m $\mu$  used in evaluation; T, titration curve used in evaluation. <sup>b</sup> Random copolymer containing 5.7 mole % leucine, etc. <sup>c</sup> Glutamic acid-5.7 leucine copolymer is anomalous in all of its properties.<sup>14</sup>

*Polymers (Fractionated and Monodisperse).* The ultraviolet absorption of high molecular weight fractionated PGA exhibited a hypochromism in going from high to low pH in agreement with that for high molecular weight unfractionated PGA. The titration curves are only slightly dependent on molecular weight as was expected in this molecular weight range. In this molecular weight range (degree of polymerization

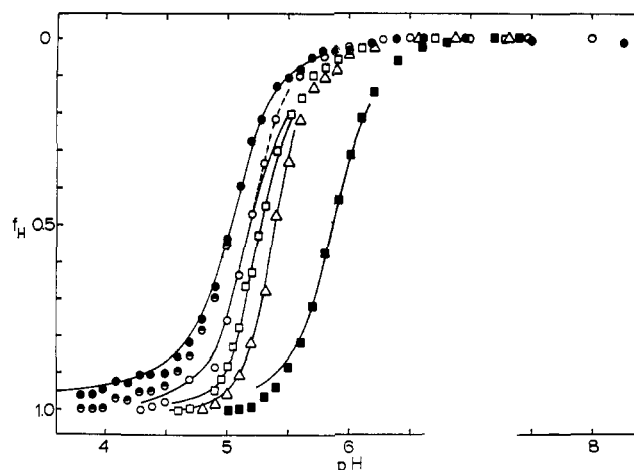


Figure 5. Comparison of theoretical curves, calculated from eq. 4;  $\sigma = 3 \times 10^{-3}$ ;  $s'$  from Figure 3) and experimental description of the helix-random coil transition in unfractionated polymers: ●, PGA, 0.2 M NaCl, maximum helical content taken to be 94%; ○, same except maximum helical content taken to be 100%; ○, glu-12.4 leu, 0.2 M NaCl; □, glu-22.9 leu, 0.2 M NaCl; △, glu-29.1 leu, 0.2 M NaCl; ■, PGA, no added electrolyte. Dashed line is explained in text.

> 70)  $\sigma$  and  $s'$  could be determined as in the preceding section. In the region of 70 to 100 residues, it made little difference whether  $\sigma$  was determined using eq. 11 or by best fit using eq. 2 and 3. A linear relationship between the absorptivity and  $f_H$  was assumed, the same as was done with the unfractionated polymer.

In investigating the lower molecular weight fractions, eventually a molecular weight was reached in which full hypochromism was not observed. Further reduction in molecular weight gave progressively smaller hypochromism indicating a lower helical content was reached at low pH. Although the contribution to the hypochromism resulting from change in carboxylate ion concentration is small for the high molecular weight polymers, it becomes relatively more important as the total hypochromism becomes smaller. In the tripeptide, a chain length at which the helical conformation is not expected, a small change in absorptivity was observed in going from pH 5 to 4. On the assumption that this change was due entirely to the carboxylate absorption, the hypochromism observed in the tripeptide at any pH was subtracted from the corresponding absorption data of all other peptides. In this way the hypochromism resulting from conformational change was separated from that due to the carboxylate absorption. The high pH absorptivity was assumed to characterize the random coil. At other pH the  $f_H$ -molar absorptivity relationship for the high molecular weight material was assumed, thus allowing a value of  $f_H$  to be assigned to each absorptivity. The data plotted in this manner are given in Figure 6.

If it is further assumed that the  $s'$ -pH curve for high molecular weight PGA (Figure 3) is applicable to the lower molecular weight material,  $\sigma$  can be treated as an adjustable parameter in eq. 2 and 3 and the value of  $\sigma$  taken as the value which gives the best fit to the experimental data. The "best fit" curves are indicated by the solid lines in Figure 6a with the corresponding  $\sigma$  values shown in Figure 7. At very low molecular weights relatively large values of  $\sigma$  are needed to fit the

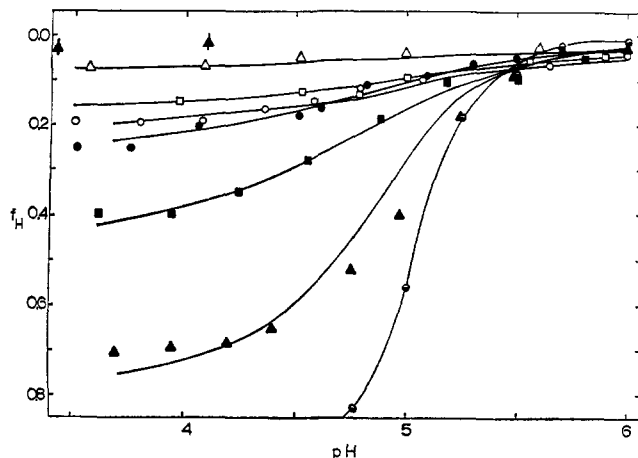


Figure 6a. Comparison of theoretical (solid lines, calculated from eq. 2 and 3,  $\sigma$  as in Figure 7;  $s'$  as in Figure 3, D) and experimental description of the helix-coil transition in oligomers and highly fractionated PGA, 0.2 M NaCl: ▲, tetramer; △, hexamer; □, octamer; ○, nonamer; ●, dodacamer; ■, heptadecamer; ▲,  $\bar{x}_n \approx 40$ ; ⊖,  $\bar{x}_n = 350$ .

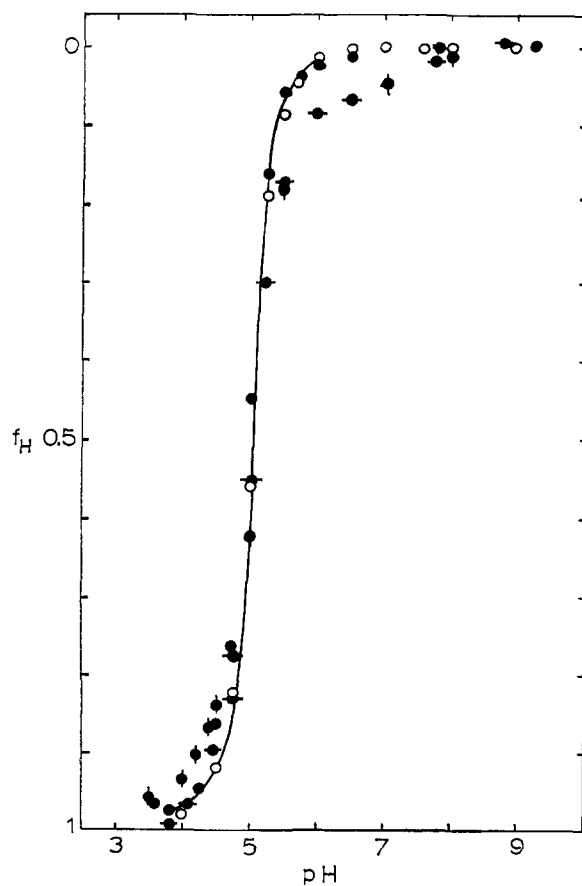


Figure 6b. Comparison of theoretical (solid lines, calculated from eq. 4,  $\sigma = 1.4 \times 10^{-3}$ ;  $s'$  as in Figure 3, D) and experimental description of the helix-coil transition in oligomers and highly fractionated PGA, 0.2 M NaCl: average number of residues ( $\bar{x}_n$ ) = 95 (●), 170 (-●-), 350 (○), and 540 (●).

data. As the chain length increases,  $\sigma$  quickly falls; further increases show only a slight trend. The trend in the molecular weight region above 10,000 is too slight to show up in the unfractionated PGA. For the largest chain length (approximately 540) reported here  $\sigma$  is  $1.4 \times 10^{-3}$ ; much higher chain lengths ( $\sim 1500$ )

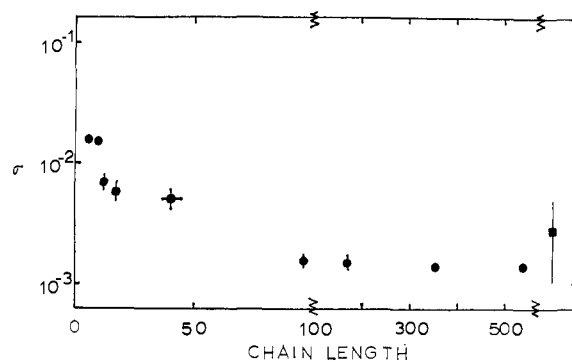


Figure 7. Dependence of the initiation parameter ( $\sigma$ ) on chain length (number of amino acid residues): ●, oligomers and fractionated polymer; ■, unfractionated polymers.

showed an anomalous double transition which is presently not understood, but may be due to a slight contamination with cupric ion.

## Discussion

*High Molecular Weight Polymer.* In treating the experimental data we have assumed that the helical content is a linear function of the hypochromism. It has been shown<sup>13,16</sup> for PGA that optical rotation, optical absorption, and the titration curve are similar functions of helical content. As Applequist and Breslow have stated, it does not follow that these quantities are linear functions of helical content. Nevertheless, it is suggestive. Tinoco and co-workers,<sup>17</sup> however, have shown that the observed amide oscillator strength in optical absorption should be a linear function of helical content so long as only a small fraction of the helical residues are located at or near ends of helical sections. At the midpoint of the transition, the average length of a helical section is about 20 residues ( $1 + 1/\sqrt{\sigma}$ ), indicating this condition is reasonably satisfied. At very small values of  $f_H$  where the average length of a helical section is small, this condition will not be met. In principle, the oscillator strength at any pH may be determined from the area under the dispersion curve. In practice, it is difficult to obtain the far-ultraviolet part of the dispersion; in addition, carboxylate absorption becomes more important below 200 m $\mu$ . By staying outside of the region where carboxylate absorption is important, we have shown that hypochromism at a selected wave length as a function of pH, when normalized, is independent of the wave lengths investigated.<sup>14</sup> Assuming this holds at all wave lengths of amide absorption, change in absorption at a single wave length will be a linear function of helical content. The use of a linear relationship seems to be justified. The value to be assigned to  $f_H$  at the low pH limit is an additional difficulty. Since  $s'$  can never be larger than  $s$ , an upper limit can be set on  $f_H$ . Using the appropriate  $s$  values in eq. 2, the maximum value of  $f_H$  ranges from 0.945 for PGA to 0.995 for the glu-29.1 leu copolymer. The experimental data for PGA ( $\bar{M}_n = 12,000$ ) are shown in Figure 5 with  $f_H$  (max.) taken as 0.94 and also as 1.00. Although the calculated curve fits the

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

(17) I. Tinoco, A. Halpern, and W. Simpson, "Polypeptides and Proteins," M. Stalhamm, Ed., University of Wisconsin Press, Madison, Wis., 1962, p. 147.

experimental data better when the lower limit on  $f_H$  is used, there is little compelling evidence to justify one limit over the other.

The calculated curve of the helix-random coil transition depends on a correct evaluation of  $\sigma$  and  $s'$ . Extrapolation of  $\alpha_h$  past the helix-random coil midpoint can be done with little certainty and consequently will affect the value of  $s'$  determined in this region. The dashed line for the glu-12.4 leu copolymer in Figure 5 indicates the effect of a slight change in  $s'$ , indicated in the corresponding curve in Figure 3. Use of eq. 11 to determine  $\sigma$  is equivalent to a curve-fitting procedure and the calculated curve must consequently fit the experimental curve in the region of the helix-coil midpoint.

Attempts were made to estimate  $\sigma$  for PGA in dioxane-water<sup>18</sup> and for polylysine in water. The difficulty in extrapolating the titration curve to yield  $\alpha_h$ ,  $\alpha_c$ , and consequently  $s'$  precluded meaningful results. For the PGA-dioxane-water system  $\sigma$  appeared to be about the same as for the systems reported here; for polylysine-water  $\sigma$  may be smaller. In the polypeptide-water systems we have studied quantitatively,  $\sigma$  is essentially constant though an order of magnitude larger than this is found in the polybenzylglutamate-dichloroacetic acid-dichloroethane system. The reason for the variation in  $\sigma$  is uncertain. As mentioned earlier, it could be due to enthalpic contributions which might vary with side chain and solvent. The type of helix formed may also affect  $\sigma$ . The possibility that not all polypeptides form the  $\alpha$ -helix in solution has recently been considered.<sup>19</sup> It is reasonable to assume that initiation of different types of helices may be characterized by differing values of  $\sigma$ .

*Chain-Length Dependence of  $\sigma$ .* The assumptions made in analyzing the short-chain data must be considered before the chain-length dependence of  $\sigma$  is taken seriously. Two major assumptions are the use of the high molecular weight  $f_H$ -molar absorptivity and  $s'$ -pH curves for the short-chain length polymers. As end effects become important, the average length of a helical section will be shorter in the small oligomers, thus forcing a greater fraction of the helical residues to be near or at ends of helical sections. Residues in very short helical sections will not exhibit full hypochromism. One turn of a helix has been calculated to have only 80% of the hypochromism of a very long helix.<sup>17</sup> Thus the observed hypochromism will *underestimate* the helical content of the oligomers. If the data were corrected to compensate for this effect, it would take an even larger value of  $\sigma$  to bring agreement between the calculated and experimental curves.

The use of the high molecular weight  $s'$ -pH curve is more difficult to justify. The titration curves for the small oligomers indicate that the intrinsic dissociation constant is not significantly chain-length dependent.<sup>12</sup>

(18) P. Doty, J. Yang, and E. Blout, *J. Polymer Sci.*, **23**, 851 (1957).

(19) (a) J. Marchal and C. Lapp, *J. chim. phys.* **61**, 999 (1964); (b) V. Luzzati, M. Cesari, G. Spach, F. Masson, and J. Vincent, *J. Mol. Biol.*, **3**, 566 (1961); (c) G. Spach, L. Freund, M. Daune, and H. Benoit, *ibid.*, **7**, 468 (1963).

End effects become important, resulting in a decrease in the electrostatic interaction in the random coil as the chain length becomes small. The corresponding titration curve of the pure helix is not available as the fully helical molecule never forms. It seems likely that end effects would also tend to decrease the electrostatic interaction in the helix. Although the  $s'$ -pH curve may be slightly altered on going to small chain lengths, it is unlikely that the uncharged helix will be more stable (larger value of  $s$ ). If  $\sigma$  is held constant and the maximum value of  $s'$  ( $=s$ ) is inserted in eq. 3,  $f_H$  is considerably underestimated in very short chains. It is also underestimated if  $f_H$  as defined by eq. 1 is considered. For this reason  $\sigma$  was adjusted until the calculated maximum  $f_H$  agreed with the experimental value. Upon adjusting  $\sigma$  in this manner not only the maximum  $f_H$  but the entire calculated curve was in reasonable agreement with experiment. The calculated curve in Figure 6b is the result for infinite chain length (eq. 4). The slight sharpening in going from the 95-mer to the 540-mer is more likely a reflection of the asymptotic approach to the infinite chain curve than to a continued trend in  $\sigma$ .

Within the framework of eq. 1 through 4 and the assumptions discussed above,  $\sigma$  increases at small chain lengths. An additional criticism is that eq. 1-4 are based on nearest-neighbor interactions, whereas longer-range electrostatic interactions certainly exist in polyelectrolytes. For long chains we found the nearest-neighbor model adequate and furthermore  $\sigma$  did not depend on electrolyte concentration. In addition, at the low pH limit electrostatic interactions become negligible and  $s$  does not reflect side-chain electrostatic interactions. If  $\sigma$  is entirely entropic, a varying value of  $\sigma$  is difficult to rationalize. If an enthalpic contribution due to bond-dipole interactions is present,<sup>6,20</sup> a spectrum of  $\sigma$  values may result which for short helices is a function of the length of the helical section. In fact, the calculations of Arridge and Cannon indicate that dipole interactions in very short helices are repulsive but become attractive in longer helices. By placing the dipole effects in  $\sigma$  and keeping a two-parameter mathematical description, a chain-length dependence of  $\sigma$  is predicted that is in the direction which we observe. A statistical mechanical treatment taking into account dipole interactions would require more than a nearest-neighbor model. For long chains the difference between the two models will be small and indeed we find a two-parameter, nearest-neighbor, nonadjustable  $\sigma$  model adequately describes our data for long chains.

*Acknowledgments.* This investigation was supported in part by Public Health Service Research Grant GM-08409. W. G. M. wishes to thank the John Simon Guggenheim Foundation for a Fellowship and Professor P. J. Flory and the Chemistry Department of Stanford University for their kind hospitality during the writing of the manuscript. We also wish to thank Dr. D. A. Brant for helpful discussions.

(20) R. G. C. Arridge and C. G. Cannon, *Proc. Roy. Soc. (London)*, **A278**, 91 (1964).