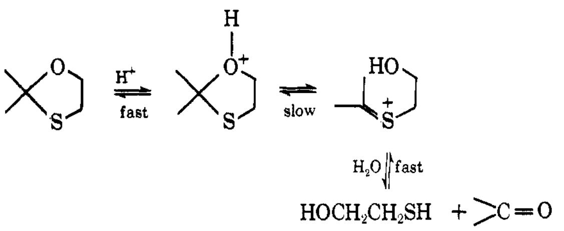
thiolane recorded in the presence of FSO_3H-SbF_5 catalyst at −60° had a triplet due to the $-OH_2^+$ grouping at 10.9 ppm, whereas the spectrum of dithiolane showed a triplet due to $-SH_2^+$ at 7.3 ppm at −30°. Reports on the mass spectrometric fragmentation of 1,3-oxathiolanes[19] and 1,3-oxathianes[20] suggest that sulfur-containing ions are predominantly formed in these fragmentation processes; *i.e.* the ring splitting occurs mainly at the C–O bond.

Recently, the relative rates of the hydrolytic decomposition of 2,2-dimethyl-1,3-dioxolane, -oxathiolane, and -dithiolane were also determined. The results pointed out that if the relative hydrolysis rate for 2,2-dimethyl-1,3-dithiolane is taken to be unity, then 2,2-dimethyl-1,3-oxathiolane hydrolyzes with a relative rate of 2×10^4 and 2,2-dimethyl-1,3-dioxolane with a relative rate of 1×10^6. The great increase in rate between dithiolane and oxathiolane when compared to the relatively low rate increase between oxathiolane and dioxolane supports also the view of a C–O bond fission in the hydrolysis of 1,3-oxathiolanes.[17,21]

Conclusion

According to the collected data, 1,3-oxathiolanes hydrolyze under acidic conditions by an A-1 mechanism, in which fission of the acetal carbon–oxygen bond occurs predominantly as shown in eq 2. This conclusion confirms the view of Fife and Jao[8] about the A-1 mechanism and that of De and Fedor[7] about selective O protonation, *i.e.*, the fission of the acetal carbon–oxygen bond in the critical transition states. Consequently, contradictions in the previous papers are removed and the experimental results are in general agreement.



(2)

The most peculiar feature of the hydrolysis of 1,3-oxathiolanes is the specific solvent deuterium isotope effect; *i.e.*, the rate is much higher when the deuterium atom fraction approaches unity than one would expect. Also, this phenomenon is best understood by assuming that a carbonium–sulfonium intermediate is formed, in which the hybridized p and d orbitals of sulfur play a significant role. This effect is worth additional studies with other model compounds.[17]

Acknowledgments. The author wishes to express his gratitude to the National Science Foundation for a fellowship which made this work possible. I am also grateful to Mr. Alpo Nikkilä for his skillful help in the experimental work and to Professor Pentti Salomaa for many valuable discussions. Also, information and comments of Professor Gerard Lamaty of the Department of Chemistry, University of Montpellier, are gratefully acknowledged.

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Kinetics of the Helix–Coil Transition in Aqueous Poly(L-glutamic acid)

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Abstract: Ultrasonic absorption measurements in dilute aqueous poly(α,L-glutamic acid)(PGA) have been carried out over the frequency range 40 kHz–35 MHz by the resonance, statistical reverberation, and pulse techniques. Data at the two PGA concentrations 2.7×10^{-3} and 1.1×10^{-3} (mol residue)/l. in 0.03 *M* NaCl at 37° exhibited relaxational absorption over the pH range 4.8–5.6. Both the relaxational amplitude (A) and relaxation time (τ) passed through maximum values at pH 5.21, the midpoint of the helix–coil transition. The maximum values of A and τ were found to be 100×10^{-17} sec² cm⁻¹ and 1.0 μsec, respectively. The value of the rate constant for helical growth in the Schwarz theory was estimated to be $(8 \pm 5) \times 10^7$ sec⁻¹.

The helix–coil transition of the poly(α,L-amino acids) has long served as a model from which it is hoped to gain insight into the structural and dynamic properties of proteins.[1] About the thermodynamics of the transition there exists a wealth of information. Very little is known about the kinetics. In fact, prior to this investigation, complete kinetic data had been taken for only one system—poly(α,L-ornithine) in water–methanol mixtures.[2]

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We report here a study of the kinetics of the helix–coil transition of poly(α,L-glutamic acid) (PGA henceforth) in aqueous 0.03 M NaCl. The work was undertaken because the relaxation time for the process had been shown by temperature-jump studies[3] to be less than 10 μsec and by ultrasonic absorption techniques[4,5] to be larger than 10^{-8} sec, and thus had to lie in a time region accessible only by the ultrasonic resonator and statistical reverberation techniques—both of which are present in these laboratories. The results of this investigation constitute the first direct observation of the kinetics of the helix–coil transition of a poly(amino acid) in water

Theoretical Section

As shown by Zimm and Bragg[6] and Zimm and Rice,[7] two parameters are necessary to describe the helix–coil transition of a charged poly(amino acid): the "equilibrium constant" s' for the conversion from coil to helix of a (charged) residue at the end of a string of helical segments; and the nucleation parameter σ, which describes the difficulty of introducing the first helical segment into a region of otherwise coil residues. The experimental observable, the fraction of helicity f_h, is given by

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

At the transition midpoint $f_h = 1/2$ and $s' = 1$. The breadth of the pH region over which f_h varies from 1 to 0 is dictated by the magnitude of σ. For PGA in aqueous salt solutions, σ has been shown[8] to be $(3 \pm 2) \times 10^{-3}$

Schwarz[9] has devised a model for the close-to-equilibrium kinetics of the helix–coil transition. The basic kinetic event, helical growth, may be represented as

$$\left.\begin{matrix}\text{hcc}\\ \text{or}\\ \text{cch}\end{matrix}\right\} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \left\{\begin{matrix}\text{hhc}\\ \text{or}\\ \text{chh}\end{matrix}\right. \qquad \text{(I)}$$

where "h" means a segment in the helical (*i.e.*, hydrogen bonded) conformation, while "c" indicates a random coil segment. The rate constant k_f is the forward rate constant for helical growth, while the equilibrium constant for helical growth $s' = k_f/k_b$. The relaxation time in the vicinity of the transition midpoint is given by

$$1/\tau = k_f[(s' - 1)^2 + 4\sigma] \qquad (2)$$

A small amplitude sinusoidal sound wave acts as a source of energy for perturbing a dynamic equilibrium by virtue of the pressure and temperature fluctuations that accompany its travel through a medium. If the frequency of the sound wave is comparable to the relaxation frequency f_r, the perturbation of the equilibrium will result in absorption of acoustic energy. By measuring acoustic absorption as a function of frequency, the relaxation time, $\tau = 1/2\pi f_r$, may be obtained.

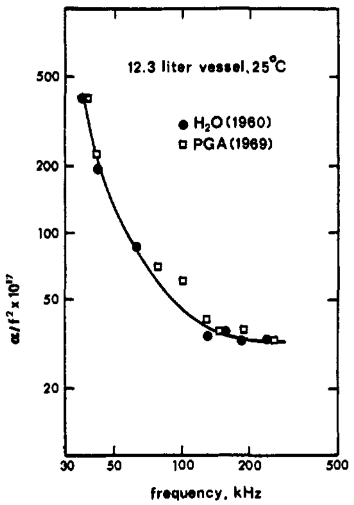

Figure 1. Calibration curve for the 12.3-l. spherical resonator using: (●) distilled water or (□) PGA outside the transition pH region.

Ultrasonic absorption occasioned by a single chemical relaxational process can conveniently be expressed in terms of the quantity α/f^2

$$\alpha/f^2 = \frac{A}{1 + (f/f_r)^2} + B \qquad (3)$$

where α = the amplitude absorption coefficient at frequency f, B = the nonrelaxing background" absorption, and A = the "relaxation amplitude"—a frequency independent term given by[10]

$$A = \frac{2\pi^2 \rho c}{1000RT}(\Delta V_{\text{eff}})^2 \Gamma \tau \qquad (4)$$

where ρ is the density of and c is the sound velocity in the solution of interest. The effective volume change is given by

$$\Delta V_{\text{eff}} = \Delta V^\circ - \frac{\kappa \Delta H^\circ}{\rho c_p} \qquad (5)$$

where ΔV°, ΔH° are the volume and enthalpy changes for the chemical process and κ and c_p are respectively the coefficient of thermal expansion and the specific heat of the system. The quantity Γ is an amplitude function which accounts for the change in the relaxation amplitude with changes in the concentrations of reactants and products and is given by

$$\Gamma = \left[\frac{\text{d} \ln K_{\text{eq}}}{\text{d}C_i}\right]^{-1} \qquad (6)$$

For a single relaxation, α/f^2 plotted *vs.* f is sigmoidal in shape and descends through an inflection point at $f = f_r$, thus permitting calculation of the relaxation time.

Experimental Section

Materials. Poly(α,L-glutamic acid) was purchased from Pilot Chemicals.[11] The manufacturer listed the molecular weight as 58,000. The material was characterized and used without further purification. Solutions were prepared by the method of Jennings, *et al.*[12] Concentrations are reported in mole residue of PGA per liter.

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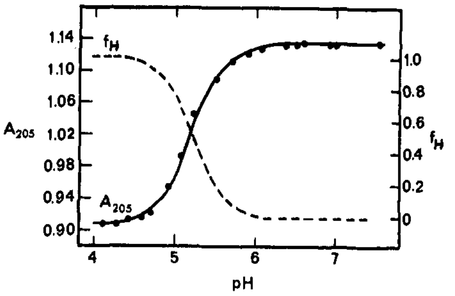

Figure 2. pH dependence of the absorbance at 205 nm (solid curve) or the fraction of helicity (dashed curve) for 3.9 × 10^{-3} (mol residue)/l. of PGA at 37°.

Ultrasonic Measurements. The attenuation of ultrasound in solutions of PGA in 0.03 M NaCl was measured as a function of pH and concentration over the frequency range 40 kHz–35 MHz, with emphasis on the region 40–1000 kHz. Three different techniques were utilized: resonator (40–250 kHz), statistical reverberation (250–1000 kHz), and pulse send-receive (15–35 MHz).

The resonator and statistical reverberation techniques are similar in nature and difficulty. A spherical glass resonator (12.3 l. in these studies) is set into oscillation by a sound wave of single frequency f (resonator) or by a statistical distribution of frequencies centered at f (statistical reverberation). The signal is then switched off and the subsequent rate of decay of acoustic energy is measured. The ultrasonic absorption may be calculated from the equation

$$\alpha/f^2 = 0.115D/cf^2 \tag{7}$$

where D = the decay rate in decibels per second and 0.115 converts from decibels to nepers.

The magnitude of the observed rate of decay in a degassed solution at thermal equilibrium in a resonator is determined not only by the absorption due to chemical relaxation, but also by the sphericity and elasticity of the glass vessel and by the amount of energy dissipated at the vessel's walls in mode coupling and frictional losses. These elevate the rate of decay above its "true" value and must be accounted for by calibrating the decay characteristics of the vessel with a nonrelaxing solution having similar sound velocity, viscosity, and density as the relaxing solution. A point-by-point subtraction yields the inherent absorption due to chemical relaxation.

The resonator apparatus was similar to that described elsewhere.[13-15] For the present studies, the equipment was modified to include temperature control (water circulation along the inside of the bell jar) and a precision frequency counter (Hewlett-Packard 5216-A). The crystals were x-cut 0.5-in. barium titanate held against the vessel by silicone grease. The PGA solutions were degassed by mild cavitation under reduced pressure, which did not result in polymer fragmentation, as evidenced by the intrinsic viscosity of PGA which remained constant at 0.4 dl/g.

Acoustic information was obtained by the resonator method from the rates of decay of radial modes of oscillation of the 12.3-l. vessel. For a given set of conditions, the radial (*i.e.*, spherical) modes have longer rates of decay than all other modes of oscillation by virtue of their low boundary losses. As in previous work with this method,[13-17] the decay of a mode was accepted for calculation only if the mode's frequency location remained constant (within ±10 Hz out of 40,000 Hz or more) on a day-to-day and system-to-system basis; furthermore, only the lowest rate of decay observed for a given mode for a given set of conditions was used. Several weeks were commonly necessary to characterize the decays of the eight reproducible spherical modes between 40 and 250 kHz.

The statistical reverberation apparatus used in these studies was entirely new and is being reported elsewhere.[18] It has these features: a pulsed Gaussian wave package was centered at the frequency of interest with a ±5-kHz bandwidth. The acoustic decays were displayed on a Tektronix 549 storage CRO equipped with a Tektronix 013-067 log adapter, and photographed. The (average) rates of decay were calculated from the photographs. The criteria for acceptability of data were as stringent for statistical reverberation as for the resonator method: reproducible lowest rates of exponential decay for each frequency between 250 and 1000 kHz.

The 12.3-l. vessel was calibrated at 37° for boundary losses with a 2.7 × 10^{-3} (mol residue)/l. of PGA solution at pH 6.0 or greater (where the helix-coil transition is absent). To test the efficacy of the choice, the rates of decay in 2.7 × 10^{-3} (mol residue)/l. of PGA in 0.03 M NaCl at 25° and pH 6.0 were compared to those obtained in water in the same vessel at room temperature As is seen in Figure 1, the boundary losses in the nonrelaxing PGA solution and in water are indistinguishable. Accordingly, the calibration was repeated at 37° and α/f^2 values for each frequency from 40 to 1000 kHz in the PGA solution at pH 6.0 were subtracted from the total α/f^2 for each frequency in the transition region to give $\Delta\alpha/f^2$—the excess absorption due to chemical relaxation.

Treatment of Ultrasonic Data. A number of methods for analyzing ultrasonic absorption data exist. The following method was chosen because of the ease with which the relaxation amplitude A and the relaxation time τ together with their uncertainties may be obtained.[19] Equation 3 may be rewritten

$$\frac{1}{\alpha/f^2 - B} = \frac{1}{A} + \frac{4\pi^2\tau^2f^2}{A} \tag{8}$$

Equation 8 is seen to have the form of a straight line with intercept $1/A$ and slope $4\pi^2\tau^2/A$ when the left-hand side is plotted *vs.* f^2. Since B, the nonrelaxing background absorption, is known from the high-frequency measurements, the desired $\Delta\alpha/f^2$ is given by $\alpha/f^2 - B$.

The values of A and τ shown in Figure 4 were obtained from plots of $1/(\Delta\alpha/f^2)$ *vs.* f^2. The "best" values of A and τ were derived from the best straight line; the uncertainties were estimated from the "worst" lines.

Other Measurements. The fraction of helicity f_h at 37° was determined from the pH dependence of the optical density at 205 nm.[8,20-22] The measurements were carried out on a Gilford-Beckman DU spectrophotometer equipped with linear digital readout. The 1-mm quartz cells were purchased from Luminon, Inc., Irvington, N. J. A titration was carried out in this manner: about 200 ml of PGA solution was thermostatted at 37°. Starting at a pH about 7.5, 1.0 M HCl was added dropwise. After each addition of acid, an aliquot was removed for measurement of the optical density. In the course of each titration, the optical density at each pH was reproducible to ±0.002 absorbance unit. A typical titration curve is shown in Figure 2. The fraction of helicity was calculated from the equation

$$f_h = (A_c - A)/(A_c - A_h) \tag{9}$$

where A_c and A_h are the absorbances of the coil and helical forms of the molecule and A is the absorbance in the transition pH region.

The densities of the PGA solutions, as measured with flask-type pycnometers, were found to be pH independent at 0.9943 g/cm^3. The sound velocity (1524 m/sec) was obtained with a 2-MHz acoustic interferometer. Coefficients of shear viscosity were determined with Ostwald viscometers. Over the pH range 4.6–6.5 at 37° the viscosity of 2.7 × 10^{-3} (mol residue)/l. of PGA increased from 7.13 to 7.24 mP.

The pH's of the PGA solutions were measured with a Beckman Research pH meter calibrated at high sensitivity with a standard millivolt source. The Thomas 4854-L60 or Corning 476050 combination electrodes were standardized against NBS buffers. All pH's reported are accurate to ±0.01 unit or better.

Results and Discussion

The fraction of helicity as a function of pH is shown in Figure 2. The shape of the curve is very similar to those obtained by previous workers at other temperatures and ionic strengths.[8,20-22] The midpoint of the

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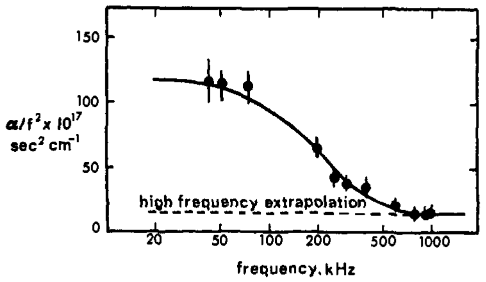

Figure 3. Typical α/f^2 *vs.* frequency curve for 2.7×10^{-3} (mol residue)/l. of PGA at 37°, pH 5.11, and I = 0.03 M NaCl. The solid line is that given by eq 3.

transition ($f = 0.5$) occurs at pH 5.21 under the present experimental conditions.

The excess acoustic absorption as a function of frequency was measured at several pH values in the transition pH region.[23] No excess absorption was observed at pH >5.8 or <4.6. A typical absorption curve is shown in Figure 3. Each set of data could be fitted within experimental error to eq 3, where B was determined by the send-receive method at high frequencies and found to be $(16.0 \pm 0.5) \times 10^{-17}$ sec²/cm, independent of pH. The B value obtained for PGA is identical with the absorption of water at 37°. For each pH in the transition region, the relaxation amplitude A and the relaxation time τ were calculated by the method described above. The A and τ values so obtained (Table I) are plotted against the fraction of helicity in Figure 4.

Table I. Relaxation Amplitudes and Relaxation Times for PGA at 37° and 0.03 M NaCl

	$C^0 = 2.7 \times 10^{-3}$		$C^0 = 1.1 \times 10^{-3}$	
pH (f_H)	A, sec² cm⁻¹ × 10¹⁷	τ, μsec	A, sec² cm⁻¹ × 10¹⁷	τ, μsec
4.84 (0.85)	30 ± 10	0.5 ± 0.1		
5.10 (0.62)			40 ± 10	0.9 ± 0.2
5.11 (0.62)	110 ± 10	0.95 ± 0.1		
5.15 (0.56)	110 ± 15	1.05 ± 0.1		
5.21 (0.50)	125 ± 20	1.0 ± 0.1	50 ± 10	1.1 ± 0.2
5.34 (0.34)	100 ± 15	0.95 ± 0.15		
5.40 (0.20)			30 ± 15	~0.7
5.55 (0.15)	30 ± 10	0.55 ± 0.15		

Typically, the relaxation amplitude near the transition midpoint was about 100×10^{-17} sec²/cm and the relaxation time was about 10^{-6} sec.

There are four noteworthy features of the data represented in Figure 4: (1) excess absorption is found only in the pH region where the helix–coil transition occurs; (2) a maximum is found in both the relaxation amplitude and the relaxation time at pH 5.21 (the transition midpoint); (3) the relaxation amplitude increases linearly with overall PGA concentration; and (4) the relaxation time is independent of concentration.

These features are exactly as predicted for the helix–coil process. On the other hand, there are at least two other possible causes of relaxational absorption in aqueous PGA solutions: proton transfer at the carbonyl side chains and a polymer–solvent interaction of unknown nature.

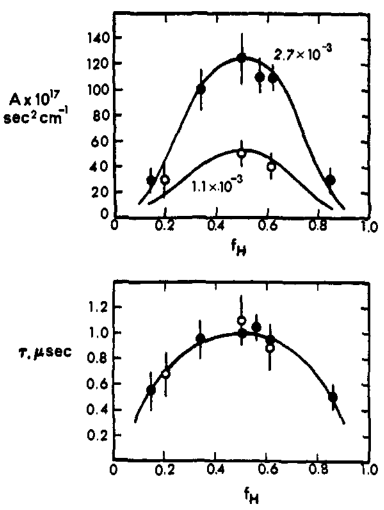

Figure 4. Upper curves: relaxational amplitude, A, *vs.* fraction of helicity. Lower curve: relaxation time *vs.* fraction of helicity. (●) 2.7×10^{-3} (mol residue)/l., (○) 1.1×10^{-3} (mol residue)/l.

Let us first consider the proton-transfer reaction

$$RCO_2^- + H^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RCO_2H \qquad (II)$$

The expression for the relaxation amplitude[10] for the protolysis reaction is given by eq 4 with

$$\Gamma = \left[\frac{1}{(RCO_2H)} + \frac{1}{(RCO_2^-)} + \frac{1}{(H^+)}\right]^{-1} \qquad (10)$$

and

$$1/\tau = k_1[(RCO_2^-) + (H^+) + K_a] \qquad (11)$$

where K_a is the intrinsic ionization constant for the γ-carboxyl groups. Equations 4 and 11 predict that both A and τ should monotonically increase with decreasing pH—not be maximized—over the narrow pH region where absorption is observed. Furthermore, the relaxation time at a given pH is expected to increase with decreasing PGA concentration—not remain constant—and finally, for a $\Delta V°$ of ~11 cm³/mol[24,25] and τ = 1 μsec, eq 4 predicts that the absorption at frequencies much less than the relaxation frequency can be no greater than 10×10^{-17} unit. We conclude therefore that the relaxational absorption observed does not result from the proton transfer reaction.

The polymer–solvent interactions observed in highly concentrated aqueous solution of poly(L-amino acids)[4,5] and other polymers[26–28] cannot account for the pH dependent excess absorption. For example, Saksena, *et al.*,[5] studied PGA in 0.2 M NaCl at a concentration of 0.15 (mol residue)/l. (more than 50 times the highest concentration employed in the present study). They observed in the megahertz frequency region relaxational

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[23] A table containing the ultrasonic absorption data will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number JACS-72-3334. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

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absorption over a broad pH range (4.0–10.0) upon which was superimposed the tail of the absorption due to the helix–coil transition. The behavior of the absorption outside the transition pH region was similar to that observed in aqueous poly(ethylene glycol)[26] and dextran,[27] in which systems polymer–solvent interactions are known to occur. Thus, the polymer–solvent interaction, were it being observed at all in the present work, should generate excess absorption across a much wider pH region than that in which an effect is seen. We find, however, that as soon as one passes the transition region for the helix–coil process, the excess absorption disappears. In fact (as shown earlier; Figure 1), one can use the polymer solution at pH 6 as a calibration solution. Furthermore, the relaxation frequencies for the polymer–solvent interactions are typically of the order of 1–10 MHz,[28] not 160 kHz, as is observed in this work. It can thus be concluded that polymer–solvent interactions are not responsible for the excess absorption in dilute solutions of PGA.

There is considerable evidence by a variety of techniques[1] (and reinforced by our own spectrophotometric measurements) that the helix-to-coil transition manifests itself in the pH region over which we observed relaxational absorption. Schwarz[9] has shown that the amplitude function Γ is given by

$$\Gamma = C^0 \frac{\sigma s'(s'+1)}{[(s'-1)^2 + 4\sigma s']^{3/2}} \tag{12}$$

where s' and σ have been defined earlier, and C^0 is the overall concentration of PGA. The relaxation time for the helix–coil transition is given by eq 2. From eq 2 and 12, it is seen that at the transition midpoint ($f_h = 0.5$ and $s' = 1$), both Γ and τ should have their maximum values and both should tend toward zero as f_h approaches 1 or 0. Such is exactly the behavior observed. Furthermore, the Schwarz theory predicts a linear concentration dependence for the relaxation amplitude—exactly as observed—and a concentration-independent relaxation time—again, exactly as observed.

Let us consider a more quantitative description of the kinetics of the helix–coil transition. We first calculate the effective volume change for the helix–coil transition (eq 5). Noguchi and Yang[25] have measured $\Delta V°$ to be 0.5–1.0 cm^3/(mol residue) for PGA. Rialdi and Hermans,[29] as well as Hagnauer,[22] have found $\Delta H° = -1100 \pm 200$ cal/(mol residue). In the present studies κ was measured to be 3.6×10^{-4} deg^{-1}. The specific heat of the system is 1 cal/(g deg). Thus, one can calculate from eq 5 that $\Delta V_{\text{eff}} = 0.9\text{–}1.4$ cm^3/(mol residue) of PGA.

At pH 5.21, the transition midpoint, the relaxation amplitude and relaxation time have their maximum values (Figure 4): $A_{\max} = (125 \pm 20) \times 10^{-17}$ sec^2/cm and $\tau_{\max} = (1.0 \pm 0.1) \times 10^{-6}$ sec. The equation for the relaxation amplitude (eq 4) may be written upon insertion of these values with $C^0 = 2.7 \times 10^{-3}$ (mol residue)/l. as

$$(\Delta V_{\text{eff}})^2\left(s'\frac{df_h}{ds'}\right)_{\max} = \frac{A_{\max}}{(3.14 \times 10^{-10})\tau_{\max}} = 4.0 \pm 1.3 \tag{13}$$

There are numerous pairs of ΔV_{eff} and τ which are within the known uncertainties of these parameters ($\Delta V_{\text{eff}} = 0.9\text{–}1.4$ cm^3/(mol residue) and $\sigma = (3 \pm 2) \times 10^{-3}$), which satisfy the equality of eq 13 and which generate calculated pH profiles for A and τ consistent with those experimentally observed. For example, the solid lines in Figure 4 were calculated with $\Delta V_{\text{eff}} = 1.0$ and $\sigma = 4 \times 10^{-3}$, $A_{\max} = 125 \times 10^{-17}$, and $\tau_{\max} = 1 \times 10^{-6}$. The fit is seen to be excellent. Thus, the helix–coil transition clearly and quantitatively accounts for the pH profile and concentration dependence of the relaxation amplitude and for the pH profile and concentration independence of the relaxation time.

From eq 2, one can calculate the forward rate constant for helical growth. At the transition midpoint, $s' = 1$; the nucleation parameter $\sigma = (3 \pm 2) \times 10^{-3}$ and $\tau_{\max} = (1.0 \pm 0.1) \times 10^{-6}$ sec. The result is $k_f = (8 \pm 5) \times 10^7$ sec^{-1}. That this rate constant, the first of its kind to be measured for a poly(amino acid) in water, should be much less than that expected if hydrogen-bond formation were the principal kinetic event in helix growth[9] may indicate that reshuffling of secondary structure on the surface of a protein, or in other regions of the protein exposed to solvent, may be slow. On the other hand, Hammes and Roberts[2] have found k_f for poly(L-ornithine) in 85% water–15% methanol to be $\sim 7 \times 10^{10}$ sec^{-1}, or diffusion controlled. The dependence of the rate constant on side-chain groups and solvent composition must be clarified before further conclusions can be drawn. To this end, more studies will be undertaken in these laboratories.

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