and sets up a $^{\delta+}C(2)-N(3)^{\delta-}$ bond polarity. Carbon magnetic resonance results show the C(2) to have a positive charge nearly that of carbon in a $C{=}O$ bond with a polarity for the C(2)-N(3) bond reminiscent of $C{=}O$ bonds.[15]

Finally, the inflexible nature of the 2-α-HETHC ion suggests that knowledge of the absolute configuration at the C(2α) atom in the side chain produced in the enzymic reaction would give desirable detail concerning the stereochemical pathway of the intermediate steps in the reaction.

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# Kinetic Studies of the Helix-Coil Transition in Aqueous Solutions of Poly(α-L-glutamic acid) Using the Electric Field Pulse Method

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**Abstract:** Relaxation phenomena were studied in aqueous solutions of poly(α-L-glutamic acid) under various conditions using the electric field pulse method with detection by conductivity change. The relaxation time has a maximum value at the midpoint of the helix-coil transition but does not depend on the polymer concentration, degree of polymerization, kind of counterion, or electric field density. Some possible mechanisms are discussed and the observed relaxation phenomenon is attributed to the helix-coil transition. Using Schwarz's theory the rate constants of the helix growth step are estimated. The activation parameters are also calculated from the temperature dependence of the rate constants. It is found that the helix growth process is not diffusion controlled but is limited by an accompanying large decrease of activation entropy.

The conformational transitions of biopolymers are known to play an important role in their functions in biological systems. The helix-coil (H-C) transition of synthetic polypeptides has been studied extensively as a useful model for conformational transitions of biopolymers. The equilibrium properties of the H-C transition have been studied in detail both experimentally and theoretically. Many kinetic studies have been made by the various relaxation methods such as the temperature-jump,[2,3] ultrasonic absorption,[4-7] and dielectric relaxation techniques.[8,9] However, the dynamic features of the H-C transition still are not clear.

Burke et al.[10] have estimated limits for the relaxation time of the H-C transition of poly(α-L-glutamic acid) (PGA) as $5 \times 10^{-8} < \tau < 10^{-5}$ by combining their result of ultrasonic absorption with that of the temperature-jump method by Lumry et al.[2] Subsequently, relaxation phenomena due to H-C transitions have been observed experimentally by using the ultrasonic absorption method for poly(α-D-glutamic acid) by Inoue[6] and for PGA by Barksdale et al.[7] The maximum relaxation times near the midpoint of the H-C transition have been estimated as $1.1 \times 10^{-6}$ sec at 30°C and $1 \times 10^{-6}$ sec in 0.03 *M* NaCl at 37°C, respectively. Recently, the present authors[11] have studied the H-C transition of PGA by a modified temperature-jump method employing optical rotation to follow the transient, and the maximum relaxation time of $3.6 \times 10^{-6}$ sec has been estimated. Previously we[12] have observed a relaxation in aqueous solutions of PGA by means of the electric field pulse (EFP) apparatus with detection by electric conductivity change and have attributed it to the H-C transition from the dependence of the relaxation time on pH and polymer concentration. The assignment of the relaxation mechanism, however, has not been conclusive enough to be confirmed.

The purpose of the present investigation is to confirm the assignment of the observed relaxation to an H-C transition and to obtain detailed information of the dynamic picture of the H-C transition.

## Experimental Section

The sodium salts of PGA with degrees of polymerization (dp) of 100, 250, and 700 were supplied from Ajinomoto Co., Inc. The samples were dialyzed against deionized water for 2 days and lyophilized and dried at 50°C for 4 hr in vacuo just prior to the preparation of the stock solutions ($5 \times 10^{-4}$ residue mole) which were stored in a refrigerator. The sample solutions were prepared by dilution of the stock solution with deionized, distilled water to the desired concentration. Since low conductivity of the solution is required for the EFP measurements in order to obtain a square wave pulse, the concentration range used is limited from $5 \times 10^{-5}$ to $2 \times 10^{-4}$ residue mole. The tetrabutylammonium PGA (TBA-PGA) was prepared by passing a given volume of NaPGA solution through the column of TBA-neutralized cation-exchange resin (Dowex 50 W-X8). Completeness of ion exchange was checked by flame test. For pH adjustment, HCl, NaOH, and tetrabutylammonium hydroxide were used. All these chemicals were of reagent grade and were used without further purification. The pH measurements were carried out with an accuracy of ±0.05 pH unit by a Hitachi-Horiba F-5 type pH meter at room temperature just before the relaxation studies and the optical rotatory dispersion (ORD) measurements.

In order to obtain the degree of dissociation, $\alpha$, the titration of PGA solution was performed with HCl or NaOH at room temperature in a nitrogen atmosphere. To avoid a large error resulting from a change in the pH value which is due to contamination by KCl from the calomel electrode during the course of the usual titration, pH measurements were carried out on a very small amount of solution taken from the large quantity of the titrated sample solution. The degree of dissociation was calculated as a function of pH by subtracting the blank curve graphically from the curve of the PGA solution.

ORD measurements were carried out in the wavelength 225–350 nm with a JASCO ORD/UV-5 spectropolarimeter using a 5-cm quartz lidded cell. The temperature was controlled to ±0.2°C by circulating thermostated water around the cell. The helix contents were evaluated from ORD data using the following equation

$$\theta = \frac{[m']_{\lambda_0} - [m']^{c}_{\lambda_0}}{[m']^{h}_{\lambda_0} - [m']^{c}_{\lambda_0}} \tag{1}$$

where $[m']_{\lambda_0}$ is the measured value of the reduced residue rotation at the minimum point of the trough in the vicinity of 233 nm, and $[m']^{h}_{\lambda_0}$ and $[m']^{c}_{\lambda_0}$ are the reduced residue rotations for completely helical and coiled states, respectively. These values were calculated from the following equations by Warashina and Ikegami[11] as a function of temperature $T$ (°C).

$$[m'(T)]^{h}_{233} = -18400 + 66T \tag{2}$$

$$[m']^{c}_{233} = -2000 \tag{3}$$

The electric field pulse apparatus used here is similar to that originally constructed by Ilgenfritz.[14] A block diagram of the apparatus is shown in Figure 1. A 1-km length of 10C-2V coaxial cable was used as a capacitor. The transients were followed by monitoring the conductance change with a Wheatstone bridge. The difference in voltage between points A and B (Figure 1) was detected by a Tektronix type W plug-in differential preamplifier. Two resistances of the lower arms of the bridge are equal and the value of resistance is selected so as to give about 10 V as input signals of the amplifier. The distance between the two electrodes of the sample cell is fixed at 0.3 cm and that of the reference cell is kept variable. The reference cell contains a NaCl or tetrabutylammonium chloride solution of approximately the same conductance as the sample solution. The resistances of both cells were matched precisely by adjusting the distance of the two electrodes of the reference cell. The duration of the high voltage pulse applied to the bridge is 10 or 20 μsec, and the rise and decay times of the applied electric field are much faster than 0.1 μsec. The electric field intensity in the cell is usually about 30 kV/cm. As a test of the apparatus, dissociation field effects for two pH indicators, bromcresol purple and phenol red, were measured spectrophotometrically, and the results agreed with those reported by Ilgenfritz.[14]

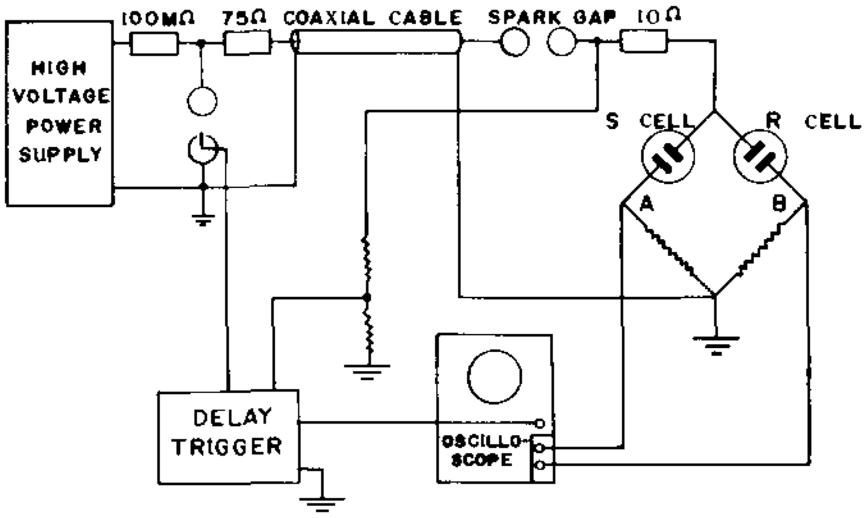

Figure 1. The block diagram of the electric field pulse apparatus.

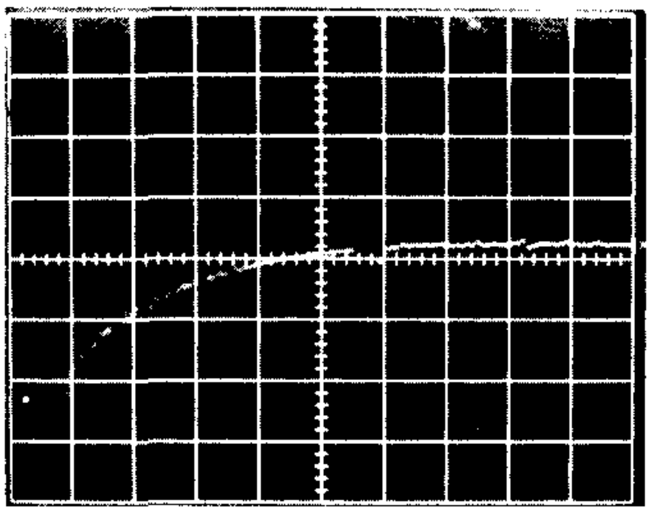

Figure 2. The typical relaxation spectrum for NaPGA (dp = 250), $1.0 \times 10^{-4}$ residue mole, pH 6.63, at 25°C; sweep 2 μsec/division.

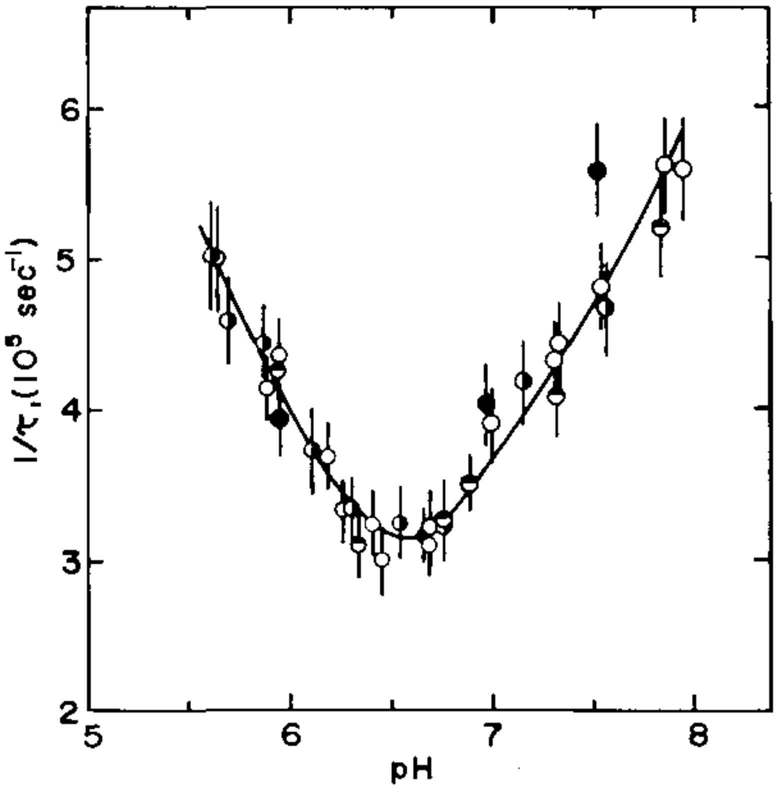

Figure 3. The concentration dependence of the reciprocal relaxation time vs. pH curve for NaPGA (dp = 250) at 25°C: (◐) $5.7 \times 10^{-5}$ residue mole, (⊖) $8.0 \times 10^{-5}$ residue mole, (○) $1.0 \times 10^{-4}$ residue mole, (●) $2.0 \times 10^{-4}$ residue mole.

The relaxation studies using an electric conductivity change as a monitor of transient concentration changes were performed mainly for NaPGA with dp = 250 over the concentration range of $5 \times 10^{-5}$–$2 \times 10^{-4}$ residue mole in the range 15–45°C. The effects of the counterion and the chain length on the relaxation behavior were also studied for TBA-PGA and NaPGA with dp = 700, respectively. Furthermore, the EFP experiments were done by spectrophotometric detection on solutions of PGA containing the pH indicator, bromcresol purple, in order to examine the effect of dissociation of the proton from the carboxyl group in the side chain.

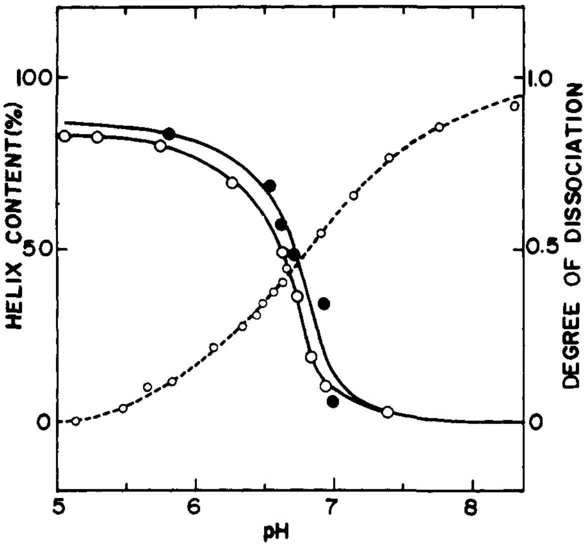

**Figure 4.** The pH dependencies of the helix content (solid curve) and the degree of dissociation (dashed curve) for NaPGA (dp = 250). Solid curve: (●) 5.0 × $10^{-5}$ residue mole, (○) 1.0 × $10^{-4}$ residue mole, at 25°C. Dashed curve: 1.0 × $10^{-4}$ residue mole at room temperature.

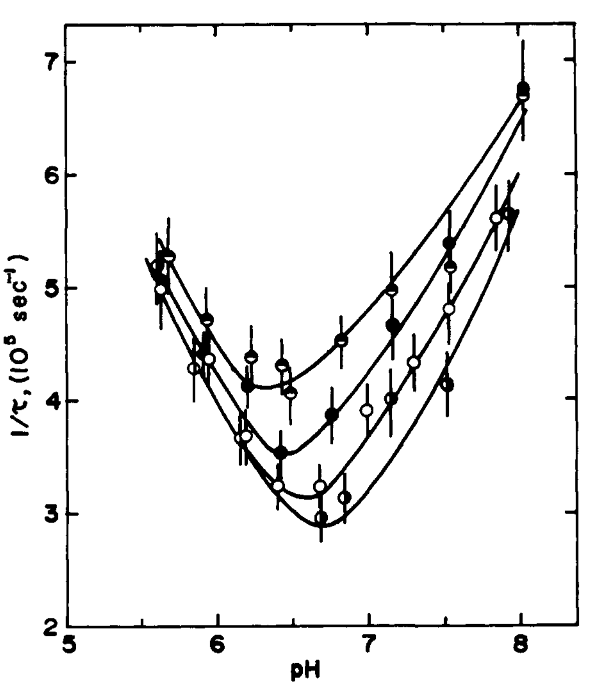

**Figure 5.** The temperature dependence of the reciprocal relaxation time vs. pH curve for NaPGA (dp = 250), 1.0 × $10^{-4}$ residue mole: (◑) 15°C, (○) 25°C, (●) 35°C, (⊖) 45°C.

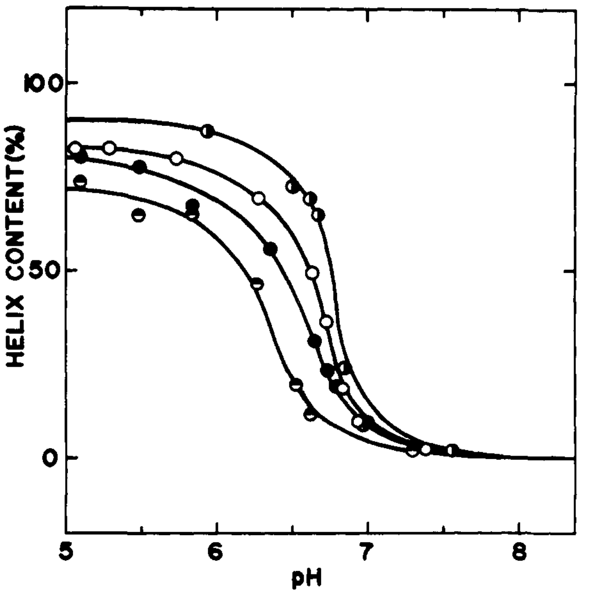

**Figure 6.** The temperature dependence of the helix content vs. pH curve for NaPGA (dp = 250), 1.0 × $10^{-4}$ residue mole: (◑) 15°C, (○) 25°C, (●) 35°C, (⊖) 45°C.

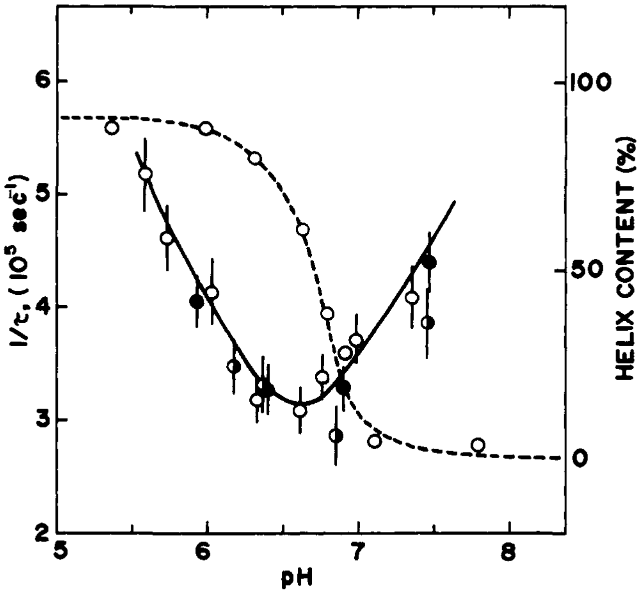

**Figure 7.** The pH dependencies of the reciprocal relaxation time (solid curve) and the helix content (dashed curve) for TBA-PGA (dp = 250) at 25°C. Solid curve: (◑) 5.0 × $10^{-5}$ residue mole, (○) 1.0 × $10^{-4}$ residue mole, (●) 2.0 × $10^{-4}$ residue mole. Dashed curve: 1.0 × $10^{-4}$ residue mole.

## Results and Discussion

The kinetic measurements were carried out in the pH range 5.5–8.0. Outside this pH range there still existed a small amplitude relaxation for which an accurate relaxation time could not be estimated because of its small magnitude, and at the extremes of pH 5.0 and 9.0 the relaxation effect disappeared completely. A typical relaxation curve is shown in Figure 2. The direction of the relaxation signal indicates an increase in the conductance of the solution by the application of the electric field. The relaxation spectra show that a rapid increase of conductance occurs with application of the electric field and is followed by the relaxation part of the conductance increase, which is characterized by a single relaxation at all pH values. Figure 3 shows the pH dependence of the relaxation time for NaPGA with dp = 250 at various polymer concentrations (5 × $10^{-5}$–2 × $10^{-4}$ residue mole). The relaxation time has a maximum value at pH 6.6 and is independent of polymer concentration. Figure 4 shows the corresponding pH dependence of the helix content from the ORD measurements. In this figure the H–C transition region shifts considerably to the higher pH side in comparison with that reported in the literature[13,15,24] and depends slightly on the polymer concentration. These shifts of the transition region can be explained as the effect of the ionic strength of the counterion.[16] It is seen from Figures 3 and 4 that the relaxation time reaches its maximum value in the vicinity of the midpoint of the H–C transition. This maximum at the midpoint of H–C transition was observed at all studied temperatures and is shown in Figures 5 and 6. The same results were obtained not only in the solution of TBA-PGA with dp = 250 but also in the solution of NaPGA with dp = 700 and shown in Figures 7 and 8, re-

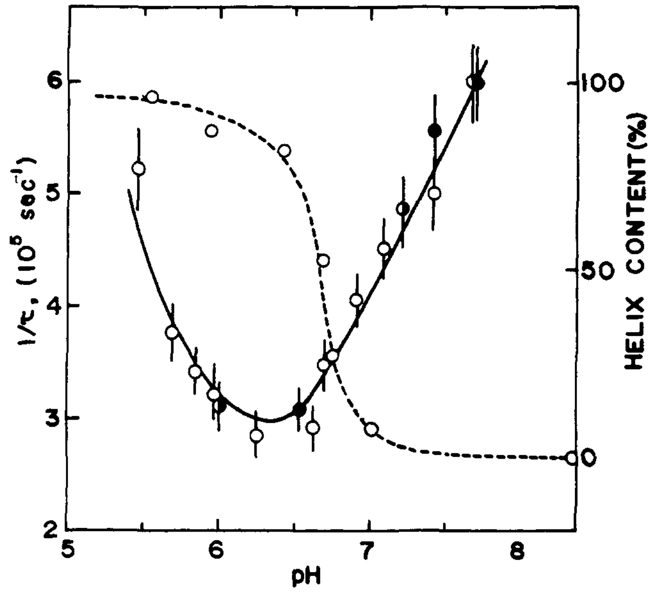

**Figure 8.** The pH dependencies of the reciprocal relaxation time (solid curve) and the helix content (dashed curve) for NaPGA (dp = 700) at 25°C. Solid curve: (◑) $5.0 \times 10^{-5}$ residue mole, (○) $1.0 \times 10^{-4}$ residue mole, (●) $2.0 \times 10^{-4}$ residue mole. Dashed curve: $1.0 \times 10^{-4}$ residue mole.

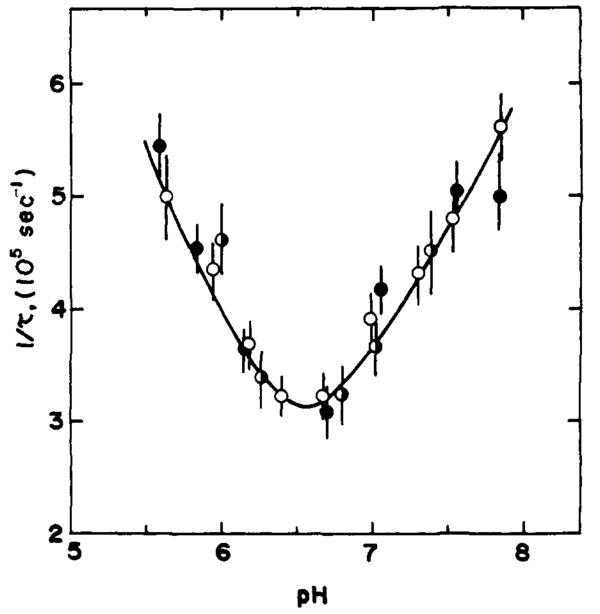

**Figure 9.** The dependence of the reciprocal relaxation time vs. pH curve on the electric field density for NaPGA (dp = 250), $1.0 \times 10^{-4}$ residue mole, at 25°C: (◑) 23 kV/cm, (○) 30 kV/cm, (●) 66 kV/cm.

spectively, along with H-C transition curves. However, in PGA with lower dp (about 100) the magnitude of the relaxation was so small that accurate estimation of the relaxation time was not possible. Figure 9 shows the pH dependence of the relaxation time under various electric field densities (EFD). Although the relaxation amplitude increased with increasing EFD, the relaxation time remained constant as seen in Figure 9.

When a high electric field is applied to the polyelectrolyte solution, the conductivity may be affected in three different ways.[17,18] One of them is due to the change in mean mobility of the charge carriers caused by orientation of the long axis of the polyion in the direction of the field (orientational field effect). The second case is the increase in conductivity due to increased dissociation of the proton of the carboxyl group in the side chain or counterion bound to the polyion by application of a high electric field (dissociation field effect). Also there is the possibility that a high electric field induces the conformational change of polypeptide which accompanies the increased dissociation of proton and counterion or the change in polyion mobility. Accordingly there exist four possible mechanisms for the observed relaxation processes: an orientation process, an ion-binding reaction of the counterion, the proton-transfer reaction, and the H-C transition. These possible mechanisms will be examined in detail.

(1) **Orientation Field Effect.**[17,18] The electric field induces electric dipoles on the polyelectrolyte particles due to the polarization of the inner ionic atmospheres of the polyions and causes the orientation of their long axis in the direction of the field. As a consequence of anisotropy of the nonspherical particle, anisotropy of the conductivity is produced in the solution. When the orientation is caused by a pure induced dipole, which may be applied to the present case, the relaxation time of the orientation process at high EFD is[17]

$$\tau_E = 1.68 \times kT/\alpha_{\parallel} D_r \times E^{-2} \tag{4}$$

where $\tau_E$ is relaxation time, $\alpha_{\parallel}$ is polarizability perpendicular to the electric field, $D_r$ is the rotational diffusion coefficient, and $E$ is the electric field density, respectively. The most characteristic property of this equation is that $\tau_E$ is inversely proportional to the square of EFD. This is not consistent with the observed results in which the relaxation time is independent of applied EFD. Furthermore, our preliminary experiments of electric birefringence show that the rise time of the electric birefringence is two times faster than the present relaxation time and decreases with EFD. These facts indicate that the present relaxation phenomena cannot be ascribed to the orientation field effect.

(2) **Ion Binding Process of the Counterion.** In a solution of polyelectrolyte, the counterion is bound around the polyion in two ways.[19] One is the specific association between counterions and the ionic groups of the polyion (site binding). The other is the association of the counterion in the vicinity of the polyion due to its large electrostatic field (ionic atmosphere binding). The application of the external field may bring about the removal of these counterions from the polyion, which leads to an increase in the conductance of the solution. The former type of binding, however, can be eliminated from the possible mechanisms by the experimental fact that no relaxation behavior changed when the counterion was converted from sodium to tetrabutylammonium ion, which is not site bound because of its large radius.[19,20] The latter mechanism is also not likely to be responsible for the observed relaxation given the following considerations. The relaxation time for this process must be closely connected with the mobility of the counterion or the environment on the polyion. Accordingly, the relaxation behavior would be expected to change when the counterion is converted from sodium to tetrabutylammonium because of the difference in mobilities of both counterions. This speculation contradicts the observed behavior. Wissbrun et al.[21] also have studied conductance changes under high electric fields for polyacrylic acid and polystyrenesulfonic acid, etc., and assigned it to the reaction associated with removal of the counterion in the ion atmosphere by application of the external field. In their experiments, the relaxation time showed no discernible variation with degree of dissociation but decreased with increasing electric field. They have also suggested that the relaxation time increases with molecular weight. These features of the relaxation time are opposite to

the present relaxation phenomena. Thus the ion-binding reaction of the counterion also can be eliminated from the possible mechanisms.

(3) **Proton Transfer Reaction of the Carboxyl Group.** For the proton-transfer equilibrium of the carboxyl group in the side chain

$$\mathrm{RCOOH} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{RCOO^-} + \mathrm{H^+} \tag{I}$$

the relaxation time is expressed by the following equations

$$1/\tau = k_{-1}[(\mathrm{RCOO^-}) + (\mathrm{H^+})] + k_1 \tag{5}$$

$$= k_{-1}[\alpha C_0 + (\mathrm{H^+}) + K_{\mathrm{app}}] \tag{6}$$

where $\alpha$ is the degree of dissociation in the carboxyl group, $C_0$ is the total polymer concentration, and $K_{\mathrm{app}}$ $(=k_1/k_{-1})$ is the apparent dissociation constant. The value of $\alpha$ obtained from the titration experiment is shown as a function of pH in Figure 4. In general, for the protolytic reaction the reciprocal relaxation time $(1/\tau)$ has also the minimum value at a certain pH ($\mathrm{pH_m}$), and $\mathrm{pH_m}$ is expected to be below 5.5 for the PGA. On the other hand, as the value of $K_{\mathrm{app}}$ is in the range $10^{-6}$–$10^{-7}$ in the present case, $\alpha C_0 + (\mathrm{H^+}) > K_{\mathrm{app}}$ is satisfied. Therefore the reciprocal relaxation time must increase monotonically with total polymer concentration at constant pH and with pH at constant polymer concentration. This expectation contradicts the present results in which the relaxation time does not show any discernible variation with polymer concentration and reaches its maximum value at pH 6.6. This fact indicates that the observed relaxation phenomena cannot be attributed directly to the proton-transfer reaction.

(4) **H–C Transition.** The observed relaxation phenomena cannot be explained by the above three mechanisms but can be satisfactorily explained with the H–C transition mechanism as will be shown below. The relaxation time goes through its maximum value in the midpoint of the transition and is independent of polymer concentration, kind of counterions, and applied EFD. These features of the relaxation time are easily understood in light of the H–C transition mechanism. Schwarz[22] has adapted the Zimm–Bragg model[23] to the kinetic theory of H–C transitions. According to his theory, the mean relaxation time of the H–C transition in the vicinity of the midpoint of the transition is expressed by the following equation

$$1/\tau = k_{\mathrm{f}}[(s' - 1)^2 + 4\sigma] \tag{7}$$

where $k_{\mathrm{f}}$ and $s'$ are the forward rate constant and the equilibrium constant for the helix growth, respectively, and $\sigma$ is the nucleation parameter. This equation predicts that the mean relaxation time has its maximum value at the midpoint of the transition and is independent of polymer concentration. At the transition midpoint, $s'$ becomes unity and the equation is simplified to

$$1/\tau = 4\sigma k_{\mathrm{f}} \tag{8}$$

The value of $\sigma$ is given as $(3 \pm 2) \times 10^{-3}$ for PGA from the titration experiments by Snipp et al.[24] and is independent of ionic strength[24] and temperature.[25] Introducing the experimentally determined maximum relaxation times into the above equation, the rate constants of helix growth, $k_{\mathrm{f}}$, are obtained at various temperatures and are given in Table I where a $\sigma$ value of $3 \times 10^{-3}$ was used. These values are comparable to those obtained by other investigators, e.g., $4.4 \times 10^7\ \mathrm{sec^{-1}}$ for poly($\alpha$-D-glutamic acid) at 30°C by Inoue[6] and $(8 \pm 5) \times 10^7\ \mathrm{sec^{-1}}$ for PGA in 0.03 *M* NaCl at 37°C by Barksdale et al.[7]

According to Eyring's absolute rate process, the rate constant $k_{\mathrm{f}}$ is related to the activation enthalpy $\Delta H^\ddagger$ and entropy $\Delta S^\ddagger$ as follows.

**Table I.** Maximum Relaxation Times, $1/\tau_{\mathrm{max}}$, and the Rate Constants, $k_{\mathrm{f}}$, of the Helix Growth Process at Various Temperatures[a]

| $T$, °C | $1/\tau_{\mathrm{max}}$, $10^5\ \mathrm{sec^{-1}}$ | $k_{\mathrm{f}}$, $10^7\ \mathrm{sec^{-1}}$ |
|---|---|---|
| 15 | 2.9 ± 0.2 | 2.4 ± 0.2 |
| 25 | 3.1 ± 0.2 | 2.6 ± 0.2 |
| 35 | 3.5 ± 0.2 | 2.9 ± 0.2 |
| 45 | 4.1 ± 0.3 | 3.4 ± 0.2 |

[a] In the estimations of $k_{\mathrm{f}}$, a $\sigma$ value of $3 \times 10^{-3}$ was used.

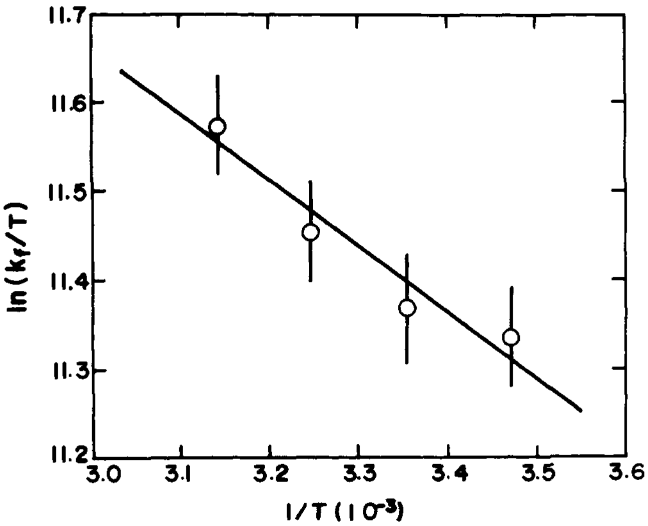

**Figure 10.** Ln $(k_{\mathrm{f}}/T)$ vs. $1/T$ for NaPGA (dp = 250). The straight line is a least-squares fit of the experimental points.

$$k_{\mathrm{f}} = (kT/h)e^{-(\Delta H^\ddagger - T\Delta S^\ddagger)/RT} \tag{9}$$

The values of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ were calculated to be 1.5 ± 0.4 kcal/mol and −20 ± 2 cal/mol, respectively, from a ln $(k_{\mathrm{f}}/T)$ vs. $1/T$ plot, which is illustrated in Figure 10. The small value of $\Delta H^\ddagger$ is to be expected for the H–C transition of polypeptides because it is connected mainly with the hydrogen bond formation which does not require any appreciable activation energy. However, an unexpectedly large value of $\Delta S^\ddagger$ cannot be explained by the diffusion-controlled formation of the hydrogen bond. Schwarz et al.[8] have estimated the maximum value of the relaxation time of poly($\gamma$-benzyl L-glutamate) in a dichloroacetic acid–ethylene dichloride mixture as $5 \times 10^{-7}$ sec, which leads to a value for $k_{\mathrm{f}}$ of $1.3 \times 10^{10}\ \mathrm{sec^{-1}}$. This value of $k_{\mathrm{f}}$ shows that in the poly($\gamma$-benzyl glutamate), the helix growth reaction must be practically diffusion controlled. Such differences in rate constants for the polypeptides with different side chains may be attributed to the nature of the interaction between the side chain and solvent. In the case of PGA, a large decrease of entropy in the activation state plays the important role in the H–C transition.

From the present experiments it was found that a high electric field may induce an H–C transition in PGA. Some theoretical considerations have been given for the effect of electric fields on H–C transitions of polypeptides.[26,27] Schwarz[8,26] has examined theoretically the electric field effect on H–C transitions of polypeptides with no dissociation group in the side chain and has applied successfully his theory to the dielectric relaxation of poly($\gamma$-benzyl glutamate) in dichloroacetic acid–ethylene dichloride mixtures. Recently Kikuchi et al.[28] have observed the transition of charged helix to charged coil in poly(L-lysine hydrobromide) solutions by application of high electric fields. The origin of these electric-field induced H–C transitions has been attributed to the difference in the magnitude of dipole moments for each conformation which leads to a difference in free energy under the electric field. In the present case, a more complicated situation may arise. From the experiment using a pH indicator, it was found that a considerable number of protons are released from carboxyl groups in the side chains by application of an electric field. Accordingly, it is expected that the dissociation of the proton plays some important role in the observed relaxation. Taking this fact into account, two possible mechanisms for the electric-field induced H–C transition can be proposed. One possibility is that the transition is induced by increasing electrostatic repulsion between the charged side chains caused by proton dissociation. The other is that it originates from the difference in overall dipole moment between each conformation of PGA.[29] At the present time it is difficult to distinguish definitely between the two mechanisms from the available data. However, the second one may be more suitable for the following reason. The molecular length may affect the magnitude of the H–C transition per unit EFD greater in the second mechanism than in the first one. In the present study, it was found that in order of the chain length (700, 250, and 100) the relaxation amplitude so decreases that at the extreme of 100 the relaxation time cannot be estimated accurately. This fact suggests that the second mechanism is preferable to the first one.

As is shown above, the observed relaxation phenomena were attributed to the H–C transition, but two problems still remain to be clarified. (1) The relaxation phenomena were still observed at considerably high pH values where helix content is close to zero according to ORD data. (2) The helix content ($\theta$) is related to $s'$ by the following equation.[23]

$$\theta = \tfrac{1}{2}\left[1 + \frac{s' - 1}{[(1 - s')^2 + 4\sigma s']^{1/2}}\right] \qquad (10)$$

Then with the known value of $\sigma$ and the value of $k_f$ estimated experimentally from the maximum relaxation time, the theoretical curve for a variation of $1/\tau$ with pH can be calculated according to eq 7 and 10. The theoretical curve obtained shows a sharper dependence of pH than was observed experimentally. The same results were obtained in recent kinetic studies of the H–C transition in PGA by the optical rotation temperature-jump method.[11] The first problem may be due to the existence of small helix content even at such a pH, but the explanation for the second one is not so simple. Barksdale et al.[7] have reported kinetic studies of PGA in aqueous 0.03 *M* NaCl by means of ultrasonic absorption, in which a pH profile of the relaxation time experimentally obtained is consistent with that calculated using Schwarz's theory in contrast with the present results. Although the deviation of the relaxation time from Schwarz's theory in the present case may result from the complicated coupling of the H–C transition reaction with the proton-transfer reaction or orientation process, the cause of this discrepancy is not clear at present. Before we attain detailed dynamic pictures of the H–C transitions of polypeptides, we must await the accumulation of more experimental information on the kinetics of H–C transitions.

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## References and Notes

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