Elongational Flow Field as a Tool for Investigating Helix–Coil Transition: Observation of Helix–Coil Transition in Poly(L-glutamic acid) Induced by pH Change

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SYNOPSIS

Elongational flow technique has been used to investigate helix–coil transition of poly(L-glutamic acid) (PGA) induced by pH change. The elongational flow field was generated by a four-roller mill apparatus of the type originally used by G. I. Taylor.[16] The flow-induced nonlocalized birefringence was observed all over the pH range measured. The birefringence, Δn_{176}, at a fixed strain rate $\dot{\varepsilon} = 176$ s^{-1} decreases rapidly within a narrow pH range with increasing pH. The Δn_{176} value is considered to contain information about both helix content, θ, of the system and the degree of orientation of polymers in the elongational flow field. From the Δn_{176} value, using rotational diffusion coefficient, D_r, the helix content, θ, was estimated at each iso-pH measurement. pH dependence of θ was obtained and was found to correspond well to those determined by optical rotary dispersion (ORD) studies. Even in a hinged-rod conformation, the PGA molecule is expected to be in an extended structure. On the basis of the hydrodynamic considerations about the molecular conformation, it is concluded that the elongational flow technique is useful to investigate conformational transitions of biopolymers. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

This article describes a new approach to structural transition of biopolymers on the basis of the response of polymeric molecules to a pure elongational flow field which has been provided valuable information on the molecular process of chain extension, relaxation, and orientation of molecules. In examining the response of flexible,[1-3] semiflexible,[4] and rigid-rod-like[5,6] polymer molecules to well-defined elongational flow fields, it has been established that the elongational flow method can distinguish between flexible polymer chains and rigid-rod-like chains.[7] For flexible molecules, flow-induced birefringence is localized along the pure elongational flow field. In a birefringence (Δn) vs. strain rate ($\dot{\varepsilon}$) plot, at small $\dot{\varepsilon}$s, Δn remains zero and then increases rapidly at a critical strain rate $\dot{\varepsilon}_c$. The phenomenon has been predicted theoretically as the coil-stretch transition of a polymer chain.[8,9] In the case of rigid-rod-like molecules, nonlocalized birefringence is observed over the whole irradiated field around the pure elongational flow field. Δn increases continuously with $\dot{\varepsilon}$.

In our previous work, molecular properties of monodisperse type I collagen from lathyritic rat tail tendon were investigated by the elongational flow technique.[10] In the thermal denaturation measurements, the possibility of the elongational flow field was suggested as a new tool for investigating conformational transition from helix to coil and vice versa.

We aimed to apply an elongational flow field as a new tool for investigating the dynamic structure of biopolymers. As a first step, we are focusing our attention on confirming the suggestion about the possibility of the elongational flow technique to in-

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vestigate a helix–coil transition, previously presented.[10] In the present work, we tried to reproduce the helix–coil transition in poly(L-glutamic acid) aqueous solution by observing responses to the elongational flow field. The results obtained by the elongational flow method were compared with those obtained by the already consolidated method of optical rotary dispersion (ORD) measurements, for example,[11] and the ability of the elongational flow field was discussed in relation to the dynamic structure of biopolymers.

EXPERIMENTAL

Materials

The polymer used for this work was poly(L-glutamic acid) (PGA) which was purchased in lyophilized sodium salt form from Sigma Chemical company, Ltd. The molecular weight (M_w) of PGA was determined to be 6.38×10^4 Da by viscosity measurement,[12,13] which accords with the value 5.37×10^4 Da reported by Sigma.[12,14] According to Sigma,[14] polydispersity was $M_w/M_n = 1.40$. The lyophilized PGA was dissolved into distilled and ion-exchanged water buffered by an acetic acid–sodium acetate aqueous solution at room temperature. Glycerol was used as a viscosity builder. In order to prepare a homogeneous solution, at least 24 h of stirring was performed by a magnetic stirrer at room temperature. The molecular weight 5.37×10^4 Da for PGA gives an α–helix of 63 nm long. The c^* for the dilute–semidilute boundary[15] of PGA as an α-helical structure is 0.42 mg/ml. In this work, 1.28 mg/ml of PGA was dissolved into 85% (v/v) of glycerol aqueous solution buffered by acetic acid–sodium acetate. pH value was changed by changing the contents of the buffer from pH 5 to pH 6.2. All of the measurements were performed at 20°C.

Apparatus

The apparatus used to generate an elongational flow field was a four-roll mill, originally utilized by G. I. Taylor for the study of liquid droplets in the flow field,[16] and already used for elongational flow studies of biopolymers.[10,17,18] Dimensions of the apparatus are as follows: roller radius $R = 5.0$ mm; surface-to-surface distance between rollers $h = 2.0$ mm; and roller length $L = 20.0$ mm. The flow-induced birefringence, Δn, was monitored as the response of polymer molecules to the elongational flow field. Optical monitoring system including laser beam is

the same as previously used in collagen experiments.[10] Strain rate $\dot{\varepsilon}$ of the elongational flow was determined by Torza's formula.[19] Measurements of Δn as a function of $\dot{\varepsilon}$ were made isothermally at each pH for strain rates over the range of 0 and 176 s^{-1}. The temperature of a solution was controlled to be 20°C, at which all measurements were performed, by a heat jacket through which water from a heat bath was circulating.

THEORETICAL

We expect it will be possible to observe the conformational change of molecules in solution from a flexible random coil to a rigid-rod-like helix, and vice versa, by observing the flow-induced birefringence pattern. In an actual system of polymer solution with molecular weight below 10^6, to realize a stretched state of a flexible polymer chain a strain rate of about 10^6 s^{-1} is required,[7,20] which is much larger than that generated by our equipment. Hence, for molecules of $M_w < 10^6$ and using our equipment, all we can monitor is the nonlocalized birefringence observed when the molecule is in helical conformation. The PGA molecule is in a helical conformation at low pH values, and with the increase in pH value it changes the conformation to a random coil. Then, by observing the intensity of the nonlocalized flow-induced birefringence (Δn) with increasing pH, the helix–coil transition of the polymer can be investigated. Figure 1(a) illustrates a three-dimensional plot of Δn against $\dot{\varepsilon}$ and pH value, where a basic concept of observing the helix–coil transition
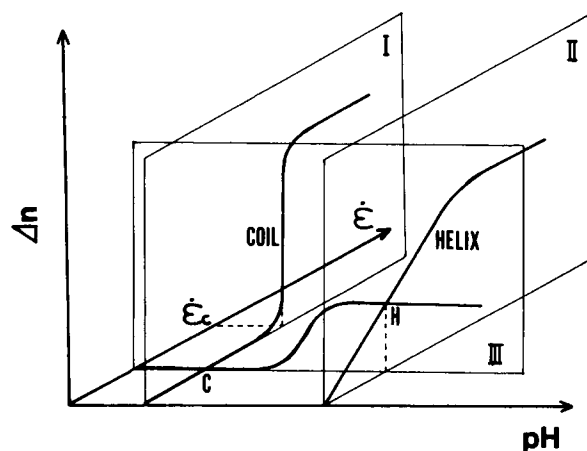

Figure 1(a) Three-dimensional schematic plot of Δn against $\dot{\varepsilon}$ and pH. (I) Δn versus $\dot{\varepsilon}$ for coil. (II) Δn versus $\dot{\varepsilon}$ for helix. (III) Δn versus pH at a fixed $\dot{\varepsilon} \ll \dot{\varepsilon}_c$.
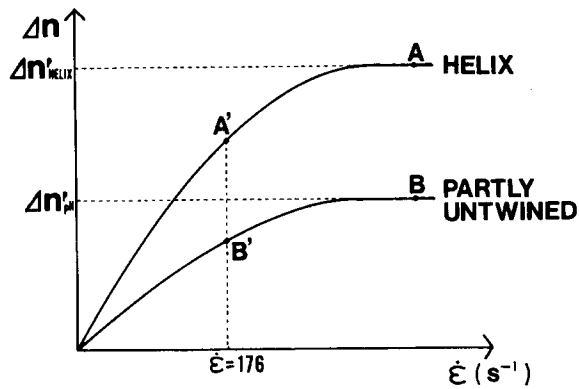
Figure 1(b)   Iso-pH plot of Δn versus $\dot{\varepsilon}$ for a complete helix, $\Delta n^p_{\text{HELIX}}$, and a helix partly untwined, Δn^p_{PH}. A and B are birefringence responses at complete orientation of molecules to elongational flow field at each conformation. A' and B' are those at imperfect orientation.

induced by pH change is shown. At a pH value of coil state, in a Δn vs $\dot{\varepsilon}$ plot, there are $\dot{\varepsilon}$ region(s) where Δn is practically zero (around the point C up to $\dot{\varepsilon}_c$ in plane I). At a pH value of the helix state, the Δn vs $\dot{\varepsilon}$ pattern for rigid-rod-like molecules is obtained (the point H in plane II). At a certain $\dot{\varepsilon}$, which is required to be large enough, changing pH, a typical transition-like sigmoidal curve in the Δn–pH plane will be observed (plane III).

The helix content, θ, in the solution is defined as

$$\theta = \Delta n^p / \Delta n^p_{\text{helix}} \qquad (1)$$

where Δn^p and $\Delta n^p_{\text{helix}}$ are flow-induced nonlocalized birefringence (plateau) values of a completely oriented polymer solution for partly untwined helices and complete α-helices, respectively, which are il-

lustrated as A and B in Figure 1(b). In an actual measurement, it was impossible to observe both values because of the limit in the strain rate that can be generated. We used the birefringence values Δn^{176}_K and $\Delta n^{176}_{\text{helix}}$ at the uppermost strain rate of our equipment, $\dot{\varepsilon} = 176$ s^{-1}, instead, at a pH $= K$ where helices are partly untwined and in the helical conformation, which are illustrated as A' and B', respectively, in Figure 1(b). The relation between the birefringence value of the completely oriented polymer solution, Δn_p, and that of the imperfectly oriented one, Δn, is[21]:

$$\Delta n = (2\Delta n_p / 15 D_r)\dot{\varepsilon}[1 - \exp(-6D_r t)] \qquad (2)$$

where D_r is the rotational diffusion coefficient of the polymer molecule in solution. Using this quantity, θ can be empirically obtained:

$$\theta = \Delta n^{176}_K D_r(\text{helix})/\Delta n^{176}_{\text{helix}} D_r(K) \qquad (3)$$

where $D_r(\text{helix})$ and $D_r(K)$ are rotational diffusion coefficients of helix and partly untwined helix at pH $= K$. These values can be obtained from the slope of Δn vs $\dot{\varepsilon}$ curve at $\dot{\varepsilon} \rightarrow 0$ or from the relaxation rate of nonlocalized birefringence after sudden stopping of the roller operation where $\Delta n \propto \exp(-6D_r t)$.[5]

RESULT AND DISCUSSION

Figure 2 shows Δn vs $\dot{\varepsilon}$ curves for pH values ranging from 5 to 6. When the polarizer and analyzer were positioned at 45° relative to the mill inlet and outlet direction, the birefringence was not localized and was homogeneous all over the area enclosed by the
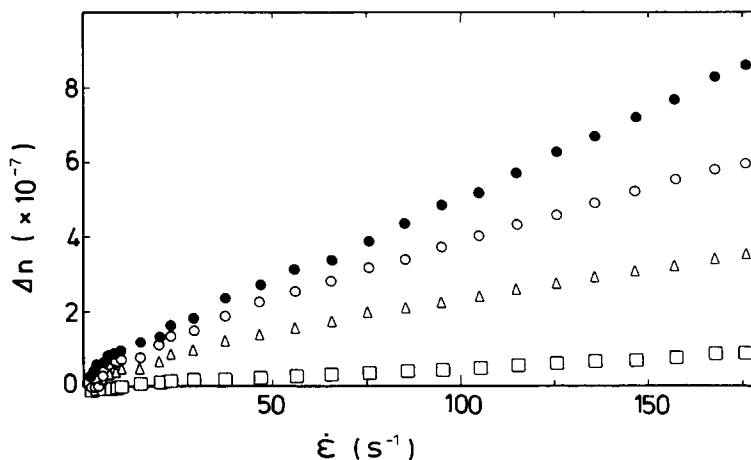

Figure 2   Flow-induced birefringence (Δn) plotted against $\dot{\varepsilon}$ for PGA aqueous solutions at pH $= 5.2$ (●), 5.6 (○), 5.8 (△), and 6.1 (□).
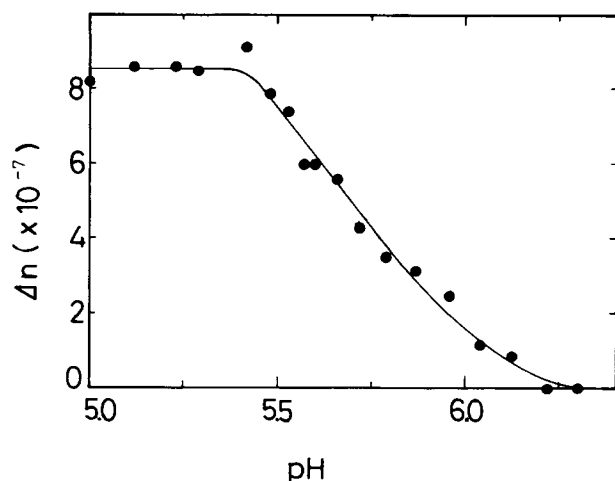
Figure 3 pH dependence of flow-induced birefringence of PGA solution at $\dot{\varepsilon}$ = 176 s^{-1}.

rollers. Nonlocalized birefringence was observed for all isothermal and iso-pH experiments. With the polarizer and analyzer positioned parallel and perpendicular to the inlet and exit directions, the same area became uniformly dark. These results suggest that PGA molecules are rigid-rod-like and that the transmission axes of the birefringent solution coincide with one of these two directions. Rigid-rod-like molecules are empirically known to be oriented parallel to the exit symmetry plane of the elongational flow field at the center of a four-roll mill.[5] In the case of PGA molecules, it is not unreasonable to recognize that the rod axis of a molecule is oriented parallel to the exit symmetry plane within the center of the four-roll mill.

Figure 3 shows pH dependence of flow-induced birefringence, Δn, at $\dot{\varepsilon}$ = 176 (sec^{-1}). Δn decreases rapidly with increasing pH at a narrow pH range. As has been discussed in the theoretical section, molecular weights for these polymers are insufficient to generate the flow-induced birefringence pattern characteristic to a flexible random coil. Then, the Δn observed, which is nonlocalized, through the entire pH range observed originates from the α-helix or the α-helical parts of the molecules in solution. Thus, the observed change in Δn against pH is considered to express the conformational change of the polymer from random coil to α-helix.

In order to estimate the helix content, θ, D_r values at each pH are needed. Figure 4(a) shows the pH dependence of D_r. From pH 5 to 5.5, the D_r value is almost constant; but it decreases beyond pH 5.5, where PGA was expected to begin to untwine into random coil. This decrease in D_r over pH 5.5 indicates the increase in hydrodynamic resistance of
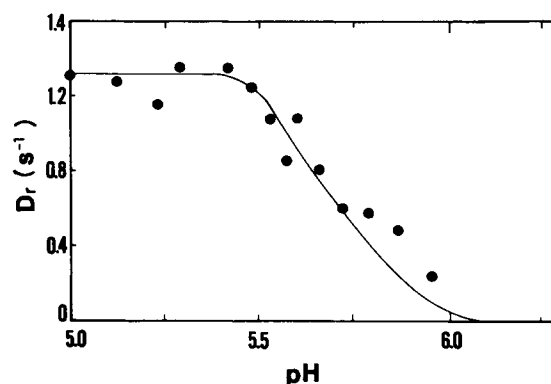

Figure 4(a) pH dependence of rotational diffusion coefficient, D_r, determined by measuring relaxation of birefringence after sudden stop of the flow.

molecules in the solution. Figure 4(b) shows the pH dependence of helix content, θ, in PGA aqueous solution. In the diagram, θ determined by ORD measurements[11] are also shown. pH dependence of θ by elongational flow method was found to accord with that determined by ORD experiments. It is notable that even in the partly untwined state, some of the results indicate PGA would be rigid-rod-like; (1) the birefringence pattern in the mill space was nonlocalized at any pH, and (2) in Δn vs $\dot{\varepsilon}$ curve at any pH has no delay of Δn appearance on $\dot{\varepsilon}$, $\dot{\varepsilon}_0$.[10,17] At pH values from 5.0 to 5.5, PGA was demonstrated to be α-helix and hydrodynamically a rigid-rod. From the birefringence measurements here and in the ORD experiments[11] beyond pH 5.5, PGA begins to untwine and is expected to be in the shape of a hinged-rod.

In previous studies, both in type I collagen and DNA in the partly untwined state, molecules are
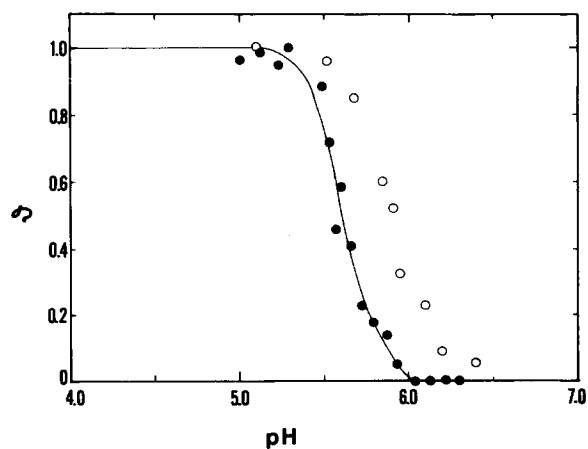

Figure 4(b) Helix content, θ, in PGA aqueous solution as a function of pH by elongational flow method (●) and by ORD experiments (○).[11]

considered to be hinged-rod.[10,17] In those polymers, at $\dot{\varepsilon} = 0$ s^{-1}, because of an entropic force, the hinged-rod molecule is in a conformation similar to a random coil with no anisotropy in optical properties, where a coiled chain of rods and hinges is compared to a molecular coil. With increasing strain rate, frictional force between solvent and polymer molecule also increases. The frictional force positively contributes to the free energy of the system. In the flow field, "random coil" deformation and deformed-"coil" orientation take place to reduce the free energy of the system. At $\dot{\varepsilon} \geqq \dot{\varepsilon}_o$, such a reduction of the free energy occurs. The arrangement of the deformed "coil" resultantly generates an optical anisotropy, then the solution becomes birefringent.

In the case of PGA, at any pH and $\dot{\varepsilon}$, the molecule has optical anisotropy and the solution is birefringent. pH dependence of D_r indicates that with the beginning of the helix loosening and untwining, hydrodynamic resistance at a molecule increases.[22,23] These results suggest that even at the partly untwined state, the PGA molecule is in an extended conformation. Such an extended conformation is considered to be originated from a polyelectrolyte nature of the PGA molecule.[11,24] At low pH, the side chain COOH group remains neutral because of excess H$^+$; then α-helix can be stable. When pH increases, concentration of H$^+$ become small and the COOH groups of side chains begin to be ionized to COO$^-$ and H$^+$. Ionized COO$^-$ groups generate strong repulsive electrostatic force to each other, the helical structure becomes unstable, and the PGA helix begins to untwine. Therefore, even in a hinged-rod structure, because of this repulsive force, a partly untwined PGA molecular chain is expected to be rather extended.

It is concluded that the change in flow-induced birefringence in a PGA aqueous solution with pH corresponds to the helix–coil transition of PGA induced by pH change in the solution. On the basis of the detailed nature of birefringence generated by the flow field, the PGA molecule was deduced to be extended and to have optical anisotropy. Thus, the elongational flow technique is proven to be useful in investigating the conformational change in biopolymers.

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