Elongational Flow Studies of Hinged Rodlike Molecules

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SYNOPSIS

Poly(amino acid) in an intermediate state of its helix–coil transition is known to be in a hinged rodlike conformation. In this work, the responses of poly(amino acids) in the hinged rodlike conformation against an elongational flow field were investigated by monitoring their flow-induced birefringence. Poly(L-glutamic acids) (PGA) and poly(γ-benzyl-L-glutamate) (PBLG) were examined as polyelectrolyte and noncharged poly(amino acids), respectively, and the results were compared. In the plots of flow-induced birefringence, Δn, against strain rate, $\dot{\varepsilon}$, for hinged rodlike PBLG, there was a critical strain rate, $\dot{\varepsilon}_0$, below which Δn was not observed. Over $\dot{\varepsilon}_0$, the birefringence pattern observed was identical with that of rodlike molecules. The Δn vs. $\dot{\varepsilon}$ plot for hinged rodlike PGA had characteristics of a rigid rod at any strain rate and there was no $\dot{\varepsilon}_0$ observed. The rotational diffusion coefficient, D_r, of PBLG in the hinged rodlike conformation was larger than that for its helical conformation, while D_r for the hinged-rodlike PGA was smaller than that for its helical conformation. It is concluded that the hinged-rodlike PGA molecule is in an extended form and that the hinged-rodlike PBLG is hydrodynamically more compact and rigid than that in its quiescent state. It is deduced that at $\dot{\varepsilon}_0$ hinged rodlike PBLG molecules collapse to a conformation optically anisotropic and mechanically rigid. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

The response of polymer molecules to an elongational flow field can provide information on the molecular nature, orientation, and relaxation of molecules and is now an established part of polymer physics.[1-4] The elongational flow technique has already been used to characterize a number of synthetic polymers.[4] It has been revealed that there are two typical responses of polymers against the elongational flow field: Flexible polymeric molecules show a transitionlike feature in a flow-induced birefringence, Δn, vs. strain rate, $\dot{\varepsilon}$, plot, in which Δn remains zero up to a certain strain rate, $\dot{\varepsilon}_c$, and increases rapidly at $\dot{\varepsilon}_c$ [Fig. 1(a)]. The birefringence which appeared beyond $\dot{\varepsilon}_c$ localized at the pure elongational flow field. These features of the response have been explained by the coil–stretch transition manifested by the flexible polymeric molecule in the elongational flow field.[5-7] On the other hand, in the case of a rigid rodlike molecule, Δn increases gradually with $\dot{\varepsilon}$ from $\dot{\varepsilon} \sim 0$ s^{-1} [Fig. 1(b)]. The birefringence is not localized and the intensity is isotropic throughout the irradiated field around the pure elongational flow field. These features are attributed to the unidirectional orientation of rigid rodlike molecules in the flow field.[3,8]

Monodisperse collagen molecules from a lathyritic rat tail tendon, however, showed a response to the elongational flow field other than the two typical responses explained above.[9] The collagen molecule manifested a conformational transition from a helix to a coil by thermal excitation. At the intermediate state of the helix–coil transition, the collagen molecule was considered to be in a hinged rodlike conformation. In the intermediate state, Δn as a function of $\dot{\varepsilon}$ has a critical strain rate, $\dot{\varepsilon}_0$, as the flexible polymer, but as in the case of the rigid rodlike molecule, the birefringence which appeared over $\dot{\varepsilon}_0$ did not localize. This response to the elongational flow was classified as the third type of response [Fig. 1(c)] and is considered to be characteristic of the hinged rodlike molecule.

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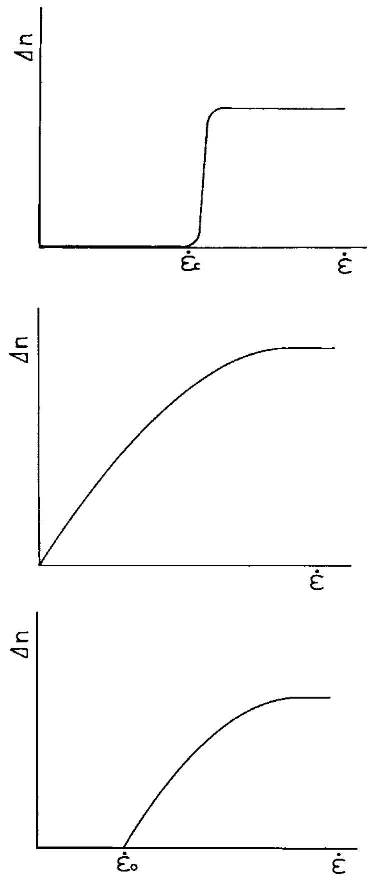

Figure 1 Schematic drawings of flow-induced birefringence as a function of strain rate for (a) flexible molecules, (b) rigid rodlike molecules, and (c) the Type 3 response.

In our previous work, the helix–coil transition of poly(L-glutamic acid) (PGA) was investigated using elongational flow studies.[10] The response to the flow field of the intermediate state of PGA, which was also regarded as a hinged rodlike conformation, was, however, typical of that of the rigid rodlike molecule. The results were discussed on the basis of the electrostatic interaction in a molecule. If we investigate the response to the elongational flow field of other poly(amino acid)s whose side-chain groups are electrostatically neutral, we can expect a Δn vs. $\dot{\varepsilon}$ plot similar to that reported for type I collagen, the third type of the response. In this article, we study the process of conformational change in the helix–coil transition of poly(γ-benzyl-L-glutamate) (PBLG). The side chain of PBLG is the esterified form of that of PGA with benzyl alcohol. Thus, the side chain of PBLG is electrostatically neutral. Comparing the results of PGA with PBLG, the nature of the hinged rodlike conformation in polyelectrolyte and noncharged polyamino acids can be examined.

EXPERIMENTAL

Materials

Samples were purchased in lyophilized form from the Sigma Chemical Co. and were closely monodisperse. The molecular weight, M_w, of the samples was determined to be 8.23×10^4 Da for PGA[11,12] and 1.10×10^5 Da for PBLG[13,14] by viscosity measurement. The lyophilized PGA was dissolved into distilled water buffered by an acetic acid–sodium acetate and 90% (v/v) of a glycerol aqueous solution. The lyophilized PBLG powder was dissolved into dichloroacetic acid (DCA) containing a 10 wt % cyclohexanol (CHL) and 80% (v/v) glycerol solution. Glycerol was used as a viscosity builder to prevent turbulence. To prepare a homogeneous solution, the solution was stirred for at least 24 h by a magnetic stirrer at room temperature. The molecular weight 8.23×10^4 Da for PGA gives an α-helix of 82 nm long. The c^* for the dilute–semidilute boundary[15] of PGA as an α-helical structure is 0.25 mg/mL. The concentration of the PGA sample solution was 0.23 mg/mL. In the case of PBLG, the molecular weight of 1.10×10^5 Da gives an α-helix of 75 nm long. The c^* for the dilute–semidilute boundary of PBLG as an α-helical structure is 0.42 mg/mL. In this work, since the transparency of the sample solution is low, the concentration of the PBLG solution was 1.0 mg/mL, larger than c^*, in order to obtain sufficient intensity of flow-induced birefringence.

Apparatus

The apparatus used to generate an elongational flow field was a four-roll mill, originally utilized by Taylor for the study of liquid droplets in a flow field[16] and previously used for elongational flow studies of biopolymers.[9,10,17-19] 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. The strain rate of the elongational flow was determined by Torza's formula.[20] Measurements of Δn as a function of $\dot{\varepsilon}$ were made isothermally for strain rates over the range of 0 and 176 s^{-1}. The temperature of the solution was controlled by a heat jacket through which water from a heat bath was circulating. The increasing rate of the temperature between two successive measuring points was about 3°C per h.

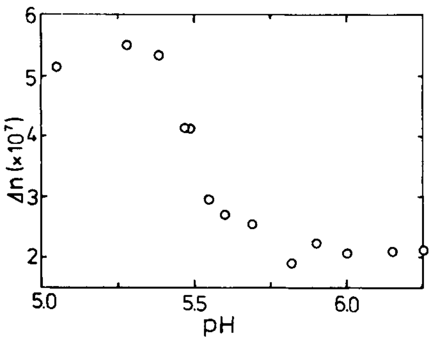

Figure 2 pH dependence of flow-induced birefringence of a PGA aqueous solution at $\dot{\varepsilon}$ = 176 s^{-1}.

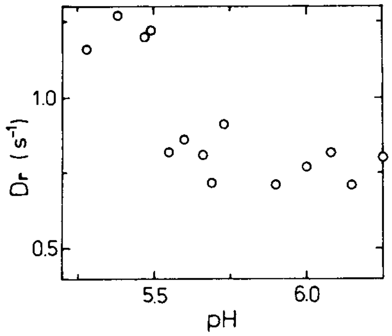

Figure 4 pH dependence of rotational diffusion coefficient, D_r, of a PGA aqueous solution.

RESULTS

Birefringence Response from Poly(L-glutamic acid)

Figure 2 shows that the pH dependence of flow-induced birefringence, Δn, at $\dot{\varepsilon}$ = 176 s^{-1} is far smaller than the $\dot{\varepsilon}_c$ of PGA used here. Δn decreases rapidly with increases in pH over a small range of pH. As reported previously,[10] a conformational change of molecules from a random coil to a rodlike helix, and vice versa, can be seen. In the coiled state of the molecule, Δn is not zero in the figure, which contrasts with our previous results. This is thought to be caused by a higher concentration of glycerol in the present system, which generates a higher viscosity of the solution and larger values of Δn. In Figure 3, Δn is plotted against $\dot{\varepsilon}$ at pH values from 5.4 to 5.9 where PGA molecules are in a hinged rodlike conformation. No $\dot{\varepsilon}_0$ is observed at each pH. The birefringence pattern was not localized at the pure elongational flow field and the intensity was isotropic throughout the irradiated field. Figure 4 shows the pH dependence of the rotational diffusion coefficient, D_r, for the PGA molecule. D_r decreased with increases in pH, which agree with the results of our previous study. This indicates that the mobility of the hinged rodlike molecule is smaller than that of a molecule in a helical conformation.

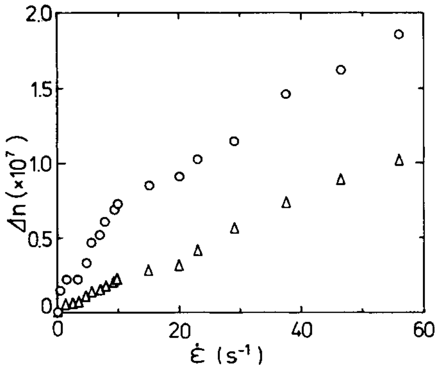

Figure 3 Δn plotted against $\dot{\varepsilon}$ for PGA aqueous solutions of pH (○) 5.49 and (△) 5.60.

Birefringence Response from Poly(γ-benzyl-L-glutamate)

PBLG in a dichloroacetic acid (DCA)–cyclohexanol (CHL) mixed solution has been known to undergo an inverse thermal helix–coil transition.[13,14,21,22] Therefore, the helix–coil transition of PBLG is observed by changing the temperature of the solution. As the transition temperature of PBLG is around room temperature, in this study, the temperature range was set between 13.9 and 30.5°C. Figure 5 shows the temperature dependence of Δn, at $\dot{\varepsilon}$ = 176 s^{-1}, for PBLG. Δn increases rapidly with increases in temperature over a small temperature range around 20°C.

Figure 6 shows Δn vs. $\dot{\varepsilon}$ curves for PBLG at 20.0 and 17.0°C. A PBLG molecule is deduced to be in a hinged rodlike conformation in these temperatures

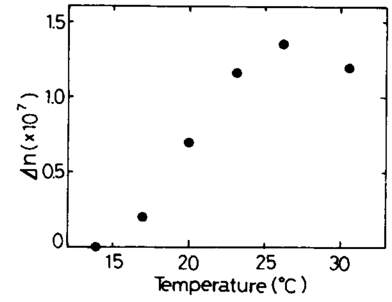

Figure 5 Temperature dependence of flow-induced birefringence of PBLG in a DCA–CHL mixed solution at $\dot{\varepsilon}$ = 176 s^{-1}.

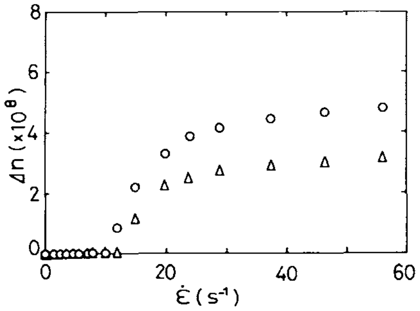

Figure 6 Δn plotted against $\dot{\varepsilon}$ for PBLG in a DCA–CHL mixed solution at (○) 20.0°C and (△) 17.0°C.

from Figure 5. There are zero Δn regions before the increase in Δn begins at a critical strain rate, $\dot{\varepsilon}_0$, and a gradual rise of Δn with $\dot{\varepsilon}$ ($\geq \dot{\varepsilon}_0$) follows. The birefringence at these temperatures did not localize and the intensity was isotropic throughout the irradiated field. This suggests that the hinged rodlike PBLG molecule has the characteristics of a rigid rod at $\dot{\varepsilon} \geq \dot{\varepsilon}_0$. This response observed for PBLG in a hinged rod conformation is clearly different from that observed for PGA, as in Figure 2, where $\dot{\varepsilon}_0$ was not observed. This result for PBLG is in accordance with the results for collagen.[9] Figure 7 shows the rotational diffusion coefficient, D_r, of PBLG as a function of temperature, which was determined from the slope of the Δn vs. $\dot{\varepsilon}$ curve at $\dot{\varepsilon} = \dot{\varepsilon}_0$ on the basis of the fact that at $\dot{\varepsilon}_0 \leq \dot{\varepsilon}$ the hinged rodlike PBLG molecule behaves as a rigid rod.[3,8,23] The flow-induced birefringence was also plotted in this figure. D_r for a hinged rodlike molecule in the elongational flow field of $\dot{\varepsilon} \geq \dot{\varepsilon}_0$ is larger than that for a helix. This result contrasts with that for PGA.

DISCUSSION

The Conformation of Hinged Rodlike PGA

The response of the hinged rodlike PGA to the elongational flow field has the characteristics of a rigid rod. In our previous work,[10] we considered that this response indicates the hinged rodlike PGA is not in the folded form like a random coil, but in an extended form even in the quiescent state and is oriented in the same way as a rigid rod in an elongational flow field. In addition, the smaller D_r of the hinged rod PGA than that of helical PGA indicates that the hinged rod PGA is more extended than, but not as flexible as, a PGA molecule in the helical structure. The side-chain COOH groups of PGA are ionized to COO^- and H^+ in the solution at certain pH values, which generates a strong electrostatic repulsive force on each other and stiffens the hinged part of the PGA molecule. This causes an extended rigid structure of the hinged rod PGA.

Type 3 Response

The results for PBLG in the hinged rod conformation was typical of a Type 3 response. Since the side chain of PBLG is electrostatically neutral, a repulsive force does not act among the side chains. In such a molecule, a hingelike part is much more flexible than in a PGA molecule. Therefore, it is considered that the hinged rod of PBLG is in a folded form in a quiescent solution due to an entropic force. At $\dot{\varepsilon} \leq \dot{\varepsilon}_0$, no birefringent response is observed in the optical signal, and, therefore, a PBLG molecule is considered to be in the folded form, where the short rods, considered to be the helical part in the spheroid, remain oriented at random. Beyond $\dot{\varepsilon}_0$, Δn increases gradually and the birefringence does not localize, which is a typical pattern for a rigid rodlike molecule. As shown in Figure 7, D_r for a hinged rod is larger than that for a helix. This fact indicates that the hinged rodlike PBLG beyond $\dot{\varepsilon}_0$ is more mobile than is the PBLG in the helical form and that the former must be in a hydrodynamically more compact form than the latter. It is deduced that at $\dot{\varepsilon} = \dot{\varepsilon}_0$ the hinged rodlike molecule collapses to a conformation which is optically anisotropic and mechanically rigid. At the critical strain rate, $\dot{\varepsilon}_0$, the elongational force overcomes the entropic force in the molecule in the flow field. Short rodlike helices in a PBLG molecule are considered to be rearranged and aggregated parallel to one another in the elongational flow field at $\dot{\varepsilon}_0 \leq \dot{\varepsilon}$. Such molecules are oriented by the flow field, which results in flow-induced birefringence.

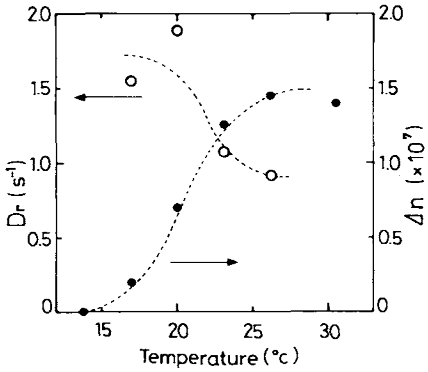

Figure 7 Temperature dependence of (○) D_r for PBLG in a DCA–CHL mixed solution. Δn values at $\dot{\varepsilon} = 176\ s^{-1}$ were also plotted for a comparison of D_r values at hinged rod and helical conformations of PBLG.

CONCLUSIONS

A hinged rodlike poly(α-amino acid) molecule, which is an intermediate state of the helix–coil transition, is in an extended form for PGA, a polyelectrolyte molecule, because of the electrostatic repulsive force acting among side chains. The response to the elongational flow field is classified as that of a rigid rod. If the side chain of the polymer is electrostatically neutral, the response of its hinged rodlike conformation to the elongational flow field is classified as a Type 3 response. Beyond the critical strain rate, $\dot{\varepsilon}_0$, the molecule is folded hydrodynamically to a more compact and rigid form than that in the quiescent state. It is deduced that at $\dot{\varepsilon}_0$ the hinged rodlike molecule collapses to a conformation which is optically anisotropic and mechanically rigid.

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