Elongational Flow Studies of the Conformation of DNA Molecules in the Globular State

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ABSTRACT: Elongational flow-induced birefringence of a T4-phage DNA aqueous solution was measured with changing NaCl and polyethylene glycol (PEG) concentrations. DNA molecules are known to manifest a coil–globule transition with increasing PEG concentration. At certain PEG concentrations near the critical concentration of the transition, the globular DNA solution, which was expected to be nonbirefringent, showed flow-induced birefringence. Strain-rate dependence of the birefringence intensity, having a critical strain rate, was similar to that of the flexible polymer chain that manifests the coil–stretch transition. The flow-induced birefringence pattern, however, suggested that the globular DNA molecules were rigid and optically anisotropic. At the critical strain rate, the globular DNA molecules in the solution of the particular PEG concentration were considered to collapse nonadiabatically to an optically anisotropic and mechanically rigid conformation. The overall shape of the collapsed conformation of the globular DNA was estimated to be an ellipsoid with an aspect ratio of about 0.7.
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Key words: elongational flow field; flow-induced birefringence; DNA; coil–globule transition; rotational diffusion coefficient

INTRODUCTION

It is known that large DNA molecules in aqueous solution exhibit a conformational change between a random coil and a more compact globular state with the addition of polyethylene glycol (PEG), as revealed by single-molecule imaging techniques by using fluorescence microscopy.[1–4] Investigation of the coil–globule transition of DNA chains is expected to provide valuable information on the folding ability of DNA chains in living cells and viruses. In our previous article, we established a method of detecting the conformational transition of DNA molecules from coil to globule structure, by making use of the response of DNA molecules to an elongational flow field.[5] DNA molecules in the random coil structure were expected to show flow-induced birefringence, Δn, whereas those in the globular state were not. The change in Δn with increasing PEG concentration corresponded closely to changes in the size of the DNA molecules observed by the fluorescent microscopic technique. There was, however, a particular PEG concentration near the transition point where the flow-induced birefringence was observed for the solution in which the DNA molecules were considered to be in a globular state on the basis of the fluorescent microscopic image.[5]

The aim of this work is to understand what kind of molecular events cause the birefringence in the globular DNA molecules. To detect the birefringent globular DNA molecules, coil–globule transition of DNA molecules was investigated by means of both single-molecule imaging tech-

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niques and the elongational flow birefringence technique. The analysis was concentrated on the strain-rate dependence of Δn and the birefringence pattern in the flow field. The hydrodynamic structure of the globular DNA was determined from the rotational diffusion coefficient. The results were discussed in terms of the flexibility of the DNA molecules.

EXPERIMENTAL

Materials

We used coliphage T4 DNA, the molecular weight of which is 1.1×10^8 Da (i.e., 167,000 base pairs). T4 DNA molecules in a solution of 10 mM Tris HCl, pH 8.0, and 1.0 mM EDTA were purchased from Sigma Chemical Company Ltd. (St. Louis, MO). DNA solutions were prepared by diluting with a mixture of water and 0.15*M* or 0.1*M* NaCl. PEG (M_w = 8000 Da) was added to induce the coil–globule conformational transition in the DNA molecules. The final concentration of DNA was 5 μg/mL. T4 DNA molecules were reported to have a radius of gyration (R_g) of about 1.5 μm in aqueous solution.[6] This R_g value indicates that the critical concentration c* for the dilute solution of T4 DNA should be 12.9 g/mL, which means that the concentration of all samples examined in this work was $< c^*$. For fluorescent microscopy, a fluorescent dye, 4′,6-diamidino-2-phenyl-indol (DAPI), and an antioxidant, 2-mercaptoethanol (2-ME), were used. These chemicals were purchased from Wako Pure Chemical Industries, Ltd. The concentrations of DAPI and 2-ME were 5.0 μg/mL and 4% (v/v), respectively.

Apparatus

The elongational flow field was generated by a four-roller mill system, originally utilized by Taylor for the study of liquid droplets in a flow field.[7] Details of the four-roller mill used in this study were described elsewhere.[8] To quantify the response of DNA molecules to the elongational flow field, the flow-induced birefringence, Δn, in the four-roller mill system was observed. Measurements of Δn as a function of strain rate, $\dot{\epsilon}$, were performed isothermally over the range of $\epsilon = 0$–176 s^{-1}. The strain-rate value was determined by Torza's formula for four-roller mill measurement.[9] Fluorescent micrograph investigation of DNA molecules was performed by using a Nikon Optiphoto-2 microscope equipped with a 100× oil-immersed objective lens, and images were recorded on video tape by using a Hamamatsu CCD camera (C3077). All measurements were carried out at room temperature, ~20°C.

RESULTS AND DISCUSSION

Observation of the Coil–Globule Transition

The birefringent globular DNA molecules were determined by comparing the flow birefringence with the directly measured molecular length of DNA as a function of PEG concentration.[5] The coil–globule transition data obtained from two different methods were also compared in this study.

Figure 1 shows the length of the long axis of DNA molecules l as a function of PEG concentration in weight, [PEG], for the solution containing 0.10*M* NaCl. At [PEG] ~ 15% (w/w), l suddenly decreased, indicating that the coil–globule transition occurred at this PEG concentration. In the shaded region in Figure 1, both coiled and globular DNA molecules were observed in the same field of the microscope. The bimodal distribution of l around the transition point is caused by the spatial fluctuation of the PEG concentration in the solution.[1–4] Figure 2 shows Δn plotted against strain rate, $\dot{\epsilon}$, for DNA aqueous solutions containing 0.10*M* NaCl at six PEG concentrations between 7.3 and 24.3%. Flow-induced birefringence was observed for solutions of [PEG] $\leq$ 20.6%, whereas no birefringence was observed for the [PEG] = 24.3% solution. The birefringence pattern was localized at the pure elongational flow area in the flow field for solutions of [PEG] $\leq$ 15.3%. On the basis of the strain-rate dependence of Δn and its pattern in the flow field, it is concluded that DNA molecules in these solutions manifest a coil–stretch transition by the flow field.[10,11] Criticality of the coil–stretch transition appears to be decreased because the DNA molecules are semiflexible.[12] The birefringence pattern for the [PEG] = 20.6% solution was nonlocalized throughout the flow field, although the solution has criticality reminiscent of the coil–stretch transition of DNA molecules. In Figure 3, Δn at $\dot{\epsilon} = 29\ s^{-1}$ was plotted against [PEG] for a DNA aqueous solution containing 0.10*M* NaCl. At low PEG concentrations, Δn increased with PEG concentration (i.e., with increases in the viscosity of the solvent); then, at about [PEG] = 13.7%, Δn began to decrease. In Figure 4, both Δn at $\dot{\epsilon} = 29\ s^{-1}$ and l were plotted against [PEG] concentration for a DNA aqueous

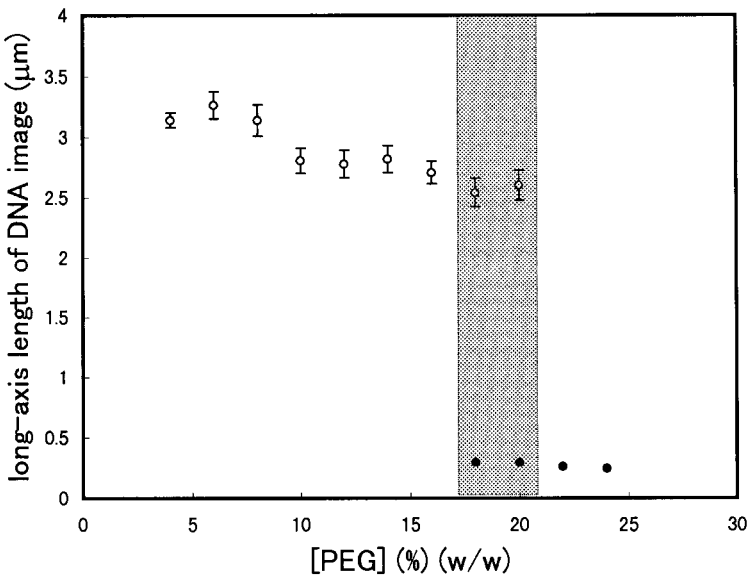

Figure 1 The long-axis length (l) of T4 DNA molecules in aqueous solution containing 0.1M NaCl plotted against PEG concentrations. The open and closed circles indicate the maximums of the distribution in l of the DNA molecules.

solution containing 0.10M NaCl. The decrease in Δn with increasing [PEG] corresponds closely to the decrease in l. It is concluded that the coil–globular transition occurred at about [PEG] = 13.7% for T4 DNA molecules in a 0.1M NaCl aqueous solution. DNA molecules in the [PEG] = 24.3% solution were nonbirefringent because all of the DNA molecules in the solution were globular and would not be deformed by the flow field. The fluorescent microscopic image recorded at [PEG] = 20.6% was completely that of a globular DNA molecular system. For [PEG] = 20.6%, however, as shown in Figure 2, a flow-induced birefringence signal was observed. The puzzling features of the [PEG] = 20.6% solu-

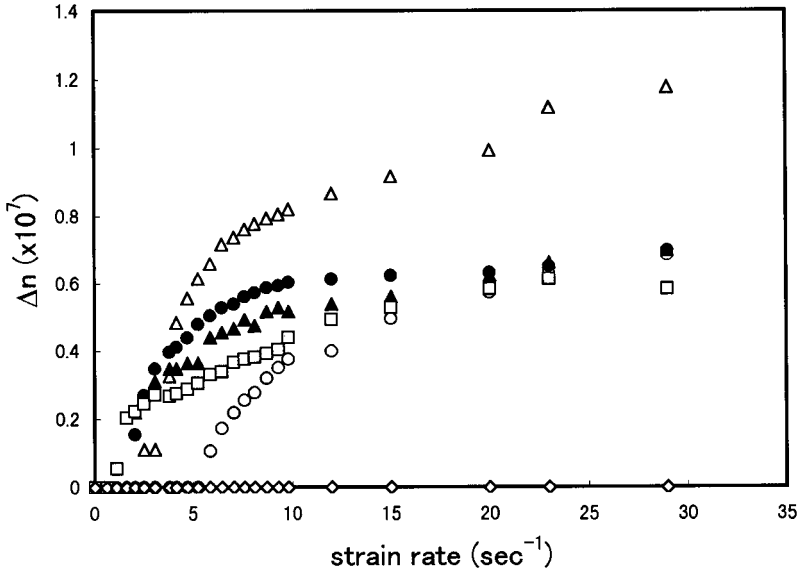

Figure 2 Δn plotted against $\dot{\epsilon}$ for a T4 DNA aqueous solution containing 0.1M NaCl at PEG weight concentrations of 7.3% (○), 10.6% (●), 13.7% (△), 15.3% (▲), 20.6% (□), and 24.3% (◇).

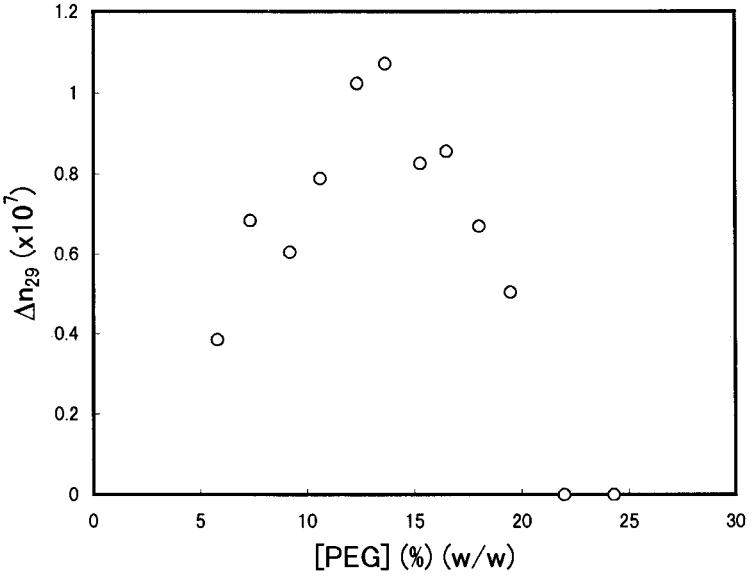

Figure 3 Δn for a DNA aqueous solution containing 0.1*M* NaCl plotted against PEG concentration at $\dot{\epsilon} = 29\ s^{-1}$.

tion will be discussed in the next section. Figure 5 shows Δn as a function of [PEG] at $\dot{\epsilon} = 9.8$, 20, and 29 s^{-1}. The [PEG] at the coil–globular transition point was not affected by any of these strain-rate values. Indeed, the critical [PEG] for the transition was not affected up to $\dot{\epsilon} = 176\ s^{-1}$, the largest strain rate that our apparatus is able to generate. Figure 6 shows Δn for a 0.10*M* NaCl solution and for a 0.15*M* NaCl solution plotted against solvent viscosity, η. Δn for a T4 DNA aqueous solution containing 0.20*M* NaCl and PEG or 0.20*M* NaCl and glycerol were also plotted.[5] In the low-viscosity solutions including ones containing glycerol, Δn as a function of η looks almost universal with no dependence on NaCl concentration or viscosity builder. In the high-viscosity solutions, the coil–globule transition occurred in the solutions containing PEG and Δn decreased with increasing [PEG]. According to the

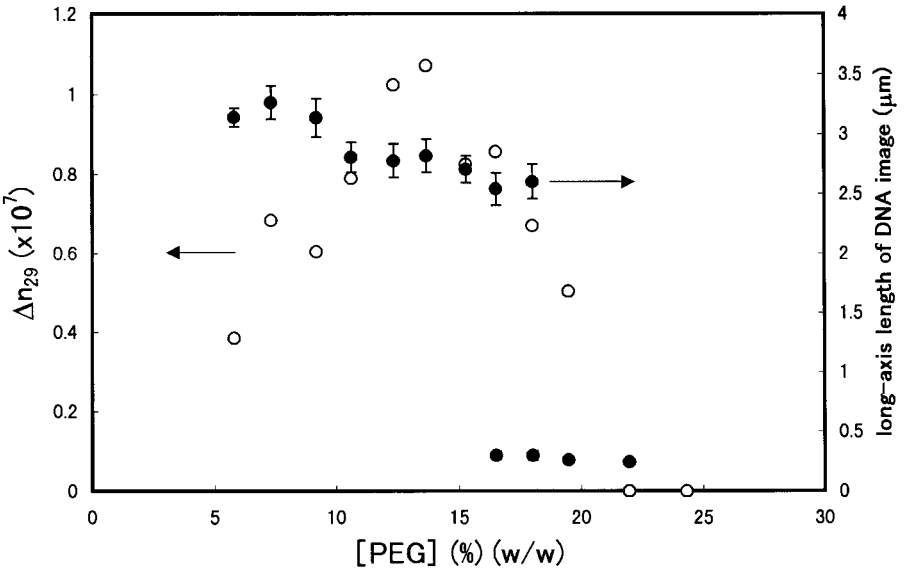

Figure 4 Comparison of Δn for a DNA aqueous solution containing 0.1*M* NaCl and the length of DNA molecules as a function of PEG concentration.

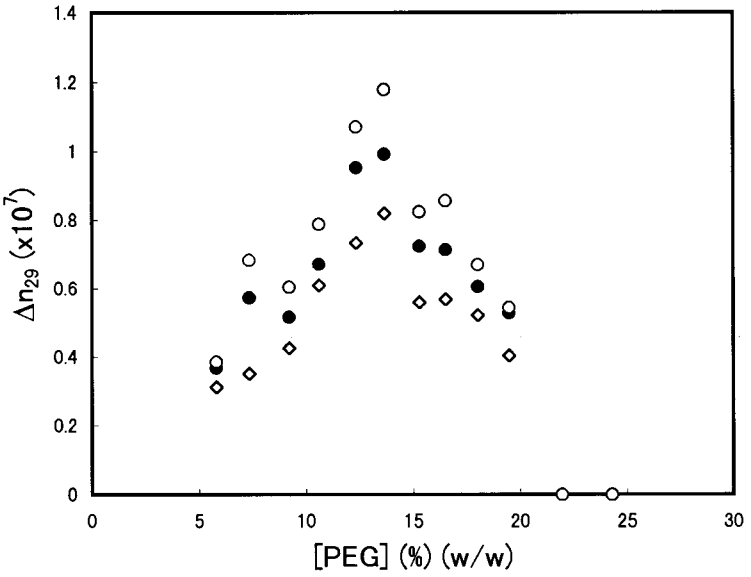

Figure 5 Δn for a DNA aqueous solution containing 0.1*M* NaCl plotted against PEG concentration at $\dot{\epsilon} = 9.8\ s^{-1}$ (◇), $20\ s^{-1}$ (●), and $29\ s^{-1}$ (○).

theoretical explanation of a coil–globule transition, the more flexible the polymer chain is, the more ambiguous is the transition.[13,14] The curve of Δn–log η plot for the 0.20*M* NaCl solution seems to have the largest half-width among the three peaks. This indicates that the DNA chain in the 0.20*M* NaCl is more flexible than that of the other two solutions. This flexibility can be explained in terms of the higher degree of screening of phosphate charges in the DNA backbone chain by Na^+ ions in the 0.2*M* NaCl solution than in the 0.1*M* and 0.15*M* NaCl solutions.

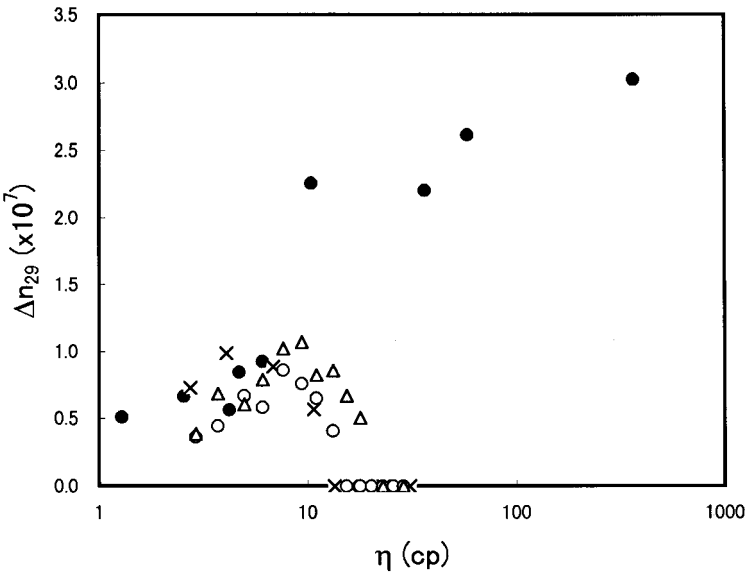

Figure 6 Δn plotted against the logarithm of viscosity of solvents η at $\dot{\epsilon} = 29\ s^{-1}$ for aqueous solutions containing PEG and 0.2*M* NaCl[5] (×), 0.15*M* NaCl (○), and 0.1*M* NaCl (△). The results for aqueous solutions containing glycerol and 0.2*M* NaCl[5] (●) was also plotted.

Structure of the Birefringent Globular DNA Molecules

As mentioned above, there is a solution that manifests flow-induced birefringence, although the DNA molecules in the solution at that PEG concentration were considered to be in the globular conformation on the basis of the results of fluorescent microscopy. Such PEG concentrations were also found for DNA solution containing 0.15 and 0.2*M* NaCl.[5] To explain the origin of the birefringence of the globular DNA molecules, the birefringence response pattern and the response against the strain rate of the flow field were investigated. As shown in Figure 2, Δn for the 0.10*M* NaCl, 20.6% PEG solution as a function of $\dot{\epsilon}$, manifests a transition-like feature; at a strain rate below the critical strain rate $\dot{\epsilon}_0$, Δn remains zero and increases rapidly for $\dot{\epsilon} > \dot{\epsilon}_0$. This type of strain–rate dependence of the birefringence is characteristic of a flexible polymer chain. According to Keller and Odell,[10,11] the birefringence pattern of a flexible polymer localizes at the pure elongational flow area, whereas that of a rigid-rod-like molecule is isotropic over the entire irradiated flow area. The birefringence that appeared beyond $\dot{\epsilon}_0$ for this solution, however, did not localize at the pure elongational flow field but appeared isotropic throughout the irradiated field. It is concluded that the DNA molecules in this condition have properties both of a flexible coil and a rigid rod, on the basis of the established classifications for elongational flow studies of polymers.[10,11] These features of flow-induced birefringence were observed for hinged-rod-like polymers and were classified as the third type of response of polymers against an elongational flow field.[8]

The third type of response of globular DNA molecules to the elongational flow field indicates that at $\dot{\epsilon} \leq \dot{\epsilon}_0$, the DNA molecules are thought to be in a folded and optically isotropic form. Beyond $\dot{\epsilon}_0$, the DNA molecules manifest the properties that are characteristic of a rigid-rod-like molecules.[10,11] Globular DNA molecules in solution at that particular PEG concentration are deduced to collapse nonadiabatically at the critical strain rate $\dot{\epsilon}_0$ to a conformation that is optically anisotropic and mechanically rigid [Fig. 7a)]. For the third type of response, the birefringence increase at about the critical strain rate, $[d(\Delta n)/d\dot{\epsilon}]_{cr}$, is attributed to the beginning of orientation of the rigid molecules along the flow line.[8] This value is

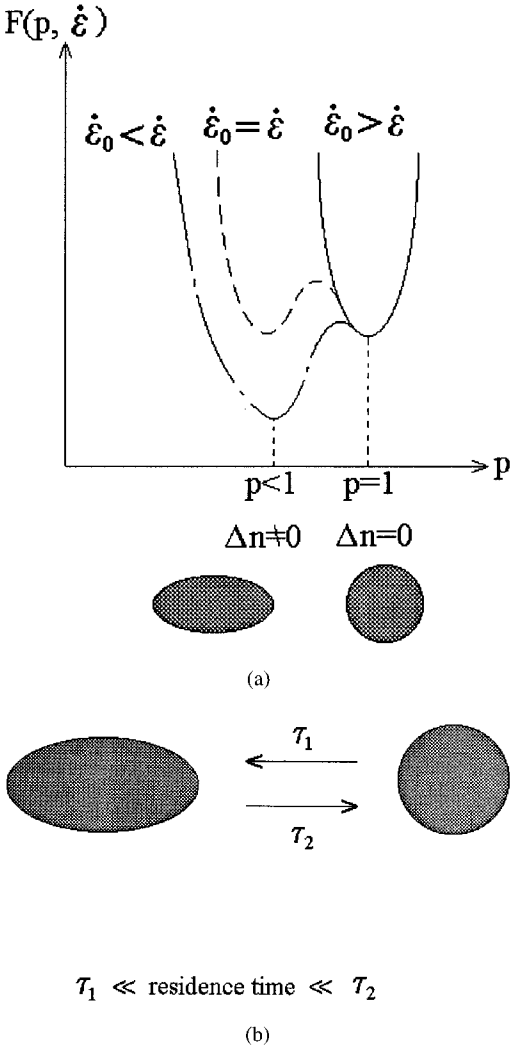

Figure 7 (a) Schematic drawing of the nonadiabatic collapse of the globular DNA molecules in an elongational flow. The Helmholtz free energy $F(p, \dot{\epsilon})$ of the globule is considered to be a function of an aspect ratio p of the molecule and the strain rate $\dot{\epsilon}$. (b) For the system to manifest the nonlocalized birefringence pattern, the relaxation time from the sphere to the ellipsoid, τ_1, is much shorter than the residence time of DNA molecules in the flow field and that from the ellipsoid to the sphere, τ_2, is much larger than the residence time in the flow field.

related to the rotational diffusion coefficient, D_r, as[10,11]

$$D_r = (2/15)\Delta n_\infty (1/[d(\Delta n)/d\dot{\epsilon}]_{cr}). \quad (1)$$

From the data in Figure 2, a D_r value of the DNA molecules in the [PEG] = 20.6% solution was preliminarily estimated to be about 0.3 s^{-1}, where

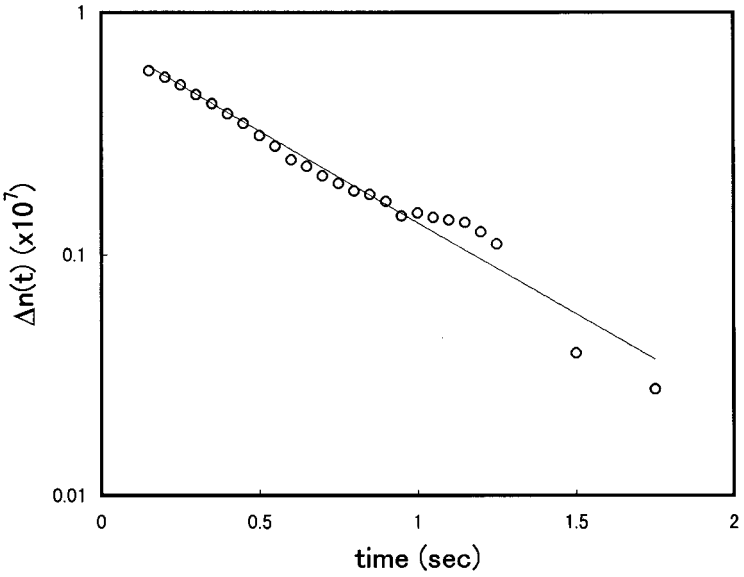

Figure 8 A typical decay curve of flow-induced birefringence for T4 DNA in a 20.6% PEG aqueous solution after the cessation of the flow at the strain rate $\dot{\epsilon} = 58\ s^{-1}$.

$[d(\Delta n)/d\dot{\epsilon}]_{cr} = 3.35$ s and Δn_∞ was assumed to be 0.65×10^{-7}.

The birefringence decay, $\Delta n(t)$, after cessation of the flow at $\dot{\epsilon} = 58\ s^{-1}$, was also investigated for DNA molecule for the [PEG] = 20.6% solution. Figure 8 shows the typical decay of the flow-induced birefringence; the logarithm of $\Delta n(t)$ holds a linear relationship with time up to about 2 s and the relaxation time was determined as $\tau = 0.60$ s. Only one relaxation was observed (i.e., there was only a single relaxation time) in this time scale. In the case of Δn decay originated from molecular disorientation, the relaxation time, τ, was related to the rotational diffusion coefficient D_r, $\tau \propto D_r^{-1}$. Here, the birefringence decay is also attributed to the disorientation of the optically anisotropic, rigid DNA globular molecules. The D_r value of the DNA molecules is determined by measuring $\Delta n(t)$ as:

$$\Delta n(t) = \Delta n_0 \exp(-t/\tau) = \Delta n_0 \exp(-6D_r t) \quad (2)$$

From Figure 8, we obtained a D_r value of 0.280 s^{-1}. The value accords well with the D_r value determined from the slope of Δn versus $\dot{\epsilon}$ plotted at the critical strain rate $\dot{\epsilon}_0$. This accordance of the two D_r values confirms that the flow-induced birefringence can be attributed to the orientation of the optically anisotropic and mechanically rigid globular DNA molecules. As Δn decay has only one relaxation attributed to the disorientation process of the molecules, the conformational relaxation time of the new structure induced in the globular DNA molecules by the flow field is considered to be far larger than the residence time of the molecules in the flow field [Fig. 7(b)].

Direct observation using a fluorescent micrograph is not suitable for determining the overall shape of the globular DNA molecules due to limitations in optical resolution, 0.5 μm. When a material in a fluid is ellipsoidal in shape, its aspect ratio p is related to D_r as,

$$D_r = 3kTf(p)/16\pi a b^2 \eta \quad (3)$$

where k is the Boltzmann's constant, T is the absolute temperature, a and b are the long- and short-axis lengths of the ellipsoid ($p = b/a$), respectively, and η is the solvent viscosity. $f(p)$ is a function of the aspect ratio[15]:

$$f(p) = p^2/(1 - p^4)[\{2 - p^2)/2(1 - p^2)^{1/2}\} \times ln\{[1 + (1 - p^2)^{1/2}]/[1 - (1 - p^2)^{1/2}]\} - 1] \quad (4)$$

The assumption that the DNA molecule is ellipsoid in shape does not decrease the generality of the discussion, because the ellipsoidal shape includes even a sphere where $p = 1$. Using the D_r value obtained above for the birefringent globular DNA molecules, the hydrodynamic structure of the molecules was estimated. p will be obtained

uniquely if we know the volume of the DNA molecules. As the packing density of segments in the globular DNA molecules is much higher than that in the random-coil DNA molecules, it is possible to assume that

$$\text{Volume of DNA}_{ellipsoid} = \text{Volume of DNA}_{chain} \quad (5)$$

By using the crystallographic data for type B DNA molecules, the diameter of double helix chain = 1.7 nm; the chain length for 10 nucleotides = 3.4 nm,[16,17] and the volume was estimated as

$$\text{Volume of DNA}_{chain} = 1.3 \times 10^{-22} m^3$$

On the basis of this value, the D_r value and eqs. [3] and [4], the aspect ratio p was determined to be $p = 0.69$. In the case of a λ-phage DNA aqueous solution containing 0.2*M* NaCl and 90% glycerol, the corresponding aspect ratio at the strain rate of 29 s^{-1}, which is larger than the critical value for the coil–stretch transition of the DNA molecule, was 0.08.[18] The deviation of the overall structure of the globular DNA from a sphere is much smaller than that for random-coil λ-phage DNA at the coil–stretch transition, indicating a drastic reduction in the flexibility of the molecule due to the conformational transition to the globular form.

CONCLUSION

By observing the flow-induced birefringence pattern, the coil–globule transition of DNA molecules was investigated in a solution of globular DNA molecules that were considered to be nonbirefringent. A comparison of the result with that obtained by using a fluorescent micrograph revealed, however, that this was a birefringent globular DNA solution. The birefringence of the globular DNA solution has characteristics both of a flexible coil and a rigid rod. An elongational flow field at the critical strain rate $\dot{\epsilon}_0$ causes the globular DNA molecules to collapse nonadiabatically to a conformation that is optically anisotropic and mechanically rigid. Then, the observed birefringence was attributed to the orientation of the globular DNA molecules by the flow field, where the overall shape of the molecules was an ellipsoid with an aspect ratio of about 0.7.

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REFERENCES

1. Minagawa, K.; Matsuzawa, Y.; Yoshikawa, K.; Khokhlov, A. R.; Doi, M. Biopolymers 1994, 34, 555–558.
2. Vasilevskaya, V. V.; Khokhlov, A. R.; Matsuzawa, Y.; Yoshikawa, K. J Chem Phys 1995, 102, 6595–6602.
3. Yoshikawa, K.; Matsuzawa, Y. Physica D 1995, 84, 220–227.
4. Mel'nikov, S. M.; Sergeyev, V. G.; Yoshikawa, K. J Am Chem Soc 1995, 117, 2401–2408.
5. Hayakawa, I.; Sasaki. N.; Hikichi, K. Polymer 1998, 39, 1393–1397.
6. Yanagida, M.; Hiraoka, Y.; Katsura, I. Cold Spring Harbor Symposium on Quantitative Biology 1983, 47, 117.
7. Taylor, G. I. Proc R Soc London 1934, 146, 501–523.
8. Hayakawa, I.; Hayashi, C.; Sasaki, N.; Hikichi, K. J Appl Polym Sci. 1996, 61, 1731–1735.
9. Torza, S. J. J Polym Sci, Polym Phys Ed 1975, 13, 43–57.
10. Keller, A.; Odell, J. A. Colloid Polym Sci 1985, 263, 181–201.
11. Odell, J. A.; Keller, A.; Atkins, E. D. T. Macromolecules 1985, 18, 1443–1453.
12. Odell, J. A.; Taylor, M. Biopolymers 1994, 34, 1483–1493.
13. de Gennes, P. G. J Phys Lett 1975, 36, L55-L57.
14. Post, C. B.; Zimm, B. H. Biopolymers 1979, 18, 1487–1501.
15. Doi, M.; Edwards, S. F. The Theory of Polymer Dynamics; Clarendon; Oxford, 1986; Chapter 8.
16. Watson, J. D.; Crick, F. H. C. Nature 1953, 171, 737–738.
17. Saenger, W. Principles of Nucleic Acid Structure; Springer: Berlin, 1984.
18. Sasaki, N.; Hayakawa, I.; Hikichi, K.; Atkins, E. D. T. J Appl Polym Sci. 1996, 59, 1389–1394.