ELSEVIER

PII: S0032-3861(97)00411-4

Polymer Vol. 39 Nos. 6–7, pp. 1393–1397, 1998

0032-3861/97/$17.00+0.00

The response of DNA macromolecules to an elongational flow field and coil–globule transition of DNA

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(Received 26 March 1997)

Large DNA molecules are known to exhibit a conformational change between the coil and globule states with the addition of polyethylene glycol (PEG). We investigated the coil–globule transition of T4DNA by observing the response of the molecule to a well-defined elongational flow field, where the response was monitored by the flow-induced birefringence. The flow-induced birefringence (Δn) increased with the strain rate for solutions of low PEG concentration, indicating that the DNA molecule is in an extended coil state. There were no Δn response at high PEG concentration solutions, where the DNA molecule is in a well-developed globule state. The dependence of Δn on PEG concentration is in agreement with the PEG concentration dependence of DNA size, which was directly measured by a fluorescence micrograph method. A comparison of the results by the flow birefringence method with those by the fluorescence micrograph method indicated that the globule of the DNA molecule near the coil–globule transition is deformed by the elongational flow field. The elongational flow technique was proven to be a useful method for investigating the coil–globule transition of large DNA molecules.

(Keywords: elongational flow; coil–globule transition; T4DNA)

INTRODUCTION

Rheological properties of polymer solutions have generally been determined by viscoelasticity measurement in a shear flow field. Frank proposed that the response of polymer molecules to an elongational flow field is intrinsically different from that to a shear flow field[1]. In a shear flow, the extensional and rotational components of the strain rate are equal, and full extension and/or alignment of molecules is not achieved. In fact, flexible polymer chains will be only slightly deformed from the coil. Most of the traditional studies on flow-induced orientation were performed in the shear flow field. Full extension of a chain polymer requires fluid elements in a continuing state of extension, i.e. the flow termed 'persistently extensional'[2]. In an elongational flow, there is no rotational component at all. When the hydrodynamic frictional force by an elongational flow field exceeds the entropic force of a polymer chain, the polymer is expected to undergo a transition from the coil state to a stretched state. This expectation by Frank was formulated theoretically as the coil–stretch transition by De Gennes[3] and was confirmed empirically by Keller and Odell[4].

The response of a chain molecule to an elongational flow provides various parameters characterizing chain properties such as flexibility. The elongational flow field in conjunction with a birefringence measuring system has been used to characterize a number of synthetic polymers[4]. We have used the elongational flow field as a new tool for studying the helix–coil transition of polyamino acids[5]. We found that the change in the birefringence response corresponds to the conformational change of the molecule. We also obtained information on the conformation and flexibility of the molecule at an intermediate state of the helix–coil transition[6]. The elongational flow technique has thus been proven to be useful for investigating viscoelastic properties of polymer molecules.

The shape of DNA molecules labelled by a fluorescent dye in an aqueous solution was directly observed under a fluorescent microscope. Direct observation and controlled deformation of individual DNA molecules give insights into a currently inaccessible, evidential feature of single-polymer dynamics, which is still a theoretical prediction[7–10]. It is known that large DNA molecules exhibit a conformational change between the coil and globule states with the addition of polyethylene glycol (PEG)[11–13] or cetyltrimethylammonium bromide (CTAB)[14], as revealed by direct observation using fluorescence microscopy. 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.

The aim of this study was to investigate the coil–globule transition of T4DNA by observing the response of the molecule to a well-defined elongational flow field, in which the response is monitored by flow-induced birefringence. The results obtained by the elongational flow technique were compared with those obtained by fluorescent micrography in order to examine the possibility of using the elongational flow field as a new tool for investigating coil–globule transition. New information concerning the sfiffness of the DNA molecule in a globule conformation is also discussed.

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EXPERIMENTAL

Materials

We used the coliphage T4DNA, whose molecular weight is 1.1×10^8 Da, i.e. 167 000 base pairs. T4DNA molecules in a solution of 10 mM Tris–HCl, pH 8.0, and 1.0 mM EDTA were purchased from Sigma Chemical Company Ltd. (USA). The DNA solutions were diluted by water containing 0.2 M NaCl. To increase the viscosity of the solution, PEG ($M_w = 20{,}000$) and glycerol were added. The concentrations of PEG in monomer units were 0.45–7.73 M, and those of glycerol were 10–95% (v/v) (1.4–1300 M). The final concentration of DNA in monomer units was 5.0 μg ml^{-1}. T4DNA molecules have been reported to have a radius of gyration (R_g) of about 1.5 μm in aqueous solution[15]. This R_g value indicates that the critical concentration c^* for the dilute solution of this T4DNA should be 12.9 μg ml^{-1}, which means that the concentration of all samples used was below c^*. For fluorescence microscopy, a fluorescent dye, 4′,6-diamidino-2-phenylindole (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^{-1} and 4% (v/v), respectively.

Apparatus

The elongational flow field was generated by a four-roller mill system, originally utillzed by Taylor for the study of liquid droplets in a flow field[16]. The flow-induced bireftingence, Δn, was monitored as the response of polymer molecules to the elongational flow field. Measurements of Δn as a function of strain rate $\dot{\varepsilon}$ were made isothermally for strain rates over the range of 0–176 s^{-1}. The strain rate was determined from flow volume per second, which has been confirmed to be useful for four-roller mill measurement[17]. Fluorescence micrograph investigation of DNA molecules was performed using a Nikon Optiphoto-2 microscope equipped with a 100 × oil-immersed objective, and images were recorded on video tape using a CCD camera. All measurements were carried out at room temperature, *ca.* 20°C.

RESULTS AND DISCUSSION

Observation by means of fluorescence microscopy

As mentioned before, DNA molecules exhibit a conformational change between the coil and globule structures upon the addition of PEG, as revealed by fluorescent microscope investigations[11–13]. At first, the coil–globule transition of T4DNA was confirmed by fluorescence microscopy. *Figure 1*(a) and (b) shows fluorescent images of fluctuating DNA molecules in solution. At low concentrations of PEG, DNA molecules exhibited a relatively extended conformation [*Figure 1*(a)], while at high concentrations, the molecules showed a contracted conformation [*Figure 1*(b)]. Considering the changes in size and intensity of the fluorescent image, these results indicate that the molecules change their conformations from coil to globule. *Figure 2* shows the size of DNA as a function of the concentration of PEG. The size of DNA was quantified as the longest distance in the outlined shape of a DNA image. Though the size of the observed DNA image was larger than the actual size of the DNA due to thermal fluctuation, it is evident from these figures that conformational change in

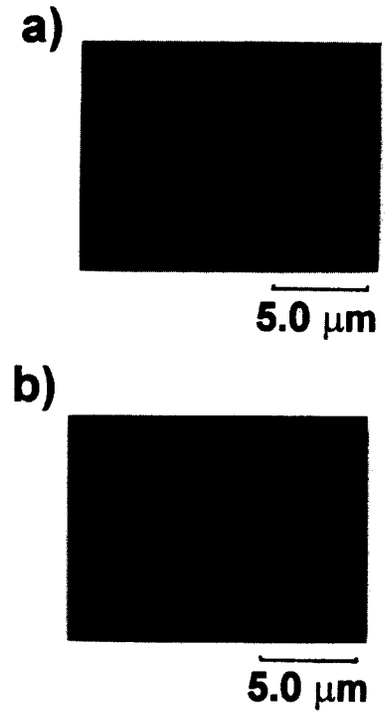

Figure 1 Fluorescent images of T4DNA molecule (a) in 0.45 M PEG solution, and (b) in 2.05 M PEG solution.

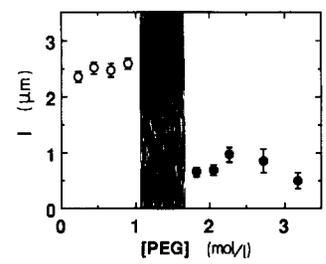

Figure 2 The long-axis length (l) of T4DNA molecules plotted against PEG concentration. The open and filled circles indicate the maximums of the DNA lengths' distribution.

DNA molecules was induced by change in the PEG concentration of the solvent.

The shaded region in *Figure 2* corresponds to the bimodal distribution region. The bimodality in the long-axis length distribution indicates the coexistence of coil and globule states. According to Yoshikawa *et al.*[11–14], the coil and globule states coexist at PEG concentrations around the transition point, due to the spatial fluctuation of PEG concentration in solution. Such a result proves that the coil–globule transition for a DNA molecule is the first-order phase transition[18,19].

Stress-induced scission of DNA molecules

A mechanical scission of large DNA molecules is known to occur in an elongational flow field. It is reported that λ-phage DNA molecules ($M_w = 3.1 \times 10^7$ Da) are fractured at $\dot{\varepsilon} = 5 \times 10^3$ s^{-1}[20]. Since we used a four-roller mill apparatus in this study to produce strain rates up to 176 s^{-1}, the λ-phage DNA molecules are not considered to be fractured in the elongational flow field. For T4DNA molecules, the molecular weights before and after the experiments in the elongational flow field were measured by pulsed-field gel electrophoresis. Data for T4DNA used in this experiment are shown in *Figure 3*. The molecular

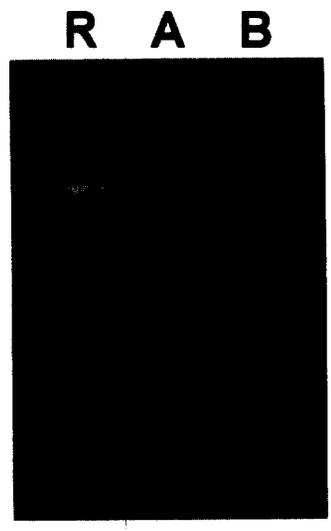

Figure 3 Agarose gel electrophoresis of T4DNA before (lane A) and after (lane B) the elongational flow experiments. Lane R shows the reference bands.

weight of T4DNA molecules did not change before and after elongational flow measurement, confirming that the T4DNA molecule was not fractured in these experiments.

Response of DNA solution to elongational flow field

The elongational flow method can distinguish rigid rod-like chains from flexible polymer chains by Δn–$\dot{\varepsilon}$ plot[21–25]. In rigid rod-like chains, Δn increases gradually with $\dot{\varepsilon}$ from $\dot{\varepsilon} \cong 0\ s^{-1}$. On the other hand, in the case of flexible polymer chains, a transition-like feature in the Δn–$\dot{\varepsilon}$ plot is observed, in which Δn remains at zero up to a certain strain rate $\dot{\varepsilon}_c$ and then increases rapidly at $\dot{\varepsilon}_c$. This response feature has been explained by the coil–stretch transition manifested by the flexible polymer in the elongational flow field. This transition, which is initiated at $\dot{\varepsilon}_c$, is sufficient for overcoming the entropic force of the flexible polymer. The relation between $\dot{\varepsilon}_c$ and the longest relaxation time of the molecule τ is[4,26]

$$\dot{\varepsilon}_c \approx 1/\tau \qquad (1)$$

In the case of DNA molecules, the coil chains in the elongational flow field are expected to be stretched and, accordingly, Δn increases with increases in $\dot{\varepsilon}$. On the other hand, the DNA globule chains are considered to be undeformed because of the strong energetic elasticity originating from the intramolecular cohesive force, which depends on the solvent strength. Therefore, no birefringencce response would be observed for globule conformation. Thus we expect that coil–globule transition can be observed as a change in Δn at fixed $\dot{\varepsilon}$.

Figure 4 shows Δn plotted against $\dot{\varepsilon}$ for DNA aqueous solution at PEG concentrations of 0.45–2.05 M. At low PEG concentrations, there was no birefringence response at $0 \leq \dot{\varepsilon} < \dot{\varepsilon}_c$, and Δn increased gradually at $\dot{\varepsilon} \geq \dot{\varepsilon}_c$. Odell *et al.* proposed that the gradual increase in Δn indicates that the DNA coil is unfolded gradually at $\dot{\varepsilon} \geq \dot{\varepsilon}_c$ because a DNA coil is a free-draining chain[27]. Different values of Δn for each PEG concentration are considered to indicate that the hydrodynamic stress applied to a DNA molecule depends on the viscosity of the solution. The increase in the viscosity of the solution is thought to be caused by an increase in the interaction of DNA molecules with solvent molecules due to the increase in PEG concentration. As a result, τ becomes longer and $\dot{\varepsilon}_c$ decreases with solvent viscosity, as shown in equation (1).

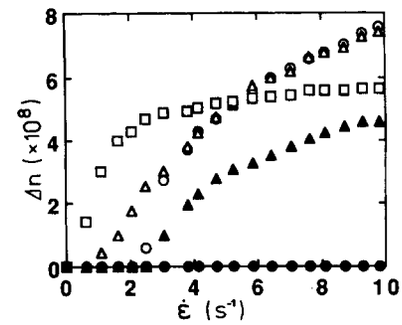

Figure 4 Δn plotted against $\dot{\varepsilon}$ for T4DNA aqueous solution at PEG concentrations of 0.45 M (▲), 0.91 M (○), 1.36 M (△), 1.82 M (□) and 2.05 M (●).

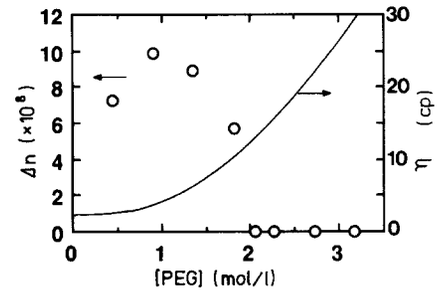

Figure 5 Δn plotted against PEG concentration at $\dot{\varepsilon} = 29\ s^{-1}$ (○) and viscosity of solvent η as a function of PEG concentration (solid line) for T4DNA aqueous solution.

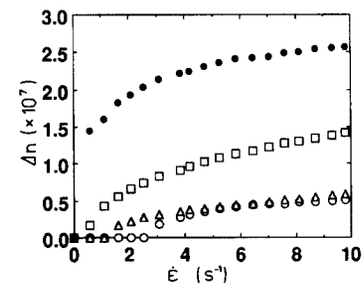

Figure 6 Δn plotted against $\dot{\varepsilon}$ for T4DNA aqueous solution at glycerol concentrations of 10% (v/v) (○), 50% (v/v) (△), 75% (v/v) (□) and 95% (v/v) (●).

Figure 5 shows Δn plotted against PEG concentration at $\dot{\varepsilon} = 29\ s^{-1}$ for DNA aqueous solution. *Figure 5* also shows the dependence of the viscosity of solvents on PEG concentration [PEG]. At low PEG concentrations, Δn increased with increases in PEG concentration, whereas Δn began to decrease at about [PEG] = 1 mol l^{-1} and Δn was zero beyond [PEG] = 2.05 mol l^{-1}. This result indicates that birefringent responses at high PEG concentrations are caused by a molecular conformation different from that at low PEG concentrations.

Figure 6 shows Δn plotted against $\dot{\varepsilon}$ for DNA aqueous solution at glycerol concentrations of 10–95% (v/v). A coil–globule transition has not been reported for DNA molecules in glycerol solution. This result indicates that at low glycerol concentrations, Δn increases gradually at $\dot{\varepsilon} \geq \dot{\varepsilon}_c$ and that at high glycerol concentrations, Δn increases at $\dot{\varepsilon} = 0$ as observed in the DNA–PEG aqueous solution system. *Figure 7* shows Δn plotted against glycerol concentration at $\dot{\varepsilon} = 29\ s^{-1}$. Δn did not decrease with

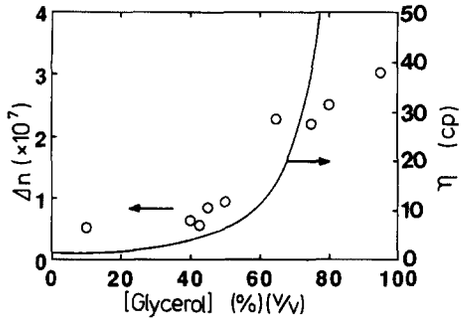

Figure 7 Δn plotted against glycerol concentration at $\dot{\varepsilon} = 29\ s^{-1}$ (O) and viscosity of solvent η as a function of glycerol concentration (solid line) for T4DNA aqueous solution.

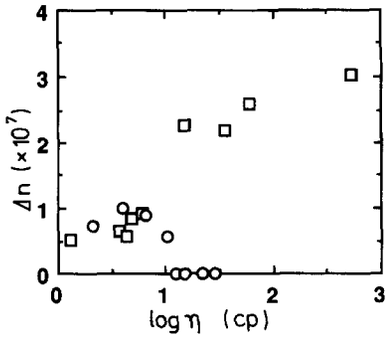

Figure 8 Δn plotted against the logarithm of viscosity of solvents η at $\dot{\varepsilon} = 29\ s^{-1}$ for PEG (O) and glycerol (□) aqueous solvents.

increases in the glycerol concentration. Δn was dependent only on the viscosity of the solvent, which suggests that DNA coil conformation is stabilized at all glycerol concentrations measured here.

Figure 8 shows Δn plotted against the logarithm of viscosity of solvents η at $\dot{\varepsilon} = 29\ s^{-1}$ for the DNA–PEG and DNA–grycerol systems. At low viscosity, Δn values are the same for both systems. A difference in Δn between solution systems appears and increases with increases in viscosity. The Δn value for the PEG system increases with $\log\eta$, and at about $\log\eta \sim 0.80$, Δn begins to decrease to zero. In the glycerol system, Δn increases with η throughout the concentration range measured. The decrease in Δn in the PEG aqueous solution indicates that there is a decrease in the flexibility of DNA molecules. This result is explained by the occurrence of a coil–globule transition at a PEG concentration of about $\log\eta \sim 1$.

Comparison of flow data with the results of fluorescence micrography

The results shown in *Figure 5* were compared with those in *Figure 2*. The beginning point of the transition is regarded as a point where the molecular length (in *Figure 2*) and Δn (in *Figure 5*) begin to decrease. In both results, the transition begins at [PEG] = 0.91 mol l^{-1}. Thus, the result by the elongational flow method (*Figure 5*) is in good agreement with that obtained using the fluorescence micrography method (*Figure 2*). Therefore, it is concluded that the elongational flow field is a useful tool for investigating the coil–globule transition of DNA molecules.

A detailed comparison revealed that at a point of [PEG] = 1.82 mol l^{-1}, DNA molecules are considered to be in the globule state in *Figure 2*, whereas $\Delta n \neq 0$ in *Figure 5*. The following explanation is offered for this discrepancy. The conformational change in DNA molecules between the coil and globule states with the addition of PEG is induced by a decrease in the affinity of the solvent to polymer segments. If coexistence of molecules in the coil and globule states still occurs at this PEG concentration, there is a possibility of observing a birefiringence response of a few remaining DNA coils. However, no molecules were observed in the coil state in fluorescent micrograph images for the solution of [PEG] = 1.82 mol l^{-1}. Thus, the coexistence of DNA coils and globular DNA molecules cannot explain the Δn observed at [PEG] = 1.82 mol l^{-1}.

Another possible explanation for the observed Δn at [PEG] = 1.82 mol l^{-1} is an actual deformation of DNA molecules in the globule state by the elongational flow field. Deformation of the polyelectrolyte globular chain in an elongational flow field was investigated by Borisov *et al*[28]. They considered that the force required for deformation of a polymer depends on the solvent strength. In a DNA globule chain, segments are closely packed at high PEG concentrations. In a DNA globule chain near the transition concentration, however, segments are thought to be packed loosely, compared with segments in a globule chain at high PEG concentrations, because of the relatively low solvent strength. This loosely packed DNA molecule in the globule state is thought to be deformed by the flow field. Therefore, it is not unreasonable to consider that the globular DNA molecule in [PEG] = 1.82 mol l^{-1} is deformed by the elongational flow field. It is concluded that the birefringence in [PEG] = 1.82 mol l^{-1} originates from deformed globule chains, which have response in a relatively weak intramolecular condensation force.

CONCLUSIONS

The response of DNA molecules in PEG aqueous solution to an elongafional flow field was revealed to be different from that of DNA molecules in glycerol aqueous solution. The large Δn values that were observed at low PEG concentrations correspond to the response where DNA molecules are in the coil state, whereas the zero-Δn which was observed at high PEG concentrations corresponds to the response of DNA molecules in the globule state. Since the change in Δn with PEG concentration agrees well with the results of the contour length of DNA molecules as a function of PEG concentration determined by fluorescence micrography, it is concluded that the elongational flow technique is useful for investigating the coil–globule transition of DNA molecules. DNA chains in the globule state near the transition concentration are considered to be deformed in the elongational flow field.

REFERENCES

1. Frank, F. C., *Proceedings of the Royal Society (London)*, 1970, **A319**, 127–136.
2. Frank, F. C. and Mackley, M. R., *Journal of Polymer Science, Polymer Physics Edition*, 1976, **14**, 1121.
3. De Gennes, P. G., *Journal of Chemical Physics*, 1974, **60**(2), 5030–5042.
4. Keller, A. and Odell, J. A., *Colloid and Polymer Science*, 1985, **263**, 181–201.
5. Hayakawa, I., Sasaki, N. and Hikichi, K., *Journal of Applied Polymer Science*, 1995, **56**, 661–665.
6. Hayakawa, I., Hayashi, C., Sasaki, N. and Hikichi, K., *Journal of Applied Polymer Science*, 1996, **61**, 1731–1735.
7. Chu, S., *Science*, 1991, **253**, 861–866.
8. Smith, S. B., Finzi, L. and Bustamante, C., *Science*, 1992, **258**, 1122–1126.

9. Perkins, T. T., Smith, D. E. and Chu, S., *Science*, 1994, **264**, 819–822.
10. Perkins, T. T., Quake, S. R., Smitli, D. E. and Chu, S., *Science*, 1994, **264**, 822–826.
11. Minagawa, K., Matsuzawa, Y., Yoshikawa, K., Khokhlov, A. R. and Doi, M., *Biopolymers*, 1994, **34**, 555–558.
12. Vasilevskaya, V. V., Khokhlov, A. R., Matsuzawa, Y. and Yoshikawa, K., *Journal of Chemical Physics*, 1995, **102**, 6595–6602.
13. Yoshikawa, K. and Matsuzawa, Y., *Physica D*, 1995, **84**, 220–227.
14. Mel'nikov, S. M., Sergeyev, V. G. and Yoshikawa, K., *Journal of the American Chemistry Society*, 1995, **117**, 2401–2408.
15. Yanagida, M., Hiraoka, Y. and Katsura, I., *Cold Spring Harbour Symposium on Quantatative Biology*, 1983, **47**, 177.
16. Taylor, G. I., *Proceedings of the Royal Society (London)*, 1934, **146**, 501–523.
17. Torza, S. J., *Journal of Polymer Science, Polymer Physics Edition*, 1975, **13**, 43–57.
18. Lifshitz, I. M., Grosberg, A. Yu. and Khokhlov, A. R., *Review of Modern Physics*, 1978, **50**, 683.
19. Grosberg, A. Yu. and Khokhlov, A. R., *Soviet Science Review Section*, 1987, **A8**, 147.
20. Atkins, E. D. T. and Taylor, M. A., *Biopolymers*, 1992, **32**, 911–923.
21. Mackley, M. R. and Keller, A., *Philosophy Transactions of the Royal Society (London)*, 1969, **A278**, 29–66.
22. Crowley, D. G., Frank, F. C., Mackley, M. R. and Stephenson, R. G., *Journal of Polymer Science, Polymer Physics Edition*, 1976, **14**, 1111–1119.
23. Pope, D. P. and Keller, A., *Colloid and Polym. Sci.*, 1977, **255**, 633–643.
24. Odell, J. A., Keller, A. and Atkins, E. D.T., *Macromolecules*, 1985, **18**, 1443–1453.
25. Odell, J. A., Atkins, E. D.T. and Keller, A., *Journal of Polymer Science, Polymer Letters Edition*, 1983, **21**, 289–300.
26. Odell, J. A., Muller, A. J. and Keller, A., *Polymer*, 1988, **29**, 1179–1190.
27. Odell, J. A. and Taylor, M. A., *Biopolymers*, 1994, **34**, 1483–1493.
28. Borlsov, O. V., Darinskii, A. A. and Zhulina, E. B., *Macromolecules*, 1995, **28**, 7180–7187.