

# Strong effects of molecular topology on diffusion of entangled DNA molecules

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When long polymers such as DNA are in a highly concentrated state they may become entangled, leading to restricted self-diffusion. Here, we investigate the effect of molecular topology on diffusion in concentrated DNA solutions and find surprisingly large effects, even with molecules of modest length and concentration. We measured the diffusion coefficients of linear and relaxed circular molecules by tracking the Brownian motion of single molecules with fluorescence microscopy. Four possible cases were compared: linear molecules surrounded by linear molecules, circular molecules surrounded by linear molecules, linear molecules surrounded by circles, and circles surrounded by circles. In measurements with 45-kbp DNA at 1 mg/ml, we found that circles diffused  $\approx 100$  times slower when surrounded by linear molecules than when surrounded by circles. In contrast, linear and circular molecules diffused at nearly the same rate when surrounded by circles, and circles diffused  $\approx 10$  times slower than linears when surrounded by linears. Thus, diffusion in entangled DNA solutions strongly depends on topology of both the diffusing molecule and the surrounding molecules. This effect also strongly depends on DNA concentration and length. The differences largely disappeared when the concentration was lowered to 0.1 mg/ml or when the DNA length was lowered to 6 kb. Present theories cannot fully explain these effects.

polymers | reptation

Among polymers DNA is rather unique in that it is naturally found in a number of different topological forms, including linear, supercoiled circular, relaxed circular, knotted circular, and branched. DNA solutions handled *in vitro* in molecular biology research are often relatively concentrated ( $\approx 1$ – $10$  mg/ml, for example, after lysis of bacterial cells during DNA isolation, or when DNA is redissolved after ethanol precipitation). According to classical theories and experiments in polymer physics, long flexible molecules form random coils that overlap and become entangled as the concentration of solutions is increased (1, 2). In the field of polymer physics and rheology there is considerable fundamental interest in understanding the effect of molecular topology on entangled polymer dynamics (3, 4). In gel electrophoresis it is well known that molecular topology strongly affects the mobility of DNA. However, with few exceptions, most theories and experiments on diffusion in concentrated polymer solutions have examined only linear molecules. Here, we investigate the effect of molecular topology on diffusion of entangled DNA. Although relaxed circular molecules differ from linear molecules only by the presence of one additional pair of phosphodiester bonds (linking head to tail) and diffuse at nearly the same rate in dilute solution (5), we observe large differences in their diffusion rates with molecules of modest size and concentration.

## Results

A 45-kbp fosmid DNA construct (pCCFOS1-45) was prepared as described (6). It was treated with topoisomerase I to prepare the relaxed circular form and with *Apa*I to prepare the linear form (5, 6). The DNA was concentrated by isopropanol precipitation and resuspended in TE buffer (Tris·HCl/EDTA, pH 8)

with 10 mM NaCl. At this ionic strength it has been predicted and experimentally confirmed that intrinsic DNA charge is moderately screened (7), and that charge repulsion results in a  $\approx 5$ -fold increase in the effective diameter (distance of closest approach) of the DNA molecule from its bare diameter of 2 nm (5, 8–10). Because we have only monovalent counterions present, the DNA–DNA interactions are purely repulsive such that excluded volume considerations are important and there is no aggregation or condensation of the DNA (11, 12). Self-diffusion coefficients were measured by labeling a small fraction of molecules with the dye YOYO-I (Molecular Probes, Carlsbad, CA) (13–15) and tracking Brownian motion with video microscopy as described (5). In all cases we found that the mean squared displacement increased linearly with time, indicating conventional diffusion. It has been shown that YOYO-I binding increases both the contour length and the persistence length of the DNA slightly (by less than a factor of 2), but the number of persistence lengths and elasticity remains essentially unchanged for molecules much longer than the persistence length (16, 17). Electrophoresis and dilute diffusion measurements show that no supercoiling is induced by the YOYO-I binding (5).

Here, we study all four possible topological combinations of linear and circular DNA molecules: linear molecules surrounded by linear molecules (L-L), circular molecules surrounded by linear molecules (C-L), linear molecules surrounded by circles (L-C), and circles surrounded by circles (C-C). These cases are depicted schematically in Fig. 1. At 1 mg/ml we observed dramatic differences in the diffusion coefficients (Fig. 2 *Top* and Table 1). Changing the topology of the surrounding molecules had the largest overall effect. Most strikingly, the diffusion coefficient ( $D$ ) of a circular molecule decreased by  $\approx 100$ -fold when the surrounding DNA was changed from circular to linear. A smaller, although still quite significant, decrease of  $\approx 6$ -fold was observed with linear DNA when the surrounding DNA was changed from circular to linear. The result also depended on the topology of the diffusing molecule. When the surrounding DNA was circular the topology of the diffusing molecule made very little difference ( $D_{C-C} \approx 1.3D_{L-C}$ ), but when the surrounding DNA was linear there was a sharp difference ( $D_{L-L} \approx 10D_{C-L}$ ). In previous dilute solution diffusion measurements (5) we found that the radius of gyration of the linear form was only 1.58 times that of the circular form, thus this 10-fold difference cannot simply be attributed to a size difference between circular and linear molecules of equal length and must be attributed to the influence of topology on entanglement effects.

To investigate the dependence of these topological effects on concentration we made additional measurements with the 45-kbp fosmid diluted to 0.1 mg/ml. In this case we found that all

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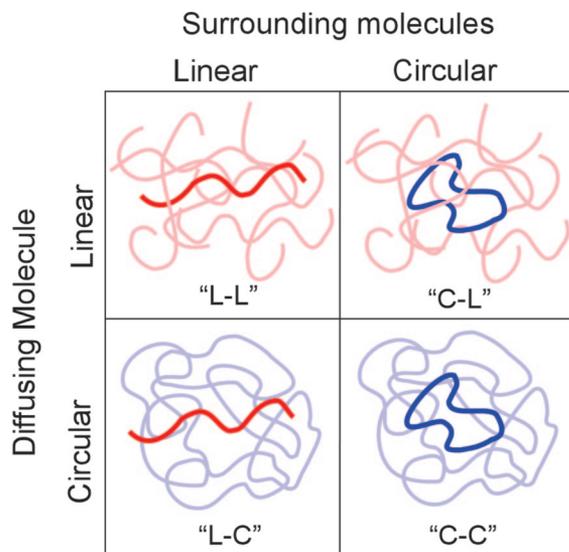
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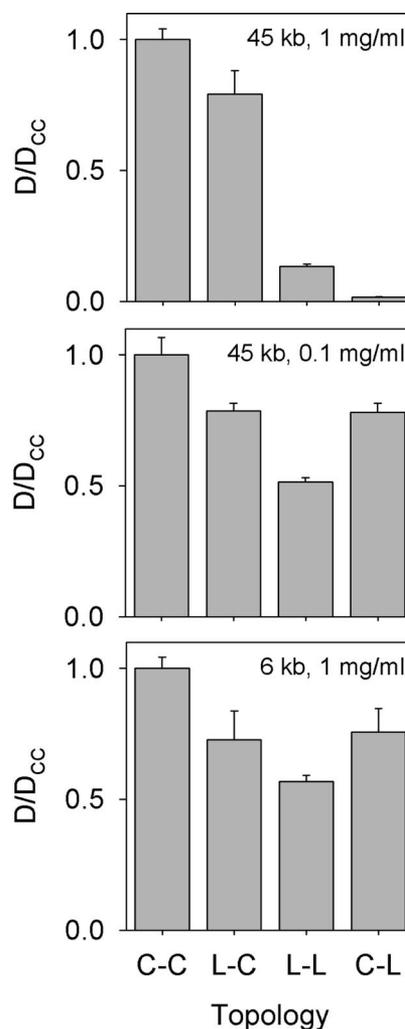
**Fig. 1.** Schematic illustration of the possible topological combinations of entangled linear and relaxed circular DNA molecules. The four cases depicted are: a linear diffusing molecule surrounded by linear molecules (L-L), circular molecule surrounded by linear molecules (C-L), linear molecule surrounded by circular molecules (L-C), and circular molecule surrounded by circular molecules (C-C). Linear molecules are shaded red, and circular molecules are blue. For clarity, the diffusing molecules are represented by brighter, thicker lines than the surrounding molecules.

four  $D$  values were within a factor of 2 of each other (Fig. 2 *Middle*). Thus, these topological effects are only important at high concentration and the onset is between 0.1 and 1 mg/ml.

To investigate the dependence on DNA length we also made further measurements with a 6-kbp plasmid DNA construct at both 1 and 0.1 mg/ml. This sample was digested with BamHI to prepare the linear form (6). With this shorter molecule there were only minor effects of topology, even at 1 mg/ml (Fig. 2 *Bottom* and Table 1). Most conspicuously, whereas  $D_{C-L}$  decreased only  $\approx 4$ -fold when DNA length was increased from 6 to 45 kbp at 0.1 mg/ml, it decreased by  $\approx 1,000$ -fold at 1 mg/ml. Interestingly, the small differences in the four topological cases with the 6-kbp sample were almost identical at both concentrations and mimicked those measured with the 45-kbp DNA at 0.1 mg/ml. Thus, reducing the DNA length had an equivalent effect as reducing the concentration. Our interpretation is that the 6-kbp molecules are too short to effectively become entangled regardless of their topology.

## Discussion

Although several previous experiments have measured DNA diffusion in dilute solutions (5, 18–23), very few have examined the effect of entanglements. Dynamic light scattering was used to study supercoiled 2.3-kbp DNA molecules at concentrations up to 4.0 mg/ml (23) and the results were in qualitative agreement with predictions for semidilute rigid rod polymers. That regime is very different from that considered here. Photobleaching recovery was used to study longer 48.5-kbp DNA solutions up to 0.3 mg/ml (24), but only a weak concentration dependence was observed and only the linear form was studied. Previously, we studied 48.5-kbp linear DNA at higher concentrations (up to 0.8 mg/ml) and a sharp decrease in  $D$  was observed above  $\approx 0.5$  mg/ml (13). In this regime, the dependence on length and concentration was found to be in accord with predictions of the reptation model of entangled polymer dynamics (1). The key postulate of this model is that the entangled molecule is confined to a tube-like region parallel to its contour. Diffusion occurs by



**Fig. 2.** Dependence of DNA self-diffusion coefficients ( $D$ ) on molecular topology. Bar graphs display normalized  $D$  values vs. topological case (shown in Fig. 1). All  $D$  values are normalized by the corresponding measured diffusion coefficient for a circular DNA surrounded by circular DNA ( $D_{C-C}$ ) (the highest value of  $D$  in each case). The DNA length and concentration is listed in each plot.

a slithering motion of the molecule parallel to this tube whereby it may escape head-first or tail-first from the tube. Motion in a perpendicular direction is highly restricted and can only occur if the surrounding molecules move out of the way, a process termed “constraint release,” which is predicted to be very slow compared with reptation.

The reptation model was originally developed to describe polymer melts, i.e., molten synthetic polymers with a polymer volume fraction of 1. This situation is potentially very different from that of our DNA solutions, where the highest volume fraction is  $\approx 10^{-3}$ . Although reptation models have been extended to describe polymer solutions by the use of scaling arguments, the case of circular molecules has not been considered to our knowledge. Thus, we can only compare our results with predictions for melts. As circular molecules have no heads or tails by which to slither out of a tube-like constraint they cannot diffuse by normal reptation. Three configurations have been proposed for circular molecules in a melt of linear molecules (25). First, the circle could be pinned or threaded such that it can only move by constraint release (Fig. 3 *c* and *d*). Second, it could be unpinned but ramified by large loops (Fig. 3*b*). Third, it could be unpinned with no large loops and move by reptation (similar to a linear molecule of half the length) (Fig. 3*a*).

**Table 1. Measured DNA diffusion coefficients for the different length, concentration, and topological combinations of molecules**

DNA length, concentration	Diffusion coefficients, $\mu\text{m}^2/\text{s}$			
	C-C	L-C	L-L	C-L
45 kbp, 1 mg/ml	$0.026 \pm 0.001$	$0.021 \pm 0.002$	$0.0035 \pm 3\text{e-}4$	$4.0\text{e-}4 \pm 5\text{e-}5$
45 kbp, 0.1 mg/ml	$0.43 \pm 0.03$	$0.33 \pm 0.01$	$0.22 \pm 0.01$	$0.33 \pm 0.01$
6 kbp, 1 mg/ml	$0.46 \pm 0.02$	$0.33 \pm 0.05$	$0.26 \pm 0.01$	$0.35 \pm 0.04$
6 kbp, 0.1 mg/ml	$1.71 \pm 0.19$	$1.22 \pm 0.11$	$1.04 \pm 0.07$	$1.45 \pm 0.16$

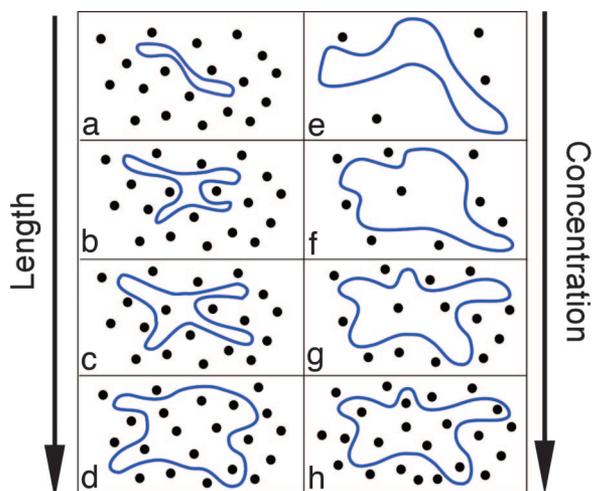
Constraint release is predicted to dominate in the limit of long length, leading to greatly hindered diffusion for a circular molecule. This prediction is thus consistent with our finding that  $D_{C-L} \ll D_{L-L}$ . It has also been speculated, although not proven, that constraint release would be negligible and reptation would dominate the diffusion of circular polymers in a circular melt (25). This conjecture is consistent with our finding that  $D_{C-C} \gg D_{C-L}$ . Several computer simulations have also predicted  $D_{C-C} > D_{L-L}$  (26, 27). However, none of these analyses of melts can fully explain our results. None apply to solutions with low volume fraction, none predict concentration dependence, and none consider all four topological combinations that we have studied.

Surprisingly, most of the experimental findings with synthetic polymer melts differ quite dramatically from our findings with DNA.  $D_{L-L} > D_{C-C}$ ,  $D_{C-C} \approx D_{C-L}$ ,  $D_{L-L} \approx D_{L-C}$ , and  $D_{L-L} > D_{C-L}$  have been reported (28–31). Only one of these results ( $D_{L-L} > D_{C-L}$ ) was in qualitative agreement with our findings on DNA solutions. However, in that study (31) a smaller difference (of  $\approx 5$ -fold) was observed than in our experiment despite the use of melt with polymers of a greater number of persistence lengths and a 1,000-fold higher volume fraction. Only one previous study, using pulsed-gradient NMR, has examined circular polymers in solution and found  $D_{C-C} > D_{L-L}$  (32). However, in that study a theta solvent was used and the measured ratio  $D_{C-C}/D_{L-L} \approx 1$ –2 was fairly constant over the entire concentration range, whereas in our measurements it increased from  $\approx 2$  to 10. No previous experiments

have investigated solutions where the diffusing and surrounding molecules had different topologies to our knowledge.

Although such topological effects on diffusion in concentrated solutions of DNA have not been previously examined to our knowledge, strong effects of molecular topology on DNA mobility in gel electrophoresis are well known (33), and numerous theories have been put forward to explain these effects (34–36). When plasmid DNA of  $\approx 3$ –10 kbp is electrophoresed at constant voltage in 0.8% agarose the supercoiled form runs the fastest, followed by the linear and relaxed circular forms. However, with  $\approx 40$ - to 300-kbp fosmids and bacterial artificial chromosomes the linear form migrates the fastest followed by the supercoiled, whereas the mobility of the relaxed circle is practically zero (37, 38). More recently, fabricated nanostructures have been used to study transport and conformation of single DNA molecules of different lengths and topologies and have led to many insights into their dynamics in confined environments (39–41). The reptation model was originally used to explain DNA mobility in both DC and pulsed-field gel electrophoresis as well as other confined environments; however, predictions did not always match experiment, and further contributions and corrections were needed to account for observed motion (34). Loop formation is predicted to play a large role in the migration of circular DNA and long linear DNA molecules (35), and the zero mobility phenomenon of long relaxed circular DNA has been attributed to the pinning of the relaxed circular DNA by free ends of the gel fibers or other impurities (36–38, 42). Further, because DNA is continually stretched by a driving electric field, tension plays an important role in its dynamics (43, 44). Those studies shed light on the mechanism by which both linear and circular DNA move through fixed obstacles. However, although the mechanism of diffusion of DNA past rigid, fixed obstacles (gel fibers) may be compared with the case of an entangled DNA solution (where the obstacles formed by the surrounding molecules are dynamic), the two cases are still quite different. Gel electrophoresis not only involves fixed obstacles but the motion of the DNA is driven by an electric field, whereas in our experiment molecules are freely diffusing in all directions. Further, our examination of how the topology of the surrounding molecules affects diffusion has no analogy in gel electrophoresis as in that case the obstacles are not DNA molecules of varying topologies.

In summary, we report strong effects of molecular topology on the diffusion of entangled DNA molecules of modest size and concentration. We found that the topology of surrounding molecules has a very strong influence and the topology of the diffusing molecule has a strong influence only when the surrounding molecules are linear. These findings suggest that free ends of molecules play a critical role in generating entanglements that retard diffusion. We propose that the strongly hindered diffusion of long circular DNA molecules surrounded by linear molecules is caused by threading of the ends of the linear molecules through the circles, as predicted for polymer melts (25) and somewhat like the pinning of DNA circles by dangling gel fibers proposed to occur during gel electrophoresis, which



**Fig. 3.** Schematic model of the effect of increasing length and concentration on the conformation and diffusion of a circular DNA molecule. Black dots represent “obstacles” formed by surrounding entangled DNA molecules, and the blue loop represents a diffusing circular DNA. (a–d) The effect of increasing the molecular length, as proposed in ref. 6. (e–h) The effect of increasing concentration. With short length (a) or low concentration (e), the molecule is most likely unthreaded and can diffuse by reptation. As length (c and d) or concentration (f–h) increases, DNA is increasingly likely to get threaded and can only diffuse by constraint release of the surrounding entangling DNA.

