Dynamical Scaling of DNA Diffusion Coefficients

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Fluorescence microscopy has been used to study many of the physical properties of DNA at the single-molecule level.[1−9] Several of our experiments[6,8,9] have focused on testing polymer physics scaling laws,[10] which predict that various properties of flexible polymers, such as relaxation times[6] and hydrodynamic drag,[8] should vary as a power of the polymer lengths. Here we present measurements of the Brownian motion of long, fluorescently labeled DNA molecules and observe power-law scaling of the diffusion coefficients (D) with chain length (L). The results are in good agreement with the prediction of the Zimm model in good solvent conditions ($D \sim L^{-3/5}$),[11] which further supports the idea of dynamical scaling.[6,10]

λ phage DNA (48 502 base pairs) (Gibco BRL) was linearized by heating to 65 °C, and restriction fragments were generated with the restriction enzyme *Hind*III (NEB). The fragments were separated by gel electrophoresis, and the largest four (F1 = 23 130 bp, F2 = 9416 bp, F3 = 6557 bp, and F4 = 4361 bp) were purified by affinity to glass beads. Molecules up to 6 times longer than λ-DNA were generated by concatenating λ-DNA molecules using T4 DNA ligase (NEB). The DNA was labeled with the dye TOTO-1 (Molecular Probes),[12] which increases the length of λ-DNA to about 22 μm from the native length of 16.3 μm.[8] The persistance length of native DNA (unlabeled, in 10 mM NaCl) is approximately 50 nm.[13]

To measure the length of the two longest molecules we studied, we manipulated them by a technique we call "optical chopsticks". In this technique, a free molecule is hooked in the crevice between two fused 2 μm polystyrene microspheres manipulated with an optical trap.[14] The hooked, "U"-shaped molecule may then be moved about, stretched out in a fluid flow, and released. By measuring the fractional extensions of the two stretched "branches" of the "U"-shaped chain in a constant-velocity fluid flow, the molecule's length may be determined.[8]

The molecules were loaded into a sample chamber made of a microscope slide and coverslip separated by two 75 μm wire spacers. The edges were sealed with epoxy. The solution was very dilute, so that there was no interaction between DNA molecules. To minimize interactions with the surfaces, the microscope was focused in a plane that was >20 μm away from the coverslip and slide. The projection of the Brownian motion in the x−y plane was tracked using video fluorescence microscopy,[15] and the increase in the mean square displacements, $\langle x^2 \rangle = \langle y^2 \rangle = 2Dt$, with time ($t$) were recorded for an ensemble of molecule paths. To determine the diffusion coefficient, $(\langle x^2 \rangle + \langle y^2 \rangle)/2$ was plotted vs time, and a linear fit yielded the slope $2D$.[16] Error estimates were made using the bootstrap method[17] to find the standard deviation in D for a thousand subensembles of molecule paths.

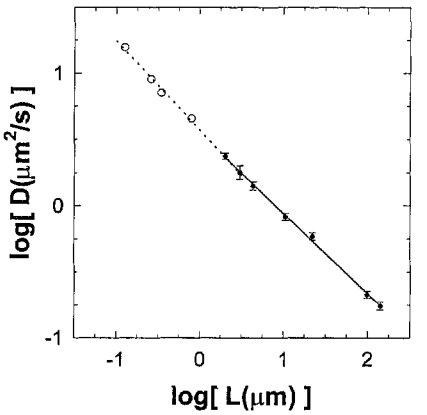

Figure 1. log−log plot of the self-diffusion coefficient versus chain length for isolated DNA molecules. The solid points are the data obtained by tracking fluorescently labeled DNA. The solid line is a linear fit to the data and the slope gives the scaling exponent $\nu = 0.611 \pm 0.016$. The plotted values were derived from the values in Table 1 by correcting for a change in persistence length due to the binding of the dye (see text). The error bars indicate σ_D from the bootstrap analysis. The open circles are the data of Sorlie and Pecora for shorter, unlabeled DNA molecules,[19] and the dotted line is a linear fit to their data (slope = −0.68).

Table 1. Diffusion Coefficients and Inferred Radii of Gyration for TOTO-1 Stained Bacteriophage DNA Molecules

source	size (bp)	length (μm)	D (μm²/s)	R_G (μm)
λ concatmer	~309 000	140	0.14 ± 0.01	2.47
λ concatmer	~216 000	98	0.17 ± 0.01	2.03
λ phage	48 502	22	0.47 ± 0.03	0.73
λ *Hind*III F1	23 130	10.5	0.66 ± 0.04	0.52
λ *Hind*III F2	9 416	4.3	1.13 ± 0.08	0.31
λ *Hind*III F3	6 557	3.0	1.43 ± 0.17	0.24
λ *Hind*III F4	4 361	2.0	1.94 ± 0.10	0.18

For molecules ranging in length from 2.0 to 140 μm, D varied as a power of L ($D \sim L^{-\nu}$) (Table 1 and Figure 1). The scaling exponent $\nu = 0.611 \pm 0.016$ was determined by a linear fit to log D vs log L. This value agrees with the prediction ($\nu = {}^3/_5$) of the Zimm model in good solvent conditions: $D = 0.196 k_B T / 6^{1/2} \eta R_G$, where η is the solvent viscosity (here 0.95 cP), $k_B T$ is the thermal energy (here $T \cong 297$ K), and $R_G \sim L^{3/5}$ is the radius of gyration. If we assume that the Zimm model with complete nondraining hydrodynamics applies, we can estimate the radii of gyration R_G (Table 1). The $\nu = {}^3/_5$ ("Flory") scaling exponent provides clear evidence for the excluded volume effect.[18]

The scaling we observed is theoretically expected for "flexible" molecules which have lengths much longer than the persistence length ($P \cong 150$ bp for native DNA). For shorter, "semiflexible" molecules, the length dependence is expected to increase. Our video camera is not able to track shorter molecules; however, dynamic light scattering was used by Sorlie and Pecora[19] to measure D for native fragments of length 367 to 2311 bp (L/P = 2.4−15.4). Their study revealed a steeper dependence ($\nu \cong 0.68$) in this region, which is well described by the theory of Yamakawa and Fujii.[20] We can therefore draw the conclusion that there is a gradual transition from flexible (scaling) behavior to semiflexible (nonscaling) behavior as L/P decreases from approximately 40 to 10.

To quantitatively compare our measurements on fluorescently labeled DNA with the previous measurements of Sorlie and Pecora on unlabeled DNA, we scaled our data to allow for a change in the persistance length

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due to the binding of the dye. The diffusion coefficient scales as $D \sim P^{-2/5}$, and we needed to multiply the diffusion coefficients in Table 1 by a factor of approximately $(1.75)^{2/5}$ to bring our data into accord with data of Sorlie and Pecora (as plotted in Figure 1). This suggests that the dye, which intercalates in the DNA, increased the persistance length by a factor of roughly 1.75 at our dye concentration.[21]

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(12) The DNA was labeled for about 1 h at a concentration of about 0.5×10^{-7} M TOTO-1 and 3×10^{-7} M double-stranded DNA base pairs and diluted by about 5–10-fold into a final solution of 10 mM Tris-HCl (pH 8), 1 mM EDTA, 10 mM NaCl, 0.8% β-mercaptoethanol, 50 μg/mL glucose oxidase, 0.1% glucose, and 10 μg/mL catalase. There is some uncertainty in the ratio of dye to nucleotides for the restriction fragments since their concentration after purification was only known within a factor of 2–3.
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(15) The microscope and video equipment used are described in ref 6.
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