Macromolecules 1994, *27*, 6808–6811

Dynamic Light Scattering from Monodisperse 2311 Base Pair Circular DNA: Ionic Strength Dependence

Jay Newman,[†] **John Tracy, and R. Pecora***

Department of Chemistry, Stanford University, Stanford, California 94305-5080

Received July 12, 1994; Revised Manuscript Received August 26, 1994[⊛]

ABSTRACT: The ionic strength dependences of the translational diffusion coefficient and the first internal mode relaxation time of monodisperse 2311 base pair relaxed circular DNA were studied by dynamic light scattering. Intensity autocorrelation functions were analyzed using the inverse Laplace transform method (CONTIN). Above the lowest scattering angle studied (33°), generally two peaks were found in the distribution of decay rates. The translational diffusion coefficient, corresponding to the slower of the peaks, at a low DNA concentration of 100 μg mL^{-1} was found to be $(4.52 \pm 0.2) \times 10^{-8}$ cm^2 s^{-1}, nearly independent of added NaCl from 1 to 0.001 M using a buffer of 10 mM Tris·HCl, 1 mM EDTA, pH 8. The mean relaxation frequency of the first internal mode, extracted from the faster of the two peaks, decreases by a factor of 2 as the added NaCl is decreased to 0 from 1 M. The amplitude of the faster mode, when plotted as a function of the square of the reduced scattering vector length, $q^2R_g^2$, lies on the same curve at all ionic strengths studied. Calculation of the radius of gyration, R_g, as a function of ionic strength based on a scaling argument shows about a 25% increase as the added salt is decreased from 1 M. An "extraordinary mode" is observed when the Tris content is reduced to 1 mM with both slow and fast diffusive modes observed.

I. Introduction

DNA is widely used as a model system for investigating the solution dynamics of linear polymer chains with varying degrees of stiffness. Short pieces of duplex B-DNA (less than about 50 nm in length) may be modeled as rods and, as the chain becomes longer, as semiflexible chains. DNAs can be made in a homologous series and can be prepared in monodisperse dispersions. In addition, different conformations of a given duplex DNA can often be prepared, including superhelical, linear, and relaxed circular forms.

In previous works, translational and internal relaxation of 2311 base pair DNA (1.53×10^6 molecular weight) in solution was studied using the dynamic light scattering technique (DLS). The 2311 base pair DNA has been studied in its superhelical form,[1–2] linear form,[3–4] and relaxed circular form.[5] The relaxed circular form (called cDNA in the following) has certain advantages as a model system for dilute solution DLS studies of the dynamics of flexible and semiflexible macromolecules. It was shown that there is more separation in relaxation times between the DLS pure translational mode and the translational/longest internal mode than for the linear form of the same DNA. For this system, in addition, the amplitudes of the translational/internal mode terms are not so large that overlapping of contributions from many internal modes occurs. Furthermore, theories for circular chain dynamics are often mathematically more tractable because of the circular boundary conditions. The major disadvantage to performing solution dynamics experiments on relaxed circles in comparison to linear or superhelical chains is the relatiely tedious preparation procedure.

Seils and Pecora[5] have previously studied dilute solutions of 2311 base pair cDNA using DLS at two ionic strengths. In this article, the work of Seils and Pecora is extended to cover a wider range of ionic strengths as well as to investigate the region of no added salt in which other investigators have observed a "slow" or "extraordinary" mode, in addition to the "ordinary" modes seen at higher ionic strengths.[6–11]

II. Methods

Sample Preparation. The pLH2311 plasmid DNA was prepared as previously described,[1] except that the purification was accomplished by banding twice with an ultracentrifuge using a double-density CsCl gradient. The plasmid was converted to the open circular form in several small batches of DNA (250 μL, 350 μg mL^{-1}) in a buffer containing 0.05 M NaCl, 0.005 M MgCl$_2$, and 0.1 M Tris·HCl (pH 7.5) by carefully nicking it with DNase I (3 μL, 0.06 μg mL^{-1}) at 0 °C for 5 min. The reaction was stopped by adding 250 μL of 0.2 N EDTA. To remove all traces of the DNase, the samples were immediately phenol–chloroform extracted, following the procedure of Maniatis et al.[12] Gel electrophoresis, using 1% agarose TPE buffer (10.8 g of Tris·HCl, 1.55 mL of 85% H$_3$PO$_4$, and 0.93 g of Na$_2$EDTA per 100 mL of solution, pH 8.0) with 200 μL of ethidium bromide (10 mg/mL) added, showed two bands which were well-separated and recovered by syringe resulting in monodisperse samples of open circular DNA. Following butanol extraction of the ethidium dye, the DNA was precipitated with ethanol twice and resuspended in TE buffer (10 mM Tris·HCl, pH 8.0, and 1 mM EDTA) made with doubly-distilled water further treated with a Millipore filtration system. Samples were initially filtered through small-volume 0.45 μm pore Acrodisc filters directly into precleaned dust-free optical glass cuvettes. All final buffers were prefiltered before use. Ionic strengths were adjusted by gravitometric addition of filtered concentrated NaCl solutions or by dilution with filtered TE buffer. Those samples with less than 0.02 M salt were placed in a 1.5 mL Eppendorf tube and spun for 1 h at 20 000 rpm in a Dupont SS-34 rotor. The samples were immediately transferred to a clean scattering cell using a precleaned pipettor tip. The optical cells were centrifuged at 5000 rpm for 16–24 h just prior to light scattering measurements. All samples were inspected visually for dust prior to measurements using a 5× eyepiece and laser illumination. Measurements were performed at 20 °C. Periodic gels were run to check for sample degradation and none was detected. DNA concentrations were typically determined after light scattering by UV absorption at 260 nm using an extinction coefficient of 50 μg mL^{-1} OD. Sample purity was checked by computing the ratio of absorbance at 280/260 nm, which was typically 0.54.

Dynamic Light Scattering. The light scattering apparatus has been described previously.[13] Time autocorrelation functions of the scattered light intensity were obtained using a Brookhaven BI2030 correlator. Measurements were typically made over a range of scattering angles from 33 to 123° with a minimum of three measurements at each angle. The laser wavelength was set at 488 nm, and typically 400 mW of power was incident on the sample. Experimental durations were set to obtain independently

---

[†] Permanent address: Physics Department, Union College, Schenectady, NY 12308.

[⊛] Abstract published in *Advance ACS Abstracts*, October 1, 1994.

0024-9297/94/2227-6808$04.50/0

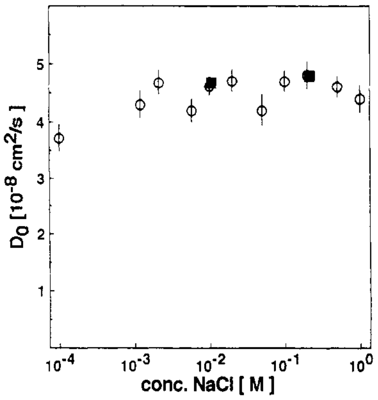

Figure 1. Translational diffusion coefficient of monodisperse 2311 bp circular DNA as a function of added NaCl. D values were obtained by dividing the slowest mean relaxation frequency determined at each angle by the square of the magnitude of the scattering vector, where $q = 4\pi n \sin(\theta/2)/\lambda$; n is the solvent index of refraction, θ is the scattering angle, and λ is the laser wavelength. D values obtained were independent of q and were averaged over scattering angles from 33 to 90° at each salt concentration. The filled squares are from Seils and Pecora.[5]

calculated autocorrelation function background levels of at least 10^7, and experimental sample times were set to span about 3 mean correlation times. After a preliminary analysis using the method of cumulants,[14] the data were transferred to a VAXStation 3200 computer and analyzed using the program CONTIN.[15] The frequency limits were chosen as the inverse first and last channel times and the chosen solutions, those with probability to reject closest to 0.5, were selected to be reported.

III. Results and Discussion

Measurements of the time autocorrelation functions of light scattered from circular 2311 bp DNA samples in TE buffer with a series of different amounts of added NaCl were performed. Salt concentrations were varied over a wide range, from no added salt to a concentration of 1 M NaCl. The DNA concentrations were kept roughly constant at 100 μg mL^{-1}, low enough that no appreciable concentration dependence is expected.[5] Frequency distributions obtained from CONTIN generally had two peaks, except at the lowest scattering angle measured, 33°, where only one peak was detected. The frequency of the slower of these scaled with the square of the scattering vector, q, as expected for diffusive motion, and was identified as the center of mass diffusion. Figure 1 shows the salt dependence of the translational diffusion coefficient, D (averaged over scattering angles from 33 to 90°), over the range of salt concentrations measured. Except for the samples with no added salt, the translational diffusion coefficient remains constant within the uncertainty of the measurement. There is a decrease in the translational diffusion coefficient of about 20% with no added salt present. These results are in good agreement with previous results from this laboratory on the same circular DNA at salt concentrations of 0.2 and 0.01 M added NaCl (shown as filled squares in the figure).[5]

A second peak was observed in the CONTIN distributions of decay rates at angles of 57° and higher for all samples in the 10 mM Tris·HCl buffer. The amplitude of this peak was seen to increase with increasing scattering angle of the experiment. After subtracting the slower, translational diffusion relaxation frequency from the faster mean relaxation frequency, the resulting frequency was determined to be independent of the magnitude of the scattering vector, q, and was thus attributed to an internal mode of motion. The results for the different salt concentrations are shown in Figure 2. A decrease of about a factor of 2 in the relaxation frequency with decreasing ionic strength is evident. This is in agreement with the earlier work of Seils and Pecora (shown as filled squares in the figure)[5] for two ionic strengths. These data are not in agreement with what would be expected if local stiffness dominated this mode. In that case, a trend toward higher frequencies would be expected as the local stiffness increased at lower ionic strengths. These relaxation frequencies are considerably higher than those from overall rotational tumbling of a rigid ring of DNA. The DNA should therefore not be picture as a rigid structure even at low ionic strength.

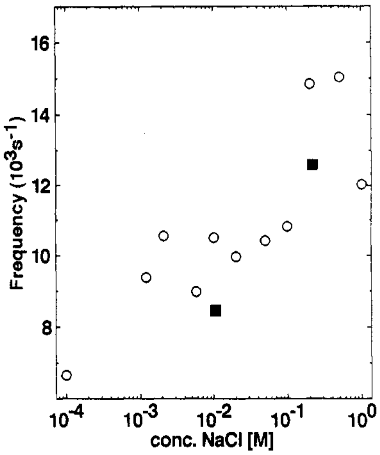

Figure 2. Mean relaxation frequency (Γ_1) corresponding to the first (longest wavelength) internal mode of motion as a function of added salt. Frequencies were obtained by subtracting the slower translational frequencies from the corresponding faster frequency at each scattering angle and averaging the results from the angles between 57 and 90° at each salt concentration. The filled squares are from Seils and Pecora.[5]

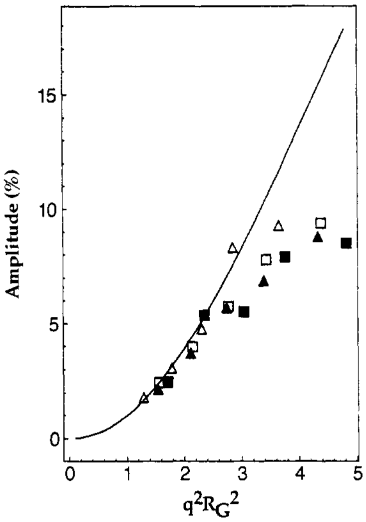

Figure 3. Relative amplitude of the first internal mode determined by CONTIN as a function of the dimensionless parameter ($q^2R_g^2$) for four different added concentrations of NaCl: (■) 0.001 M; (□) 0.01 M; (▲) 0.1 M; (△) 1.0 M. R_g values were determined as outlined in the text and are shown in Figure 4. The solid line is calculated from the Rouse–Zimm model including only the longest internal mode.[16]

The relative amplitudes of the internal mode are plotted in Figure 3 as a function of the dimensionless parameter $q^2R_g^2$, where R_g is the radius of gyration of the DNA. The amplitudes measured at the different salt concentrations all lie on the same curve. The radius of gyration for the c2311 DNA samples in different ionic strengths was calculated using a scaling relation for the internal relaxation frequency Γ_1 and the measured diffusion coefficient, as first proposed by Seils and Pecora.[5] It has been shown

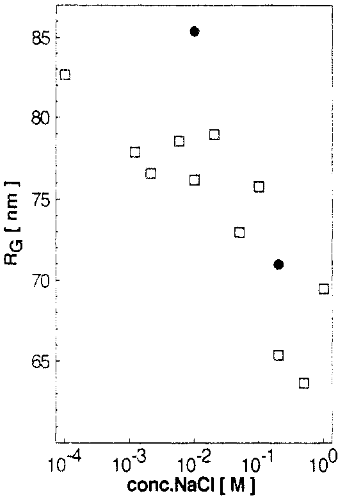

Figure 4. Calculated radius of gyration of 2311 bp circular DNA as a function of the added NaCl concentration. Values were obtained from the scaling relation suggested by eq 1 and by substituting a value of 45 nm for the persistence length of DNA in 0.2 M salt into eq 3 to arrive at a value of 71 nm for R_g. From this, values at other ionic strengths were calculated using eq 1 and the measured D and Γ_1 at each salt concentration. The filled circles are from Seils and Pecora.[5]

that for a circular Rouse model, the first internal decay time τ_1 (equal to the inverse of Γ_1) is given by[16]

$$\tau_1 = \frac{R_g^{\,2}}{\pi^2 D_0} \tag{1}$$

This suggests that $R_g^2 \propto D_0/\Gamma_1$ for the Rouse model. Seils and Pecora[5] also showed that τ_1 for the Soda–Berg model[17–19] scales in the same way as the Rouse model, but multiplied by a function $\sigma(y,d)$, where y is the ratio of the contour length (L) to the persistence length (a) and d is the chain diameter, which is due to the bending force term and hydrodynamic interactions. Seils and Pecora also showed that for the c2311 DNA σ does not significantly change in the range of ionic strengths used and thus the same proportionality holds approximately for the Soda–Berg model. The proprotionality constant was found from the measured D_0 and Γ_1 and a calculated R_g value at 0.2 M salt. This calculated value was determined by substituting the persistence length a (taken as 45 nm) and the known contour length L for the 2311 bp DNA of 799.2 nm and using the standard relation for a wormlike ring (near the flexible limit)[20]

$$R_g^{\,2} = \frac{a^2}{2}\left(\frac{L}{3a} - 1 + 2\left(\frac{a}{L}\right) - 2\frac{1 - \exp\left(-\frac{L}{a}\right)}{\left(\frac{L}{a}\right)^2}\right) \tag{2}$$

The 45 nm persistence length value was found by Seils and Pecora[5] to give good agreement between the measured D_0 at 0.2 M salt and that predicted by the Fujii–Yamakawa model for a wormlike cylinder with a diameter of 2.0–2.6 nm. At 0.2 M salt the value obtained from eq 2 is 71 nm. Values for R_g at other salt concentrations were obtained using the measured translational diffusion coefficient and internal mode relaxation time and the scaling relation contained in eq 1. The R_g values obtained from this procedure, shown in Figure 4, increase steadily as the salt concentration is decreased from 1 to 0.02 M; there is a possible indication of a plateau as the salt is further decreased to 0.001 M salt.

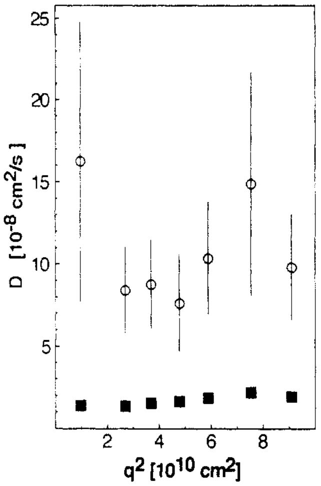

Figure 5. Slow and fast diffusion coefficients determined by CONTIN as a function of q^2 for a solution of 160 μg/mL monodisperse circular DNA in 1 mM Tris, 1 mM EDTA, pH 7.6. The larger scatter in the faster D values is probably due to the much smaller relative amplitudes of the faster mode.

As was already mentioned, in TE buffer at all salt concentrations studied, no additional modes were detected. We find no evidence for a slow mode at the fairly low DNA concentrations used even with no added NaCl. In a more limited series of measurements, we studied the frequency spectrum of 160 μg/mL circular DNA in a buffer of 1 mM Tris·HCl, 1 mM EDTA, pH 7.6. These results are shown in Figure 5. In this case, when the data were analyzed using CONTIN, two peaks were obtained, both of which scaled with q^2, indicating diffusive behavior. The major peak, with relative amplitudes of 85–90%, was about a factor of 2 slower than the normal self-diffusion coefficient measured at higher salts. The second peak (relative amplitude 10–15%) also appears to scale with q^2, although there is more scatter in these data, and is some 3–4 times faster than at 10 mM Tris. An even faster peak, which seems to correspond to the peak attributed to an internal mode for the samples measured in 10 mM Tris, was also present in very small amounts. The scatter seen in the second peak amplitude is probably due to the problem CONTIN has placing peaks with small relative amplitudes. The relative amplitude of the faster peak appears to increase with the magnitude of q, ranging from approximately 5% at an angle of 33° to approximately 15% at an angle of 123°. Samples were run with DNA concentrations as low as 25 μg/mL. Other than the expected increase in noise, no effects from dilution were seen.

Since Lin, Lee, and Schurr[6] first reported that the apparent diffusion coefficient measured by DLS for polylysine dropped in value by over an order of magnitude at a precise concentration of salt, the issue of a slow or extraordinary mode in very low ionic strength polyelectrolyte systems has created much controversy. Slow modes have been observed in DNA samples by several workers.[7–11,21] Previous reports of slow modes in DNA have generally indicated that the slow mode appears below about 0.01 M NaCl in a buffer with typically 1 mM Tris. A number of theories have been proposed for the existence of slow modes (reviewed in Ferrari and Bloomfield[11]), but none have yet been accepted as complete. It is currently thought that the slow mode is the result of the formation of multichain domains in solution. Schmitz et al.[22] first proposed the existence of "temporal aggregates" resulting from fluctuating forces due to counterion dynamics. Recently, it has been reported that the slow mode is caused by entanglements or impurities that are "removable" by proper filtration.[23-25] Schmitz,[26] however, has argued that the "filterable impurity or aggregate" model is not in accord with experimental data. Furthermore, Schmitz and Singh[27] present results of a study using bovine serum albumin (BSA), which, being a globular protein, is not expected to form entanglements. A slow mode was observed in this system. In another recent study, Sedlak[28] filtered a charge neutral solution of PMA through 0.01 μm pore filters. The neutral samples showed no evidence for a slow mode, but upon the addition of filtered, pure NaOH solution, a slow mode was evident. These experiments appear to be inconsistent with the entanglement or impurity theory.

Acknowledgment. This work was supported by National Science Foundation Grants CHE-9119676 to R.P. and DMB-8905906 to J.N. and by the NSF-MRL program through the Center for Materials Research at Stanford University. We are grateful to J. Seils and A. Patkowski for helpful discussions.

References and Notes

(1) Lewis, R. J.; Huang, J. H.; Pecora, R. *Macromolecules* **1985**, *18*, 944.
(2) Seils, J.; Pecora, R. *Macromolecules*, submitted.
(3) Sorlie, S. S.; Pecora, R. *Macromolecules* **1988**, *21*, 1437.
(4) Sorlie, S. S.; Pecora, R. *Macromolecules* **1990**, *23*, 487.
(5) Seils, J.; Pecora, R. *Macromolecules* **1992**, *25*, 354.
(6) Lin, S. C.; Lee, W. I.; Schurr, J. M. *Biopolymers* **1978**, *17*, 1041.
(7) Fulmer, A. W.; Benbaset, J. A.; Bloomfield, V. A. *Biopolymers* **1981**, *20*, 1147.
(8) Schmitz, K. S.; Lu, M. *Biopolymers* **1984**, *23*, 797.
(9) Nicolai, T.; Mandel, M. *Macromolecules* **1989**, *22*, 2348.
(10) Wang, L.; Garner, M. M.; Yu, H. *Macromolecules* **1991**, *24*, 2368.
(11) Ferrari, M. E.; Bloomfield, V. A. *Macromolecules* **1992**, *25*, 5266.
(12) Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning, A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1982.
(13) Tracy, M. A. Ph.D. Thesis, Stanford University, 1992.
(14) Koppel, D. E. *J. Chem. Phys.* **1972**, *57*, 4814.
(15) Provencher, S. W. *Comput. Phys. Commun.* **1982**, *27*, 213, 229.
(16) Seils, J.; Pecora, R. *Macromolecules* **1992**, *25*, 350.
(17) Berg, O. G. *Biopolymers* **1979**, *18*, 2861.
(18) Soda, K. *Macromolecules* **1984**, *17*, 2365.
(19) Huber, K.; Stockmayer, W. H.; Soda, K. *Polymer* **1990**, *31*, 1811.
(20) Shimada, J.; Yamakawa, H. *Biopolymers* **1988**, *27*, 657.
(21) Goinga, H. Tj.; Pecora, R. *Macromolecules* **1991**, *24*, 6128.
(22) Schmitz, K. S.; Lu, M.; Gauntt, J. *J. Chem. Phys.* **1983**, *78*, 5059.
(23) Ghosh, S.; Li, X.; Reed, W. F. *Biopolymers* **1991**, *30*, 1101.
(24) Li, X.; Reed, W. F. *J. Chem. Phys.* **1991**, *24*, 6189.
(25) Peitzsch, R. M.; Burt, M. J.; Reed, W. F. *Macromolecules* **1992**, *25*, 806.
(26) Schmitz, K. S. *Biopolymers* **1993**, *33*, 953.
(27) Schmitz, K. S.; Singh, N. *Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.)* **1993**, *35*, 1050.
(28) Sedlak, M. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1993**, *35*, 1044.