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Salt effects on internal motions of superhelical and linear pUC8 DNA

Dynamic light scattering studies

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The plasmid pUC8 (2717 bp) has been studied in its native superhelical and *Eco*RI-linearized forms by dynamic light scattering at NaCl concentrations from 1.1 mM to 1 M. The data were analyzed using the biexponential model for the dynamic structure factor described by us in a previous paper (J. Langowski, U. Giesen and C. Lehmann, Biophys. Chem. 25 (1986) 191). As before, we could identify two decay components corresponding to the center-of-mass diffusion and to internal motions of the DNA, where the fast component could be identified as a rotational diffusion contribution in the case for superhelical, but not for linear DNA. We found that the conformation of superhelical pUC8 is not affected by changing the ionic strength, while the amplitude of the internal relaxation increases approx. 2-fold when [NaCl] is raised from 1.1 mM to 1 M. The linearized DNA shows an increase of the diffusion coefficient with ionic strength which is, however, not quite as pronounced as that found by others (Z. Kam, N. Borochov and H. Eisenberg, Biopolymers 20 (1981) 2671), and, together with the unchanged conformation of the superhelical DNA, suggests a persistence length which is not strongly dependent on ionic strength. In contrast to the increasing amplitude of internal relaxation for the superhelical DNA, this amplitude remains constant or decreases slightly for linear DNA on going from 1.1 mM to 1 M salt. Our findings are further discussed with respect to possible models of the interwound form of superhelical DNA.

1. Introduction

As for any polyelectrolyte, the solution configuration of DNA depends strongly on the ionic environment. Electrostatic effects on DNA conformation and dynamics play an important role in maintaining the shape of DNA in its native environment and have therefore been the subject of numerous recent investigations; we refer readers to a review by Schurr and Schmitz [1] for an overview on the subject. Controversies persist regarding such fundamental questions as to how the persistence length of DNA depends on ionic strength, how superhelix configuration changes with salt concentration, and what role such changes may play for the DNA in order to maintain its biological function.

The objective of the study described here is to exploit further the observations we made previously [2] on the dynamics of superhelical and linearized pUC8 DNA, where we could extract four fundamental parameters from a biexponential analysis of the dynamic light scattering (DLS) autocorrelation functions of DNA solutions. At low scattering vectors K ($K = 4\pi n/\lambda \sin(\theta/2)$, where n is the refractive index of the solution, λ the wavelength and θ the scattering vector), we measured the translational diffusion coefficient, and a relaxation time that was K-independent. The latter was tentatively assigned to rotational

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diffusion of the plasmid as a whole. At high K, we found that the fast relaxation time became proportional to K^2 and could therefore be expressed as an 'internal diffusion coefficient'; its amplitude increased with K and was substantially larger for the linear than for the supercoiled DNA.

We thus proceeded to characterize the dependence of those parameters: the translational diffusion coefficient D_t, rotational contribution τ_{rot}, internal diffusion coefficient D_i and amplitude of internal relaxation a_2 as a function of ionic strength by varying the Na^+ concentration between 1.1 mM and 1 M.

2. Materials and Methods

pUC8 plasmid DNA was prepared from *E. coli* HB101 harboring the plasmid. Cells were grown at 37 °C under aeration in 10 l LB medium (10 g/l tryptone, 10 g/l yeast extract, 5 g/l NaCl) containing 50 μg/ml ampicillin to a cell density of $A_{550} = 1.0$–1.5. 150 μg/ml chloramphenicol was then added for amplification and the incubation continued for another 15 h. The cells were harvested by centrifugation and resuspended in 200 ml TES buffer (50 mM Tris-HCl (pH 8.0), 50 mM EDTA, 15% sucrose). 0.5 g lysozyme (Boehringer, Mannheim) were then added and the mixture incubated for 30 min at room temperature, thereafter the cells being completely lysed by addition of 10% (v/v) of 3 M sodium acetate solution and 5% (v/v) of 10% SDS solution. The lysate was centrifuged for 60 min at 35 000 rpm in a Beckman Ti 45 rotor. The supernatant was incubated for 30 min at 37 °C with 100 μg/ml of an RNAse A solution which had previously been incubated at 100 °C for 10 min to destroy DNAse. Subsequently, proteinase K (Boehringer) was added to 10 μg/ml and the incubation continued for another 30 min. Crude DNA was precipitated by adding NaCl to 0.5 M and 20% (v/v) of a 50% solution of polyethylene glycol 10 000 (PEG) (Merck, Darmstadt) and keeping the solution on ice for 60 min. The precipitate was collected by centrifugation, washed with 80% ethanol, and redissolved in 20 ml TE buffer (50 mM Tris-HCl (pH 8.0), 50 mM EDTA). This solution was extracted twice with phenol equilibrated in TE buffer, and three times with chloroform. NaCl was again added to 0.5 M and the polyethylene glycol precipitation repeated as above. The final precipitate was almost pure plasmid DNA with 80–90% in the superhelical form and only traces of chromosomal DNA, as judged by 1% agarose gel electrophoresis. On the gels, no small RNA fragments could be detected by ethidium fluorescence at the position expected, viz., approximately twice the mobility of the bromophenol blue band. This prepurified plasmid was then further purified in a CsCl/ethidium density gradient by adding 0.954 g CsCl and 0.1 ml of 10 mg/ml ethidium bromide solution per ml plasmid solution and centrifuging in a Beckman VTi50 rotor for 48 h at 36 000 rpm. The lower fluorescent plasmid band was collected under ultraviolet illumination (wavelength, 330 nm) by puncturing the tube with a syringe needle and the plasmid solution diluted 1 : 2 (v/v) with water. 2 vols. ethanol were then added and the solution kept at −80 °C for 60 min. The DNA precipitate was collected by centrifugation, washed with ethanol, dried, resuspended in TE buffer and re-precipitated twice with ethanol from 0.3 M sodium acetate to remove traces of CsCl. The CsCl gradient centrifugation was repeated if the DNA was not sufficiently pure (> 95% superhelical). After the last precipitation, the DNA was resuspended in water; we then added buffer stock solution as needed to 1 mM sodium phosphate (pH 7.0), 0.1 mM Na-EDTA, and NaCl for $[Na^+]$ 1.1 mM to 1 M.

pUC8 DNA was linearized by incubation with restriction endonuclease *Eco*RI (Boehringer, Mannheim) in 0.1 M NaCl, 0.01 M Tris-HCl (pH 7.5), 0.02 M $MgCl_2$, checking that digestion was complete by electrophoresis on 1% agarose gels. After digestion, the DNA solution was extracted with phenol to remove the enzyme, the plasmid precipitated with ethanol and adjusted to the experimental buffer conditions above.

To ensure that no protein contamination was present, we measured the A_{260}/A_{280} ratio which, for all DNA solutions, was 2.0 or higher.

For photon correlation spectroscopy experiments, DNA solutions were prepared in 15-mm diameter cylindrical cuvettes at concentrations of

75–100 μg/ml. Filtering of solutions has been described before [2]; after the experiments, which took approx. 1–2 days each at room temperature (22 ± 0.2 °C), the DNA samples were checked for integrity by electrophoresis on 1% agarose gels. No degradation could be detected; superhelical samples contained a maximum of 10% of the open circular form after the experiment.

Light scattering was carried out using an Ar^+ laser operating at 400–600 mW at 488 nm, an Amtec goniometer and a Malvern K7023 single-bit correlator with 88 + 4 delay channels. The data accumulation was performed in two different ways: for the majority of runs, we collected for each scattering vector 8–10 autocorrelation functions at a count rate of approx. 10^5 s^{-1} with 4–6 min collection time, using two different sampling times. Then, only those measurements were used for the final analysis in which the relative difference between calculated total squared intensity (from the total count rate) and measured total squared intensity (from the delay channels) was less than 0.005. To the accumulated measurements, the function

$$G^{(2)}(\tau) = A + \left[\sum_{i=1}^{N} a_i e^{(\lambda_i \tau)}\right]^2 \quad (1)$$

was fitted with the number of independent exponential decay components $N = 2$ or 3. The data permitted a stable reproducible fit with three exponential components, varying all parameters. However, since going from two to three components did not result in any improvement in quality of the fit, and for the reason mentioned earlier [2] that an inverse Laplace transform on selected sets of data showed a bimodal distribution, we used the biexponential fit in our analysis.

2.1. Electronic dust filter

Some sets of data were taken using an alternate procedure implemented on an Apple Macintosh computer with a data collection routine written in Mach2™ Forth. We collected sets of autocorrelation functions over periods of 2 s, deciding for each individual data set as to whether to include it in the accumulation according to two criteria. First, the relative difference between the calculated and measured baselines was to be less than a particular level, usually 0.005; second, a threshold was set for the total counts accumulated during the 2 s period, and any function exceeding that threshold was discarded. Both thresholds were set in such a way that only obvious intensity 'spikes' due to occasional dust particles resulted in rejection of the data set. Data were taken by this procedure up to a total accumulation time of approx. 10–15 min. The calculated/measured baseline difference of the accumulated data was then always 0.0005 or lower. No difference was found in the analysis of autocorrelation functions taken with this 'software dust filter' and the method mentioned above, where each data set was checked after the accumulation, except for a slightly increased 'dust term' for the latter case (A in eq. 1); however, approx. 2–3-times less time was required to collect data of comparable signal-to-noise levels. A quantitative account of the dust elimination method will be published elsewhere (Langowski and Wolling, manuscript in preparation).

All diffusion coefficients reported here have been reduced to the standard state of 20 °C, corrected for buffer viscosity. Viscosities taken were those reported for NaCl solutions (CRC Handbook of Chemistry and Physics).

3. Results

3.1. Translational diffusion

The effect of changing the NaCl concentration on the translational diffusion coefficient of superhelical pUC8 plasmid DNA can be seen in fig. 1, where the slow relaxation time τ_1 from the biexponential fit has been converted to a diffusion coefficient $D_1 = (K^2\tau_1)^{-1}$ and plotted vs. K^2. D_1 increases slightly with K^2; this increase is the same for all ionic strengths within experimental error. Linear regression lines for D_1 vs. K^2 extrapolate for $K \to 0$ within an error of 0.1×10^{-12} m^2 s^{-1} to the same translational diffusion constant, $D_t = 4.9 \times 10^{-12}$ m^2 s^{-1}. The variation between these extrapolations is smaller than the

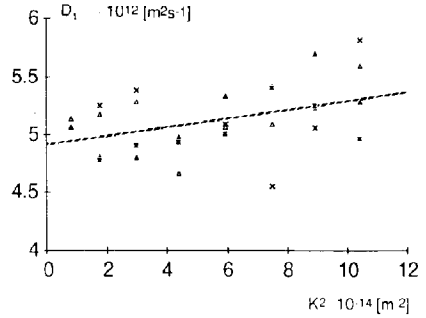

Fig. 1. Translational diffusion coefficient of superhelical pUC8 from the slow relaxing component of the autocorrelation function at 1.1 mM (*), 10 mM (△), 0.1 M (▲), and 1 M Na^+ (×). The dashed line represents the least-squares fit through all the data points; fits through the data sets at each individual salt concentration do not deviate significantly from this line (see text). The translational diffusion coefficient is obtained from an extrapolation of this line to $K^2 = 0$.

statistical error for each extrapolation, and the line shown in fig. 1 is therefore taken through all points simultaneously. We conclude that, between ionic strengths of 1 mM and 1 M NaCl, there is no large-scale conformational change of the superhelical form of pUC8, which would manifest itself as a change in the overall hydrodynamic friction factor.

The corresponding measurements on linearized pUC8, on the other hand, do extrapolate to different values of the translational diffusion constant

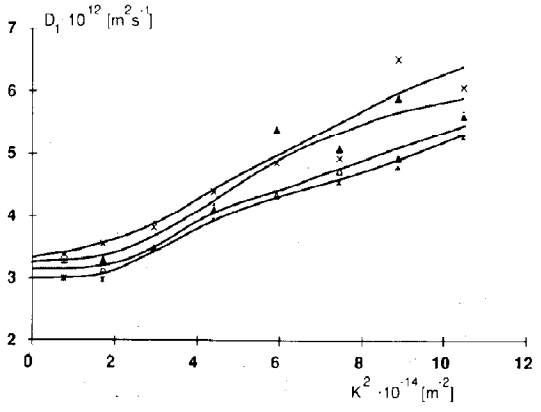

Fig. 2. Translational diffusion coefficient of linear pUC8 from the slow relaxing component of the autocorrelation function at 1.1 mM (*), 10 mM (△), 0.1 M (▲), and 1 M Na^+ (×).

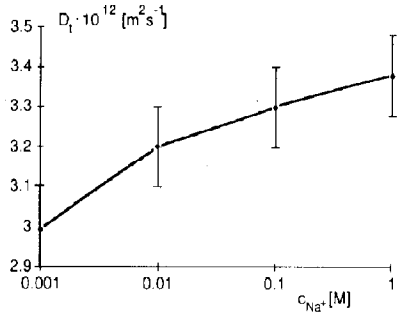

Fig. 3. Variation of the translational diffusion coefficient of linear pUC8 DNA with salt concentration. Points were obtained from an extrapolation of the data of fig. 2 to $K^2 = 0$.

D_t at different ionic strengths (fig. 2). Although the change is not very large, there is clearly a trend in the D_{app} values for the two smallest K values to increase with ionic strength. Since D_1 in this case increases much more strongly and nonlinearly with K^2 than for superhelical DNA, the curves were manually extrapolated to $K = 0$; we estimate the error to be of the order of 0.1×10^{-12} $m^2\ s^{-1}$. D_t for linear pUC8 DNA is plotted as a function of ionic strength in fig. 3. Its value increases from 3.0×10^{-12} $m^2\ s^{-1}$ at 1.1 mM Na^+ to 3.4×10^{-12} $m^2\ s^{-1}$ at 1 M Na^+.

An increase in D_t of linear DNA with increasing ionic strength has been reported for several other systems [3,4]. It corresponds to changes in excluded volume due to screening of electrostatic interactions and to changes in persistence length of the DNA [3].

As is evident from the increase of D_1 with K for the linear DNA, we can use the slow relaxation as a measure of the translational diffusion coefficient of linear DNA of this size only for rather low K ($K^2 < 2 \times 10^{14}$ m^{-2}). In this region, several theories of flexible polymer chains or semiflexible rods [6–11] as well as other empirical data [5,12] do in fact show a double-exponential form for the dynamic structure factor, with the slow relaxation corresponding to translational diffusion. For the superhelical form, the range over which D_t can be approximated by D_1 seems to be much larger.

Compared to the 'apparent diffusion coefficient' D_{app} from a force-fitted single-exponential

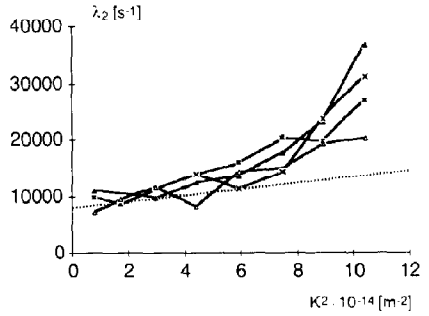

Fig. 4. Fast relaxation component of the autocorrelation function of superhelical pUC8. The rate is displayed as a function of the square of the scattering vector for 1.1 mM (*), 10 mM (▲), 0.1 M (△) and 1 M Na^+ (×). The straight line represents the expected behavior of a rigid rod with the same rotational diffusion coefficient as that of the superhelical DNA.

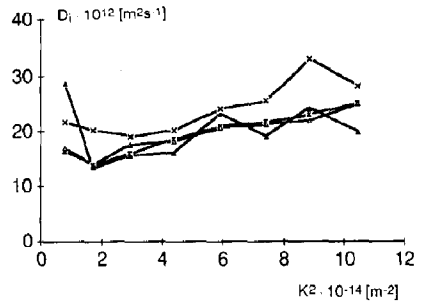

Fig. 6. Internal diffusion coefficient of linear pUC8 as a function of scattering vector and Na^+ concentration.

or first-cumulant fit to the intensity autocorrelation function, a biexponential fit with extrapolation of the slow relaxing component D_1 in any case leads to more reliable values of D_t, since the effects of internal motions act to increase D_1 only at rather high K compared to D_{app}.

3.2. Internal motions

Figs. 4 and 5 give the relaxation rates of the fast component $1/\tau_2 = \lambda_2$ of the biexponential fit as a function of K^2 and salt concentration for superhelical and linear pUC8. For low K and anisotropically shaped molecules, this component is K-independent and corresponds to the rotational relaxation rate of the molecule [6,11].

The λ_2 vs. K^2 plot for superhelical pUC8 shows a nonzero intercept at all ionic strengths. This indicates an anisotropic shape of the DNA and allows us to calculate its rotational relaxation time, which is the inverse of λ_2 extrapolated to $K^2 \rightarrow 0$. The position of the intercept does not vary with ionic strength beyond the limits of experimental error. The average rotational relaxation time calculated from the four curves is $\tau_r = 130\ \mu s$.

This constancy of τ_r of the superhelical form with ionic strength is also – like the data on the translational diffusion coefficients – in support of an ionic strength-independent solution shape of pUC8.

At higher K, we find that the relaxation rates for both linear and superhelical forms are similar regardless of ionic strength. This is reflected in our finding (figs. 6 and 8) that the internal diffusion coefficient D_i at any particular K^2 is constant for all samples investigated. We also find that for all ionic strengths the relaxation rates are nearly pro-

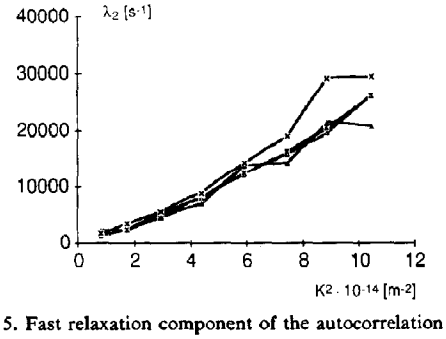

Fig. 5. Fast relaxation component of the autocorrelation function of linear pUC8. Symbols as in fig. 4.

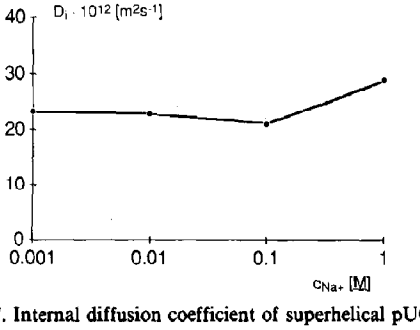

Fig. 7. Internal diffusion coefficient of superhelical pUC8 as a function of Na^+ concentration; values shown are averages of the four highest K values in fig. 6.

portional to K^2 above $K^2 = 4 \times 10^{14}$ m^{-2}, D_i being independent of the scattering vector in this region for superhelical DNA and increasing slightly for linear DNA.

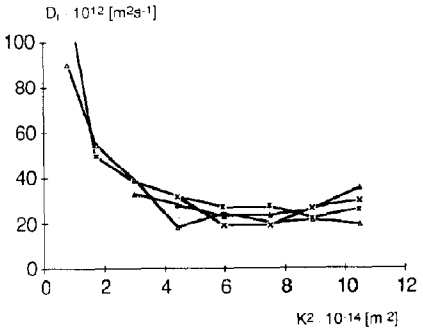

Fig. 8. Internal diffusion coefficient of superhelical pUC8 as a function of scattering vector and Na^+ concentration.

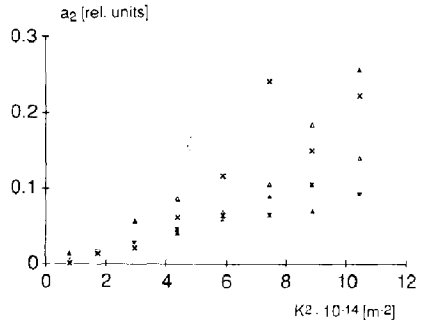

Fig. 9. Relative amplitudes of the fast relaxing component of supercoiled pUC8 DNA as a function of scattering vector at 1.1 mM (*), 10 mM (△), 0.1 M (▲), and 1 M Na^+ (×).

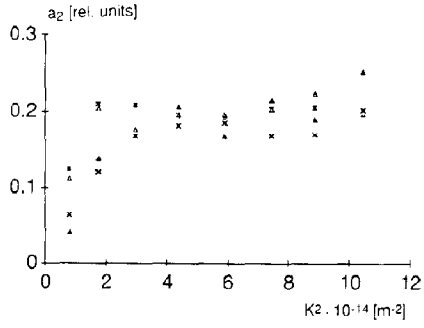

Fig. 10. Relative amplitudes of the fast relaxing component of linear pUC8 DNA as a function of scattering vector at 1.1 mM (*), 10 mM (△), 0.1 M (▲), and 1 M Na^+ (×).

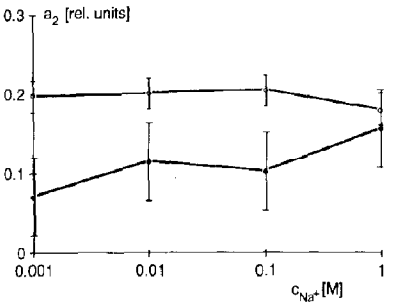

Fig. 11. Relative amplitudes of the fast relaxing component as a function of Na^+ concentration for superhelical (●) and linear (○) pUC8 DNA. Values displayed are the average of the four highest K values in figs. 9 and 10.

The ionic strength dependence of internal motions is reflected in the amplitude a_2 of the fast relaxation time. Figs. 9 and 10 show the relative amplitudes of the second component of the biexponential fit for superhelical and linear pUC8 as a function of salt concentration.

Taking the average of a_2 at the four highest K values as a measure of the amplitude of internal motions, we find an increase with salt concentration for the superhelical form and an almost constant value, decreasing slightly for the linear form. A decrease of the fast relaxation amplitude with ionic strength had been observed earlier by Caloin et al. [5], who interpreted it as resulting from an expansion of the linear worm-like coil at low ionic strength due to excluded volume effects.

4. Discussion

4.1. Superhelix structure

Our results lead to the conclusion that the overall solution shape of pUC8 DNA in its native superhelical form does not depend on salt concentration between 1.1 mM and 1 M NaCl. The DNA exists as an interwound superhelix; no transition between interwound and toroidal forms, as described for PM2 phage DNA [13], was found. The structure has a translational diffusion coefficient of 4.9×10^{-12} m^2 s^{-1} and a rotational relaxation time of 130 μs. The rotational relaxation

time equals that of a cylinder of length 220 nm [14], assuming an effective hydrodynamic radius of 1.75 nm, $\sqrt{2}$-times the hydrodynamic radius of the DNA.

A simple double-spiral model can be used to calculate the theoretical translational diffusion coefficient for a superhelical DNA. Two strands of half the contour length of pUC8 each, consisting of adjacent beads of radius 1.25 nm, are assumed to be wound around a cylinder of radius 4 nm (since the radius and DNA contour length are fixed, variation of the number of superhelical turns will change the total length of the model). The diffusion coefficient of such a structure, calculated according to the approximated Kirkwood formula with the corrections proposed by De Haën et al. [16], is 4.95×10^{-12} m^2 s^{-1} for 11 superhelical turns. Changing the radius of the structure will affect the number of superhelical turns at which our measured diffusion coefficient is matched by the model; since a priori it is not known how the difference in linking number of pUC8 is distributed between twist and writhe, it is difficult to make an exact estimate of the superhelix radius. However, referring to calculations by Benham [17], Le Bret [18], or Levine and Crothers [19], we may assume that the superhelical elastic energy is distributed about halfway between twist and writhe. Since the linking number difference of pUC8 is 18 in its native state, the assumption of 11 superhelical 'writhe' turns and a radius of 4 nm is probably not far from the truth. The parameter which critically determines the translational friction factor is the total length of the model structure, which is approx. 350 nm for the diffusion coefficient measured, very much independent of the assumed radius.

The rotational diffusion coefficient would be in agreement with an end-to-end distance which is shorter by a factor of 1.6. A tentative explanation for this discrepancy is as follows. We have argued in a previous paper [20] that the ratio of contour length to persistence length of an interwound pUC8 structure is very similar to that of a 587 bp linear DNA (assuming that the overall bending rigidity of the interwound structure is twice that of a linear DNA double strand). Therefore, the rotational dynamics of pUC8 may be described – after appropriate scaling – by the correlation function that has been determined empirically by Elias and Eden [21] for 587 bp DNA.

In the above mentioned paper [20], the slowest rotational relaxation time for pUC8 was estimated to be 240 μs, which was in agreement with the NMR data. Other, faster components contribute to the tumbling correlation function, and will contribute to the rotational part of the dynamic structure factor at low $\boldsymbol{K}$ [21], therefore making the observed rotational relaxation time faster than that expected for a rigid structure. The measured value of 130 μs would then be a weighted average over the contributions from the slowest tumbling time and the empirical faster terms (eq. H1 in [20]), which cannot be determined separately from our data.

We may conclude that the order-of-magnitude agreement between the observed rotational contribution to the dynamic structure factor and τ_{rot} expected for a 350 nm long rigid cylinder supports the general model of an interwound structure of the superhelical form of pUC8.

4.2. Ionic strength effects on the structure of pUC8 linear DNA

The diffusion coefficient of linear pUC8 DNA increases from 3.0×10^{-12} m^2 s^{-1} at 1.1 mM to 3.4×10^{-12} m^2 s^{-1} at 1 M Na^+. Although the errors in the diffusion coefficients at each ionic strength are of the order of 0.1×10^{-12} m^2 s^{-1}, the increase is significant. Kam et al. [3] reported for linearized ColE1 DNA a diffusion coefficient that increased with ionic strength at low salt concentrations and changed only slightly at higher salt concentrations. Soda and Wada [22] found no significant change in $D_{25,w}$ of the same DNA when [Na^+] was varied between 0.15 and 1.5 M. Results obtained by other groups [5,23] seem to indicate that their DNA samples show a slight change of persistence length with ionic strength above 0.01 M [Na^+ +] and a stronger dependence below that concentration.

Assuming a Gaussian chain (to a first approximation), the hydrodynamic radius of the DNA will vary according to the square root of the persistence length. Taking 50 nm for the per-

sistence length of DNA at 0.1 M salt, a value which is generally accepted [24], we obtain 61, 53 and 47 nm for the persistence lengths at 1.1 mM, 0.01 M and 1 M Na^+, respectively, from our measured diffusion coefficients. These values agree with recent magnetic birefringence measurements made by Maret and Weill (fig. 5 of ref. 24) as well as with data of several other groups reviewed in the same paper.

Although the existing work on the ionic strength dependence of the persistence length of DNA agrees on the qualitative fact that the dependence is quite large below 0.01 M and small above 0.01 M salt [24], quantitative discrepancies still persist between the various results referenced (see fig. 5 of ref. 24 for an overview). At present, we have no straightforward explanation for such differences. In the case of superhelical DNA, there is evidence for metastable conformational states of the DNA which vary in their static and dynamic properties [25]; one might find similar behavior in linear DNAs, but any such explanation can only be very speculative for the time being.

4.3. Ionic strength effects on the internal dynamics of pUC8 DNA at higher $\boldsymbol{K}$

The dependence on ionic strength of the relaxation amplitudes for the linearized form of pUC8 is qualitatively similar to that observed earlier by Caloin et al. [5] for chicken erythrocyte DNA. A slight decrease of approx. 10% in the amplitude of internal relaxation is seen when the ionic strength is changed from 1.1 mM to 1 M (figs. 10 and 11). In ref. 5 the decrease in internal relaxation amplitude with increasing ionic strength was assumed to be due to overall shrinking of the polymer coil; in fact, on a scale of KR_g the data could be represented by one curve independent of ionic strength. Although we are not trying to show a quantitative agreement, we note that our measurements show the same qualitative behavior. Therefore, we may conclude that, similar to the findings in ref. 5, the dynamics of linear DNA do not vary greatly with ionic strength.

For either form of the DNA, the internal relaxation rate at high $\boldsymbol{K}$ is independent of ionic strength but proportional to the square of the scattering vector and can therefore be expressed as an internal diffusion coefficient. This diffusion coefficient reflects internal motion of sub-segments of the superhelical structure with respect to each other; its average is $D_i = 2.2 \pm 0.2 \times 10^{-11}$ $m^2\ s^{-1}$. We note that a rigid DNA segment of length 50 nm would have a diffusion coefficient of 2.5×10^{-11} $m^2\ s^{-1}$; taking into account that DNA is already flexible on this length scale, we may conclude as before [2] that the internal diffusion coefficient describes a diffusion process of segments of the order of 50–100 nm (1–2 persistence lengths) within the DNA coil. A changing amplitude of the fast relaxation would then correspond to a change in the amplitudes of these segmental motions.

The amplitude of internal relaxation of supercoiled pUC8 behaves very differently from that of the linear form (figs. 9 and 11). We note first that the amplitudes are lower in general, and increase much more smoothly with $\boldsymbol{K}$ than for linear DNA, where the increase is steepest in the $0 \leq \boldsymbol{K}^2 \leq 2 \times 10^{14}$ m^{-2} region and then reaches a plateau. Changing from 1.1 mM to 1 M Na^+ increases the relative contribution of the fast component to the total relaxation amplitude by a factor of 2. This change is rather smooth (fig. 9) and therefore does not indicate a sudden structural transition at a particular salt concentration, as was found for PM2 DNA [13].

Even though the overall solution shape of superhelical pUC8 seems to be independent of ionic strength, screening of electrostatic repulsions seems to increase local segmental motion. Since there exists no theory on the dynamic structure factor for a closed circular interwound superhelical DNA – even the static structure being in fact far from well-characterized – our explanation for this effect may only be very tentative. A possibility to explain our findings would be that the balance between internal tension and electrostatic repulsion of opposing double strands of the superhelical DNA leads to a structure which is very rigid against lateral motion of opposing DNA segments, while for higher ionic strengths the segmental mobility perpendicular to the superhelix axis increases because of the lower repulsion.

The superhelical diameter of 8 nm that was

assumed above is of the same order of magnitude as the Debye screening lengths for the ionic strengths employed in our measurements. Fluctuations in those parts of the superhelix, which are wound more tightly than others contribute almost certainly to the internal relaxation that we observe; it is very plausible that such fluctuations would occur much more frequently in a structure having less repulsion between the phosphates.

Acknowledgments

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