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ORIENTATION RELAXATION OF DNA RESTRICTION FRAGMENTS AND THE INTERNAL MOBILITY OF THE DOUBLE HELIX

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The orientation relaxation of 15 DNA restriction fragments (43–4361 base-pairs) is characterized by measurements of linear dichroism using high electric field pulses. The off-field relaxation of fragments of 84 base-pairs or less can be described by single exponentials, which are related to the transverse rotational diffusion of the helix. Fragments of 95 base-pairs or greater exhibit an additional fast component with time constants around 100 ns for fragments of approx. 100 base-pairs, increasing with chain length to about 700 ns for a fragment with 258 base-pairs. The amplitude of this process increases from virtually zero at low fields (≈ 10 kV) to a substantial limit contribution at high fields. According to these results, we suggest that electric fields induce stretching of the DNA fragments from a weakly bent to a more straight form and that the fast component reflects the internal mobility of the DNA chain. The slow off-field components of the orientation are discussed in terms of different models. The data up to helix lengths of about 400 base-pairs can be described by the 'weakly bending rod' model from Hearst using 3.4 Å rise per base-pair and 13 Å axial radius of the helix. Both the weakly bending rod according to Hearst and the 'wormlike chain' according to Hagerman and Zimm provide a persistence length of 500 Å. The on-field relaxation is slower than the corresponding off-field process at low field strengths, but the on-field process is accelerated substantially at high electric fields. These observations are compared with model calculations of Schwarz.

1. Introduction

Orientation relaxation curves observed in field pulse experiments may provide detailed information about the structure of macromolecules [1,2]. The analysis of orientation processes induced by field pulses is particularly useful in the case of DNA molecules, since orientation effects can be observed with extremely high sensitivity due to the high polarizability and large optical anisotropy of DNA. The interpretation of the experimental data has been very difficult in the past, because only DNA samples with a relatively broad distribution of chain lengths were available. Now this problem can be avoided by using restriction fragments of defined chain lengths. Recently, several groups have investigated the orientation relaxation of DNA restriction fragments by birefringence measurements [3–5]. We have performed a corresponding investigation by linear dichroism. The methods are complementary and the time constants obtained should be identical. However, the linear dichroism is more appropriate for our purpose, since the solvent does not contribute to the measured signal. In the case of birefringence, the contribution of water to the measured signal may be relatively large [4] and thus the characterization of fast components in the orientation curve is more difficult. The linear dichroism detection has been used previously for the analysis of restriction fragments by Hogan et al. [6] but these authors could only measure the relaxation in the presence of the electric field, which has a strong influence on the time constants.

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We have analyzed our orientation curves by numerical procedures developed for the characterization of chemical relaxation curves. These procedures have been used to determine the number of exponentials, their time constants and amplitudes to a high degree of accuracy. The analysis has been performed over a wide range of chain lengths both for the 'in'- and 'out'-field relaxation. Since the various models for the orientation relaxation of DNA fragments have been reviewed in previous investigations [3–5], we will not discuss the basis of these models again and we simply present our results in terms of some selected models.

2. Materials and methods

The preparation of the DNA fragments has been described previously [7]. The orientation of the fragments was measured in the standard buffer 1 mM NaCl, 0.2 mM EDTA, 1 mM sodium cacodylate, pH 7.1, at 21°C, unless specified otherwise.

The field jump technique has also been described previously [8,9]. The rise and decay times of the electric field were about 10 ns, i.e., more than a factor of 10 below the fastest recorded orientation relaxation time. The rise time of the optical detection system was measured with the aid of a fast photodiode as a light source. Most of the orientation data were recorded by a detection system with a rise time of 145 ns.

The data were initially stored on a transient recorder (DL 920 from Datalab, 50 ns sample time, 8-bit amplitude resolution, 4 Kb storage) and then transfered to the Univac 1108 of the Gesellschaft für Wissenschaftliche Datenverarbeitung mbH (Göttingen), where all calculations have been carried out. Our orientation curves have been partly 'convoluted' by passing the measured signal through a detection system with a limited rise time [10]. Due to the convolution an orientation curve with m components

$$A(t)=\sum_{k=1}^{m} A_K(1-e^{-t/\tau_K}) \tag{1}$$

is measured in the form

$$A'(t)=\sum_{k=1}^{m} A_K\left(1+\frac{\tau_K}{\tau_D-\tau_K}\cdot e^{-t/\tau_K}-\frac{\tau_D}{\tau_D-\tau_K}\cdot e^{-t/\tau_D}\right) \tag{2}$$

where τ_D is the (exponential) rise time of the detector. When τ_D is equal to τ_K the terms in brackets is replaced [10] by $(1-(1+t/\tau_K)e^{-t/\tau_K})$. For m components $2m+1$ parameters have to be fitted: m relaxation times $+\ m$ amplitudes and the baseline. The optimal parameters are calculated according to the 'least squares' using either a simplex [11] or a quasi-Newton method. The decision on the number m of components contributing to the observed curve is mainly based on the magnitude of the error sum or the signal-to-noise ratio, but also on the plot of residuals and the autocorrelation of residuals. The signal-to-noise ratio S is defined by

$$S=y_{\max}/\sqrt{\frac{\sum_{i=1}^{n}(f(t_i)-y_i)^2}{(n-\nu)}} \tag{3}$$

where $y_{\max}$ is the maximum amplitude of the theoretical function, $f(t_i)$ the theoretical function at time t_i, y_i the measured value at time t_i, n the number of measured points and ν the number of fitted parameters.

3. Results and discussion

3.1. Assignment of rotation relaxation processes

In order to avoid repetition we will not describe the tests again that have already been reported in previous papers [3–5]. For example, it will be sufficient to mention that our data were obtained at DNA concentrations (≈ 20 μM phosphate residues) where the orientation curves are not dependent upon DNA concentration. We have checked by measurements at the magic angle that under our conditions, the electric field pulses did not induce denaturation of the double helices. Furthermore, we did not find any evidence for photodamage in the DNA fragments induced by our measurements.

The critical step in the present investigation is the assignment of time constants. Since the number of time constants and their magnitude is not only dependent upon the fragment length but also for a given fragment upon the conditions of the pulse, we will first describe our analysis and its results in detail for a given fragment having 194 base-pairs.

At low field strengths the off-field relaxation can be described with high accuracy by a single exponential. With increasing field strength E an additional, fast component appears. The appearance of the second component is indicated by the fact that the signal-to-noise ratio of the fit is increased by the addition of a second exponential. The amplitude of the fast process grows continuously from essentially zero at low E to a limit percentage of about 40% relative to the total amplitude at high E (cf. fig. 1). The time constants of both off-field processes do not depend upon E, whereas the time constant of the on-field process strongly decreases with increasing field strength (cf. fig. 2). The on-field process can be described by a single exponential up to the highest field strengths.

When the orientation curves obtained for our short fragments from 43 to 84 base-pairs were subjected to a corresponding analysis, we did not find evidence for the existence of two exponentials in the off-field relaxation. Both on- and off-field relaxation could be described by single exponentials with high accuracy and fits with two exponentials did not increase the signal-to-noise ratio.

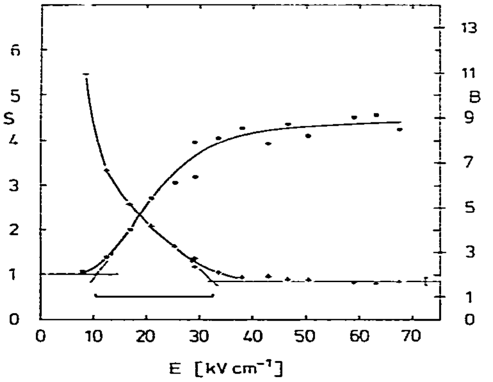

Fig. 1. Signal-to-noise ratio S of fits with two exponentials relative to fits with one exponential as a function of the field strength (●, left scale); the amplitude B of the slow process relative to that of the fast process is also shown (+, right scale).

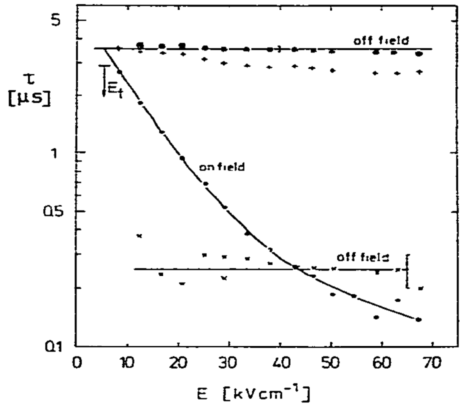

Fig. 2. Relaxation time constants as a function of the field strength for a 194 base-pair fragment measured at different field strengths. Off-field relaxation: fit with one (+) and with two exponentials: fast (×) and slow (∗) process. On field relaxation (●).

The shortest fragment, which under our conditions exhibits the additional fast process in the off-field relaxation, has 95 base-pairs. In this case the fast component becomes apparent at field strengths above 20 kV/cm. With increasing chain length the field strength E_α required for the observation of the fast component decreases. This is illustrated in fig. 3. The amplitude of the fast component increases with field strength and ap-

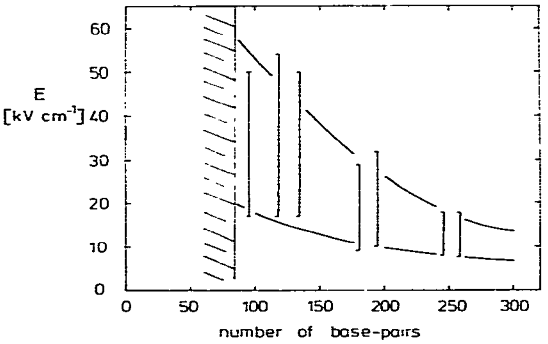

Fig. 3. Appearance field range for the fast off-field process (cf. text): lower line indicates limit field strength, which has to be exceeded for the observation of the fast process; upper line indicates field strength, where the fast amplitude approaches its limit relative contribution.

proaches a constant level at field strengths higher than a limit value E_w. The magnitude of E_w also decreases with increasing chain length. The values of E_α and E_w as a function of chain length define a 'range of appearance' for the fast component. According to the data shown in fig. 3, it should be expected that the fast component is also present in our fragments with less than 95 base-pairs. However, we are not able to trace this component in the short fragments, because the relative amplitude of the fast component decreases with decreasing chain length (cf. fig. 4). Furthermore, the total amplitude of the orientation curves also decreases with decreasing chain length, which results of course in a decrease in the overall signal-to-noise ratio.

The number of individual components detected in our off-field relaxation curves becomes more than two for chain lengths of 603 base-pairs or greater and more than three for our longest fragment with 4361 base-pairs. For these long chains it is open to question whether it is reasonable at all to assign individual components. Probably the orientation relaxation of these long chains should be described by a continuum of relaxation 'components'. Due to the uncertainty in the assignment of the longest component for the long fragments, these values are not listed.

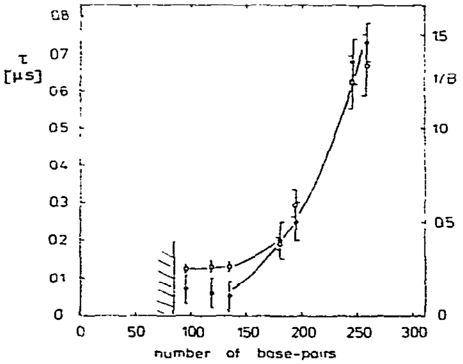

Fig. 4. Relaxation time τ_f (●) and amplitude $1/B$ (○) of the fast relative to the slow off-field process versus the number of base-pairs. $1/B$ is given at high field strengths, where $1/B$ approaches a limit value. τ_f is independent of the field strength: presented are mean values of about 15 measurements at different field strengths.

Due to experimental conditions the on-field relaxation could not be characterized with the same precision as the off-field process. For all the fragments and field strengths the on-field relaxation could be described by single exponentials, except for the two longest fragments with 1450 and 4361 base-pairs, showing a second on-field component at high field strengths, which appears to be independent of the field strength.

3.2. *Interpretation of slow components in terms of hydrodynamic models*

The slow off-field relaxation process is associated with the rotational diffusion of the long axis of the molecule. The time constant of the rotation is related to the diffusion constant D by

$$\tau = 1/(6D) \tag{4}$$

Several authors have developed theories for the rotational motion. According to Broersma [12,13] the diffusion coefficient of a rigid rod is given by

$$D_R = (3kT/\pi\eta_0 L^3)\{\ln(L/r) - 1.45 + 7.5[1/\ln(L/r) - 0.27]^2\} \tag{5}$$

where kT is the thermal energy, η_0 the solvent viscosity, L the length and r the radius of the rod. For the case of a weakly bending rod, Hearst [14] calculated the following diffusion coefficient

$$D_{BR} = \frac{kT}{\eta_0\pi\cdot L^3}[3\ln(L/b) - 7 + 4(b/a) + \lambda L\{2.25\ln(L/b) - 6.66 + 2(b/a)\}] \tag{6}$$

where $1/\lambda$ is the statistical length corresponding to twice the persistence length, b the distance between frictional elements, a the Stokes' diameter of each element and L the contour length.

According to Hearst [14], the diffusion constant of a wormlike coil is given by

$$D_{WC} = \frac{kT\lambda}{\eta_0\cdot L^2}[0.716(\lambda L)^{1/2} - 3.288 + 1.150\{(b/a) - \ln(b\cdot\lambda)\}] \tag{7}$$

It is possible, of course, to fit the various parameters of these models to the experimental data. For example, we may fit the rise per base-pair and the axial radius according to the Broersma equation. From a fit of our data obtained in the range 43–134 base-pairs we obtain 3.2 Å rise and 11 Å radius for the double helix. However, in view of the approximations inherent in this procedure the fit parameters cannot be reliable. Thus, we decided to introduce those parameters which are known for B-DNA from the X-ray analysis (cf. also data from sedimentation measurements [15]): rise per base-pair 3.4 Å and axial radius 13 Å. With these parameters the orientation times calculated according to Chen et al. [12] using the formalism of Broersma are clearly larger than the measured values for all chain lengths. The orientation times calculated by eq. 4 with the numbers originally deduced by Broersma [13] are even slightly larger than those calculated according to Chen et al. [12]. When we apply the weakly bending rod model, we get an accurate representation of our experimental data with a persistence length of 500 Å (cf. fig. 5). For comparison we have also included in fig. 4 the chain length dependence according to the wormlike coil model. This model is expected to be valid for chain lengths greater than 650 base-pairs. In the range from 430 to 600 base-pairs, we see already increasing deviations of our experimental data from the values expected from the weakly bending rod. According to the trend of our data the wormlike coil model may be more appropriate for fragments greater than 650 base-pairs. Due to the uncertainty in the experimental data for the long fragments we did not pursue any detailed studies on this point.

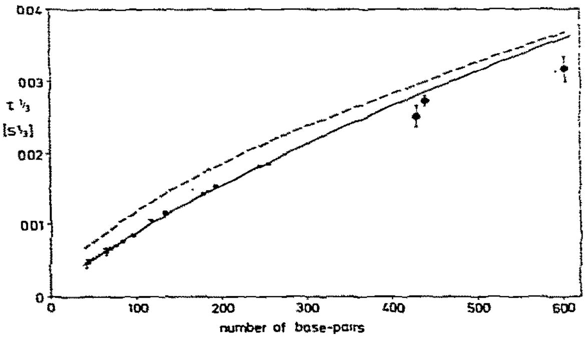

Fig. 5. Third root of the slow off-field relaxation process as a function of chain length. The lines represent the theoretical dependence using the following parameters: rise per base-pair 3.4 Å, axial radius 13 Å, persistence length 500 Å, bead diameter 50 Å. Broersma (· · · · · ·); weakly bending rod (———) and wormlike coil (-------). Points indicated by O are the slowest component from an analysis with three exponentials. Some points are presented with error bars. In most cases the errors are too small to be shown. The probe with 430 base-pairs is a pool.

All theories discussed above predict the same temperature dependence of the orientation relaxation, which is determined by the factor η/T. We have measured the temperature dependence of the off-field relaxation for the 95 base-pair fragment. Using the measured value at 21°C as a starting point for calculation, we get an excellent agreement between theory and experiments (cf. fig. 6). This result indicates that the DNA structure does not change in the temperature range from 2.5 to 41°C under our experimental conditions.

Recently, Hagerman and Zimm [16] analyzed the rotational diffusion of short wormlike chains by a Monte Carlo procedure. These authors define a ratio R of the longest rotational relaxation time of a wormlike chain to the transverse rotational relaxation time of a rigid cylinder having the same axial length L and segmental volume. The ratio R is essentially independent of the axial ratio of the cylinder for any value of L/P, where P is the persistence length. The range of validity for this analysis is $0.1 < L/P < 5$ and axial ratio greater than 20. We have calculated the experimental ratios

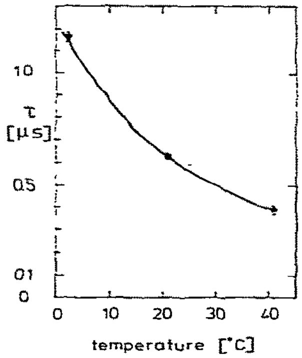

Fig. 6. The slow relaxation time (off-field process) as a function of the temperature for the 95 base-pair fragment. Solid line indicates theoretical expectation.

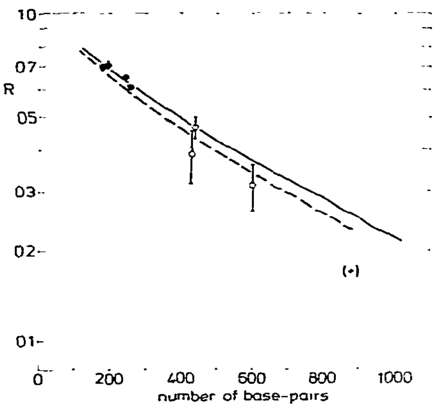

Fig. 7. Ratio R calculated according to Hagerman and Zimm as a function of the chain length. The theoretical dependence is given for the persistence length $P=500$ Å (———) and 450 Å (------). Slowest component of an analysis with two components (●); with three components (○); with four components (+).

R using 3.4 Å rise per base-pair and 13 Å axial radius. These values were compared with the ratios calculated according to the equation given by Hagerman and Zimm using P values of 450 and 500 Å (cf. fig. 7). According to these calculations the persistence length is approx. 500 Å, which is in close agreement to the value given by Hagerman [5] for corresponding experimental conditions.

3.3. The nature of the fast off-field relaxation

The rotational motion of a rigid rod may be reflected by as many as five separate relaxation components [17]. From this point of view the observation of a second, fast component in the off-field relaxation for fragments with more than 84 base-pairs is not surprising at all. However, the attributes of this component make it very special. We observe a characteristic increase in its amplitude from virtually zero at low field strength to a substantial limit contribution at high field strength. This dependence suggests that the electric field induces some change in the form of the polymer chain. It is known that the orientation of the DNA molecules by the electric field is induced by a polarization of the ion atmosphere of the polyelectrolyte. The polarization can be considerable with very high effective dipole moments. It is obvious that these dipole moments will not only lead to an orientation of the DNA molecules but also to some elongation of the chains. We conclude from our analysis of the long orientation relaxation time that the DNA is not present as a straight rigid rod but is weakly bent. We arrived at a corresponding conclusion from our analysis of the steady-state dichroism [7]. Thus, we should expect that the DNA molecules, which are weakly bent in the absence of the field, are straightened in high electric fields due to the polarization of the ion atmosphere. We suggest that the reverse of this process is reflected in the fast component of the off-field relaxation. Various observations are consistent with this interpretation. For example, the decrease in E_a and E_w with increasing chain length may be explained by the increase in the polarizability which will result in a stronger 'elongation force'. We do not have independent information on the time constants of the internal mobility and thus cannot compare our time constants with any corresponding processes. However, the values of the observed time constants and also their increase with increasing chain length seem to be consistent with our interpretation.

3.4. The on-field process

Schwarz [18] has developed a theory for the anisotropy of electrical conductance and its time dependence in the presence of electric fields. We are using his equations describing the time dependence for a comparison with our experimental data. At low field strength the time dependence of the signal is given by

$$\phi=\tfrac{2}{15}\gamma(1-e^{-6Dt})+\tfrac{4}{315}\gamma^2(1-e^{-6Dt}-6D\cdot t\cdot e^{-6Dt}) \tag{8}$$

with

$$\gamma=pE^2/(2kT) \tag{9}$$

where ϕ is the degree of orientation, p the polarizability, E the electric field strength, D the rotation diffusion coefficient and t the time. According to eq. 7, the on-field relaxation time should increase with increasing field strength to values larger than the off-field relaxation time as long as the field strength remains relatively low. The theory of Matsumoto et al. [19] leads to the same prediction. As shown in fig. 8 the prediction is in agreement with our observations.

At higher field strength we observe an acceleration of the on-field process. Finally, at very high field strength, the on-field process is much faster than the off-field process. For these conditions the orientation diffusion of the particles can be neglected. According to Schwarz [18], the time dependence of the orientation can then be described by

$$\phi=\frac{1}{1-e^{-x}}\left(1+\frac{1}{2}e^{-x}-\frac{3}{2}\cdot\frac{\arctan\sqrt{e^{x}-1}}{\sqrt{e^{x}-1}}\right) \tag{10}$$

with

$$x=4\gamma\cdot D\cdot t \tag{11}$$

In a previous investigation [7], we have shown that at high field strengths, the energy factor γ is changed from that for an induced dipole mechanism to the one for a constant dipole mechanism. The energy factor is described by

$$\gamma=p\cdot E\{E\cdot\theta+E_0(1-\theta)\}/2k_b\cdot T \tag{12}$$

with

$$\theta=\frac{1}{e^{(E-E_0)/E_m}+1} \tag{13}$$

where E_0 and E_m are the saturation field strength and the saturation field range, respectively. Since D is known from the off-field relaxation analysis and all the other parameters from the analysis of the amplitudes, the on-field relaxation can be calculated. According to eq. 9 the time dependence cannot be described by an exponential, whereas all the on-field data have been fitted by single exponentials. However, a comparison can be made at various amplitudes compared to the amplitude at the steady state. We have indicated the times required to arrive at 48 and 91% of the total amplitude as a function of the field strength in fig. 8. The agreement with the experimental relaxation times is not satisfactory. Some deviation may be due to the fact that the orientation diffusion was neglected in the theoretical calculation. However, the theoretical values and the experimental data also do not converge at high field strength, where the contribution due to orientation diffusion is expected to be very small.

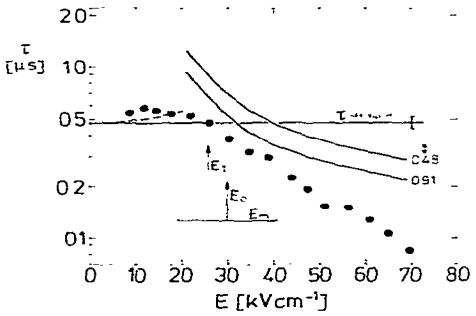

Fig. 8. The on-field relaxation time as a function of the field strength for the 84 base-pair fragment. The experimental value for the off-field relaxation is indicated as horizontal line. The meaning of E_t, E_0 and E_m is explained in the text. The theoretical dependence according to Schwarz is shown for low field strengths (-------) and for high field strength (two different Φ values: 0.48 and 0.91; cf. text).

From a detailed analysis of the steady-state dichroism [7] we have defined a saturation field strength E_0 and a saturation field range E_m. The transition described by our saturating induced dipole mechanism is also reflected by the on-field relaxation times. Within the saturation field range E_m the on-field relaxation time becomes faster and continues to decrease with increasing field strength. The slope of this decrease observed for different fragments is correlated with the value of E_m: a steep decrease in the on-field relaxation time indicates a small E_m value. Furthermore, the on-field relaxation which goes through a maximum with increasing field strength, becomes equivalent to the off-field relaxation at a well defined field strength E_t (cf. fig. 8). The E_t value is slightly smaller but very close to the E_0 value for all measured fragments. The close correspondence of these effects may be useful in the further elucidation of the molecular processes induced by electric fields in linear polyelectrolytes.

As discussed in section 3.1 the on-field relaxation could be described by single exponentials, even when the off-field relaxation clearly showed separated processes. This phenomenon may be due to various effects. First of all, under the conditions of high field strengths, where the fast components are associated with particularly high amplitudes in the off-field relaxation, the orientation of the helices into the field is strongly accelerated. It is possible that the internal movements of the helices are not accelerated to the same extent and thus cannot be separated as easily from the overall orientation in high electric fields. Another effect may come from the coupling of orientation and stretching. It is expected that the stretching force increases with the degree of orientation and thus, to a large extent, orientation is required before stretching. However, when the field is turned off, the DNA may relax with respect to internal movements, before the overall orientation becomes equally distributed. Finally, it is possible that the polarization of the ion atmosphere is rate limiting in the on-field stretching process, whereas the reverse relaxation of the internal modes will show only very limited coupling to the equilibration of the ion atmosphere.

3.5. General comments

The analysis of nucleic acid structures by measurements of the orientation relaxation can be very useful. For example, the strong dependence of the relaxation time constants upon the length of the DNA helix can be used to characterize changes, e.g., due to intercalation [20], with very high sensitivity. However, the method has to be applied carefully. For example, an extrapolation of on-field relaxation times towards zero field strength may not always provide the relaxation time, which is characteristic of the field free state.

The determination of hydrodynamic parameters providing information about the structure of macromolecules in solution has been the main application for rotation relaxation measurements. However, as our present results indicate, the experimental data also contain useful information about the internal mobility. Since it is difficult to characterize internal mobilities by other methods, electro-optical procedures will prove to be useful in the further analysis of this important property of nucleic acid helices.

Acknowledgements

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