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FLEXIBILITY OF NUCLEOSOMAL DNA

Christian MARION and Bernard ROUX

Laboratoire de Chimie biologique
Université Claude Bernard- Lyon I
69622 VILLEURBANNE CEDEX - FRANCE

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Summary

In this study transient electric birefringence (TEB) has been used to investigate the molecular flexibility of short fragments of DNA. Nucleosomal DNA always exhibits negative birefringence and Kerr behavior was observed up to high field strengths (6 KV/cm). The value of the Kerr constant is 3.5 10^{-2} e.s.u.. Birefringence decays were single exponentials and a field dependence of the molecular orientational relaxation time τ was found : it is explained by an inherent flexibility of the DNA molecule. A 20 % decrease in the calculated length was observed with fields applied as low as 2 KV/cm. The results obtained at very low fields establish TEB as a method well suited to calculate accurate values for the length of small fragments of DNA : the τ value of 4.3 μsec corresponds to a DNA length of 660 Å.

Eukaryotic chromatin is organized into repeating subunits called nucleosomes (for reviews see 1-6). Nucleosomes consist of a stretch of DNA, 200 base-pairs long (680 Å in the B-form), condensed around a protein core having the dimensions of about 100 Å in diameter. DNA must then be folded considerably since its length is contracted to about one-seventh. There are two extreme models to account for this folding of DNA. One is a smooth, continuous bending of the DNA (7-9) ; the other is localized distorsion at regular points ("kinks") with straight regions of multiples of 10 base-pairs (10-12). Although both models are energetically possible, numerous experimental results support the nucleosome model with DNA smoothly wrapped around the histone core without interruption of base-stacking interactions (13-17). It does not seem however that a definitive choice can yet be made and recent theorical considerations have shown that both models represent only a small fraction of the many paths that can reduce the DNA to the required compaction (18). The flexibility of the DNA double helix, which is directly related to its packaging is then a topic of considerable current interest. Many studies, which are characterized by a wide divergence of experimental results, have however already been reported. These are summarized in recent papers (19, 20).

In order to apprehend the inherent flexibility of DNA, small monodisperse fragments are required. We present here a transient electric birefringence (TEB) study of nucleosomal DNA. This electro-optical method is well suited to such problems as change in DNA length and DNA or nucleosomes orientation (21-29).

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Methods

Preparation of nucleosomal DNA

Native chromatin was prepared from rat liver nuclei (30). Purified nuclei suspended at a concentration of 2 10^8 nuclei/ml were digested with micrococcal nuclease (300 units/ml ; 37°C) for 3 min. The digestion was stopped by the addition of EDTA which also serves to lyse the intact nuclei. The digest chromatin was layered on an isokinetic sucrose gradient 5-28.2 % (31) and fractionated by a 20 hours centrifugation (SW 27 rotor ; 26 000 rpm).

DNA was extracted from monomer particles by incubation with 100 µg/ml of proteinase K (1 M NaCl, 1 % SDS ; 37°C) for several hours, followed by phenol-chloroform-isoamylalcohol treatments and precipitation with ethanol. Estimation of proteins by thin layer chromatography on cellulose showed less than 0.5 ‰. A DNA length of 195 base-pairs was determined by electrophoresis on a 3 % acrylamide gel (32). Hae III restriction digest of SV 40 DNA was used as size marker. DNA solutions were prepared by an overnight dialysis against 1 mM NaCl. At this low salt concentration, the melting temperature T_m was 43°C with an hyperchromicity at 260 nm of 38 %. DNA concentrations were calculated from the absorbance at 260 nm with $E_1^{1\,cm}_{\%}$ of 200.

Electric birefringence measurements

The electric birefringence apparatus was similar to that previously described (33). Single rectangular pulses (from 100 to 1200 volts) were applied to the solution. Birefringence signals are displayed on a storage oscilloscope and photographed.

The general principles of TEB have been reviewed elsewhere (21, 23). At low fields, the steady state birefringence Δn_{eq}, induced in a solution by an electric field E, is proportional to E^2 (Kerr's law) and a specific Kerr constant B can be defined

$$B = (\Delta n_{eq} / \lambda c\, E^2)_{E \to o} \qquad (1$$

where λ is the wave length of the incident light in vacuum (here 6328 10^{-7} cm : He-Ne laser) and c is the solute concentration.

The molecular orientational relaxation time τ is studied through the analysis of the birefringence decay after a sudden removal of the field. This decay is given for a monodisperse solution by (34)

$$\Delta n\,(t) = \Delta n_{eq}\, e^{-t/\tau} = \Delta n_{eq}\, e^{-6D_r t} \qquad (2$$

D_r is the rotational diffusion coefficient which is given for rods by Broersma (35)

$$D_r = \frac{3\,k\,T}{\pi\eta\,L^3} \left\{ \ln\left(\frac{L}{b}\right) - 1.57 + 7\left(\frac{1}{\ln(L/b)} - 0.28\right)^2 \right\} \qquad (3$$

where L and b are respectively the length and the radius of the rod, η is the solvent viscosity and kT is the Boltzmann constant times temperature.

Results and Discussion

The birefringence of DNA always presents a negative sign, the origin of which is well-known (36) : the molecule is oriented with its long axis in the direction of the field, the base planes being perpendicular to the helix axis. It has been verified that a number of pulses (about twenty) can be applied to the solutions without any detectable change in the optical signal. Even at very low fields, DNA has never shown positive signals as observed for low molecular weight sonicated DNA (24, 37). This reversal of the birefringence sign was certainly due to a separation of DNA strands (38) which was perhaps field induced (39)

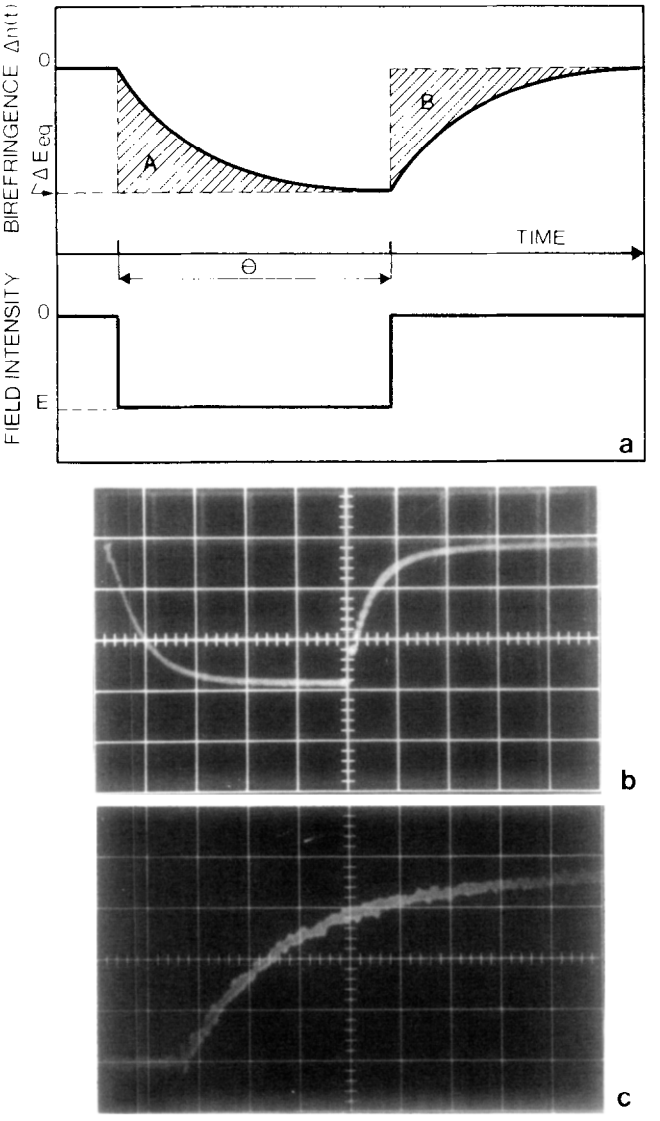

Figure 1 : (a) Schematic illustration of a birefringence signal.
The upper trace is the optic response curve, the lower one is the variation with time of the applied electric field. The transition times of the apparatus are about 50 nsec ; the time required to obtain an orientation equilibrium depends on the applied voltage. Here θ from 20 to 300 μsec are applied. The ratio of areas A and B can be related to the polarization mechanism. A/B is equal to 4 if the orienting dipole is a permanent dipole whereas A/B = 1 for an induced dipole.

(b) Typical oscilloscope tracing of a TEB signal. DNA concentration was 50 mg/l in 10^{-3} M NaCl. Electric field intensity was 1 000 V/cm. The time scale was 5μsec/div.

(c) Birefringence decay curve. E = 2 500 V/cm and 1 div. = 1 μsec. A linearity was observed on more 3 neperian units in the log $\Delta n(t)/\Delta n_{eq}$ versus time plot : this indicate that the birefringences were recorded down to 4-5 % of their initial values.

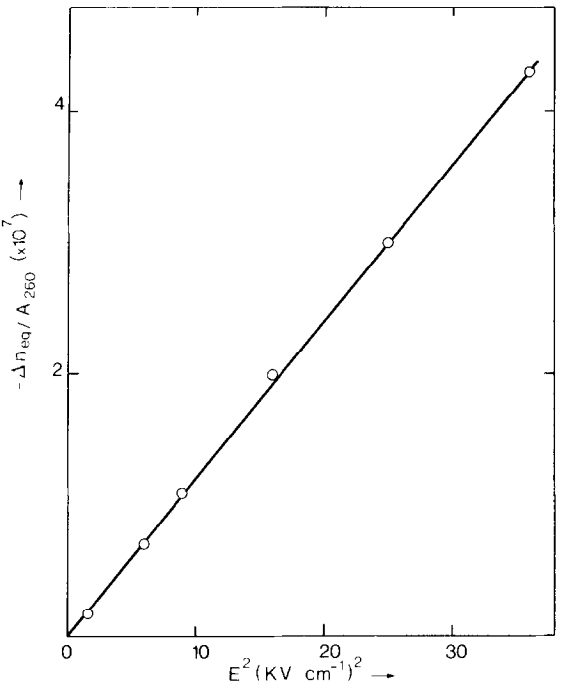

Figure 2 : Dependence of the specific steady state birefringence on E^2. These data are for a Na^+ concentration of 10^{-3} M at 18°C.

A typical oscilloscope trace of a birefringence signal is shown in figure 1b ; figure 1a showing a schematic illustration of this signal for an orienting field applied as a single pulse. In the concentration c range studied (40 to 300 µg/ml), the values of Δn_{eq} are linearly related to c whatever the fields are and Δn_{eq} could be expressed in term of specific birefringence. In figure 2, $\Delta n_{eq}/A_{260}$ is plotted versus the square of the applied field. Up to the higher fields used (6000 $V.cm^{-1}$), the Kerr law applies and a linear dependence of the birefringence on E^2 is found. Both effects of electric field and concentration are quite different from those we have previously reported for higher molecular weight DNA (40, 41) : Kerr's law was only obeyed for very low fields (E < 30 $V.cm^{-1}$) and DNA concentration (A_{260} < 0.4). Inserting the slope value into equation (1, the specific Kerr constant B is $-3.5\ 10^{-2}$ e.s.u.. This value is lower than all values precedently summarized (25) even for sonicated samples (22). B seems strongly to depend on the molecular weight and we have recently observed that the ratio B/base-pairs was constant up to a 400 base-pairs length (unpublished results). The usefulness of the Kerr constant for studying the chromatin structure is demonstrated elsewhere (29).

The mechanism of DNA orientation is still obscure. Information on the relative contribution of two standard mechanisms for field induced orientation can

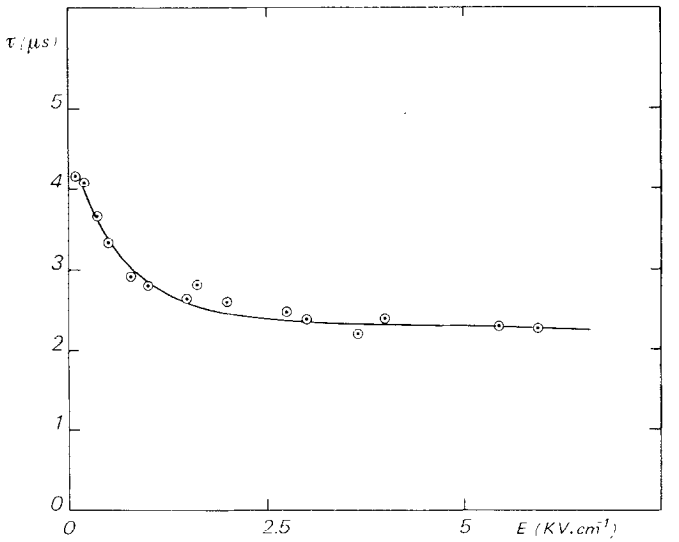

Figure 3 : Dependence of relaxation times on field strength. Each point is the average of at least three determinations. τ was not dependent on DNA concentration.

be obtained from the shape of both rise and decay curves (23). In this work, the ratio of the two areas marked A and B in figure 1a was always about 1 and the curves are symmetrical, showing the same exponential behaviour. These two facts indicate an orientation due to a pure induced electric dipole ; that is generally admitted (23, 25). First reversing pulse experiments are however consistent with the presence of a small permanent dipole moment. Such a presence has been recently reported both on small fragments of DNA (42) and on T7 viral DNA (28).

The transient decay curves were analysed in terms of equation (2. Figure 1c shows such a typical curve. The orientational relaxation time τ was determined from the slope of this normalized experimental curve. For all fields applied straight lines were obtained : this shows a perfect fitting of the decay curves with a single exponential. It should be emphasized that a single relaxation time is always observed even if the higher fields are applied. The dependence of τ on the field strength has been investigated. The relaxation is slower for low fields than for high field strengths and τ seems to reach a minimum value for $E > 4\ 000\ V.cm^{-1}$ (figure 3). Very low fields (smaller 150 $V.cm^{-1}$) are thus required to determine the length of DNA. With the constant value $\tau = 4.3$ μsec, a length of 660 Å was calculated (the rod radius was taken to be 13 Å). A good agreement was then found with electrophoretic measurements, considering a spacing of 3.4 Å between 2 base-pairs (195 bp).

The field dependence of τ originates either from the polydispersity of the solutions or (and) from the flexibility of the DNA molecule : an increase in the amplitude of the field causes in the first case the smaller molecules to

orient themselves ; in the second case, it causes the rotation of segments within the macromolecules (23). The monodisperse fragments used here allow to dismiss the first assumption to explain our results. It is then clearly demonstrated experimentally that DNA molecules as short as about 660 Å in length exhibit a significant molecular flexibility. A decrease in length of about 20 % was observed at high fields (with a relaxation time of 2.2 µsec). This flexibility and then the field dependence of τ thus observed for very small DNA fragments explain why DNA lengths determined using TEB were always smaller than theorical values (25).

Recent electric dichroism measurements on such nucleosomal fragments (42) have shown no detectable field dependence of τ. This result may be however explained by the high fields applied ($E \geq 2KV.cm^{-1}$). Future TEB studies of polyamines -and ethidium bromide- DNA interactions should provide additional information about the flexibility of nucleosomal DNA.

Acknowledgements

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