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THRESHOLDS IN FIELD-INDUCED REACTIONS OF LINEAR BIOPOLYMERS

STRONG CHAIN-LENGTH DEPENDENCE OF FIELD EFFECTS IN DNA

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We have analysed the field-induced conformation change of DNA by absorbance measurements at the magic angle. Conformation changes are observed when the electric field strength exceeds a clearly defined threshold value. The threshold values increase with increasing salt concentration and show a linear dependence upon the logarithm of the ionic strength. Measurements with homogeneous DNA samples of different chain lengths N show that the threshold increases with decreasing N; at a given ionic strength the threshold is a linear function of the logarithm of N. The threshold value observed for a circular DNA molecule with a chain length N_c fits to these data with an effective length $N_c/2$. This result indicates that the length of maximal extension is important for the field-induced reaction and suggests, together with the other results, that the field-induced reaction is mainly driven by a polarization of the ion atmosphere along the axis of DNA. Some data are also given for the dynamics of the reaction: at high electric field pulses the first step is a fast destacking and tilting of the bases followed by a slow unwinding process. For short pulses the reaction is almost completely reversible with a characteristic time constant of about 3 μs for the back reaction.

1. Introduction

Field-induced conformation changes have been observed in various biopolymers [1–9]. However, in most cases the field-induced reactions have not been analysed quantitatively. This seems to be mainly due to experimental difficulties in the unequivocal quantitative assignment of the conformation changes, since field-induced orientation effects in biopolymers are usually much larger than the effects due to field-induced conformation changes. A separate analysis of field-induced conformation changes is only possible by measurements with polarized light oriented at 54.8° with respect to the electric field vector [10,11]. Using this technique the extent of the conformation change can be measured directly as a function of the field strength.

In a previous investigation on single-stranded polynucleotides, electric fields were observed to induce conformation changes when the field strength exceeded a threshold value [7]. According to these measurements, the threshold increases with the ionic strength and the degree of counterion association at the polyelectrolyte chain. An unscreened high charge density of the polymer favours the field-induced conformation change.

In the present investigation we have analysed similar field-induced reactions in double-stranded DNA [1,8]. Both single-stranded polynucleotides and double-stranded DNA show a helix-coil transition. However, in the case of double-stranded DNA the transition is highly cooperative. The results obtained for DNA may be used as a test for some theoretical calculations on field-induced reactions in polyelectrolytes presented by Man-

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ning [12]. Another argument for using DNA molecules as a model system for the analysis of field-induced reactions is the fact that DNA samples can be prepared over a wide range of chain lengths with an exactly defined number of base-pairs. Using these DNA samples we characterize the chain-length dependence of the field-induced helix-coil transition.

2. Materials and methods

Calf thymus DNA was purchased from Boehringer, Mannheim, and was dialyzed extensively against 1 mM Tris, pH 8.0, 50 μM EDTA (buffer A). Part of the calf thymus DNA was sonicated at 0°C for 20 min in 0.1 M NaCl, 1 mM Tris, pH 8.0, and subsequently dialyzed extensively against buffer A. The average molecular weights of these samples were determined by analytical zone sedimentation. The evaluation was based upon the data of Studier [13]. By this procedure we found 30000 base-pairs for the original and 1000 base-pairs for the sonicated sample. The restriction fragments with 194 and 880 base-pairs (from *Hae*III digestion of pVH51 DNA) as well as the vector pBR322 with 4362 base-pairs and its linearized form (*Bam*HI digestion) were kindly provided by Dr. W. Hillen. The GC content of the various samples is: 194 base-pairs, 51.0%; 880 base-pairs, 51%; 4362 base-pairs, 54%; calf thymus DNA, 42.4%. Poly(dA) · poly(dT) and polyd(G-C) were obtained from Boehringer, Mannheim. All samples were dialyzed extensively against buffer A.

The field-jump measurements were performed with an apparatus constructed by Grunhagen [14] with modifications of the optical detection system [15]. The field-jump cell contained about 0.7 ml with an optical path length of 9.7 mm and an electrode distance of 6.3 mm.

Transmission changes were recorded with polarized light at 0, 54.8 and 90° orientation of the light vector with respect to the field vector. We checked that the absorbance changes ΔA_0, $\Delta A_{54.8}$ and ΔA_{90} measured at the different orientation always fulfilled the condition

$$\frac{\Delta A_0 - \Delta A_{54.8}}{\Delta A_{90} - \Delta A_{54.8}} = -2.0$$

within an experimental accuracy of a few percent. The absorbance changes were recorded at 248 nm (mercury line) and at a standard temperature of 12°C unless stated otherwise).

3. Results

3.1. The 'magic angle' conditions

The experimental results on the field-induced conformation change of DNA can only be obtained in a quantitative manner, when the conditions for the selective measurements at the magic angle [10,11] are fulfilled very carefully. Since the orientation effects of DNA are very large compared to the absorbance changes associated with the conformation change, a small deviation from the magic angle conditions will lead to relatively large perturbations of the data obtained for the conformation change. Nevertheless, it is possible to characterize the field-induced transition selectively when various precautions are taken. For example, the observation windows of the cell should be strain free and remain so under the action of high-field pulses. The orientation of the polarizer corresponding to the magic angle can be determined most accurately by field pulse experiments with a sample, which does not show any field-induced reaction. According to our experience, DNA samples of low molecular weight at a relatively high salt concentration (cf. below) are most appropriate. The DNA experiments at the magic angle can only be performed at low concentrations such that the absorbance changes due to orientation remain below a limit value. For the present investigation it was sufficient to adjust the experimental conditions such that the transmission changes at parallel orientation of polarizer and electric field remained below about 10%.

3.2. Measurements

When a DNA sample is subjected to electric field pulses the absorbance at the magic angle remains virtually unchanged as long as the field strength does not exceed a certain limit value. When this limit is exceeded, absorbance changes

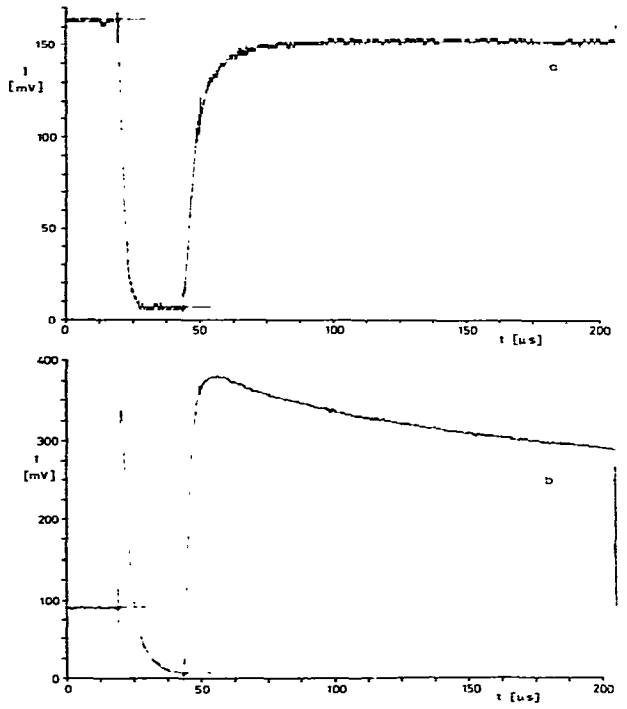

Fig. 1. Change of transmission for calf thymus DNA (30000 base-pairs) induced by a field pulse of 60 kV/cm for 19 µs (ionic strength 0.24 mM). (a) Recorded at the magic angle; (b) recorded at parallel orientation of polarizer and electric field. The arrows indicate the beginning and end of the field pulse.

can be clearly detected. As shown in fig. 1a, the electric field induces a decrease in the transmission corresponding to an unstacking of the bases and a denaturation of the double helix. The time course of the field-induced reaction is quite characteristic and clearly different from that of the orientation. This difference is also a useful indication that the effects observed at the magic angle are not simply due to physical orientation.

When the process is recorded at a parallel orientation of the polarizer and the electric field, the absorbance change results from the superposition of the field-induced reaction and orientation effects (cf. fig. 1b). In the first moment of the field pulse the DNA molecules start to be oriented, which results in an increase in transmission. As soon as the field-induced reaction contributes to the signal, the transmission is decreasing again. Finally, a plateau value is attained with a rather small change of the transmission relative to the starting value. The small transmission change cannot be explained by a counterbalance between the increase in the 'intrinsic' DNA absorbance and the decrease in the absorbance due to a usual orientation effect, since the orientation of native DNA results in much larger absorbance changes than that found for the field-induced reaction at the magic angle. Thus, the observed effect can only be explained by a field-induced reaction, which is accompanied by the reduction of the DNA orientation effect. This may be due to a general disorder of the bases or to a decrease in the angle between the bases and the long axis of the helix.

The time required for the field-induced reaction depends upon the field strength, the length of the DNA and other factors. For long DNA with 30000 base-pairs it may take about 1 ms to approach the stationary state. Due to the conductance of the solutions the field pulses can be maintained at a constant field strength only for a limited time. At very low ion concentrations (ionic strength 0.5

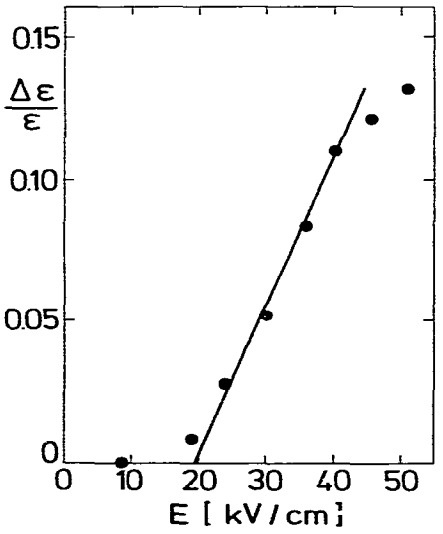

Fig. 2. Absorbance change relative to the total absorbance ($\Delta\epsilon/\epsilon$) of an 880 base-pair DNA fragment as a function of the field strength (0.035 mM Tris, 2 µM EDTA).

mM) the maximal pulse time is about 1 ms. For this reason it is not always possible to measure the absorbance at the stationary state directly. In some cases, it is necessary to extrapolate the observed time course of the absorbance change to 'infinite' time. Thus, the absorbance changes corresponding to the stationary state cannot be determined with high accuracy in all cases. Nevertheless, the accuracy is sufficiently high to characterize the functional relationship between the field strength and the absorbance change, which is a measure of the extent of the reaction. An example is given in fig. 2 for a DNA with 880 base-pairs. In all cases investigated the absorbance change is a linear function of the field strength E for a range of E values above a clearly defined threshold. The slope of this linear function is about 5×10^{-3} cm/kV and is almost independent of various factors like length of the DNA, its GC content, ionic strength and temperature. The threshold field strength E_t is defined by extrapolation of the linear part to the abscissa. Usually some absorbance changes are observed at field strengths below E_t, but these changes are rather small compared to those found above E_t. At high field strengths the absorbance changes no longer follow the linear relation and start to level off.

Measurements at different salt concentrations demonstrate that the threshold field strength E_t increases with increasing ionic strength I. As shown in fig. 3, E_t is a linear function of the logarithm of the ionic strength. We find similar dependences for all DNA molecules investigated. Also, the slope $dE_t/d \log I$) is quite similar for DNA pieces of various length (about 15 kV/cm). Nevertheless, the threshold value is a strong function of the chain length. Short DNA fragments show much higher thresholds than long DNA chains. When we plot the thresholds observed for various DNA molecules at a fixed ionic strength as a function of the logarithm of chain length we again arrive at a linear relationship. Only one experimental point obtained for a circular DNA sample does not fit directly (fig. 4). However, the circular DNA clearly cannot be extended to the same length as a linear DNA having the same number of base-pairs. Thus, it appears justifiable to plot the point obtained for the circular DNA at half the number of its base-

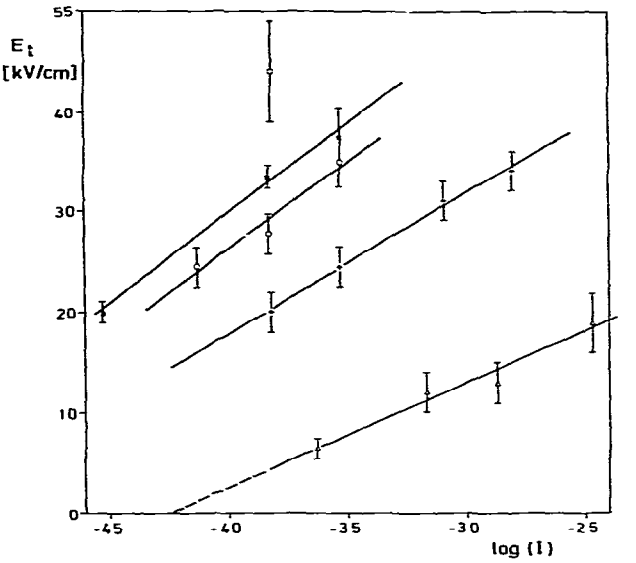

Fig. 3. Threshold field strength E_t as a function of the logarithm of the ion concentration for various chain lengths: (Δ) 30000 base-pairs; (+) 4362 base-pairs, linear; (○) 4362 base-pairs, circular; (*) 880 base-pairs; (□) 194 base-pairs.

pairs. With this correction the value obtained for the circular DNA fits to those obtained for linear DNA molecules. This result indicates that the length of maximal extension is an important factor

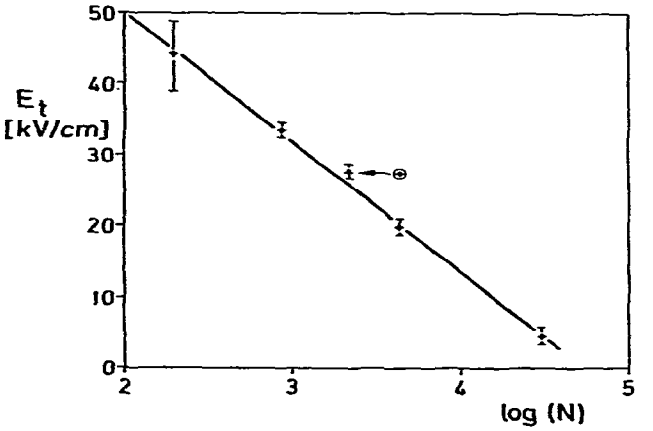

Fig. 4. Threshold field strength E_t at 0.15 mM ionic strength as a function of the logarithm of the chain length N. The point for the circular DNA with a chain length N_c (○) is also shown at $N_c/2$ (cf. text).

for the threshold value in the field-induced conformation change of DNA.

Some additional experiments have been performed with DNA samples of a relatively broad length distribution. These samples were obtained either by sonication of high molecular weight DNA or by enzymatic methods, which did not provide DNA molecules of uniform length. According to the strong chain-length dependence found from the analysis of restriction fragments, the results obtained for non-homogeneous DNA samples should be interpreted with some caution. Nevertheless, some of the additional results are also useful:

The temperature dependence of the threshold effect was analysed with a sonicated DNA sample (about 1000 base-pairs) in the temperature range from 2 to 22°C. The threshold field strength (measured at a constant ionic strength of 0.29 mM) decreased slightly with increasing temperature. The apparent activation enthalpy was close to 1 kcal/mol. A comparable, even smaller temperature dependence was found for the linearized DNA from pBR322 (4362 base-pairs).

The dependence of the threshold effect upon the GC content was analysed with the following samples: (1) poly(dA) · poly(dT), (2) sonicated calf thymus DNA (43% GC) and (3) polyd(G-C). All these samples had approx. 1000 base-pairs. The threshold field strength found at an ionic strength of 0.29 mM increased from 23 kV/cm for 0% GC to 33 kV/cm for 43% GC and to 38 kV/cm for 100% GC. Although these results show an increase in the threshold effect with the GC content, the values should be interpreted with caution, since some difference in the average molecular weights of our samples may obviate a quantitative comparison.

3.3. The dynamics of the transition

It is not the main goal of the present investigation to analyse the dynamics of the DNA denaturation in high electric fields. However, the field-jump experiments provide some useful information about the kinetics, which also gives an indication of the nature of the observed processes. The kinetics of the DNA denaturation [16,17] are notoriously complicated, at least for long DNA molecules. Thus, we will not attempt to evaluate a mechanism quantitatively, but will discuss the observed phenomena in a qualitative manner.

The time course of the reaction during an electric field pulse is very much dependent upon the field strength. At field strengths just above the threshold value, the reaction of a long DNA (several thousand base-pairs) usually takes a few milliseconds, whereas electric field pulses, which are 'high' compared to the threshold, may accelerate it up to a few microseconds. When a high-field pulse is applied to a long DNA molecule for a time of about 20 μs, which is sufficient to attain a stationary level of absorbance, the reaction is almost completely reversible (cf. fig. 1). Most of the back-reaction is observed within 10 μs; part of the back-reaction takes considerably more time. When a high-field pulse is applied to the same DNA for a longer time, the reversible part of the amplitude decreases. Pulses in the millisecond time range cause a denaturation, which is almost irreversible. From these observations it may be concluded that the electric field pulses induce two consecutive reactions: in the first reaction the bases are unstacked, as indicated by the increase in the absorbance, and also tilted, as indicated by the decrease in the dichroism. Although the molecular arrangement of the bases seems to be completely changed, the bases of the complementary strands remain close to each other during the first few microseconds after the formation of the new structure. Thus, the double helix may be renatured when the electric field is turned off after some microseconds. If the electric field is maintained for a longer period of time, however, the complementary sites are removed from each other by diffusion until finally the reaction is essentially irreversible.

We did not analyse the time constants of the different processes as a function of the various parameters in a systematic manner. This would be the subject for a separate investigation. However, we have found that one of the processes shows relatively small variations for different helices and also for different conditions: the time constant for the first part of the renaturation process is usually around 3 μs. A process associated with this time

constant was found in poly(dA) · poly(dT), polyd(G-C) and natural DNA. The time constant shows very little dependence on the chain length; only in the case of the 194 base-pair fragment, the time constant decreased to a value of about 1 μs.

The close correspondence of the time constants suggests that the process reflects the same type of reaction in all cases. According to the assignment of the processes discussed above, it should be associated with a stacking reaction of the base-pairs. The rate of the present stacking reaction in double helices is slightly lower than that found previously for the stacking of bases in single-stranded polynucleotides [18] like poly(A). This may be explained by the fact that the stacking of base-pairs is a more complex reaction, which will be associated with a higher entropy of activation.

4. Discussion

Electric fields may induce conformation changes by various mechanisms. In the present case, the dependence of the field-induced reaction upon the ion concentration indicates that the electric field perturbs the ion binding to the polymer. It is well known that electric fields may reduce the degree of ion binding by a 'dissociation field effect' [19]. The linear dependence of the denaturation amplitude upon the field strength (fig. 2) suggests the existence of a dissociation field effect. Recently, Manning [12] developed a theory for a dissociation field effect in polyelectrolytes on the basis of his ion condensation model. He applied his theory to the field-induced helix-coil transition of DNA and predicted a dependence of the threshold field strength E_t upon the ion concentration c with a slope $dE_t/d \log c = 372$ kV/cm. This value is more than an order of magnitude higher than that found experimentally.

This discrepancy may be due to different reasons. The theory is based upon a simplified model of the field effect and may require further development. However, it is more likely that the main reason for the discrepancy comes from a difference in the basic mechanism assumed by the theory from that producing the field effects in DNA. This view is supported by the observation of the strong chain-length dependence in the field-induced reaction. A simple dissociation field effect is not expected to show the observed strong chain-length dependence; at least not in our range of chain lengths, where simple end effects should not contribute too much to the overall polyelectrolyte potential. The chain-length dependence suggests that the polarization of the ion atmosphere along the axis of the polymer is the main cause of the denaturation [8]. The special role of the chain length is directly illustrated by the data obtained for the circular DNA, which suggest that the length of maximal extension is an important factor for the field-induced reaction. The data for the circular DNA indicate that the field-induced reaction is coupled to the orientation of the molecules. Thus, we should compare our present results with those obtained previously for the orientation of restriction fragments [20]. A careful analysis of orientation data over a wide range of chain lengths showed that the induced dipole of DNA helices is saturated in a transition range to give a constant dipole moment at high field strengths. The field-induced conformation changes are observed in the field range where the orientation follows a constant dipole mechanism. The constant dipole moments of DNA are extremely high (≈ 10000 debye). Compared to these high values the change in the dipole moment required to explain our field-induced reaction is relatively small. It is possible that an extended form of the DNA helix has a higher dipole moment than the usual Watson-Crick helix and/or that the total moment of the separated strands is higher than that of the native helix. At first glance, the observed linear dependence of the amplitude upon the field strength seems to argue against this interpretation, since reactions induced in electric fields by dipole changes usually have a quadratic dependence of the amplitude upon the field strength [21]. However, in the case of very high dipole moments the orientation function (cf. refs. 20 and 21) becomes saturated and the quadratic dependence is reduced to a linear one. Thus, at low electric fields the amplitude may increase with the square of the field strength. When the amplitude is large enough to be clearly detected, however, the orientation function is close to saturation already and thus a

linear relation between amplitude and field strength is observed. The saturated dipole moment of the double helix has been measured as a function of the chain length N and was found to be almost independent of N for fragments with more than 200 base-pairs (corresponding approximately to the persistence length). Thus, the electric parameter derived from orientation parameters is only very slightly dependent upon N for $N > 200$ base-pairs, whereas the field-induced conformation change shows a logarithmic dependence in the same range of N. Since the reaction in the electric field is induced by a difference in the dipole moments of the reactants, a quantitative description of our reaction would require measurements of the dipole moments of single-stranded DNA as a function of the chain length.

Our present results indicate that there are different domains of field-induced reactions. In the case of long polyelectrolytes, electric fields will induce a strong reaction driving force due to a polarization mechanism along the axis. For short chains or spherical molecules of limited dimensions, however, it is likely that the 'classical' dissociation field effect becomes more important.

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