Biophysical Chemistry 20 (1984) 225–235
Elsevier

BPC 00879

INTERACTIONS OF NUCLEIC ACID DOUBLE HELICES INDUCED BY ELECTRIC FIELD PULSES

Dietmar PORSCHKE, Hans-Josef MEIER and Jorg RONNENBERG

Max-Planck-Institut für biophysikalische Chemie, 34 Göttingen, F.R.G.

Received 13 October 1983
Revised manuscript received 27th January 1984
Accepted 22nd May 1984

Key words: DNA; Electric field effect; Helix interaction; Dipole reaction; Light scattering; Electric dichroism

Electric field pulses induce a substantial increase of the light scattering intensity of double-helical DNA. The relative change of light scattering and also the reciprocal relaxation time constants under electric field pulses increase with increasing nucleotide concentration. These observations, together with a large difference between dichroism orientation time constants and light scattering time constants under electric field pulses, demonstrate that the main part of the light scattering effect is due not to field-induced orientation but to interactions between DNA helices. From the concentration dependence of the light scattering time constants we obtain, according to an isodesmic reaction model, association rate constants in the range 3×10^{10} M^{-1} helices s^{-1} for DNA with approx. 300 base-pairs. These values are at the limit of a diffusion-controlled DNA association and do not show any dependence upon the field strength. The dissociation rate constants k_d decrease strongly with increasing field strength E and thus demonstrate that the interactions between the helices are induced by the electric field. This conclusion is consistent with independent measurements which do not reveal any DNA association at zero field strength. The observed linear relation between $\log(k_d)$ and E^2 suggests a field-induced reaction driven by dipole changes. According to this interpretation the change of dipole moment should be in the range of approx. 1400 debye. The dissociation rates for DNA helices with approx. 300 to approx. 800 base-pairs strongly increase with increasing salt concentration (measured in the range 1–5 mM ionic strength), whereas the association rate constants remain virtually unchanged. Measurements of the linear dichroism in the same range of DNA chain length demonstrate that for long field pulses of e.g., 40 μs, the amplitude approaches a maximum value and then decreases. The dichroism relaxation curves observed after long field pulses exhibit a component with a positive dichroism and an increased decay time. These observations suggest the formation of a DNA aggregate with an unusual arrangement of the bases.

1. Introduction

Electro-optical methods have been widely used for the analysis of biological macromolecules. The data obtained from electro-optical experiments, e.g., rotational diffusion coefficients or the reduced dichroism, provide valuable information on the structure of macromolecules in dilute solution [1,2]. Electro-optical data proved to be particularly useful for the characterization of structures with some symmetry such as nucleic acids [3–10]. However, application of electric field pulses to biological macromolecules requires special caution, since the field pulses may induce conformation changes [11–16]. Some of these conformation changes may be induced already at moderate field strength. In most of the cases investigated the reaction is driven by the dissociation of ion complexes accompanied by a polarisation of the ion atmosphere around the polymer molecules. The ion displacement may result in the dissociation of ordered structures, for example, the transition from the α-helix to the coil form in polypeptides or from double helices to single strands in polynucleotides. According to these examples it seems that the action of electric fields upon biopolymers is always 'destructive'.

However, in the present communication we present evidence for a 'constructive' action of electric fields resulting in interactions between nucleic acid helices. This reaction was found during investigations of the DNA conformation by measurements of light scattering changes under the influence of electric fields.

2. Materials and methods

DNA from calf thymus was obtained from Boehringer GmbH, Mannheim. Part of this DNA was sonicated and the fragments separated by Sepharose 4B column chromatography. The average length of the resulting DNA samples was determined by analytical zone sedimentation using a Beckman model E centrifuge equipped with a photoelectric scanner. The evaluation was based upon the data given by Studier [17]. DNA from the bacteriophages λ, PM2, plasmid pBR322, poly[d(AT)] and poly[d(GC)] were also obtained from Boehringer gmbH, Mannheim. T2 and T4 DNA were purchased from Miles Laboratories, Inc. T7 DNA was isolated by phenol extraction from phages, which were kindly provided by Professor M. Schweiger, Innsbruck. All DNA samples were dialysed extensively against 'NCE7' buffer containing 1 mM NaCl, 1 mM sodium cacodylate (pH 7), 0.2 mM EDTA. A buffer with a 2-fold concentration of the same components is denoted 2 × NCE7 and correspondingly a buffer with half the concentration of NCE7 is denoted 1/2 × NCE7. For measurements of light scattering all the samples were filtered through 0.45 μm membrane filters. Concentrations were determined after filtration by absorbance measurements using the extinction coefficients given by Allen et al. [18].

The field pulses were generated with an instrument constructed by Grunhagen [19]. Changes in the light intensity resulting from absorbance or light scattering changes were measured with the optical setup described previously [20]. The cell used for measurements of light scattering was similar to the fluorescence temperature jump cell described by Rigler et al. [21]. The cell body was constructed from black dynal with electrodes of platinum or gold at a distance of 14.4 mm. The intensity of scattered light was recorded by two photomultiplyers both at right angles with respect to the incident beam and their signals averaged by a photometric control unit. The electric field strength and the optical signal as a function of time were stored on a Tektronix 7612D programmable transient digitizer interfaced to an LSI 11/23 from Digital Equipment. Amplitudes were evaluated with the aid of graphic routines using the LSI 11/23. For the evaluation of time constants the data were transferred to the computer centre of the Gesellschaft für wissenschaftliche Datenverarbeitung, Göttingen. The convolution of relaxation curves by the detection system was considered by a simple exponential deconvolution procedure [9]. Since the length of field pulses was not exactly reproducible, the calculation of average relaxation curves after termination of field pulses from several individual curves required a special numerical procedure: first, the time of pulse termination was determined from the record of the electric field strength and then the various curves were projected on the curve resulting from the longest pulse, with time scale zero at pulse termination. When the data were recorded with a change in the sampling interval, the curves were projected via linear interpolation.

3. Results

When DNA molecules in a dilute buffer solution are subjected to an electric field pulse, the intensity of scattered light recorded at right angles with respect to an incident light beam is strongly increased. As shown in fig. 1 the scattered intensity approaches a stationary value during a field pulse of about 150 μs. After termination of the field pulse the scattered intensity relaxes back to its original level. The field-induced increase of scattered intensity is observed for DNA preparations from various organisms such as bacteriophages λ, PM2, T2, T4, T7, plasmid pBR322 and calf thymus. Since the effect remains after repeated phenol extraction, it is not likely to be associated with some contamination. Measurements at different wavelengths in the range 313–436 nm demonstrate that the relative change

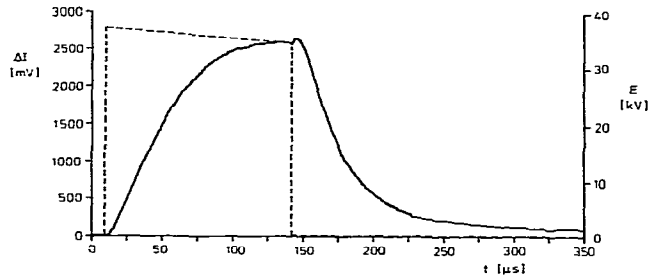

Fig. 1. Light scattering intensity ΔI of a DNA sample with approx. 430 bp as a function of time (continuous line, left scale) under an electric field pulse E (broken line, right scale) (452 μM nucleotide residues; 1/2 × NCE7 buffer: 20 °C; rise time of the detection unit 10 μs).

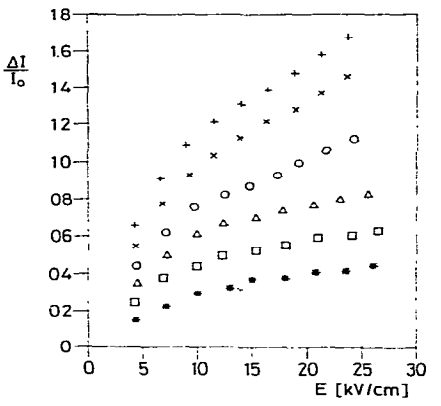

Fig. 2. Light scattering amplitudes $\Delta I/I_0$ for calf thymus DNA as a function of the electric field strength: (+) 896 μM, (×) 658 μM, (○) 308 μM, (△) 151 μM, (□) 77 μM, (*) 37.4 μM; 20 °C, NCE7 buffer.

of light intensity induced by a given field pulse is not dependent upon the wavelength. This result may be expected for, e.g., a field-induced reaction with a change in the molecular weight: in a first approximation both the scattered intensity I_0 and its change ΔI depend upon $(1/\lambda)^4$ and thus $\Delta I/I_0$ remains constant. For a further analysis of the field-induced effect we use two different types of information obtained from field-jump experiments as shown in fig. 1: (1) the amplitude given by the difference in the light intensity between the field free state and the stationary state approached during a sufficiently long electric field pulse; (2) the time constant(s) describing the approach to the stationary state in the electric field and the reverse reaction to the field free state.

3.1. Amplitudes

The relative increase of scattering intensity $\Delta I/I_0$ has been measured as a function of field strength E for many DNA samples under different conditions. Although $\Delta I/I_0$ always increases with E (cf. fig. 2), the functional relationship between these parameters can be very different. In some cases the increase appears to be almost linear, yet there are also cases where the slope $d[\Delta I/I_0]/dE$ increases or decreases with E. Thus, it is not simple to fit the dependence of the amplitudes upon the field strength with a general model. As shown in fig. 2, the relative amplitudes $\Delta I/I_0$ increase with increasing DNA concentration. This effect – observed for all different DNA samples used in the field-jump experiments – indicates that the field-induced reaction involves interactions between DNA molecules [22,23]. For a simple orientation phenomenon or a field-induced intramolecular reaction $\Delta I/I_0$ should be independent of the DNA concentration. Usually, it is possible to evaluate thermodynamic parameters from the concentration dependence of jump amplitudes. However, light scattering intensities may be strongly influenced by various factors (cf. textbooks of biophysical chemistry). Thus, our quantitative evaluation was mainly based upon relaxation time constants. Nevertheless, the amplitudes can be quite instructive, at least for a qualitative comparison. For example, the amplitude induced by a given field pulse at a constant nucleotide concentration and a DNA chain length of 430 basepairs (bp) increases with decreasing buffer concentration. This observation indicates that the interactions between DNA molecules in the presence of electric fields increase with decreasing salt concentration. An alternative explanation of this observation by a simple orientation phenomenon with an increasing dipole moment at decreasing salt concentrations can be excluded again owing to the dependence of the effect upon the nucleotide concentration.

While the salt dependence of the amplitudes is relatively strong for low chain lengths around 400 bp, the salt dependence observed in the range 1–5 mM is rather small for DNA molecules with approx. 800 bp. A high-molecular-weight DNA from calf thymus with approx. 10000 bp even showed a reverse salt dependence with an increase of amplitudes at increasing salt concentrations in the range 1–10 mM. Due to the conductivity of the solutions it is difficult to apply defined field pulses of sufficient length at high salt concentrations. Under these conditions the field jump is always accompanied by a sizable temperature jump. Thus, it was not possible to follow the reaction of high-molecular-weight DNA to still higher salt concentrations. Experiments performed at a constant salt and nucleotide concentration (2.4 mM and 150 μM, respectively) demonstrate that the relative amplitude increases strongly with chain length N for helices with $N \leq 1000$ bp. However, for chain lengths $N > 1000$ bp the further increase of the amplitude with N is comparatively small.

As shown in fig. 1 the 'off'-field reaction curve has a small 'blip' indicating the presence of a fast relaxation process with an amplitude opposite to that of the slow one. Usually the fast relaxation effect is more clearly seen in the 'off' than the 'on' relaxation curve. However, in the case of a DNA sample with approx. 250 bp, for example, the fast process is also seen in the on relaxation curve (fig. 3). The relative amplitude of both the fast and the slow process depends upon the polarization of the incident light beam. The relative amplitudes of both processes are maximal for an incident light beam polarised parallel to the electric field vector. When the incident light is polarised perpendicular to the electric field the sign of the amplitude associated with the fast process is reversed, whereas the amplitude of the slow process is reduced but does not change its sign. The reversal of the amplitude suggests by its similarity to the reversal of dichroism amplitudes that the fast process reflects the orientation of double helices by the electric field. This interpretation is supported by a correspondence of the time constant found for the fast process detected by measurements of light scattering with the orientation time constant found by dichroism measurements. However, the time constant of the fast process is close to the limit of time resolution of our light scattering detection and thus cannot be characterised with high accuracy. Some questions remain concerning the interpretation of the fast process. It is difficult to reconcile the presence of a fast amplitude in the off relaxation and its absence in the on relaxation, as found, for example, for a 430 bp DNA in 1/2 NCE7 (cf. fig. 1), with an interpretation by a simple orientation phenomenon. The dependence of the fast amplitude upon field strength and nucleotide concentration also does not seem to be consistent with a simple orientation phenomenon in all cases. These problems will be investigated in more detail with an improved detection system.

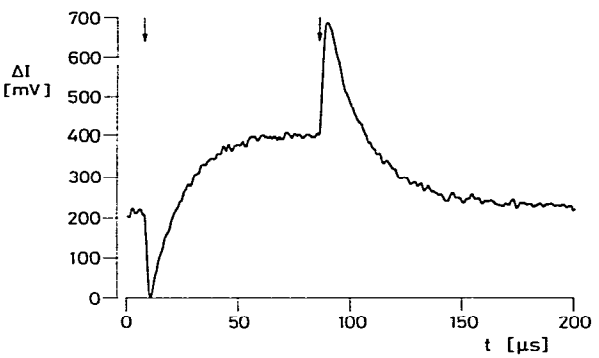

Fig. 3. Light scattering intensity of a DNA sample with approx. 250 bp as a function of time t under an electric field pulse of 36 kV/cm; the beginning and end of the pulse are indicated by arrows (585 μM nucleotide residues; NCE7 buffer; incident light beam polarised parallel to the electric field; rise time of the detection unit 1.2 μs; average of 4 experiments).

3.2. *Time constants*

When electric field pulses induce a small perturbation, the on- and off-field relaxation curves should be equivalent. For the present systems we observe a difference between the on and off relaxation curves, which is particularly large at high field strengths indicating large perturbations. Under these conditions the on-field relaxation proves to be particularly instructive. The data obtained for a DNA sample with 430 bp in 1/2 × NCE7 buffer are used as an example. In this case the

on-field relaxation curves can be accurately represented by a single exponential; from the data shown in fig. 1, for example, we evaluate a time constant of 38 μs. This process is clearly slower than the slowest component (0.54 μs) detected in the on-field orientation curve measured for this DNA sample by linear dichroism under corresponding experimental conditions. Thus, the process detected by measurements of light scattering does not simply reflect the orientation of the DNA helices by the electric field. The different nature of the phenomena detected by light scattering and by linear dichroism is also demonstrated by a strong difference in the field dependence of the relaxation process. While the on-field time constants observed by linear dichroism decrease with increasing field strength, the opposite dependence is found in light scattering experiments. For the DNA with 430 bp in 1/2 × NCE7 buffer the on-field time constant increases with field strength from 12 μs at 4.5 kV/cm over 20.5 μs at 13 kV/cm to 38 μs at 24 kV/cm. This dependence upon the field strength is quite unusual and indicates a special mechanism of the field-induced reaction.

More information on this reaction may be obtained by measurements at different DNA concentrations. As shown in fig. 4 the reciprocal time constants measured at a given field strength increase linearly with the nucleotide concentration.

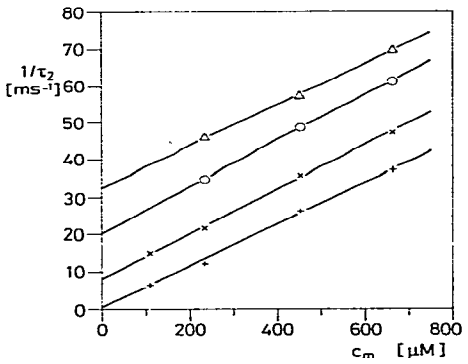

Fig. 4. Reciprocal relaxation time constants $1/\tau_2$ measured for the on-field process as a function of the nucleotide concentration c_m at different field strengths: (+) 24 kV/cm, (×) 18.6 kV/cm, (○) 13.0 kV/cm, (△) 10.2 kV/cm; average length of DNA 430 bp; 1/2×NCE7 buffer; 20 °C).

This result clearly demonstrates that the field-induced relaxation is due to an intermolecular process of DNA helices. The most simple mechanism to describe the observed concentration dependence of relaxation time constants is a dimerisation according to

$$\text{Monomer} + \text{monomer} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \text{dimer} \qquad (1)$$

with a reciprocal relaxation time constant [22,23]

$$1/\tau = 4k_a[\text{monomer}] + k_d \qquad (2)$$

However, the simple dimerisation mechanism is probably not correct. It is difficult to envisage a DNA-dimer structure, which would prevent association of further DNA helices. It is more likely that the reaction proceeds to higher degrees of association. Evidence for this is provided by the amplitude data. The relative changes of light scattering induced by electric fields do not approach saturation at high DNA concentrations as may be expected for a simple dimerisation. Instead, the amplitudes continue to increase at high DNA concentrations as may be expected for an infinite association reaction. In the absence of information about details of the infinite association we use a simple 'isodesmic' association model, which assumes equivalent equilibrium constants for consecutive association steps. This model has been used, for example, to describe the stacking of nucleic acid bases [24]. The reciprocal relaxation time constant is given by

$$1/\tau = 2k_a\Sigma c + k_d \qquad (3)$$

where Σc is the sum of the concentrations of monomer, dimer, trimer, etc., assuming that each of these aggregates has the same potential for association. As can be seen from eqs. 2 and 3 an evaluation according to the infinite, isodesmic association model provides the same rate constant for dissociation as the simple dimerisation model, whereas the rate constants of association differ by a factor of 2. The magnitude of the association rate constants is obviously dependent upon the concentration units used for the evaluation. Although polynucleotide concentrations are usually given in monomer units, in the present case con-

centrations in terms of mol helices/dm^3 are more appropriate, since the process described is an association reaction of helices. Using the isodesmic reaction model we obtain an association rate constant $k_a = 2.4 \times 10^{10}$ (M helices)$^{-1}$ s^{-1}. The order of magnitude indicates a diffusion-controlled association process (cf. section 4). As shown in fig. 4 the association rate constants are virtually independent of the field strength, whereas the dissociation rate constants decrease strongly with increasing field strength. This result obtained from the concentration dependence of relaxation time constants clearly demonstrates that the degree of association increases with increasing field strength. The decrease of k_d with increasing field strength (at constant k_a values) shows that the electric field pulses induce association of DNA helices. Dissociation rate constants have been evaluated for field strengths ranging from 5 to 28 kV/cm. As shown in fig. 5 for the case of a DNA with 430 bp the logarithm of k_d is a linear function of the square of the electric field strength. From the extrapolation of $\log k_d$ to $E^2 = 0$ it is apparent that the degree of association in the absence of electric fields is very small under the present experimental conditions.

The on-field relaxation curves have been evaluated for various DNA samples using pulses

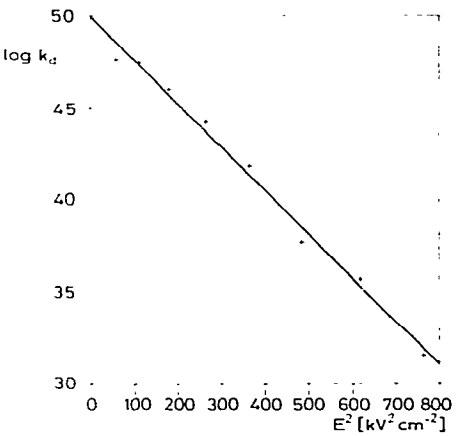

Fig. 5. Logarithm of dissociation rate constant $\log k_d$ as a function of the square of the electric field strength E^2 for a DNA with approx. 430 bp in 1/2×NCE7 buffer at 20 °C.

with an average field strength of 24 kV/cm. The parameters obtained for various chain lengths and salt concentrations are compiled in table 1. At a given DNA and buffer concentration the relaxation time constants tend to decrease with increasing chain length. Since the faster processes are more difficult to characterise quantitatively, the accuracy decreases with increasing chain length. Due to these difficulties it also cannot be excluded that the field-induced 'association' process is partly intramolecular for DNA samples with a high number of base pairs.

According to the data available the rate constants of 'intermolecular' association are almost independent of the chain length, when calculated on the basis of monomer concentration units. A rather clear increase with chain length appears when these rate constants are given in units of helix concentrations. A smaller increase with chain length is observed for the rate constants of dissociation. The chain length variation of the 'equilibrium' constants calculated from the rate constants is rather small and remains within the limits of experimental accuracy. The dependence of the field reaction upon the ionic strength is mainly reflected by the rate constants of dissociation, which show a strong increase with ionic strength.

All the kinetic parameters discussed above have been evaluated from the on-field relaxation curves. Obviously, the off-field relaxation curves should be consistent with the interpretation given above on the basis of the on-field process. We are using again the results obtained for the DNA with 430 bp as an example. The off relaxation is accurately represented by two exponentials with time constants of $\tau_1 = 1 \pm 0.5$ and $\tau_2 = 26.5 \pm 2$ μs (1/2 × NCE7 buffer). The amplitude of the fast process is opposite to that of the slow process and is usually much smaller (cf. fig. 1). Since the time constant of the fast process is at the limit of time resolution of our light scattering detection, this process could not be analysed with sufficient accuracy (cf. section 3.1). Measurements over a range of concentrations revealing a strong concentration dependence for the on relaxation time (cf. fig. 3) did not show any concentration dependence of the time constant τ_2 associated with the slow off-field relaxation process. Apparently, the term describing the

Table 1

Rate parameters obtained from concentration dependences of the on-field relaxation process using electric field pulses of 24 kV/cm Evaluation according to an isodesmic model. Estimated accuracy of rate constants ±20%

Buffer	Average chain length	k_a^m [M^{-1} monomer s^{-1}]	k_a^h [M^{-1} helices s^{-1}]	k_d [s^{-1}]	K [M^{-1} helices]
1/2×NCE7	800	7.2×10^7	13×10^{10}	5.8×10^3	20×10^6
	500	5.0×10^7	5.0×10^{10}	3.7×10^3	13×10^6
	430	2.8×10^7	2.4×10^{10}	1×10^3	24×10^6
	370	1.9×10^7	1.4×10^{10}	0.8×10^3	17×10^6
NCE7	800	10.3×10^7	16×10^{10}	68×10^3	2.4×10^6
	500	6.9×10^7	6.8×10^{10}	34×10^3	2.0×10^6
	430	8.2×10^7	7.1×10^{10}	26×10^3	2.7×10^6
	370	4.4×10^7	3.3×10^{10}	29×10^3	1.1×10^6
	255	4.5×10^7	2.3×10^{10}	24×10^3	1.0×10^6
2×NCE7	430	6.8×10^7	5.8×10^{10}	135×10^3	0.43×10^6
	370	5.5×10^7	4.0×10^{10}	110×10^3	0.37×10^6

association for this process is negligible compared to that for the dissociation. This interpretation is supported by the dependence of the dissociation rate constants k_d upon the field strength. The k_d value extrapolated for zero field strength (10^5 s^{-1}) is rather close to the value $1/\tau_2 = 3.8\times10^4$ s^{-1}. Thus, the off-field relaxation essentially represents the dissociation of complexes formed during the field pulses. Similar data have been obtained for other DNA samples. An additional component in the off-field relaxation curves with time constants in the range of 100 μs to 1 ms is observed for longer DNA samples. The nature of this process has not yet been elucidated. It may result from the dissociation of large DNA aggregates.

3.3. Dichroism measurements

It may be expected that the field-induced interactions of DNA are also reflected in the electric dichroism and the orientational relaxation time of the DNA samples. Thus, we measured the dichroism of some samples under conditions corresponding to those of the light scattering experiments. It is well known that electric fields accelerate the orientation of DNA helices in the direction of the electric field vector, when the field strength is above a certain limit value (cf. ref. 9). Due to this acceleration effect the dichroism of a DNA sample with, e.g., 430 bp, induced by a field pulse of 14 kV/cm appears to reach a stationary, maximal level already within a few microseconds. However, application of longer field pulses reveals a decrease of the electric dichroism after approx. 10 μs. Part of the decrease in the electric dichroism may be explained by a decrease in the field strength due to conductivity of the sample. However, a quantitative comparison demonstrates that the decrease of the dichroism cannot be explained by the decay of the field strength alone. Apparently, the electric field induces some reaction of the DNA sample. This interpretation is supported by the relaxation curve observed after termination of the field pulse. The light intensity passes a minimum before relaxing towards the level observed before the field pulse. This peculiar effect in the off-field relaxation curve is not observed after short field pulses. An exponential analysis of the relaxation curves in the field free state reveals a time constant of 23 ± 3 μs as the slowest component appearing after short field pulses (e.g., up to 10 μs at 14 kV/cm). This time constant corresponds to the value expected according to the average length of the DNA helices used in the experiment. The slowest component detected after long field pulses is about 50 ± 10 μs and is associated with an amplitude opposite to the conventional one (fig. 6). The increase in the orientation relaxation time

induced by long field pulses indicates some association of DNA strands to a complex with larger molecular dimensions and probably also with reduced flexibility. The positive component of the dichroism decay curve suggests an unusual orientation of DNA bases in the complex. The length of field pulses required for the induction of the unusual dichroism effect is compatible with the times required for induction of the light scattering effects. Thus, it is likely that the light scattering and dichroism effects reflect the same molecular process.

Measurements at the magic angle do not provide evidence for any field-induced change of the DNA absorbance under the conditions of the present experiments. Thus, the field-induced reaction does not involve any large extent of DNA denaturation. It is known from independent investigations that DNA denaturation is only induced when the electric field strength exceeds a threshold value which is dependent upon DNA chain length and ionic strength [14]. The measurements described in the present investigation were performed well below the corresponding denaturation thresholds except for some experiments with DNA of particularly high chain length.

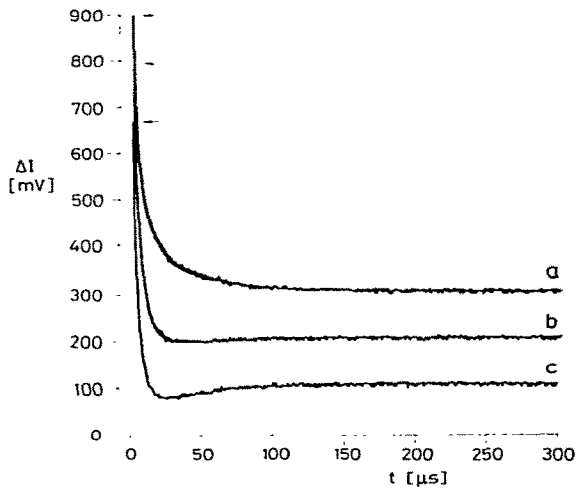

Fig. 6. Dichroism relaxation curves measured for a DNA sample with approx. 430 bp at 248 nm after electric field pulses of constant field strength (14.1 kV/cm) but different lengths: (a) 5.1 μs, (b) 15.7 μs and (c) 44 μs. The intensity level at the end of the field pulses are indicated by arrows; curves a and b are shifted against c by 200 and 100 mV, respectively.

Measurements of the electric dichroism at various wavelengths, λ, with strongly different absorbance of DNA (e.g., 289.4, 296.7, and 302.2 nm) exhibit very different absorbance changes resulting from field-induced orientation (factor of approx. 15 between 289.4 and 302.2 nm), but show an almost constant relative contribution of the positive dichroism effect. Thus, it is unlikely that the positive dichroism merely is a side effect resulting from a change in turbidity, since the observed amplitudes are not at all consistent with a $(1/\lambda)^4$ dependence which should be expected for effects due to light scattering in a first approximation.

3.4. Control experiments

The rate constants of dissociation measured at different field strengths (fig. 5) indicate that there should be very little association at zero field strength. This prediction has been checked by various experiments. The scattering intensity of DNA measured at a detection geometry equivalent to that used in the field-jump experiments increases linearly with the concentration and thus does not provide evidence of any strong association. More detailed measurements of DNA light scattering by various authors [25–27] showed that the second virial coefficient derived from the concentration dependence is positive over a broad range of salt concentrations and thus does not provide any evidence for DNA association. Measurements of dynamic light scattering performed by C. Koitz on one of our samples (= 430 bp in 1/2 × NCE7 buffer) also did not provide any evidence for strong association. Finally, we characterised the sedimentation velocity of one of our DNA samples (≈ 500 bp in buffer NCE7) over a wide range of concentrations and also did not find any indication of association.

4. Discussion

The influence of electric fields upon light scattering of DNA has been studied by various authors [28–33]. In general, changes in the light

scattering induced by electric fields have been attributed to the orientation of DNA helices. In the present investigation the main part of the observed scattering changes is attributed to an association reaction. Evidence for our interpretation comes from measurements over a wide range of concentrations. Apparently, the concentration dependence of field-induced scattering effects has not been studied over a sufficiently wide range in previous investigations. However, the main difference between the present and earlier investigations is in the range of field strengths applied to the DNA solutions. The field strengths applied in previous investigations apparently did not exceed 200 V/cm, whereas the range of field strengths used for the present measurements was 4–28 kV/cm. According to the strong dependence of the rate parameters upon the field strength (cf. fig. 5), it may be difficult to characterise a field-induced interaction of DNA at low field strengths. However, it is expected that at least some of the field-induced DNA interaction remains at low field strengths.

The main arguments in favour of the field-induced DNA interaction may be summarised as follows:

(1) The relative change of light scattering for a given field pulse strongly increases with increasing nucleotide concentration. If the scattering change were merely due to an orientation of the DNA, the relative effect $\Delta I/I_0$ should not depend upon the DNA concentration in the limit of ideal conditions. However, from the present amplitude measurements alone it is difficult to exclude contributions to non-ideal light scattering intensities from effects other than DNA association. For example, the scattering intensity may be affected by changes of the polymer conformation and/or the second virial coefficient (cf. textbooks of biophysics). More information on the interactions of large particles may be obtained by measurements of scattered light at different angles. However, our measurements were restricted to a fixed geometry due to the construction of our field-jump apparatus. In the absence of more detailed intensity measurements our conclusions are mainly based upon the information resulting from relaxation time constants.

(2) According to dichroism measurements the orientation of a DNA sample by an electric field pulse is faster by factors up to 70 than the time constant of the scattering effect observed under the same experimental conditions (cf. section 3.2). This result clearly demonstrates that at least for DNA samples with approx. 400 bp the process of orientation can be clearly separated on the time scale from the main part of the scattering effect.

(3) The concentration dependence of the time constants associated with the scattering changes is not compatible with a simple orientation mechanism. The increase of the reciprocal on-field relaxation time with increasing nucleotide concentration provides clear evidence for interactions between DNA molecules under electric field pulses. This concentration dependence also shows that the observed scattering effect does not result from 'orientational interactions' described recently by Marion et al. [34] for 'congested solutions'.

(4) The rate constants of dissociation measured as a function of the field strength clearly show the direction of the field-induced reaction: the interactions are relatively small at zero field strength, but increase strongly with increasing field strength. This conclusion is consistent with results from independent investigations showing the absence of interactions between DNA helices (positive second virial coefficient) at zero field strengths [25–27].

According to previous investigations electric fields usually induce the dissociation of macromolecular structures [11–16]. Thus, a field-induced association reaction of DNA has hardly been expected, since the high negative charge density of DNA helices should lead to repulsion especially at low ionic strength. Under these conditions a very special force is required to drive an association reaction. Some information on the driving force is available from the dependence of the rate constants upon the electric field strength. The linear relation between the logarithm of the dissociation rate constant and the square of the electric field strength suggests that the reaction is induced by some change of dipole moments [18,35]. It is well known that equilibrium constants K depend upon the electric field strength E according to

$$\frac{\partial \ln K}{\partial |E|} = \frac{\Delta M}{RT} \qquad (4)$$

where ΔM is the change of the molar dipole moment upon reaction, R the gas constant and T the absolute temperature. The change of the molar dipole moment upon a reaction between components i is given by

$$\Delta M = N_A f(\epsilon, n) \frac{\Sigma \nu_i p_i^2}{3kT} E \quad (5)$$

where N_A is Avogadro's number, p_i molecular dipole moments of the reactants with their stoichiometric coefficients ν_i and $f(\epsilon, n)$ a correction factor for the 'internal' and 'directing' field [36]. Eq. 5 is valid in the range $p_i E/kT < 1$. Integration of eqs. 4 and 5 provides

$$\ln k = f(\epsilon, n) \frac{\Sigma \nu_i p_i^2}{6k^2T^2} E^2 + \ln K_0 \quad (6)$$

where K_0 is the equilibrium constant at zero field strength. As a simple approximation we use the correction factor $f(\epsilon, n)$ derived for spherical molecules [36,37]

$$f(\epsilon, n) = \frac{(n^2+2)^2 \epsilon (2\epsilon + 1)}{3(2\epsilon + n^2)^2} \quad (7)$$

According to these equations we may evaluate an approximate value for the change of dipole moment required to explain our experimental data. From the slope -2.36×10^{-13} m^2/V^2 of the plot of $\log k_d$ vs. E^2 we obtain a change of dipole moment $\Sigma \nu_i p_i^2$ in the range of 1400 debye. This is a large change and almost seems to be beyond the range of possible molecular dipole changes. However, it has been demonstrated that the dipole moments of DNA helices induced by electric fields can be as high as 5000 debye [3,10]. Compared to these dipole magnitudes the change required to explain the field-induced association appears to be accessible.

It is generally accepted that the DNA dipole moment results from a polarisation of its ion atmosphere. However, a detailed molecular model of the polarisation and in particular of its saturation at relatively low field strength remains to be developed. Under these circumstances it is difficult to postulate a model for the apparent increase of the dipole moment in the associated state, especially in the absence of detailed information about the structure of the associated state. The DNA helices may form side-by-side aggregates [38], but an end-to-end association similar to the phenomenon of pearl chain formation [39,40] is also possible. Some information on the structure of the aggregates may be obtained from dichroism measurements using DNA restriction fragments of uniform length (unpublished results). These experiments should also provide more information on the unusual DNA aggregate associated with a positive dichroism.

The rate constants for the field-induced association of DNA are very high, when calculated in units of M^{-1} helices s^{-1}. We may compare these values with an estimate for a diffusion-controlled reaction. For this purpose we use a translation diffusion coefficient of 2.5×10^{-7} cm^2 s^{-1}, evaluated from measurements of dynamic light scattering for a DNA with 190 bp [41]. When we assume a reaction radius corresponding to the length of the DNA and a steric factor of 1 we arrive at a rate constant of 2.4×10^{10} M^{-1} s^{-1}. This value is of the same order of magnitude as observed for our short DNA samples. The increase of the experimental association rate constants with chain length can hardly be explained in terms of translational motion of whole polymer spheres, but is apparently associated with segmental motions of the polymer chains.

The field-induced interaction of nucleic acids is probably not essential for their biological function. Nevertheless, it is important to know about this reaction for several reasons. First of all it may strongly affect the results obtained in the analysis of nucleic acid structures by electro-optical methods. Recently, these methods have been widely used to investigate the structures of different double helices, chromatin and various intercalation complexes, for example. All these applications require great caution to avoid any field-induced association reaction. The new reaction demonstrates an activity of electric fields which has not yet been described in this form at the molecular level and may be important for bioelectric phenomena. It may also be useful for a further understanding of the nature of electric field effects in polyelectrolytes.

Acknowledgements

The authors are indebted to Professors H. Eisenberg and M. Mandel for discussions.

References

1 E. Fredericq and C. Houssier, Electric dichroism and electric birefringence (Clarendon Press, Oxford, 1973).
2 C.T. O'Konski, Molecular electro-optics (Marcel Dekker, New York, 1976).
3 M. Hogan, N. Dattagupta and D.M. Crothers, Proc. Natl. Acad. Sci. U.S.A. 75 (1978) 195.
4 P.J. Hagermann, Biopolymers 20 (1981) 1503.
5 N.C. Stellwagen, Biopolymers 20 (1981) 399.
6 C.H.Lee and E. Charney, J. Mol. Biol. 161 (1982) 289.
7 P.J. Ridler and B.R. Jennings, FEBS Lett. 139 (1982) 101.
8 X. Emonds-Alt, C. Houssier and E. Fredericq, Biophys. Chem. 10 (1979) 27.
9 S. Diekmann, W. Hillen, B. Morgeneyer, R.D. Wells and D. Porschke, Biophys. Chem. 15 (1982) 263.
10 S. Diekmann, W. Hillen, M. Jung, R.D. Wells and D. Porschke, Biophys. Chem. 15 (1982) 157.
11 C.T. O'Konski and N.C. Stellwagen, Biophys. J. 5 (1965) 607.
12 E. Neumann and A. Katchalsky, Proc. Natl. Acad. Sci. U.S.A. 69 (1972) 993.
13 D. Porschke, Biopolymers 15 (1976) 1917.
14 S. Diekmann and D. Porschke, Biophys. Chem. 16 (1982) 261.
15 K. Yoshioka, K.Kikuchi and M. Fujimori, Biophys. Chem. 11 (1980) 369.
16 G. Schwarz and U. Schrader, Biopolymers 14 (1975) 1181.
17 F.W. Studier, J. Mol. Biol. 11 (1965) 373.
18 F.S. Allen, D.M. Gray, G.P. Roberts and I. Tinoco, Biopolymers 11 (1972) 853.
19 H.H. Grunhagen, Meßtechnik (1974) 19.
20 D. Porschke, Nucleic Acids Res. 8 (1980) 1591.
21 R. Rigler, C.R. Rabl and T.M. Jovin, Rev. Sci. Instrum. 45 (1974) 580.
22 M. Eigen and L. DeMaeyer, in: Techniques of organic chemistry, eds. S.L. Friess, E.S. Lewis and A. Weissberger, (Interscience, New York, 1963) vol. 8, part 2.
23 C.F. Bernasconi, (Relaxation kinetics, Academic Press, New York, 1976).
24 D. Porschke and F. Eggers, Eur. J. Biochem. 26 (1972) 490.
25 M. Mandel and J. Schouten, Macromolecules 13 (1980) 1247.
26 N. Borochov, H. Eisenberg and Z. Kam, Biopolymers 20 (1981) 231.
27 Z. Kam, N. Borochov and H. Eisenberg, Biopolymers 20 (1981) 2671.
28 A. Scheludko and S. Stoylov, Biopolymers 5 (1967) 723.
29 C. Hornick and G. Weill, Biopolymers 10 (1971) 2345.
30 B.R. Jennings and H. Plummer, Biopolymers 9 (1970) 1361.
31 T. Fujikado, R. Hayakawa and Y. Wada, Biopolymers 18 (1979) 2303.
32 B.R. Jennings, in Molecular electro-optics, ed. C.T. O'Konski (Marcel Dekker, New York, 1976) p. 275.
33 B.R. Jennings, in: Molecular electro-optics, ed. S. Krause (Plenum Press, New York, 1981).
34 C. Marion, B. Roux and M. Hanss, Biopolymers 22 (1983) 2353.
35 L. DeMaeyer and A. Persoons, in: Techniques of chemistry, ed. A. Weissberger (Wiley, New York, 1974) Vol. VI, part 2.
36 C.J.F. Bottcher, Theory of electric polarisation, vol. I (Elsevier, Amsterdam, 1973).
37 L. Onsager, J. Am. Chem. Soc. 58 (1936) 1486.
38 M. Mandelkern, N. Dattagupta and D.M. Crothers, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 4294.
39 H.P. Schwan and L.D. Sher, J. ELectrochem. Soc. 116 (1969) 22C.
40 U. Zimmermann, Biochim. Biophys. Acta 694 (1982) 227.
41 M. Mandelkern, J.G. Elias, D. Eden and D.M. Crothers, J. Mol. Biol. 152 (1981) 153.