Biophysical Chemistry 22 (1985) 237–247
Elsevier

BPC 01010

THE MECHANISM OF ION POLARISATION ALONG DNA DOUBLE HELICES

Dietmar PORSCHKE

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

Received 3rd January 1985
Revised manuscript received 20th May 1985
Accepted 20th May 1985

Key words: DNA; Electric field effect; Polarisation; Ion binding; Electric dichroism

The orientation curves of short DNA fragments induced by electric field pulses are measured with high time resolution and analysed by efficient deconvolution techniques. A small, but clearly detectable delay of the 'on-field' orientation can be described accurately by the superposition of two exponential processes with opposite amplitudes. The time constant of the faster process is around 10 ns and the slower one in the range 50–1000 ns depending upon the electric field strength and chain length of the DNA fragment. The relation between amplitudes and time constants observed for each curve corresponds exactly to that expected for a convolution of two processes, where the first process is without optical response and becomes detectable only via the optical response of the second process. These results indicate that the first process reflects the polarisation of the ion atmosphere required for the second process of the orientation. Measurements at different ion concentrations c demonstrate that the reciprocal time constant of the fast process is a linear function of c and thus is consistent with an association reaction. The association rate constant evaluated from this dependence according to a simple bimolecular reaction model is 8×10^9 M^{-1} s^{-1} for a 95 base-pair fragment and is consistent with binding of Na^+ to the helix, a reaction close to the limit of diffusion control. The association rate constant is almost independent of the electric field strength E, while the dissociation rate constant k^- strongly increases with E, indicating dissociation of ions at high E values. The data suggest a linear correlation between $\log(k^-)$ and E^2 corresponding to a reaction driven by a dipole change. The apparent dipole change evaluated from this dependence is in the order of magnitude estimated for an elementary step of ion dissociation at one end of the helices. The combined results obtained from the polarisation and the orientation mechanism can be explained by dissociation of surprisingly few counterions biased towards one end of the helices. The experimental data obtained for a 76 base-pair fragment are analogous to those for the 95 base-pair fragment, whereas the 'slow' ion polarisation has not been detected for a fragment with 27 base-pairs. This result together with those obtained for the longer fragments at low field strengths indicate that there is a fast polarisation mechanism without 'ion dissociation' at low chain lengths and for low electric field strengths. This mechanism is replaced at high chain lengths and/or high electric field strengths by the ion dissociation mechanism. The change in the polarisation mechanism is paralleled by a change in the orientation mechanism deduced previously from measurements of the orientation equilibrium.

1. Introduction

Measurements of electric field effects induced in polyelectrolytes have been very useful for the investigation of their structure [1,2] and their interactions with ions [3]. Many of these investigations have been conducted with DNA molecules due to their biological importance and also due to their well defined structure which is advantageous for a quantitative comparison with theoretical models. The large degree of DNA orientation already observed at relatively low field strengths has been attributed to a high polarisability of the counterion atmosphere. A quantitative analysis of orientation data obtained for long DNA molecules suggested the existence of a large permanent dipole moment [4–6]. Since a large permanent dipole moment cannot be explained by the molecular structure of DNA itself, it has been postulated that the polarisability of the ion atmosphere is

already saturated at relatively low field strengths. Using an orientation model with a saturating polarisability [7–9] it has been possible to describe a large amount of experimental data obtained over a wide range of chain lengths with satisfactory accuracy. However, the molecular mechanism leading to the saturation phenomenon and, for example, its dependence upon the DNA chain length remain unexplained.

An experimental approach to the problem of these electric field effects is an analysis of the dynamics of ion polarisation. Information about this process may be obtained by a careful analysis of the field-induced orientation of DNA molecules. Since the ion polarisation is expected to be very fast, its analysis requires an experimental technique with high time resolution. Using a fast pulse generator [10] together with a detection system of high time resolution [11] and an efficient deconvolution procedure [12] it has been possible to characterise the dynamics of ion polarisation and its dependence upon various parameters including the ion concentration and electric field strength.

2. Materials and methods

The DNA fragments were kindly provided by W. Hillen. Cacodylic acid was purchased from Sigma, München. All other reagents were of analytical grade. The DNA fragments were dialysed extensively against the standard buffer containing 1 mM NaCl, 1 mM sodium cacodylate, pH 7.0, and 0.2 mM EDTA.

The orientation of the DNA fragments by electric field pulses was recorded by measurements of the linear dichroism. The field pulse generator [10] and the system for detection of the electric dichroism [11,12] were as described in the cited references. The distance between the electrodes in the measuring cell was 5.9 mm. The temperature of the samples was maintained at 20 ± 0.1°C by a thermostatted cell holder and controlled by a Pt100 sensor inserted into the upper electrode. The duration of the electric field pulses usually did not exceed 1 μs and thus heating of the sample was negligible. Some data were recorded by a Tektronix 7612 D transient digitizer, whereas most of the data obtained in the fast time range were transiently stored by a Tektronix R7912. All data were transmitted to the computation centre of the Gesellschaft für wissenschaftliche Datenverarbeitung mbH, Göttingen, for analysis of the time constants by an efficient deconvolution procedure [12].

3. Results

3.1. Time constants and amplitudes associated with the on-relaxation process

The field-free relaxation curves of short DNA restriction fragments observed after electric field pulses can usually be described by a single exponential [13]. Similar results were obtained for the on-relaxation process recorded in the presence of the electric field, i.e. when the electric field pulse is applied to the sample. However, a more detailed analysis of the on-process reveals a more complex relaxation. As shown in fig. 1 an accurate description of the dichroism data requires a second process with a time constant much shorter than that of the main process and with an opposite amplitude. This process has not been detected in previous investigations, but can now be demonstrated very clearly owing to improvements of our experimental techniques and data analysis. Our present detection system [11,12] has a much higher time resolution than that used previously and in addition the convolution of the orientation curves arising from the still limited response time of our experimental device is now considered more accurately by appropriate numerical procedures [12].

The additional fast relaxation process with an amplitude opposite to the main process essentially reflects a delay of the DNA orientation. A corresponding delay effect is known for the orientation of permanent dipoles [14]. It is obvious, however, that double helices should not be associated with any large permanent dipole moment. Since the dipoles observed for DNA molecules result from the polarisation of their ion atmosphere, it is likely that the additional process reflects the polarisation itself. The polarisation requires movements of ions

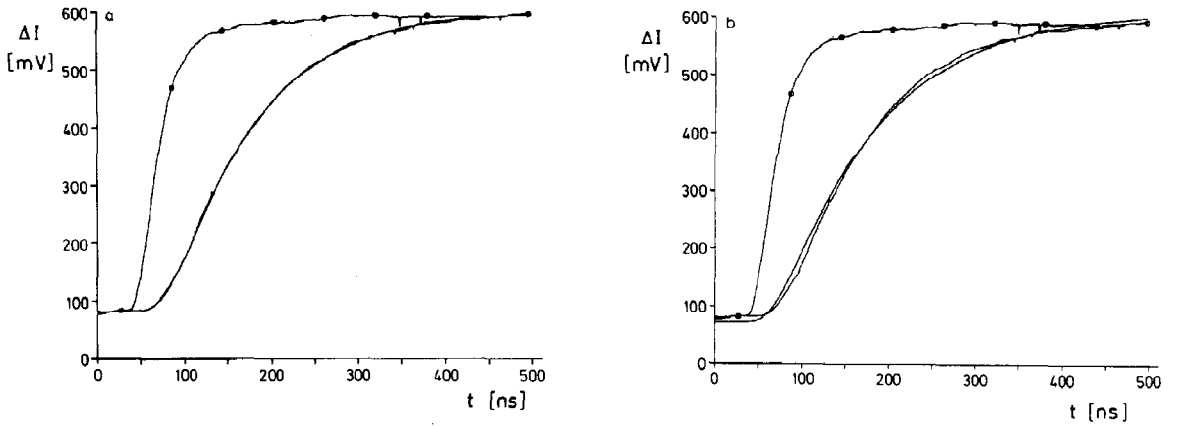

Fig. 1. On-field orientation curve for a 76 base-pair fragment induced by a field pulse of 61.9 kV/cm in 0.5 mM NaCl, 0.5 mM sodium cacodylate, pH 7.0, 0.1 mM EDTA (average curve obtained from 10 dichroism experiments). The line marked by circles is a birefringence curve of H_2O (induced by a corresponding field pulse and recorded with the same adjustment of the detector and transient recorder) and serves as a reference for deconvolution. (a) A fit with two exponentials ($\tau_p = 14$ ns and $\tau_r = 85.4$ ns with respective amplitudes of -104 and 620 mV) cannot be distinguished on this scale from the experimental curve. (b) A least-squares fit with a single exponential is shown for comparison. In this case the sum of squared residuals is larger by a factor of 20. (The lag period in the curve representing the single exponential fit results from convolution with the detector response curve. Note that this lag period is clearly not sufficient for an accurate description of the experimental curve.)

and thus will be associated with a limited time constant. As a first approximation we may assume that the polarisation is described by a simple exponential according to

$$P = P_\infty(1 - e^{-t/\tau_p}) \tag{1}$$

where P is the polarisation at time t and P_∞ the limit degree of polarisation at $t \to \infty$. The orientation of DNA observed by dichroism measurements is also described by a simple exponential

$$\Delta A = \Delta A_\infty(1 - e^{-t/\tau_r}) \tag{2}$$

where ΔA is the absorbance change at time t, ΔA_∞ the limit absorbance change at $t \to \infty$ and τ_r the rotation time constant

$$\tau_r = \frac{1}{6D} \tag{3}$$

where D is the rotational diffusion coefficient. Since the orientation of DNA helices requires polarisation of their ion atmosphere, the polarisation and the orientation process are convoluted with each other. The ion polarisation is not directly reflected by changes of the optical parameters, but only indirectly by the DNA orientation. Thus, the absorbance change resulting from the orientation [15,16] is given by

$$\Delta A = \Delta A_\infty\left(1 + \frac{\tau_p}{\tau_r - \tau_p}e^{-t/\tau_p} - \frac{\tau_r}{\tau_r - \tau_p}e^{-t/\tau_r}\right) \tag{4}$$

The form of eq. 4 suggests a simple test of the experimental data. Amplitudes and time constants have been fitted to the orientation curves as free variables without the restriction imposed by eq. 4. Provided that the model is correct the ratio of the amplitudes should be defined by the time constants as in eq. 4. Examination of a large number of on-field relaxation processes showed that the amplitude ratio always corresponds to the value expected from eq. 4 within experimental accuracy. Since eq. 4 only involves the assumption that polarisation and orientation can be described by simple exponentials, it is expected to be valid over a wide range of conditions. The experimental data, including those obtained at the highest electric

field strengths accessible by our instrument, do not indicate any deviations as long as short DNA fragments are concerned. Deviations observed for longer fragments are discussed in section 3.3.

It may be useful to compare the result obtained from the 'slow polarisation' model with a model assuming the presence of both a permanent and an induced dipole moment. According to Benoit [14] the rise curve is given in the limit of low electric field pulses by

$$\frac{\Delta A}{\Delta A_\infty} = 1 - \frac{3P/Q}{2(P/Q+1)} e^{-2\theta t} + \frac{P/Q-2}{2(P/Q+1)} e^{-6\theta t} \qquad (5)$$

with

$$P = \mu_p^2/k^2T^2$$

and

$$Q = \Delta\alpha/kT$$

where μ_p is the permanent dipole moment, $\Delta\alpha$ the polarisability and kT the thermal energy. According to this model the exponential time constants should be in a given ratio of 3 and the ratio of the amplitudes should also be fixed. The observed time constants and amplitudes are not consistent with eq. 5. The possibility cannot be excluded that the disagreement is partly due to the fact that the experimental data were obtained at rather high electric field strength, while eq. 5 is derived for low electric field pulses. However, even when the experimental data are extrapolated to zero field strength, there is no agreement with eq. 5, since the ratio τ_r/τ_p extrapolated for the 95 bp fragment, for example (by procedures described in the following sections), is much larger than 3.

All the on-field relaxation curves observed in the present investigation were found to be consistent with the ion polarisation model. Thus, it was not necessary to fit all the experimental data by a general exponential fitting routine allowing for a free variation of both amplitudes. Since the ratio of the amplitudes is defined by the time constants as described in eq. 4, the fitting procedure was adapted as follows: The two time constants τ_p and τ_r were fitted by a non-linear fitting procedure and the total amplitude by linear regression. The individual amplitudes were calculated from their ratio defined by the time constants and the total amplitude. This procedure led to a satisfactory representation of all the experimental data.

3.2. Dependence of the time constants on concentration and electric field strength

The nature of the polarisation process found in the on-field relaxation curves may be characterised in more detail by measurements of concentration dependences. For a control the polarisation time constant was measured at different helix concentrations. As should be expected for this process, the time constant is not dependent upon the DNA concentration (cf. fig. 2), at least in the range 5–20 μM, where interactions between helices are avoided.

Since the polarisation process involves motions of ions in the counterion atmosphere of the polyelectrolyte, it is possible that the polarisation time constant depends upon the ion concentration. As

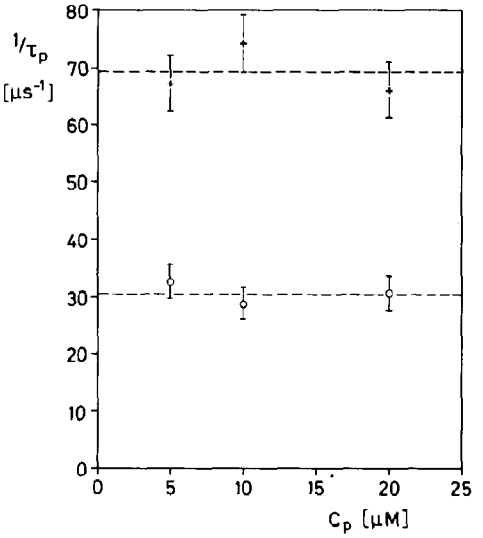

Fig. 2. Reciprocal polarisation time constant observed for the 95 base-pair fragment in 0.5 mM NaCl, 0.5 mM sodium cacodylate, pH 7.0, 0.1 mM EDTA as a function of the DNA concentration given in units of nucleotide residues at two different field strengths: (O) 39.3 kV/cm, (+) 69.8 kV/cm.

shown in fig. 3, the reciprocal of the polarisation time constant $1/\tau_p$ determined at a given electric field strength shows a linear correlation with the Na^+ concentration c_s. According to the experimental data the slopes of the plots of $1/\tau_p$ vs. c_s are virtually independent of the electric field strength, whereas the intercepts at $c_s = 0$ strongly increase with the electric field strength. An interpretation of these data in terms of a simple model is given in section 4.

The separation of two different processes in the on-field orientation curves has been mainly used in the present investigation to characterise the 'slow' polarisation mechanism. However, the separation also provides new information on the rotation of rod-like DNA molecules under electric field pulses. The dependence of the rotation time constants upon the electric field strength found in previous investigations appeared to be relatively complex [13]. This may have been partly due to the fact that the convolution of the polarisation and the orientation process has not been recognised. When the rotation time constant τ_r is evaluated correctly according to eq. 4, a very well defined and simple dependence upon the electric field strength is detected. As shown in fig. 4, $1/\tau_r$ increases with the square of the electric field strength.

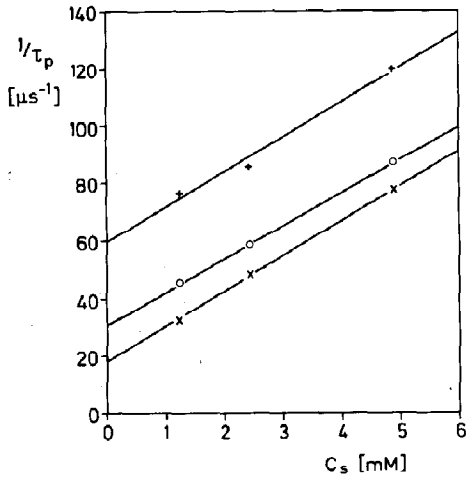

Fig. 3. Dependence of the reciprocal polarisation time constant $1/\tau_p$ upon Na^+ concentration for a helix with 76 base-pairs at three different field strengths: (×) 24.6 kV/cm, (○) 54.1 kV/cm, (+) 68.8 kV/cm.

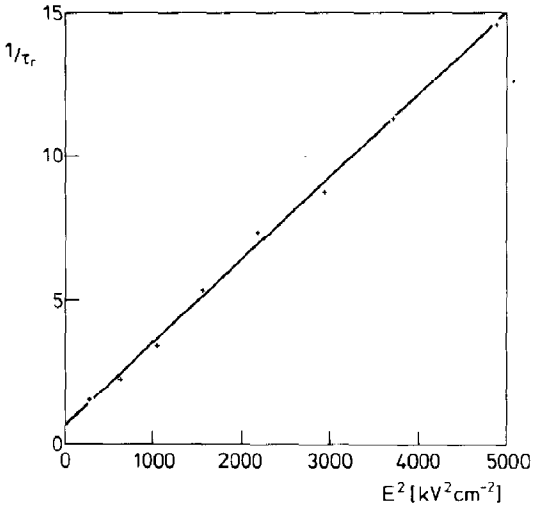

Fig. 4. Reciprocal rotation time constant $1/\tau_r$ for the 95 base-pair fragment as a function of the square of the electric field strength E^2 (0.5 mM NaCl, 0.5 mM sodium cacodylate, pH 7, 0.1 mM EDTA).

Measurements at different DNA concentrations c_p in the range 5–20 μM nucleotide residues reveal that τ_r does not depend on c_p. However, τ_r increases with the salt concentration c_s. This may be illustrated by a set of τ_r values observed for the 95 base-pair fragment at an electric field strength of 61 kV/cm, where the rotation time constant τ_r is 88.5 ns at $c_s = 1.22$ mM, 108 ns at $c_s = 2.44$ mM and 124 ns at $c_s = 4.88$ mM. The linear correlation of $1/\tau_r$ with E^2 is observed at all the different salt concentrations. The slopes of the graphs of $1/\tau_r$ vs. E^2 decrease with increasing salt concentration.

3.3. Dependence upon chain length

As described above, the observed polarisation effects are very similar for the fragments with 76 and 95 base-pairs. Since experimental data for an extended range of chain lengths may provide more detailed information on the polarisation mechanism, the on-field orientation curves have been characterised for two additional DNA fragments.

The chain length of one of these fragments with 187 base-pairs is close to the persistence length [13] and thus an influence resulting from bending of these molecules cannot be excluded. The on-field curves observed for these fragments show a clear delay effect similar to that observed for the 76 and 95 base-pair fragments. However, the fits obtained with two exponentials according to eq. 4 are not as exact as found for the shorter fragments. Although the reciprocal values of the polarisation time constants increase with Na^+ concentration, the correlation is not as high as observed for the shorter fragments. Apparently these difficulties result from the fact that the 187 base-pair fragment can no longer be regarded as a rigid rod. Since the off-field orientation curves observed for this fragment require two exponentials for a reasonable representation, the on-field orientation will also be more complex than described by eq. 4. It may be concluded that the polarisation mechanism for the 187 base-pair fragment is probably similar to that observed for the 76 and 95 base-pair fragment, but cannot be determined with sufficient accuracy due to difficulties resulting from DNA bending.

Another fragment with 27 base-pairs was investigated to explore the polarisation mechanism in the limit of low chain lengths. The on-field orientation curves were measured at a nucleotide concentration of 30 μM with a satisfactory signal-to-noise ratio. The rotation time constant τ_r was found in the range around 40 ns with a slight decrease at increasing field strengths. A delay effect was not detectable. The polarisation time constants τ_p obtained by least-squares fits of the on-field curves according to eq. 4 were in the range below 0.5 ns and thus clearly smaller than those found for the longer fragments. Since the binding of ions to the 27 base-pair fragments and its kinetics are expected to be similar to those for the longer fragments, the on-field curves observed for the short fragment indicate the existence of a different polarisation mechanism. This result seems to be related to the fact that the stationary dichroism as a function of the field strength observed for this fragment can be represented by the induced dipole mechanism over the whole range of accessible electric field strengths (cf. ref. 7).

Another observation for the orientation curves of longer fragments appears to be related to this phenomenon. The polarisation time constants τ_p found for the 76 and 95 base-pair fragments increase with decreasing electric field strength (cf. fig. 3) provided that the electric field remains above a limit range of electric field strengths around 25 kV/cm. In spite of a relatively high variation of the τ_p values at low field strengths, the available data indicate a decrease of τ_p for field strengths lower than 25 kV/cm. This decrease may result from a superposition of two different polarisation mechanisms. One of them is reflected by the relatively slow polarisation process, which is dominant at medium and high chain lengths together with high electric field strengths. The other mechanism prevails at low chain lengths and/or low electric field strengths.

4. Discussion

The effects of electric fields upon biopolymers are usually induced by a polarisation of their ion atmosphere. Thus, an understanding of these electric field effects requires knowledge on the mechanism of ion polarisation. Up to now the knowledge available on this mechanism mainly came from rather indirect observations, for example, the stationary degree of orientation as a function of electric field strength. The present approach provides more direct information by an analysis of the polarisation dynamics. Due to the high rate of the ion polarisation its analysis requires an experimental technique with a particularly high time resolution. Since the polarisation time constants are very close to the limit of time resolution of both the pulse generator and the detection unit used for the measurements, an analysis was only possible by an efficient deconvolution technique. Another essential for the present investigation was the availability of DNA restriction fragments. It has been demonstrated that these fragments in the range of chain lengths up to 100 base-pairs exhibit hydrodynamic properties like rigid rods and that their orientation can be described by a single time constant [13]. Thus, the identification of an additional process in the on-field orientation curves cannot be attributed to any heterogeneity of the samples.

In addition to the different and well defined chain lengths available for nucleic acids, these molecules are particularly suitable for the present investigation because of their large optical anisotropy.

The polarisation of the ion atmosphere along DNA helices has usually been envisaged as resulting from motions of ions along the long axis of the molecules [17–21] corresponding to a mechanism with relatively small 'displacements' of individual ions along the double helix. The results obtained in the present investigation provide new information on the polarisation process which cannot be explained quantitatively by any of the published theories of ion polarisation along polyelectrolytes. In the absence of a complete theory it will be useful to explain the results in a semi-quantitative manner by a relatively simple model.

4.1. Polarisation by ion dissociation

The linear dependence of the reciprocal polarisation time constant $1/\tau_p$ upon the ion concentration c_s suggests a very simple interpretation by an ion dissociation reaction according to

$$HI \underset{k^+}{\overset{k^-}{\rightleftharpoons}} H^- + I^+ \qquad (6)$$

For this reaction the reciprocal relaxation time constant $1/\tau$ is given by [15]

$$1/\tau = k^+([I^+] + [H^-]) + k^- \qquad (7)$$

where k^+ and k^- are the rate constants of association and dissociation, respectively. $[I^+]$ and $[H^-]$ represent the concentration of ions and helices, respectively. Since $[I^+]$ is much larger than $[H^-]$ (and [HI]) in all experiments, the contribution of $[H^-]$ may be neglected, which is in agreement with the polarisation time constant being independent of the helix concentration (cf. fig. 2).

The linear dependence of $1/\tau_p$ upon the ion concentration may not be regarded as a proof for a polarisation mechanism via ion dissociation. The parameters evaluated according to the ion dissociation mechanism provide a more stringent test for its validity. The rate constant of ion association evaluated according to eq. 7 from the data given in fig. 3 for a helix with 76 base-pairs is 1.1×10^{10} M^{-1} s^{-1}. A similar value (8×10^9 M^{-1} s^{-1}) is found for a helix with 95 base-pairs. These rate constants are at the limit of a diffusion-controlled reaction as should be expected for the association of Na^+ to DNA double helices. Furthermore, the measured rate constants are in close agreement with those obtained previously for the formation of complexes between Na^+ and simple ligands [22]. This agreement can hardly result by accident and provides strong evidence for a polarisation mechanism involving ion dissociation.

4.2. Dependence upon electric field strength and the mechanism of the field-induced reaction

Measurements at different field strengths E show (cf. fig. 3) that the k^+ value is almost independent of E, whereas the dissociation rate constant k^- strongly increases with E. This dependence demonstrates that high electric field pulses induce dissociation of ion complexes HI. It is well known that high electric field pulses lead to the dissociation of ion pairs. This reaction was discovered by Wien [23] and a corresponding theory was developed by Onsager [24] for the case of simple electrolytes. A 'dissociation field effect' has also been demonstrated for polyelectrolytes [25] including DNA [26]; however, an exact theory for this case remains to be developed. Various measurements indicate that the linear dependence of the dissociation rate constant upon the electric field strength E, which is characteristic of a dissociation field effect of simple weak electrolytes, is also valid for the case of polyelectrolytes [27]. Thus, evidence for a dissociation field effect may be obtained by an analysis of the dependence of the dissociation rate constant k^- on the field strength E. Due to the limited accuracy of the experimental data an unequivocal decision on the functional relationship between k^- and E is relatively difficult. A linear regression of the k^- values obtained for the 76 base-pair fragment, for example, with the electric field strength provides a reasonable correlation. However, extrapolation of the straight line obtained by linear regression to zero field strength leads to a negative k^- value, which is without any physical sense. A corresponding result is obtained for the 95 base-pair fragment. Thus, the present experimental data can

hardly be explained by a dissociation field effect.

Another simple mechanism to explain the field-induced effect is a reaction driven by a change of the dipole moment [15,28]. According to this mechanism the logarithm of the dissociation rate constant is given by

$$\ln(k^-) = f(\varepsilon,n)\frac{\sum \nu_i p_i^2}{6k^2T^2}E^2 + \ln(k_0^-) \qquad (8)$$

where k_0^- is the rate constant at zero field strength, p_i the dipole moments of the reactants with their stoichiometric coefficients ν_i, kT the thermal energy, $f(\varepsilon,n)$ a correction factor for the 'internal' and 'directing' field [29] and E the electric field strength. It should be noted that eq. 8 is valid for the case of changes of permanent dipole moments. Application of this equation can be justified by the fact that the polarisation dynamics is resolved in the range of field strengths where the orientation of DNA molecules follows a permanent dipole mechanism (cf. sections 3.3 and 4.3).

An unequivocal decision concerning this mechanism is again difficult due to the limited accuracy of our data. The correlation between $\log k^-$ and E^2 of the data obtained for the 76 and 95 base-pair DNAs is slightly higher than that found in the interpretation of the same data according to the dissociation field effect (cf. fig. 5). This increase would certainly not be sufficient for a decision in favour of the dipole-driven mechanism. However, the interpretation by the dipole-driven mechanism is strongly supported by the fact that in this case the extrapolation of $\log(k^-)$ to zero field strength leads to reasonable k_0^- values. For example, the dissociation rate constant of Na^+ from the 76 base-pair fragment k_0^- is about $1.3 \times 10^7\ s^{-1}$. A corresponding rate constant of $1.2 \times 10^7\ s^{-1}$ was evaluated for the 95 base-pair fragment.

The experimental data discussed above demonstrate a field-induced dissociation of ions. Obviously, this effect would not lead to a dipole if the ions are dissociated homogeneously along the double helix. Apparently, counterions are dissociated preferentially at one of the ends of the double helix, where both ends of the double helix are equivalent in this respect. According to this model the dipole of the helix arises stepwise and thus cannot be described by the usual polarisability. The dipole moment resulting from the dissociation of a counterion at one end of a double helix is $0.5le_0$, where l is the length of the double helix and e_0 the elementary charge. This 'elementary dipole' for a helix with 76 base-pairs is 2.1×10^{-27} C m and may be compared with the change of the dipole moment evaluated from the slope $d(\log k^-)/d(E^2)$ according to eq. 8. When the correction factor $f(\varepsilon, n)$ is assumed to be 1, the dipole change obtained for the 76 base-pair fragment is 1.7×10^{-27} C m and for the 95 base-pair fragment 1.9×10^{-27} C m. The close agreement between the elementary dipole and the experimental dipole change indicates that the simple model applied for the interpretation of the experimental data is a useful approximation. However, some additional factors have to be considered. Since the ion dissociation will hardly be restricted to a single elementary step, a simple correspondence between elementary and experimental dipole changes cannot be valid due to the dependence of $\ln(k^-)$ upon $\Sigma \nu_i p_i^2$ (cf. eq. 8). Provided that the dissociation of each ion can be regarded as a separate elementary step, the averaged correction factor resulting from the quadratic dependence remains in the range of 2 as long as the number of elementary steps does not exceed 5–6. According to the overall dipole moment evaluated from the stationary degree of orientation by the saturating induced dipole model (8.9×10^{-27} C m for the 76 base-pair fragment [7]), the ion dissociation is limited to a surprisingly low number of elementary steps. All these considerations remain qualitative for various reasons; e.g., shielding of charges by counterions will tend to decrease the magnitude of an elementary dipole below the maximal value. Nevertheless, qualitative considerations based on the present experimental results may be useful for the future development of a quantitative model.

A polarisation mechanism via ion dissociation can most easily be envisaged when the ions in the bound state are attached to sites at the helix surface. However, according to the present state of knowledge, it appears to be more likely that in the case of ions such as Na^+ the major part of the ions associated with the helix are not attached to the helix via 'site binding', but surround the helix as a highly mobile ion cloud usually described by the

term 'ion atmosphere binding'. The present results do not provide evidence for one of these modes of binding. Although a polarisation mechanism via ion dissociation can be envisaged more easily for site-bound counterions, a corresponding mechanism for the case of ion atmosphere binding is also possible.

The equilibrium constant K of ion binding obtained from the rate constants extrapolated to zero field strength is about 800 M^{-1}. Due to the complexity of ion binding to polyelectrolytes the model used for the evaluation of the data and also the values resulting from this model can only be regarded as a first approximation. However, a comparison with data obtained for single-stranded polynucleotides, where ion binding can be followed by measurements of absorbance changes [30], indicates that the equilibrium constant obtained from the polarisation time constants is at least consistent with binding of ions to the double helix. The equilibrium constant may be regarded as an approximate value that can be used to describe the dissociation of a few ions from the double helix within a limited range of the ionic strength.

4.3. The polarisation mechanism at low chain lengths and / or low field strengths

The experimental data demonstrate that ion dissociation provides a dominant contribution to the polarisation mechanism only in a restricted range of chain lengths and electric field strengths. The polarisation mechanism involving dissociation of ions is indicated by a relatively slow polarisation time constant. The absence of a slow polarisation effect for the 27 base-pair fragment demonstrates that the polarisation mechanism is different in this case and probably involves only minor displacements of the ion positions along the DNA. It is very likely that this observation on the polarisation mechanism is directly related to independent observations on the orientation mechanism [7]. It has been shown previously that the orientation of small fragments (e.g., with 27 base-pairs) follows an induced dipole mechanism over the whole range of field strengths investigated, whereas the orientation of larger fragments (e.g., with 76 and 95 base-pairs) follows a permanent dipole mechanism, when the electric field strength exceeds the range of about 25 kV/cm. Thus, it is likely that the fast polarisation mechanism is related to the induced dipole orientation and that the slow ion dissociation mechanism is related to the permanent dipole orientation. If this relation is correct, it should be possible to detect indications of a fast polarisation mechanism for the longer fragments, when the electric field strength remains below the transition range [7]. Unfortunately it is rather difficult to determine τ_p values at low field strengths with satisfactory accuracy. Nevertheless, the available data indicate a decrease of the τ_p values found for the 76 and 95 base-pair fragments when the electric field strength decreases below 25 kV/cm, which is in contrast to the opposite dependence found at high electric field strengths. These results suggest a direct relation between the two polarisation and orientation mechanisms. A close relationship between these mechanisms should be expected anyway, since the orientation is a consequence of the polarisation. Thus, the demonstration of two different polarisation mechanisms should also be useful for the development of appropriate orientation functions.

4.4. The acceleration of DNA rotation at high electric field strengths

The separation of two processes in the on-field orientation curves provides new information not only on the polarisation dynamics but also on the rotation of DNA fragments under high electric field pulses. As shown in fig. 4, the reciprocal rotation time constant $1/\tau_r$ is a linear function of the square of the electric field strength.

A corresponding relation was predicted a long time ago by Schwarz [31]

$$1/\tau_r = \frac{2\alpha_r D_r}{kTx_0} E^2 \qquad (9)$$

where α_r is the polarisability, D_r the rotation diffusion coefficient and x_0 a factor which is approx. 3.35. Although this equation results from a rather simple model, it may be useful to estimate the polarisability α from the observed slope

$d(1/\tau_r)/d(E^2)$. The value $\alpha_r = 7.5 \times 10^{-29}$ C cm^2 V^{-1} obtained by this procedure for the 95 base-pair fragment, for example, in the standard buffer is much higher than that obtained previously [7] from the saturating induced dipole model $\alpha_s = 4.5 \times 10^{-33}$ C cm^2 V^{-1}. The difference is apparently due to the fact that α_s is derived from the orientation equilibrium at relatively low field strengths, whereas α_r is determined from data measured at relatively high electric field strengths. According to the data obtained for the 27, 76 and 95 base-pair fragments the polarisability α_r increases approximately with the square of the chain length.

The intercept $1/\tau_r^0$ obtained by extrapolation to $E = 0$ is relatively small and thus cannot be determined with high accuracy. However, in all cases investigated, τ_r^0 was larger than the rotation time constants obtained from the off-field orientation curves. This is apparently due to the fact that eq. 9 is valid in the limit of high electric field strength and thus extrapolation to $E = 0$ is not expected to provide a value corresponding to that evaluated from off-field orientation curves.

4.5. Limits of the simple polarisation model and general conclusions

The model discussed above for the polarisation of double helices by ion dissociation should be regarded as a rough approximation, since the various elements of the model do not describe the complexity of the molecular processes expected for polyelectrolytes in high electric fields in sufficient detail. The polarisation of polyelectrolytes is known to be an extremely complex process, which is very difficult to describe quantitatively [17–21]. Even sophisticated models can hardly be developed without serious approximations. From this point of view the simple model described above is surprisingly successful, since it provides a reasonable value for the rate constant of ion association and also a realistic magnitude for the change of the dipole moment. These results demonstrate that at least the essential elements of the model are not too far from reality and thus should be considered in the future development of more detailed polarisation models.

The experimental data obtained in the present investigation indicate the existence of two limit mechanisms of counterion polarisation along DNA helices. The mechanism observed in the limit of low electric field strengths and/or low chain lengths probably involves only small displacements of the counterions, whereas strong evidence for the dissociation of ions is provided in the limit of high electric field strengths and/or high chain lengths. Dissociation of ions induced by high electric fields is a process well known from many different investigations [27] and thus it is not surprising that field-induced dissociation of ions contributes to polarisation. A general field-induced dissociation of ions may also be a reason for the experimental observation that the dipole moments obtained for DNA helices at high electric field strengths are limited to relatively low magnitudes [7,32].

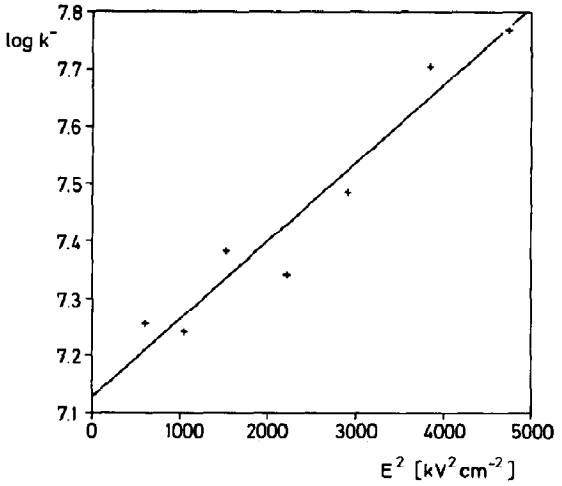

Fig. 5. Logarithm of the dissociation rate constant $\log(k^-)$ as a function of the square of the electric field strength E^2 for the 76 base-pair fragment. The straight line was obtained by linear regression.

The identification of the ion dissociation mechanism from orientation curves is not only useful for an understanding of polarisation and orientation mechanisms. The quantitative parameters obtained for the ion dissociation can be very useful to characterise the mode of ion binding. This approach is particularly convenient, since the ion dissociation can be followed without addition of any indication system.

Acknowledgements

The expert technical assistance of J. Ronnenberg is gratefully acknowledged. The author is also indebted to Professor W. Hillen for a generous gift of restriction fragments, to M. Jung for an adaption of an exponential fitting program for the present project and to Professor M. Mandel for comments on the manuscript.

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 D. Porschke in: Molecular electro-optics, ed. S. Krause (Plenum Press, New York, 1981) p. 269.
4 D. Ding, R. Rill and K.E. Van Holde, Biopolymers 11 (1972) 2109.
5 X. Emonds-Alt, C. Houssier and E. Fredericq, Biophys. Chem. 10 (1979) 27.
6 S. Sokerov and G. Weill, Biophys. Chem. 10 (1979) 161.
7 S. Diekmann, W. Hillen, M. Jung, R.D. Wells and D. Porschke, Biophys. Chem. 15 (1982) 157.
8 K. Yoshioka, J. Chem. Phys. 79 (1983) 3482.
9 S. Diekmann, M. Jung and M. Teubner, J. Chem. Phys. 80 (1984) 1259.
10 H.H. Grünhagen, Thesis, University of Braunschweig (1974).
11 C.R. Rabl, Thesis, University of Konstanz (1984).
12 D. Porschke and M. Jung, J. Biomol. Struct. Dyn. (1985) in the press.
13 S. Diekmann, W. Hillen, B. Morgeneyer, R.D. Wells and D. Porschke, Biophys. Chem. 15 (1982) 263.
14 H. Benoit, Ann. Phys. 6 (1951) 561.
15 M. Eigen and L. De Maeyer, in; Techniques of organic chemistry, eds. S.L. Friess, E.S. Lewis and A. Weissberger (Interscience, New York, 1963) vol. 8, part 2.
16 I. Tinoco, Jr and K. Yamaoka, J. Phys. Chem. 63 (1959) 423.
17 M. Mandel, Mol. Phys. 4 (1961) 489.
18 A. Morita and H. Watanabe, Macromolecules 17 (1984) 1545.
19 W. van Dijk, F. van der Touw and M. Mandel, Macromolecules 14 (1981) 792.
20 M. Fixman and S. Jagannathan, J. Chem. Phys. 75 (1981) 4048.
21 M. Mandel and T. Odijk, Annu. Rev. Phys. Chem. 35 (1984) 75.
22 M. Eigen and G. Maass, Z. Phys. Chem. 49 (1966) 165.
23 M. Wien, Phys. Z. 32 (1931) 545.
24 L. Onsager, J. Chem. Phys. 2 (1934) 599.
25 K.F. Wissbrun and A. Patterson, Jr, J. Polym. sci. 33 (1958) 235.
26 N. Ise, M. Eigen and G. Schwarz, Biopolymers 1 (1963) 343.
27 D. Porschke, Annu. Rev. Phys. Chem. 36 (1985) in the press.
28 D. Porschke, H.J. Meier and J. Ronnenberg, Biophys. Chem. 20 (1984) 225.
29 C.F.J. Böttcher, Theory of electric polarisation, vol. I (Elsevier, Amsterdam, 1973).
30 D. Porschke, Biophys. Chem. 4 (1976) 383.
31 G. Schwarz, Z. Phys. 145 (1956) 563.
32 M. Hogan, N. Dattagupta and D.M. Crothers, Proc. Natl. Acad. Sci. U.S.A. 75 (1978) 195.