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DNA double helices with positive electric dichroism and permanent dipole moments: Non-symmetric charge distributions and "frozen" configurations

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Abstract

The stationary electric dichroism of DNA restriction fragments with chain lengths of 399, 587 and 859 base pairs (bp) is shown to be positive at low electric field strengths in the range up to about 1 kV/cm; the dichroism is changed to values with the usual negative sign at higher electric field strengths. A positive dichroism at low field strengths has been observed in buffers with Na^+-cations of various ionic strengths from 1 to 11 mM. Experiments with reversal of the electric field vector indicate that the alignment is induced by a permanent electric moment at low field strengths; when the field strength is increased, the contribution from an induced dipole moment increases and becomes dominant at high field strengths. Thus, the permanent dipole mechanism is prevailing in the range with positive values of the dichroism found at low electric fields, whereas the induced dipole mechanism is dominant in the range with negative values of the stationary dichroism found at high electric fields. In contrast to these results obtained in buffers containing only monovalent ions, the electric dichroism is negative in the whole accessible range of electric field strengths, when the buffer contained 100 μM Mg^{2+}. The stationary values of the dichroism are negative under all tested conditions of buffer concentrations and electric field strengths for DNA double helices with 95, 179, 256, 4361 and 48502 bp. The positive dichroism found in the intermediate range of chain lengths is reduced and finally changed to the standard negative dichroism by UV irradiation. Positive values of the linear dichroism are predicted by Monte Carlo simulations for ensembles of wormlike chains with the charge density and optical parameters of DNA double helices, when the polarizability along the chain axis is negligible and when the internal mobility is frozen. The positive dichroism is due to the fact that virtually all DNA fragments are bent by thermal agitation; most of these bent DNA's are asymmetric and, thus, are associated with permanent dipole moments; the majority of dipole vectors in a given range of chain lengths is perpendicular to the end-to-end vector and, thus, leads to positive values of the linear dichroism at low degrees of bending. The model predicts a chain length dependence consistent with the experimental one due to a superposition of chain length dependences of the dipole moment and of the dichroism. The change from positive to negative values of the linear dichroism at increasing field strengths suggests some increase of the polarizability with the field strength, but may also be partly due to field induced stretching. The absence of the special effect in the presence of Mg^{2+} indicates a particularly high compensation of the phosphate charges by this ion. The time constants of electrooptical rise curves observed in the range with the positive stationary dichroism are rather close to those of the decay curves. It is shown by Brownian dynamics simulations that this result is consistent with the existence of a permanent dipole moment. The deviation from the standard expected for permanent dipoles is due to the fact that in the present case the dipole vectors are mainly perpendicular to the long axis

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of the molecules. The decrease of the positive dichroism by UV irradiation is consistent with the interpretation of the data by a frozen ensemble of configurations and indicates that photoproducts introduce flexible joints into the DNA chains.

Keywords: Bending dynamics of DNA; DNA restriction fragments; Electrooptics; Photoreaction; Wormlike chain model

1. Introduction

Double helical DNA has been a favored object for electrooptical investigations [1–15], partly because of its biological relevance but also because of its high electrical and optical anisotropy leading to large electrooptical effects, which can be characterized most accurately. According to the structure of the DNA double helix, its electric dichroism and birefringence are expected to be of negative sign. This expectation has been confirmed throughout the available scientific literature. In the present investigation it is shown that double helical DNA in a given range of chain lengths and salt concentrations exhibits both stationary electric dichroism and stationary birefringence of positive sign under conditions where the double helices are clearly native. Moreover, the electric field strengths applied in the present experiments are lower than those used in most previous investigations and, thus, the results of the present experiments are less likely to be affected by field induced changes of conformation and should be more representative of the native state of these molecules than the results of any previous investigations.

The present results might have caused considerable irritations if obtained some decades ago. Now, the structure of DNA has been established beyond doubt and owing to progress in the theoretical interpretation of electrooptical data, positive values of the linear dichroism can be explained without claiming an unusual molecular structure of DNA or unconventional electrical parameters. In this context, the information obtained from the new experiments is on a more special level: as shown below, the data tell us new details about the structure and the dynamics of DNA double helices and also reveal information on the mode of ion binding.

2. Materials and methods

The DNA fragment with 587 base pairs (bp) was prepared from the plasmid pUCBM20 (obtained from Boehringer Mannheim) by the restriction nuclease *Hae*III. The fragments with 179, 256 and 859 bp were prepared from the plasmid pRW574 (kindly provided by W. Hillen) by *Hae*III digestion; the fragment with 95 bp was obtained from the same plasmid by *Eco*RI digestion. The fragment with 399 bp was cut from a pUC18/19 plasmid with an insert from the DNA of *Chironomus thummi thummi* by *Eco*RI and *Hin*dIII. The fragments were separated by ion exchange HPLC. λDNA was obtained from Pharmacia, pBR322 DNA was from Boehringer Mannheim. All DNA samples were dialyzed extensively, first against 1 *M* NaCl, 10 m*M* Na-cacodylate pH 7, 1 m*M* EDTA and finally against 1 m*M* NaCl, 1 m*M* Na-cacodylate pH 7.0, 0.2 m*M* EDTA (buffer A) or against 1 m*M* NaCl, 1 m*M* Na-cacodylate pH 7.0, 100 μM $MgCl_2$ (buffer B).

The samples were exposed to electric field pulses in standard cuvettes of 1 cm optical pathlength with an insert machined from teflon, holding platinum electrodes at a distance of 4.8 mm. Electric field pulses were generated by a Cober pulse generator model 606; two of these generators were combined for pulse reversal experiments. Linear dichroism signals were recorded by an optoelectronic detection system, which has been developed for measurements of temperature jump relaxation [16]: a 600 W Hg/Xe lamp was used as a light source together with a Schoeffel GM250 grating monochromator; the dichroism was measured at the 248.2 nm mercury line; the light was polarized by a glan prism obtained from Halle (Berlin); part of the light beam was reflected by a quartz plate on a reference multiplier; the reference signal was used for compensation of lamp fluctuations transmitted into the signal of the dichroism detector. The output signal was recorded together with the enforcing electric field by a Tektronix 7612D programmable digitizer. The data were transferred via an LSI 11/23 to the facilities of the Gesellschaft für wissenschaftliche Datenverarbeitung mbH Göttingen and were analyzed by various fitting procedures, including an exponential fitting routine with an efficient deconvolution algorithm [17].

3. Results

3.1. Electrooptical experiments

When solutions of DNA fragments with 587 bp or with 859 bp are exposed to electric field pulses of e.g. 500 V/cm, the transmission of light polarized parallel to the electric field vector is clearly decreased (cf. Fig. 1). Measurements at the magic angle orientation of the polarized light do not reveal any field induced conformation changes under these conditions and, thus, the experiments demonstrate a positive electric dichroism for native double helical DNA. Measurements at different electric field strengths reveal a maximum of the positive electric dichroism at rather low field strengths, usually in the range below 1 kV/cm (cf. Fig. 2). At higher field strengths the electric dichroism is changed to values with the standard negative sign. The dependence of the reduced linear dichroism $\Delta\epsilon/\epsilon$ on the electric field strength is strongly influenced by the salt concentration (cf. Fig. 2). Positive values of the dichroism have been observed for the fragment with 587 bp in the range of salt concentrations from ~ 1 mM to ~ 11 mM in buffers containing monovalent ions. A maximal value in the positive dichroism is observed at an ion concentration corresponding to 2.4 mM. In contrast to the results found in buffers containing monovalent ions exclusively, no indication of any positive dichroism effect has been observed in a buffer containing 100 μM Mg^{2+} ions (buffer B).

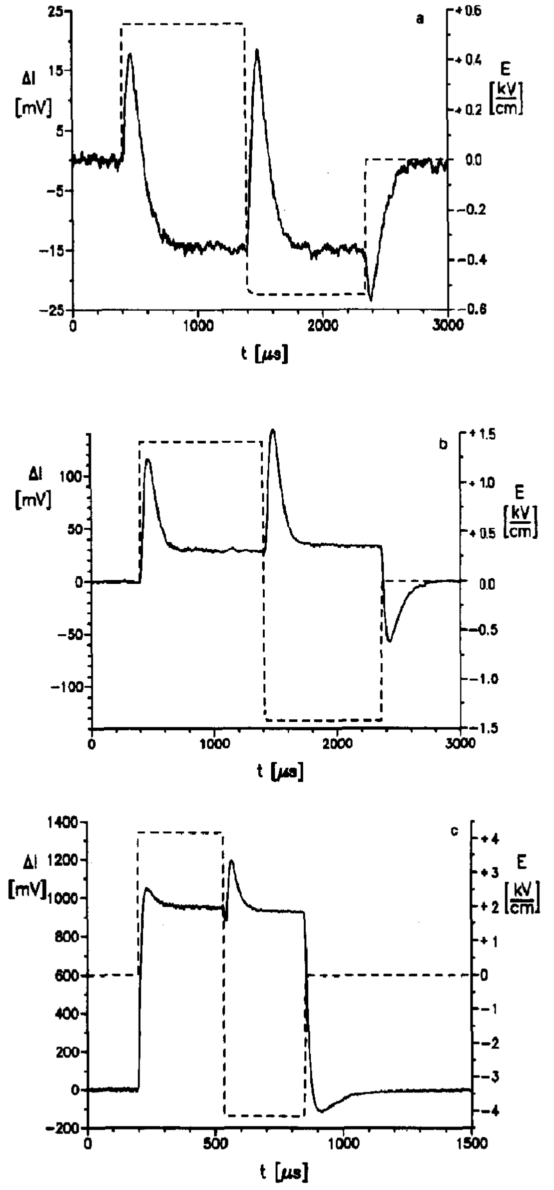

Fig. 1. Intensity changes ΔI of light, which is polarized parallel to the electric field vector and is transmitted through a solution of a 587 bp fragment, as a function of time t during exposure to an electric field pulse E (dashed line) of 3 different electric field strengths (a, b and c; buffer A at 2°C, DNA helix concentration 0.121 μM).

More information on the nature of the dichroism effect is provided by the dichroism risecurves and by the transients observed upon pulse reversal (cf. Fig. 1). Pulse reversal leads to a large change of the electrooptical effect with a transient reversal of the sign of the dichroism; this change is particularly large in the range of electric field strengths, where the stationary values of

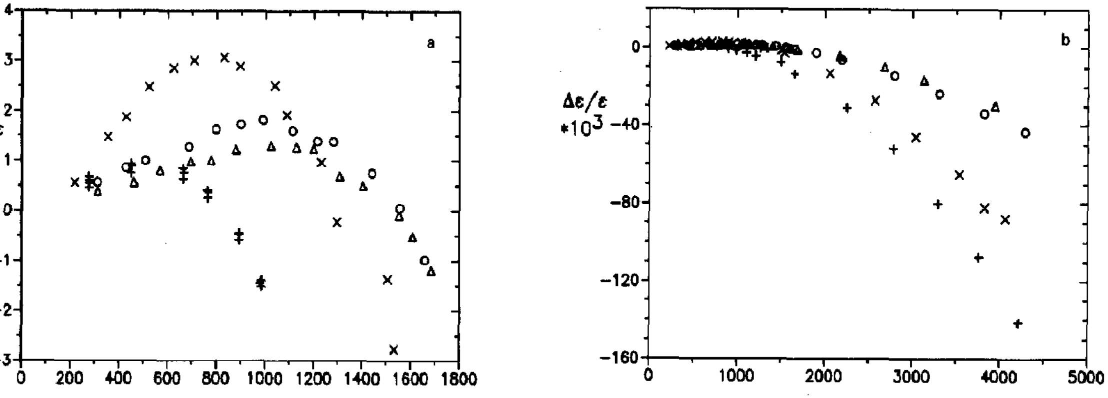

Fig. 2. Stationary values of the electric dichroism $\Delta\epsilon/\epsilon$ for the DNA fragment with 587 bp at 20°C as a function of the electric field strength E in two different ranges of E and in buffers of different salt concentrations: (+) 1/2 buffer A (ionic strength $I \approx 1.2$ mM), (×) buffer A ($I \approx 2.4$ mM), (○) buffer A+5 mM NaCl ($I \approx 7.4$ mM), and (△) buffer A+10 mM NaCl ($I \approx 12.4$ mM).

the dichroism are positive. The changes observed upon pulse reversal decrease with increasing electric field strength and are relatively small—but still clearly existent—at the highest field strengths, which can be generated by the electrooptical instrument used for the present measurements. According to the standards known in the literature [18–22], the special effects, which are observed most clearly at low field strengths, would be assigned to the presence of a permanent electric moment and/or to some polarizability in the direction perpendicular to the long axis of the double helix.

More detailed information may be expected from a quantitative analysis of the time constants. All transients require at least two relaxation processes with amplitudes of opposite sign. In the case of the risecurves and the curves induced by pulse reversal, the time constants associated with the relaxation processes are rather close to each other and the amplitudes obtained from fitting procedures are particularly high, but compensate each other to a large degree. It is remarkable that the largest time constant of the dichroism rise curves and also the largest time constant of the transients observed upon pulse reversal are not larger than the largest time constant observed in the dichroism decay (cf. Fig. 3). For permanent dipoles it may be expected according to Benoit [20] that the largest time constant associated with electrooptical risecurves in the limit of low field strengths is three times larger than the corresponding time constant of the decay curves. Thus, the present observations appear to argue against an orientation mechanism involving a permanent dipole moment. However, as shown below, this argument is not conclusive in the present case.

The special effects described above for the case of a DNA fragment with 587 bp have also

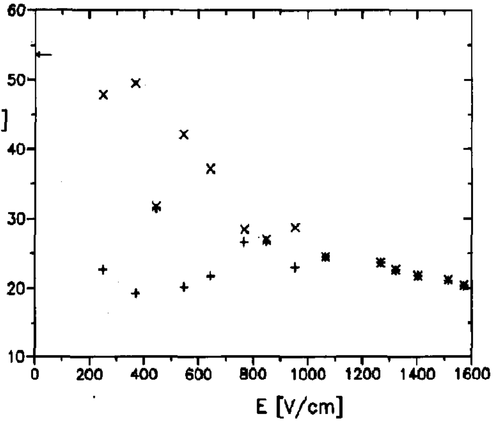

Fig. 3. Time constants τ_r^1 (+) and τ_r^2 (×) obtained by fitting of dichroism rise curves measured for the DNA fragment with 587 bp at different electric field strengths E. The average value of the slow decay time constants found for the same solution is indicated by an arrow (buffer A, 20°C, DNA helix concentration 0.176 μM).

been observed for DNA fragments with 399 bp and with 859 bp. According to its reduced electrophoretic mobility in polyacrylamide gels, the fragment with 399 bp has been classified as "curved", but its electrooptical parameters proved to be rather close to those of standard DNA fragments in an independent investigation; it has been demonstrated that the close correspondence is due to fluctuations of the long range structure [23]. Positive values of the electric dichroism have neither been observed for DNA fragments with 95 bp, 179 bp and 256 bp nor for pBR322 DNA (4361 bp) or λDNA (48502 bp). Thus, positive values of the electric dichroism are only found in a restricted range of intermediate chain lengths.

According to theory it should be expected that the birefringence effects for a given solution are analogous to those observed by dichroism detection. This has been verified by measurements with the 859 bp fragment in buffer A: the birefringence is positive at low field strengths and is negative at high field strengths; the birefringence transients are also in close analogy with those observed by dichroism detection.

Because of its high negative charge density, double helical DNA is not expected to aggregate under the conditions of the present experiments. Nevertheless, it is important to check, whether the special effects described above might be due to some association reaction between the double helices. Some concentration dependence is found in the positive stationary dichroism of the 587 bp fragment: at high DNA concentrations the magnitude of the positive dichroism is somewhat larger than at low DNA concentrations. However, a constant level of the positive dichroism is found at low DNA concentrations. A constant value of the positive dichroism has been found also for the fragment with 859 bp in the range from 28 nM to 88 nM helix concentration (Fig. 4). These results demonstrate that the positive electric dichroism is characteristic of the *internal* structure of the DNA fragments. The enhancement of the positive dichroism at increasing DNA concentrations may be due to some aggregation or due to some long range interactions between DNA fragments.

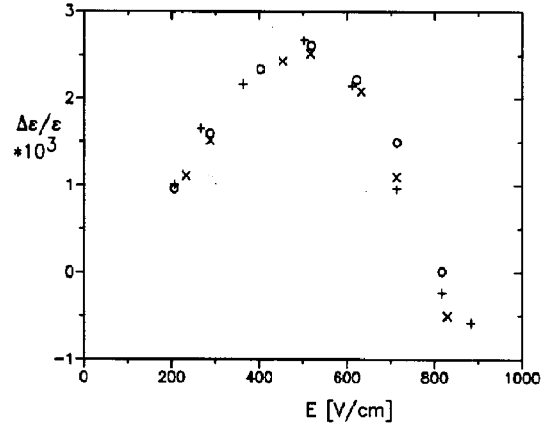

Fig. 4. Reduced electric dichroism $\Delta\epsilon/\epsilon$ for the DNA fragment with 859 bp as a function of the electric field strength E at different DNA concentrations: (+) 28.3 nM, (×) 57.1 nM and (○) 87.7 nM (concentrations in units of double helices; buffer A; 20°C).

The dichroism effects described above have been recorded at rather low electric field strengths, where orientation effects are expected to be far from saturation. The rather complex dependence of the measured stationary values of the dichroism cannot be described by any of the available orientation functions. Under these conditions it is of interest to analyze the field strength dependence of the amplitude A_2 associated with the slow relaxation process. The A_2-values can be determined at a satisfactory accuracy by fitting of dichroism decay curves. As shown for the example of the fragment with 859 bp in Fig. 5, this amplitude saturates at a rather low electric field strength. The field strength dependence of A_2 is consistent with an orientation function for a permanent dipole and cannot be fitted by an orientation function for an induced dipole. The magnitude of the permanent dipole resulting from a least squares fit is remarkable: 70400 D. The ratio of the amplitudes A_1/A_2 associated with the fast and with the slow relaxation process is a non-linear function of the electric field strength (data not shown). Extrapolation to field strength $E = 0$ leads to a ratio $A_1/A_2 = -0.44$ (for the fragment with 859 bp; experimental conditions cf. legend to Fig. 5).

The experiments showing a positive electric dichroism may be repeated many times for a given solution, but the amplitude decreases gradually with an increasing number of shots. For a

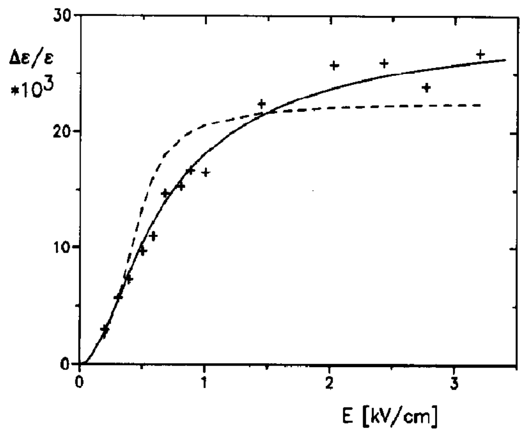

Fig. 5. Dichroism amplitude associated with the slow relaxation process found in the dichroism decay of the DNA fragment with 859 bp in 1 mM NaCl, 1 mM Na-cacodylate pH 7.0, 100 μM EDTA at 2°C. The continuous line represents a least squares fit by a permanent dipole model with a dipole moment 2.35×10^{-25} Cm (≡ 70400 D) and a limit reduced dichroism of 0.00305; the dashed line represents a least squares fit of the data by an induced dipole model (polarizability 1.34×10^{-29}, limit reduced dichroism 0.00226).

typical series of experiments with 40 shots over the whole range of electric field strengths up to 4 kV/cm, the decrease of the positive dichroism amplitude was about 15%. A decrease of the amplitude has not been observed, when the orientation effects were recorded by birefringence detection at 365 nm. Independent experiments showed that the decrease is due to a photoreaction and is not due to any effects caused by the electric field pulses. When the DNA samples are irradiated at 248 nm for longer periods ($\geqslant$ 60 s in our instrument), the positive electric dichroism is finally changed to a negative one. As discussed below, the denaturation of a very small region of the double helix may be sufficient already for suppression of the positive dichroism.

3.2. *Wormlike chain model*

The experiments described above demonstrate a positive electric dichroism for double helices in a given range of chain lengths and salt concentrations. Furthermore, the transients observed upon pulse reversal indicate that the field induced alignment in the range with a positive electric dichroism is caused either by some permanent electric moment or by a polarizability, which is dominant in the direction perpendicular to the helix axis. Now it has to be shown, whether these observations are consistent with the known molecular structure of DNA double helices.

It is expected that straight DNA double helices with a B-type structure exhibit a negative electric dichroism. Thus, the special effects described above are probably due to the fact that the double helices are subject to bending by thermal agitation. It has been shown by model calculations that bent forms of DNA usually bear considerable "permanent" electric moments, which result from asymmetric distributions of phosphate charges [24]. For an explanation of the results presented above, it should be demonstrated that this effect may lead to positive values of the electric dichroism for the distributions of bent DNA forms, which are expected to exist under the given experimental conditions.

Distributions of DNA configurations have been generated according to the standard wormlike coil model by algorithms described in the literature [25–27]. The dipole moments due to the phosphate charges associated with the non-symmetric DNA configurations must be referred to the center of diffusion, which has been computed by bead model simulations [28,29]. These simulations also provide the diffusion coefficients of any given configuration. The tensor of the extinction coefficients is obtained by addition of the contributions from the chain segments. These parameters of a given configuration are used for the calculation of the electrooptical transient for this configuration according to the procedure described Wegener et al. [30]. Transients have been calculated for large numbers of configurations, which have been generated independently; summation of these provides the electrooptical transient for distributions of configurations. It should be noted that this procedure is valid, when the transition between individual configurations is slow compared to rotational diffusion [23,27]: the time constant(s) for configurational transitions τ_c should be larger than the time constant(s) of rotational diffusion τ_r. Thus, the model describes a "frozen" ensemble of configurations.

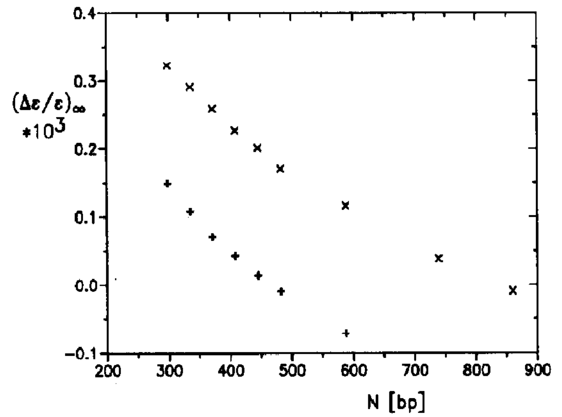

Fig. 6. Limit electric dichroism $(\Delta\epsilon/\epsilon)_\infty$ simulated according to the DNA wormlike chain model for different chain lengths N in bp; the effective charge associated with phosphate residues is 0.15 × [elementary charge]; polarizability terms are assumed to be negligible; (+) persistence length $p = 2000$ Å, (×) p = 5000 Å.

In order to simplify the analysis and to demonstrate the influence of permanent moments resulting from asymmetric charge distributions, a major part of the simulations has been restricted to the case, where the contributions from polarizability terms are negligible. As a first step, it is useful to analyse limit values of the electric dichroism $(\Delta\epsilon/\epsilon)_\infty$ predicted for "frozen" distributions of wormlike chains. These values are given for complete orientation of the ensembles expected at infinitely high electric field strength. As shown in Fig. 6, the $(\Delta\epsilon/\epsilon)_\infty$-values are positive in a broad range of chain lengths. For an explanation of the numerical results it must be recalled that virtually all DNA molecules are bent by thermal agitation—including the short ones (cf. Fig. 7). Most bent forms of short DNA's are similar to circular arcs; their dipole moment arising from the phosphate charges is in radial direction [24]; this dipole vector leads to a positive dichroism, provided that the bending angle of the "arc" remains small; obviously, in the case of short DNA's the bending angle remains limited for most configurations. The limitation in the magnitude of the dipole moment expected for short DNA's is not reflected in the $(\Delta\epsilon/\epsilon)_\infty$-values (cf. Fig. 6), because these values are given for infinitely high electric field strengths. For long DNA molecules the dipole vector is more often in the direction of the long axis of the molecules and, thus, the limit dichroism decreases with increasing chain lengths; finally, at high chain lengths the dichroism is negative.

For a comparison with the present experimental results, the dichroism contributions of individual configurations have to be weighted according to the magnitude of the dipole moment. These weighted contributions are calculated according to the procedure of Wegener et al. [30] using the "alignment tensor", which provides the contributions for the limit of low electric field strengths, i.e. in the regime of the Kerr law. The $\Delta\epsilon/\epsilon$-values calculated by this procedure are positive at low chain lengths, show a maximum for interme-

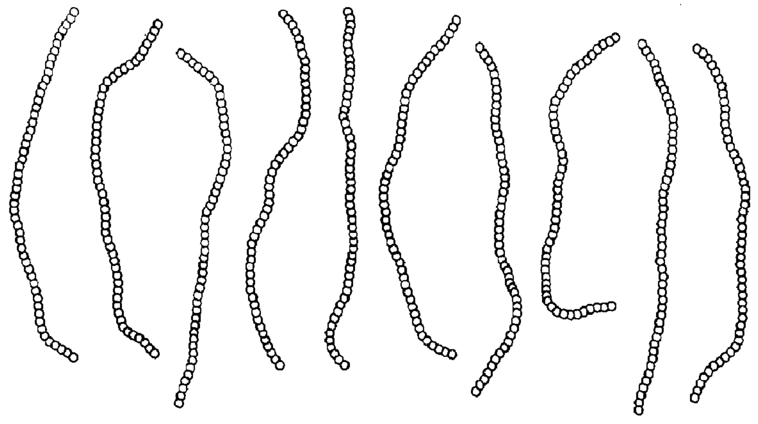

Fig. 7. Configurations generated according to the wormlike chain model for a contour length corresponding to the persistence length.

diate chain lengths (cf. Fig. 8) and finally decrease to negative values at high chain lengths.

The average values shown in Fig. 6 and 8 represent broad distributions of different configurations with widely different values of the linear dichroism and of the dipole moment. The distribution resulting from a simulation for the fragment with 587 bp at a persistence length of 4500 Å (cf. Fig. 9) demonstrates that the average positive dichroism results from a large contribution of configurations with a electric dichroism in the range around +0.4 and with dipole moments around 10000 D.

The simulations show that the electric dichroism of DNA wormlike chains within a given range of chain lengths is positive, provided that the electric parameters are determined by the location of the phosphate charges and that contributions from polarizability terms are negligible. It remains to be shown that positive values of the dichroism may be expected for the fragments used in the present investigation. According to the wormlike chain model, the $\Delta\epsilon/\epsilon$-value for the fragment with 587 bp is positive only, when the persistence length is as high as 4500 Å. The dichroism decay simulated under these conditions is close to the experimental one observed at low electric field strengths and requires two time constants for fitting; in view of the approximations and in the absence of any systematic fitting of the data, the values $\tau_1 = 6.3$ μs and $\tau_2 = 72.2$ μs are in satisfactory agreement with the experimental ones ($\tau_1 = 9.1 \pm 0.4$ μs, $\tau_2 = 48.3 \pm 2$ μs in buffer A; all values at 20°C).

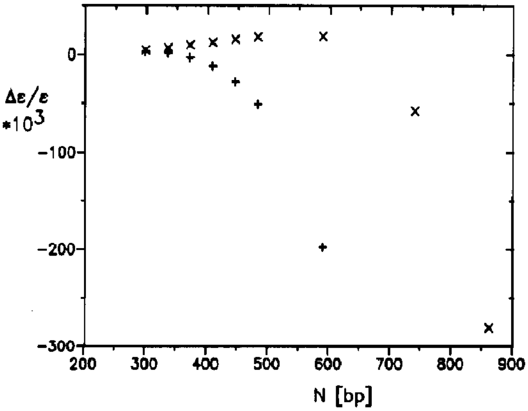

Fig. 8. Stationary electric dichroism $\Delta\epsilon/\epsilon$ simulated according to the DNA wormlike chain model for different chain lengths N in bp; the $\Delta\epsilon/\epsilon$-values are weighted according to the dipole moment of individual configurations (average of 6000 to 10000 configurations); polarizability terms are assumed to be negligible; (+) persistence length $p = 2000$ Å, (×) $p = 5000$ Å.

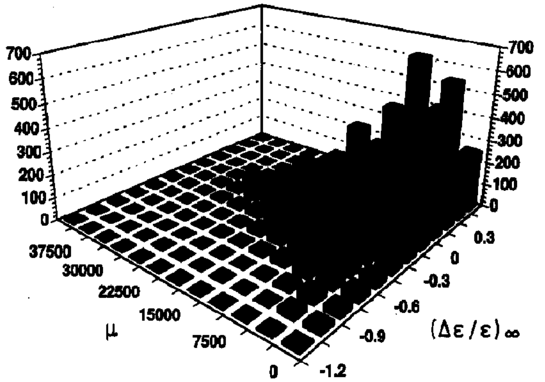

Fig. 9. Number of configurations found in different ranges of the dipole moment μ and of the limit electric dichroism $(\Delta\epsilon/\epsilon)_\infty$; from a simulation for a DNA double helix with 587 bp with a total number of 8000 random configurations; persistence length $p = 4500$ Å; the average limit reduced dichroism is +0.0863; the stationary reduced dichroism (at low field strengths) is $+2.43 \times 10^{-3}$; the average decay time constants are $\tau_1 = 6.3$ μs, $\tau_2 = 72.2$ μs; the maximal dipole moment in the ensemble is 43460 D.

3.3. Permanent dipoles with electrooptical rise curves different from the case of Benoit

The interpretation of the experimental data given above on the basis of permanent dipole moments arising from asymmetric distributions of charges appears to be in contrast with the experimental dichroism rise curves. According to Benoit [20] it is expected that the electrooptical rise of permanent dipoles is much slower than their electrooptical decay. However, Benoit has calculated electrooptical transients by an analytical treatment for ellipsoids of revolution with the dipole vector along the axis of revolution and has not considered cases with special combinations of dipole moments and diffusion coefficients. A special feature of the permanent dipoles described above is the perpendicular direction of the dipole vector with respect to the long axis of the molecule, which determines the slowest mode of

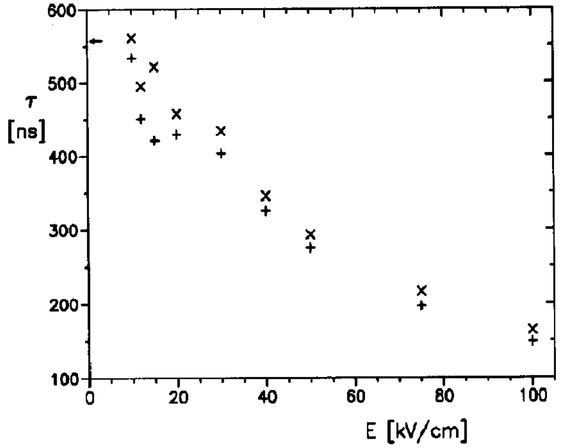

Fig. 10. Dichroism risetime constants τ_r^1 (+) and τ_r^2 (×) from Brownian dynamics simulations for a circular arc of 300 Å length and with a bending angle of 0.3×2π (corresponding to 30% of a circle) at different electric field strengths E. The larger exponential time constant of the dichroism decay is indicated by an arrow.

rotational diffusion. Dichroism risecurves have been calculated for this special case by Brownian dynamics simulations. The procedure used for the simulations has been described [31,32]; the input parameters were generated by the procedures for bead model simulations described above.

Although the Brownian dynamics approach can be applied for any combinations of parameters, simulations are given below for a relatively simple case, which should be sufficient to demonstrate the principle. A circular arc of DNA is used with a contour length of 300 Å corresponding to 88 bp and a bending angle of 0.3 × (2π), i.e. the overall shape corresponds to 30% of a circle. Electrooptical rise curves simulated for various electric field strengths can be represented by two exponentials with amplitudes of opposite sign; the decay curves also require two exponentials with amplitudes of opposite sign. As shown in Fig. 10, the larger one of the risetime constants approaches the value found for the larger decay time constant at low field strengths. In spite of some limitations in the accuracy of the simulated time constants due to a limit in the number of trajectories, it is clear that the rise time constants for low field strengths are far from the values expected according to Benoit.

In order to check the validity of the simulation procedure, rise curves have also been simulated for the other limit case, where the permanent dipole is directed parallel to the long axis of the molecule. In this case the rise curves obtained in the limit of low fields strengths are consistent with the predictions of Benoit.

4. Discussion

Although the electrooptical parameters of DNA molecules have been investigated repeatedly in many different laboratories, positive stationary values of the electric dichroism have not been reported yet in the literature. In the present laboratory, electrooptical effects used to be characterized mainly in the range of high electric field strengths, where the stationary electric dichroism of DNA always appeared with the standard negative sign. Some reports [33,34] have been given on electrooptical transients induced by pulse reversal, which suggested the existence of permanent dipole moments or the existence of a strong polarizability perpendicular to the helix axis. However, a quantitative interpretation of such transients has not been described in the literature yet. Some authors have tried to explain evidence for a permanent dipole associated with double helices by fluctuations of ion binding on straight DNA models [35]; as discussed recently [36], this interpretation is not consistent with the experimental observations. Further evidence against the interpretation by fluctuations has been obtained by pulse reversal experiments on sufficiently short DNA fragments, which have been evaluated by an appropriate deconvolution routine (in preparation).

The interpretation given above on the basis of a wormlike chain model appears to be consistent with all essential experimental informations available. Obviously, this interpretation should be subject to further experimental tests and simulations should be extended to more complex models. Nevertheless, some major conclusions appear to be justified already at the present stage:

First of all, positive values of the electric dichroism for DNA double helices appear together with permanent dipoles. The special shape of the dichroism rise curves and of the dichroism transients upon pulse reversal may also be attributed to a strong polarizability component perpendicular to the long helix axis, but there is hardly any evidence for this interpretation: first of all, the polarizability of objects like DNA is known to be maximal in the direction of maximal extension. Furthermore, various simulations during the present and previous projects [23,24,27] never revealed the unusual electrooptical transients, when the alignment was only due to standard polarizability terms. Finally, a strong argument against the interpretation by a polarizability perpendicular to the long axis comes from the disappearance of the positive electric dichroism in the presence of Mg^{2+} ions. It is known that the condensation of Mg^{2+} ions to DNA double helices is much more extensive than that of monovalent ions. Thus, the effective charge of phosphate residues is expected to be much lower in the presence of Mg^{2+} than in the presence of Na^+, whereas the polarizability of double helical DNA is known to be hardly affected upon addition of 100 μM Mg^{2+} [9]. According to the predictions of polyelectrolyte theory [37,38], the charge density of a line charge modelled in analogy to the DNA double helix is reduced to 12% in the presence of Mg^{2+}-ions. This prediction is based on electrostatic interactions and does not consider coordination effects. The present experiments suggest that the charge density of DNA is reduced by Mg^{2+}-ions to values below 12%; the reduction beyond the value expected from polyelectrolyte theory may be due to coordination effects.

The main experimental observations are shown to be consistent with a "frozen" ensemble of configurations simulated according to the wormlike chain model. Simulations on ensembles of configurations with a high internal mobility should be useful for comparison and should facilitate a more detailed assignment of processes, but these simulations require more extensive computations [39,40]. According to a straightforward interpretation of the available experimental data, the evidence is against a high internal mobility of DNA under the present experimental conditions. This evidence comes from the time constants of the dichroism transients observed upon pulse reversal: if the internal mobility would be high (i.e. the time constants for internal transitions would be clearly lower than the overall rotational diffusion time constant), the time constants of the electrooptical transients should be much shorter than observed. This expectation is based on the fact that the direction of the dipole of a bent DNA configuration with a high internal flexibility may be turned around by motion of the terminal segments; such motion should be fast for flexible chains.

The present evidence on low rates for configurational transitions appears to be in contradiction with previous observations on bending time constants [9,41]. The contradiction is reconciled by the fact that the bending time constants were obtained under special experimental conditions: the DNA fragments were exposed to high electric field strengths leading to particularly high stretching forces. It is very likely that stretching at high field strengths is mainly in the "elastic" limit; this type of stretching is expected to revert quickly with time constants determined by hydrodynamic friction. The other limit case with relatively high barriers of activation for transitions between different configurations leads to distributions, which are virtually "frozen" during the time of the relatively short electrooptical transients. Various modes of internal transitions including the extreme ones may be present simultaneously in a given polymer chain. A close analogy exists in the case of protein folding, where both fast and slow modes have been observed; in proteins the slow modes are represented by e.g. the *cis–trans* transition of proline residues. It is very likely that activation barriers exist also in configurational transitions of DNA double helices. It should be added that the time limit of configurational transitions, which may be resolved by measurements of the electric dichroism, is defined by the rate of overall rotational diffusion: thus, the time constants of internal transitions within DNA for a frozen ensemble need not be larger than ≈ 50 μs at 20°C and may be even somewhat smaller than that value.

The hydrodynamics of frozen configurations is expected to be strongly affected by perturbations like local denaturation. For example, the dissociation of a few base pairs may introduce a flexible joint, which may change the rotational diffusion of a long DNA chain entirely, if this joint is located somewhere in the central part of the chain. From this point of view, it is not at all surprising that the unusual electrooptical response described in this communication is more sensitive than standard electrooptical response curves towards damages accumulating with an increasing number of experiments (cf. Section 3). This sensitivity may be useful for future experiments on the influence of various ligands on the long range structure of DNA. The example of the photoreaction, which induces a strong decrease of the positive dichroism and finally a change to a negative one, is consistent with the present interpretation. The standard UV-photoproducts of DNA nucleotide residues interrupt base stacking in the double helix and, thus, it must be expected that these photoproducts introduce flexible joints.

Some of the new experimental results, which are not yet explained by the wormlike chain model with frozen configurations and with non-symmetric charge distributions, require a separate discussion: The model is very useful to explain the permanent dipole moments observed at low electric field strengths, but the change of the orientation mechanism from the permanent to the induced dipole type remains unexplained. It is possible that this change is partly due to field induced stretching, which is more efficient at higher field strengths. Stretching decreases the permanent dipole moment and, thus, contributions from polarizability terms may become dominant. According to model calculations on field induced stretching [42], however, major contributions from stretching would not be expected at the low field strengths, where the change of the orientation mechanism is observed. In the absence of simple and complete explanations on other levels, the change in the electric response may be attributed to an increase of the polarizability with the field strength. Due to the complexity of polyelectrolytes it is conceivable that shielding by counterions leads to an induction range of the polarizability at low field strengths. This hypothesis may be tested by further experiments.

The interpretation of the new electrooptical data by the wormlike chain model implies unexpectedly high values of the "dynamic" persistence length. Recent independent experiments indicated that the dynamic persistence length is in the range around 1000 Å [23], under conditions where electrostatic shielding is quite efficient due to the presence of Mg^{2+} ions or of high mononovalent ion concentrations. Most of the present experimental data have been collected at rather low ionic strengths, where a strong contribution from "electrostatic persistence" must be expected [43–45]. A particularly large electrostatic persistence together with a strong chain length dependence of this parameter has been computed recently by Fenley et al. [45] for a helical and constant dielectric model. At 1 m*M* salt the electrostatic persistence length calculated for a 600 bp fragment is about 1200 Å according to this model and, thus, the effective persistence length is at least in the order of magnitude required for explanation of the present results. Fenley et al. [45] do not favour the "constant dielectric" model, because "it possesses a strong dependence of the binding fraction on salt, which is not found experimentally". It may be noted that the present experimental results on the salt dependence of the positive dichroism may be explained by a combination of an increase in the degree of ion binding and a decrease of the polarizability with increasing salt. According to the chain length dependence of the electrostatic persistence predicted by Fenley et al., the persistence lengths compiled recently for low salt concentrations [14] have to be revised, because most of the experimental data were collected for relatively low chain lengths. Another reason for a reinterpretation of the persistence lengths found at low salt concentrations in the absence of Mg^{2+} are the special effects [27] resulting from the existence of permanent dipole moments. Finally, a further problem for a quantitative assignment of the dynamic persistence length comes from the fact that DNA double helices are not only bent by thermal agitation but also are inherently curved. Even DNA fragments without anomalous gel mobility are expected to be curved to some degree. According to the model of Bolshoy et al. [46], the DNA fragments with 587 and 859 bp are both somewhat curved: model calculations on the predicted equilibrium configurations provide a positive electric dichroism for the 859 bp fragment, but a negative one for the 587 bp fragment (calculated for the case of negligible polarizability). Due to the limitations in the prediction of DNA curvature and also in the calculation of electrooptical data from given structures, small deviations from predictions must be interpreted with caution. Both procedures for prediction of DNA curvature and for calculation of electrooptical transients should be refined for a more detailed assignment of structures in solution.

The electric moment leading to a positive electric dichroism of DNA double helices appears to be a permanent one according to standard electrooptical criteria. In fact, this special dipole moment is permanent in the sense that it does exist in the absence of an external electric field. However, the special dipole moment is clearly not permanent for individual molecules with respect to time and, thus, does not correspond to the type of dipoles which have been defined as permanent ones originally. The special type of dipole moment described in the present investigation may be denoted as a dipole moment due to configurational fluctuations. A related dipole moment resulting from fluctuations has been described by Kirkwood [47] for the case of proteins: protons may fluctuate between different sites of binding and, thus, may generate an anisotropy of the charge distribution; this may be reflected by dielectric effects, which are hard to distinguish from those due to standard permanent dipoles. It may be added that dipoles of macromolecules may also result from fluctuations in the binding of other ligands [48]. Furthermore, it should be mentioned that still another type of permanent dipole moment has been found previously in DNA double helices: the polarizability of DNA may approach saturation in a given range of field strengths [8] and above that range the dipole moment remains independent of the field strength as in the case of permanent dipoles. Thus, a rather large number of different dipole moments must be distinguished in the case of macromolecules; obviously, the wide variety of different electric moments is due to the complexity of these macromolecules with respect to their structure and dynamics.

In summary, the unexpected experimental results have been explained by a model, which describes the fluctuations of double helical DNA and their inference on electric parameters in terms of a frozen ensemble. Unusual electric properties of polyelectrolytes, including DNA, have been discussed frequently, but have not been explained in any sufficient detail previously. The present results, obtained from experiments and from simulations, contribute to a better understanding of these complex electric phenomena. In addition, the electrooptical results prove to be useful for the characterization of the internal DNA dynamics. The experimental data indicate that bending and/or curvature of the DNA fragments used in the present investigation is limited. Furthermore, the data indicate that double helices are less flexible with respect to the dynamic persistence and also with respect to the rate of internal configurational transitions than implied in the past.

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