Deconvolution of Electrooptical Data in the Frequency Domain: Relaxation Processes of DNA from Rigid Rods to Coiled Spheres

Dietmar Porschke* and Gerhard Nolte

Max Planck Institut für biophysikalische Chemie, D-37077 Göttingen, Germany

Received August 30, 1993®

ABSTRACT: The effects of sinusoidal electric fields on various DNA samples are characterized by parallel recording of the "enforcing" electric field and of the resulting dichroism effects; these data are then evaluated quantitatively by a deconvolution technique. The linear dichroism of a restriction fragment with 256 base pairs under sinusoidal electric fields shows the effects expected from rotational diffusion of an induced dipole with a short polarization time constant: a dispersion of the optical sine amplitude and of the phase difference in the frequency range around 20 kHz, but no dispersion of the average stationary value of the negative dichroism. The experimental data can be described with high accuracy as a convolution product of the squared forcing function with a single relaxation process over a broad range of frequencies. Measurements with plasmid DNA's of various chain lengths reveal that both the sine amplitude and the average stationary amplitude of the negative dichroism effect almost disappear at high frequencies; the data show a distribution of time constants; both mean value and width of the distribution increase with the chain length. The dispersion data for the long DNA molecules are attributed to a dipole resulting mainly from field induced deformation of the polymer spheres. The deconvolution procedure is applied also for analysis of reverse pulse experiments. The dichroism induced by a reverse pulse in a solution of a 256 bp restriction fragment can be fitted with high accuracy by a single time constant reflecting overall rotational diffusion. This experiment demonstrates a high rate of polarization and the absence of any substantial contribution to the orientation from a permanent moment. In general, the deconvolution procedure for analysis of electrooptical data obtained in the frequency domain proves to be superior to conventional procedures, because the required information, which used to be extracted from frequency dependences of stationary amplitudes, sine amplitudes, or phases, is obtained in a single step; furthermore, transients, which are observed at the start or the end of enforcing wavetrains and contain valuable information, may be included in the analysis without problems.

Introduction

Electrooptical data of macromolecular or colloidal systems have been generated preferentially by application of rectangular electric field pulses to the samples and have been recorded as transients of the linear dichroism, birefringence, or light scattering.[1-4] The closely related experimental procedures based on application of sinusoidal electric fields have not been applied as frequently.[4-10] Apparently, this is at least partly due to a lack of convenient procedures for a quantitative analysis of experimental data. Transients generated by rectangular electric field pulses may be analyzed conveniently by simple exponential fitting routines under most experimental conditions. Fitting of electrooptical response curves generated by sinusoidal electric fields requires more sophisticated routines involving deconvolution. We have developed an appropriate procedure for the evaluation of electrooptical data recorded in the frequency domain and demonstrate its application for the analysis of various DNA samples. We have used data obtained for a 256 bp restriction fragment as a reference and show that the electrooptical effects found for long DNA chains mainly result from field induced deformation of polymer spheres. The deconvolution procedure is shown to be very useful also for the evaluation of reverse pulse experiments, because transients observed during pulse reversal can be analyzed quantitatively. By this technique we have demonstrated the absence of any substantial contribution from a permanent dipole moment to the field induced orientation of a 256 bp restriction fragment.

® Abstract published in *Advance ACS Abstracts*, December 15, 1993.

Materials and Methods

The DNA fragment with 256 base pairs was prepared from the plasmid DNA pVH27 (kindly provided by W. Hillen) by the restriction nuclease *Hae*III and was separated by ion exchange chromatography.[11] The plasmid DNA's SP65 (3005 bp) and PM2 (~9800 bp), the cosmid DNA pHC79 (6524 bp) and λDNA (48502 bp) were obtained from Boehringer Mannheim. All DNA's were dialyzed extensively, first against 1 M NaCl, 10 mM Na-cacodylate pH 7, 1 mM EDTA and finally against 0.5 mM NaCl, 0.5 mM Na-cacodylate pH, 7.0, 0.1 mM EDTA (standard buffer). DNA concentrations are given in units of nucleotide residues.

The samples were exposed to electric field pulses in standard cuvettes of 1-cm optical path length with an insert machined from Teflon, holding platinum electrodes at a distance of 4.8 mm. Sinusoidal electric fields of various frequencies were generated by a Fluke 5700A calibrator. Rectangular field pulses with reversal of the field vector were produced by two Cober pulse generators Model 606, which were tuned for a minimal pule reversal time. Linear dichroism signals were recorded by an optical detection system, which has been developed for measurements of temperature jump relaxation:[12] we used a 600-W Hg/Xe lamp and a Schoeffel GM250 grating monochromator as a light source; 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 a lamp fluctuations in the signal of the dichroism detector by a device based on analog electronics. 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 the procedure described below.

Procedure for Evaluation

The experimental data discussed in our present contribution are generated by application of sinusoidal electric

0024-9297/94/2227-0590$04.50/0

fields to solutions of macromolecules. Thus, the sinusoidal electric fields are used as *forcing functions F*. The response of the solution is modeled by a sum of p exponentials:

$$\sum_{k=1}^{p} \frac{a_k}{\tau_k} e^{-t/\tau_k} \tag{1}$$

The measured data $Y_o(t_i)$ are then expected to be equivalent to the convolution product of the model (1) with the forcing function F (cf. refs 13–15):

$$Y_o(t_i) \approx Y_c(t_i) := a_0 + \int_{-\infty}^{t_i} \left[F(s) \sum_{k=1}^{p} \left(\frac{a_k}{\tau_k} e^{-(t_i-s)/\tau_k} \right) \right] ds \tag{2}$$

The parameter s is used as an integration variable. Because we want to evaluate the model parameters a_k and τ_k from the experimental data, we have to find the minimum for the following function:

$$\Phi(a_0, ..., a_p, \tau_1, ..., \tau_p) = \sum_{i=1}^{NP} (Y_o(t_i) - Y_c(t_i))^2 \tag{3}$$

where NP is the number of data points. By interchange of summation and integration eq 2 is converted to

$$Y_c(t_i) = a_0 + \sum_{k=1}^{p} a_k E(i,k) \tag{4}$$

with

$$E(i,k) = \frac{1}{\tau_k} \int_{-\infty}^{t_i} F(s) e^{-(t_i-s)/\tau_k} ds \tag{5}$$

For a simplified calculation of (5) we assume that

- $F(t) = 0$ for $t < 0$; i.e. the system is in equilibrium or in a stationary state before perturbation.
- F is piecewise linear; i.e. it has the form $F(t) = \alpha_i t + \beta_i$ for $t_{i-1} \le t \le t_i$. t_i denotes the times of data acquisition.
- the measured data and the points of F are sampled at the same instant.

If the experiments are conducted in agreement with these conditions, which do not impose any serious restriction, then the evaluation may be based on the following procedure: we may split the integral in (5) and calculate each part separately:

$$E(i,k) = \sum_{j=1}^{i} e^{(t_j-t_i)/\tau_k} [\alpha_j (t_j - \tau_k - e^{(t_{j-1}-t_j)/\tau_k} (t_{j-1} - \tau_k)) + \beta_j (1 - e^{(t_{j-1}-t_j)/\tau_k})] \tag{6}$$

which leads to an easy to compute recursion formula.

For minimization of (3) we use the following procedure: for a given set τ_k, the optimal set of coefficients a_j is calculated either by a fitting procedure or by a generalized regression analysis; the resulting value of the error function Φ is then introduced into a simplex routine and the optimal set of τ_k values (≡minimal value of Φ) is evaluated by iteration.

When the model function (1) is folded with a steplike forcing function F ($F(t) = 0$ for $t \le 0$ and $F(t) = f$ for $t > 0$), we obtain

$$Y_c(t) = a_0 + f \sum_{k=1}^{p} (a_k (1 - e^{-t/\tau_k}) \quad \text{for } t \ge 0 \tag{7}$$

and the following sum of amplitudes

$$(a_1 + ... + a_p) f = Y_c(\infty) - Y_c(0) \tag{8}$$

Although the assumptions on the forcing function F described above constitute some boundary conditions, there is hardly any restriction in the choice of forcing functions. Thus, we may consider different functional relationships between the measured data Y_o and the forcing function F, for instance a dependence on the square of the forcing function F.

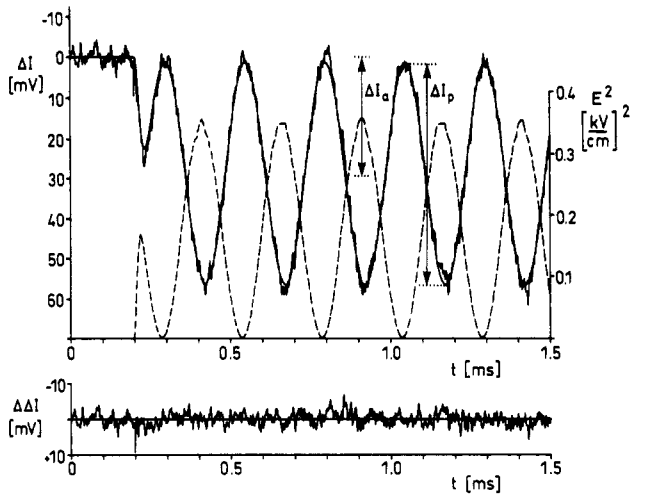

Figure 1. Change of the intensity ΔI of light polarized parallel to the field vector in a solution of a 256 bp fragment induced by a sinusoidal electric field pulse of 2 kHz; the squared electric field strength E^2 (dashed line) has been used for deconvolution; the least squares fit by a single exponential (τ = 11.2 μs) can hardly be distinguished from the experimental data; the residuals $\Delta\Delta I$ are shown in the lower panel. The peak to peak amplitude ΔI_p and the average stationary amplitude ΔI_a are indicated by arrows; these values are converted to the corresponding amplitudes of the reduced linear dichroism $(\Delta\epsilon/\epsilon)_p$ and $(\Delta\epsilon/\epsilon)_a$ (definition cf. refs 1 and 2; examples of $(\Delta\epsilon/\epsilon)$ values cf Figures 2a and 5) Experimental conditions: standard buffer; 2 °C; c(DNA) = 204 μM; light intensity before pulse application, 8.2 V. The rise time constant of the detector 4.8 μs has been included in the deconvolution.

In many cases the measured data are determined not only by the forcing function but also by the response function of the detector system. Usually, the detector function may be described by a simple exponential. Because convolution is known to be associative and commutative,[16] the influence of the detector may be included by convolution of the detector exponential function $[(1/\rho)e^{-1/\rho}$ with rise time $\rho]$ and the forcing function. The convolution procedure is analogous to the one described above. When the effect of the detector function is included in the evaluation, it is possible to record the experimental data with a limited bandwidth of the detector and, thus, with an increased signal to noise ratio.

Results

DNA Restriction Fragment. For testing of the procedure and setting up of a reference, it is useful to start with a system where a relatively simple response may be expected. We have used a DNA restriction fragment with 256 base pairs for this purpose. At low electric field strengths the dichroism decay of this fragment may be described by a single exponential at a reasonable accuracy. Application of sinusoidal electric field pulses with an effective field strength of 417 V/cm leads to a response which can be recorded at a satisfactory signal to noise ratio (cf. Figure 1). The response curve may be described (1) by the peak to peak dichroism amplitude in the stationary state, (2) by the average dichroism amplitude in the stationary state—corresponding to the mean displacement from the level observed in the absence of the electric field (definition of amplitudes cf. Figure 1), and (3) by the phase shift of the optically recorded signal

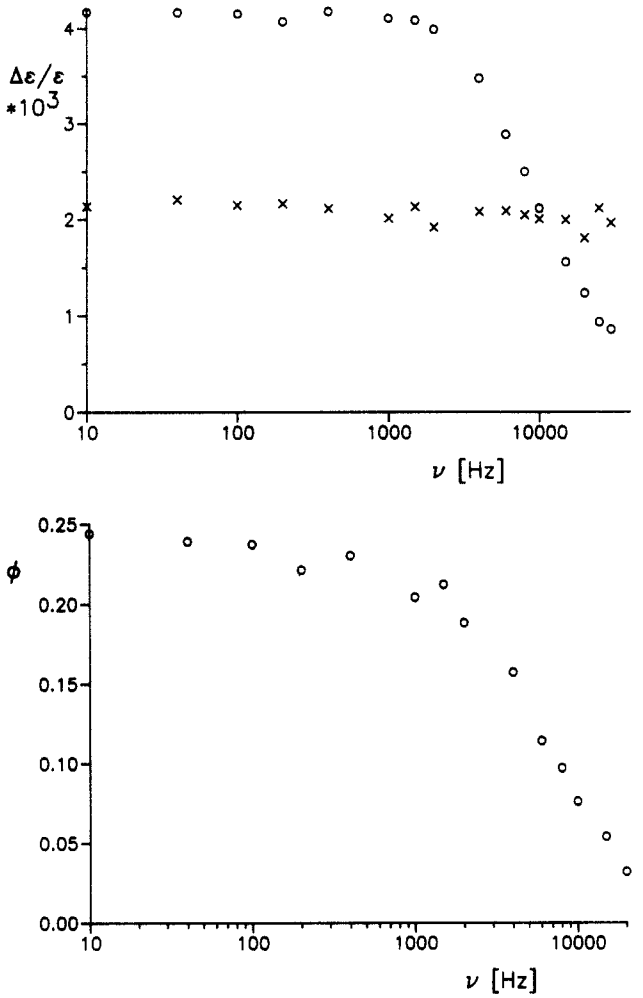

Figure 2. Electrooptical effects induced in a solution of a 256 bp fragment by sinusoidal electric field pulses of different frequencies ν at a constant effective field strength of 417 V/cm (standard buffer; 2 °C): (a) peak to peak amplitudes $(\Delta\epsilon/\epsilon)_p$ (O) and average stationary amplitudes $(\Delta\epsilon/\epsilon)_a$ (×); (b) phase ϕ of the optical signal with respect to the enforcing sinusoidal field pulse.

with respect to the electrical forcing function. The first two parameters have been evaluated by a simple computer graphics procedure at different frequencies of the forcing function. As shown in Figure 2a, the average amplitude observed for the DNA fragment remains constant over the frequency range from 10 Hz to 100 kHz, whereas the peak amplitude shows a clear decrease in the frequency range around 10 kHz. A least squares fit of the dispersion curve according to the standard expression of Debye provides a time constant for rotational relaxation of 14 μs. The phase of the optical signal with respect to the enforcing sinusoidal electric field starts from a limit value of 0.25 at low frequencies (cf. Figure 2b); this value results from the fact that the optical effect is proportional to the square of the forcing function. A dispersion of the phase is observed in the same frequency range as found for the peak amplitude.

A particularly accurate value of the rotational time constant can be obtained by a direct numerical analysis of the forcing function and the optical response curve by the deconvolution procedure described above. This procedure may also be used to establish the functional relationship between the forcing function and the optical signal. Doubling of the frequency in the optical response curve suggests that the linear dichroism is a linear function of the squared forcing function, but this observation does not exclude yet the possibility that the dichroism increases

Table 1. Time Constant τ and Amplitude $\Delta\epsilon/\epsilon$ Obtained by Deconvolution of Dichroism Response Curves Induced in a Solution of a 256 bp Fragment by Sinusoidal Electric Field Pulses of Various Frequencies ν (Standard Buffer, 2 °C; Deconvolution with Squared Forcing Function)

ν (kHz)	τ (μs)	$(10^3\Delta\epsilon/\epsilon)/E^2$ [(cm/kV)^2]
2	11.1	11.6
4	11.8	11.6
6	11.1	11.2
8	12.0	11.3
10	11.2	10.9
15	11.6	11.2
20	11.0	11.5
25	11.1	11.8
30	10.9	11.5

linearly with the *absolute* value of the forcing function. These alternatives may be distinguished from a single recorded dichroism wavetrain of sufficient signal to noise ratio by simple visual inspection. A more quantitative basis for decision is provided by our numerical evaluation procedure. In the case of the DNA fragment with 256 bp, analysis of the data demonstrates that the dichroism increases with the square of the forcing function.

The time constants of rotational diffusion can be determined by our evaluation procedure from data obtained with forcing functions over a broad range of frequencies. It is possible to record the optical data with a rise time of the detection unit close to the time constant of the process under investigation; the detector rise time can be measured independently and then can be included in the deconvolution process. As shown in Table 1, the accuracy of the resulting time constant can be very high. Another parameter provided by the deconvolution procedure is the stationary value of the dichroism per unit forcing function; in the case of the DNA fragment with 256 bp, the values given in Table 1 are in units of (cm/kV)^2.

Reverse Pulse Experiments. Rectangular electric field pulses with reversal of the field vector may be used for an assignment of the nature of dipole moments.[17] However, pulse reversal by standard pulse generators requires a relatively long time and, thus, transients of the dichroism or of the birefringence observed during pulse reversal may simply result from the time period required for reversal of the field vector. Any contribution due to this effect is included, when the data are analyzed by an appropriate deconvolution procedure.

For our experiments we have used a reverse pulse generator tuned for a particulary short reversal time. As shown in Figure 3, the dichroism transient during pulse reversal observed for a restriction fragment with 256 bp remains very small. For a quantitative analysis we have to consider the fact that the optical signal increases with the square of the electric field strength and, thus, the squared forcing function has to be used for deconvolution. According to this procedure, the dichroism transient can be fitted with high accuracy by a single time constant τ = 11.9 μs, which is in excellent agreement with the time constant obtained by deconvolution of the electrooptical data measured in the frequency domain.

DNA Molecules with Chain Lengths >1000 bp. For DNA chains with an increasing number of base pairs, the number of exponentials associated with rotational diffusion is expected to increase. This expectation is verified by measurements with various plasmids DNA's. As shown in Figure 4 for the example of the DNA from the cosmid pHC79, the optical signal induced by a sinusoidal electric field pulse requires at least two exponentials for a satisfactory representation. The dispersion curves of the

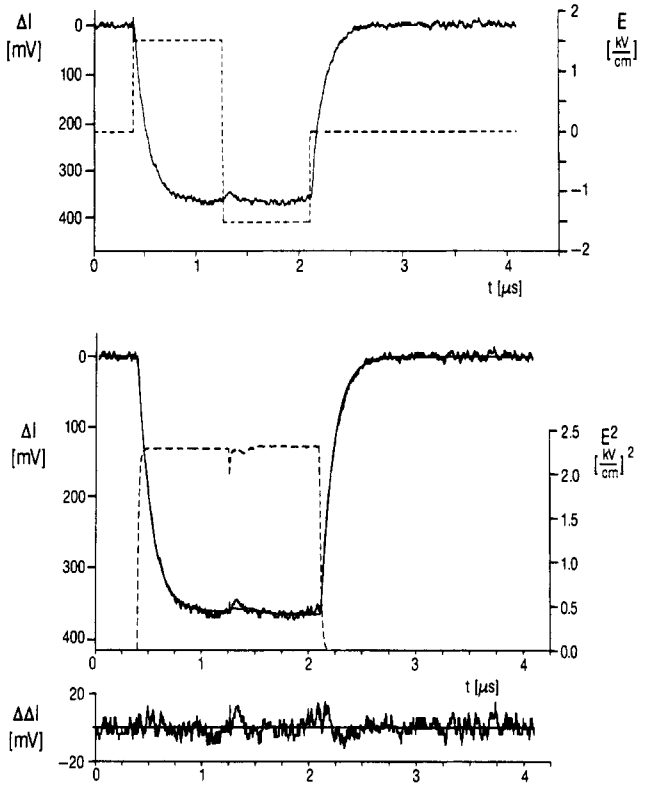

Figure 3. (a) Change of the intensity ΔI of light polarized parallel to the field vector in a solution of a 256 pb restriction fragment induced by a rectangular electric field pulse with reversal of the field vector (dashed line). (b) Least squares fit of the experimental data by the deconvolution procedure described in the text. The deconvolution is based on the squared forcing function, which has been convoluted with the rise time constant of the detector (1.45 μs; dashed line); the residuals of the least squares fit (τ = 11.9 μs) is shown in the lower panel.

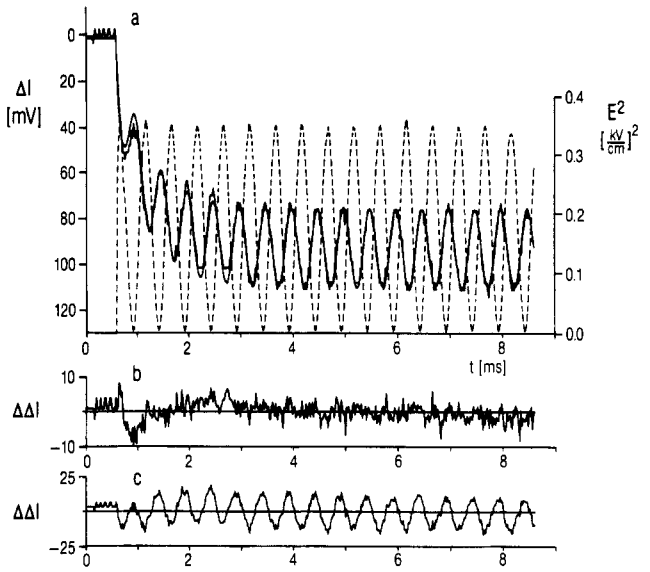

Figure 4. Change of the intensity ΔI of light polarized parallel to the field vector in a solution of the DNA from the cosmid pHC79 induced by a sinusoidal electric field pulse of 1 kHz. The squared electric field strength E^2 (dashed line) has been used for deconvolution. The least squares fit by two exponentials (τ_1 = 2.7 μs; τ_2 = 570 μs) can hardly be distinguished from the main part of the experimental data. The residuals $\Delta\Delta I$ of the fit by two exponentials are shown in the panel b. Panel c shows the residuals of a least squares fit by a single exponential (τ = 470 μs) (Experimental conditions: standard buffer; 2 °C; c(DNA) = 98 μM; light intensity before pulse application, 8.2 V. The rise time constant of the detector 12.6 μs has been included in the deconvolution).

peak amplitudes (cf. Figure 5) found for DNA's with chain lengths in the range from 3005 to 48502 bp are much broader than that observed for the restriction fragment with 256 bp. A more remarkable difference is observed in the frequency dependence of the average stationary dichroism (cf. Figure 5): this parameter exhibits a clear decrease with increasing frequency in the range from 10 Hz to 100 kHz for the long DNA chains, whereas it does not show any dependence in the same frequency range for the fragment with 256 bp. The difference indicates a change in the mechanism of polarization. For the short DNA fragment the dipole moment is generated with a time constant τ_p much below the time constant corresponding to the maximal frequency used in this investigation ($\tau_p \ll 2$ μs). In the case of the plasmid DNA molecules, a dominant part of the dipole moment is generated with time constants in the range from 2 to 200 μs; for λDNA the spectrum of time constants extends to ≈2 ms. As shown in the Discussion, it is very likely that the spectrum of time constants reflects the spectrum of the internal mobility of the wormlike DNA chains.

Discussion

In many cases the effects induced by electric fields in biological macromolecules are rather complex and, thus, it is often quite difficult to assign the nature of a given response. Under these conditions the assignment may be facilitated by application of different procedures for analysis. In our present investigation we have used sinusoidal electric field pulses for a quantitative electrooptical analysis of double helical DNA. Sinusoidal field pulses have rarely been used for electrooptical investigations, which is at least partly due to a lack of appropriate quantitative procedures for data evaluation. This problem has been solved in our present investigation by development of a numerical procedure. The case of the DNA restriction fragment has been used as a test of this procedure. All the data obtained for the restriction fragment are consistent with field induced orientation by a standard induced dipole mechanism: polarization is much faster than rotational diffusion and rotational diffusion can be described by a single time constant.

For DNA molecules with chain lengths ≥3005 bp, the electrooptical response is clearly more complex. This complexity is not only due to the fact that there is a spectrum of time constants for rotational motion of long polymer chains. The experimental data indicate that there is a change in the mechanism of polarization, when the chain length increases. The strong decrease of the average stationary amplitude with increasing frequency demonstrates that there is a slow process involved in the generation of the optical signal. We have to check whether this slow process corresponds to polarization.

The standard model of polarization used for molecules like DNA implies motion of counterions along the chain. Such motion should be easily possible within a sufficiently short time, when the chain length remains limited. For high chain lengths, motion of ions along the chain is clearly not the most efficient mechanism of polarization. Large dipoles of molecules with high chain lengths can be generated much more efficiently by changes in the degree of ion binding at the ends of the chain. Redistribution of monovalent ions is known to proceed in the nanosecond time range;[18,19] thus, polarization by redistribution of ions should be a fast process. Among various potential processes toward a given state, the one with the highest rate must be operating, and thus, it is rather unlikely that the slow process identified in the electrooptical signal of

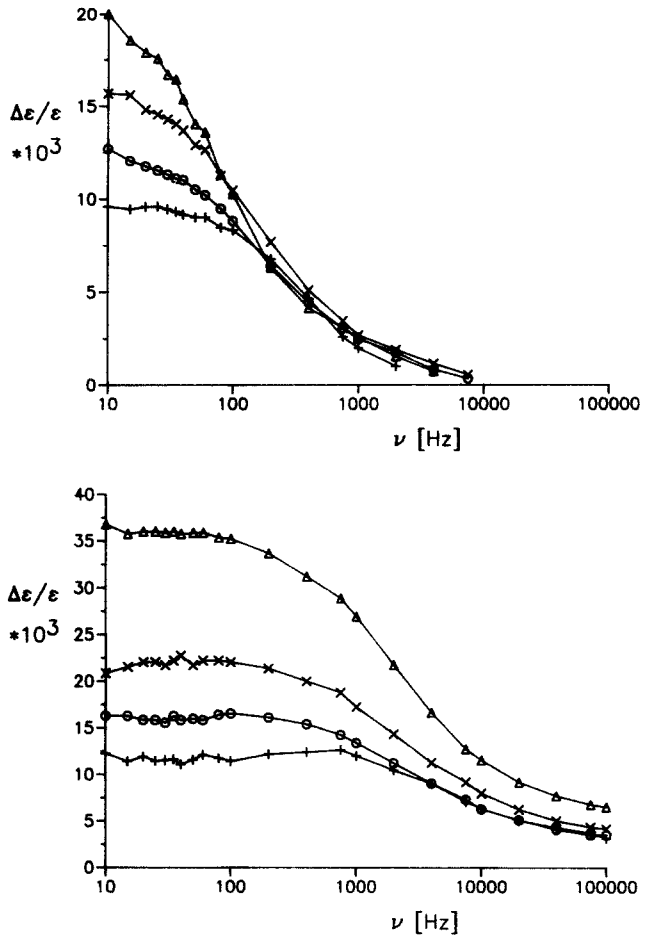

Figure 5. Amplitudes $\Delta\epsilon/\epsilon$ induced by sinusoidal electric field pulses of different frequencies ν in solutions of λDNA (Δ), pHC79–DNA (×), PM2–DNA (○) and SP65–DNA (+) at a constant effective field strength of 417 V/cm (standard buffer, 2 °C, DNA concentrations from 51 to 109 μM): (a) peak to peak amplitudes $(\Delta\epsilon/\epsilon)_p$; (b) average stationary amplitudes $(\Delta\epsilon/\epsilon)_a$.

long DNA chains corresponds to a redistribution of ions for generation of a dipole.

Long polymer chains are known to exist in a large number of different configurations. On average the configuration of polymers with a chain length much beyond the persistence length may be considered in a first approximation as a polymer sphere. If a polymer chain is distributed randomly within a sphere, its optical anisotropy is zero. In this case an electrooptical signal may be generated by deformation of the spherical configuration. Due to the flexibility of the double helix, this is easily possible either by fluctuations of the polymer configuration or by stretching effects resulting in the presence of an electric field from charge fluctuations. It is expected that deformation of long polymer chains is characterized by a spectrum of time constants, which extends to increasing times with increasing chain lengths.

Polymer spheres may be deformed to configurations with ellipsoidal shape, which should be associated with an optical anisotropy. A dipole moment of such ellipsoids may be generated by redistribution of ions. According to the arguments given above, this redistribution should be a relatively fast process and, thus, the optical anisotropy of ellipsoidal configurations should be reflected by a virtually constant average stationary dichroism amplitude up to frequencies of at least 100 kHz.

Polymer spheres may also be deformed to configurations without symmetry (or with a symmetry lower than that of ellipsoids). Due to the high charge density given in the case of DNA, these configurations would be associated with high "permanent" dipole moments.[20] If the electrooptical effects result from these "permanent" dipole moments, the average stationary dichroism should decrease to zero at high frequencies. For rigid molecules with a standard permanent dipole moment, the average stationary dichroism should be zero over the whole range of frequencies.[8] In the case of flexible molecules, however, the distribution of charges is affected by the presence of an electric field: if the field vector remains in a given direction for a time comparable to or larger than the time constant(s) of internal motion, flexible molecules are subject to deformation. If deformation leads to a dipole moment, it is favored in the presence of an external electric field. In summary, field induced deformation leads to a measurable stationary dichroism at frequencies below the frequency range of internal motions and to a zero value of the stationary dichroism, when the frequency is above the range of internal motions. A comparison with the experimental data indicates that most of the electrooptical amplitude is due to the "deformation dipole" effect and only a minor contribution results from an induced dipole effect.

Compared to previous investigations of DNA by electrooptics in the frequency domain,[6,7,9,10] the present analysis is different in various respects. First of all, the DNA samples used previously were not of homogeneous chain length, whereas all the samples used in the present investigation are monodisperse. Then, the DNA concentrations used previously[9,10] were clearly higher (by factors of 10 or more) than those used in the present investigation. Furthermore, in several cases the DNA's were analyzed in solutions without pH buffer and, thus, the pH was not under control. A rigorous control of the pH is clearly required, especially at low salt concentrations. Finally, we have used a more elaborate technique for recording and analysis of our experimental data. Our present data were obtained by measurements of the linear dichroism. More sensitive measurements are possible by using the birefringence technique, but conclusions on molecular structures may be derived more easily from dichroism data.

The data obtained for the restriction fragment with 256 bp are very useful as a reference. On the basis of this reference, some interpretations of experimental data given previously appear to be unlikely. Moreover, some of the experimental data presented in the literature are probably affected by artifacts. Because there are several differences both in the samples and in the procedure used for the analysis, it is hardly possible to identify the source of the discrepancy in more detail.

The detailed recording and deconvolution of the experimental data used in our present investigation provides information more conveniently and more directly than is accessible by frequency selective amplification techniques using, e.g., lock-in detectors or network analyzers. All the information, which has been extracted separately from frequency dependences of peak to peak amplitudes, average stationary amplitudes, or phases in the past, is extracted by deconvolution in a single step. Pertubations from a limited bandwidth may be corrected easily. The number of relaxation time constants, for example, may be determined from experimental data recorded at a single frequency. Unusual response curves (in preparation) can be identified by simple visual inspection, and the relaxation components leading to a given response may be determined quantitatively. Finally, an important advantage of the deconvolution technique is the fact that transients, which are observed at the start or the end of an enforcing

wavetrain and contain valuable information, may be included in the analysis without problems.

Acknowledgment. The technical assistance of Hartmut Dangendorf is gratefully acknowledged. The facilities of the Gesellschaft für wissenschaftliche Datenverarbeitung mbH Göttingen have been used for fitting of experimental data and for model calculations. We thank Dr. F. Eggers for valuable comments on the manuscript.

References and Notes

(1) Fredericq, E.; Houssier, C. *Electric Dichroism and Electric Birefringence*; Clarendon: Oxford, U.K., 1973.
(2) O'Konski, C. T. *Molecular Electrooptics, I Theory and Methods, II Applications to Biopolymers*; Dekker: New York, 1976, 1978.
(3) Charney, E. *Q. Rev. Biophys.* **1988**, *21*, 1.
(4) Stoylov, S. P. *Colloid Electro-optics—Theory, Techniques, Applications*; Academic Press: New York, 1991.
(5) Jennings, B. R. In *Light scattering from polymer solutions*; Huglin, M. B., Ed.; Academic Press: London, 1972; p 529.
(6) Jennings, B. R.; Plummer, H. *Biopolymers* **1970**, *9*, 1361.
(7) Stoimenova, M.; Radova, T.; Stoylov, S. P. *Colloid Polym. Sci.* **1979**, *257*, 1226.
(8) Thurston, G. B.; Bowling, D. I. *J. Colloid Interface Sci.* **1969**, *30*, 34.
(9) Wilkinson, R. S.; Thurston, G. B. *Biopolymers* **1976**, *15*, 1555.
(10) Ookubo, N.; Hirai, Y.; Ito, K.; Hayakawa, R. *Macromolecules* **1989**, *22*, 1359.
(11) Colpan, M.; Riesner, D. *J. Chromatogr.* **1984**, *296*, 339.
(12) Rabl, C. R. Dissertation, Universität Konstanz, 1985.
(13) Porschke, D.; Jung, M. *J. Biomol. Struct. Dyn.* **1985**, *2*, 1173.
(14) Eigen, M.; DeMaeyer, L. *Techniques of Organic Chemistry VIII*; Weissberger, A., Ed.; Interscience: New York, 1963; Part II.
(15) Grinvald, A.; Steinberg, I. Z. *Anal. Biochem.* **1974**, *59*, 583.
(16) Cooper, G. R.; McGillen, C. D. *Methods of Signal and System Analysis*; Holt, Rinehard and Winston Inc.: New York, 1967.
(17) Tinoco, I., Jr.; Yamaoka, K. *J. Phys. Chem.* **1959**, *63*, 423.
(18) Eigen, M.; Maass, G. *Z. Phys. Chem.* **1966**, *49*, 165.
(19) Porschke, D. *Biophys. Chem.* **1985**, *22*, 237.
(20) Antosiewicz, J.; Porschke, D. *Biophys. Chem.* **1989**, *33*, 19.