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A resonance-like effect induced by sinusoidal electric fields in a macromolecular dipole

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Abstract

The linear dichroism of DNA fragments with 587 and 859 base pairs has been measured under sinusoidal electric fields over a broad range of frequencies. The sine-amplitude of the dichroism shows a distinct maximum at frequencies around 1 kHz at an effective electric field strength of ≈ 400 V/cm and at a salt concentration around 1 mM; this maximum does not appear at a field strength of ≈ 200 V/cm. At low frequencies, the initial period of the linear dichroism induced by the electric field shows a characteristic dependence on the phase of the enforcing sinusoidal electric field. The essential part of the system response is described by a deconvolution procedure with a quadratic dependence of the optical effect on the field strength and by two relaxation processes with amplitudes of opposite sign. These relaxation processes are virtually identical with those found independently in the relaxation response induced by rectangular field pulses under equivalent experimental conditions; under these conditions the stationary electric dichroism is positive. Additional processes found in the relaxation response induced by sinusoidal field pulses indicate some field-induced conformation change. The 'resonance' has been recorded by the electric dichroism, which reflects the physical process of molecular alignment, but it is concluded that this process is accompanied by a resonance effect in the molecular configuration. The resonance effect observed in the present case clearly does not correspond to resonance as defined in physics. Nevertheless, the maximum of a system response at a given frequency due to superposition of relaxation effects – corresponding to e.g. activation and inhibition – may be useful for a selective control of molecular processes.

Key words: DNA double helix; Frequency domain electrooptics; Permanent/induced dipole; Phase dependence

1. Introduction

Resonance effects in molecular systems are of particular interest, because resonance may be very useful for selective control of processes. However, resonance effects have not been observed yet in 'simple' macromolecules. A claim was presented by Edwards et al. on resonance effects in nucleic acid double helices detected by dielectric relaxation [1]; subsequently the data of Edwards et al. have been interpreted extensively by van Zandt [2–4]. However, the 'resonance' effect could not be confirmed by other groups [5,6] and had to be attributed to some technical artefact.

In the present communication a special form of resonance is demonstrated for DNA restriction fragments. The resonance effect was found acci-

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dentally during an investigation of electrooptical effects in the frequency domain. Although the relaxation processes contributing to this resonance are shown to be directly related to special modes of orientational relaxation, it is concluded that the orientational 'resonance' is coupled to some resonance in the mode of ion distribution and in the macromolecular conformation.

2. Materials and methods

The DNA fragments with 587 base pairs (bp) and with 859 bp were prepared from the plasmids pUCBM20 (obtained from Boehringer Mannheim) and pRW574 (kindly provided by W. Hillen), respectively, by the restriction nuclease HaeIII. The fragments were separated by HPLC on ion exchange resins. All DNA samples 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 c(DNA) are given in units of nucleotide residues and have been calculated from the absorbance measured at 260 nm with an extinction coefficient 6500 M^{-1} cm^{-1}.

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 with sine waves of various well-defined frequencies were generated by a Fluke calibrator 5700 A. The phase of these sine-wave pulses has been controlled by a simple relay circuit, which has been initiated by the trigger circuit of an oscilloscope via its gate signal. Linear dichroism signals were recorded by an opto-electronic detection system, which has been developed for measurements of temperature jump relaxation [7]: 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 fluctuations in the lamp emission. 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 a special deconvolution procedure [8].

3. Results

The series of various electrooptical response curves induced in solutions of DNA fragments by sinusoidal electric field pulses is introduced by the limit case, where the experimental results are consistent with a standard orientational relaxation process, as shown in Fig. 1a for a restriction fragment with 587 bp. The amplitudes of the response curve in the stationary range may be characterized by (1) the average peak to peak value ΔA_p in the stationary part of the signal and (2) by the stationary average displacement ΔA_a from the level before application of the pulse. For a molecule with a standard induced dipole subjected to pulses of constant effective voltage, the ΔA_a value is expected to be independent of the frequency; the ΔA_p value should be constant at low frequencies $\nu \ll (2\pi\tau_r)^{-1}$, but should decrease to zero with increasing frequency within the dispersion range centered at $\nu = (2\pi\tau_r)^{-1}$, where τ_r is the rotational relaxation time constant. The existence of the simple standard response over the whole accessible range of frequencies has been verified for a DNA restriction fragment with 256 bp [8]. In the case of the fragment with 587 bp a deviation from the standard response is found at a frequency of 1 kHz (cf. Fig. 1b), where the optical response function exhibits particularly large amplitudes, but is almost symmetrical with respect to the zero level. The third example given in Fig. 1c has been measured at a frequency of 80 Hz (applied effective electric field strength 417 V/cm in all examples Figs. 1a–1c) and shows an amplitude of the optical response, which is clearly lower than that found at 1 kHz. Furthermore, the response induced by the 80 Hz field pulse shows a clear

deviation from the standard sine function. This deviation indicates some process(es) in addition to standard orientational relaxation. The dichroism response curves measured in the frequency range from 10 Hz to 100 kHz reveal a distinct

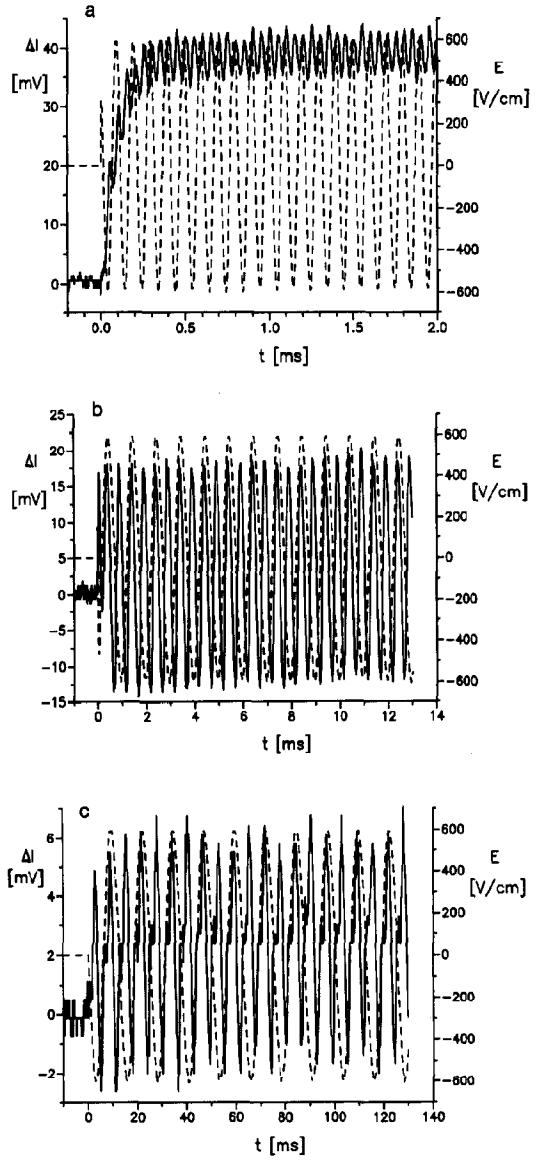

Fig. 1. Change of the intensity ΔI of light polarized parallel to the field vector in a solution of a 587 bp fragment induced by sinusoidal electric field pulses E (dashed lines) of different frequencies: (a) 10 kHz, (b) 1 kHz and (c) 80 Hz (standard buffer, 2°C, c(DNA) = 106 μM, the light intensity before pulse application corresponds to 8.2 V).

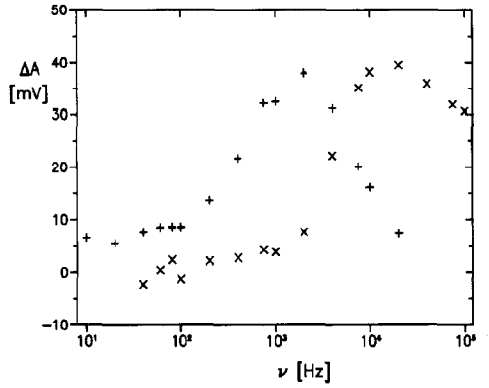

Fig. 2. Amplitudes ΔA of light polarized parallel to the electric field vector in a solution of a 587 bp fragment induced by sinusoidal electric field pulses of different frequencies ν: (+) peak to peak amplitudes ΔA_p and (×) average stationary amplitudes ΔA_a (conditions as described in legend to Fig. 1).

maximum of the ΔA_p values in the range around 1 kHz (cf. Fig. 2). The average displacement ΔA_a is close to zero at low frequencies, increases in a dispersion range centered around 5 kHz and finally decreases again at frequencies above 20 kHz.

Similar results have been obtained for a DNA fragment with 859 bp. A maximum of the ΔA_p values corresponding to a resonance effect is observed at a frequency of 1 kHz, when the effective electric field strength is 417 V/cm (cf. Fig. 3). The maximum is still visible at a field strength of 313 V/cm, but can hardly be detected anymore at 208 V/cm. The frequency dependence of the ΔA_p values found at 208 V/cm is very close to that expected for standard orientational relaxation with a dispersion range centered at about 3 kHz (cf. Fig. 3). The stationary average displacement ΔA_a is negative at low frequencies corresponding to a positive electric dichroism and positive at high frequencies corresponding to a negative electric dichroism (cf. Fig. 4).

Another remarkable result is obtained, when the onset of the optical response curve is recorded as a function of the phase of the applied sinusoidal electric field pulse. When the sinusoidal pulse starts with a phase delay of $\pi/4$ or $-\pi/4$, i.e. when the electric field strength 'immediately'

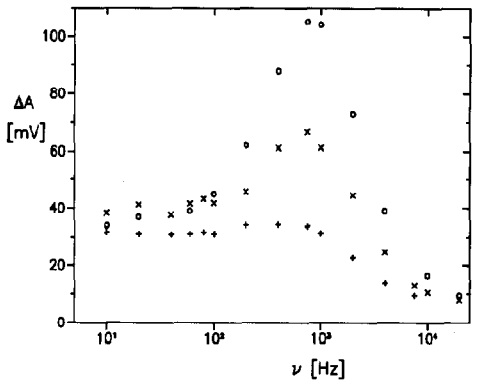

Fig. 3. Peak to peak amplitudes ΔA_p of light polarized parallel to the field vector in a solution of a 859 bp fragment induced by sinusoidal electric field pulses of different frequencies ν at the effective field strengths 208 V/cm (+), 313 V/cm (×) and 417 V/cm (○) (standard buffer, 2°C, c(DNA) = 152 μM, the light intensity before pulse application corresponds to 8.2 V).

jumps to its maximal value, the initial dichroism amplitude is much larger than the values observed, when the electric field strength returns to identical values later during the same pulse (cf. Fig. 5). Effects of this type are not observed, when the sinusoidal pulse starts without phase delay or with a phase delay of $\pi/2$, i.e. when the electric field strength increases smoothly from the zero level.

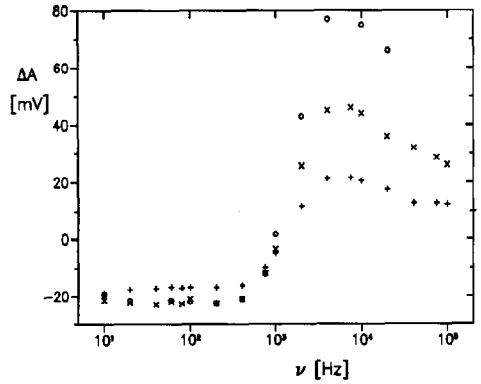

Fig. 4. Average stationary amplitudes ΔA_a of light polarized parallel to the field vector in a solution of a 859 bp fragment induced by sinusoidal electric field pulses of different frequencies ν at the effective field strengths 208 V/cm (+), 313 V/cm (×) and 417 V/cm (○) (conditions as described in the legend to Fig. 3).

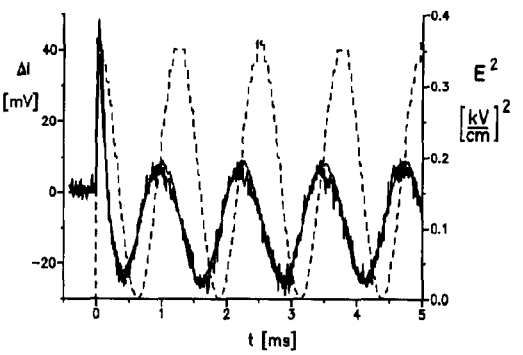

Fig. 5. Change of the intensity ΔI of light polarized parallel to the field vector in a solution of a 587 bp fragment induced by a sinusoidal electric field pulse E of 400 Hz. The least-squares fit (continuous line) with 2 relaxation processes obtained by a deconvolution procedure using the squared electric field strength E^2 (dashed line) as forcing function can hardly be distinguished from the measured data ($\tau_1 = 20$ μs, $\tau_2 = 120$ μs, $A_1 = -209$ mV/(kV/cm)^2, $A_2 = 256$ mV/(kV/cm)^2; the amplitudes are given per unit change of the forcing function; standard buffer, 2°C, c(DNA) = 174 μM, the light intensity before pulse application corresponds to 8.2 V).

The special dependence of the dichroism response curve on the phase of the electric field can be represented by two exponentials with amplitudes of opposite sign. This has been demonstrated by deconvolution of the experimental data using the square of the electric field strength as the forcing function (cf. Fig. 5). The time constants evaluated by this procedure are very close to those derived from independent data obtained by application of rectangular field pulses under equivalent experimental conditions. Under these conditions the stationary dichroism is negative and all transients require two exponentials with amplitudes of opposite sign for fitting.

Application of rectangular pulses over a broad range of field strengths E demonstrated [9] that the amplitude A_1 associated with the fast relaxation process increases strongly with E, whereas the amplitude A_2 associated with the slower process does not increase as strongly and also saturates at relatively low E values in the range around 2.5 kV/cm. Thus, the absolute value of the ratio A_1/A_2 increases strongly with E. Simulations of the relaxation response induced by sinusoidal forcing functions show that the ab-

sence of a resonance effect at low E values and its existence at higher E values is mainly due to the strong increase of $|A_1/A_2|$ with E. The peak to peak amplitudes A_p (calculated for sinusoidal pulses with an effective field strength of 417 V/cm) do not show any resonance effect, when A_1/A_2 is -0.63. However, a clear resonance effect is found, when $A_1/A_2 = -1.12$ was used in the simulations (cf. Fig. 6). As shown in a separate investigation [9], the increase of $|A_1/A_2|$ with the field strength is due to an increase of the induced dipole moment in the direction of the long axis of the molecules.

Some test experiments have been performed, in order to confirm that the relaxation response results from intramolecular processes. First of all, the amplitude of the measured dichroism signal increases linearly with the concentration up to ≈ 250 μM (in units of nucleotide residues/dm^3). Thus, the reduced electric dichroism is independent of the concentration and the dichroism effects reflect intramolecular processes without contributions from intermolecular ones. Experiments with detection of the light scattering intensity did not show any effects induced by sinusoidal electric field pulses; for technical reasons the effective electric field strength was limited to ≈ 200 V/cm in these tests. Measurements at the magic angle adjustment of polarized light did not show any effects under the conditions, where the experimental data discussed above were obtained. However, pulses of 417 V/cm and 10 Hz applied for a period $\geqslant 0.5$ s lead to some changes in the absorbance of the solutions. Samples exposed under these conditions also showed changes in the melting curves, indicating some field-induced denaturation. Some limited, local denaturation seems to be the reason for special dichroism response curves with clear deviations from a simple sine function found e.g. for the fragment with 859 bp at low frequencies (cf. Fig. 7). The same response was observed when the phase of the applied sinusoidal pulse was shifted by π, whereas the dichroism response was different, when the phase of the applied pulse was changed by $+\pi/2$ or $-\pi/2$.

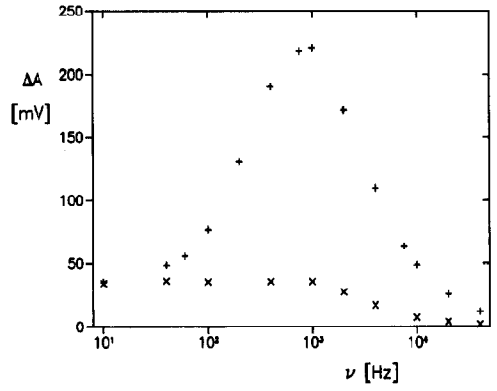

Fig. 6. Peak to peak amplitudes ΔA_p obtained by numerical simulation of the relaxation response induced by forcing functions of different frequencies ν. The response is calculated for the squared forcing function with the following relaxation time constants: $\tau_1 = 44$ μs, $\tau_2 = 225$ μs. The data denoted by (×) are calculated with the amplitudes $A_1 = -155.8$ mV/(kV/cm)^2, $A_2 - 247.9$ mV/(kV/cm)^2 and simulate the conditions at low electric field strengths; the data denoted by (+) were calculated with $A_1 = -836.8$ mV/(kV/cm)^2 and $A_2 = +744.6$ mV/(kV/cm)^2 and simulate the conditions at a higher electric field strength (both sets of data were calculated with forcing functions of an effective electric field strength of 0.417 kV/cm).

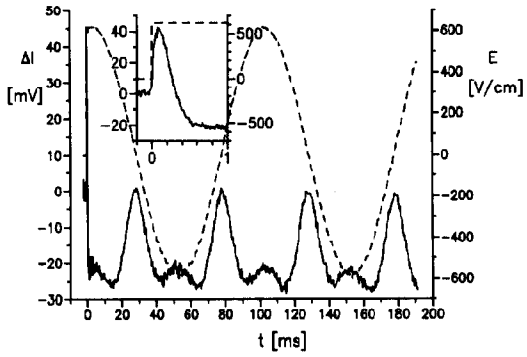

Fig. 7. Change of the intensity ΔI of light polarized parallel to the field vector in a solution of a 859 bp fragment induced by a sinusoidal electric field pulse E (dashed line) of 10 Hz with an effective field strength of 417 V/cm (standard buffer, 2°C, c(DNA) = 90 μM, the light intensity before pulse application corresponds to 8.2 V). The insert shows the initial part of the data at a higher time resolution.

4. Discussion

The experimental data obtained for the DNA fragments with 587 and 859 bp clearly show a

distinct maximum of the dichroism peak to peak amplitude in the frequency range around 1 kHz. This effect is well reproducible and the main part of the effect can be explained in terms of properties of the DNA molecules, which have been characterized independently in a recent investigation [9]. According to the independent results, the DNA molecules bear a considerable permanent dipole moment under the experimental conditions, where the resonance effect has been observed. The permanent dipole moment is due to the fact that DNA molecules are subject to bending: the loss of symmetry due to bending together with the high charge density and the large dimensions of DNA lead to dipole moments of considerable magnitude. Model calculations predict that the permanent dipole moment of bent double helices, which is mainly directed perpendicular to the long axis in a given range of chain lengths, should lead to positive values of the electric dichroism for these chain lengths [9]. Experiments verify the prediction in a restricted range of low electric field strengths. When the electric field strength is increased to values above 1 kV/cm, the electric dichroism is negative. This change in the sign of the dichroism is attributed to an increase of the polarizability along the long axis of the molecules with the electric field strength.

As described above, the resonance effect appears in the range of electric field strengths, where the stationary dichroism is positive, but it also requires a minimal field strength. Thus, the resonance effect is due to coupling of the permanent and the induced dipole moment, which results in a special type of rotational diffusion reflected by two major relaxation processes. The amplitude of the rotational relaxation with the smaller time constant is enhanced with increasing electric field strength, which is attributed to an increase of the induced dipole moment. The amplitude of the other relaxation process with the larger time constant is maximal in the limit, where the relaxation response is dominated by the permanent moment. As shown by the simulations given in Fig. 6, the contributions of both the permanent and the induced moment are essential for the existence of the resonance effect. The amplitudes associated with the modes of rotational diffusion are determined by the magnitudes of the permanent and the induced dipole moments. The maximum of the electric dichroism amplitude A_a in the frequency range around 1 kHz is expected to be coupled with a maximal change in the polarization of the molecules. Because of this coupling, the resonance effect in the

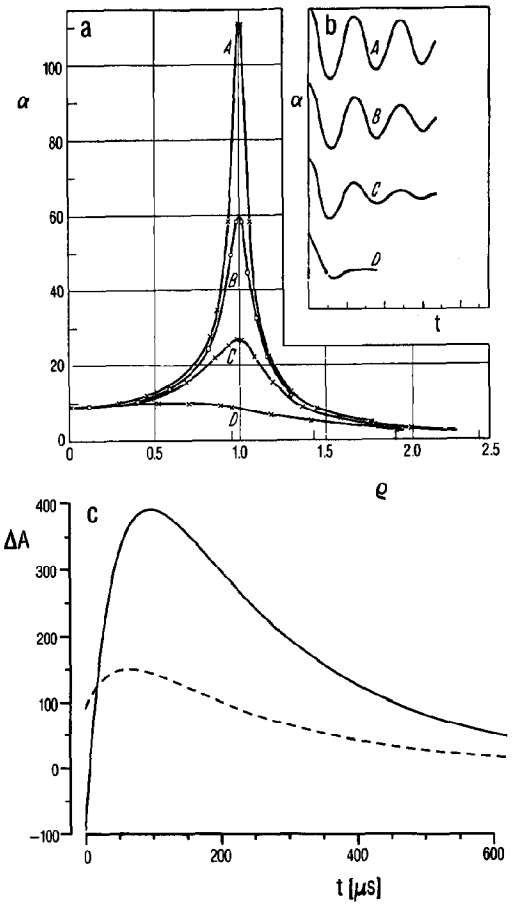

Fig. 8. (a) Amplitude α of a resonator at different excitation frequencies ρ, given relative to the natural frequency of the resonator, and at different damping constants: (A) 1.29, (B) 1.60, (C) 2.90 and (D) ≈ 50. (b) Amplitude α of the same resonator as a function of time after a stepwise perturbation at different damping constants K (values A to D as in (a); (a) and (b) reproduced with permission from [11]. (c) Relaxation curves calculated for the two exponential relaxation responses used in Fig 6; $\tau_1 = 44$ μs, $\tau_2 = 225$ μs with the amplitudes $A_1 = -155.8$ mV, $A_2 = 247.9$ mV (dashed line) and $A_1 = -836.8$ mV, $A_2 = 744.6$ mV (continuous line).

electric dichroism should be connected at least with a resonance effect in the ion distribution around the double helices. Furthermore, the ion distribution should be coupled with the degree of bending, i.e. with the configuration of the DNA, and, thus, a resonance effect in the ion distribution should also lead to some resonance effect with respect to the DNA configuration.

An effect closely related to that described in the present communication has been included in the theoretical part of a paper on "the frequency dependence of the Kerr effect for suspensions of rigid particles" by Thurston and Bowling [10]. These authors considered rigid ellipsoidal particles and found a maximum in the frequency dependence of the amplitude of the alternating component for the case, where a permanent dipole is directed along the long axis of the ellipsoid and the induced dipole is perpendicular to the permanent one. As shown by the evidence given in ref. [9], the directions of the dipoles are just opposite in the present case. Furthermore, Thurston and Bowling considered a case with a single electrooptical decay time constant, whereas two decay time constants corresponding to two modes of rotational diffusion are found in the present case.

The maximum of the amplitude at a given frequency suggests the existence of a resonance, but the mechanism in the present case is different from that of a resonance as defined in physics. The difference may be illustrated by the response resulting from a stepwise perturbation. In the case of a system with a true resonance, e.g. an elastic spring, the equilibrium state is approached with oscillations (cf. Fig. 8). For high values of the damping constant, the amplitudes of subsequent oscillation periods decrease rapidly to zero. In the present case a stepwise perturbation does not induce oscillations, but a relaxation effect composed of two processes with amplitudes of opposite sign, which mimic an oscillation response with a high damping constant. The superposition of these two processes is sufficient to cause a maximum of the amplitude at a given frequency.

Resonance effects may be very useful for a selective control of processes. At a first glance, the effect described above does not appear to be suitable for this purpose. However, coupling to the conformation of the DNA is easily possible and must be expected to occur. A field-induced change of the DNA conformation is indicated by the special dichroism response shown in Fig. 7, for example. Under these conditions, subsequent steps for amplification of signals are clearly possible.

Acknowledgement

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