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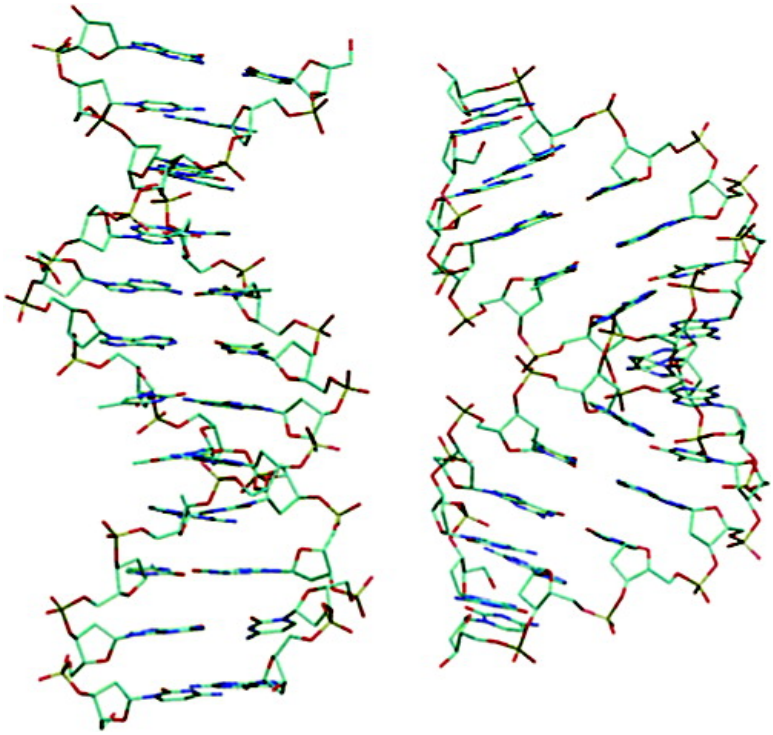

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The Dynamics of the B−A Transition of Natural DNA Double Helices

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Abstract: The dynamics of the B−A transition of DNA double helices with different GC contents and various chain lengths has been characterized by an electric field pulse technique. The field-induced B−A reaction is separated from orientation effects using the magic angle technique. Amplitudes reflecting the B−A reaction are observed selectively in the limited range of ethanol contents, where CD spectra demonstrate the B−A transition. The maximum amplitude appears at 1−2% higher ethanol content than the center of the B−A transition observed by CD because electric field pulses induce a relatively large perturbation from the A- toward the B-form. The relaxation curves measured after pulse termination reflect a spectrum of up to three relaxation processes. For DNA's with ∼50% GC, the main part of the amplitude (∼75%) is associated with time constants of ∼2 μs, and another major component appears with time constants of 50−100 μs. These relaxation effects have been observed for DNA samples with 859, 2629, 7160, and 48501 bp. The time constant associated with the main amplitude increases with decreasing GC content from ∼2 μs at 50% GC to ∼3 μs at 41% GC and ∼10 μs at 0% GC at the center of the B−A transition. Model calculations on the kinetics of cooperative linear Ising lattices predict the appearance of a distinct maximum of the mean relaxation time at the center of the transition. The absence of such maximum in our experimental data indicates a low cooperativity of the B−A transition with a nucleation parameter of ∼0.1. The rate of the B−A transition is lower by ∼3 orders of magnitude than that predicted by molecular dynamics simulations.

Introduction

The B−A transition of DNA double helices has been characterized initially by X-ray analysis of DNA fibers.[1−4] Over the years, the information on B- and A-structures was extended by the determination of many crystal structures with high resolution.[5,6] Analysis of protein−nucleic acid complexes revealed that the standard B-form of DNA is converted to A-type structures upon binding of proteins in many cases[7−11] and, thus, confirmed the biological relevance of the B−A transition. The equilibrium transition between the B- and the A-form of DNA has also been studied extensively in solution.[12−14] However, the dynamics of this transition has not been characterized, until very recently first results[15] were obtained for the case of poly[d(A−T)]. This model polymer was selected because its B−A transition is much narrower than that of natural DNA. Thus, a given perturbation by a change of an external parameter may generate relatively large relaxation amplitudes. Using the electric field pulse technique,[16,17] it was possible for the first time to resolve this reaction. The observed time constants in the range around 10 μs are in stark contrast with predictions by molecular dynamics simulations. On the basis of the available force-fields, many different groups[18−24] predicted that the B−A transition is complete within a few nanoseconds.

The discrepancy raises questions both on the molecular dynamics simulations and on the experimental data. In the present investigation, we will not discuss any potential problems associated with the simulations, but try to extend the experimental evidence and eliminate any arguments against the available experimental data. For example, it may be argued that poly[d(A−T)] double helices are special and not representative

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of natural DNA. Thus, experimental data on the dynamics of the B−A transition of natural DNA samples with a mixed composition of base pairs are required. The B−A transition of these DNA's is not as narrow because the affinity toward the A-form is dependent on the GC content; GC-rich sequences have a higher tendency to form the A-structure than do AT-rich sequences.[14] For this reason, the reaction amplitudes induced in DNA's with mixed sequences by a given perturbation are smaller, and the sensitivity had to be increased for measurements of sufficient accuracy. By selection of optimal experimental conditions, we managed to get data for various natural DNA samples. The results demonstrate that the main features of the B−A dynamics in natural DNA are similar to those of poly[d(A−T)], but that there is some difference in detail, revealing a special signature of GC base pairs in the B−A dynamics.

Materials and Methods

The DNA fragment with 859 bp was prepared by HaeIII digestion from the plasmid pVH51, kindly provided by Wolfgang Hillen. The plasmid DNA pF012 was obtained from Plasmid Factory, Bielefeld; this plasmid DNA was linearized by EcoRI and contained 7160 bp in the linear form. A plasmid DNA sample derived from pUC18 covered the sequence between the EcoRI and HindIII restriction sites and, thus, contained 2629 bp. λ-DNA (48501 bp) was obtained from Fermentas. DNA samples subjected to digestion by restriction nucleases were purified by phenol extraction. Poly[d(A−T)] with an average chain length of 1600 bp and salmon testes DNA with an average chain length of 8000 bp were obtained from Sigma. The poly[d(A−T)] sample with an average chain length of 100 bp was generated by sonication and subsequent fractionation on a sephacryl S500 column. Chain lengths were determined by gel electrophoresis. All DNA samples were dialyzed extensively first against 1 M NaCl, 1 mM cacodylate pH 7, 1 mM EDTA, and finally against 250 μM NaCl, 250 μM cacodylate pH 7, and 50 μM EDTA. The GC content is 52.6, 49.4, 47.8, and 50.1% for the DNA's with 859, 2629, 7160, and 48501 bp, respectively. The GC content of salmon testes DNA is 41.1%. DNA concentrations are given in monomer units.

The samples were subjected to electric field pulses using a generator originally constructed by Grünhagen.[26] Parts of this pulse generator were adapted to reduce perturbations and optimize operation. The optical detection system was reconstructed for optimal signal-to-noise ratio and clean measurements under magic angle conditions.[17,27] For most of the measurements, we used a cell machined from macrolon with an optical path-length of 2 cm and Pt electrodes at a distance of 5.5 mm. The magic angle was calibrated by dichroism measurements using a sonicated DNA sample at angular orientations of the polarizer in the range from −10 to 100° with respect to the field vector. The resulting data were fitted to the expected standard dependence, providing the value for the magic angle at an accuracy of ±0.1°. The magic angle technique, which is crucial for the present study, has been discussed extensively, both in general[17,27] and with special emphasis to measurements of the B−A transition.[15]

Transients were fitted by a combined linear (amplitudes) and nonlinear (time constants) least-squares fitting procedure. Convolution with a limited bandwidth of the detector was included either by representing the detector response by an exponential and application of analytical convolution equations[28] or by using the directly measured detector response with a numerical convolution procedure.[29]

When relaxation curves show a broad distribution of time constants and/or the time constants cannot be separated with sufficient accuracy, it is useful to characterize the relaxation process by its integral time constant

$$\tau_i = \sum \tau_n \times A_n/A_t$$

where τ_n and A_n are the time constant and the amplitude of the nth relaxation process, respectively; A_t is the total relaxation amplitude.

Ethanol contents are given in volume %. The ethanol content of solutions subjected to degassing for field jump experiments was determined by density measurements using a DMA60 density meter with a DMA602 cell (Anton Paar, Graz, Austria).

Results

Search for Optimal Conditions. The possibilities to induce the B−A reaction at a sufficient time resolution are limited. To the best of our knowledge, the electric field pulse technique is the only one that can be used (1) to apply sufficiently large perturbations and (2) to follow the reaction at the required time resolution. The first attempts[15] to characterize the B−A transition of natural DNA by this technique were not successful. Now we continue our efforts by careful optimization of reaction conditions, using the experience obtained with the model system poly[d(A−T)].

Studies of the B−A reaction *in solution* are restricted to low salt concentrations because reduction of the water activity by ethanol induces precipitation at higher salt. Thus, there are hardly any degrees of freedom in the solution parameters, and the only chance remaining for signal improvement is careful selection of the parameters of optical detection. The standard difference spectrum observed during the B−A transition of natural DNA shows maximal values of absorbance decrease around 245 nm and of absorbance increase around 265 nm. The other wavelength ranges seem to be without interest; wavelengths below 240 nm are not useful because light sources of sufficiently high intensity are not available in this range, whereas changes appear to be too small at $\lambda > 280$ nm. However, a change of the presentation from the standard *absolute* to *relative* absorbance changes (cf. Figure 1) reveals that the range $\lambda >$ 290 nm is of particular interest. Although the absolute changes are small in this range, the relative changes are clearly higher than in other wavelength ranges. Obviously higher concentrations are required for measurements at $\lambda > 290$ nm, but these concentrations are still in a range around 100 μM and remain quite low compared to the requirements of other techniques.

For optimal results, we want to maximize the amplitude of the B−A reaction, but at the same time, we have to avoid any unwanted side reaction. It is known that electric field pulses may induce denaturation of DNA double helices. Detailed studies of this reaction demonstrated that DNA denaturation remains negligible below thresholds of the electric field strength, which are dependent on the DNA chain length and the ionic conditions.[30] We have checked the thresholds for our samples under the conditions used for the studies of the B−A transition. In general, we used pulses for induction of the B−A transition with field strengths remaining ~5 kV/cm below the threshold for denaturation. Thus, any residual field-induced denaturation

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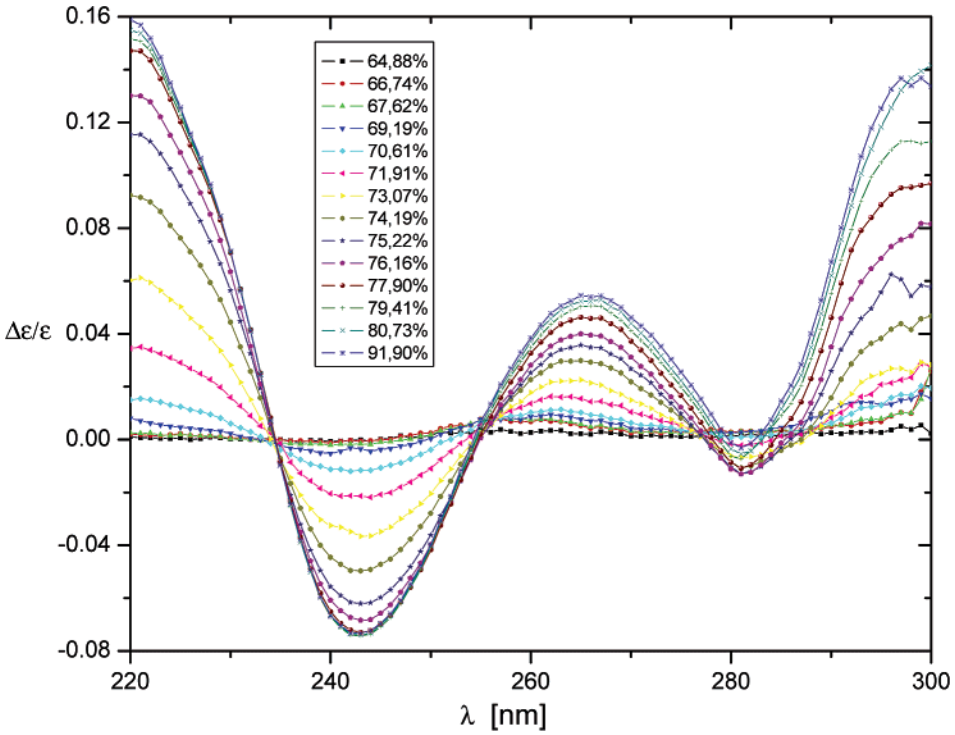

Figure 1. Relative absorbance difference spectra of plasmid DNA (2629 bp) as a function of the ethanol content (v/v). The spectrum measured at 62.88% is subtracted from the spectra measured at higher ethanol content and then divided by the spectrum measured at 62.88% (8 °C, 75 μM NaCl, 75 μM Na cacodylate, 15 μM EDTA).

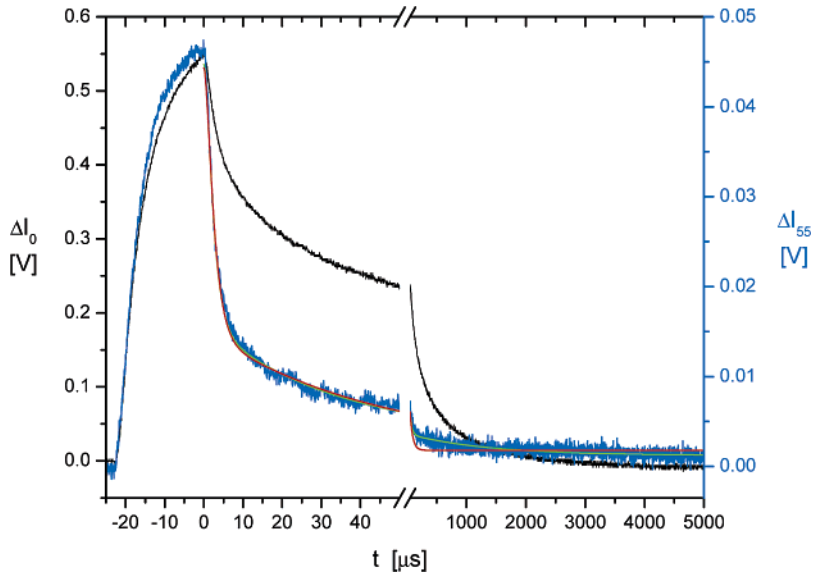

Figure 2. Transients of the electric dichroism (ΔI_0, black line, left scale) and of the field-induced B−A reaction (ΔI_{55}, blue line, right scale); $t = 0$ of the time axis has been fixed to pulse termination. Least-squares fits of the magic angle decay curve by two and three exponentials are shown in red and green, respectively ($\tau_1 = 1.87$ μs, $A_1 = 29.5$ mV, $\tau_2 = 45$ μs, $A_2 = 13$ mV, and $\tau_1 = 1.7$ μs, $A_1 = 28.8$ mV, $\tau_2 = 31.5$ μs, $A_2 = 12.2$ mV, $\tau_3 = 1.3$ ms, $A_3 = 2.4$ mV). Plasmid DNA with 2629 bp in 75 μM NaCl, 75 μM Na cacodylate, 15 μM EDTA, 8 °C, 75.77% EtOH, electric field pulse = 27 kV/cm for 20 μs.

should be negligible under our experimental conditions. Moreover, if there should be some residual denaturation, its effect on the dynamics of the B−A transition is expected to be hardly detectable because the cooperative length of the B−A transition[31] is much shorter than that of the helix-coil transition.[32]

Relaxation Data for Plasmid and λ DNA. Electric field jump experiments using a plasmid DNA with 2629 bp confirm the expectation described above and reveal effects at the magic angle that can be characterized at a sufficient accuracy. The electric field pulses induce an increase of the light intensity at wavelengths around 300 nm (Figure 2), demonstrating a reaction from the A- to the B-form under the field pulses. The spectrum of time constants found in the magic angle decay curves is clearly different from that observed in the decay of the dichroism and, thus, confirms that separation of chemical and physical effects is successful. The amplitudes observed under magic angle conditions show a clear maximum at an ethanol content of

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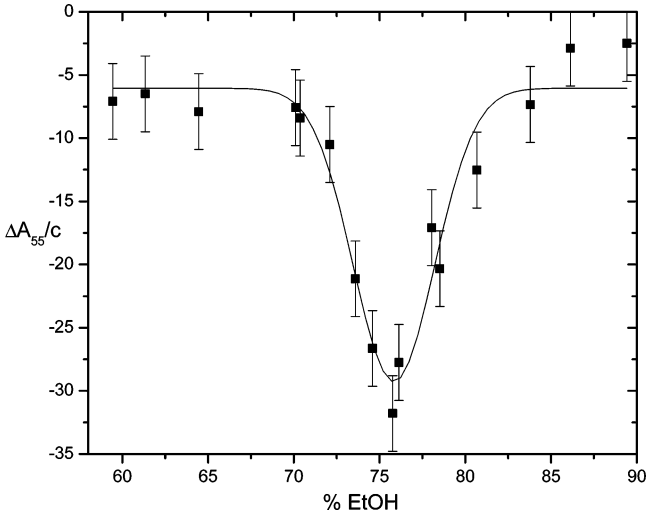

Figure 3. Magic angle amplitudes $\Delta A_{55}/c$ induced at 296 nm for the plasmid DNA with 2629 bp by electric field pulses of 34.5 kV/cm as a function of the ethanol content (in v/v %). The $\Delta A_{55}/c$ values are given in absorbance units normalized by the nucleotide concentration c (75 μM NaCl, 75 μM Na cacodylate, 15 μM EDTA, 8 °C, 80 μM plasmid DNA). The continuous line represents a fit by a Gaussian with a center at 75.84% ethanol v/v and a width of 4.74.

75.8% (Figure 3). Thus, the B−A amplitudes are observed in the same range of ethanol contents, where the B−A transition is found by other methods. However, a more detailed examination demonstrates a noticeable difference. When the CD band at 268 nm is used as a measure of the B−A equilibrium, the center of the B−A transition is observed at an ethanol content of 73.9%. Thus, the maximum of the field jump amplitudes appears at a higher ethanol content than that corresponding to the midpoint of the equilibrium transition. This shift of the maximum amplitude is expected because the electric field pulses induce a relatively large perturbation of the equilibrium from the A- toward the B-form. Identical values for the ethanol content at these points are expected only in the limit of very low extents of perturbation.

The decay of the magic angle effect, observed after pulse termination, requires at least two exponentials for a satisfactory fit. In some cases, fits are improved by a third exponential (cf. Figure 2). We cannot distinguish whether the relaxation response reflects a continuous spectrum of time constants for the B−A reaction or separate normal modes of this reaction. The rise curves, observed in the presence of the electric field pulse, usually require two exponentials for a satisfactory fit. The time constant of the process reflecting almost all of the rise amplitude shows a clear increase with the ethanol content, whereas the time constants associated with the dominant part of the amplitude for the decay curves are almost independent of the ethanol content (Figure 4). The time constants associated with the main components of the rise and the decay curves are identical at ∼73.8% ethanol (cf. Figure 4), which is close to the center of the B−A equilibrium indicated by CD data. At this center, the rates for forward and backward reactions are expected to be identical, provided that the perturbation by the electric field on these rates can be neglected.

As a test for a potential chain length dependence, a sample of λ DNA with a chain length of 48501 bp was analyzed under closely corresponding conditions. In this case, the center of the B−A transition was observed by CD at 73.7% ethanol, whereas the maximum of the field jump amplitudes induced by pulses of 27.3 kV/cm appeared at 74.8%. The spectrum of time constants associated with the magic angle rise and decay curves is very similar to that observed for the plasmid DNA with 2629 bp. The relaxation time constants associated with the dominant part of the amplitudes observed for the rise and the decay curves (Figure 4) are identical at ∼73% ethanol, which is again close to the center of the equilibrium transition. Another set of field jump data was obtained for a plasmid DNA with 7160 bp, which showed results very similar (cf. Figure 4) to those observed for the DNAs with 2629 and 48501 bp.

DNA Restriction Fragment with 859 Base Pairs. The analysis of the B−A dynamics in natural DNA is facilitated by DNA samples with a particularly narrow B−A transition. Hillen et al.[33] reported such a narrow transition for a restriction fragment with 95 bp. We have used a longer restriction fragment with 859 bp because reaction amplitudes induced by electric field pulses are expected to be more extensive for longer DNA's. Measurements at 248 nm revealed a field-induced increase of the absorbance under magic angle conditions in the restricted range of ethanol contents from 72 to 77% ethanol. The maximum amplitude appeared at 74.5% ethanol. The transients require two exponentials for satisfactory fitting. Most of the amplitude observed in the field free state is represented by the fast process with time constants in the range from 2 to 5 μs. As shown in Figure 5, the time constants associated with the main amplitudes do not show much variation upon changes of the ethanol content. Furthermore, these time constants are very similar—within experimental accuracy—for the rise and the decay process, indicating that the extent of perturbation is relatively small.

Another set of data was obtained by measurements at 265 nm, where electric field pulses induce a decrease in the magic angle absorbance within the B−A transition range. The transients are very similar again, but there is a trend to somewhat shorter time constants at 248 nm compared to those at 265 nm. The difference is relatively small and close to the current level of experimental uncertainty. Thus, it is not clear yet whether there is uneven weighting of the relaxation spectrum at different wavelengths.

Salmon Testes DNA. All the data described above were obtained for DNA's with a GC content very close to 50%. To test for a dependence on the GC content in natural DNA, we have analyzed salmon testes DNA. The chain length distribution of the commercially available sample is relatively broad. However, the data obtained for DNA's with 50% GC demonstrate that the B−A time constants are not dependent on the chain length in the range from 859 to 48501 bp. The relaxation transients induced by electric field pulses in salmon testes DNA are very similar to those found for the other DNA samples. A difference was observed for the time constant associated with the main amplitude in the transients recorded after pulse termination; this decay time constant was ∼3 μs for salmon testes DNA, whereas DNA samples with 50% GC showed values of ∼2 μs for the corresponding process (cf. Figure 4). The difference is relatively small, but combined with the data obtained for poly[d(A−T)], we find a continuous increase of this decay time constant with decreasing GC content.

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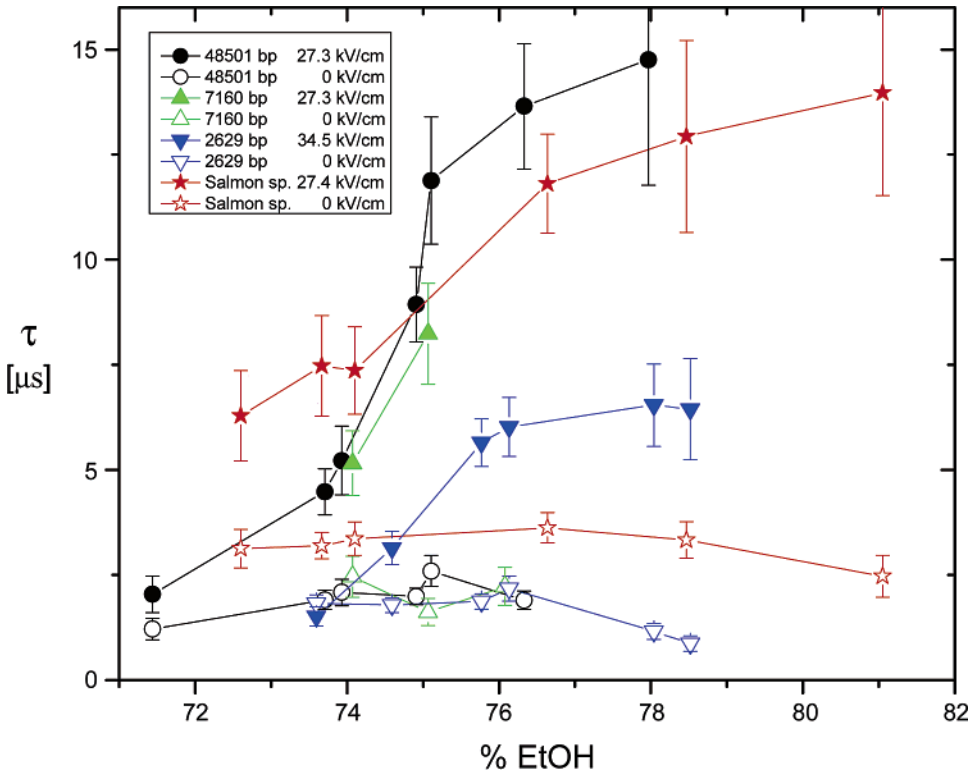

Figure 4. Time constants of natural DNA associated with the main amplitudes of the relaxation effects observed in the field free state (empty symbols) and under the influence of electric fields (filled symbols) as a function of the ethanol content (v/v): 8 °C, 75 μM NaCl, 75 μM Na cacodylate, 15 μM EDTA.

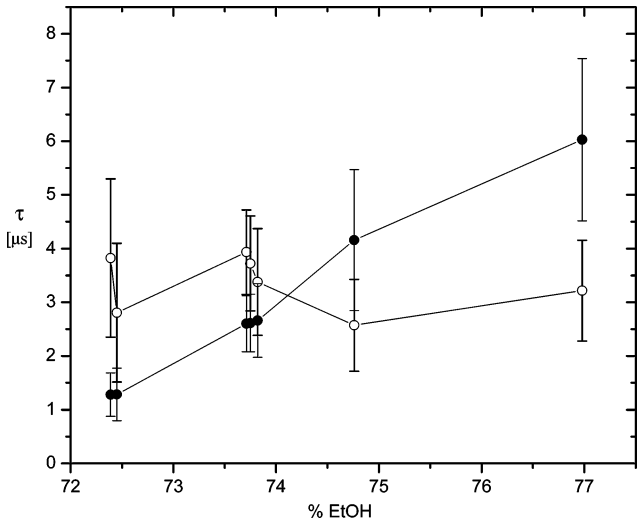

Figure 5. Magic angle time constants associated with the main amplitude of the 859 bp fragment measured at 248 nm as a function of the ethanol content in % units (v/v) at field strength $E = 0$ (○) and at $E = 34.5$ kV/cm (●). 8 °C, 75 μM NaCl, 75 μM Na cacodylate, 15 μM EDTA.

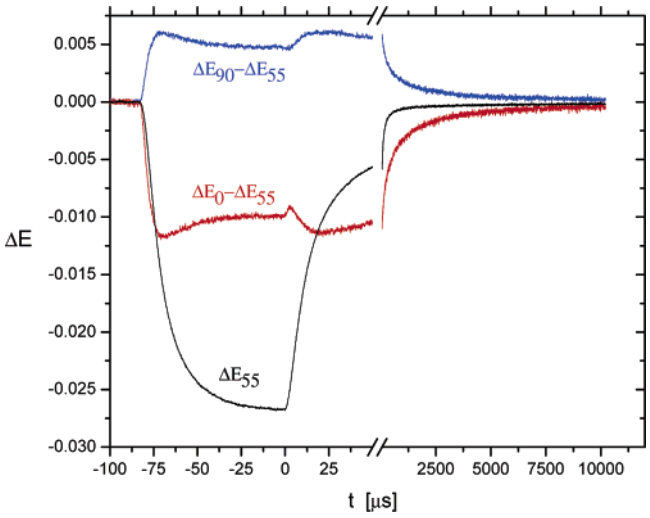

Figure 6. Field-induced change of the absorbance ΔE at 302 nm in poly-[d(A−T)] at different angular orientations φ of the polarized light with respect to the field vector. The transient at $\varphi = 55$ is the average of five shots; the transients $\Delta E_0 - \Delta E_{55}$ and $\Delta E_{90} - \Delta E_{55}$ are single shots and are corrected by the transient at $\varphi = 55$. The transient ΔE_{55} requires at least three exponentials for satisfactory fitting ($\tau_1 = 12.2$ μs, $\tau_2 = 124$ μs, $\tau_3 = 1.6$ ms, $A_1 = 74.1\%$, $A_2 = 23.0\%$, $A_3 = 2.9\%$): 8 °C, 70.68% EtOH v/v, 160 μM poly[d(A−T)], 75 μM NaCl, 75 μM Na cacodylate, pH 7, 15 μM EDTA; field pulse = 34.5 kV/cm.

Poly[d(A−T)]. The first experimental data on the B−A dynamics[15] were obtained for poly[d(A−T)] at wavelengths around the absorbance maximum ($\lambda = 248$ and 280 nm) using a relatively low concentration of the polymer. The wavelength range $\lambda > 290$ nm proves to be particularly attractive also for measurements with poly[d(A−T)] because the absorbance increases upon the reaction from the B- to the A-form by factors of more than 3. Under these conditions, the amplitudes due to the B−A transition are much higher than the dichroism amplitudes (Figure 6), and the B−A transients are of clearly higher quality than any other transients observed for this reaction in previous experiments. Thus, the B−A reaction of poly[d(A−T)] can be studied with increased accuracy. The time constants (cf. Figure 7) observed at a polymer concentration of 160 μM are in close agreement with those obtained previously[15] at a polymer concentration of 8.5 μM. These experimental data clearly show the absence of a dependence on the polymer concentration, as expected for an intramolecular reaction. Furthermore, the data indicate that the observed time constants are not affected by aggregation effects. Finally, the transients

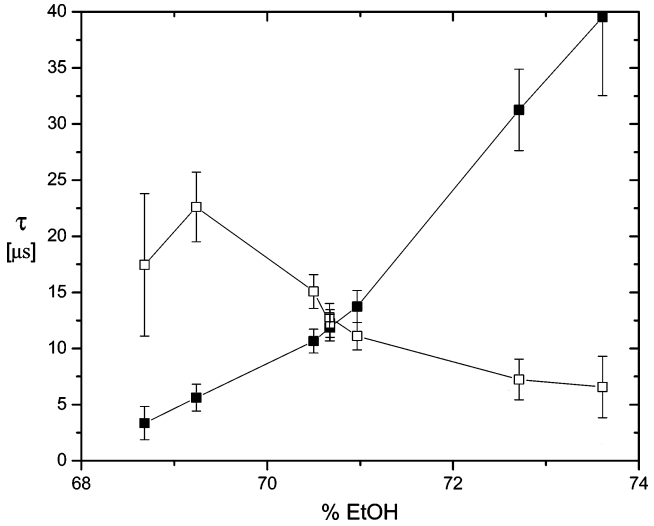

Figure 7. Time constants of poly[d(A−T)] associated with the main amplitudes of the relaxation effects observed in the field free state (empty symbols) and under the influence of electric fields (filled symbols, 34.5 kV/cm) as a function of the ethanol content (v/v): 8 °C, 75 μM NaCl, 75 μM Na cacodylate, 15 μM EDTA.

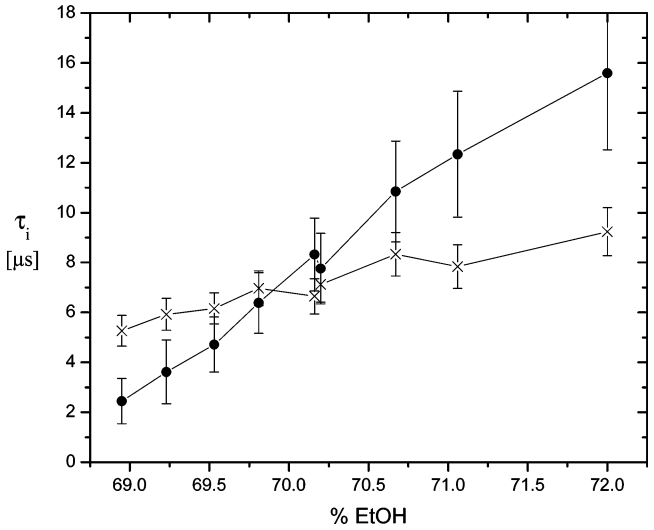

Figure 8. Integral time constants for magic angle rise (●) and dichroism rise (×) of poly[d(A−T)] as a function of the ethanol content in % units (v/v): 8 °C, 75 μM NaCl, 75 μM Na cacodylate, 15 μM EDTA, 34.5 kV/cm.

in Figure 6 with their dominant reaction amplitude and the small dichroism amplitude reconfirm the successful separation of reaction effects from orientation processes by the magic angle technique.

The case of poly[d(A−T)] has also been used to obtain more information about the mechanism of the field-induced reaction. In our first report,[15] we presented evidence for field-induced stretching as the major force driving the reaction from the A- to the B-form. We have now compared the spectra of time constants observed for the A→B transients and for the dichroism rise. At 70.32% ethanol, the integral time constants for these processes are identical within experimental accuracy (cf. Figure 8). However, at lower ethanol contents the A→B reaction tends to be faster than the dichroism rise, and at higher ethanol contents the opposite relation is observed (cf. Figure 8).

More information on the relation between the time constants of the A→B reaction and the molecular orientation can be

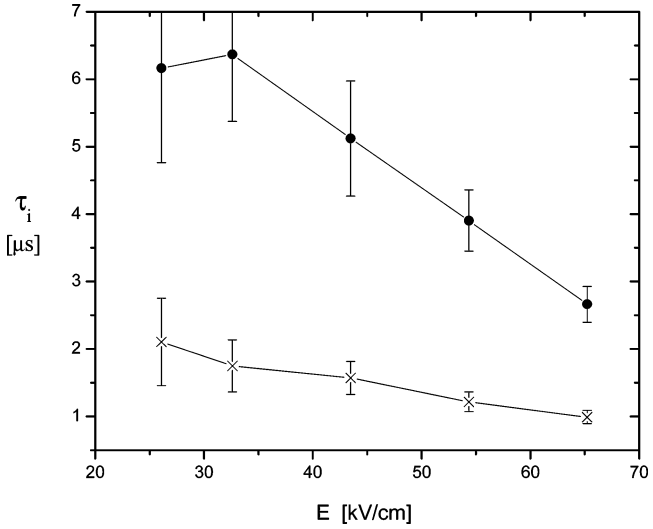

Figure 9. Integral time constants for magic angle rise (●) and dichroism rise (×) of poly[d(A−T)] with ∼100 bp as a function of the electric field strength: 70.60% ethanol (v/v), 8 °C, 75 μM NaCl, 75 μM Na cacodylate, 15 μM EDTA, 34.5 kV/cm.

obtained from samples of different chain lengths. When the chain length of the polymer is reduced, the rotation time constants are decreased considerably, but the reaction time constants remain almost constant. This is reflected in the integral time constants of the rise curves measured for a poly[d(A−T)] sample with ∼100 bp, where the time constants for the A→B reaction are larger by factors of ∼3 than those of the dichroism (cf. Figure 9).

Thus, field-induced orientation is faster than field-induced reaction for samples with a relatively low chain length. The opposite relation is observed for DNA samples with high chain lengths, as should be expected from the strong decrease in the rate of rotational diffusion with increasing chain length. This general trend is also reflected in the reaction time constants compiled in Figures 4 and 7. For samples with a relatively low chain length, like poly[d(A−T)] and 2686 bp plasmid DNA, the rise time constants are smaller than the decay constants at low degrees of the B→A transition. For samples with higher chain lengths, the rise time constants remain larger than the decay time constants even at low degrees of the B→A transition. This seems to be due to the fact that the orientation of long molecules and, thus, the full rise of their dipole takes more time.

A Relaxation Effect Outside the B−A Transition Range. The absorbance at wavelengths of $\lambda > 290$ nm indicates the B−A transition with a particularly high sensitivity. Apparently, this high sensitivity results from $\pi^* \leftarrow n$ transitions contributing in this wavelength range, which reflect changes in the environment, such as solvation, more directly than $\pi^* \leftarrow \pi$-transitions. Thus, the absorbance at $\lambda > 290$ nm may also reflect more subtle changes of the helix structure than the B−A transition. Indeed, we find a relatively small but nevertheless reproducible relaxation effect outside the range of ethanol contents for the B−A transition. This effect is reflected by a background amplitude appearing in the plots of the B−A amplitudes as a function of the ethanol content measured at $\lambda > 290$ nm (Figure 3). This background amplitude was not observed in the measurements of the B−A transition at $\lambda = 248$ and 280 nm. A detailed description of the new relaxation effect is in preparation.

Discussion

Interpretation of the Relaxation Effects. The reaction from the standard B-form of double helices to the A-form is induced by reduction of the water activity.[3,12−14] In vivo, this type of effect is associated with binding of proteins to DNA. Compilations of crystal structures clearly demonstrate that the structure of DNA fragments is shifted toward the A-form in many protein−DNA complexes.[7−11] Because the kinetics of protein binding to DNA usually is relatively complex, it is hardly possible to separate the B−A transition from the other steps of protein binding by any experimental technique. Thus, the conditions of the B−A transition must be simplified for a separate analysis of the B−A reaction. Addition of ethanol is quite convenient for this purpose, but restricts the reaction conditions to low salt because otherwise aggregation and/or precipitation cannot be avoided. Furthermore, the restriction to low salt, the absence of any sufficiently large temperature dependence, and a relatively high rate of the B−A reaction impose limits with respect to the technique. Currently, the electric field jump technique appears to be the only one that can be used reasonably well for this analysis.

Our present set of experimental data demonstrates that the B−A dynamics of natural DNA with mixed GC/AT base pairs is similar to that observed previously for poly[d(A−T)]. In all cases investigated up to now, we observed a relaxation response with at least two time constants, and in some cases, a third one is required for satisfactory fitting. Thus, the B−A reaction may be reflected by a broad spectrum of time constants rather than a limited number of normal modes. A broad spectrum may be expected because we have studied polymers with chain lengths much above the cooperative length. Even if we neglect long-range effects, resulting, for example, from changes of the contour length during the reaction, the coupling of many individual B−A reaction steps in sequences of B- and A-structures of different lengths may lead to a relatively broad spectrum of time constants. In the following, we do not discuss this problem any further and simplify our discussion by assigning major and minor relaxation responses based on the amplitudes obtained from least-squares fits of the experimental transients by sums of exponentials.

A distinctive feature of our experimental data results from the fact that the electric field pulses induce large perturbations in most cases. This is indicated by the large amplitudes recorded at the magic angle and by the clear difference between the relaxation transients observed under electric field pulses from those in the field free state. The electric field pulses induce large changes of at least one of the rate parameters, which is then reflected by changes of the relaxation time constant(s). In the present case, large perturbations may also affect the result by inducing different degrees of contributions from nucleation processes. Finally, the orientation of the polymer molecules into the direction of the electric field may affect the force and, thus, the extent of perturbation experienced by these molecules (see below).

Because of these special problems associated with the interpretation of the data measured under electric field pulses, we discuss first the results obtained at zero electric field strength. In all cases of natural DNA's with ∼50% GC, we observed a fast process representing ∼75% of the amplitude with time constants around 2 μs. It is likely that this process reflects mainly chain growth, whereas the slower processes are probably associated with nucleation. The combined set of time constants measured for the natural DNA's with chain lengths 2629, 7160, and 48501 bp shows a shallow maximum in the range of 74−75% ethanol content (Figure 4)—close to the center of the B−A transition. The data measured for the sample with 859 bp are consistent with this set, but were not included in Figure 4 because of their higher error level (cf. Figure 5).

The decay time constants observed for DNA samples with ∼50% GC are very similar over a wide range of different chain lengths. Thus, the clear increase in the decay time constants observed for the case of salmon testes DNA to ∼3 μs is due to the change in the GC content from ∼50 to 41.1%. A dependence of the B−A dynamics on the GC content is expected because it is known that CC/GG contacts facilitate the B to A transition.[14]

The time constant for poly[d(A−T)] associated with the main portion of the amplitude is about 10 μs at the center of its B−A transition. This is about a factor of 5 higher than the corresponding time constant observed for DNA samples with ∼50% GC. However, the center of the B−A transition for poly[d(A−T)] is at ∼69% ethanol and for DNA with ∼50% GC at ∼73.5% ethanol. Although there is a trend toward reduced time constants at higher ethanol content, the time constants for poly[d(A−T)] remain higher by a factor of ∼3 in the ethanol range around 73.5%, where data have been measured for both types of polymers. Thus, the different affinity of AT and GC base pairs toward the A-form is reflected in the B−A dynamics by an increase of the decay time constants with decreasing GC content.

Effect of Electric Fields on the B−A Reaction. The experimental data clearly show that the electric field pulses induce a reaction from the A- toward the B-form. Because electric fields drive reactions toward the state associated with a higher dipole moment,[16] the experiments indicate that the dipole moment of the B-form is higher than that of the A-form. This is consistent with the fact that the contour length of B-DNA is higher than that of the A-form and that the magnitude of dipoles measured for DNA double helices increases strongly with their length.[34] Thus, the A→B reaction seems to be driven by "dipolar stretching". However, the field-induced reaction may also be driven by a dissociation field effect. This effect detected by Wien[35] and its theoretical basis developed by Onsager[36] leads to dissociation of ion pairs and is known to be particularly extensive in polyelectrolytes.[37,38] In general, field-induced dissociation of ions is expected to favor the state with a lower degree of electrostatic repulsion, which is the B-form with a spacing of 3.4 Å between adjacent base pairs compared to ∼2.8 Å for the A-form. The dissociation field effect is expected to be a linear function of the electric field strength, *E*, but this is also expected for field-induced stretching because the dipoles are usually saturated under the conditions of the present experiments. In agreement with these expectations, the reciprocal rise time constants increase linearly with the field strength.[15]

In most cases, electric field pulses accelerate reactions, but in the present case, some time constants observed under electric field pulses are larger than those found at zero field strength,

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particularly at high ethanol contents (cf. Figures 4 and 7). This effect may be due to a field-induced decrease of the rate constant for the reaction from the B- to the A-form. However, we have to consider another interpretation, as well, which is suggested by a comparison of the rise curves measured for the reaction at the magic angle with those recorded for the molecular orientation (Figure 8). Over a wide range of conditions, the time constants for the magic angle rise curves are very similar to the time constants of the dichroism rise curves. For the interpretation of this result, it should be noted that the transients measured at the magic angle clearly reflect the time course and the extent of the B−A reaction. Nevertheless, it may happen that the time constant of the B−A reaction is determined by the orientation. This type of coupling appears to be very plausible for the case of dipolar stretching. The dipolar stretching force increases with increasing field strength. The effective field strength experienced by individual DNA molecules depends on their orientation with respect to the field vector. Thus, the field strength acting on these molecules is expected to increase with an increasing degree of orientation. Under these conditions, the response observed at the magic angle may be slow, even if the reaction itself is much faster, provided that the overall time constant is determined by a relatively slow rotation of the molecules into the direction of the field vector. A comparison of the rise time constants found for the reaction and for the dichroism at high ethanol contents (Figure 8) suggests that the time constants for the reaction in the presence of the electric field are indeed determined by molecular rotation in the case of chain lengths ≥859 bp.

In summary, we must expect a rather complex composition of different effects acting in the presence of electric field pulses. Because the time constants obtained from the rise curves of the dichroism and the magic angle effect are almost identical for the DNA samples with chain lengths ≥859 bp, we must expect close coupling of orientation and reaction in these cases. However, rotational diffusion is strongly accelerated at lower chain lengths. Thus, orientation and reaction are not coupled anymore in the rise curves of poly[d(A−T)] samples with chain lengths ≤120 bp. In these cases, the time constants of the magic angle rise curves are clearly larger than the time constants of the dichroism rise (Figure 9) and, thus, are determined by the rate of the B−A reaction.

Coupling of orientation with reaction processes may affect and even may determine the time course of magic angle rise curves, but this type of coupling cannot occur in the absence of electric fields. The decay curves measured at the magic angle cannot be influenced by rotational processes because there is no vectorial driving force in the absence of the electric field anymore. The molecules are still partly oriented, when the electric field E is turned off, but the reaction of each molecule is independent of its orientation at $E = 0$.

Comparison of Experimental Data with Results from Model Calculations. The B−A transition of DNA double helices is a reaction with many equivalent reaction steps of the base pairs that are coupled with each other in a cooperative manner. The thermodynamics and kinetics of this type of reaction are described by the linear Ising model.[39] This model has been analyzed mainly with reference to the α-helix coil transitions of polypeptides. Because the elementary reaction steps in polypeptides are quite different from those of the B−A transition in double helical DNA, the reaction parameters are expected to be different. However, the formal reaction schemes are identical, and thus, general features of the reactions should be equivalent.

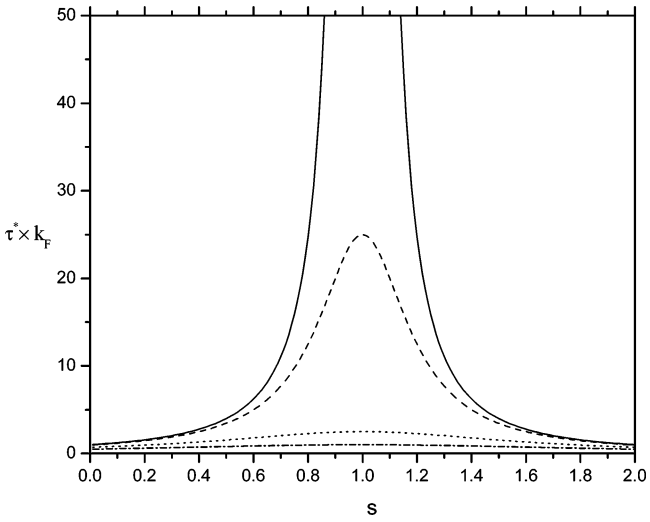

Figure 10. Mean relaxation time τ^* according to Schwarz[40] for a linear Ising model as a function of the chain growth parameter s for different values of the cooperativity σ: (—) 10^{-4}; (- - -) 10^{-2}; (• • •) 0.1; (- • - • -) 0.25.

The first detailed model on the kinetics of the linear Ising model was developed by Schwarz,[40] who derived a simple expression for the mean relaxation time τ^*

$$\frac{1}{\tau^*} = k_f[(s-1)^2 + 4\sigma]$$

where σ is the nucleation parameter, s the chain growth parameter, and k_f the rate constant for helix growth. Using the parameters known for α-helix coil transitions of polypeptides, this equation predicts a distinct maximum of the mean relaxation time at the center of the α-helix coil transition. This maximum has also been observed in experiments.[41] In the case of the B−A transition, there is no indication of a distinct maximum of the relaxation time at the transition midpoint. Thus, we have to check where this difference comes from. A comparison of the helix coil parameters shows that the α- helix coil transition is much more cooperative than the B−A transition. A simple calculation of the relaxation time τ^* as a function of s for different nucleation parameters σ (Figure 10) shows that the maximum is reduced very much at σ values around 0.1. The magnitude of the nucleation parameter σ for the B−A transition is not known exactly, but the cooperative length has been estimated[31] to be in the range of 10−30 base pairs. According to the linear Ising model, the cooperative length corresponds to $1/\sqrt{\sigma}$, and thus, the estimated cooperative lengths correspond to nucleation parameters of 10^{-2} to 10^{-3}. Our kinetic data for natural DNA indicate that the nucleation parameter is in the range around 10^{-1}. A relatively low cooperativity is also indicated by the observation of coexisting A- and B-forms in short oligomer double helices.[10,42] We note that the maximum

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value of the time constants for poly[d(A−T)] at zero field strength appears at an ethanol content close to that found at the center of the B−A transition. Probably, the cooperativity is higher in the case of poly[d(A−T)], but a final conclusion in this respect is not possible yet because the experimental accuracy is not sufficiently high.

The absence of a clear maximum in the $\tau = f$(ethanol) dependence in natural DNA samples may be partly due to a heterogeneity in the distribution of base pairs, resulting in a broadened B−A transition. For a more direct comparison between the B−A kinetics of AT and GC base pairs, it would be useful to study double helices formed from GC homopolymers. Unfortunately, there is too much conformational heterogeneity in GC polymers, preventing a straightforward analysis of their B−A kinetics. Poly[d(G−C)], the direct analogue to poly[d(A−T)], shows a B−A transition, but it is coupled with a B−Z transition.[43] The other candidate poly(dG)·poly(dC) is well-known to be a difficult case because of its strong tendency for aggregation. This aggregation is enhanced under the conditions of the B−A transition.[44]

Because the α-helix coil transition is one of the key steps for protein folding, the dynamics of this transition has been analyzed for various reaction conditions. For example, Craig and Crothers[45] presented numerical simulations for very large perturbations. They found a reaction progress curve of sigmoidal shape for a very large perturbation from the completely helical state. Response curves of sigmoidal shape were also found for the field-induced reaction from the A- to the B-form.[15] This shape was interpreted[15] as evidence for a two-step reaction, where the first step, representing the rise of the dipole, is required for induction of the second step corresponding to the A→B reaction. The simulations indicate that the sigmoidal shape may not reflect the dipole rise but may be due to the A→B reaction itself.

Slowdown of the B−A Reaction due to Special Reaction Conditions? Because of the limitations discussed above, the B−A reaction could be studied only under special solvent conditions at a low salt concentration. The B−A transition was induced by electric field pulses, which perturb the ion atmosphere around the polymer, and thus, the kinetics of ion equilibration is essential. Binding of ions is expected to be diffusion controlled, when the mode of binding corresponds to that of an ion atmosphere. In agreement with this expectation, the time constants of monovalent ion binding to double helical DNA, derived from polarization time constants,[25] were found to be around 10 ns down to the range of low salt concentrations used in the present investigation. Thus, binding of monovalent ions is much faster than the B−A reaction and is not rate-limiting.

Nevertheless, it is possible that the B−A dynamics is affected by electrostatic repulsion between the phosphate charges. An argument against this possibility is the observation that the B−A dynamics of poly[d(A−T)] remained constant[15] within experimental accuracy, when the ion concentration was increased from 0.18 to 0.7 mM. Obviously, it would be useful to extend the measurements to higher salt concentration, but this has not been possible yet because of technical problems (cf. above).

Another process that may slowdown the B−A reaction is aggregation. As a very sensitive indicator for aggregation, we have measured the time constants of dichroism decay. These decay time constants, obtained over the full range of the B−A transition of a poly[d(A−T)] sample with 70 bp, did not show any evidence for aggregation.[15]

A strong argument for the conclusion that our data reflect the B−A dynamics without serious interference by any side effect is the close relation to previously published data on very similar processes in nucleic acids. The B−A transition can be considered as a stacking rearrangement. This type of reaction has been studied under various conditions, including high salt concentrations. The time constants for stacking rearrangements[46,47] in single-stranded oligo- and polynucleotides were found in the range up to ∼1 μs, clearly demonstrating the existence of activation barriers. This result was not commonly expected, as shown by the example of the oscillating dimer model,[48,49] which implied the absence of activation barriers. The information on stacking rearrangements has been extended, meanwhile, by cases of hairpin loops,[50,51] where several residues are rearranged together with time constants up to 100 μs. In summary, the time constants observed for the B−A transition are in the expected time domain.

General Conclusions. Our present experimental results demonstrate that the B−A reaction of natural DNA is slower by about 3 orders of magnitude than predicted by molecular dynamics.[18−24] For DNA samples with 50% GC, a spectrum of time constants is observed with the main amplitude at ∼2 μs and with minor amplitudes at 50−100 μs, showing the existence of activation barriers in the transition, in contrast with interpretations[5,6] of crystal structures claiming the absence of activation barriers. Our time constants are consistent with previous experimental data[46,47,50,51] obtained for various elementary steps of base stacking and for conformation changes in hairpin loops. The different affinity of AT and GC sequences to the A-form is reflected by the fact that the time constant associated with the main amplitude increases from ∼2 μs at 50% GC to 3 μs at 41% GC and to ∼10 μs at 0% GC at the center of the transition. The time constant associated with the main amplitude shows a relatively small dependence on the degree of transition, indicating a low cooperativity of the B−A transition with a nucleation parameter of ∼0.1.

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Supporting Information Available: Complete ref 10. This material is available free of charge via the Internet at http://pubs.acs.org.

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