B-DNA's Conformational Substates Revealed by Fourier Transform Infrared Difference Spectroscopy

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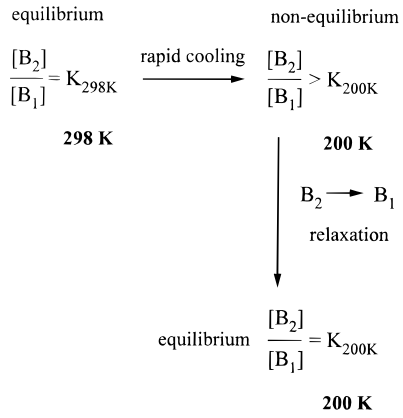
Abstract: Conformational substates (CSs) of deoxyribonucleic acid (DNA), essential for its dynamics and function, are spectroscopically revealed by a new low-temperature method. Hydrated films of the sodium salt of DNA containing up to ≈100% of the B-form were quenched into the glassy metastable state at 77 K. Attainment of equilibrium in the glass → liquid transition region by interconversion of CSs was followed isothermally by Fourier transform infrared spectroscopy. Kinetic analysis of difference spectra revealed two sequential relaxation processes and the spectral patterns of two distinct CSs of B-DNA, with exponential interconversion kinetics for each vibrational mode. The characteristic spectral features of the two CSs are also found in the spectrum of B-DNA in aqueous solution. The relevance of these CSs is discussed with respect to the crystalline state and DNA−protein interaction.

Introduction

In DNA, dynamics are essential for its biological function and, as in proteins,[1] conformational substates (CSs) seem to play a decisive role. At physiological temperatures, these CSs are assumed to interconvert rapidly on the nanosecond or even subnanosecond time scale. For the biologically active B-form of DNA, the existence of two major CSs called B_I and B_{II} and their biological role in the interaction of DNA with proteins has been invoked from studies of DNA oligomers by single-crystal X-ray analysis,[2−4] NMR spectroscopy,[5−8] and molecular modeling.[9,10] However, it is not clear whether the B_{II} state actually exists in solution and it has been suggested that its appearance in single-crystal X-ray studies may be linked to crystal packing effects.[10−13] ^{31}P NMR data of solutions of regular oligonucleotides have been interpreted in terms of populations of the two thermodynamically stable B_I and B_{II} states, where the phosphate is assumed to make rapid jumps between the two states on the time scale of the NMR experiment. These analyses of time-averaged NMR spectra are hindered by the fact that the proposed changes of dihedral angles ϵ and ζ involved in the B_I to B_{II} transition cannot be determined unambiguously.[10]

Here we report for the first time the spectral features of *distinct* conformational substates revealed at low temperatures by Fourier transform infrared (FT-IR) spectroscopy. Scheme 1 gives an outline of our approach: two major conformational substates called B_2 and B_1, in analogy to B_{II} and B_I for reasons given below, are at ambient temperature, say 298 K, in equilibrium, with an equilibrium constant K_{298}, interconverting on the nanosecond or subnanosecond time scale. Since the equilibrium constant decreases with decreasing temperature, nonequilibrium distribution of B_2/B_1 conformer population is generated on rapid cooling to, for example, 200 K. At this temperature which is in the glass → liquid transition region of hydrated B-DNA,[14,15] isothermal structural relaxation toward equilibrium occurs by interconversion of $B_2 \rightarrow B_1$ on time scales of between minutes and hours.[14,15] Because of that slow time scale, interconversion can be followed by conventional FT-IR spectroscopy.[16] The spectral changes on isothermal annealing are reported in form of FT-IR difference spectra, where positive peaks indicate the formation of one (or more) CS and negative peaks their disappearance. Analysis of these difference spectra reveals the distinct spectra for each CS and the kinetics of conformer interconversion. We then show that the characteristic spectral features of these CSs are also contained in the spectrum

Scheme 1



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of B-DNA in aqueous solution. By comparison with studies of protein−DNA interactions by vibrational spectroscopy reported in the literature, we surmize that it is the B_1 state which interacts with a protein.

We emphasize that studies of hydrated fibers by vibrational spectroscopy are also of relevance for DNA in aqueous solution because, as pointed out by Thomas and Peticolas,[17] "the fact that DNA in solution shows essentially the same Raman spectrum as DNA in the fiber at 95% relative humidity is probably the most rigorous experimental proof that ordered DNA in solution is of the same B-genus conformation as that in the fiber". The short time scale of vibrational transitions enables the observation of distinct conformer species even for the fastest conformer interconversion processes, whereas for NMR studies of fast-time-scale conformational dynamics of biomolecules, "significant practical and interpretative issues remain unresolved".[18]

This study is an extension of our previous studies for investigating DNA's dynamics by differential scanning calorimetry (DSC),[14,15] namely by enthalpy relaxation of vitrified NaDNA from ≈153 to ≈263 K and its recovery on reheating. Structural relaxation became observable in the form of endothermic enthalpy recovery for $\Gamma > 3-4$ (water molecules per nucleotide), and the effects were attributed to the conformational flexibility of B-DNA. The large width of the glass → liquid transition region and nonexponential kinetics were interpreted in terms of superposition of a large number of relaxation modes of different parts, or conformational substates, each with a single relaxation time.[14,15]

Experimental Section

Materials and Sample Preparations. Films of hydrated NaDNA from salmon testes (from Fluka, No. 31163, used as received, containing 0.08 wt % protein) were obtained by keeping the NaDNA solutions on AgCl discs over saturated salt solutions for several weeks. The well-known dependence of A- and B-conformer populations on the hydration level was used to prepare films of hydrated NaDNA with varying ratios of B- and A-DNA.[19−24] The hydrated NaDNA films were thereafter covered with a second AgCl disc, the two discs were taped, and the sample was positioned in a self-made copper holder for the cryostat. The FT-IR spectrum of the film was first recorded at 293 K to characterize the film and to determine the relative A- and B-DNA populations via band areas of the two bands centered at 864 cm^{-1} (from A-DNA) and at 838 cm^{-1} (from B-DNA) and, for the film consisting only of B-DNA, by the absence of the sharp band at 1188 cm^{-1} from A-DNA. FT-IR spectra of the A- and B-forms recorded at 293 K were on the whole identical with those reported in Figure 2 of ref 24. The Γ values of the films were determined from the ratios of the measured absorbance at 3400 cm^{-1} (due to OH groups) and at 1220 cm^{-1} (due to phosphate groups of DNA).[25] Special care was taken to avoid orientation of the film, and the method of preparation ensures unoriented films.

The sample and sample holder were quenched into the glassy state outside of the cryostat, by immersion into liquid N_2. The cooling rate obtained in this way was ≈500 K min^{-1}. For the film with $\Gamma \approx 20$, a weak sharp band in the OH stretching band region at ≈3200 cm^{-1} indicated formation of a small amount of crystalline ice, whereas for the film with $\Gamma = 12$, this was absent. This is consistent with Falk et al.'s[25] infrared study of ice formation in hydrated NaDNA films. Sample and holder were thereafter transferred into the precooled cryostat under a cover of dry N_2 to avoid condensation of water vapor. This transfer is the critical step in the procedure, and after several trials, it became routine with heating of sample only up to ≈110 K and minimal condensation of water vapor during the transfer. FT-IR spectra of the films were recorded thereafter isothermally at selected temperatures (constant to ±0.1°), and spectral changes were followed in the form of difference spectra recorded at the same temperature to avoid temperature-dependent changes in band profiles. The samples were studied in vacuo at ≤190 K and under an atmosphere of dry Ar at >190 K to avoid dehydration of the films. The absence of dehydration or irreversible spectral changes was confirmed by comparing FT-IR spectra recorded at 293 K before and after the low-temperature experiment. The temperature of the sample was calibrated by melting of ethyl acetate[26] and was accurate within 0.5 K.

The same procedure was applied to a hydrated mononucleotide (2′-deoxyguanosine 5′-monophosphate disodium salt, from Fluka, No. 31080). Spectral changes in a quenched film on isothermal annealing recorded in the same manner as for DNA from salmon testes were not observable.

FT-IR Spectra. FT-IR spectra were recorded on a Bio-Rad FTS-45 model at 4 cm^{-1} resolution by coadding 64 scans (UDR1) and using a DTGS detector. The collection time of 200 s constitutes the time resolution. For NaDNA in aqueous solution (Figure 4, curve a), 1024 scans were coadded. The spectra are displayed in the figures on the same ordinate scales. Vertical bars indicate the ordinate scale in absorbance units.

The "Ratio Method". The "ratio method" allows resolution of "the spectrum of a mixture of an unknown small number of unknown constituents in unknown proportions into the spectra of these constituents without isolating them", provided that each unknown constituent absorbs dominantly in a particular spectral region.[27,28] The difference spectrum depicted in Figure 1 for 84-1 min was resolved into two components which are shown in Figure 4 as curves c and d. The scaling coefficients needed for resolving into the constituents were obtained from second derivatives and their difference curve, at 1283, 1218, 1086, 1062, and 1051 cm^{-1}, respectively. The percentage of changes of peak heights is 13% for the film with $\Gamma \approx 20$ kept at 200 K for 84 min and 16% for the film with $\Gamma = 12$ kept at 200 K for 112 min. Therefore, subtraction of curve c from d (Figure 4) multiplied by 0.13 gives the difference spectrum shown in Figure 1, whereas addition of curves c and d gives spectrum b of Figure 4.

Kinetic Analysis of Spectral Changes. The data points displayed in Figure 3 were fitted according to the stretched exponential relation $I(\Delta t) \propto 1 - \exp[-(t_a/\tau_a)^{\beta}]$, where $I(\Delta t)$ is the intensity change of the bands obtained from the difference spectra, t_a the annealing time, τ_a the relaxation time, and β an empirical parameter with a value between zero and 1. $\beta = 1$ corresponds to exponential relaxation, and the solid lines shown in Figure 3 were obtained from fits with this value.

Results and Discussion

Interconversion of Conformational Substates Seen in FT-IR Spectra. Three films of hydrated native NaDNA (from salmon testes) films were studied in detail which contained ≈100, ≈31, and ≈20% B-DNA, with Γ values of ≈20, 12, and 6. For these studies, it is essential to quench the samples into the glassy state as rapidly as possible to maximize immobilization of a nonequilibrium population of conformers.[29] This then

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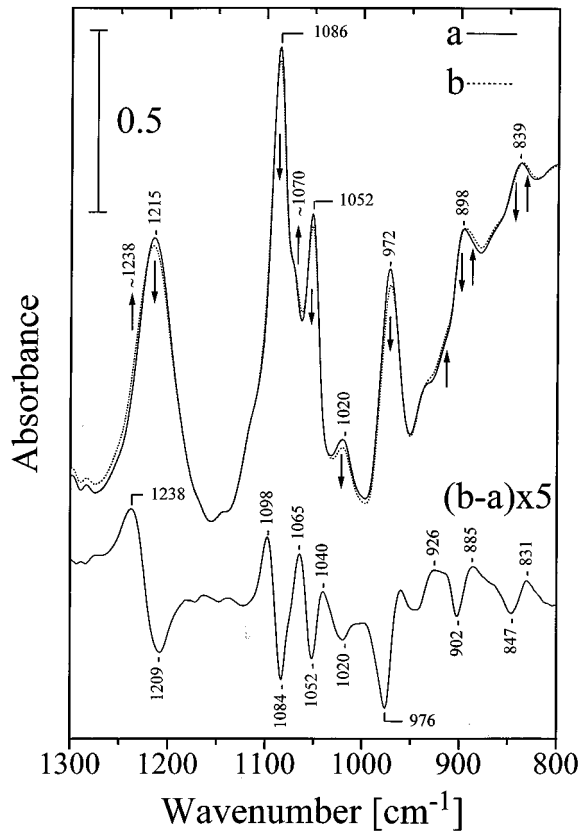

Figure 1. (top) Structural relaxation seen in FT-IR spectra of a quenched NaDNA film with $\Gamma \approx 20$ on isothermal annealing at 200 K: (a) after 1 min, (b) after 84 min. Arrows indicate decrease or increase of band intensity. (bottom) Difference spectrum (b − a) 5-fold enlarged.

gives pronounced spectral changes on isothermal relaxation toward equilibrium. Therefore special care was taken to maximize the cooling rate, and a rate of ≈500 K min^{-1} was used throughout.

FT-IR spectra of a quenched film of NaDNA with ≈100% B-DNA show on isothermal annealing at 200 K, the most pronounced spectral changes between 1300 and 800 cm^{-1} (Figure 1, curves a and b). Arrows indicate where a decrease or increase of intensity had occurred. These intensity changes do not necessarily occur at the peak maximum because the original band contour is a composite of several highly overlapping bands. However, in second- or fourth-derivative curves of the orignal composite bands (not shown), distinct peaks can be separated and correlated with these intensity changes. The spectral changes become more pronounced in form of a difference curve (Figure 1, curves (b−a), enlarged 5-fold). This subtraction gives positive bands for spectral features developing with time and vice versa, and the same type of subtraction will be used throughout. Peak positions of the major spectral changes in the original spectra (e.g., at 1215, 1086, 1052, 1020, 972, and 898 cm^{-1}) are very similar to the most pronounced features in the difference curve. This is an important aspect, and it ensures that the spectral features of the difference spectrum are not simply due to changes in half-bandwidth (HBW) and/or peak position with annealing time[30] but that they indicate conversion of at least one species into another one. Here it is most important to compare spectra recorded isothermally because temperature-dependent changes in peak position and HBW are avoided. We will show in the following that the

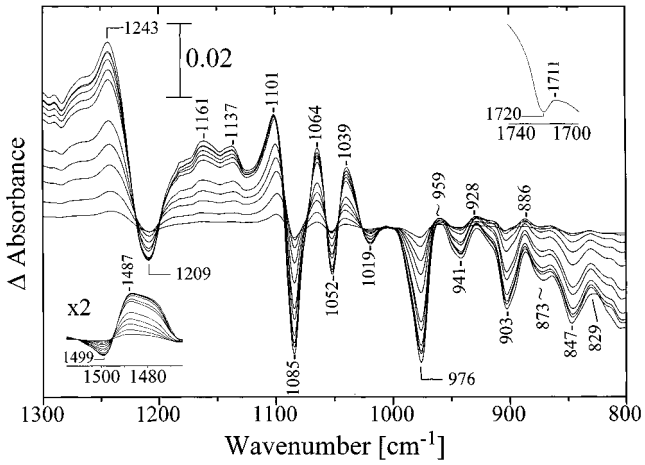

Figure 2. Difference curves of a NaDNA film with $\Gamma = 12$ where the spectrum recorded at 200 K after *n* min had been subtracted from that recorded after 1 min (n = 4.0, 7.8, 12.8, 18.0, 25.3, 46.6., 56.3, 72.4, 82.5, 85.9, and 112.2 min, respectively). The spectral changes are drawn on the same scale. The sloping background is caused by base line instability, and partial correction was made by shifting difference spectra vertically until at 1000 cm^{-1} the same absorbance value was obtained.

spectral changes on structural relaxation and attainment of equilibrium are caused by interconversion of a high-energy CS of B-DNA into its low-energy CS. Involvement of A-DNA can be ruled out because intensity changes are absent in spectral regions where A-DNA has characteristic bands (e.g., at 1188 and 868 cm^{-1}). This is consistent with the reported slow conversion of the A-to-B transition (and vice versa) at ambient temperatures, taking up to several days.[22,31]

A second film of hydrated NaDNA with $\Gamma = 12$ and containing ≈31% B-DNA was studied in the same manner. For this hydration level, formation of ice can be excluded.[25] The spectral changes on isothermal annealing at 200 K are shown from 800 to 1300 cm^{-1} in the form of difference curves (Figure 2), and weak spectral changes in other spectral regions are shown as inserts. These spectral changes are on the whole the same as those observed for the film with ≈100% B-DNA (Figure 1, curve b − a), and this proves that formation of a small amount of ice on quenching the film containing ≈100% B-DNA had no influence on the spectral features of the difference spectra.

Plots of these spectral changes with time are shown for eight prominent peak maxima or minima (Figure 3). Adjacent peak maxima and minima were chosen where interconversion of the band of one CS into that of another CS seems to be coupled (i.e., for bands centered at cm^{-1}: 1209 → 1243, 1085 → 1101, 1052 → 1064, 904 → 887). For each plot, the data points were exponentially fitted and the fit is indicated by the solid line. The relaxation times (τ) obtained from these fits fall into two groups, one with τ values between 16 and 35 min and a second one with τ values between 35 and 46 min (Figure 3, top and bottom plots). This is a strong indication that structural relaxation and attainment of equilibrium involves *two* relaxation processes where interconversion by a fast process is followed sequentially by a slower one.

Difference curves were also obtained for both samples containing ≈100 and 31% B-DNA from spectra recorded at other temperatures in steps of 20°. For similar annealing times, spectral features indicating interconversion of CS were absent below 150 K, became weakly observable at 180 K and were most pronounced at 200 and 220 K. Weak spectral features

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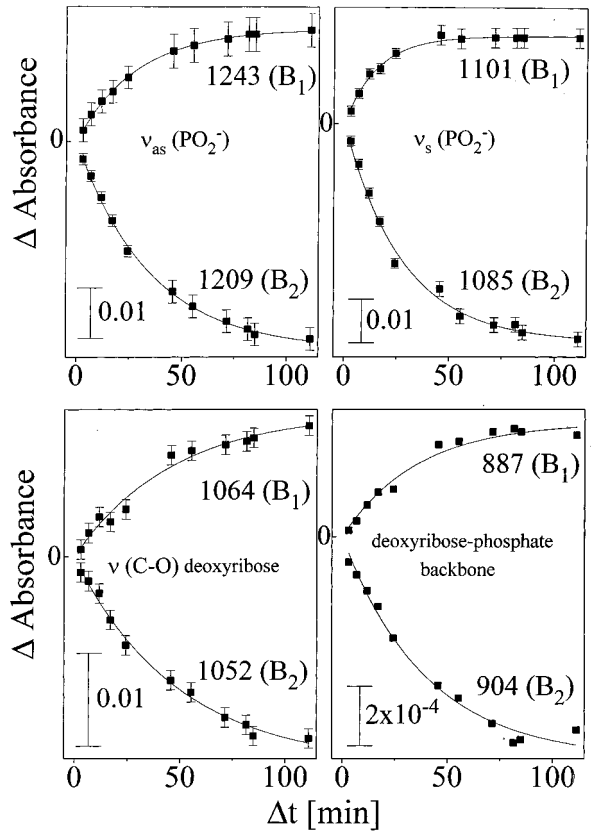

Figure 3. Evaluation of the time dependence of spectral changes shown in Figure 2. The data points were exponentially fitted (solid lines) and relaxation times in minutes, with errors in brackets, are as follows: 28(7) for 1243 cm^{-1}, 35(3) for 1209 cm^{-1}, 16(2) for 1101 cm^{-1}, 28(2) for 1085 cm^{-1}, 46(10) for 1064 cm^{-1}, 37(1) for 1052 cm^{-1}, 40(4) for 904 cm^{-1}, and 35(5) for 887 cm^{-1}, respectively.

were also observed on isothermal annealing at 240 K, but these were about 1 order of magnitude smaller than those shown here. In a third film containing only ≈20% B-DNA ($\Gamma = 6$) and studied in the same manner, spectral features assignable to interconversion of CS were too small to be evaluable. These results are consistent with our previous DSC study[14,15] in that only B-DNA is involved in relaxation and that the time and temperature of relaxation are similar. Nonexponential kinetics observed in the DSC study must be caused by the sum of a number of relaxing regions, each with its own exponential relaxation time as observed here, and not by intrinsically nonexponential relaxation.[32]

In a next step the difference spectra of the NaDNA film containing ≈100% B-DNA were separated by the "ratio method" [27,28] into the spectral components of the disappearing and formed CS (Figure 4, curves c and d). Their sum (Figure 4, curve b) is identical with the spectrum of the film recorded at 200 K. The CSs corresponding to the disappearing and formed species (Figure 4, curves c and d) are called B_2 and B_1. This is simplified because isothermal relaxation kinetics indicate two relaxation processes (Figure 3). The interconversion of $B_2 \rightarrow B_1$ is exothermic because the equilibrium constant as defined in Scheme 1 must have decreased with decreasing temperature. Since B_2 interconverts on structural relaxation at 200 or 220 K into B_1, B_1 must be thermodynamically more stable at low temperatures than at ambient temperature.

Assignment. Discussion of the spectral changes in going from the B_2 state to the B_1 state (Figure 4, curves c and d) is based on infrared and Raman band assignments of NaDNA fibers, films, and solutions reported in the literature[24,33−36] and on the kinetics of disappearance and formation of bands of the B_2 and B_1 states (Figure 3). The most pronounced changes occur for the intense bands in the symmetric and antisymmetric stretching band region of the PO_2^- group (ν_s, ν_{as}, from 1085 to 1091 cm^{-1} and from 1212 to 1230 cm^{-1}) and for the C−O stretching band (from 1052 to 1067 cm^{-1}, frequencies from difference spectra in Figure 3 are shifted at most by several wavenumbers). The increase in frequency in going from the B_2 to the B_1 state is attributed to increasing bond strength, which could be caused by a decrease in hydrogen-bond interaction with the water molecules.[37] However, we cannot exclude changes in vibrational coupling between the PO_2^- and C−O stretching modes as an alternative explanation.[38]

The second type of pronounced spectral changes in going from the B_2 to the B_1 state occurs between 1030 and 800 cm^{-1}, which is the region of the phosphodiester backbone and sugar moieties vibrations. In this spectral region, all B_1 bands are shifted to a lower frequency in comparison to those of B_2 and their intensity is less (e.g., from 1020 to 1015 cm^{-1}, from 975 to 966 cm^{-1}, from 900 to 888 cm^{-1}, and from 843 to 832 cm^{-1}). The decrease in frequencies can be attributed to increasing hydrogen-bond interactions. Both types of spectral changes pointed out above, i.e., increase in peak frequencies in the PO_2^- and C−O stretching band region in going from B_2 to B_1 and simultaneous decrease in peak frequencies below ≈1030 cm^{-1}, seem to be concerted with respect to changes in hydrogen bonding. The weak band centered in the spectrum of the B_1 state at ≈863 cm^{-1} is not the marker band of A-DNA[24,33] because the characteristic sharp band of A-DNA centered at 1188 cm^{-1} is absent. Spectral changes are much smaller in the other spectral regions and are not shown here. The IR spectrum of the B_1 state resembles in many respects that of the C-form which belongs to the B-family.[35,39]

Kinetics of Conformer Interconversion. Insight into molecular aspects of relaxation is obtained from the τ values. The fastest relaxation processes involve the stretching vibrations of the PO_2^- group, with τ values of between 16 and 35 min for relaxation at 200 K (Figure 3). From evaluation of all other bands including those of the bases (not shown), we had obtained τ values about a factor of 2 larger than the fastest ones, and only two of these are shown here (Figure 3). These involve a C−O stretching band and a band (904 → 887 cm^{-1}) possibly involving a deoxyribose phosphate backbone vibration.[33] These results seem to be consistent with Saenger's notion that the P−O bonds may be considered the "major pivots affecting polynucleotide structure".[39]

Comparison with B-DNA in Aqueous Solution. The spectral features of the B-DNA film recorded at 200 K and of the separated B_1 and B_2 states are next compared with the spectrum of B-DNA in aqueous solution recorded at 293 K (Figure 4, curves a− d). Band narrowing by deconvolution allows separatation of overlapping bands into their components (curves a′−d′). Comparison of the film spectrum recorded at

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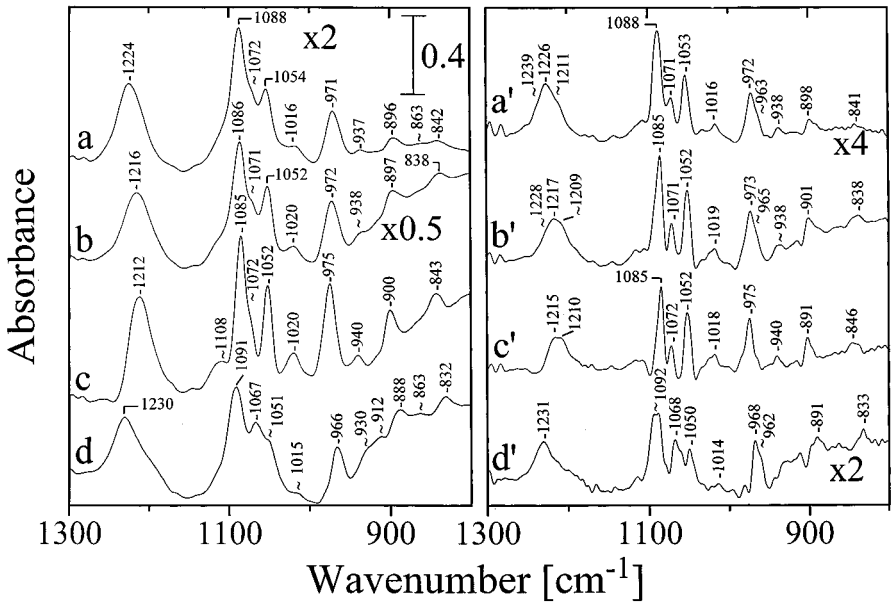

Figure 4. (d and c) Spectra of the B_1 and B_2 states separated by the "ratio method" [27,28] and (b) their sum. (a) For comparison, the spectrum of a 6 wt % aqueous solution of NaDNA (from salmon testes) where for clarity the spectrum of the solvent had been subtracted. The spectra are shown on the same scale. (a′−d′) Deconvolved spectra (HBW 22 cm^{-1}, 65% smoothing).

200 K (which is identical with the sum of the spectra of the B_1 and B_2 states) with that of B-DNA in aqueous solution (Figure 4, curves b and a) shows that except for small temperature-dependent reversible shifts of peak positions, the features of both spectra are remarkably similar. This becomes even more obvious by comparison of their deconvolved spectra (curves a′ and b′), where resolution enhancement led to development of similar peak asymmetry and shoulders for bands centered at 1224 (1216) and 971 (972) cm^{-1} and to a distinct peak centered at 1071 cm^{-1}. Characteristic spectral features of the B_1 and B_2 state in the spectrum of B-DNA in aqueous solution are best seen by comparison of deconvolved spectra, first, for ν_{as} of the PO_2^- group where the bands of the B_1 and B_2 state appear in the composite deconvolved spectrum in the form of shoulders (compare c′ and d′ with a′), and second, in form of band asymmetry of the band centered at 972 cm^{-1} (curve a′) which indicates contributions from the B_1 and B_2 bands centered at 968 and 975 cm^{-1} (curves d′ and c′). We conclude that the spectral features of the B_1 and B_2 state are contained in the spectrum of B-DNA in aqueous solution, although at somewhat differing conformer populations and shifted slightly by temperature effects. By curve resolution methods of highly overlapping bands developed recently,[40] ratios of band areas in the spectrum of B-DNA in aqueous solution can give the populations of the B_1 and B_2 states at ambient temperature.

Comparison with the B_I/B_{II} States. It is possible that our B_1 and B_2 CS are the same as the B_I and B_{II} states reported and discussed in the literature.[4−10] The B_I and B_{II} states have first been observed in single-crystal X-ray analysis of B-DNA oligomers and involve phosphate backbone conformations about the C3′−O3′−P segment of the backbone chain, the B_I conformation being much more common than the B_{II}.[4] In crystals the B_I or B_{II} conformation is frozen-in. Solution studies of B-DNA oligomers by NMR spectroscopy[7,8] and molecular modeling[9,10] also point at the presence of two thermodynamically stable B_I and B_{II} conformations of about equal population which interconvert on a subnanosecond time scale, with B_I being slightly more stable than B_{II}.[7−10] We do not know yet the relative populations and stabilities of our B_1 and B_2 states at ambient temperature, and we chose to call the CS developing at low temperatures on isothermal annealing the B_1 state. Whether or not the B_I and B_{II} states and our B_1 and B_2 states reported here are the same can be tested by obtaining for B-DNA oligomers distinct FT-IR spectra of CSs in the same manner as reported here for DNA from salmon testes. These can then be compared with FT-IR spectra of the same B-DNA oligomers either in the crystalline state or in solution and the presence of one or more CS be ascertained. In a next step, equality between the two sets of CS can be tested by comparison with B_I and B_{II} populations obtained from single-crystal X-ray and from solution studies. Until then we prefer to refer to our CS as B_1 and B_2. However, it is remarkable that an oligomer which in solution is of B-type has a very intense band at 1065 cm^{-1} in the FT-IR spectrum of the crystal which is consistent with enhanced population of the B_1 state (see Figure 9, curves a and b in ref 36). We note that the kinetics of B_2 to B_1 interconversion clearly indicate two (or more) relaxation processes, whereas for the kinetics of B_{II} to B_I interconversion, a cooperative process involving one relaxation only has been assumed.[7,8] Since conformer interconversion is expected to be very fast at ambient temperature, where B_{II} and B_I states usually are studied in solution, it might be impossible to separate the two relaxation processes.[18]

Implications for Protein−DNA Interaction. Finally, the interaction of B-DNA with proteins is discussed in the context of participation of the B_1 or B_2 state. Raman spectroscopy is mainly used for studies of protein−DNA interactions by vibrational spectroscopy, and infrared spectroscopic studies are rare. These studies demonstrated that a B-type or a modified B-form of DNA is present in chromatin and in specific protein−DNA interactions.[41−44] From comparison of the distinct spectral features of the B_1 and B_2 states (Figure 4, curves d and c) with vibrational spectroscopic studies of chromatin and protein−DNA

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interaction, we surmize that the B_1 state, or a state with similar spectral features as the B_1 state, seems to be involved. Spectroscopic arguments for participation of the B_1 state are collected as follows: (i) Comparison of the Raman spectrum of an aqueous suspension of intact salmon sperm with that of purified salmon sperm shows in the former enhanced intensity of the band at $\approx$1060 cm^{-1} and, for several bands below 1000 cm^{-1}, a shift to lower frequency (read from Figure 7 in ref 42), which is consistent with changes from the fiber spectrum to that of the B_1 state (Figure 4, curves b and d). (ii) In an infrared spectroscopic study of the conformation of DNA in chromatin protein−DNA complexes, shift of the band at 835 to 830 cm^{-1} was observed in the complex (see Figure 1 in ref 44), which is consistent with the change observed here (Figure 4, curves d and b). (iii) Observation of a weak infrared band at $\approx$855 cm^{-1} in the spectrum of chromatin[44] is also consistent with the weak band at similar frequency in the B_1 spectrum (Figure 4, curve d). These comparisons are at present highly speculative and neglect, among other things, differences in selection rules and band intensities for infrared and Raman bands. Nevertheless, these coincidencies are also sufficiently encouraging to pursue further the comparison of the characteristic vibrational spectral patterns of B_1 and B_2 states with those of B-DNA in chromatin. In particular, studies of the B_1 and B_2 states by Raman spectroscopy and the comparison with the many Raman spectroscopic studies of protein−DNA interactions are important for further elucidating participation of the B_1 (or B_2) conformational substate. The latter studies are related to those mentioned above for the crystalline state because, as emphasized by El Hassan and Calladine,[45] "one might expect to see in naked (crystallized) DNA oligomers the range of conformations that are normally available for DNA when it is bound to protein".

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