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A new conformation-specific infrared band of A-DNA in films

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ABSTRACT

A band at 1185 cm^{-1} occurs in the infrared spectra of nucleic acids in their A-type conformation which has been reproduced in the literature, too. The absorbance of this band is proportional to the fraction of the A form of DNA samples containing a mixture of different forms. The 1185 cm^{-1} band has been assigned tentatively to a vibration of the sugar-phosphate backbone with a fairly high contribution from the sugar moiety.

Drastic dehydration of the DNA films is accompanied by a continuous intensity decrease of the 1185 cm^{-1} band indicating a collapse of the A form.

INTRODUCTION

In addition to X-ray diffraction analyses, the infrared and Raman spectroscopy represent reliable methods to characterize the conformational state of solid DNA. In two pioneering papers, PILET and BRAHMS have introduced the infrared linear dichroism of oriented films of DNA as a tool to describe the B, A and C forms of DNA under several conditions of relative humidity (r.h.) and salt /1, 2/. The B-A transition of DNA is reflected by dramatic changes of the dichroic behavior of the antisymmetric and symmetric stretching vibration bands at about 1230 cm^{-1} and 1090 cm^{-1}, respectively /1/. These results have been reproduced by further authors /3-5/. Based upon these results, unambiguous assignments of some infrared and Raman bands in the spectral range of 900 - 750 cm^{-1} to related DNA forms have been made. Especially, an infrared band near 840 cm^{-1} has been found in B and C forms of DNA. On the other hand, the A form is characterized by bands at about 880 cm^{-1} and 810 cm^{-1} /6, 7/. Our findings have completely confirmed

these results /8/. Furthermore, we have observed consistently an infrared absorption band at about 1185 cm^{-1} in all DNA films containing the A form. This paper describes the spectroscopic features, the tentative assignment and the eventual application of this band. All experimental procedures have been the same as reported elsewhere /3, 5/.

RESULTS

In figure 1, polarized infrared spectra of oriented films of DNA in different conformational states are presented. The B form of DNA is characterized by dichroic ratios $R_{1230} \approx 1$ and $R_{1090} > 1$, respectively /1/ (fig. 1a). (The dichroic ratio R_ν at the wave number ν is defined by $R_\nu = A_\perp^\nu / A_\parallel^\nu$, the ratio of the absorbances measured with the infrared radiation polarized perpendicularly and parallely, respectively, with respect to the orientation direction of the sample.)
Both the B form (fig. 1a) and the C form of DNA (not shown) do not produce an infrared band at 1185 cm^{-1}. On the other hand, the A form of DNA is characterized by $R_{1230} > 1$ and $R_{1090} < 1$ /1/. The B-A transition of DNA films is completed at 76 % r.h. Under these conditions, the band at about 1185 cm^{-1} is well pronounced in the infrared spectrum (fig. 1d). The wave number of the band is 1183 ... 1185 cm^{-1}, the dichroic ratio R_{1185} is 0.8 ... 0.9, i.e. the polarization of the band is weakly parallel. The absorbance of the 1185 cm^{-1} band has been measured by the standard base-line procedure and has been normalized by means of the absorbance A_{1230} of the antisymmetric PO_2^- stretching vibration band. The normalized absorbance $A^N_{1185} = A_{1185} / A_{1230}$ results in values up to 0.25.

Spectra 1b and 1c refer to conditions of coexisting B and A forms of DNA. The coexistence of both forms has been induced by the removal of very small amounts of water from the high r.h. B form (fig. 1b), and the presence of caffeine as a ligand which prevents the completeness of B-A transition /9/ (fig. 1c), respectively. The coexistence of the A and B form is indicated by intermediate values of both dichroic ratios of the PO_2^- stretching vibration bands. The relative absorbances of the 1185 cm^{-1} band in figure 1 are within the interval $0 < A^N_{1185} <$

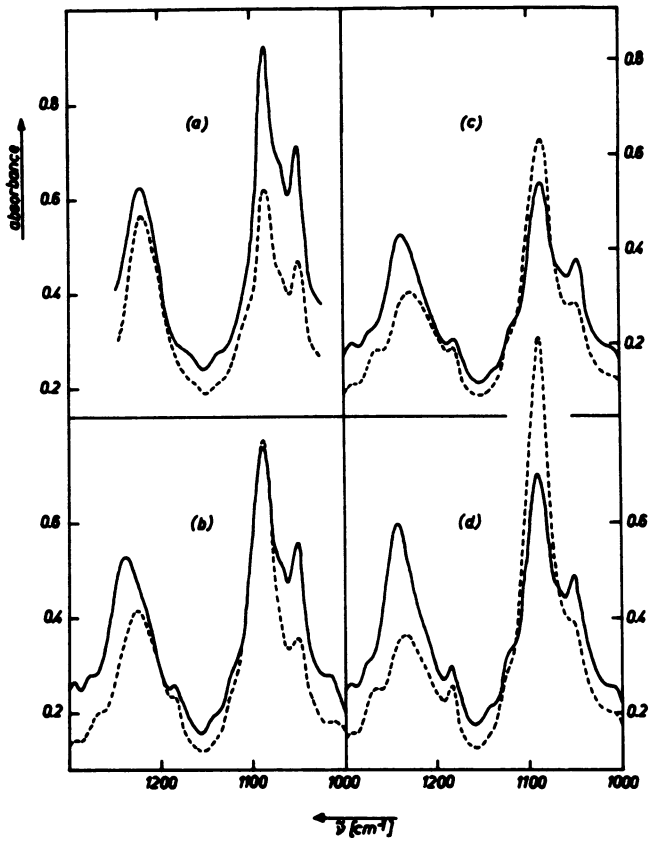

Fig. 1: Infrared spectra of oriented films of calf thymus DNA (a, b, d) and the DNA-caffeine complex (c) measured with polarized light; solid and broken lines indicate that the electric vector of the incident light is perpendicularly and parallely polarized with respect to the orientation direction of the sample (= DNA helix axis), respectively. Spectra (a) and (d) show "pure" B- and A-DNA at 98 % and 76 % r.h., respectively; (b) is obtained by careful partial drying of B-DNA by short-time infrared irradiation; (c) refers to DNA-caffeine, 76 % r.h.; (b) and (c) demonstrate spectral characteristics of coexisting B and A conformations.

0.25.

We have proved whether the relative absorbance A^N_{1185} reflects the fraction x_A of the A form of the investigated DNA samples. When a mixture of two forms is present in the DNA film, the

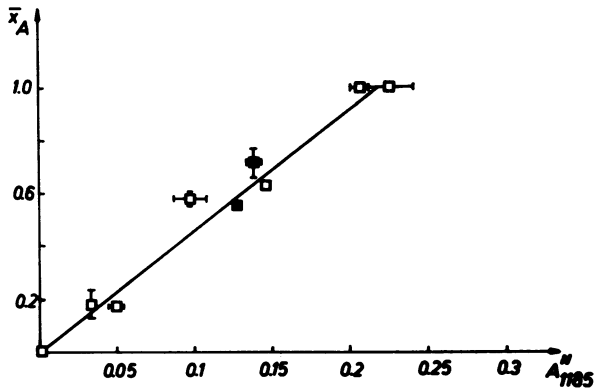

Fig. 2: Correlation between the normalized absorbances of the band at 1185 cm^{-1} and $\bar{x}_A$, the fraction of A form in mixtures with B and C form, respectively. The different symbols originate from data of different DNA samples.

fraction of one of them can be calculated by linear interpolation between (i) the phosphate angles Θ_{yr} (calculated from the dichroic ratios, cf. ref. 1) /5, 10/; (ii) the wave numbers of the antisymmetric PO_2^- vibration at about 1230 cm^{-1} /5/ of each of the two coexisting forms. The exact parameters of the related equations will be published elsewhere /11/. By means of these equations, we have calculated the fraction of A form, x_A, in mixtures with B form of DNA and C-like form of ultraviolet-irradiated DNA, respectively. The individual fractions of A form obtained by the several methods described above have been averaged. The resulting mean values, $\bar{x}_A$, have been plotted against the normalized absorbance A^N_{1185} of the 1185 cm^{-1} band (fig. 2). The linear relationship clearly demonstrates that the intensity of the 1185 cm^{-1} band reflects the fraction x_A of the A form of the investigated DNA films.

DISCUSSION

Inspection of infrared and Raman spectra of DNA in the literature reveals further occurrence of bands in the region at about 1185 cm^{-1}. DNA's from several sources as well as other nucleic acids and DNA complexes with several ligands show the

1185 cm^{-1} band in their infrared spectra under conditions which favor the existence of an A-type conformation (films at 60-85 % r.h., sodium chloride content 3-5 % w/w).

In this paper, we have demonstrated the existence of the 1185 cm^{-1} band in the infrared spectra of calf thymus DNA (fig. 1). The same band can be observed in the spectra of DNA's from salmon sperm /6/ and Micrococcus lysodeikticus /1, 12/ and, moreover, in the spectrum of poly [d(A-T)] . poly [d(A-T)] /7, 13/. The latter polynucleotide forms a very pronounced A-type conformation, called A^{H}, accompanied by an essentially strong band at 1185 cm^{-1} (see fig. 3 of ref. 13).

The intensity of the 1185 cm^{-1} band in the infrared spectra of films of DNA complexes depends on the type and the concentration of the ligand. The complex formation is frequently connected with a more or less pronounced inhibition of the transition from the B to the A form of the DNA films /9, 10, 14, 15/. The more the B-A transition is inhibited the less intense is the band at about 1185 cm^{-1}. This can be noticed in the infrared spectra not only of the DNA-caffeine complex (fig. 1c and /9/), but also of several DNA-polypeptide complexes, for instance DNA - poly(L-lysine) and DNA - poly(L-arginine) /16/ as well as complexes of histones /10,14/ and non-histone proteins with DNA /15/, respectively. Furthermore, the infrared spectrum of a DNA-RNA hybrid (film at 92 % r.h.) reveals a weak band at 1184 cm^{-1} under conditions which indicate a coexistence of B and A forms by the dichroic behavior of the phosphate bands /17/. In the infrared spectra of RNA both in oriented films /18/ and in solution /19, 20/, a band near 1175 cm^{-1} occurs consistently. On the other hand, a band at about 1180 cm^{-1} reported in numerous Raman spectra of nucleic acids and nucleotides may not be attributed to a special conformation because of its occurrence in the Raman spectra of both B and A forms of DNA films /21/. Summarizing all these facts, the 1185 cm^{-1} band in the infrared spectra of nucleic acids unambiguously refers to the presence of the A form in the investigated sample.

Several authors have attributed a Raman band at 1180 cm^{-1} to external C-N stretching motions of the bases /22, 23/.

This assignment, however, seems to be inadequate for the infrared band at 1185 cm^{-1} because (i) a conformation specificity of a base vibration is not well to understand, and (ii), the intensity of this band is obviously independent of the base composition of the DNA, which can be checked by inspection of the infrared spectra of refs. 1 and 13.

On the other hand, a normal coordinate analysis of the nucleic acid backbone has resulted in a normal mode at 1187 cm^{-1} for A-DNA, 1168 cm^{-1} for A-RNA and 1160 cm^{-1} for B-DNA, respectively /24/. These three bands have been attributed to stretching vibrations of the sugar-phosphate backbone /24/. Accordingly, the experimental results show a higher wave number of the band for the A-type deoxyribonucleotides (~1185 cm^{-1}) than for ribonucleotides (~1175 cm^{-1})/18-20/. However, we were unable to detect a corresponding B-type band of significant intensity in the infrared spectra of DNA films expected at about 1160 cm^{-1}. Only a very weak inflection centered at 1165...1170 cm^{-1} has been observed (cf. fig. 1a).

Furthermore, tetrahydrofurane as well as deoxyribose and deoxyribosephosphate have infrared bands at 1178 cm^{-1} /24/ and 1190 cm^{-1} /23/, respectively. Therefore, we propose to assign the band at 1185 cm^{-1} to stretching vibrations of the sugar-phosphate chain with a fairly high contribution from the sugar residue. This tentative assignment is confirmed by the results of a further normal coordinate analysis which ascribes a band at 1179 cm^{-1} to sugar vibrations including predominantly the C(3)-O(1) stretching motion /25/.

The A-conformation specificity of the 1185 cm^{-1} infrared band is based most probably upon a distinct difference between the respective intensities of the A and B forms. The existence of such an intensity difference is plausible taking into consideration the different sugar puckering in A and B forms of DNA /26/. Otherwise, the intensities of the respective Raman bands of B-DNA and A-DNA are obviously nearly the same /21/.

Finally, we would like to illustrate the diminution of the 1185 cm^{-1} band in the course of the dehydration of DNA films. It is well known that the ordered conformation of a DNA film

collapses to a disordered state as a concequence of the removal of water down to 0 % r.h. /1, 5/. In the disordered state, the regularity of the DNA secondary structure is lost in a reversible manner. This is accompanied by a decrease of the orientation degree of an oriented DNA film. This process can be monitored by the decrease of the dichroic ratio R_B of the in-plane vibrations of the DNA bases at 1710 cm^{-1} as a function of decreasing r.h. (dehydration of the film) shown in fig. 3.

R_B is related to the orientation degree of the sample, f, by $f = 2 (R_B-1) / (2R_B+1) (3 \sin \Theta_B-2)$ where is Θ_B the angle of the bases relative to the helix axis of DNA. Complete disorganization of the sample (f = 0) is attained when $R_B = 1$.

Simultaneously, the collapse of the A form is indicated independently by an intensity decrease of the 1185 cm^{-1} band. Figure 3 demonstrates the closed similarity of the R_B and the

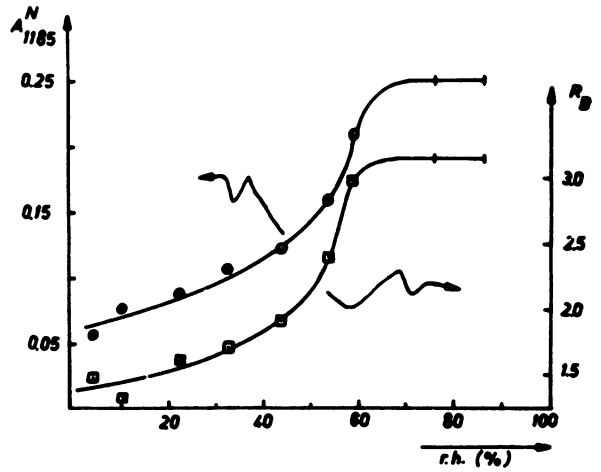

Fig. 3: Dependence of the normalized absorbance of the band at 1185 cm^{-1}, left side, (●), and the dichroic ratio of the band at 1710 cm^{-1} attributed to the DNA bases, right side, (⊡), on the relative humidity in the r.h. range of A form and disordered form of DNA in oriented films, respectively. Both sets of data refer to the same sample, and to decreasing humidities (dehydration) only, in each case. The A form plateaus are only schematically drawn.
schematically drawn.

A^N_{1185} plots vs. the relative humidity of DNA films. This finding suggests the A-form specificity of the 1185 cm^{-1} band not only in the humidity region of the B and A forms, but also in the region of low humidity where the regular DNA structure is gradually transferred into a disordered state.

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