BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 47 (4), 1043—1044 (1974)

Spectral Difference of the A and B Forms of Deoxyribonucleic Acid

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(Received November 19, 1973)

Synopsis. Infrared absorption spectrum of an oriented film of deoxyribonucleic acid from *Micrococcus lysodeikticus* has been examined in its A and B forms. In the A form a weak band was observed at 807 cm^{-1}, while in the B form this is absent and a broad and weak band appears at 832 cm^{-1}.

The primary purpose of this note is to report on some differences in the infrared absorption spectra in the 1000—750 cm^{-1} region of the A and B forms of deoxyribonucleic acid (DNA). Our experiment was directed by two reports,[1,2] which recently appeared. Erfurth, Kiser, and Peticolas[1] showed that in the Raman spectrum of calf thymus DNA in the A form there is a strong Raman line at 807 cm^{-1}, while in the B form this line is absent and a new line appears at 835 cm^{-1}. For characterizing these frequencies, it is desirable to examine infrared absorption spectra of the A and B forms of DNA in this frequency region, because the selection rule (or intensity rule) in the Raman effect and infrared absorption are different. Pilet and Brahms[2] made a detailed investigation on the infrared spectra of A and B forms of DNA's with various base compositions, and found a marked difference between the two forms in the dichroic properties of the  antisymmetric and symmetric stretching bands (at about 1230 and 1090 cm^{-1}, respectively). They did not show, however, any bands in the frequency region lower than 1000 cm^{-1}.

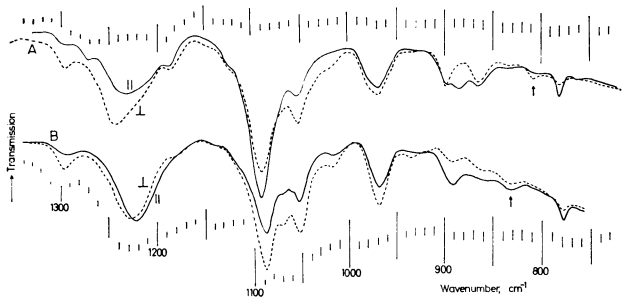

Fig. 1. Infrared absorption curves of oriented films of DNA from *Micrococcus lysodeikticus* in its A and B forms, observed with the polarized radiation. Full line, electric vector of the incident radiation is parallel to the fiber axis. Broken line, electric vector is perpendicular to the fiber axis.

Figure 1 shows the infrared spectra of an oriented DNA film from *Micrococcus lysodeikticus* in its A and B forms observed with the polarized radiation. The film was prepared from an aqueous solution of DNA with 10^{-5} M NaCl onto an AgCl plate. This film was soaked overnight in 73% ethanol with 0.1 M NaCl to add a proper amount of NaCl, and then washed only once by 80% ethanol. The spectrum A was obtained on keeping the film in the air of 75% humidity for a long time, and the spectrum B at 92% humidity. The A—B conversion readily takes place, but for regaining the spectrum A we need to keep the film in the air of 75% humidity for a week or so.

In the 1300—1000 cm^{-1} region, we have observed almost exactly what Pilet and Brahms reported:[2] in the A form the  antisymmetric stretching band (whose // component is at 1230 cm^{-1} and ⊥ component at 1243 cm^{-1}) shows a perpendicular dichroism and the symmetric stretching band (both // and ⊥ components are at 1092 cm^{-1}) a parallel dichromism, whereas in the B form the antisymmetric stretching band (// at 1220 cm^{-1} and ⊥ at 1230 cm^{-1}) is almost non-dichroic and the symmetric stretching band (both // and ⊥ are at 1088 cm^{-1}) shows a perpendicular dichroism. In the 800—700 cm^{-1} region, we found practically the same frequencies with what Erfurth *et al.*[1] observed in the Raman spectra both for the A and B forms, but with greatly different relative intensities. Thus, in the A form a weak band is observed at 807 cm^{-1}, while in the B form this is absent and a broad and weak band appears at 832 cm^{-1}. The former shows a perpendicular dichroism whereas the latter is almost non-dichroic.

The Raman or infrared frequency, 807 cm^{-1} of the A form or 832 cm^{-1} of the B form, is probably caused by a vibration in which mainly P–O single-bond stretching takes place, because of the following bases:

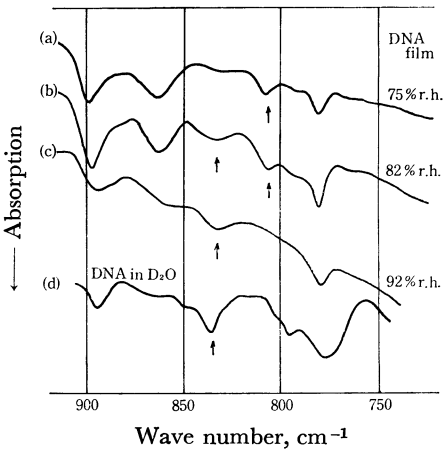

Fig. 2. Infrared absorption spectra in the 920—750 cm^{-1} region with unpolarized radiation. (a) Film of DNA from *M. lysodeikticus* at relative humidity (r.h.) 75%. (b) The same film at 82% r.h. (c) The same film at 92% r.h. (d) Calf thymus DNA in D_2O solution, pD=6 (observed with a Digilab FTS-14 Fourier Transform Infrared Spectrometer).

(1) The Raman line of nucleic acids at about 810 cm^{-1} is in general very strong and its appearence and absence are so sensitive to the mainchain conformation that it is not probable to be caused by a vibration of a base residue.[1,3]

(2) The 807 and 832 cm^{-1} bands of DNA are found to remain almost unchanged when the film is exposed to D_2O vapor. Many other data[3] indicate that the frequency now in question is insensitive to the deuteration of the amino and hydroxy groups of the nucleic acid.

We have conducted a number of experiments in an attempt to establish a correlation (if any) between the P–O stretching frequency and the conformation around this bond in the C–O–[PO_2^-]–O–C system. Unfortunately, however, what was attempted has not so far come out in any neat form. Nevertheless, this band may be useful for finding a gross mainchain-conformation around the phosphate group in a given sample of nucleic acid; it is favorable that we only need to find the position of the band and do not need to examine infrared dichroism here. As seen in Fig. 2 (b), the *M. lysodeikticus* DNA film shows both of the 807 and 832 cm^{-1} bands in the air of 82% humidity. It may be concluded, therefore, that such a film contains both of the A and B forms. This conclusion is reached in a less ambiguous manner than that from a dichroic measurement in the 1250—1000 cm^{-1} region. On the other hand, calf thymus DNA in neutral D_2O solution shows a well-defined band at 840 cm^{-1} but not at 807 cm^{-1} (Fig. 2 (d)), and this fact supports the idea that the DNA molecule is in the B-form in its aqueous solution.

It may be pointed out here that each of the double-helical ribonucleic acid (RNA)[4] and DNA–RNA hybrid[5] shows a medium intensity perpendicular band at 810 cm^{-1} but not at 832 cm^{-1}. By X-ray diffraction studies[5] both of these are known to have structures similar to that of DNA A form, but quite different from the B form. The 810 cm^{-1} band of RNA was once assigned to a nearly symmetrical stretching vibration of the $\overset{C}{C}>$C–O structure at the position 2′ of the ribose residue.[6] We now consider that at least a part of this band is caused by the P–O single bond stretching mode. It is not yet excluded, however, that the band due to the ribose vibration is superposed here. Perhaps it is worthwhile to mention here our Raman spectrum of RNA from cytoplasmic polyhedrosis virus (CPV) of silk worm in comparison with that of DNA in their aqueous solutions. CPV RNA is a natural RNA with a complete double-helical structure. As may be seen in Fig. 3, CPV RNA shows a srtong and sharp Raman line at 810 cm^{-1} while DNA does not. This line is insensitive to deuteration. This fact suggests that this RNA has, in its aqueous solution, a structure similar to that of DNA A form.

Lastly, a few comments are to be added on some other band observed in the A- and B-forms of DNA.

In the 900—880 cm^{-1} region, there is another marked difference found between the A and B forms. In the A form, there is a medium intensity band at 881 cm^{-1} which is strongly polarized along the parallel direction with the fiber axis (Fig. 1). This is missing in the B-form; instead, there is a parallel band at 891 cm^{-1}. This is also unaffected by deuteration, and is assignable to another mainchain stretching vibration.

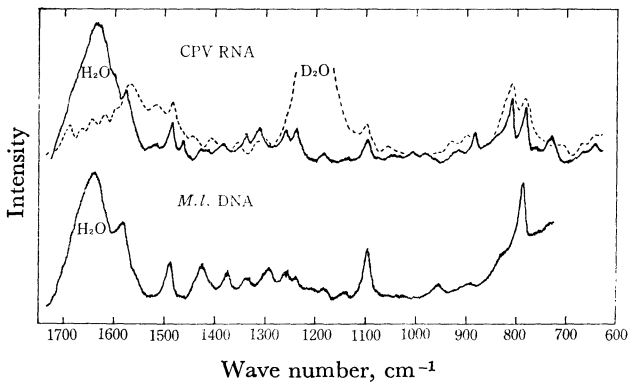

Fig. 3. Upper: Raman spectra of CPV RNA, excited by the 514.5 nm beam of an Ar^+ laser (Coherent Radiation, 52G-A) and observed with a JRS-Ul Raman spectrophotometer of Japan Electron Optics Laboratory, Company (JEOL). — in H_2O, --- in D_2O. The RNA sample was prepared under the direction of Dr. K. Miura, National Institute of Genetics. We thank Dr. Miura for his guidance. We also thank Mr. S. Muraishi, JEOL, for his kindness in the spectroscopic measurements.
Lower: Raman spectrum of DNA from *Micrococcus lysodeikticus* (GC 72%) in H_2O solution (0.02M Tris+ 0.1M NaCl, pH 7.6). The spectrum was observed by the use of a JRS-UlS Raman spectrophotometer of JEOL and 488.0 nm beam of an Ar^+ laser of coherent radiation (52G-A).

The strong Raman line at 780 cm^{-1} and the medium-intensity infrared band at 780 cm^{-1} should be assigned to different vibrations, in spite of the coincidence in their frequency values. The Raman line is assignable to the ring breathing vibration of the pyrimidine base residues. While, the infrared band which shows a strong parallel dichroism is assignable to an out-of-plane vibration in some base-residues.

A strong band at 1120 cm^{-1} of RNA, which was assigned to the $\overset{C}{C}>$C–O "degenerate" stretching,[6] is absent in DNA even in its A form (see Fig. 1). Therefore, the assignment of this band in RNA has been supported by our present work.

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