A NEW APPROACH TO THE CHARACTERIZATION OF THE B and A FORMS OF DNA by I.R. SPECTROSCOPY

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SUMMARY : The DNA conformational changes of B, A and C forms are reflected in the infrared absorption spectra in the region of 800 cm^{-1} to 900 cm^{-1} and allow one to investigate unoriented samples. The transition to the A form is characterized by the appearence of bands at about 870 cm^{-1} and at 813 cm^{-1} whereas the B and the C forms exhibit a band at 837 cm^{-1}, these bands undoubtedly arise from phosphate diester stretching vibrations and yield information about backbone conformation. The presence of these infrared bands provides a criterion for testing the simultaneous presence of two coexisting forms of DNA. It represents a useful method for structural studies of nucleic acid complexes such as protein-DNA for which it is difficult to obtain orientation.

INTRODUCTION

The structure of DNA at high degree of relative humidity (r.h.) is considered to be in the B form (1,2) or to belong to the family of the B form (3). The transition to the A form at lower relative humidity has been most precisely characterized by X-ray diffraction (1,4), and more recently by infrared linear dichroism (5,6). The investigation of the B $\longrightarrow$ A structural change of DNA is particularly important since it may have many biological implications as a model for DNA-protein interactions(7, 8) and for other DNA structural changes occuring at lower humidity. Both techniques, X-ray diffraction and infrared linear dichroism, require orientation of the sample, which may sometimes be difficult to achieve in biological complexes such as DNA-protein complexes, chromatin, viruses and others. We wish to report here that the B —— A structural transition is correlated with a characteristic change in the infrared absorption spectrum in the region of 800 cm^{-1} to 900 cm^{-1} and is detectable even on unoriented samples.

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MATERIAL AND METHODS

T4, $T4^+$ phage DNA samples were prepared by the method of THOMAS and ABELSON (9) ; T5 DNA was a gift of Dr. J. LEGAULT. Bacterial DNA's : B. SUBTILIS and SPHAEROPHORUS VARIUS and other DNA's were prepared by the method of MARMUR (10). Salmon sperm and calf thymus DNA were purchassed from WORTHINGTON, and Micrococcus Luteus from MILES LABORATORIES. All DNA samples were tested for their purity and particularly for absence of protein ; when necessary additional phenol extractions were performed. The purity tests involved ultraviolet spectra measurements and thermal denaturation curves. These tests were also applied to a DNA sample after the exposure to infrared measurements and indicated the absence of denaturating changes.

Infrared measurements were performed on oriented DNA samples prepared by two methods, previously established by PILET and BRAHMS (5,6) : firstly by equilibrating an oriented DNA in a solution containing 0.1 M NaCl 73 % alcohol or secondly by an extensive dialysis against low ionic strength and by adding an appropriate amount of 10^{-2} M NaCl. DNA films were prepared from aqueous DNA solution on a BaF_2 or AgCl plate ; NaCl content was of 3 % - 4 % as described by PILET and BRAHMS (5,6). The plate with the DNA film was mounted in a constant humidity cell (For all other details see ref. 5,6). The infrared spectra were recorded on a Beckman I.R.9 spectrophotometer equiped with a wire grid polarizer.

RESULTS

Figures 1^a and 1^b show a part of the infrared polarized spectra of two oriented DNA samples, T4 (fig. 1^a) and salmon sperm (fig. 1^b) at high relative humidity (98 % r.h.). Infrared spectral criteria established by PILET and BRAHMS (5,6) i.e. the presence of perpendicular polarization of the 1090 cm^{-1} band and almost complete absence of dichroism

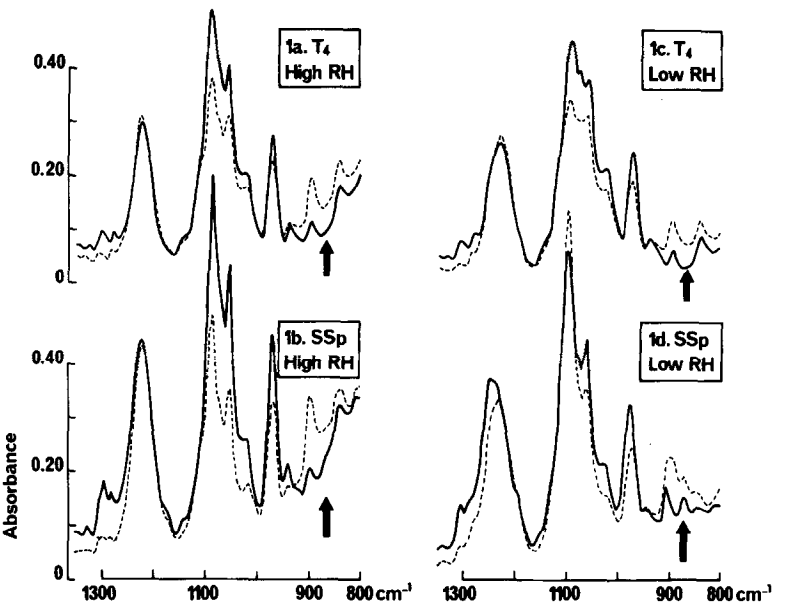

Figure I : Infrared spectra of oriented salmon sperm DNA (1b and 1d) and T4 DNA (1a and 1c) measured at 98 % r.h. (1a and 1b) and at 58 % r.h. (1c and 1d) in the spectral region between 800 cm^{-1} and 1300 cm^{-1} ; (Sodium chloride content 3-4 %)

——— electric vector of the I.R. polarized light perpendicular to the orientation axis of DNA chain
----- electric vector parallel to this DNA axis.

of the band at 1230 cm^{-1}, indicate that these DNA's are in the B form ; both bands are assigned to stretching vibrations of the phosphate group (11,12,13). In the region of 800 cm^{-1} to 900 cm^{-1} one observes the presence of two bands at about 837 cm^{-1} and 896 cm^{-1}.

Figures 1^c and 1^d show the same T4 and salmon sperm DNA's samples at low relative humidity (58 % r.h.). The T4 DNA (Fig. 1^c) and also $T4^+$ remains in a B-kind form as indicated by the perpendicular dichroism of the 1090 cm^{-1} band (5,6,11). One observes also the presence of the 837 cm^{-1} band. In contrast, the salmon sperm DNA (fig. 1^d) adopts the A form, which is indicated by the inversion of dichroism of the 1090 cm^{-1} band (parallel polarization) and by the appearence of a perpendicular dichroism of the 1230 cm^{-1} band (see ref. 5,6). Between 800 cm^{-1} and 900 cm^{-1}, new bands appear at about 863 cm^{-1} (see arrow) and 883 cm^{-1} of parallel

polarization, whereas the 837 cm^{-1} band disappears ; at 813 cm^{-1} another new band of perpendicular polarization appears. In Table I, the characteristic infrared band frequencies of B and A forms of DNA measured in the region of 800 cm^{-1} to 900 cm^{-1} are indicated. They are compared with RNA spectral characteristics previously invetigated on oriented double stranded and single stranded polynucleotides ; poly A acid form and poly 2'-O-methyl A (poly Am) neutral form (14).

The observations of the characteristic DNA bands in the 800 cm^{-1} - 900 cm^{-1} region were also confirmed by the investigation of eleven DNA samples of different origins and of different base composition. In all cases where the B $\longrightarrow$ A structural changes took place, they were reflected in the changes in the 800 cm^{-1} - 900 cm^{-1} region of the spectrum. Salmon sperm DNA Li salt (at 2-3 % by weight), when in the C form at lower humidity (below 76 % r.h.), yields the band at 837 cm^{-1}, alone as in the B form.

The assignment of these bands will be considered separately (BRAHMS et al. to be published). These bands are probably characteristic of the phosphate-ribose backbone vibrations, particularly of the -OPO- diester stretching vibrations. The following observations are in favour of this assignment : 1/ The position of the characteristic bands of the A form i.e. bands in the region of 860 cm^{-1} - 880 cm^{-1} and 813 cm^{-1} is very little if any shifted after deuteration. This is in good agreement with some previous observations on the 813 cm^{-1} infrared band by TSUBOI (13) an on Raman bands observed by THOMAS (15) and by SMALL and PETITCOLAS (16,17). 2/ In poly 2'-O-methyl A the 815 cm^{-1} band in the I.R. spectrum is weak but present, thus the main contribution arises from phosphate diester in agreement with the assignment by SMALL and PETITCOLAS (16). It is possible that in polyribonucleotides another band arising from C_2,-OH vibrations may also contribute at similar frequency (see TSUBOI (13)). 3/ Table I shows that the 813 cm^{-1} band and bands in the region of 870 cm^{-1} are present

TABLE I

INFRARED FREQUENCE CHARACTERISTICS
OF DNA A and B FORMS and of RNA
IN THE SPECTRAL REGION of 800 cm^{-1} - 900 cm^{-1}

	FORM A (deuterated)		FORM B (deuterated)	
DNA ...	898 cm^{-1}	s.	898 cm^{-1}	v.s.
	883	m.		
	863	m.		
	813	w.	837	m.

	POLY A double stranded (acid pH) deuterated	
RNA^{+}...	880 cm^{-1}	m.
	860	s.
	813	m.
	POLY Am single stranded (neutral pH) deuterated	
	870 cm^{-1}	m.
	815	v.w.

(+) Taken from PILET, ROTTMAN and BRAHMS (1973).

in various polynucleotides DNA's and in RNA regardless of the nature of the bases. This seems to exclude the possibility to assign these bands to the base vibrations. This 813 cm^{-1} band is strong and highly depolarized in the Raman spectrum and weak in the infrared, whereas the 860 cm^{-1} - 880 cm^{-1} bands are strong in the infrared and weak in the Raman spectrum. It will be shown that in the A form the 813 cm^{-1} band can be assigned to symmetric stretching vibrations of the phosphate diester, whereas we tend

to assign the corresponding antisymmetric stretching to the bands in the region of 860 cm^{-1} - 880 cm^{-1} (BRAHMS et al. 1973, to be published).

In conclusion, the A, B and C DNA forms are reflected in the infrared spectrum in the region of 800 cm^{-1} to 900 cm^{-1} by the presence of the following I.R. bands : the 837 cm^{-1} characterizes the presences of the B form which is in agreement with Raman assignment (17), or of the C form. The transition to the A form at lower r.h. is indicated by the appearence of a weak 813 cm^{-1} band and of bands between 860 cm^{-1} - 880 cm^{-1}, which in the unpolarized spectrum yield a broad band at about 870 cm^{-1}. These changes in the region of 800 cm^{-1} to 900 cm^{-1} parallel the changes in the polarized I.R. spectrum at 1090 cm^{-1} and 1230 cm^{-1} which are characteristic of the A and B forms as demonstrated by PILET and BRAHMS (5,6). It was found that measurement of the characteristic bands in this spectral region appears to provide a very sensitive conformational test for the simultaneous presence of two coexisting forms of DNA. The advantage of investigation in the region of 800 cm^{-1} - 900 cm^{-1} is the possibility of using unoriented samples. Thus, infrared investigation appears to provide a useful method for structural characterization of nucleic acids and particularly protein-nucleic acids complexes, for which it is difficult to obtain orientation and which therefore cannot be easily investigated by the X-ray diffraction method.

REFERENCES

1. Franklin, D.F. and Gossling, R.G., Acta Crystal., 6, 673, (1953).
2. Arnott, S., Progress in Biophys., 21, 265, (1970).
3. Bram, S. and Tougard P.·Nature New Biol., 239, 128, (1970)
4. Fuller, W., Wilkins, M.H.F., Wilson, M.R., Hamilton, L.D. and Arnott, S., J. Mol. Biol., 12, 60, (1965).
5. Pilet, J. and Brahms, J., Nature New Biol., 236, 99, (1972).
6. Pilet, J. and Brahms, J., Biopolymers, 12, 387, (1973).
7. Arnott, S., Fuller, W., Hodgson, A. and Prutton, I., Nature 220, 561, (1968).

8. Florentiev, V.L. and Ivanov, V.I., Nature, 228, 519, (1970).
9. Thomas, C.A. and Abelson, J., in "Procedures in Nucleic Acid Research" ed. Cantoni, G.L. and Davies, D.R., 553 Harper and Row (1967).
10. Marmur, J., J. Mol. Biol., 3, 208, (1961).
11. Bradbury, E.M., Price, W.C. and Wilkinsons, G.R., J. Mol. Biol. 3, 301, (1963).
12. Tsuboi, M., J. Amer. Chem. Soc., 79, 1351, (1957).
13. Tsuboi, M., Appl. Spectr. Rev. (1970).
14. Pilet, J., Rottman, F. and Brahms, J., Biochem. Biophys. Res. Comm. 52, 517, (1973).
15. Thomas, G.J., J. Biochem. Biophys. Acta, 213, 417, (1970).
16. Small, E.W. and Peticolas, W.L., Biopolymers, 10, 1377, (1971).
17. Erfuhrt, S.C., Kiser, E.J. and Peticolas, W.L., Proceed. Nat. Ac. Sci., 69, 938, (1972).