Conformational flexibility in the right-handed and in the left-handed double helices of Na⁺-d(m5C-G-T-A-m5C-G) studied by I R spectroscopy

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Received April 7, 1988; Accepted May 23, 1988

ABSTRACT

The X ray diffraction study of a d(m5C-G-A-T-m5C-G) crystals has shown the existence of a Z conformation while the Raman spectroscopy study of the same fragment in solution showed that then the oligonucleotide adopted a B geometry. Infrared spectroscopy has allowed us to study this sequence in films in a wide range of hydrations and to vary the water content of the sample at different rates. We have thus obtained four I.R. spectra, of the B and Z conformations respectively at 100% and 98% relative humidities, of another Z form with a different geometry of the phosphate groups at relative humidities below 98% and in addition a fourth spectrum recorded after a rapid dehydration of the sample which is then blocked in a right-handed form at low R.H.. In this case the structure of the phosphodiester chain may be not uniform. The results are discussed by comparison with previously obtained I.R. spectra of poly d(G-C), poly d(A-C).d(G-T), d'm5C-G-A-m5C-G-T-G-C-G) d(m5C-G-C-G-m5C-G) and d(C-G-m5C-G).

INTRODUCTION

The DNA hexamer d(m5C-G-T-A-m5C-G) has been studied by X-ray diffraction (1) and also by Raman spectroscopy (2). The atomic resolution single crystal X-ray diffraction analysis has shown that d(m5C-G-T-A-m5C-G) crystallizes as Z-DNA. This study shows that the two central adenines are in syn conformation and that the backbone conformation is similar to the backbone of the initial (dC-dG)₂ left-handed structure (3). Raman spectra have been obtained from single crystals and the Z to B transition has been followed by spectral changes which occur upon dissolving the crystal in H₂O. In this work we have studied d(m5C-G-T-A-m5C-G) film by infrared spectroscopy. By this technique we can vary the water content of the sample in a more gradual way than by simply dissolving the crystal into a solution. We have followed the modifications of the hexamer spectra at different relative humidities (between 100% R.H. and that corresponding to the disorganization of the double helix). When the relative humidity of a Na⁺-poly d(G-C) film is decreased from 100% to 98% a B→Z transition is observed (4,5). A B→Z transition has been detected in similar conditions in d(C-n2A-C-G-T-G) and d(m5C-G-C-A-m5C-G-T-G-C-G) films (6). On the contrary, for native DNAs containing 50% A-T and 50% G-C base pairs, for Na⁺-poly d(A-C).poly d(G-T) and Na⁺-poly d(A-T) a B→A transition occurs when the relative humidity is decreased from 100% to 76% R.H.. In the case of

d(m5C-G-T-A-m5C-G) films, as could be expected after the results of X-ray and Raman spectroscopy studies, a classical B form I.R. spectrum has been obtained at 100% R.H. and the B→Z transition has been observed by decreasing the relative humidity to 98% R.H.. However unexpected results have been obtained at lower relative humidity. For a water content of the film, (typically 81% to 76% R.H.) it is shown that it is possible to observe the hexamer either in a Z family form conformation or blocked in a right-handed form. Thus four different types of I.R. spectra are recorded for Na⁺-d(m5C-G-T-A-m5C-G) films, two of them can be interpreted in terms of right-handed structures and the two others ones in terms of left-handed structures. While the I.R. spectrum obtained at 100% relative humidity seems to reflect an uniform B double helical structure, variations of the phosphodiester chain structure at the level of the A-T and G-C base pairs could explain the I.R. spectrum of d(m5C-G-T-A-m5C-G) blocked in a right-handed form at low relative humidity. The changes in the left-handed form I.R. spectra of Na⁺-d(m5C-G-T-A-m5C-G) which occur between 98% and 81% R.H. are compared to those observed in the Z form spectra of methylated d(C-G)_n sequences.

MATERIAL AND METHODS

The oligonucleotide is a gift from Dr A.H.J. WANG.

The samples were prepared by dialysing the solution of the oligonucleotide in 10 mM Tris, 0.1 M NaCl against an NaCl solution so as to obtain 2 Na⁺ in excess per nucleotide. The pH was adjusted between 7 and 8 by adding small amounts of NaOH. Then the solutions were gently dried on infrared ZnSe transparent supports in order to obtain films. The hydration of the samples was monitored by the relative humidity present in a sealed cell, in a range between 58 and 100% (H₂O or D₂O).

The I.R. spectra have been recorded with a Perkin Elmer 180 double beam ratiorecorder spectrophotometer coupled to a Hewlett-Packard 9825 A calculator allowing systematic data treatment.

RESULTS AND DISCUSSION

As shown in fig. 1a, at very high relative humidity (100%), a B-type infrared spectrum is observed, similar to that given by the sodium salt of natural DNAs or of poly d(A-C), poly d(G-T) at the same relative humidity (7,8). After extended exposure to 98% R.H., the oligonucleotide undergoes a transition to the Z form which then persists with some modifications when the relative humidity is further reduced. Fig.1b and fig.2a are shown the two Z family type spectra of Na⁺-d(m5C-G-T-A-m5C-G) recorded respectively at 98% R.H. and below 81% R.H. respectively. A very different infrared spectrum is observed at low R.H. when the water content of the Na⁺-d(m5C-G-T-A-m5C-G) films is decreased from 100% R.H. by direct exposure to 81% or 76% R.H. The I.R. spectrum obtained in these conditions (fig.3a) will be interpreted as

Figure 1 : Infrared absorption spectra of d(m5C-G-T-A-m5C-G) film at :
 (a) 100% R.H., B form
 (b) 98% R.H., Z form

reflecting a right-handed double helix. The hexamer becomes blocked in a right-handed form which is stable for considerable periods. Transitions from this form to a member of the Z family are only observed after prolonged exposure to high humidities (up to 98%). The I.R. data associated with this right-handed low humidity form of Na⁺-d(m5C-G-T-A-m5C-G) differ from those obtained for the conventional B or A DNA secondary structures.

We will now describe in detail the spectral features which support the foregoing interpretation, and at first discuss the B→Z transition.

B→Z transition

The I.R. spectra of these two conformations have been identified by comparison with previously studied B and Z infrared spectra of poly d(G-C), poly d(m5C-G), poly d(A-C), poly d(G-T) and different oligonucleotides (4,5,6,8,10). In the spectral region of the in-plane double-bond stretching vibrations of the bases, between 1750 cm^{-1} and 1550 cm^{-1} , the decrease from 100% to 98% of the relative humidity of Na⁺-d(m5C-G-T-A-m5C-G) films is followed by the shift of the 1708 cm^{-1} band (B form fig. 1a) to 1696 cm^{-1} (Z form, fig. 1b). A similar shift from 1710 cm^{-1} to 1693 cm^{-1} had been observed upon the B→Z transition monitored by decreasing the water

Figure 2 : Infrared absorption spectra of left-handed conformations at low R.H.

- (a) d(m5C-G-T-A-m5C-G), Z form observed after a slow decrease of the R.H. between 98% and 81% R.H.
 (b) d(C-G-m5C-G), 76% R.H.

content in films of Na⁺-poly d(G-C).poly d(G-C) (4,5) or of Na⁺-d(C-n2A-C-G-T-G) and of Na⁺-d(m5C-G-C-A-m5C-G-T-G-C-G) (6). The 1750 cm⁻¹ - 1550 cm⁻¹ region is considered as being sensitive to base pairing and to base stacking. In polynucleotides, the existence of transition dipole coupling for strong I.R. absorptions involving double-bond stretching vibrations of the bases has been pointed out (7). X-ray diffraction studies have shown that the base stacking in the Z form is very different from the base stacking in the B form (1,3). Thus the dipolar interactions can be modified upon B→Z transition and this is detected by shifts of the corresponding I.R. bands. The similarity of the shifts for DNAs, seems in agreement with the weak modification shown to exist by X-ray diffraction studies at the level of base stacking of A-T base pairs in the crystal of the d(m5C-G-T-A-m5C-G) hexamer (1).

In the spectral region between 1550 cm⁻¹ and 1250 cm⁻¹ coupling may occur between the purine vibrations and some of the deoxyribose vibrations. Thus, the wavenumbers of the I.R. bands may vary depending upon whether the glycosidic linkage is syn or anti and the deoxyribose C2'-endo or

Figure 3 : Infrared absorption spectra of right-handed conformations at low R.H.

(a) d(m5C-G-T-A-m5C-G), B family form observed after direct exposure of the film to 81% R.H.
 (b) poly d(A-C).poly d(G-T), 66% R.H. (A form)

C3'-endo (11,12). As the same gross structural features have been determined by X-ray diffraction studies in d(C-G)_n, d(m5C-G)_n and d(m5C-G-T-A-m5C-G) crystals (1,3,13), it is reasonable to expect some similar I.R. absorptions in this spectral region for Z DNA containing G-C base pairs as well as A-T base pairs. The modifications of the I.R. spectrum of d(m5C-G-T-A-m5C-G) between 1550 cm⁻¹ and 1250 cm⁻¹ upon reduction of the water content from 100% to 98% are the following: shifts of the 1422 cm⁻¹ band to 1410 cm⁻¹ and of the broad band around 1370 cm⁻¹ to 1355 cm⁻¹; presence of new bands at 1433 cm⁻¹, 1320 cm⁻¹ and of a shoulder at 1264 cm⁻¹ (fig. 1a, fig.1b and fig. 4a). In this spectral region, the d(m5C-G-T-A-m5C-G) spectrum remains quite unchanged upon further decrease of the R.H. from 98% to 84%. We have reported the same modifications of the I.R. spectra of poly d(G-C).poly d(G-C) upon the B→Z transition (4,10). Likewise, the I.R. spectra of the d(m5C-G-C-G-m5C-G) hexamer, which has been found in films in Z form, whatever the water content conditions, present bands at 1410 cm⁻¹, 1354 cm⁻¹, 1320 cm⁻¹ and 1264 cm⁻¹ (fig.4b). Taking into account the results of force field calculations performed on the dG residue (12), the bands located at

Abs.

Figure 4 : Infrared spectra between 1550 and 1250 cm^{-1} of :

- (a) Z form of d(m5C-G-T-A-m5C-G), 98% R.H.
- (b) Z form of d(m5C-G-C-G-m5C-G), 76% R.H.
- (c) Z form of Ni^{2+} - poly d(A-C).poly d(G-T), 76% R.H.

1410 cm^{-1} , 1355 cm^{-1} and 1320 cm^{-1} may be assigned to a purine bound to a sugar in C3'-endo/syn configuration. The band located at 1433 cm^{-1} does not exist in the Z type spectra of DNAs which contain only G-C base pairs but is detected in the Ni^{2+} -poly d(A-C).poly d(G-T) Z form spectrum (fig. 4c) (8). This absorption may be assigned to the dA residue in C3'-endo/syn configuration.

Fig 5 shows the I.R. spectra between 1030 cm^{-1} and 750 cm^{-1} of d(m5C-G-T-A-m5C-G) (Fig.5a) and Z forms of d(m5C-G-C-G-m5C-G) (Fig. 5b) and d(m5C-G-C-A-m5C-G-T-G-C-G) (Fig. 5c), in D_2O . All three spectra exhibit the I.R. absorption profile associated with a sugar phosphate backbone in Z form (5,7), that is to say a strong band around 1015 cm^{-1} , a medium intensity

Abs.

Figure 5 : Infrared spectra in the range ($1030\text{--}750\text{ cm}^{-1}$) of deuterated samples of :

- (a) Z form of d(m5C-G-T-A-m5C-G), 81% R.D.
- (b) Z form of d(m5C-G-C-G-m5C-G), 76% R.D.
- (c) Z form of d(m5C-G-C-A-m5C-G-T-G-C-G), 66% R.D.

band around 925 cm^{-1} only a weak band at 892 cm^{-1} and the simultaneous occurrence of the 863 cm^{-1} and 834 cm^{-1} bands. This result is in agreement with the X-ray data since the sugar phosphate backbone determined for d(C-G)_n, d(m5C-G)_n and d(m5C-G-T-A-m5C-G) is the same in the three Z DNA crystals and does not depend on the presence of A-T base pairs. In contrast the spectral region between 800 cm^{-1} and 750 cm^{-1} is clearly different for the three DNAs in the same conformation, as the absorption bands are involving vibrations specific of each base (798 cm^{-1} : adenine, 785 cm^{-1} : guanine, 778 cm^{-1} : cytosine, around 775 cm^{-1} : thymine, 770 cm^{-1} : m5cytosine). The spectral region between 700 cm^{-1} and 600 cm^{-1}

is extremely favorable to characterize the right- and left-handed geometries by Raman spectroscopy. In the case of d(m5C-G-T-A-m5C-G) the sharp Z form Raman lines have been detected at 622 and 673 cm^{-1} by THOMAS and coworkers (2). Unfortunately the I.R. absorptions of these vibrational modes are extremely weak and can scarcely be detected.

The second left-handed form observed at lower than 98% R.H.

Upon decrease of the R.H. from 98% to 81%, the I.R. spectrum of d(m5C-G-T-A-m5C-G) (fig. 2a) reflects a left-handed structure since all the characteristic bands which appear at 98% R.H. during the B- $\rightarrow$ Z transition remain present (1692 cm^{-1} , 1630 cm^{-1} , 1410 cm^{-1} , 1354 cm^{-1} , 1320 cm^{-1} , around 1015 cm^{-1} and 925 cm^{-1}), but important perturbations are observed especially in the intensities of the bands between 1250 cm^{-1} and 1000 cm^{-1} . In this spectral region is found the strong absorption assigned to the antisymmetric stretching vibration of the PO_2^- groups. It is shifted from 1221 cm^{-1} (98% R.H.) to 1215 cm^{-1} (81% R.H.) but its intensity can be taken as a reference. The most striking feature is the strong hypochromism of the 1090 cm^{-1} band involving mainly the symmetric stretching vibration of the phosphates coupled to C5'-O5' stretching and to a lesser extent with other vibrations of the backbone chain (4,5,7). Its intensity is reduced by a factor of 0.73 and the absorption at 1090 cm^{-1} becomes less intense than $\nu_{\text{as}} \text{PO}_2^-$ at 1215 cm^{-1} . In contrast the intensity of the band at 1017 cm^{-1} which is split into two components at 1011 cm^{-1} and 1018 cm^{-1} , is enhanced by a factor of 1.22 and its intensity is very similar to that of the $\nu_{\text{as}} \text{PO}_2^-$. The 1017 cm^{-1} absorption is mainly assigned to C-O vibrations of the phosphodiester linkage and/or of the sugar. Thus, this suggests that the conformation of the PO_2^- and C-O-P-O-C groups (or a more extended part of the backbone at low R.H.) may be modified. This spectral profile is not observed in the classical Z form spectrum of poly Na $\cdot$ d(G-C) films whatever their R.H., however in the case of a methylated tetramer d(C-G-m5C-G) a similar I.R. spectrum is obtained (Fig.2b). The existence of two Z conformations for the same oligonucleotide has been found in crystals studied by X-ray. Two different conformations were observed for the phosphate in the GpC sequences (16). Two different Z forms have also been reported for d(C-G) $_2$ stabilized in high salt or in low salt crystals respectively (17,18) and for d(br5C-G) $_3$ in solution depending of the temperature (19). We have observed that it is also possible to stabilize two different Z forms of the d(m5C-G-T-A-m5C-G) sequence in films as an depending of the relative humidities.

Right-handed form observed at low R.H.

The conformational transitions classically observed by decreasing the relative humidity are B- $\rightarrow$ A and B- $\rightarrow$ Z. The spectrum fig. 3a does not present any of the Z form characteristic absorptions previously described. It does neither correspond to an A geometry as can be seen by comparison with the spectrum presented fig. 3b. The absorption which in B form was observed at 1708 cm^{-1} is shifted to higher wavenumbers (1722 cm^{-1}) in this spectrum recorded at 81% R.H. instead of being shifted to lower wavenumbers

as in the case of the B→A transition. Moreover, the 1185 cm^{-1} band, characteristic of the A form (20) is not present and the profile of the symmetric stretching vibration of the phosphates around 1086 cm^{-1} is strongly perturbed. In the A family form I.R. spectrum absorptions around 895 cm^{-1} , 879 cm^{-1} and 860 cm^{-1} are observed but the relative intensities are quite different from those observed on this new I.R. spectrum. However this new spectrum reflects a double stranded structure (in unpaired DNAs no sharp band is detected above 1700 cm^{-1}). It is thus clear that this spectrum does not reflect neither an A nor a classical B type helix. Nevertheless we propose to assign this spectrum to a right-handed double stranded structure for the following reason. This spectrum is obtained by rapidly decreasing the hydration of a right-handed B conformation. Further decrease of the relative humidity fails to induce a Z form, which can be recovered only by rehydrating the DNA at very high relative humidity (98% R.H.). The right-handed left-handed interconversion of the helical sense involves a rotation of the base planes, which in films is favoured by a high water content and cannot occur at low relative humidity. Therefore, if the structure corresponding to this new low hydration I.R. spectrum belonged to the left-handed family, the obtention of a Z form spectrum would have been possible without imperatively returning to high R.H.. The low relative humidity right-handed form spectrum of d(m5C-G-T-A-m5C-G) might reflect a non-uniform conformation of the phosphodiester chain. Infrared absorption bands located near 895 cm^{-1} and 835 cm^{-1} are associated with a B family conformation. In the low relative humidity right-handed form spectrum are observed the splitting of these bands ($847\text{--}880\text{ cm}^{-1}$ and $838\text{--}823\text{ cm}^{-1}$). These splittings could be explained by the existence of slightly different conformations of the sugar phosphodiester backbone at the level of the different base pairs. The B conformation is the favoured DNA geometry in solution or at high relative humidity, but nevertheless some DNA fragments have been found in B form in crystals (21,22). Our results are in agreement with X-ray diffraction data which show that in the case of the B form crystal of d(C-G-C-G-A-A-T-T-C-G-C-G) (21) a great structural variability at the level of each base. The study by I.R. spectroscopy of the d(m5C-G-T-A-m5C-G) sequence confirms the conformational flexibility of this DNA and clearly shows the very important role of the sample water content in stabilizing the different conformations.

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