Volume 13 Number 6 1985 Nucleic Acids Research

The poly dA strand of poly dA.poly dT adopts an A-form in solution: a UV resonance Raman study

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Received 29 January 1985; Accepted 22 February 1985

ABSTRACT

The study by resonance Raman spectroscopy with a 257 nm excitation wavelength of adenine in two single-stranded polynucleotides, poly rA and poly dA, and in three double-stranded polynucleotides, poly dA.poly dT, poly(dA-dT).poly(dA-dT) and poly rA.poly rU, allows one to characterize the A-genus conformation of polynucleotides containing adenine and thymine bases.

The characteristic spectrum of the A-form of the adenine strand is observed, except small differences, for poly rA, poly rA.poly rU and poly dA.poly dT. Our results prove that it is the adenine strand which adopts the A-family conformation in poly dA.poly dT.

INTRODUCTION

It is now well established that the structure of DNA is much more polymorphic than the Watson and Crick model let foresee and numerous works have attempted to determine the influence of the sequence of the bases on structure. Oligonucleotides and polynucleotides of known sequences were used in that purpose.

The more astonishing results were obtained with oligonucleotides or polynucleotides containing alterning guanine-cytosine sequences. However, the synthetic polynucleotides constituted with the adenine and thymine bases, either the alternating copolymer poly(dA-dT).poly(dA-dT) or the homopolymer poly dA.poly dT, seem to adopt particular conformations. At first, Klug et al.[1] proposed an "alternating B structure" for poly(dA-dT).poly(dA-dT) but this model does not seem to give a good account for the fiber data[2]. Right -or left- handed helix, Watson-Crick or Hoogsteen base-pairing, the structure of this polynucleotide is not yet totally elucidated (see Gupta et al.[2] for discussion).

The structure of poly dA.poly dT was less studied. The first X-ray diffraction patterns of fibers brought about Arnott and Selsing[3] to propose a minor variant of B-DNA as structure of the polymer. In a recent study Arnott et al.[4] proposed a heteronomous secondary structure for poly dA.poly dT. One chain, probably poly dA, has C3'-endo puckered furanose rings characteristic

of the A family of polynucleotide secondary structure while the other, probably poly dT, has the C2'-endo puckered rings of the B family. Classical Raman spectroscopy of poly dA.poly dT in solution revealed the simultaneous existence of both C2'-endo and C3'-endo Raman marker bands[5,6]. It is then not yet possible to know what strand has C3'-endo puckered rings.

In view of the recent interest in the secondary structure of deoxyribonucleic acids with a high dA-dT content, we have undertaken a study by resonance Raman spectroscopy with a 257 nm excitation wavelength of poly dA, poly rA, poly(dA-dT).poly(dA-dT), poly dA.poly dT and poly rA.poly rU. The information obtained by resonance Raman spectroscopy concern exclusively the bases of the nucleic acids. With a 257 nm excitation wavelength, one can see preferentially the adenine bases (as compared to thymine and uracil). This allows one to demonstrate that the stacking of the adenines is similar in poly dA.poly dT and poly rA and comparable to the stacking of the adenine residues in poly rA.poly rU. Therefore the stacking of the poly dA strand is probably of the A-type with a C3'-endo ribose ring pucker.

MATERIALS AND METHODS

Deoxyadenosine, deoxythymidine and deoxyuridine were purchased from Sigma, poly rA, poly dA.poly dT and poly(dA.dT).poly(dA-dT) from Boehringer, poly dA and poly rA.poly rU from P.L. Biochemical. All solutions were prepared in 50 mM phosphate buffer, pH 7. In some cases, the polynucleotides were dialyzed against the same buffer. The final concentrations in nucleotides were about 10^{-4} M.

The resonance Raman spectra of oligonucleotides were obtained in the range 400 cm^{-1} - 1800 cm^{-1}. Details of the Raman set-up have been described previously[7,8]. The spectra are recorded step by step in a CBM Commodore laboratory computer. It is then possible to proceed to a numerical treatment of the data, such as smoothing by the Savitsky-Golay method[9] or normalization using the 3400 cm^{-1} water band as internal standard. The temperature of the sample was controlled by using a thermostated cell-holder. Several stirring systems were utilized to minimise the photodegradation[8,10].

RESULTS AND DISCUSSION

Fig. 1 shows normalized spectra of deoxyadenosine, deoxythymidine and deoxyuridine with the 257 nm excitation wavelength. The resonance Raman spectra of deoxyadenosine-5'-monophosphate, uridine-5'-monophosphate and deoxythymidine-5'-monophosphate as well as the attribution of the Raman lines have been

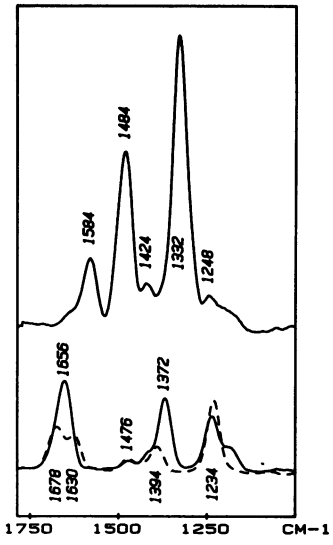

Figure 1 : Normalized resonance Raman spectra of deoxyadenosine (upper), deoxythymidine, ——— ; and deoxyuridine, ----- (lower) in the 1000 - 1800 cm^{-1} region.
Phosphate buffer 50 mM, pH 7. Excitation wavelength : 257 nm.

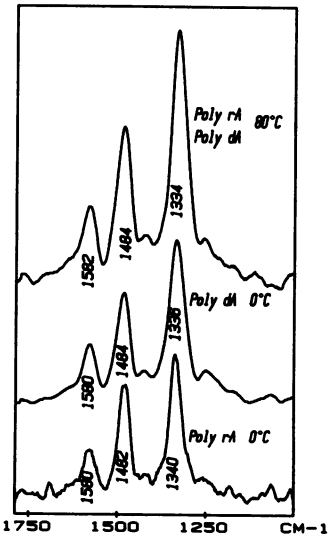

Figure 2 : Resonance Raman spectra of poly rA, (0°C) ; poly dA, (0°C) ; poly dA and poly rA (80°C) in the 1000 - 1800 cm^{-1} region.
Phosphate buffer 50 mM, pH 7. Excitation wavelength : 257 nm.

Table I. Intensity ratio of the adenine bands in the native and denaturated forms (I denaturated/I native) for the 5 studied polynucleotides.

	1334 cm^{-1}	1482 cm^{-1}	1580 cm^{-1}
Poly rA	1.6	1.3	1.3
poly dA	1.75	1.6	1.5
Poly rA.poly rU	1.65	1.25	1.3
Poly dA.poly dT	1.4	1.2	1.3
Poly (dA-dT).poly(dA-dT)	1.95	1.84	2.0

already published[7,11-14] with 300 nm or 257 nm excitation wavelengths. With the 257 nm excitation, the spectra of the nucleosides are almost similar to those of the corresponding nucleotides, allowing the assignment of the Raman lines.

Poly dA, poly rA. At 0°C, poly rA adopts in solution a A-type structure and poly dA a B-type structure comparable to that of DNA in solution. Fig. 2 shows the resonance Raman spectra of poly dA and poly rA in their native form at 0°C and thermally destacked form at 80°C. The spectra of destacked poly rA and poly dA are quite similar. Thus the differences observed between the spectra of the two native forms are due to differences in their secondary structures.

In the case of poly rA at 0°C, the adenine bands are found at 1340 cm^{-1}, 1482 cm^{-1} and 1580 cm^{-1}. In the case of poly dA at 0°C they are located at 1336 cm^{-1}, 1484 cm^{-1} and 1580 cm^{-1}. The variation of the intensities of the Raman bands following the denaturation process (hyperchromism) has been already studied in ordinary Raman spectroscopy, particularly in the case of poly rA (10 - 12). In resonance Raman spectroscopy with the 257 nm excitation wavelength, the hyperchromism is different for each single-stranded polynucleotide (Table I). It is more important in the case of poly dA, showing for the native form a stronger interaction between adjacent bases caused by a smaller interbase distance or a more important stacking interaction of the bases in the B-form (poly dA) compared to the A-form (poly rA).

Poly dA.poly dT, poly(dA-dT).poly(dA-dT), poly rA.poly rU. Fig. 3 shows the resonance Raman spectra (with the 257 nm excitation wavelength) of poly dA. poly dT and poly(dA-dT).poly(dA-dT) in their native (0°C) and denaturated forms (80°C). The spectra of the two denaturated polynucleotides are similar. Let us remind that only the vibrations of the bases are observable. Our result confirms that the Raman spectra are not sensitive to the primary structure. The

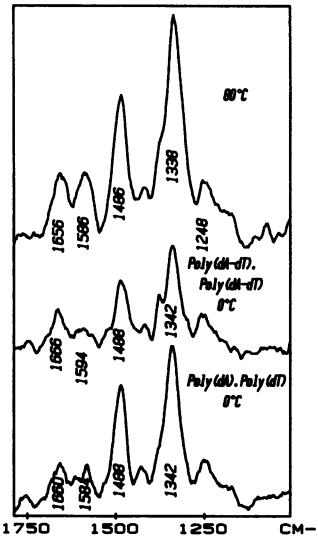

Figure 3 : Resonance Raman spectra of poly dA.poly dT, (0°C) poly(dA-dT).poly(dA-dT), (0°C) ; poly dA.poly dT and poly(dA-dT).poly(dA-dT), (80°C) in the 1000 - 1800 cm^{-1} region.
Same conditions as in figure 2.

differences observed between the spectra of the two native structures indicate differences in the secondary structures, particularly in the stacking interactions of the bases since the interstrand bonds are similar for poly dA.poly dT and poly(dA-dT).poly(dA-dT).

As one can see in Fig. 1, the bands observed with a 257 nm excitation wavelength are essentially those of adenine. Only the line located around 1660 cm^{-1} is a mere line of thymine. The 1250 cm^{-1} line is partially due to thymine. The thymine line around 1370 cm^{-1} is observable as a marked shoulder in the high frequency limb of the 1338 cm^{-1} line, particularly in the spectrum of the native form of poly(dA-dT).poly(dA-dT). For poly dA.poly dT and denaturated polynucleotides, this shoulder is weaker and merely widens the adenine line around 1360 cm^{-1}.

We studied as well the native and denaturated poly rA.poly rU (Fig. 4). This polynucleotide was chosen since its structure seems now to be well known : Arnott et al.[19] showed that the structure adopted by a fiber was of the A-family. In solution, this A-family form is conserved, as indicated by the A-type specific line at 814 cm^{-1} observed in ordinary Raman spectroscopy[20]. One can see in the spectrum of poly rA.poly rU the adenine lines located at 1332 cm^{-1},

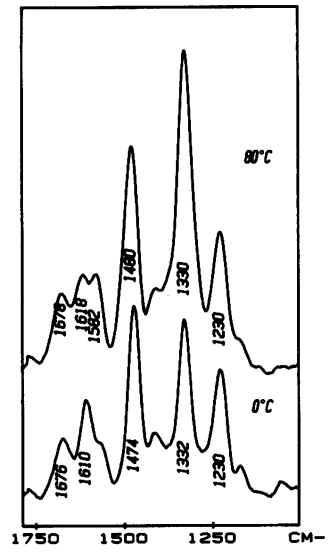

Figure 4 : Resonance Raman spectra of poly rA.poly rU at 0°C and 80°C in the 1000 - 1800 cm^{-1} region.
Same conditions as in figure 2.

1474 cm^{-1} and 1582 cm^{-1}. In the case of the native polynucleotide, the adenine band at 1582 cm^{-1} is largely hidden by the doublet of uracile at 1610 cm^{-1} and 1676 cm^{-1}.

It is possible to compare directly the spectra of the double-stranded polynucleotides poly dA.poly dT and poly(dA-dT).poly(dA-dT) to that of the single-stranded poly dA and poly rA and to that of poly rA.poly rU since, as described previously, the presence of thymine or uracil does not modify significantly the spectrum of adenine. Thus we subtracted the spectrum of thymine or uracil from the spectra of the double-stranded polynucleotides (Fig. 5) using a linear regression method.

This subtraction is defensible : (i) the intensity of the Raman lines of thymine or uracil is low compared to those of the adenine lines, (ii) the thymine or uracil lines are not located at the same frequencies as the lines of adenine. Even if the spectra of thymine or uracil are modified when the pyrimidine bases are included in a structured polynucleotide, this would not alter in a significant amount the spectra obtained after subtraction. However, we have to keep in mind that, because of the interstrand hydrogen bonds, the presence of pyrimidines may alter the Raman spectrum of adenine, particularly in shifting some of its lines. These hydrogen bonds are get more or less identical

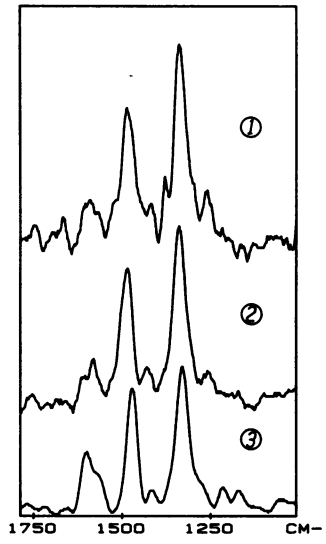

Figure 5 : Resonance Raman spectra of the adenine strand. Spectrum of poly rA.poly rU from which uridine spectrum has been subtract, **1** ; spectra of poly dA.poly dT, **2** and poly(dA-dT).poly(dA-dT), **3** from which thymidine spectrum has been subtract.
Same conditions as in figure 2.

for the three double-stranded polynucleotides and this probably induce about the same effects.

We are now in position to compare the resonance Raman spectra of adenine within the various polynucleotides. First we compare the hyperchromism (I denaturated/I native) of the adenine lines (Table I). In the case of the two polynucleotides of the A-family form (poly rA and poly rA.poly rU), this value is about 1.6 for the 1334 cm^{-1} frequency line and about 1.3 for the lines at 1482 cm^{-1} and 1580 cm^{-1}. These values are significantly different from those obtained with the polynucleotides of the B-family, i.e. poly dA and poly(dA-dT).poly(dA-dT). This suggests that the bases stacking interaction is comparable, on the one hand for poly rA and poly rA.poly rU, on the other hand for poly dA and poly(dA-dT).poly(dA-dT).

In contrast, the hyperchromism ratios measured in the case of poly dA.poly dT are quite different. That of the 1580 cm^{-1} line is the same as that of poly rA and poly rA.poly rU, i.e. in the A-form. For the 1482 cm^{-1} line the ratio value is very close to that of the A-form. For the 1334 cm^{-1} frequency, the value is closer to that of the A-form than that of the B-form. Hence one can consider that the hyperchromism ratio of the adenine lines in poly dA.poly dT

Table II. Relative intensities of the lines located around 1332 cm^{-1} and 1584 cm^{-1}, taking the 1484 cm^{-1} line as reference.

	Poly rA	Poly rA.poly rU	Poly dA	Poly(dA-dT). poly(dA-dT)	Poly dA.poly dT
$\frac{I_{1332}}{I_{1484}}$	1.37	1.22	1.6	1.6	1.35
$\frac{I_{1584}}{I_{1484}}$	0.36	0.34	0.48	0.42	0.34

is comparable to that of the adenine lines in the A-family polynucleotides.

One can give another argument in comparing for each polynucleotide the relative intensities of the lines, taking the 1484 cm^{-1} line as internal reference (Table II).

It appears that these values are similar on the one hand for the polynucleotides of the A-family, i.e. poly rA and poly rA.poly rU, on the other hand for the polynucleotides of the B-family, i.e. poly dA and poly(dA-dT).poly(dA-dT). The values found for the polynucleotides of A and B types are significantly different. An interesting result is obtained when the lines of poly dA.poly dT

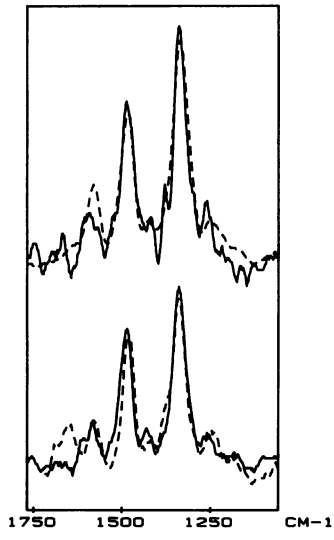

Figure 6 : Compared resonance Raman spectra of :
Upper : poly(dA-dT).poly(dA-dT) minus thymidine (——) and poly dA (-----).
Lower : poly dA.poly dT minus thymidine (——) and poly rA (-----).
Same conditions as in figure 2.

are studied in a same way. The ratios are then similar to those of the A-family polynucleotides, and are different from that of the B-family polynucleotides.

Figure 6 shows the normalized spectra of adenine in poly dA.poly dT and poly rA as well as the normalized spectra of adenine in poly(dA-dT).poly(dA-dT) and poly dA. We observe a good agreement between the spectra of poly dA.poly dT and poly rA, except the small shift of the line at 1484 cm^{-1}. The slight differences observed in the 1600 cm^{-1} region are due to the subtraction of the thymine lines from the spectrum of the copolymer. Excepting this little mismatch we can admit that the adenine spectrum in poly dA.poly dT is similar to the spectrum of poly rA. The same assumptions can be made in comparing the spectrum of poly(dA-dT).poly(dA-dT) to that of poly dA. With the 257 nm excitation wavelength no information leads to a conformation different from that of the classical B-family for both polynucleotides.

CONCLUSION

We studied the spectrum of adenine in two single-stranded polynucleotides, poly rA and poly dA, and in three double-stranded polynucleotides, poly dA.poly dT, poly(dA-dT).poly(dA-dT) and poly rA.poly rU.

In solution, poly rA and poly rA.poly rU are known to adopt a A-family structure. The Raman spectra show comparable line intensities and hypochromisms of adenine within these two polynucleotides.

With the 257 nm excitation wavelength, resonance Raman spectroscopy allows one to characterize the A-type structure of polynucleotides containing adenine and thymine bases (i) by a lower hyperchromism of the lines around 1482 cm^{-1} and 1584 cm^{-1} following the thermal unfolding (Table I), compared to the results obtained for the B-type structure. (ii) by a lower intensity of the line around 1334 cm^{-1} than in the B-form, comparatively to two other lines of adenine (Table II).

When the polynucleotides are in fiber, the stacking of the adenines does not seem different for A- and B-type structures (Fig. 5 in ref. 4). Presently no information exists about the stacking in solution. However the difference of hypochromism of two of the three lines of adenine between A- and B-forms indicates that staking is probably not the same for the the two forms in solution. There is a great variation of the intensity of the line of adenine at 1334 cm^{-1} between A- and B-form. This line was attributed to a C_8-N_9, C_2-N_3, C_8 H vibration[7] could then be sensitive to the presence of the neighbouring sugar.

Arnott et al.[4] were the first to show that the fibrous form of poly dA.po-

ly dT has a heteronomous secondary structure, one chain -probably poly dA- has C_3' endo-puckered furanose rings characteristic of the A-family while the other -probably poly dT- has the C_2' endo-puckered rings of the B-family. Thomas and Peticolas[6] showed that in solution at 5°C or lower one of the strand of poly dA.poly dT has the C_3' endo-furanose ring conformation characteristic of the A-form whereas the other has a classical B-conformation. But they could not determine what chain has a A-type structure.

The results that we presented here indicate that the resonance Raman spectroscopy with a 257 nm excitation wavelength allows us to determine a spectrum characteristic of the A-form of a polynucleotide chain containing adenines. It is this spectrum, except small differences, which is observed for poly rA, poly rA.poly rU and poly dA.poly dT, which proves that it is the adenine strand which adopts a A-conformation in this last case. It confirms the hypothesis of Arnott et al.[4].

Resonance Raman spectroscopy does not allow one to obtain new information about the structure of poly(dA-dT).poly(dA-dT) in solution. Some studies indicated (see above) that the T-A stacking is probably different from A-T stacking. For instance, Thomas and Peticolas[6] showed that, under certain conditions, some C_3' endo-conformation may exist for that polynucleotide. However, the contribution of adenine to the Raman spectrum of poly(dA-dT).poly(dA-dT) is not significantly different from the spectrum of poly dA. So the technique used here does not allow to detect a particular structure in the case of the alternating polymer.

ACKNOWLEDGEMENTS

Béatrice Jollès is a fellow of "Ligue Nationale Française Contre le Cancer".

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