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Evidence for Z-form RNA by vacuum UV circular dichroism

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ABSTRACT

Circular dichroism (CD) spectra in the vacuum UV region for different conformations of poly d(G-C)•poly d(G-C) and poly r(G-C)•poly r(G-C) are very characteristic. The CD of the RNA in the A-form (6 M $NaClO_4$ and 22°C) is very similar to that of the DNA in 80% alcohol where it is believed to be in the A-form. With the exception of the longest wavelength transition, the CD of the RNA in 6 M $NaClO_4$ at 46°C is similar to the CD of the DNA under conditions where it is believed to be in the Z-form (2 M $NaClO_4$). This substantiates that poly r(G-C)•poly r(G-C) assumes a left-handed Z-conformation in 6 M $NaClO_4$ above 35°C. CD spectra for the left-handed Z-forms of both the RNA and DNA are characterized by an intense negative peak at 190-195 nm, a crossover at about 184 nm, and an intense positive peak below 180 nm. The right-handed A- and B-forms of RNA and DNA all have an intense positive peak in their CD spectra near 186 nm. The large difference in CD in the range 185-195 nm for right- and left-handed conformations of nucleic acids can be used to identify the sense of helix winding.

INTRODUCTION

Recently, a number of spectroscopic techniques were used to show that poly r(G-C)•poly r(G-C) can be made to undergo a transition from the A-form to a left-handed Z-form (1). Under conditions of moderate salt and temperature, this double-stranded RNA molecule is in the A-form in solution (2,3). In 6 M $NaClO_4$ and at temperatures above 35°C the polynucleotide experiences a transition to a conformation that has spectroscopic characteristics of the DNA Z-form. The two peaks in the phosphorous NMR spectra show an increase in separation from 0.50 ppm to 1.3 ppm for this transition; a similar large increase occurs for the B- to Z-form transition in DNA (4,5). Measurements of the proton nuclear Overhauser effect show that the guanosine residues changed from the anti to the syn conformation, as expected from X-ray crystallography of the Z-form (6,7). Absorption spectra of the RNA in high salt (6 M $NaClO_4$) and high temperature reveal a shoulder at 290 nm, which is characteristic of the spectrum for Z DNA (8).

The circular dichroism (CD) spectrum measured for the Z-RNA was not so

definitive. This technique is extremely sensitive to secondary structure and the CD of Z-form poly r(G-C)•poly r(G-C) was certainly very different from that measured for the A-form. Indeed, the bands between 200 and 240 nm change from negative for A-RNA to positive for Z-RNA. However, the CD of Z-RNA has a positive band at 280 nm (1) rather than the negative band at 290 nm that is characteristic of Z-DNA (5,9).

Interactions among the neighboring bases in a helical structure are expected to produce CD bands of large intensity. This is seen in the CD of many repeating polynucleotides such as poly rA (10,11) and poly rC (12). The CD bands exhibited by nucleic acids at wavelengths longer than 220 nm are anomalously low in intensity. The reason that the CD bands for natural nucleic acids lack the expected intensity at longer wavelengths is unknown at this time, but one can speculate that the many intense CD bands present because of the large number of transitions cancel to produce the low intensity CD that is measured. Such cancellation would mean that these CD bands would be abnormally sensitive to small changes in conformation and thus would not be a reliable indicator of the basic helical structure.

In contrast, CD bands measured for nucleic acids at wavelengths shorter than 220 nm have the expected high intensity for base-base interactions, and are sensitive to the conformation of the nucleic acid (13). Sprecher *et al.* showed that the short wavelength CD bands found in the vacuum UV can distinguish the A and B forms of DNA. They also showed that the high salt form of DNA (then thought to be the C form) had the same base-base interactions as the B form, implying that these two forms had nearly the same number of base pairs per turn. The low intensity CD bands at longer wavelengths were quite different for these two forms of DNA. Baase and Johnson (14) then used hydrodynamic techniques and covalently-closed, double-stranded DNAs to show that the B-form and "C-form" differed by only 0.22 base pairs per turn. This was strong evidence that DNA was in the B-form in high salt as well as low salt. Subsequently, Zimmerman and Pheiffer (15) used X-ray diffraction of nucleic acid fibers to demonstrate that both the high and low salt forms of DNA were indeed in the B conformation.

Sutherland and Griffin (16) provide another example which shows that vacuum UV CD bands of nucleic acids are a reliable indicator of helical structure while CD bands measured above 220 nm are unreliable. These workers showed that the shorter wavelength CD bands of poly d(I-C)•poly d(I-C) are characteristic of the B form, even though the long wavelength CD is inverted

in sign and thus might be attributed to the Z form. Proton NMR measurements (17) also demonstrated that this polynucleotide was in the B form.

In this paper we report CD spectra measured for poly r(G-C)•poly r(G-C) in the vacuum UV region. These high intensity CD bands are quite similar to those measured for Z form poly d(G-C)•poly d(G-C), confirming that the RNA does indeed assume the Z form in 6 M sodium perchlorate at temperatures above 35°C.

MATERIALS AND METHODS

Samples of poly d(G-C)•poly d(G-C) were purchased from both Sigma and PL Biochemicals, and used without further purification. Samples from the two companies gave identical results and our spectra agreed with those previously published at longer wavelengths (5,9,11). Samples were dissolved in 10 mM sodium phosphate buffer, pH 7, and dialyzed to remove unwanted ions. The salt solutions were obtained either by direct mixing with 4 M sodium perchlorate, 10 mM sodium phosphate buffer, pH 7, or by dialysis against the final solvent composition of 2 M sodium perchlorate, 10 mM sodium phosphate buffer, pH 7. The 80% alcohol solutions were obtained by dialyzing into 3.33 mM sodium phosphate buffer, pH 7, and then adding 1,1,1-trifluoroethanol dropwise. Concentrations were determined in the low salt solvents by absorption spectroscopy using ε(255 nm) of 7,100 (8). With this as a basis, we confirmed that ε(255 nm) for the Z-form is 6,800 (8). Measurements were made in standard cylindrical quartz cells of 2.0 to 0.01 mm pathlength and at concentrations of 0.025 to 2.5 mg ml^{-1}. Conditions were chosen to meet the requirements of the wavelength range studied.

The poly r(G-C)•poly r(G-C) was synthesized in the Berkeley laboratory (12) and shipped to Corvallis in 6 M sodium perchlorate, 10 mM sodium phosphate buffer, pH 7, 0.1 mM EDTA, at a concentration of about 10 mg/ml. Measurements were made on this stock solution in nominal 0.01 mm pathlength cells to 176 nm and in thin cells (where 1 drop of solution is pressed between two clean quartz plates without a spacer) to 168 nm. The solution was also diluted 6 fold with 6 M sodium perchlorate, 10 mM sodium phosphate buffer, 0.1 mM EDTA for measurements to 178 nm in a cell with a nominal 50 μ pathlength. An ε(260 nm) of 6560 was used to determine the concentration of the solutions (2).

Spectra were measured as far as 181 nm on a Jasco J-40. They were repeated and extended to the limits of transparency (total optical density = 1.0) on a vacuum UV CD spectrometer described elsewhere (3). The temperature of the cells was controlled for all measurements.

RESULTS AND DISCUSSION

The CD spectra of poly d(G-C)•poly d(G-C) in A, B and Z conformations is given in Fig. 1. They have been measured in the long wavelength region by a number of workers (see for instance refs. 8, 11, and 14), and the B-form and Z-form spectra have also been extended to 180 nm by Sutherland et al. (9). Our spectra are in good agreement with previous measurements.

Figure 2 gives the CD spectra for poly r(G-C)•poly r(G-C) in the A- and Z-forms. Both spectra are measured in 6 M sodium perchlorate, 10 mM sodium phosphate buffer, pH 7, 0.1 mM EDTA. The RNA is in the A-form at 22°C (3), and its CD is remarkably similar to the DNA in the A-form (see Fig. 1). This comparison shows that 80% alcohol does indeed induce the A-form in DNA's. Both have very intense positive CD intensity at about 186 nm flanked on either side by negative CD bands that are still fairly intense. The longer wavelength bands are slightly different, but these differences are

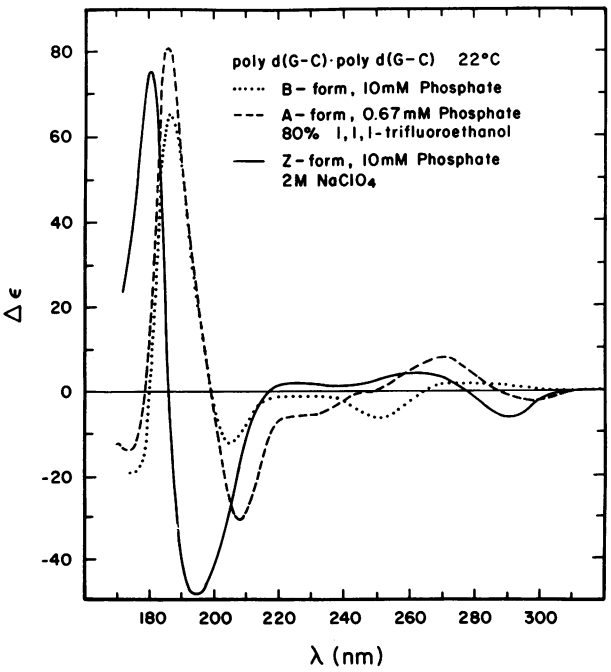

Fig. 1. The circular dichroism of poly d(G-C)•poly d(G-C) at 22°C as the B-form in 10 mM sodium phosphate buffer, pH 7 (•••), as the A-form in 80% 1,1,1-trifluoroethanol, 0.67 mM sodium phosphate buffer, pH 7 (---), and as the Z-form in 2 M sodium perchlorate, 10 mM sodium phosphate buffer, pH 7 (——).

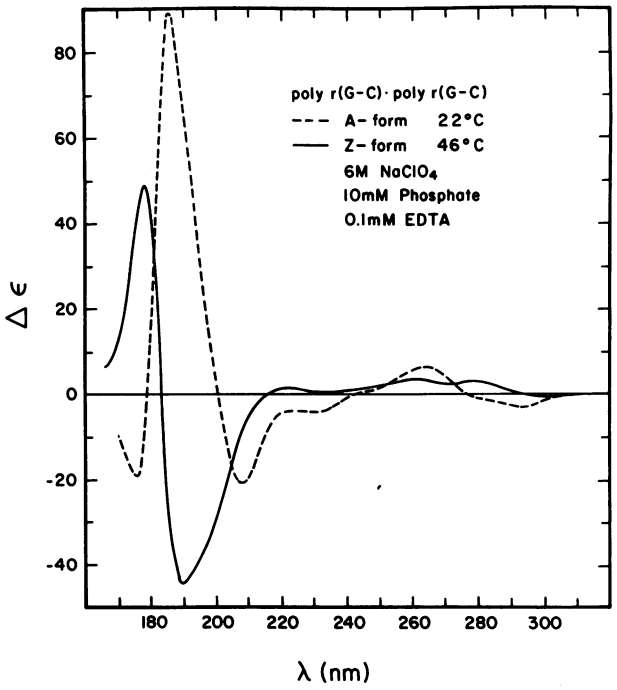

Fig. 2. The CD spectrum of poly r(G-C)•poly r(G-C) in 6 M sodium perchlorate, 10 mM sodium phosphate buffer, pH 7, and 0.1 mM EDTA as the A-form at 22°C (---), and as the Z-form at 46°C (——).

consistent with those seen when comparing native RNA to native DNA in the A-form (see for instance refs. 10, 15-18). Beginning at long wavelength, both this synthetic RNA and native RNA show (1) a more prominent negative CD band, (2) a blue-shifted and less intense positive CD band, and (3) less intense negative bands at about 230 and 208 nm, when compared to the corresponding synthetic and native DNAs in A-form.

With the exception of the longest wavelength CD band, the spectra for the RNA and DNA polynucleotides in the Z-form are quite similar. Both have positive CD bands of low intensity at about 262 and 224 nm followed by an intense negative CD band and an intense positive CD band at shorter wavelengths. These intense bands are blue-shifted about 4 nm for the RNA as compared to the DNA.

The clearest hallmark for the Z-form is the intense pair of CD bands measured at wavelengths shorter than 210 nm. This causes a substantial shift in the crossover from about 200 nm for all of the right-handed confor-

mations to roughly 185 nm for the left-handed conformations. This shift in crossover provides a sensitive means for monitoring the formation of the Z-form that is independent of the concentration of the sample. The similarity in the CD spectra between the deoxy and ribo polymers clearly substantiates that poly r(G-C)•poly r(G-C) is in the Z-form in 6 M sodium perchlorate at temperatures greater than 35°C.

The CD spectra below 220 nm are clearly characteristic of the sense of the polynucleotide helix. All the right-handed forms (A-RNA, A-DNA and B-DNA) have a positive peak near 185 nm and have a positive CD from 280 nm to 200 nm. The two left-handed forms (Z-DNA, Z-RNA) have a negative CD between 185 nm and 200 nm. If this result is found to be independent of sequence, it will be a very useful method to establish the right- or left-handed sense of nucleic acids.

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