Vacuum UV circular dichroism is diagnostic for the left-handed Z form of poly [d(A−C)·d(G−T)] and other polydeoxynucleotides

Jeannine H.Riazance, W.Curtis Johnson, Jr, Lawrence P.McIntosh*[1] and Thomas M.Jovin[1]

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, USA and [1]Max-Planck-Institut für biophysikalische Chemie, Abteilung Molekulare Biologie, Postfach 2841, D-3400 Göttingen, FRG

ABSTRACT

Circular dichroism spectra are extended into the vacuum UV to about 178 nm for four polydeoxynucleotides of various sequences capable of assuming the left-handed Z form. It is found that each of these polymers, including those with brominated bases and those with the four different bases, have a characteristic negative feature at short wavelengths when in the Z form. In contrast, the B form only has a positive band between 180 and 200 nm. Furthermore, a blue shift of the short wavelength crossover is diagnostic of the B- to Z-form transition for all polymers studied so far. These results confirm that poly[d(A-C)·d(G-T)] can assume the Z form in solution at low concentration.

INTRODUCTION

Numerous studies have shown that the DNA molecule can adopt helical configurations in solution different from the classical Watson–Crick model, now called the right-handed B-form helix (1). It is convenient to study the conformations that result from the polymorphism of DNA using synthetic polymers with known base sequences. For example, poly[d(G-C)] adopts the B-form under moderate salt conditions, but in concentrated salt solutions has been shown to convert to the left-handed Z form (2-6).

Since the discovery that poly[d(G-C)] in solution can undergo a salt-dependent transition from a right-handed to a left-handed form (2), which has subsequently been shown to be the Z-form (see, for instance, 5, 6, and references therein), investigators have studied intrinsic (base and backbone modification) and extrinsic (salt, solvent, temperature, etc.) conditions which induce the Z form in poly[d(G-C)] and its derivatives, as well as testing if other polymers with an alternating purine-pyrimidine sequence can undergo a similar transition. Many of these studies have used circular dichroism (CD) spectroscopy, a technique that is extremely sensitive to secondary structure, requires as little as 50 µg of material, and can be applied to polymers in solution. However, most of these CD studies were truncated at 220

nm, and the matching of a CD spectrum covering the 300 to 220 nm region to the helical sense of a polynucleotide can be ambiguous. For example, a negative CD band at about 290 nm does not necessarily signify the Z-form. In contrast, all the CD spectra measured into the vacuum UV region to date, give unambiguous results as to helix sense (3,7,8,9). Indeed, vacuum UV CD of poly[d(I-C)] was used to show that this polymer was right-handed while the CD in the 300 to 220 nm region indicated the possibility of the left-handed Z form (10).

In this work we extend vacuum UV CD studies by reporting spectra for four synthetic DNA's, two in the poly[d(G-C)] sequence family and two in the poly [d(A-C)·d(G-T)] family, all showing spectroscopic characteristics of the Z-form DNA. The spectrum of poly[d(G-br⁵C)] is very similar to poly[d(G-C)], demonstrating that bromination of the bases does not affect the CD, even in the vacuum UV. In the case of poly[d(I-br⁵C)], IR spectra and ^{31}P NMR confirm the existence of the Z conformation in concentrated salt solutions and at room temperature (11). We have extended the spectrum into the vacuum UV to show the characteristic negative band and blue shift of the crossover for the Z form of this polymer.

In the case of the two DNAs composed of all four bases, we confirm that they can assume the Z form by finding characteristic CD spectra in the vacuum UV. This is particularly important for poly[d(A-C)·d(G-T)] where there is evidence for the Z form (12-16) in fibers and films, but where solution studies have been ambiguous (5-8,17-22). This alternating purine-pyrimidine sequence is particularly important since it is widely distributed in eukaryotic genomes and therefore of potential importance in genetic expression via a B-Z equilibrium (14).

MATERIALS AND METHODS

Poly[d(I-br⁵C)] and poly[d(A-C)·d(G-T)] were purchased from Pharmacia and used with no further purification. Our CD spectra in the near UV agreed with previously published work, as described below, testifying to the purity of these commercial polymers. Poly[d(G-br⁵C)] and poly[d(A-br⁵C)·d(G-br⁵U] were synthesized with M. luteus DNA polymerase (P. L. Biochemicals) using poly[d(I-C)] and poly[d(A-C)]·[d(G-T)] as templates, in a manner very similar to the standard procedures of Gill et al. (23). In the reaction with the latter DNA, Tanden, a synthetic quinoxaline, was added to inhibit de novo poly[d(A-T)] synthesis (24). The products were deproteinized with proteinase K and phenol extraction. The products were characterized by nearest neighbor

analysis, and melting point determinations in 1 mM Tris-HCl, 0.1 mM EDTA, pH

7.6 were c. 95°C for poly[d(G-br^{5}C)] and 65°C for poly[d(A-br^{5}C)·d(G-br^{5}U)].

The spectral characteristics and properties of the B to Z transitions for

these polymers are given in McIntosh and Jovin (in preparation).

Samples of poly[d(I-br^{5}C)] were dissolved in glass-distilled H$_2$O, then

dialyzed on a 47-mm-diameter filter (Millipore Type VS, 0.025 μ) against 2.5 M

NaClO$_4$, 10 mM Na$_{3/2}$H$_{3/2}$PO$_4$, 0.1 mM EDTA over ice. The first two dialysis

solutions were at pH 11 to prevent aggregation, the last two were at pH 7.2.

Absorption spectra were recorded from 340 to 180 nm, and the concentration of

each sample was measured by UV absorption on a Cary 15 spectrophotometer using

a molar extinction coefficient ε(246) = 4600 ℓ M^{-1} cm^{-1} (11). Measurements

were made in standard cylindrical quartz cells of 0.005 cm pathlength.

Poly[d(G-br^{5}C)] was lyophilized, re-dissolved in glass-distilled H$_2$O,

then filter dialyzed at room temperature as above against 10 mM Na$_{3/2}$H$_{3/2}$PO$_4$,

0.1 mM EDTA, pH 6.7. The concentration of the sample was measured spectro-

scopically using ε(260) = 6,000 ℓ M^{-1} cm^{-1} (25), and scans were taken in

cells of 1.0 to 0.005 cm pathlength. All measurements were made at room tem-

perature.

Poly[d(A-br^{5}C)·d(G-br^{5}U)] was lyophilized, dissolved in glass-distilled

H$_2$O, then filter dialyzed at room temperature against 0.01 M NaClO$_4$, 10 mM

Na$_{3/2}$H$_{3/2}$PO$_4$, 0.1 mM EDTA for the low-salt form, and against 3.2 M NaClO$_4$, 10

mM Na$_{3/2}$H$_{3/2}$PO$_4$, 0.1 mM EDTA for the high-salt form. The first dialysis solu-

tions were at pH 11 to facilitate solvation, the last at pH 7.3. Concentra-

tions were determined spectroscopically using ε(260) = 5,500 ℓ M^{-1} cm^{-1}

(L. P. McIntosh and T. M. Jovin, unpublished results). Measurements were made

in cells of 1.0 to 0.01 cm pathlength.

Samples for the low-salt form of poly[d(A-C)·d(G-T)] were lyophilized,

re-solvated with glass-distilled H$_2$O, then filter dialyzed as previously

described against 0.01 M NaClO$_4$, 10 mM Na$_{3/2}$H$_{3/2}$PO$_4$, 0.1 mM EDTA, pH 7.2.

Samples run in concentrated salt solutions were purchased from Pharmacia and

used with no further purification. These were solvated with glass-distilled

H$_2$O, filter dialyzed over ice first against 1 M NaClO$_4$, 10 mM Tris-HCl, pH

7.6, then for a longer time against 6 M NaClO$_4$, 10 mM Tris-HCl, pH 7.6. 100%

ethanol was added dropwise to the samples for a mixture of 80% H$_2$O/20% ethanol

(v/v), making the solution 4.8 M in NaClO$_4$. All samples were run at 17°C and

in quartz cells with a pathlength of 0.01 to 0.001 cm. An ε(260) of 7,100 ℓ

M^{-1} cm^{-1} (L. P. McIntosh and T. M. Jovin, unpublished results) was used to

calculate concentration of the solutions.

CD measurements in the range 340-180 nm were recorded on a Jasco J-40 spectrophotometer. The instrument was calibrated with (+)-10-camphorsulfonic acid using $\varepsilon(290.5) = +2.42$. All spectra were recorded at a spectral band-width of 2 nm, sensitivity of 1-2 m°/cm, and 1 to 16 sec time constant. Measurements were repeated and extended to 178 nm using a vacuum ultraviolet CD spectrophotometer (26), calibrated as above. Those spectra were measured with a 10-20 sec time constant and a scan rate of 2-1 nm per min. The spectral slitwidth was a constant 1.6 nm.

Transmission spectra were taken in the same vacuum UV instrument following the CD scan. CD spectra were terminated at the point that the signal-to-noise ratio was less than 10, usually 178 nm. At no point in the CD spectrum did the total optical density of the sample and cell exceed 1.0.

The pathlength of all sample cells used in both instruments was measured precisely using an infrared or visible spectrophotometer (27). The temperature of the cells was controlled for all measurements.
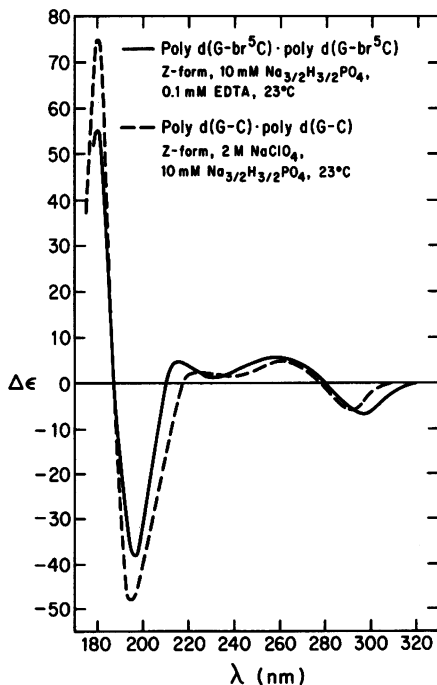

Fig. 1. The CD spectrum of poly[d(G-br^{5}C)] at 23°C as the Z form in 10 mM Na$_{3/2}$H$_{3/2}$PO$_4$, 0.1 mM EDTA, pH 7.2 (——), and poly[d(G-C)] as the Z form at 23°C in 2 M NaClO$_4$, 10 mM Na$_{3/2}$H$_{3/2}$PO$_4$, pH 7 (---), redrawn from reference (9).

RESULTS AND DISCUSSION

The CD spectrum of poly[d(G-br^{5}C)] is shown in Fig. 1. The longer wavelength spectrum agrees well with previous measurements (25,28). In contrast with poly[d(G-C)], this brominated polymer exists in the Z form under dilute salt conditions (10 mM Na$_{3/2}$H$_{3/2}$PO$_4$) at room temperature (23°C). Comparing its CD with the Z form of poly[d(G-C)] (Fig. 1), the shape is quite similar, with a small negative peak around 295 nm, two positive ones at 260 and 215 nm, followed by an intensive negative peak at 197 nm and another intense positive one at 180 nm. We conclude that bromination of the cytosine does not significantly alter the CD for the Z form, although the intensity of the two short wavelength bands is decreased.

As in the case of Z-form poly[d(G-C)] (3), the brominated Z form exhibits a substantial blue shift in the short wavelength crossover, from about 200 nm for right-handed conformations to roughly 187 nm for the present left-handed sample. This shift is characteristic of the Z form.

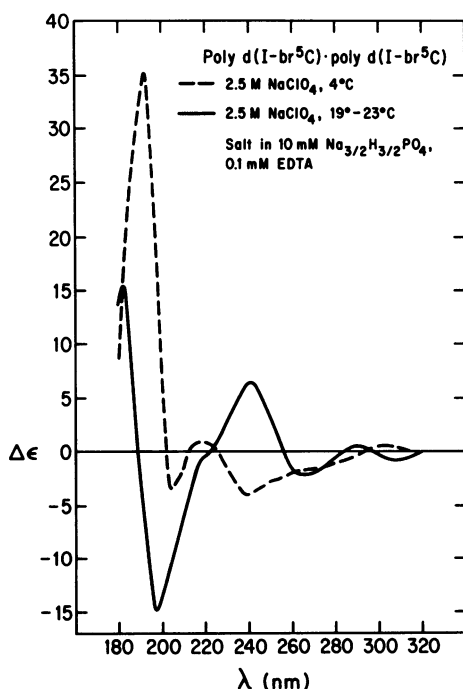Fig. 2 gives the CD spectra of poly[d(I-br^{5}C)] in the B and Z forms.

Fig. 2. The CD spectrum of poly[d(I-br^{5}C)] in 2.5 M NaClO$_4$, 10 mM Na$_{3/2}$H$_{3/2}$PO$_4$, 0.1 mM EDTA, pH 7.2, as the B form at 4°C (---), and the Z form at 19-23°C (——).
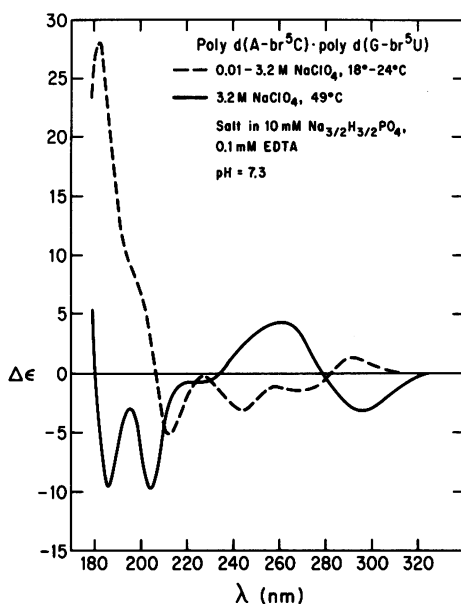
Fig. 3. The CD spectrum of poly[d(A-br^{5}C)·d(G-br^{5}U)] in 0.01 M or 3.2 M
NaClO$_4$, 10 mM Na$_{3/2}$H$_{3/2}$PO$_4$, 0.1 mM EDTA, pH 7.3, as the B form at
18-24°C (---) and the Z form at 49°C (——).

Both spectra are measured in 2.5 M NaClO$_4$, 10 mM Na$_{3/2}$H$_{3/2}$PO$_4$ buffer, pH 7.2,
0.1 mM EDTA. The DNA is in the B form at 4°C, and in the Z form when heated
to room temperature (19-23°C) (11). The concentration of Na$^+$ necessary to
effect the B to Z transition is less when using NaClO$_4$ than in previous work
using NaCl (11,29).

The B form of poly[d(I-br^{5}C)] has a very small positive peak at 302 nm,
followed by a larger negative peak at 240 nm, a positive one at 220, a similar
negative peak at 205, then an intensive positive peak near 192 nm. It bears
only slight similarity to poly[d(G-C)] in the B form (3), the negative peak at
205 and crossover around 200 nm being common points. For the Z form, the
spectrum begins with small negative and positive peaks at 306 and 290 nm,
respectively, then shows a negative peak at 265 nm, followed by a fairly
intense one at 240 nm. These features are not found in either poly[d(G-C)] or
in the brominated derivative. As expected, the intense negative peak at 197
nm and the intense positive peak at 180 nm, which are characteristic of Z form
poly[d(G-C)], are found for Z-form poly[d(I-br^{5}C)] at 197 and 182 nm.
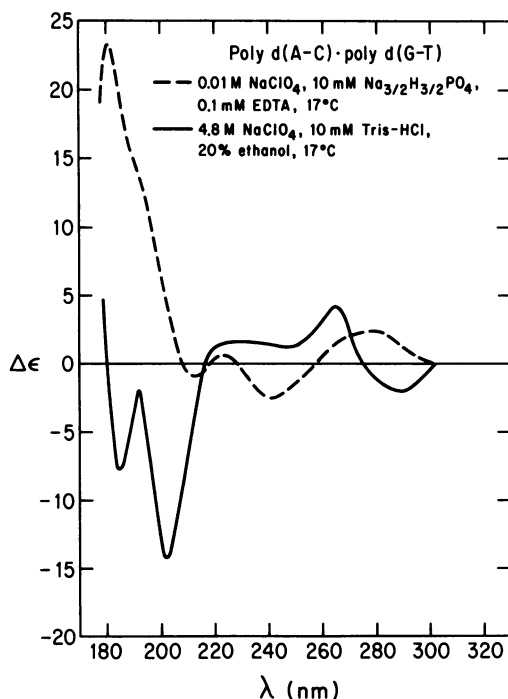However, the substitution of inosine for guanine substantially reduces the

Poly d(A-C)·poly d(G-T)

- - - 0.01 M NaClO$_4$, 10 mM Na$_{3/2}$H$_{3/2}$PO$_4$,
0.1 mM EDTA, 17°C

—— 4.8 M NaClO$_4$, 10 mM Tris-HCl,
20% ethanol, 17°C

Fig. 4. The CD spectrum of poly[d(A-C)·d(G-T)] in 0.01 M NaClO$_4$, 10 mM
Na$_{3/2}$H$_{3/2}$PO$_4$, 0.1 mM EDTA, pH 7.3 at 17°C as the B form (---), and in
4.8 M NaClO$_4$, 10 mM Tris-HCl, 20% ethanol, pH 7.6 as the Z form
(——).

intensity of these short wavelength bands. Thus, although the CD above 220 nm
contains no features diagnostic of the B- to Z-form transition, the blue
shift of the short wavelength crossover, indicative of the Z form, is seen to
change from 202 to 189 nm for poly[d(I-br^{5}C)].

Turning to the poly[d(A-C)·d(G-T)] sequence family, the spectra of B-
and Z-form poly[d(A-br^{5}C)·d(G-br^{5}U)] are presented in Fig. 3. Data on this
particular compound have not been previously published, but our results show
fairly good agreement in the long wavelength region with a similar poly-
nucleotide, poly[d(A-br^{5}C)·d(G-T)] (30). The B form is found in the range
0.01 to 3.2 M NaClO$_4$ at room temperature (18-24°C), while the Z form appears
at higher temperatures (~49°C). The B form shows a small positive CD region
until 282 nm, followed by an extensive region of negative CD until the cross-
over at 206, with small peaks around 270, 245, and 212 nm. A shoulder appears
around 200, then a positive peak at 182 nm. By comparison with B-form

poly[d(A-C)•d(G-T)] (Fig. 4), we see that the spectra are similar, with the difference that the first CD band stays positive longer (until the cross-over at 257 nm).

The Z form of poly[d(A-br^{5}C)•d(G-br^{5}U)] starts out with a broad negative band centered around 295 nm, followed by a broad positive band around 260. At short wavelength, there is a very interesting pair of negative peaks near 204 and 186 nm, with the short wavelength cross-over at 180 nm. This spectrum is very similar to the Z form of unbrominated poly[d(A-C)•d(G-T)] discussed below, again showing little effect of bromine on the CD. Thus, these polymers with a full complement of bases exhibit the same blue shift in cross-over on transition from the B form (206 nm) to the Z form (180 nm), as was seen previously in the poly[d(G-C)] sequence family.

Our B-form spectrum (Fig. 4) of poly[d(A-C)•d(G-T)] at the longer wavelengths agrees well with previous measurements (17-19), and also with work extended into the vacuum UV (8). There is a broad positive peak around 280 nm, a negative one centered at 240 nm, a small positive and negative couplet near 220 and 213 nm, respectively, and an intense positive band near 180 nm. The Z form shows a negative peak at 290 nm, cross-over at 275 nm to a positive peak around 264 nm, then a positive region until the cross-over at 216 nm, followed by a pair of negative bands at 202 and 185 nm similar to those seen in poly[d(A-br^{5}C)•d(G-br^{5}U)], and a cross-over at 180 nm. This spectrum is quite similar at longer wavelengths to that reported (31) for poly[d(A-m^{5}C)• d(G-T)] at 68°C, which differs only in having a methyl substitution on the cytosine.

In our work poly[d(A-C)•d(G-T)] was induced to change to the Z form under previously reported (5) conditions of concentrated salt (4.8 M NaClO$_4$), lowered water activity (20% ethanol), and at room temperature, but only in the absence of EDTA. This suggests that small concentrations of multivalent metal ions as impurities facilitate the transition. A fairly similar CD spectrum in the long wavelength region was obtained by Taboury and Taillandier (19) with 4 M NaCl and 40 mM NiCl$_2$, prompting their statement that Ni^{++} stabilizes the Z form under concentrated salt conditions. Another similar spectrum was produced in dilute salt solutions using just a zinc complex (22).

In previous work, we (9) and others (3,8) have noted that all the right-handed forms of DNA and RNA studied have a positive peak near 185 nm, while left-handed deoxy and ribo poly[(G-C)] have negative CD between 185 nm and 200 nm. If this result is independent of sequence, it would be a useful method to establish the handedness of nucleic acids. In the present work we see that

the intense positive CD band found for B-form DNA has its maximum over the range 192 to 180 nm. Some Z-form polymers also have a positive band at 180 nm. However, all polynucleotides studied so far show a negative CD in the region 185 nm to 200 nm for the Z form, regardless of sequence; therefore, that range seems to be truly diagnostic of a left-handed Z form.

*Present address: Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, OR 97403, USA

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