Vacuum UV CD spectra of homopolymer duplexes and triplexes containing A·T or A·U base pairs

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Received February 26, 1991; Revised and Accepted March 29, 1991

ABSTRACT

Vacuum UV circular dichroism (CD) spectra were measured down to 174 nm for five homopolymers, five duplexes, and four triplexes containing adenine, uracil, and thymine. Near 190 nm, the CD bands of poly[d(A)] and poly[r(A)] were larger than the CD bands of the polypyrimidines, poly[d(T)], poly[d(U)], and poly[r(U)]. Little change was observed in the 190 nm region upon formation of the duplexes (poly[d(A)·d(T)], poly[d(A)·d(U)], poly[r(A)·d(T)], poly[r(A)·d(U)], and poly[r(A)·r(U)]) or upon formation of two of the triplexes (poly[d(T)·d(A)·d(T)] and poly[d(U)·d(A)·d(U)]). This showed that the purine strand had the same or a similar structure in these duplexes and triplexes as when free in solution. Both A·U and A·T base pairing induced positive bands at 177 and 202 nm. For three triplexes containing poly[d(A)], the formation of a triplex from a duplex and a free pyrimidine strand induced a negative band centered between 210 and 215 nm. The induction of a band between 210 and 215 nm indicated that these triplexes had aspects of the A conformation.

INTRODUCTION

The binding of a polypyrimidine strand into the major groove of a double-stranded polypurine·polypyrimidine DNA sequence has been used to locate and cleave polypurine·polypyrimidine sequences.¹⁻⁴ Attempts are being made to use the specificity of third-strand binding to alter gene activity.⁵ Improving binding specificity and expanding the range of target sequences will require knowing the structures of polypurine·polypyrimidine sequences and triplexes. The structures of DNA·RNA hybrids are also important since they are integral intermediates formed during transcription.

X-ray crystallographic structures of two DNAs containing oligoadenine·oligothymine tracts have been solved.^{6,7} The (A·T)-tracts are in an unusual conformation that has been designated B_p.⁸ The B_p conformation differs from the canonical B conformation in that the bases are propeller twisted to be

stacked optimally. This arrangement allows the adenine N6 to form bifurcated hydrogen bonds with the O4 atoms of the adjacent thymines.

The vacuum UV CD spectrum of poly[d(A)·d(T)] is unusual in that a large positive band occurs at 190 nm. This band is not represented in the spectra of other DNAs with only A·T base pairs, but without long (A·T)-tracts. Gray *et al.*⁹ conclude that this band may be diagnostic for the conformation expressed by poly[d(A)·d(T)]. The 190 nm band could be related to the B_p conformation.

X-ray fiber diffraction analysis indicates that poly[r(A)·r(U)] exists in the A conformation.¹⁰ The CD spectrum of poly[r(A)·r(U)] is quite different from that of poly[d(A)·d(T)] in both the near UV region and in the vacuum UV region.^{9,11,12} Steely *et al.*¹² exploited differences in the near UV CD spectra to characterize the structures of hybrids and triplexes made of homopolymers containing adenine, uracil and thymine. In this work, we used vacuum UV CD spectra to further explore the structures of most of these same hybrids and triplexes. The spectrum of poly[r(A)·r(U)] was used as a reference A conformation spectrum, and the spectrum of poly[d(A)·d(T)] was used as a reference B_p conformation spectrum.

MATERIALS AND METHODS

Poly[d(U)] (S_{20,w} = 3.64) was purchased from Pharmacia. Poly[r(A)], poly[r(U)], poly[d(A)], and poly[d(T)] (M_r > 100,000) were purchased from Sigma. All five single-stranded homopolymers were exhaustively dialyzed against 10 mM Na⁺ (phosphate), 1 mM EDTA, pH 7.0, against 100 mM Na⁺ (phosphate), pH 7.0, and against 100 mM NH₄HCO₃ (a volatile salt), pH 7.8. Using the extinction coefficients listed by Steely *et al.*¹², we mixed the single-stranded homopolymers in the appropriate ratios to form double-stranded and triple-stranded complexes. Before lyophilization, NaHCO₃ was added to give 80% of the molar concentration of the bases. The NaHCO₃ was added to help with the resolubilization of the polymers.¹¹ The lyophilized samples were resolubilized by adding D₂O

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containing the KF salt concentrations listed in Table I. Concentrations of the polymers were about 5 mM in nucleic acid phosphates. Resolubilized samples containing mixtures of the homopolymers were allowed to stand for several days at 4°C to give sufficient time for complex formation. The samples were loaded into short path-length cells with 0.12-mm spacers.¹³ We confirmed that the expected complexes had formed by comparing the near UV CD spectra to published near UV CD spectra.¹²

Vacuum UV CD measurements were made at room temperature ($25 \pm 1^\circ\text{C}$) as described previously.¹¹ Digitized CD data at 1-nm intervals were smoothed using a sliding 7-point quadratic-cubic function.¹⁴ Spectra were scaled to the magnitudes of published spectra¹² using the measured magnitudes of prominent near UV CD bands. The absolute magnitudes of CD spectra shown in this paper should therefore be considered as approximate.

The random error for the CD data was estimated from the error in repeated baselines to be $\pm 1.9 \times 10^{-5}$ ($A_L - A_R$), which was about the same as in previous work.⁹ The error in the baselines showed no wavelength dependence. The random error for a molar CD spectrum would thus be $\pm 0.3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ ($\epsilon_L - \epsilon_R$) for samples of 5 mM concentration in 0.12 mm path-length cells. This error was negligible on the molar CD scales used to display spectra in this work. A more serious problem was sample spectra reproducibility. The magnitudes of vacuum UV CD bands in repeat spectra of the same sample varied by up to $\pm 10\%$. This was about the same reproducibility as in our previous work.⁹

RESULTS AND DISCUSSION

Single-stranded polymers

The vacuum UV CD spectrum of poly[r(A)] in 0.1 M KF, pD 7.4 (Figure 1), resembled previous vacuum UV CD spectra of poly[r(A)] at lower salt concentrations.^{11,15} The primary features of the vacuum UV CD spectrum of poly[r(A)] were a large negative band at 205 nm, a positive band at 189 nm, and a shoulder at about 180 nm. The shape of the poly[d(A)] spectrum was similar to that of the poly[r(A)] spectrum from 250 nm to 190 nm. However, the spectrum of poly[d(A)] had more positive CD values relative to the poly[r(A)] spectrum, and the positive band at 191 nm in the poly[d(A)] spectrum was much larger than the 189 nm band in the poly[r(A)] spectrum. The large positive band at 191 nm in the poly[d(A)] CD spectrum indicated: (1) that the 190 nm band previously described as unique to the spectrum of poly[d(A)·d(T)]⁹ was due to the poly[d(A)] strand and (2) that the aspects of the B_p conformation that cause this band in poly[d(A)·d(T)] were embodied by free poly[d(A)]. The spectrum of poly[d(A)] also had a large negative band at 177 nm that was not in the spectrum of poly[r(A)].

The vacuum UV CD spectra of poly[r(U)], poly[d(U)], and poly[d(T)] (Figure 2) had similar shapes. These spectra had much smaller magnitudes than those of poly[r(A)] and poly[d(A)]. The smaller magnitudes reflected the smaller degree of stacking of the polypyrimidines.¹² In the region between 240 and 300 nm, the spectrum of poly[d(T)] had bands shifted to longer wavelengths relative to the bands in the spectra of poly[r(U)] and poly[d(U)]. Thus, the spectral region between 240 and 300 nm reflected more the identity of the pyrimidine base than whether the polymer was a DNA or an RNA. This contrasted with the region between 174 and 200 nm, where the DNA spectra were very similar and the spectrum of the RNA was somewhat different. In this region, all three spectra had a positive band at

Figure 1. Vacuum UV CD spectra of poly[d(A)] (---) and poly[r(A)] (—).

Figure 2. Vacuum UV CD spectra of poly[d(T)] (---), poly[d(U)] (▲▲), and poly[r(U)] (—).

about 181 nm and a shoulder or band at about 194 nm; however, the bands were smaller for poly[r(U)] than for the DNAs. The mononucleotides dUMP, dTMP, and UMP have spectra with a positive band near 194 nm,¹⁶ but these spectra lack the band at 181 nm found in the spectra of poly[d(U)], poly[d(T)], and poly[r(U)].

Double-stranded polymers

The spectra of two DNA duplexes, poly[d(A)·d(T)] and poly[d(A)·d(U)], were very similar between 240 nm and 185 nm (Figure 3). The spectra of both duplexes contained large positive bands at about 216 nm and 189 nm, with a negative band between them at 205 nm. The bands in the spectrum of poly[d(A)·d(U)] were shifted to shorter wavelengths by about 1 nm relative to the spectrum of poly[d(A)·d(T)]. The high degree of similarity in their spectra between 240 and 185 nm agreed with X-ray diffraction studies that poly[d(A)·d(T)] and poly[d(A)·d(U)] have very similar structures.¹⁷ The large bands at about 190 nm in both spectra suggested that poly[d(A)·d(U)] was in the B_p conformation as was poly[d(A)·d(T)]. At wavelengths longer than 240 nm, the spectra were quite different, reflecting the difference between the spectra of poly[d(U)] and poly[d(T)] at these wavelengths. Between 185 nm and 174 nm,

Figure 3. Vacuum UV CD spectra of the duplexes poly[d(A)·d(T)] (+++++) and poly[d(A)·d(U)] (▲▲)

Figure 4. Vacuum UV CD spectra of the duplexes poly[r(A)·r(U)] (—), poly[r(A)·d(T)] (×××), poly[r(A)·d(U)] (---), and a calculated spectrum for poly[r(A)·d(U)] (●●). The spectrum calculated for poly[r(A)·d(U)] was calculated as: $CD_{\text{calculated}}(\text{poly}[\text{r(A)} \cdot \text{d(U)}]) = CD(\text{poly}[\text{r(A)} \cdot \text{d(T)}]) + CD(\text{poly}[\text{d(A)} \cdot \text{d(U)}]) - CD(\text{poly}[\text{d(A)} \cdot \text{d(T)}])$.

the spectrum of poly[d(A)·d(T)] was smaller than the spectrum of poly[d(A)·d(U)]. This difference may be ascribed to the different spectral properties of uracil and thymine in a duplex (see below).

The spectrum of poly[r(A)·r(U)] (Figure 4) was quite different from the spectra of the DNA duplexes. At short wavelengths, it had a small positive band at 222 nm, a large negative band at about 208 nm, and a very large positive band at 177 nm with a shoulder at about 190 nm. The spectra of the hybrid duplexes poly[r(A)·d(U)] and poly[r(A)·d(T)] also had bands or shoulders at 222, 208, 190, and 177 nm (Figure 4). Spectra of the hybrids were thus more similar to the spectrum of poly[r(A)·r(U)] than to the spectra of the DNAs, indicating that their structures were also more similar to that of poly[r(A)·r(U)]. The 177 nm band in the spectrum of poly[r(A)·d(T)] was substantially smaller than the 177 nm bands for poly[r(A)·r(U)] or poly[r(A)·d(U)]. As in the case of the DNA duplexes (above), this difference was likely due to differences between the thymine and uracil chromophores. If so, the difference between the spectra of poly[d(A)·d(T)] and poly[d(A)·d(U)] should have been similar

Figure 5. CD difference spectra for duplexes calculated by subtracting the average of the spectra of the constituent single strands from the spectrum of the duplex, poly[d(A)·d(T)] (+++++), poly[d(A)·d(U)] (▲▲), poly[r(A)·r(U)] (—), poly[r(A)·d(T)] (×××), and poly[r(A)·d(U)] (---).

Figure 6. Vacuum UV CD spectra of the triplexes poly[d(T)·d(A)·d(T)] (++++), poly[d(U)·d(A)·d(U)] (▲▲), poly[r(U)·d(A)·r(U)] (---), and poly[r(U)·r(A)·r(U)] (—).

to the difference between the spectra of poly[r(A)·d(T)] and poly[r(A)·d(U)]. To test this, we subtracted the spectrum of poly[d(A)·d(T)] from that of poly[d(A)·d(U)]. We added the resulting difference to the spectrum of poly[r(A)·d(T)] (the calculation is shown in the legend for Figure 4). The new spectrum (Figure 4, circles) was close to the spectrum of poly[r(A)·d(U)] (Figure 4, dashed curve) in the regions near 177 nm, 197 nm, 250 nm, and 280 nm. This suggested that poly[r(A)·d(T)] and poly[r(A)·d(U)] indeed had similar structures, with the minor differences in their spectra being largely due to the differences between thymine and uracil.

Difference spectra of duplexes

To study the CD induced by base pairing in the duplexes, we calculated difference spectra by subtracting the average of the CD of the single-strand constituents from the CD of the double strands. Each of the difference spectra (Figure 5) had a positive band near 202 nm and a large positive band at 175–178 nm. The 202 nm bands were almost completely obscured by the large bands due to the purine strands in the CD spectra of the duplexes.

Figure 7. CD difference spectra for triplexes calculated by subtracting two thirds of the spectrum of the constituent duplex plus one third of the spectrum of the constituent single strand from the spectrum of the triplex, poly[d(T)·d(A)·d(T)] (++++), poly[d(U)·d(A)·d(U)] (▲▲), poly[r(U)·d(A)·r(U)] (- -), and poly[r(U)·r(A)·r(U)] (—). To calculate the difference spectrum for poly[r(U)·d(A)·r(U)], we subtracted two thirds of the spectrum of poly[d(A)·d(U)] plus one third of the spectrum of poly[r(U)] from the spectrum of poly[r(U)·d(A)·r(U)].

Gray *et al.*⁹ found positive bands at about 178 nm and 202 nm in the CD spectra of poly[d(A-T)·d(A-T)], poly[d(A-A-T)·d(A-T-T)], and poly[d(A-A-T-T)·d(A-A-T-T)]. Johnson *et al.*¹¹ found that A·U base pairing in poly[r(A)·r(U)] and poly[r(A-U)·r(A-U)] induces bands at these wavelengths. Thus, these bands occurred in polymers with A, B, or B_p conformations, with A·T or A·U base pairs, and with a range of sequences. The spectra of poly[d(T)], poly[d(U)], and poly[r(U)] each had a positive band at about 181 nm. The interactions that produced the 181 nm bands in the spectra of the single-stranded polypyrimidines could have been enhanced by duplex formation, giving rise to the bands seen in the difference spectra at 175–178 nm. This argument was supported by the enhancement of the 175–178 nm band upon binding of a second polypyrimidine strand to form a triplex (see below). The lack of a strong sequence dependence for the 175–178 nm bands showed that they were not due to pyrimidine-pyrimidine base stacking.

Above we showed that the 175–178 nm bands in spectra of the hybrids were similar to this band in the spectrum of poly[r(A)·r(U)] (Figure 4). Since these bands appeared to be due to the polypyrimidine strands, we concluded that the polypyrimidine strands of the hybrids had features of the A conformation.

The difference spectra for the DNAs, poly[r(A)·r(U)], and the hybrid duplexes were very small at 190 nm and 220 nm. Poly[r(A)] and poly[d(A)] had large bands at 190 and 220 nm. Therefore, the conformation of the purine strand in these duplexes appeared to be much the same as for the free purine strands. That is, single-stranded poly[d(A)] and the purine strands of the poly[d(A)]-containing duplexes were in the B_p conformation, as described above. Likewise, single-stranded poly[r(A)] and the purine strands of the poly[r(A)]-containing duplexes may all have been in the same conformation (the A conformation). By Raman spectroscopy, O'Connor and Bina²⁰ also found free poly[r(A)] to be in the A conformation.

The difference spectra for poly[r(A)·r(U)], poly[r(A)·d(T)], and poly[r(A)·d(U)] each had a negative band at 215 nm that

was absent from the difference spectra for the DNAs. A negative band near 210 nm is common among double-stranded RNAs.¹⁸ This band is induced in the spectrum of poly[d(A-C)·d(G-T)] concomitant with a shift to the A conformation (by the addition of ethanol).¹⁹ The appearance of this band was an indication that poly[r(A)·d(U)] and poly[r(A)·d(T)] shifted to the A conformation upon duplex formation. Since single-stranded poly[r(A)] was already in the A conformation, the adoption of features of the A conformation by the polypyrimidine strands of poly[r(A)·d(U)] and poly[r(A)·d(T)] could have accounted for the appearance of the 215 nm band.

Our results from the vacuum UV CD spectra for the hybrids poly[r(A)·d(T)] and poly[r(A)·d(U)] showed three things: (1) the hybrids had very similar structures, (2) the polypurine strands were in the A conformation, and (3) the polypyrimidine strands were at least partially in the A conformation. These results can be compared with the results of Krueger *et al.*,²¹ Benevides and Thomas,²² and Steely *et al.*¹² Krueger *et al.*²¹ found that CC-1065 binds to both poly[r(A)·d(T)] and poly[r(A)·d(U)], indicating that these duplexes are in the B conformation. However, Benevides and Thomas²² used Raman spectroscopy to show that the purine strand of poly[r(A)·d(T)] is in the A conformation while the pyrimidine strand has a B-like phosphodiester backbone and a C2'-endo pucker. From an analysis of the near UV CD spectra, Steely *et al.*¹² found the rA strands of poly[r(A)·d(T)] and poly[r(A)·d(U)] to have the A conformation, while the pyrimidine strands had aspects of the B conformation. Our results substantiate those of Benevides and Thomas²² and Steely *et al.*¹² in providing evidence for an A conformation of the polypurine strands.

Triple-stranded polymers

The vacuum UV CD spectra of the three-stranded complexes had major similarities and major differences (Figure 6). All of the spectra were similar in having a negative band at about 208 nm and a large positive band at about 177 nm. The 177 nm bands for the triplexes containing uracil, poly[r(U)·r(A)·r(U)], poly[r(U)·d(A)·r(U)], and poly[d(U)·d(A)·d(U)], all had a magnitude of 24–28 L·mol⁻¹·cm⁻¹. The smaller magnitude for poly[d(T)·d(A)·d(T)] could be accounted for by differences between the thymine and uracil chromophores (not shown), as was the case for the duplexes. The magnitude of the 177 nm band for the triplexes did not depend greatly on whether the attendant purine strand was poly[d(A)] or poly[r(A)]. In this regard, the polypyrimidine strands of these triplexes were all similar.

The spectra of the triplexes containing poly[d(A)] (poly[d(T)·d(A)·d(T)], poly[d(U)·d(A)·d(U)], and poly[r(U)·d(A)·r(U)]) all had a positive band or shoulder near 190 nm. The presence of this band indicated that the purine strands of these triplexes, like those of the duplexes, had aspects of the B_p conformation. However, the 190 nm band was smaller for poly[r(U)·d(A)·r(U)] than for poly[d(T)·d(A)·d(T)] and poly[d(U)·d(A)·d(U)]. Thus, the poly[d(A)] strand of poly[r(U)·d(A)·r(U)] may have been only partially in the B_p conformation. The other triplex containing poly[r(U)], poly[r(U)·r(A)·r(U)], had a negative band at 189 nm. The spectra of both poly[r(U)·d(A)·r(U)] and poly[r(U)·r(A)·r(U)] were decreased relative to the spectra of their constituents near 190 nm, as shown by the difference spectra in Figure 7. Above 200 nm (Figure 6), the spectra of poly[r(U)·d(A)·r(U)] and poly[r(U)·r(A)·r(U)] were quite similar. Thus, these two triplexes were probably similar in structure, at least with regard

Table I. Polymer conformations and solution conditions

Polymer	Conformation		Buffer ^a
	Purine Strand	Pyrimidine Strand(s)	
Single Strands			
Poly[d(A)]	B _p		0.1 M KF, pD 7.4
Poly[r(A)]	A		"
Poly[d(T)]		Relatively Unstacked	"
Poly[d(U)]		"	"
Poly[r(U)]		"	"
Duplexes			
Poly[d(A) · d(T)]	B _p	B _p	0.1 M KF, pD 7.4
Poly[d(A) · d(U)]	B _p	B _p	1 M KF, pD 8.0
Poly[r(A) · d(T)]	A	A and B	0.1 M KF, pD 7.4
Poly[r(A) · d(U)]	A	A and B	"
Poly[r(A) · r(U)]	A	A	"
Triplexes			
Poly[d(T) · d(A) · d(T)]	B _p	A	1 M KF, pD 8.0
Poly[d(U) · d(A) · d(U)]	B _p	A	"
Poly[r(U) · d(A) · r(U)]	B _p to A (?)	A	0.1 M KF, pD 7.4
Poly[r(U) · r(A) · r(U)]	A (?)	A (?)	1 M KF, pD 8.0

^a The buffers were brought to the listed pD using H₃PO₄.

to the poly[r(U)] strands. This is supported by a lack of binding of CC-1065 to both poly[r(U)·d(A)·r(U)] and poly[r(U)·r(A)·r(U)].²¹ In fibers, both triplexes are in the A conformation.²³

Difference spectra of triplexes

To better understand the changes occurring upon binding of the third strand, we calculated difference spectra for each of the triplexes. The difference spectra were calculated by subtracting 2/3 of the CD of the constituent duplex plus 1/3 of the CD of the constituent pyrimidine strand from the CD of the triplex. In the case of poly[r(U)·d(A)·r(U)], the spectrum of poly[d(A)·r(U)] was not available for the calculation. Instead, we used the spectrum of poly[d(A)·d(U)]. This seemed to be a reasonable substitution since vacuum UV CD spectra of other duplexes depended primarily on the purine strand and on whether the pyrimidine strand contained uracil or thymine. The difference spectra are shown in Figure 7.

The difference spectra for poly[d(T)·d(A)·d(T)], poly[d(U)·d(A)·d(U)], and poly[r(U)·d(A)·r(U)] had a large positive band at or below 175 nm, a small negative band at about 190 nm, a small positive band (or negative trough) close to 200 nm, and a negative band centered between 210 and 215 nm. A negative band at about 210 nm is induced by the formation of poly[d(C⁺-T)·d(A-G)·d(C-T)] from poly[d(A-G)·d(C-T)] and poly[d(C⁺-T)]²⁴, and also by the formation of a triplex from two oligonucleotides.²⁵ The negative band between 210 and 215 nm indicated that the triplexes, poly[d(T)·d(A)·d(T)], poly[d(U)·d(A)·d(U)], poly[r(U)·d(A)·r(U)], poly[d(C⁺-T)·d(A-G)·d(C-T)], and the oligonucleotide triplex were at least partially in the A conformation.

In the previous section, we indicated that the purine strands of poly[d(T)·d(A)·d(T)], poly[d(U)·d(A)·d(U)], and poly[r(U)·d(A)·r(U)] seemed to be wholly or partially in the B_p conformation. If the purine strands of poly[d(T)·d(A)·d(T)], poly[d(U)·d(A)·d(U)], and poly[r(U)·d(A)·r(U)] were in the B_p conformation, it is possible that the negative band at 210 nm arose from one or both of the pyrimidine strands being in the A conformation. Therefore, these triplexes may have been

heteronomous. A heteronomous structure for poly[d(T)·d(A)·d(T)] and poly[d(U)·d(A)·d(U)] would be in agreement with drug-binding data that indicate that these two triplexes have some aspects of the B conformation.²¹

The CD spectrum of the poly[r(U)·r(A)·r(U)] triplex showed that its conformation in solution was not like that of the DNA triplexes, with poly[r(U)·d(A)·r(U)] being intermediate (Figure 6). The difference spectrum for poly[r(U)·r(A)·r(U)] was small at 210 nm but had a broad negative band at 180–200 nm (Figure 7). This latter CD change on triplex formation was probably not restricted to changes in the CD of the polypurine strand. Results from Raman spectroscopy indicate that the Watson–Crick base paired strands of poly[r(U)·r(A)·r(U)] are in the A conformation, while the second poly[r(U)] strand has a C2'-endo ribose pucker.²⁰

X-ray diffraction studies show that poly[d(T)·d(A)·d(T)], poly[r(U)·r(A)·r(U)], and poly[r(U)·d(A)·r(U)] can all be in the A conformation in fibers.^{23,26,27} Our data show that the solution structures of these triplexes are not identical.

CONCLUSIONS

Table I gives a summary of our interpretation of the strand conformations in the free single strands, duplexes, and triplexes that we have studied.

1. A·T and A·U base pairing induced a large band at 177 nm and a smaller band at 202 nm. The 177 nm band was probably due to enhancements of bands in the spectra of the pyrimidine strands.

2. The 177 nm band for the poly[r(U)·r(A)·r(U)] triplex was similar to the 177 nm bands for the poly[d(A)]-containing triplexes, suggesting that the pyrimidine strands had similar conformations.

3. The CD spectra of the duplexes and two of the triplexes contained major bands from the constituent purine strands in the region near 190 nm.

4. Free poly[d(A)], poly[d(A)]-containing duplexes, and poly[d(A)]-containing triplexes had similar CD magnitudes near 190 nm, indicating that the purine strands all had aspects of the B_p conformation.

5. Free poly[r(A)] and the poly[r(A)]-containing duplexes had similar CD magnitudes near 190 nm, indicating that the purine strands were in a similar A conformation.

6. From this work and previous work,¹² we concluded that the pyrimidine strands of the hybrid duplexes, poly[r(A)·d(T)] and poly[r(A)·d(U)], had aspects of both the A and B conformations. Overall, the hybrid duplexes were closer to the duplex RNA than to the duplex DNAs in conformation.

7. For poly[d(A)]-containing triplexes, triplex formation induced a negative band between 210 and 215 nm, an indicator of the A conformation. The DNA triplexes poly[d(T)·d(A)·d(T)] and poly[d(U)·d(A)·d(U)] were similar to each other, but different from the RNA triplex poly[r(U)·r(A)·r(U)] in conformation. The hybrid poly[r(U)·d(A)·r(U)] was intermediate.

ACKNOWLEDGEMENTS

We are grateful for support from NIH Research Grants GM-19060 (to DMG) and GM-35662 (to JCS), Grant AT-503 from the Robert A. Welch Foundation (to DMG), the Office of

Health and Environmental Research, USDOE (JCS), and the NSLS-HFBR Faculty-Student Support Program. The NSLS is supported by the Office of Energy Research, USDOE.

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