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Raman Spectroscopic Study of Left-Handed Z-RNA†

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ABSTRACT: The solvent conditions that induce the formation of a left-handed Z form of poly[r(G-C)] have been extended to include 6.5 M NaBr at 35 °C and 3.8 M $MgCl_2$ at room temperature. The analysis of the A → Z transition in RNA by circular dichroism (CD), ^1H and ^{31}P NMR, and Raman spectroscopy shows that two distinct forms of left-handed RNA exist. The Z_R-RNA structure forms in high concentrations of NaBr and $NaClO_4$ and exhibits a unique CD signature. Z_D-RNA is found in concentrated $MgCl_2$ and has a CD signature similar to the Z form of poly[d(G-C)]. The loss of Raman intensity of the 813-cm^{-1} A-form marker band in both the A → Z_R-RNA and A → Z_D-RNA transitions parallels the loss of intensity at 835 cm^{-1} in the B → Z transition of DNA. A guanine vibration that is sensitive to the glycosyl torsion angle shifts from 671 cm^{-1} in A-RNA to 641 cm^{-1} in both Z_D- and Z_R-RNA, similar to the B → Z transition in DNA in which this band shifts from 682 to 625 cm^{-1}. Significant differences in the glycosyl angle and sugar pucker between Z-DNA and Z-RNA are suggested by the 16-cm^{-1} difference in the position of this band. The Raman evidence for structural difference between Z_D- and Z_R-RNA comes from two groups of bands: First, Raman intensities between 1180 and 1600 cm^{-1} of Z_D-RNA differ from those for Z_R-RNA, corroborating the CD evidence for differences in base-stacking geometry. Second, the phosphodiester stretching bands near 815 cm^{-1} provide evidence of differences in backbone geometry between Z_D- and Z_R-RNA.

Although RNA adopts a wide variety of tertiary structures in vivo, only the A-form secondary structure has customarily been observed for double-stranded regions. DNA, by contrast, has been shown to exist in vitro in a variety of double-helical conformations, e.g., A, B, C, D, and Z forms, although most natural DNA is thought to exist in the right-handed B-conformational family under physiological conditions (Wang, 1979). However, under conditions such as high salt concentration (Pohl & Jovin, 1972), low concentrations of certain metal complexes (Behe & Felsenfeld, 1981; Woisard et al., 1985), and superhelical torsional stress (Peck et al., 1982), DNA of certain base sequences, most notably alternating purines and pyrimidines, can adopt the left-handed Z conformation. It has recently been shown that double-stranded RNA consisting of alternating cytidines and guanosines, poly[r(G-C)], can also adopt a conformation similar to the left-handed Z form of DNA; this form has been called Z-RNA (Hall et al., 1984).

The formation of Z-RNA was originally documented by

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circular dichroism (CD) and NMR spectroscopy (Hall et al., 1984; Cruz et al., 1986). A distinct change in the near-UV CD spectrum of the synthetic polynucleotide poly[r(G-C)] was observed on raising the concentration of $NaClO_4$ to 6.0 M and the temperature to 35 °C. It was found that elevated temperature and perchlorate concentration were complementary promoters of the A → Z transition; lower ionic strength increased the transition temperature, and perchlorate concentrations above 6 M stabilized the Z conformation even at room temperature.

Although the near-UV CD spectrum of poly[r(G-C)] in $NaClO_4$ was not similar to that of Z-DNA, this new conformation was identified as a left-handed Z form because the proton and ^{31}P NMR data, as well as the UV absorption spectrum, were similar to those for Z-DNA. CD spectra in the vacuum UV are a more sensitive indicator of handedness than the near-UV. The vacuum UV CD spectra of A- and B-form poly[d(G-C)] and A-form poly[r(G-C)] are similar. Likewise, the vacuum UV spectra of Z-RNA and Z-DNA are similar to each other (Riazance et al., 1985). Although spectroscopic data obtained for Z-RNA and Z-DNA indicate a gross similarity between these two structures, a more detailed comparison requires further spectroscopic characterization.

Raman spectroscopy has played an important role in the characterization of nucleic acid structure. A large number of studies have established empirical correlations between the positions of Raman bands and the conformation of the double-helical nucleic acid (Erfurth et al., 1975; Nishimura et al., 1983; Thomas & Peticolas, 1983a,b; Benevides et al., 1984; Thomas et al., 1986). The localization of the normal modes makes different Raman bands sensitive to structural changes in specific regions of the double helix repeat unit. Theoretical calculations have been moderately successful in assigning this structural sensitivity to variations in phosphodiester torsion angles, sugar pucker, glycosidic torsion angles, and stacking geometry, although the technique has remained primarily a sensitive fingerprint of secondary structure. This effort has been significantly advanced by combined Raman and X-ray diffraction studies of oligonucleotide crystals (Nishimura et al., 1983; Thomas et al., 1986). Raman spectroscopy provided a link between the structure of DNA in hydrated fibers and in solution (Erfurth et al., 1975) and played a crucial role in demonstrating the similarity between the crystal structure of Z-DNA and the high salt solution structure of alternating dG-dC polymers (Thamann et al., 1981).

In the present study, the Raman spectra of poly[r(G-C)] in both right-handed and left-handed helical forms are reported. CD, proton, and ^{31}P NMR spectra are used to correlate the structure of poly[r(G-C)] in both concentrated NaBr and $MgCl_2$ with the structure previously obtained in $NaClO_4$. Unlike perchlorate, solutions of these ions do not exhibit a strong Raman scattering background. This has enabled us to obtain Raman spectra of RNA in a double-helical form other than the A form. The Raman data for poly[r(G-C)] in NaBr and $MgCl_2$ are compared to the spectra of Z-form poly[d(G-C)] and to A-form poly[r(G-C)]. Systematic differences are seen between the Raman spectra of Z-RNA in $MgCl_2$ and NaBr, which are interpreted in terms of two forms of left-handed RNA, denoted Z_R and Z_D. Spectral differences between the species in this study are interpreted in light of the previous normal coordinate assignments of polynucleotides.

Materials and Methods

Poly[r(G-C)] was synthesized and purified according to previously published methods (Hall et al., 1985). The polymer length averaged 100 base pairs. Reagent-grade salts were used with no further purification. Deuteriated samples were prepared by using 99.8% D_2O (Bio-Rad).

Circular Dichroism and UV Absorption Spectroscopy. Ultraviolet spectra were measured on a Cary 118 spectrophotometer. Concentrated samples were run in 2-mm path length cells with 1.9-mm spacers, yielding a final path length of 0.1 mm. CD spectra were measured with a Jasco J500C spectropolarimeter, with a Zeiss thermoelectric cell block. An extinction coefficient of 6560 M^{-1} cm^{-1} at 260 nm was used for poly[r(G-C)] (Gray et al., 1981).

NMR Spectroscopy. The ^{31}P NMR spectrum was acquired at 202.40 MHz on a Bruker AM-500, using broad-band decoupling of protons. The spectra represent the sum of 200 scans, with 5-Hz line broadening on a sample 30 mM in nucleotides. Chemical shifts are referenced to internal trimethyl phosphate.

Preparation of Raman Samples. The low solubility of poly[r(G-C)] in concentrated salt solutions required special sample preparation techniques, as attempts to suspend poly[r(G-C)] directly into high-salt solutions resulted in aggregation and/or precipitation. Thus, samples were prepared as follows: approximately 100 A_{260} units of polymer were lyophilized and suspended in 300 μL of a solution at 10% of the desired final concentration of salt. The solutions were then concentrated 10-fold by evaporation down to ca. 30 μL. This evaporation resulted in a drop in pH to near 4. For NaBr solutions, the pH was adjusted to 7.0 by addition of NaOH solution while the solution was monitored with a micro pH probe. For $MgCl_2$ solutions, the pH could only be adjusted to pH 6.0 by addition of aqueous NaOH. Attempts to further raise the pH resulted in precipitation of $Mg(OH)_2$. The pH-adjusted solutions were then reevaporated to give a final volume of 30 μL. Final RNA concentrations were determined by dilution of a 5.0-μL aliquot into 1.0 mL of H_2O and measuring the absorbance at 260 nm. Typical samples were 30–50 mM in nucleotides.

Samples were sealed in 1-mm diameter capillaries and centrifuged for 10 min to remove suspended particles. Raman spectra were taken with excitation at 514.5 nm from a Spectra Physics 2020 argon ion laser. Incident power ranged from 80 to 150 mW at the sample. The beam focus was achieved with a 50-mm focal length microscope objective. The spectra were taken with a Spex Model 1401 double monochromator with photon-counting detection. Slit widths of 300 μm resulted in spectral resolution of 6 cm^{-1}. Typical spectra were averages of 10–20 scans with a 2-s dwell time and 1–2-cm^{-1} step size for a total accumulation time of 3–12 h. The low solubility (1–5 mg/mL) of the ribopolymers in concentrated salt solutions resulted in a large solvent-scattering background, which was reduced by subtraction of a solvent blank spectrum. However, due to the method of sample preparation, it was not possible to prepare solvent blanks of exactly the same salt concentration as the samples. Because of the sensitivity of the solvent scattering to salt concentration, the inability to prepare a matched blank solution resulted in background subtraction artifacts which were removed by a cubic spline base line fitting procedure. Sample temperature was controlled by placing the capillary in a thermostated brass block. With this arrangement, the sample could be held within 0.1 °C of the desired temperature.

Results

In concentrated sodium perchlorate solutions the strong scattering of the perchlorate ion masks the majority of the nucleic acid peaks, but fortunately the region most diagnostic of backbone conformation remains uncovered. The Raman

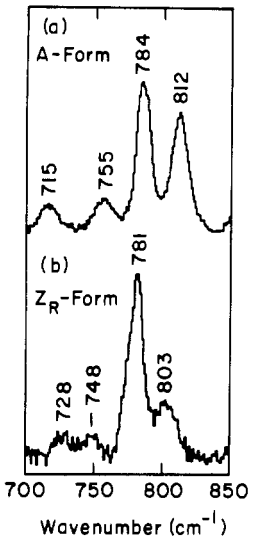

FIGURE 1: Raman spectra of poly[r(G-C)] in sodium perchlorate solutions (514.5 nm, 160 mW): (a) 1.0 M $NaClO_4$, T = 22 °C; (b) 6.2 M $NaClO_4$, T = 22 °C.

spectrum of poly[r(G-C)] in 6 M $NaClO_4$ between 700 and 850 cm^{-1} is presented in Figure 1. Here, the transition to the Z form has been induced by an increase of ionic strength rather than by elevated temperature. The low-salt spectrum in Figure 1a is a typical A-form RNA spectrum, almost indistinguishable from the spectrum of poly(rG)·poly(rC) in low salt (LaFleur et al., 1972) and poly[r(G-C)] at low NaBr concentration. The strong peaks at 784 and 812 cm^{-1} arise from cytosine ring breathing and phosphodiester stretching vibrations, respectively. The dramatic loss of phosphodiester intensity at 812 cm^{-1} in Figure 1b parallels the behavior of poly[d(G-C)] in 4 M NaCl (Pohl et al., 1973) and Z-form crystals of $[d(G-C)]_3$ (Thamann et al., 1981), although the loss observed here is not as complete and the remaining intensity is at slightly higher wavenumber.

The interference of ClO_4^- Raman bands made it necessary to establish other salt conditions which stabilize the Z form of poly[r(G-C)] as a prerequisite to further Raman studies. CD spectroscopy was the initial assay for large-scale conformational changes. In Figure 2 we present the CD spectrum of poly[r(G-C)] in 3.0 M NaCl. The shape and intensity of this spectrum are characteristic of A-form poly[r(G-C)], whether in simple aqueous buffer or NaCl or NaBr solutions. Also shown in Figure 2 are the CD spectra of poly[r(G-C)] in 6.5 M NaBr and in 6.0 M $NaClO_4$ at 35 °C. ^{31}P and proton NMR spectroscopies have demonstrated that the species in $NaClO_4$ is a left-handed Z form (Cruz et al., 1986). The similarity of the CD spectra of poly[r(G-C)] in 6.5 M NaBr to the spectrum of the polymer in 6 M $NaClO_4$ suggests that the polymer is in the Z form under these solvent conditions.

The effect of this salt-induced transition on the ^{31}P NMR spectrum is illustrated in Figure 3. The spectrum of poly[r(G-C)] in 3.5 M NaBr (Figure 3a) has two distinct resonances corresponding to the two classes of phosphates. The chemical shift difference of 0.5 ppm is similar to the splitting seen in A-form poly[r(G-C)] in 3.0 M $NaClO_4$ (Hall et al., 1984) and B-form poly[d(G-5MeC)] (Patel et al., 1982). This splitting increases to 1.5 ppm in 5.7 M NaBr, 50 °C, as shown in Figure 3b. The 1.5 ppm splitting is characteristic of both Z-DNA (Patel et al., 1982) and Z-RNA (Hall et al., 1984). The larger difference in phosphate chemical shifts in the Z forms is a consequence of the zigzag phosphodiester backbone. In addition to the ^{31}P results, a nuclear Overhauser effect between the H_8 and H_1' positions of guanosine (not shown)

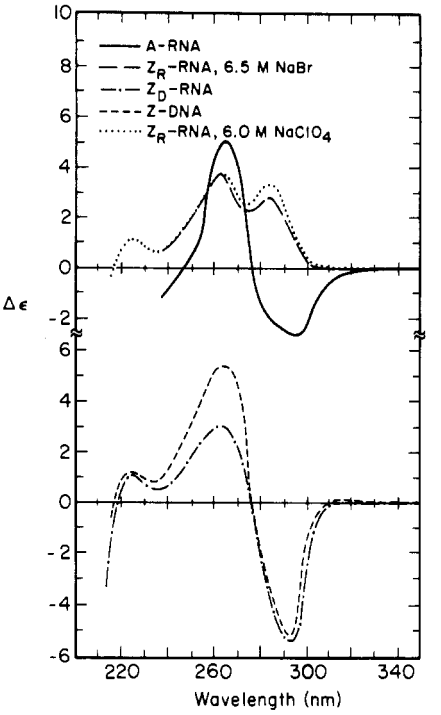

FIGURE 2: CD spectra of alternating G-C polymers: (—) A-RNA, poly[r(G-C)], 3.0 M NaCl, pH 7, T = 20 °C; (– –) Z_R-RNA, poly[r(G-C)], 6.5 M NaBr, pH 7, T = 35 °C; (–·–) Z_D-RNA, poly[r(G-C)], 3.8 M $MgCl_2$, pH 6, T = 20 °C; (- - -) Z-DNA, poly[d(G-C)], 3.0 M NaBr, pH 7, T = 20 °C; (···), Z_R-RNA, poly[r(G-C)], 6.0 M $NaClO_4$, pH 7, T = 35 °C.

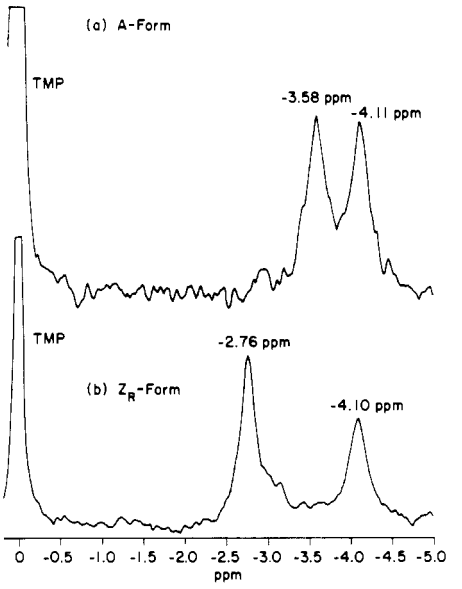

FIGURE 3: ^{31}P NMR spectra of poly[r(G-C)]: (a) A form, 3.5 M NaBr, T = 25 °C; (b) Z_R form, 5.7 M NaBr, T = 50 °C.

reveals that the guanosine residues adopt a syn conformation about the glycosidic bond. The proton chemical shifts are likewise similar to those found previously in $NaClO_4$ (Hall et al., 1984). This evidence also leads us to conclude that concentrated NaBr at elevated temperature is an alternative to $NaClO_4$ for inducing Z-RNA formation.

Shown in Figure 2 is the CD spectrum of poly[r(G-C)] in 3.8 M $MgCl_2$. Although the spectrum still has the negative band at 295 nm and the positive band at 260 nm, the intensities are very different from the A-form spectrum of poly[r(G-C)]. The spectrum of poly[r(G-C)] in 3.8 M $MgCl_2$ is very similar to the spectrum of Z-form poly[d(G-C)] in 3.0 M NaBr. (The

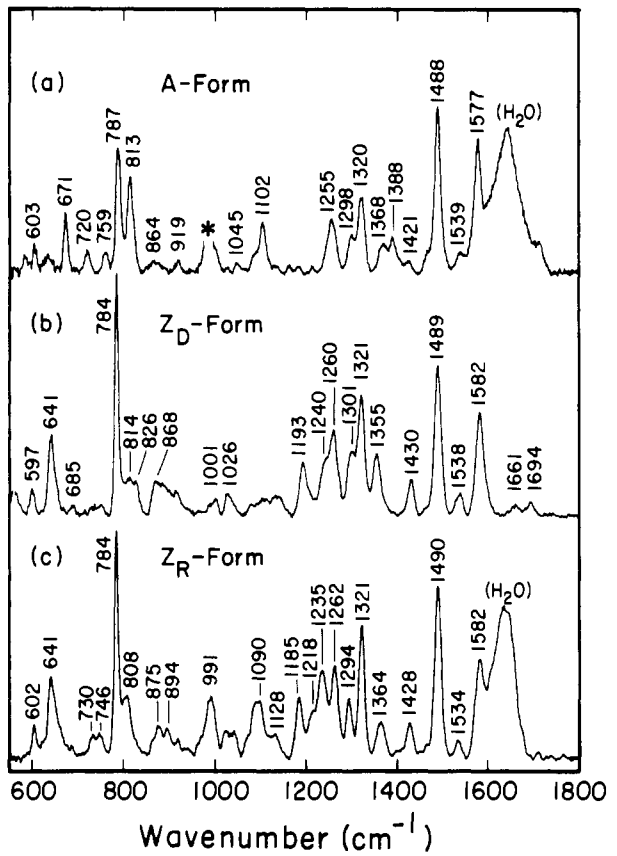

FIGURE 4: Raman spectra of poly[r(G-C)] (514.5 nm, 150 mW): (a) A form, 5.5 M NaBr, T = 20 °C (the gap marked with an asterisk at 980 cm^{-1} is an excised SO_4^{2-} peak); (b) Z_D form, 4.0 M $MgCl_2$, T = 22 °C; (c) Z_R form, 5.5 M NaBr, T = 70 °C.

CD spectrum of Z-DNA is the same in NaCl or NaBr.) Thus, the CD data indicate the existence of at least two spectroscopically distinct high-salt forms of poly[r(G-C)]. We therefore adopt the following nomenclature for these two conformational species of poly[r(G-C)] consistent with the CD data. Z_D denotes the form in $MgCl_2$ showing a Z-DNA-like CD spectrum, and Z_R refers to the species in NaBr or $NaClO_4$ showing a Z-RNA-like CD spectrum. The NMR line widths and absence of long-wavelength CD anomalies indicate that these Z-form conformational species are not forming aggregates under the salt conditions and RNA concentrations employed in this study.

The observation of two spectroscopically distinct forms of Z-RNA prompted a Raman study of these solution structures. The absence of discrete Raman scattering by monoatomic ions permitted the acquisition of full Raman spectra of the A, Z_R, and Z_D species shown in Figure 4. The Raman spectrum of the Z_R form was taken by using 5.5 M NaBr and elevated temperature, rather than 6.5 M NaBr, to enhance the solubility of the polymer.

The most apparent spectral change accompanying the transition to the Z-RNA forms in $NaClO_4$, NaBr, and $MgCl_2$ is the dramatic loss of intensity at 813 cm^{-1}. This peak has been assigned to a normal mode comprised primarily of phosphodiester bond stretching, on the basis of its relative insensitivity to base composition, its appearance in model compounds such as dimethyl phosphate, and its large demonstrated sensitivity to backbone conformation. The behavior of this band in the A → Z transition of RNA parallels the loss of 835-cm^{-1} intensity in the B → Z transition of poly[d(G-C)].

Despite the gross similarity in spectral behavior of the Z-RNA species, the 800–1000-cm^{-1} region of the Raman

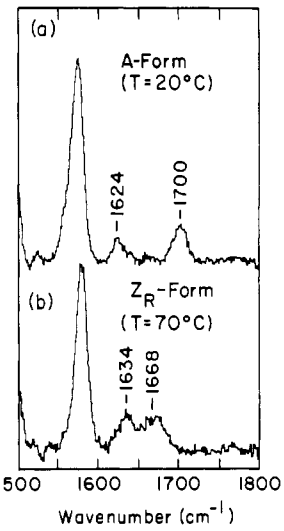

FIGURE 5: Carbonyl scattering of poly[r(G-C)] in D_2O: (a) A form, 5.5 M NaBr, T = 20 °C; (b) Z_R form, 5.5 M NaBr, T = 70 °C.

spectrum shows distinct differences between Z_D- and Z_R-RNA. The Z_R structure shows a weak peak at ~805 cm^{-1}, in both $NaClO_4$ and NaBr solutions. This feature appears doubled at 814 and 826 cm^{-1} in the Z_D-RNA spectrum. Spectral differences also appear in the ribose backbone stretching region between 850 and 900 cm^{-1}. These Raman bands support the CD evidence for two distinct forms of left-handed RNA. Further support for this conclusion comes from the dramatic loss of intensity at ~1100 cm^{-1} in the Z_D form (Figure 4b). This peak is attributed to symmetric stretching of the free P–O bonds (LaFleur et al., 1972), on the basis of its appearance in model compounds, its insensitivity to base composition, and the invariance of the frequency with backbone conformation.

A mode attributed to the guanine residue in the 600–700-cm^{-1} region has been the object of much interest, since its frequency is exceptionally sensitive to conformation. We observed a shift from 671 cm^{-1} in the A form to 641 cm^{-1} in both the Z_R and Z_D forms of poly[r(G-C)], accompanied by some broadening. This behavior parallels the shift from 682 to 625 cm^{-1} seen in the B → Z transition of poly[d(G-C)], in which the guanine residues adopt the syn glycosidic orientation (Thamann et al., 1981).

The Raman spectrum between 1150 and 1600 cm^{-1} is dominated by scattering of skeletal stretching and hydrogen in-plane rocking motions of the bases. The assignment of a peak to a particular base comes from comparison of polynucleotide spectra to those of mononucleotides. Most peaks in this region show only small shifts in position and moderate changes of intensity with conformation. An apparent one-to-one correlation of peaks holds with two exceptions. First, a peak due to cytidine at 1255 cm^{-1} is replaced by two peaks in the 1235–1265-cm^{-1} region. Second, a guanosine peak near 1390 cm^{-1} disappears in both Z-form spectra.

The carbonyl stretching modes of guanine and cytosine give rise to Raman scattering between 1620 and 1730 cm^{-1}. The broad HOH bending band of water at 1640 cm^{-1} normally obscures these peaks in aqueous solutions, and this problem is aggravated by the enhancement of water Raman scattering by the presence of halide ions (Wall & Hornig, 1967). This enhancement is most prominent with larger ions. For this reason, the poly[r(G-C)] carbonyl peaks are unobservable in aqueous NaBr even after digital subtraction of the majority of the water background. In aqueous $MgCl_2$, however, we have been able to fully subtract the H_2O scattering and the carbonyl bands are seen at 1661 and 1694 cm^{-1} in Figure 4b. To

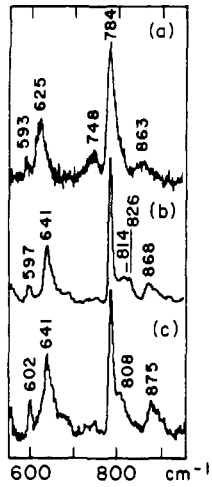

FIGURE 6: Low-wavenumber region of Z-form polymers: (a) poly[d(G-C)], 4 M NaCl [from Benevides and Thomas (1983)]; (b) poly[r(G-C)], 4 M $MgCl_2$, T = 22 °C; (c) poly[r(G-C)], 5.5 M NaBr, T = 70 °C.

observe carbonyl scattering from Z_R-RNA, samples were prepared in D_2O, which scatters near 1200 cm^{-1} rather than 1640 cm^{-1}. The carbonyl bands of A and Z_R poly[r(G-C)] in D_2O are shown in Figure 5.

DISCUSSION

Backbone Geometry. The loss of intensity at 813 cm^{-1} in the A → Z_D and A → Z_R transitions strongly resembles the loss of a peak near 835 cm^{-1} in the B to Z transition of DNA, as seen in the hexanucleotide crystal $[d(C\text{-}G)]_3$ (Thamann et al., 1981) and in poly[d(G-C)] in aqueous NaCl (Pohl et al., 1973; Benevides & Thomas, 1983; see Figure 6). The sensitivity of phosphodiester stretching modes to the torsional angles about the P–O bonds is aptly demonstrated by the normal-mode calculations of dimethyl phosphate (Shimanouchi et al., 1964). In the more complex polynucleotide backbone, other conformational parameters will influence the mode frequency as well, since this nominal phosphodiester stretching mode involves lesser motion of atoms throughout the backbone, as shown by normal-mode calculations of a truncated phosphate–ribose backbone (Brown & Peticolas, 1975) and the full polynucleotide backbone (Lu et al., 1977). This loss of intensity in the 810–840-cm^{-1} region thus reflects a large conformational change in the vicinity of the phosphodiester group, presumably due to the conversion of the GpC phosphodiester from a gauche–gauche to a gauche–trans conformation upon the A to Z transformation.

The difference between Z_D- and Z_R-RNA in phosphodiester (800–830-cm^{-1}), phosphate–ribose (850–900-cm^{-1}), and phosphodioxy (~1100 cm^{-1}) stretching regions suggests that Mg^{2+} binds specifically to the dianion PO_2 sites of the backbone, leading to conformational changes throughout the backbone, particularly around the phosphate groups. Indeed, this same trend is observable in the Z-DNA data of Thamann et al. (1981). In 3 M $MgCl_2$, the 1090-cm^{-1} PO_2 stretch of poly[d(G-C)] disappeared, and the weak remaining phosphodiester intensity shifted to slightly higher wavenumber.

Base Stacking. In-plane C–C and C–N stretching and H rocking modes of the bases are found almost exclusively between 1180 and 1600 cm^{-1}. The intensities of the Raman peaks in this region have long been known to be sensitive to the stacking interactions between bases. Nearly all of these bands become hypochromic on double helix formation from random coil (Small & Peticolas, 1971). It was shown that this Raman hypochromism is directly related to the near-UV absorption hypochromism. Numerous, though less striking, changes have been observed in this region during secondary structural transitions, such as the A–B transition of DNA fibers (Erfurth et al., 1975; Martin & Wartell, 1982), the B–Z transition of DNA in solution (Pohl et al., 1973), and premelting transitions in DNA (Erfurth & Peticolas, 1975). The CD spectrum is sensitive to stacking geometry as well, so that the CD spectra should correlate with the intensity pattern between 1180 and 1600 cm^{-1} in the Raman spectra.

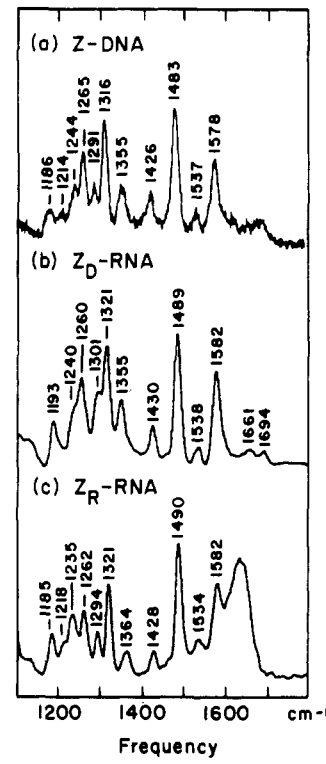

FIGURE 7: Base stretching region of Z-form polymers: (a) poly[d(G-C)], 4.0 M NaCl [from Benevides and Thomas (1983)]; (b) poly[r(G-C)], 4.0 M $MgCl_2$; (c) poly[r(G-C)], 5.5 M NaBr.

Figure 7 compares the base stretching region of the three Z-form GC polymers. In parallel with the CD spectra, *the Raman intensities of Z_D poly[r(G-C)] more closely resemble those of Z-DNA than do those of the Z_R form.* Note especially the relative intensities of the 1240- and 1260-cm^{-1} bands and the intensity of the 1355-cm^{-1} band.

Base Pairing. Whereas the intensities of peaks in the base stretching and rocking region reflect stacking geometry, the positions of these peaks are sensitive primarily to changes in strength and geometry of bonding. The conformational dependence of base stretching frequencies is illustrated in Figure 8 for several alternating G-C conformational species. Approximate normal-mode descriptions for cytidine (Nishimura & Tsuboi, 1985) and guanine (Majoube, 1985; Nishimura et al., 1985) appear in the last column.

Figure 8 shows the conformational sensitivity of the guanine mode found between 1355 and 1385 cm^{-1}. Normal-mode analyses have found a strong component of in-plane rocking of the imino proton in this normal mode, on the basis of its downshift upon ^{15}N substitution at positions N_1 and N_3 and upon deuteriation of imino and amino groups (Delabar & Majoube, 1978). The position of this peak shows a wide conformational variation (including an apparent doubling in A and B conformations) which arises because the normal mode involves the motion of atoms in the base-pairing region. Figure 7 shows the similarity of this spectral feature for the Z-DNA

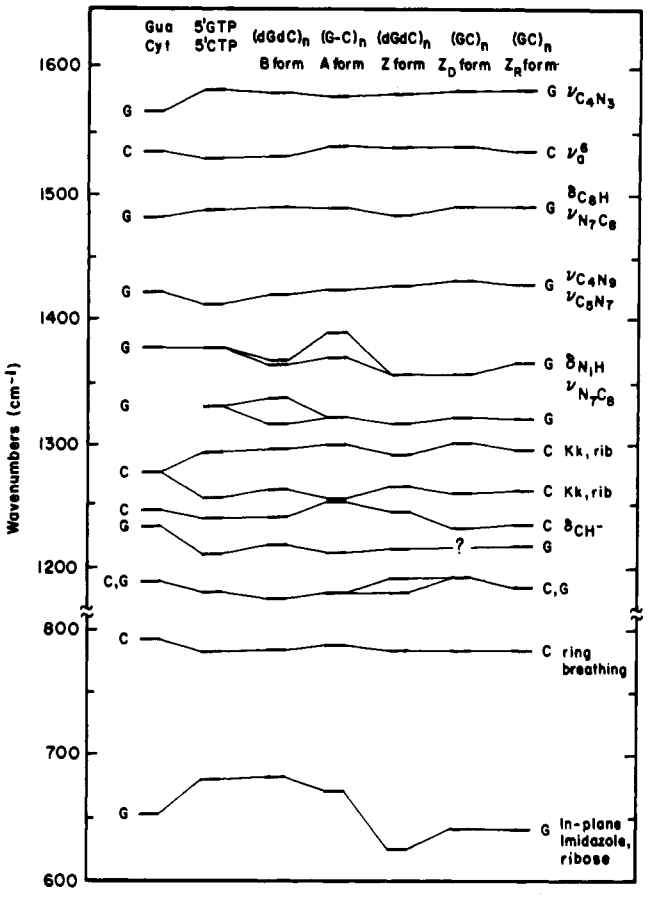

FIGURE 8: Correlation diagram for base modes of alternating G-C polymers. Base and nucleotide data are from Lord and Thomas (1967), the deoxy polymer data from Benevides and Thomas (1983), and the ribo polymer data from this study. Approximate normal-mode assignments, where reasonable agreement exists, are taken from Nishimura and Tsuboi (1985), Majoube (1985), and Nishimura et al. (1985). (ν = bond stretch, δ = in-plane rocking, and Kk = "Kekulé" motion.)

Table I: Carbonyl Stretching Frequencies (cm^{-1}) in Alternating G-C Polymers

		D_2O solutions		H_2O solutions	
species	form	Cyt	Gua	Cyt	Gua
poly[d(G-C)]	B	1635[a]	1688[a]	1630[b]	1713[b]
poly[d(G-C)]	Z	1634[a]	1662[a]	1645[b]	1681[b]
poly[r(G-C)]	A	1624	1700	N.O.[c]	1731
poly[r(G-C)]	Z_R	1634	1668	N.O.[c]	<1700
poly[r(G-C)]	Z_D			1661	1694

[a] From Benevides and Thomas (1983). [b] From Thamann et al. (1981). [c] N.O., not observed.

and both Z-RNA species, all of which lack the doubling seen in the A and B conformations. In Z_D-RNA and Z-DNA the peak appears at 1355 cm^{-1}, 9 cm^{-1} below the position in Z_R-RNA.

The carbonyl stretching peaks are sensitive reporters of base-pairing geometry and bonding. Table I summarizes the positions of these bands for several G-C-containing polymers in D_2O. Unfortunately, we have been unable to prepare poly[r(G-C)] at sufficient concentration in $MgCl_2/D_2O$ to obtain a Raman spectrum of these weak peaks, and the H_2O scattering obscures the cytosine carbonyl peaks in the $NaBr/H_2O$ samples. We must therefore restrict our comparison to the data in Table I, which demonstrates the similarity of the Z_R form to Z-DNA in D_2O (mean difference of 3 cm^{-1}) and the much larger difference between Z_D-RNA and Z-DNA in H_2O (mean difference of 14 cm^{-1}), in contrast to the pattern in the base stretching modes. Thus, although we are unable to directly compare the carbonyl scattering of the Z_D and Z_R forms in a single medium, the data in Table I strongly suggest a difference in their base-pairing environments.

Glycosidic Torsional Orientation. It has been established that guanine residues in Z-DNA adopt a syn conformation; for $[d(C-G)]_3$, the glycosidic angle, as defined by Saenger (1984) changes from $\sim -110°$ in B form to $+70°$ in Z form (Wang, 1979). This reversal in orientation is reflected in the Raman spectrum by a shift in a guanine band from 682 (B form) to 625 cm^{-1} (Z form). Figure 8 illustrates the behavior of this band in the three Z-form species. The downshift of this peak from 671 to 641 cm^{-1} in the A to Z transition of RNA is therefore consistent with the nuclear Overhauser NMR data that demonstrated a change from an anti to a syn glycosidic torsion in the guanosine residue (Cruz et al., 1986).

The normal-mode assignment of the $\sim$680-cm^{-1} guanine mode is controversial, having been ascribed to the imidazole ring breathing (Tsuboi et al., 1973) and N_9 ribose stretching and in-plane rocking (Majoube, 1985). In any case, the mode appears to be localized in the imidazole ring and couples with the ribose motion via the glycosidic bond, conferring to it the sensitivity to the orientation about this bond and, to a lesser extent, the type and conformation of the sugar. Recent normal-coordinate calculations (Letellier et al., 1986) of deoxyguanosine show the systematic dependence of this mode frequency on glycosyl and sugar pseudorotation angles. It is impossible at this time, however, to extract conformational detail from the difference in this spectral feature between Z-DNA and Z-RNA. This shift may arise from any combination of differences in glycosidic torsion angle, sugar pucker, or sugar type.

A pair of cytidine bands between 1250 and 1300 cm^{-1} have been assigned to a so-called Kekulé motion of the ring, i.e., alternating stretching and compression of adjacent bonds, coupled to motions in the ribose moiety (Nishimura & Tsuboi, 1985). These bands are derived from a single Kekulé mode at 1276 cm^{-1} in unsubstituted cytosine. The vibrational coupling between the pyrimidine ring and the ribose, and hence the positions of the coupled Kekulé–ribose bands, should depend on glycosyl bond orientation and sugar conformation and type. The normal-mode calculations on cytidine by Letellier et al. (1986) bear out this qualitative prediction. A Kekulé mode was found to vary in frequency from $\sim$1270 to 1285 cm^{-1} as the glycosidic bond angle was varied over the conformationally accessible range. The positions of these modes in the Z-DNA spectrum more closely resemble the Z_R frequencies than the Z_D frequencies, suggesting a difference in cytidine glycosyl angle between Z_D- and Z_R-RNA.

CONCLUSIONS

Solvent conditions required to induce the Z form of RNA in poly[r(G-C)] at elevated temperatures have been extended from the originally reported $NaClO_4$ to include 5.5 M NaBr and 4.0 M $MgCl_2$. The characteristic loss of Raman intensity near 813 cm^{-1} has been used to confirm the existence of Z-RNA in these solvents. NMR line widths and the absence of long-wavelength CD anomalies establish that this structural transition is taking place without significant aggregation of the polymer.

The Raman and CD data provide clear evidence for two distinct left-handed forms of RNA, which we have called Z_D and Z_R. The former conformation is found in $MgCl_2$ solutions and the latter in NaBr or $NaClO_4$ solutions. The Raman spectrum corroborates the CD evidence for differences in

base-stacking geometry between Z_D and Z_R and further supports the similarity of stacking in Z_D-RNA and Z-DNA. In addition, the Raman data indicate that the base-pairing geometries and backbone conformations of Z_D and Z_R are different. Further interpretation of these results requires more extensive normal-mode analysis and/or comparison to the Raman spectra of crystallographically characterized oligonucleotides.

The conformationally sensitive guanine band between 600 and 700 cm^{-1} shows the expected shift to lower wavenumbers accompanying the A → Z transition, although this band assumes the unique value of 641 cm^{-1} in both Z-RNA species. This implies a different glycosidic torsion angle and possibly sugar pucker for Z-RNA vs Z-DNA.

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