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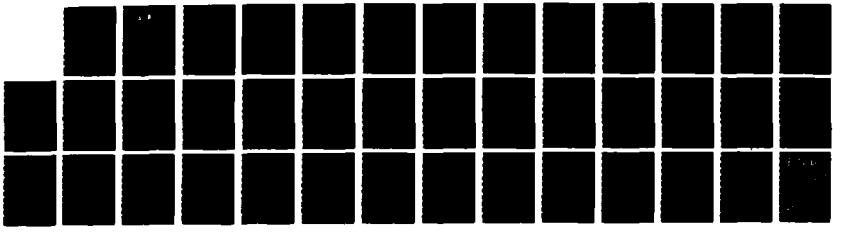

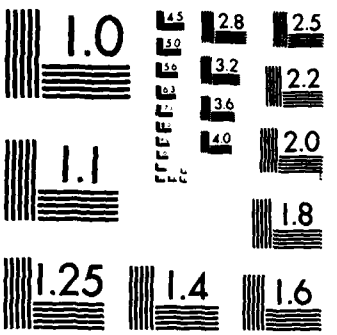

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Stabilization of Z-RNA Under Physiological Conditions and Recognition by Anti-Z-DNA Antibodies.[+] [†]

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Running Title: Stabilization of Z-RNA and Antibody Binding

[1]Abbreviations: CD, circular dichroism; csa, chemical shift anisotropy; EDTA, ethylenediaminetetraacetic acid; FID, free-induction decay; IgG, immunoglobulin G; NOE, nuclear Overhauser effect; RIA, radioimmunoassay; TMP, trimethylphosphate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; TSP, 3-(trimethylsilyl)propionate.

Limited chemical bromination of poly[r(C-G)] results in partial modification of guanine C8 and cytosine C5, producing a mixture of A- and Z-RNA forms. The Z conformation in the brominated polynucleotide is stabilized at much lower ionic strength than in the unmodified polynucleotide. More extensive bromination of poly[r(C-G)] results in stabilization of a form of RNA having a Z-DNA (Z_D)-like CD spectrum in low salt, pH 7.0-7.5 buffers. Raising the ionic strength to 6 M NaBr or $NaClO_4$ results in a transition in Br-poly[r(C-G)] to a Z-RNA (Z_R) conformation as judged by CD spectroscopy. At lower ionic strength Z-DNA-like (Z_D) and A-RNA conformations are also present.

^1H NMR data demonstrate a 1:1 mixture of A and Z-RNAs in 110 mM NaBr buffer at 37°C. Nuclear Overhauser effect (NOE)[1] experiments permit complete assignments of GH8, CH6, CH5, GH1' and CH1' resonances in both the A- and Z-forms. Distance estimates obtained from GH8 ↔ GH1' NOEs demonstrate the presence of both A- and Z-form GH8 resonances in slow exchange on the NMR timescale. The NMR results indicate that unbrominated guanine residues undergo transition to the syn conformation (Z-form).

Raman scattering data are consistent with a mixture of A- and Z-RNAs in 100mM NaCl buffer at 37°C. Comparison with the spectrum of Z-DNA indicates that there may be different glycosidic torsion angles in Z-RNA and Z-DNA (Tinoco et al., 1986). ^{31}P NMR spectra show 6 - 8 resonances spread over a 1.8 ppm range whose chemical shifts are also consistent with an equilibrium mixture of A- and Z-RNAs.

Radioimmunoassay and nitrocellulose filter-binding competition experiments were performed to determine the extent of recognition of Br-poly[r(C-G)] by anti-Z-DNA antibodies. The polyclonal rabbit anti- Br-poly[d(C-G)] IgG preparations T4 and Z6 (Zarling, et al., 1984a,b) specifically recognize the

Z-form of poly[r(C-G)], although the binding affinities are 10- to 100-fold lower for Z-RNA than for various forms of Z-DNA. Competition RIA experiments verify the presence of a Z-DNA-like determinant in left-handed Br-poly[r(C-G)] at physiological NaCl concentration. Phosphate buffer or high ionic strength conditions inhibit recognition of Z-RNA by anti-Z-DNA IgGs, consistent with recognition of a phosphodiester backbone determinant. In summary, these spectroscopic and immunochemical studies demonstrate that under conditions of conformational stress (i.e. containing brominated nucleosides) left-handed Z-RNA is stable and is specifically recognized by proteins at physiological temperature and ionic strength.

Introduction

The right-handed (A-form) to left-handed (Z-form) transition in linear double-stranded RNA was first described by Hall et al. (1984b) for the synthetic alternating copolymer poly[r(C-G)] in 6 M $NaClO_4$ buffer at 45°C. Proton and ^{31}P NMR studies demonstrated that guanine residues were in a syn conformation and that there were very different Z-form CpG and GpC phosphodiester conformations in the polynucleotide under these conditions. The CD bands centered at 282 and 230 nm were shown to undergo temperature- and salt-dependent inversion. Taken together, these data are consistent with the characteristics of Z-DNA (Rich et al., 1984; Jovin et al., 1983). The vacuum UV CD spectra of Z- and A-form poly[r(C-G)] were shown to match spectra for left- and right-handed forms of DNA at wavelengths below 220 nm (Riazance, et al., 1984). Calculated spectra for A- and Z-RNA and A-, B- and Z-DNA in this wavelength region were also consistent with experiment (Williams, et al., 1986). Thus, these intense bands are most descriptive of helix handedness. Raman scattering spectra are also consistent with the left-handed conformation (Tinoco et al., 1986).

However, the spectroscopic data and the different salt-dependences for the transition to Z-form in DNA and RNA point to significant structural, kinetic and thermodynamic differences between the two types of left-handed nucleic acid (Cruz et al., 1986a).

In 4 M $MgCl_2$, poly[r(C-G)] adopts a conformation with a Z-DNA-like CD spectrum. In 6M NaBr or $NaClO_4$ buffers, poly[r(C-G)] has a CD spectrum distinctly different in the 240-300 nm region from the 4M $MgCl_2$ form of the polynucleotide. These conformations thus differ in the relative base-stacking orientations and have been termed Z_D- and Z_R-RNA, respectively (Cruz et al., 1986b).

Stabilization of Z-DNA requires either high ionic strength, base or backbone modification, topological stress or protein binding (reviewed by Leng, 1985; Rich et al., 1984; Jovin et al., 1983). Prior to the studies described here, only high ionic strength conditions have been shown to stabilize Z-RNA (Cruz et al., 1986a).

In this work we demonstrate that chemical bromination of poly[r(C-G)] has a profound effect on the conformational properties of the RNA duplex. Characterization of the solution spectral properties of the polynucleotide confirms that it undergoes a cooperative transition between right- and left-handed conformations. Most importantly, the left-handed Z-form is shown to be present under physiological conditions of temperature, pH and ionic strength. Finally, the anti-Z-DNA polyclonal antibodies T4 and Z6 (Zarling et al., 1984a,b) are shown to specifically recognize the Z-form of RNA under physiological conditions, indicating the presence of a Z-DNA-like structural element in Z-RNA and demonstrating specific recognition of Z-RNA by a protein.

Experimental Procedures

Materials

RNA polymerase was kindly provided by Professor Michael Chamberlin (Dept. of Biochemistry, UC, Berkeley). Deoxyribonuclease I, mung bean nuclease and polynucleotide kinase were from Pharmacia Biochemicals. Nuclease S1, 5S rRNA, and calf intestinal phosphatase (Mol. Biol. Grade) were Boehringer-Mannheim products. 5.8S rRNA was the kind gift of Dr. Harold Kammen (School of Public Health, UC, Berkeley). RNase T_1, poly[d(A)•d(T)] and 16S/23S rRNA were from Sigma. Poly[d(C-G)], poly[d(br^{5}C-G)], poly[d(m^{5}C-G)], poly[r(A)•r(U)], poly[r(I-C)], and poly[r(A-U)] were from Sigma or Pharmacia Biochemicals, or were synthesized enzymatically. Polynucleotides were [^{32}P]5'-end-labeled for RIA experiments using polynucleotide kinase (Silberklang et al., 1979). Adenosine [γ-^{32}P]triphosphate was from Amersham. Kinase reaction products were purified by NENsorb chromatography (New England Nuclear) as described by the manufacturer. Residual [γ-^{32}P]ATP and [^{32}P]$_i$ were removed by ethanol precipitation and dialysis into 10 mM Tris-HCl, pH 7.5, 0.5 mM EDTA. Affinity-purified goat anti-rabbit IgG preparations were from Miles Labs or Jackson Immunoresearch. All other reagents were of analytical grade or higher.

Methods

Preparation of Br-poly[r(C-G)]. Poly[r(C-G)] was prepared by transcription from the synthetic template poly[d(I-C)] as described by Hall et al. (1984b). The extinction coefficient for poly[r(C-G)] was taken as $\varepsilon_{260} = 6760\ M^{-1}\ cm^{-1}$ (Gray et al., 1981). The purified polynucleotide was chemically brominated by modifications of the method of Möller et al. (1984). The polynucleotide (1.57 mM in nucleotides) was preequilibrated for 10 min. at 45 or 55^{o}C in a reaction mixture containing 10 mM sodium phosphate (pH 7.0), 6 M NaBr or $NaClO_4$ and 1

mM EDTA in order to facilitate the A → Z transition. Bromine-saturated H_2O was prepared at room temperature and diluted 4-fold with 6 M NaBr or $NaClO_4$ buffer. The reaction volume was adjusted to 45 µl by rapid addition of Br_2/H_2O reagent to achieve the desired Br_2/nucleotide ratio. Incubation was continued at 45 or 55°C for 10 min. Reactions were terminated by rapid addition of 755 µl ice cold 10 mM sodium phosphate (pH 7.0), 1 mM EDTA. Excess bromine was then removed by bubbling air through the reaction mixture (on ice) for 30 min. followed by dialysis at 4°C against excess 110 mM NaCl buffer, and finally 0.5 mM EDTA. Samples were lyophilized, dialyzed versus several 500 ml changes of glass-distilled H_2O in a BRL 1200-MA microdialysis apparatus (M_r cutoff = 12-14 kdal), and resuspended into the appropriate buffers. Brominated polynucleotide samples were protected from light during all manipulations. Proton NMR samples were lyophilized twice from 99.8% D_2O (Stohler) then resuspended into 99.998% D_2O (Stohler) in a glove bag under N_2(g). The polymer size distribution ranged from 40 - 800 nucleotides as determined by polyacrylamide gel electrophoresis (Hall et al., 1984b). Approximately 80% of the polymer was in the 70 - 90 nucleotide range as determined by agarose gel electrophoresis using E. coli tRNA, 5S, 16S and 23S rRNA, and yeast 5.8S rRNA as size markers. Based on ^1H NMR peak integrals (Figure 5A) and HPLC analysis (as in Möller et al., 1984), we estimate that approximately 35-50% of the guanine and 25-40% of the cytidine residues in Br-poly[[r(C-G)] were brominated. The extinction coefficient for Br-poly[r(C-G)] was taken as 5770 M^{-1} cm^{-1}.

CD, absorbance and Raman scattering spectroscopy. CD spectra were recorded on a JASCO J500C spectropolarimeter in 1 cm pathlength quartz cuvettes surrounded by a temperature block controlled by a Zeiss model P/N 00 80 thermoelectric unit. Absorbance spectra were recorded on a Cary 118 UV/VIS scanning spectrophotometer. Raman samples were sealed in 1 mm diameter

capillaries and centrifuged for 10 min. to remove suspended particles. Raman scattering spectra were obtained at a power output of 50 mW from the 514.5 nm line of a Spectra Physics 2020 argon ion laser. The spectra were accumulated using a Spex model 1401 double monochrometer with photon counting detection.

^{1}H and ^{31}P NMR spectroscopy. Proton NMR spectra were obtained at 500 MHz on a Bruker AM 500 spectrometer as described in the legend of Figure 5. Phosphorus NMR spectra were obtained at 202 MHz on the same spectrometer or at 121 MHz on the Nicolet BVX 300 spectrometer housed in the Berkeley NMR facility, as detailed in Figure 6. Protons were broadband decoupled using a power of ca. 1 W during acquisition and with sufficient power to maintain the ^{31}P{^{1}H}NOE during the delay between pulses.

Radioimmunoassays. RIAs and nitrocellulose filter-binding assays were performed in sterile 1.5 ml polyethylene (Eppendorf) tubes. The reaction mixtures (25 µl final volume) contained either 5, 10 or 50 ng (RIA), or 5 or 20 ng (filter binding assays), RNA or DNA substrate in 50 mM Tris-HCl (pH 7.45), and 5 mM EDTA. Unless otherwise stated, samples containing either 110 mM NaCl (RIA), or 220 mM, 1.5M or 4M NaCl (filter binding assays) were preequilibrated for 1 h at 37^{o}C. Next, 5 µl of the purified rabbit anti-Br-poly[d(C-G)] IgG T4 or Z6 (Zarling et al., 1984a,b) was added. Purified normal rabbit IgG, or buffer alone, were used as negative controls. Reactions between antibody and nucleic acid were incubated for 90 min. at 37^{o}C. Second antibody (1 µl goat anti-rabbit IgG was then added and incubation was continued for an additional 1 h at 37 ^{o}C. Immune complexes were collected by centrifugation at 13K rpm (20^{o}C) and most of the supernate was removed by aspiration. Immune complexes were then resuspended and washed 3 times as above with 200 µl aliquots of the appropriate buffer (including salt). The washed precipitates were resuspended in 0.2 ml 0.2 N NaOH and counted in 5 ml Aquasol-2 (New England Nuclear) in a Beckman 8000

liquid scintillation counter.

Nitrocellulose filter-binding assays. For filter binding experiments, immune complexes were prepared as above except that no second antibody was added. The immune complexes were diluted to 200 µl with the appropriate buffer and filtered through a Millipore Millititer-STHA09610 96-well filtration plate presoaked with buffer as described by the manufacturer. Filters were washed 6 times with buffer, dried, transferred to 5 ml Aquasol-2 using a Millititer filter punch and counted as above.

Results

Effect of chemical bromination on the A ↔ Z RNA equilibrium: CD and UV spectroscopy. The effects of chemical bromination on the solution structure of the alternating RNA copolymer poly[r(C-G)] was monitored by its effects on the optical spectra of the polynucleotide (Figures 1-4). The CD spectra of poly[r(C-G)] obtained using molar ratios of 0, 1, 2, and 3 Br_2/nucleotide in the bromination reaction mixture are shown in Figure 1A. Previously, Hall et al. (1984a) demonstrated that 6M $NaClO_4$ at temperatures above ca. 35°C was required to induce the A → Z_R transition in unmodified poly[r(C-G)]. Figure 1A shows a comparison of the CD spectra of A-RNA and the brominated polynucleotides formed using 1, 2, and 3 Br_2/nucleotide in a 45°C reaction mixture. These data show that at 22°C in 6M $NaClO_4$ buffer, with higher Br_2/nucleotide levels in the reaction mixture, the CD spectra of the polynucleotides adopt progressively more Z_R-RNA-like character.

When poly[r(C-G)] undergoes the A → Z_R transition there is an increase in the A_{295}/A_{260} UV absorbance ratio (Hall et al., 1984a). The increase in the A_{295}/A_{260} ratio paralleled the A → Z transition induced by chemical bromination as monitored by CD (Figure 1B). Circular dichroism spectra

corresponding to polynucleotides obtained from reactions containing 2-2.5 Br_2/nucleotide (T = 45°C) are similar to the Z_R-RNA spectra of Hall et al. (1984b). Note the plateau in the A_{295}/A_{260} ratio at this level of bromination. Using levels >3 Br_2/nucleotide at 45°C or >2 Br_2/nucleotide at 55°C resulted in spectra with negative CD at 295 nm in 6 M NaBr buffer at 22°C. Brominated poly[r(C-G)] prepared using 3 Br_2/nucleotide at 45°C retains a Z_R-form CD spectrum on lowering the ionic strength to 2 M $NaClO_4$ as judged by CD (Figure 2). Characteristic Z_R-form positive CDs are observed in these spectra at 230 and 280 nm. Thus, bromination of poly[r(C-G)] stabilizes the Z_R-form at much lower ionic strengths than is necessary for the unmodified polynucleotide. Moreover, a higher degree of bromination results in a CD spectrum progressively more similar to that found in Z-DNA. This conformation of left-handed RNA is called Z_D-RNA.

Lowering the ionic strength below 2M $NaClO_4$ or NaBr resulted in a CD spectrum intermediate between that of Z_R- and A-RNA (Figures 2B and 3). Raising the temperature did not completely convert the polymer to Z_R-form at these salt concentrations. For example, in 500 mM $NaClO_4$ or NaBr this polymer is ca. 20% Z_R-form as judged by the positive CD at 280 nm. Raising the temperature to 60°C only partially converts the spectrum to Z_R-form (ca. 50%). In contrast, if any indication of inversion is seen at 280 nm in the A-form spectrum of unmodified poly[r(C-G)], it is readily converted to Z_R-RNA by a 10-15°C increase in temperature (Cruz et al., 1986a). Thus, bromination of poly[r(C-G)] apparently stabilizes intermediate states in the A⇌Z equilibria (Z_R- and/or Z_D-RNA) at lower ionic strengths. Lowering the ionic strength to 110 mM NaBr or NaCl (Figures 2B and 3, respectively) results in CD spectra for Br-poly[r(C-G)] very much like that of Z-DNA (Figure 3). Significant differences can be seen between the CD spectra of Br- poly[r(C-G)] and Br-poly[d(C-G)]

(Figure 3) including an inflection in the RNA spectrum at 280 nm, a slight decrease in negative CD at 290 nm, and a small increase in positive CD at ca. 268 nm. These data show that the CD spectrum of Br-poly[r(C-G)] at 110 mM NaCl has features in common with the spectra of A-, Z_R- and Z_D-RNA (Figures 1-3).

^{1}H Nuclear Magnetic Resonance Experiments. Proton NMR studies provide useful insights into the types of conformational states present in Br-poly[r(C-G)] in low ionic strength solution (Figure 4). The 500 MHz ^{1}H NMR spectrum of the polynucleotide in 110 mM NaBr/D_2O buffer is shown in Figure 4A. Samples were chemically brominated using a molar ratio of 3 Br_2/nucleotide in a 55°C reaction. Downfield peaks (7.1-7.9 ppm) were present with chemical shifts clearly corresponding to those of GH8 and CH6 resonances in both the A- and Z_R-forms of poly[r(C-G)] (Hall et al., 1984a). Peaks are also present in the 5.1-6.0 ppm region corresponding to CH5 and H1' resonances for both cytosine and guanine in the A- and Z-forms. Resonances upfield from HDO (3.8-4.5 ppm) correspond to aliphatic protons on the ribose rings of the nucleotides in Br-poly[r(C-G)] (Uesugi et al., 1984). Thus, according to chemical shift and integral data, it is apparent that the ^{1}H NMR spectrum of Br-poly[r(C-G)] in 110 mM NaBr represents approximately a 1:1 equilibrium mixture of normal A- and Z-form RNA nucleotide units. This conclusion was further verified by nuclear Overhauser effect experiments.

The nuclear Overhauser effect (NOE) results from a transfer of dipolar spin energy between two or more spatially proximal nuclei (Solomon, 1955; Noggle and Schirmer, 1971). One dimensional NOE connectivity patterns were studied in the A- and Z_R- conformations of poly[r(C-G)] by Hall et al. (1984a). In order to verify the A Z RNA conformational equilibrium in Br-poly[r(C-G)] at 120 mM Na^+ concentration, NOE experiments were performed using 200 and 500 msec irradiation times prior to acquiring the FIDs (Figure 4C and D, Table 1). With a

500 msec irradiation time, spin-diffusion effects (domino-like NOEs, see Kalk and Berendsen, 1976) will be effective for Br-poly[r(C-G)] (average molecular weight ca. 50 kdal). The Z-form GH8↔GH1' and CH5↔CH6 reciprocal NOEs are clearly visible at 5.83 and 4.98 ppm (Figure 5B). The guanine NOE intensities (25-30%) are in good agreement with the expected syn-glycosidic torsional conformation; much lower NOE intensities are consistent with the expected anti- conformation in A-form guanine residues (7.42 ↔ 5.44 ppm).

These data show that brominating poly[r(C-G)] produces a transition to a polynucleotide which includes G8-protonated and brominated Z-form guanine residues. Peak integrals show that approximiately 50% of the unmodified residues in the polynucleotide are in the Z-RNA conformation at physiological ionic strength and temperature (compare CH6 and GH8 integrals to those of the H1' and CH5 protons).

^{31}P NMR and Raman Scattering Experiments. Additional evidence for a left-handed Z-form of Br-poly[r(C-G)] at 120 mM Na^+ concentration was obtained from ^{31}P NMR spectroscopy. ^{31}P resonances corresponding to the A- and Z_R-form phosphodiesters of poly[r(C-G)] were originally assigned by Hall et al. (1984) as follows: Z-form (-2.74 and -4.07 ppm); A-form (-3.64 and -4.14 ppm). The 202 MHz and 121 MHz ^{31}P NMR spectra of Br-poly[r(C-G)] in 110 mM NaBr buffer are shown in Figures 5A and B. Similar chemical shift patterns in the ^{31}P NMR spectra of r(C-br^{8}G-C-br^{8}G) and r(C-m^{8}G-C-m^{8}G) in 110 Na^+ mM buffer were assigned by Uesugi et al. (1984) to left-handed (presumably Z) conformations. Möller et al. (1984) previously noted in ^{31}P NMR studies of brominated poly[r(C-G)], that peaks corresponding to CpG and GpC in brominated tracts of the polymer resonate in the -3.6 to -3.9 ppm region. Thus, the ^{31}P NMR data shown in Figure 5 are consistent with approximately equal representations

of unmodified A- and Z-form RNAs.

Raman scattering spectroscopy confirmed that the Z-DNA conformation observed in single crystal x-ray crystallography studies (Wang et al., 1979; Drew and Dickerson, 1981) was the same as observed in solution (Thamann et al., 1981). The Raman spectrum of Br-poly[r(C-G)] in 110 mM NaCl buffer is shown in Figure 6. CD spectra of Br-poly[r(C-G)] in 110 mM NaCl and NaBr buffers are essentially identical (Figures 2B and 3). Thus, if the increase in concentration does not greatly perturb the A⇌Z equilibrium, the Raman spectrum should represent a ca. 1:1 mixture of A- and Z-form RNAs.

The most characteristic changes seen in the Raman spectrum of poly[r(C-G)] during the A → Z transition is the loss of intensity at 813 cm^{-1} due to loss of the anti-symmetric phosphodiester stretch mode (Tinoco et al., 1986). The intensities of the peak at 814 cm^{-1} and the shoulder at 807 cm^{-1} are consistent with equal amounts of unmodified A- and Z-form RNA. The peaks at 674 cm^{-1} and 643 cm^{-1} correspond to guanine imidazole ring-breathing modes in unmodified A- and Z-forms, respectively. Their intensities are considerably lower than those of the corresponding peaks in the spectra of normal A- and Z_R-RNA. The narrower distribution and smaller intensity in the Z-form band at 642 cm^{-1} resembles the Z_D-RNA Raman spectrum (Tinoco et al., 1986). This band is sensitive to the glycosidic torsional angle. As observed for Z_R-RNA (Cruz et al., 1986a,b), this band is at a frequency intermediate between the right-handed B-DNA (682 cm^{-1}) and A-RNA (671 cm^{-1}), and left-handed Z-DNA (625 cm^{-1}) peaks. No indication of Z-DNA intensity at 625 cm^{-1} can be seen in Figure 6. Bands at 1194 cm^{-1}, 1264 cm^{-1} and in the 1337-1363 cm^{-1} range are consistent with both Z_R- and Z_D-RNA Raman spectra. A higher relative intensity at 1296 cm^{-1} is peculiar to the brominated RNA. This may correspond to the strong base-stacking-sensitive guanine mode at 1320 cm^{-1} in unbrominated

Z-RNA. Broadening of the band at 783 cm^{-1} toward lower wavenumbers and the presence of the 719 cm^{-1} peaks in Figure 6 are consistent with an equal representation of normal A- and Z-RNAs. Although a complete analysis of Raman assignments has not been made, the spectrum obtained is entirely consistent with a mixture of A- and Z-RNAs. Characteristics of both Z_R- and Z_D-RNA are observed, and the Raman evidence is consistent with a Z-RNA guanine glycosidic torsional conformation different from that of Z-DNA. As pointed out by Cruz et al. (1986a,b) this may be an important difference between the two left-handed forms of nucleic acid.

Recognition of Z-RNA by Anti-Z-DNA Antibodies. Figure 7A shows results from RIA and filter-binding studies which demonstrate the recognition of Br-poly[r(C-G)] by the purified rabbit polyclonal anti-Br-poly[d(C-G)] IgG preparation T4 (Zarling et al. 1984a,b) in 110 mM NaCl/Tris buffer. Unmodified A-form or single-stranded RNAs poly[r(C-G)], poly[r(A)•r(U)], poly[r(A-U)], poly[r(I-C)] and 16S/23S rRNA were not recognized under these conditions in direct binding assays. Similar data were obtained in both RIA and nitrocellulose filter binding experiments; the amount of Z-RNA bound depended on both the antibody and substrate concentrations (Figure 7A).

Figure 7B shows the results of a more extensive characterization of the recognition of Br-poly[r(C-G)] by two rabbit polyclonal anti-Br-poly[d(C-G)] IgGs, T4 and Z6, in 220 mM NaCl/Tris buffer using a second antibody-independent nitrocellulose filter-binding assay. For comparision, data obtained from parallel reactions with the Z-DNAs poly[d(br^{5}C-G)] in 220 mM NaCl, poly[d(m^{5}C-G)] in 1.5M NaCl and poly[d(C-G)] in 3M NaCl are also shown. These data show that T4 IgG has about a 50% higher affinity for Br-poly[r(C-G)] than does Z6 IgG, and that these antibodies bind ca. 10- to 50-fold tighter to Z-DNA than to Br-poly[r(C-G)]. Note that these antibodies do not require C5- or G8-bromination in order to

recognize the polyclonal antigenic determinant on Z-DNA.

Figure 8A shows the specificity in the binding of T4 IgG to Br- poly[r(C-G)] as determined by competitive filter binding assay. Since Z-DNA in the form of poly[d(br^{5}C-G] can successfully compete with radiolabeled Br-poly[r(C-G)] in binding to T4 IgG, the same antibody populations in the polyclonal preparation are probably binding to the brominated Z-RNA. The data shown in Figure 8B demonstrate that this is the same population that binds to unmodified Z-DNA in 4M NaCl/Tris buffer. Thus, both T4 and Z6 IgG populations apparently bind to unmodified Z-DNA-like determinants present in Br-poly[r(C-G)]. This provides complementary immunochemical support for the conclusion drawn from the spectroscopic analysis that chemical bromination of poly[r(C-G)] stabilizes a Z-DNA-like conformation under physiological conditions.

Figure 9 shows the effects of NaCl (A) and sodium phosphate (B) on the binding of anti-Br-poly[d(C-G)] IgGs T4 and Z6 to Br-poly[r(C-G)]. Higher NaCl concentration reduced the binding levels significantly. For example, at 1 M NaCl, the binding levels were reduced to about 80% of those obtained in the 50-100 mM NaCl range (Figure 9A). In contrast, at 220 mM NaCl, raising the sodium phosphate concentration above 10 mM reduced the binding levels to less than 70% of those obtained in the absence of the phosphate anion (Figure 9B). A direct comparison between the NaCl and sodium phosphate data at 1 M Na^+ concentration indicates that the phosphate anion is at least 2-4-fold more effective in inhibiting the binding reaction than the Cl^- ion. This general dependence on ionic strength and the specific inhibition by the phosphate anion strongly suggest the recognition of a phosphodiester backbone determinant common to the Z-forms of RNA and DNA. In addition, since binding of Z6 IgG to Br-poly[r(C-G)] is much more sensitive to the phosphate ion concentration (Figure 9B), these data suggest that Z6 IgG binding is more dependent on

recognition of phosphodiester backbone determinants.

Discussion

Since RNA was shown to adopt a left-handed conformation under the extreme conditions of 6M $NaClO_4$ (Hall et al., 1984a), a prime question has been whether this phenomenon is biologically significant (Tinoco et al., 1986; see also Usher et al., 1984; Zwieb and Ullu, 1986). Tinoco et al. (1986) suggested a possible role for Z-RNA as a molecular switch in the ribosome or in other ribonucleoprotein complexes (e.g., Grabowski et al., 1985). The first fact to be established in investigating this hypothesis is whether Z-RNA can be stabilized at physiological ionic strength.

Topological stress (ie. negative supercoiling) can effectively induce the B → Z transition in DNA (reviewed by Rich et al., 1984). Chemical bromination (Möller et al., 1984) and high ionic strength also favor the Z-form of DNA. These conditions effectively serve to alter the energetics of the polymer: bromination of guanine residues at C8 favors transition to the higher energy syn conformation, while increased ionic strength neutralizes the unfavorable ionic repulsion of backbone phosphate moieties in the Z-form. Biologically relevant ligands contacted by the polynucleotide in its natural environment (see Rich et al., 1984, pp. 828-840) may thus temper the conformation of the polynucleotides. Presumably a knowledge of the effects produced by stressed conditions on the A ↔ Z transition will help us to better understand the possible functional roles of this transition in native RNA.

The most important result presented in this study is that bromination of guanine residues, which alters the energetics of the syn ↔ anti glycosidic equilibrium, can stabilize the Z-RNA conformation in 110 mM NaCl buffer at sites proximal to the site of modification (Figures 5-8). As noted with Z-DNA

(reviewed by Rich et al., 1984), altering the energetics in one portion of the polymer can induce structural transitions in adjacent regions of the polynucleotide.

Möller et al. (1984) showed that 38% bromination of guanine residues and 18% bromination of cytosine residues in poly[r(C-G)] results in an inversion in the CD spectrum from B- to Z-form (compare with Pohl and Jovin, 1972) and in an increase in the absorbance ratio (A_{295}/A_{260}). From ^1H NMR GH8 integration data (Figure 4A) and HPLC analyses (data not shown) we estimate that 35-50% of the guanine residues, and 25-40% of the cytosine residues in Br-poly[r(C-G)] are brominated. Thus, the studies reported in this paper show that a higher degree of chemical modification is required to stabilize Z-RNA, in slow-exchange equilibrium with A-RNA (see Figures 4-6), under physiological conditions. Again, as shown by Hall et al. (1984a) for the salt-dependence of the $A \leftrightarrow Z_R$ transition in RNA, more rigorous conditions are required to stabilize Z-RNA than Z-DNA.

Two clues regarding the structural differences between Z-RNA and Z-DNA were noted in these studies (see also Cruz et al., 1986a, Tables II and III). Raman scattering evidence indicates that Z-RNA may have a different guanine glycosidic torsional angle than Z-DNA (see also Cruz et al., 1986b); CD evidence demonstrates that different structures are present in Z_R-RNA and Z-DNA. CD data indicate that in 4M $MgCl_2$, a Z-DNA-like (Z_D) structure may be present in poly[r(C-G)] (Tinoco et al., 1986). Since both Z_R- and Z_D-RNA-like characteristics were seen in the CD spectra of Br-poly[r(C-G)] in low salt buffers (Figures 2 and 3), these data may suggest heterogeneity in the population of Z-RNA forms in this polynucleotide under these conditions.

Due to their inherent specificity and sensitivity, immunoglobulins have been used as indirect probes for DNA conformations, both in solution and in cytological preparations (Stollar, 1980). This was shown to be a useful approach

in the case of Z-DNA by several investigators (Lafer et al., 1981; Malfoy and Leng, 1981; Nordheim et al., 1981; Zarling et al., 1984a). The constitutive Z-DNA Br-poly[d(C-G)] was used as the immunogen in eliciting the rabbit polyclonal anti-Z-DNA IgGs T4 and Z6 (Zarling et al., 1984a) . A variety of control experiments demonstrated that anti-Br-poly[d(C-G)] antibodies are directed against left-handed conformational determinants and not, for example, the brominated nucleoside (Zarling et al., 1984a; Nordheim et al., 1981). Thus, the extent of recognition of Z-RNA by T4 and Z6 IgGs is an indirect measure of the conformational relatedness between Z-DNA and the left-handed RNA characterized in this study.

The anti-Br-poly[d(C-G)] IgG-binding studies (Figures 7 and 8) demonstrate that some immunological (and therefore protein-binding) properties of Z-RNA are similar to those of Z-DNA under physiological conditions. Inhibition of anti-Z-DNA IgG-Z-RNA complex formation by phosphate and high ionic strength (Figure 9) strongly suggests the presence of a common phosphodiester determinant on the left-handed forms of RNA and DNA. ^{31}P NMR (Figure 5) and Raman scattering data (Figure 6) are also consistent with a zig-zag backbone conformation.

As shown in Figure 7B, T4 IgG binds more strongly and to a higher extent than Z6 IgG to C5-brominated Z-DNA. If Z-RNA has a structure similar to the crystal structure of Z-DNA (see Rich et al., 1984), the bromine atom at C5 closely contacts the guanine imidazole ring, and the edge of the hydrophobic convex surface of the polynucleotide is composed of the following group of substituents (...G2'-hydroxyl, GpC-phosphate, G8-bromine, C6-bromine...). Thus, the most obvious difference between Z-RNA and Z-DNA, the 2'-OH, is certainly at least partially responsible for the substantially lower binding affinity of the anti-Z-DNA IgGs T4 and Z6 for Z-RNA (Br-poly[r(C-G)]) when compared to Z-DNA.

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We thank John Peitso and Barbara Dengler for assistance in synthesizing poly[r(C-G)] and Kathy Mielke for typing the manuscript. We also thank Connie Calhoun for assistance with the RIA experiments and Drs. Phillip Cruz and Thomas Jovin for helpful discussions.

References

Cruz, P., Hall, K., Puglisi, J., Davis, P., Hardin, C. C., Trulson, M. O., Mathies, R., Tinoco, Jr., I., Johnson, Jr., W. C. and Neilson, T. (1986a) in Proceedings of the Fourth Symposium in Biomolecular Stereodynamics III, Adenine Press, New York, 1986.

Cruz, P., Hall, K., Leonhardt, E., Puglisi, Jr., Trulson, M. O. and Tinoco, Jr., I. (1986b) Biophys. J. 49, 425a.

Drew, H. R. and Dickerson, R. E. (1981) J. Mol. Biol. 152, 723-736.

Grabowski, P. J., Seiler, S. R. and Sharp, P. A. (1985) Cell 42, 345-353.

Gray, D. M., Liu, J.-J., Ratliff, R. L. and Allen, F. S. (1981) Biopolymers 20, 1337-1382.

Hall, K., Cruz, P., Tinoco, Jr., I. Jovin, T. M. and van de Sande, J. H. (1984a) Nature (London) 311, 584-586.

Hall, K., Cruz, P. and Chamberlin, M. J. (1985b) Arch. Biochem. Biophys. 236, 47.

Jovin, T. M., McIntosh, L. P. Arndt-Jovin, D. J., Zarling, D. A., Robert-Nicoud, M., van de Sande, J. H., Jorgenson, K. F. and Eckstein, F. (1983) J. Biomol. Struct. Dynam. 1, 21-57.

Kalk, A. and Berendsen, H. J. C. (1976) J. Mag. Resonance 24, 343-366.

Lafer, E. M., Möller, A., Nordheim, A., Stollar, B. D. and Rich, A. (1981) Proc. Nat. Acad. Sci., USA 78, 3546-3550.

Leng, M. (1985) Biochim. Biophys. Acta 825, 339-344.

Malfoy, B. and Leng, M. (1981) FEBS Letters 132, 45-48.

Möller, A., Nordheim, A., Kozlowski, S. A., Patel, D. J. and Rich, A. (1984) Biochemistry 23, 54-62.

Noggle, J. H. and Schirmer, R. E. (1971) in The Nuclear Overhauser Effect,

Academic Press, New York, NY.

Nordheim, A., Pardue, M. L., Lafer, E. M., Stollar, B. D. and Rich, A. (1981) Nature (London) 294, 417-422.

Pohl, F. M. and Jovin, T. M. (1972) J. Mol. Biol. 67, 375-396.

Riazance, J. H., Baase, W. A., Johnson, Jr., W. C., Hall, K., Cruz, P. and Tinoco, Jr., I. (1985) Nucl. Acids Res. 13, 4983-4989.

Rich, A., Nordheim, A. and Wang, A. H.-J. (1984) Annu. Rev. Biochem. 53, 791-846.

Robert-Nicoud, M., Arndt-Jovin, T. J., Zarling, D. A. and Jovin, T. M. (1984) The EMBO Journal 3, 721-731.

Silberklang, M., Gillum, A. M. and RajBhandary, U. L. (1979) Meth. Enzymol. 59, 58.

Solomon, I. (1955) Phys. Rev. 99, 559-565.

Stollar, B. D. (1980) Immunochem. Tech. 70, 70-85.

Thamann, J. J., Lord, R. C., Wang, A. H.-J. and Rich, A. (1981) Nucl. Acids Res. 9, 5443-5457.

Tinoco, Jr., I., Cruz, P., Davis, P., Hall, K., Hardin, C. C., Mathies, R. A., Puglisi, J. D., Trulson, M. O., Johnson, W. C. and Neilson, T. (1986) in Structure and Dynamics of RNA, Plenum Press.

Uesugi, S., Ohkubo, M., Urata, H., Ikehara, M., Kobayashi, Y. and Kyogoku, Y. (1984) J. Am. Chem. Soc. 106, 3675-3676.

Usher, D. A., Profy, A. T., Walstrum, S. A., Needels, M. C., Bulack, S. C., and Lo, K. M. (1984) Origins of Life 14, 621-628.

Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G. and Rich, A. (1979) Nature (London) 282, 680-686.

Zarling, D. A., Arndt-Jovin, D. J., Robert-Nicoud, M., McIntosh, L. P., Thomas, R. and Jovin, T. M. (1984a) J. Mol. Biol. 176, 369-415.

Zarling, D. A., Arndt-Jovin, D. J., McIntosh, L. P., Robert-Nicoud, M. and Jovin, T. M. (1984b) J. Biomol. Struct. Dynam. 1, 1081-1107.

Zwieb, C. and Ullu, E. (1986) Nucl. Acids Res. 14, 4639-4657.

Figure Legends

Figure 1 - (A.) Circular dichroism spectra of poly[r(C-G)] in the A-form (-----) and brominated poly[r(C-G)] obtained using bromine/nucleotide ratios of 1.0 (-•-•-•), 2.0(••••) and 3.0(——), respectively, in 45°C reaction mixtures, in 10 mM sodium phosphate (pH 7.0), 6M $NaClO_4$ and 1 mM EDTA at 22°C. (B.) Absorbance ratios A_{295}/A_{260} plotted versus the bromine/nucleotide ratio in the 45°C (○) and 55°C (△) reaction mixtures. Conditions were the same as those listed in (A).

Figure 2 - (A.) (----) Circular dichroism spectrum of brominated poly[r(C-G)] in 10 mM sodium phosphate (pH 7.0), 2M $NaClO_4$ and 1 mM EDTA at 22°C. The bromine/nucleotide ratio was 3.0 in a buffered 6M $NaClO_4$ reaction mixture maintained at 45°C for 10 min. (----) CD spectrum was obtained from a more extensively brominated sample of poly[r(C-G)] in 10 mM sodium phosphate (pH 7.0), 6M NaBr and 1 mM EDTA at 22°C. The bromine/nucleotide ratio was 3.5 in a 6M NaBr reaction mixture maintained at 55°C for 10 min. (B.) Effects of NaBr concentration on the CD spectrum of brominated poly[r(C-G)] in 1 mM sodium phosphate (pH 7.0) and 0.1 mM EDTA at 22°C. Conditions labeled on the abscissa are as follows: (0) following extensive dialysis into H_2O, (✱) after addition of concentrated sodium phosphate (pH 7.0)/EDTA solution to yield 0.5 mM sodium phosphate (pH 7.0), 0.05 mM EDTA and (✱) 1 mM sodium phosphate (pH 7.0), 0.1 mM EDTA. Ellipticities were

obtained at 230 nm (◇), 263 nm (□), 280 nm (Δ) and 296 nm (○) upon dissolving lyophilized aliquots from a 6M NaBr solution in the sample. (✱) Ellipticities obtained upon raising the temperature to 60°C in 1 mM sodium phosphate (pH 7.0), 6M NaBr and 0.1 mM EDTA. For reference, standard ellipticities obtained from A-RNA and Z_R-RNA are plotted at the low and high NaBr concentration extremes of the plot, respectively.

Figure 3 - Circular dichroism spectra of extensively brominated poly[r(C-G)] (----) and poly[d(C-G)] (----) in 10 mM sodium phosphate (pH 7.0), 110 mM NaCl and 1 mM EDTA at 22°C. The concentration was approximately 0.2 mM in nucleotides. The brominated DNA is known to adopt the Z conformation under these conditions.

Figure 4 - (A.) and (B.) 500 MHz ^{1}H NMR spectrum of extensively brominated poly[r(C-G)] in 10 mM sodium phosphate (pH 7.0), 110 mM NaBr and 0.5 mM EDTA at 37°C. The concentration was approximately 55 mM in nucleotides. ^{1}H NOE difference spectra obtained on preirradiating resonances at (C.) 7.13 ppm and (D.) 7.79 ppm. The spectra were obtained in an interleaved experiment (256 scans, 8 scans/cycle) included an off-resonance preirradiation control (ν_{irrad}=6.79 ppm). A digital linebroadening of 2 Hz was applied prior to Fourier transforming the NOE difference FIDs. Chemical shifts are reported relative to internal standard TSP.

Figure 5 - (A.) 202 MHz ^{31}P NMR spectrum of Br-poly[r(C-G)] in 10 mM sodium phosphate (pH 7.0), 110 mM NaBr, and 0.5 mM EDTA at 37°C. A digital linebroadening of 8 Hz was applied prior to Fourier transforming the FID. Chemical shifts are reported relative to internal standard TMP. (B.) 121 MHz ^{31}P NMR spectrum of the same sample described in (A.). A- and Z_R-form ^{31}P chemical shifts for poly[r(C-G)] (Hall et al., 1984) are also shown in the figure.

Figure 6 - Raman spectrum of Br-poly[r(C-G)] in 10mM sodium phosphate (pH 7.0), 100mM NaCl. The spectral resolution was 6 cm^{-1}; the spectrum was the average of ca. 10-12 hours of accumulation.

Figure 7 -Binding of anti-Br-poly[d(C-G)] polyclonal IgGs to [^{32}P]Br-poly[r(C-G)] in 40 mM Tris-HCl (pH 7.5), 110 mM NaCl and 4 mM EDTA. (A) T4 IgG dilutions were tested by RIA using goat anti-rabbit IgG as second antibody as described in Methods. Second antibody-independent nitrocellulose filter-binding assay results demonstrating recognition of Br-poly[r(C-G)] by T4 IgG are also shown. Poly[r(C-G)] (A-RNA,*) and poly[d(C-G)] (B-DNA) were not recognized under these conditions. Specific anti-Z-RNA IgG recognition was verified in both types of assay using the IgG fraction of normal rabbit serum (NR IgG→) as first antibody. Background levels were obtained by adding 5 µl buffer to the preincubated polynucleotide solutions in place of antibody (→). (B) Filter-binding assay results comparing the recognition of Z-RNA and Z-DNA by anti-Br-poly[d(C-G)] IgGs T4 and Z6. Dilution curves

are labeled as follows: Recognition of Br-poly[r(C-G)] in 220 mM NaCl/buffer by (a) T4 and (b)Z6 IgGs; (c) poly[d(br^{5}C-G)] in 220 mM NaCl/buffer, (d) poly[d(m^{5}C-G)] in 1.5 M NaCl/buffer and (e) poly[d(C-G)] in 3 M NaCl/buffer by T4 IgG; (f) poly[d(br^{5}C-G)] in 220 mM NaCl/buffer and (g) poly[d(m^{5}C-G)] in 1.5 M NaCl/buffer by Z6 IgG.

Figure 8 - Specificity in the binding of anti-Br-poly[d(C-G)] IgGs T4 and Z6 to [^{32}P]Br-poly[r(C-G)] in 220 mM NaCl (Z-RNA) and [^{32}P]poly[d(C-G)] in 4 M NaCl (Z-DNA). (A) Filter-binding competition assay results obtained by preincubating 2.5 ng ^{32}P-labeled and 17.5 ng unlabeled polynucleotide in 40 mM Tris-HCl (pH 7.5), 4 mM EDTA with the specified amounts of unlabeled competitor polynucleotide prior to addition of T4 IgG. Polynucleotides were as follows: Z-RNA, Br-poly[r(C-G)]; Z-DNA, poly[d(br^{5}C-G); A-RNA, poly[r(C-G)]; B-DNA, poly[d(C-G)]. A-RNA (◇, ◆) and B-DNA (△, ▲) did not compete with Z-RNA or Z-DNA in these assays. Due to the considerably reduced affinity of T4 IgG for Z-RNA, Br-poly[r(C-G)] does not compete with Z-DNA at the competitor levels tested in these assays (●). (B) Results obtained by preincubating Br-poly[r(C-G)] (2.5 ng ^{32}P- and 17.5 ng unlabeled) with unlabeled, unmodified poly[d(C-G)] Z-DNA competitor in 4 M NaCl/buffer prior to adding T4 or Z6 IgGs.

Figure 9 - Effects of sodium and phosphate ions on the binding of anti-Br-poly[d(C-G)] antibodies to Br-poly[r(C-g)] (Z-RNA) in 40 mM Tris-HCl (pH 7.5), 4 mM EDTA. 2.5 ng ^{32}P-labeled and 17.5 ng

unlabeled Br-poly[r(C-G)] were incubated with 5 µl T4 (○) or Z6 (△) IgG at the specified NaCl (A) or sodium phosphate (B) concentrations. Filter binding assays were performed as described in Methods. The initial NaCl concentration was 220 mM in the phosphate inhibition experiments.

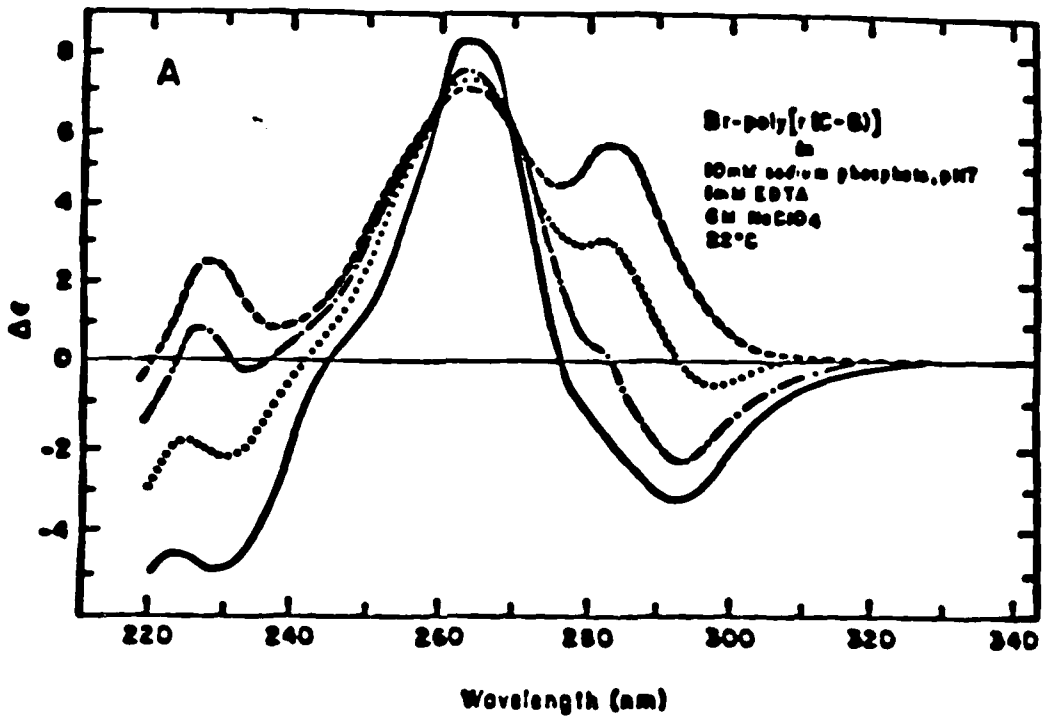
Table I - NOEs Observed in the ^{1}H NMR Spectrum of Br-poly[r(C-G)].

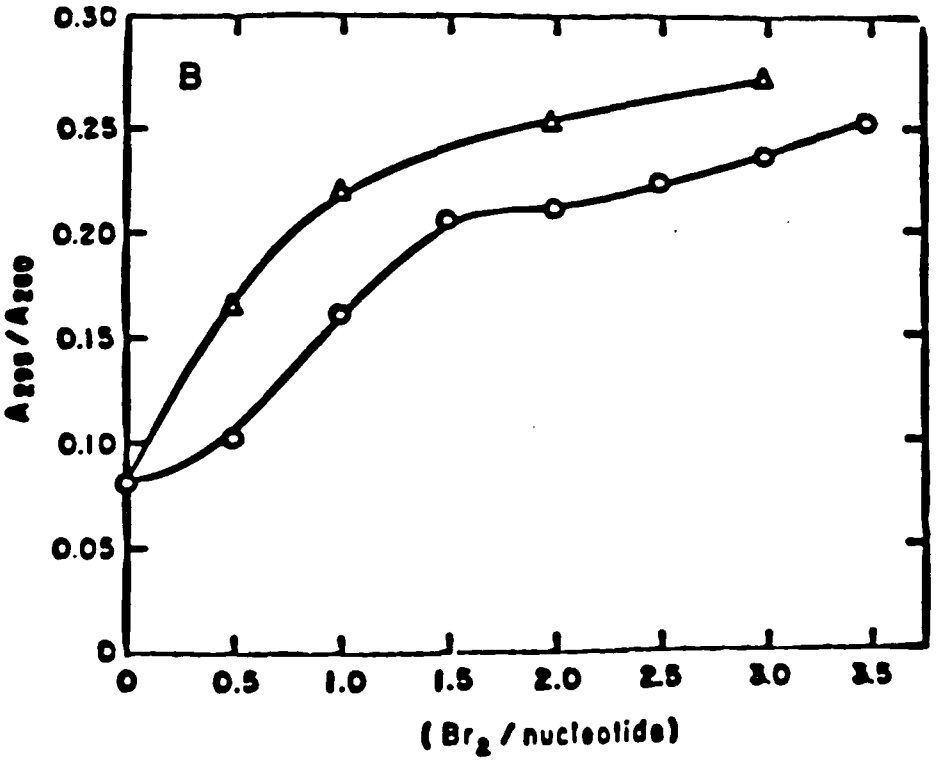
Preirradiation frequency (ppm)	NOE frequency (ppm)	%NOE[a] 500 msec preirradiation	%NOE[a] 200 msec preirradiation	NOE[b] contact
7.79 (GH8Z)	7.13	<5%	<5%	GH8Z→CH6Z
	5.83	30%	25%	→GH1'Z
	5.65	<15%	<10%	→CH1'Z
7.59 (CH6A)	5.65	20%	<10%	CH6A→GH1'Z(*[c])
	5.44	15%		→CH1'A
	5.07	30%	30%	→CH5A
7.42 (GH8A)	5.65	15%	<10%	GH8A→CH1'Z(*[c])
	5.44	15%	<10%	→CH1'A
	5.07	20%	<15%	→CH5A
7.13 (CH6Z)	5.92	<10%	<5%	CH6Z→GH1'A(*[c])
	5.83	<10%	<5%	→GH1'Z
	5.65	20%	<15%	→CH1'Z
	4.98	30%	30%	→CH5Z

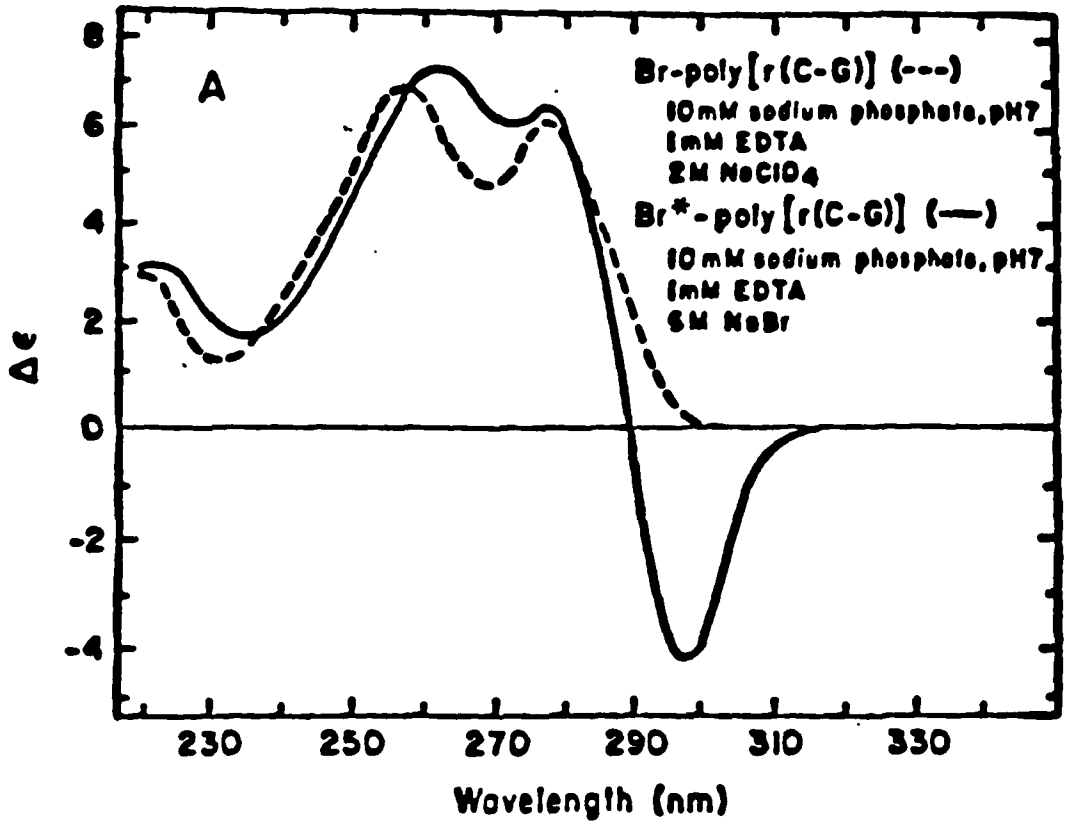
[a]Percentages accurate to ±5%.

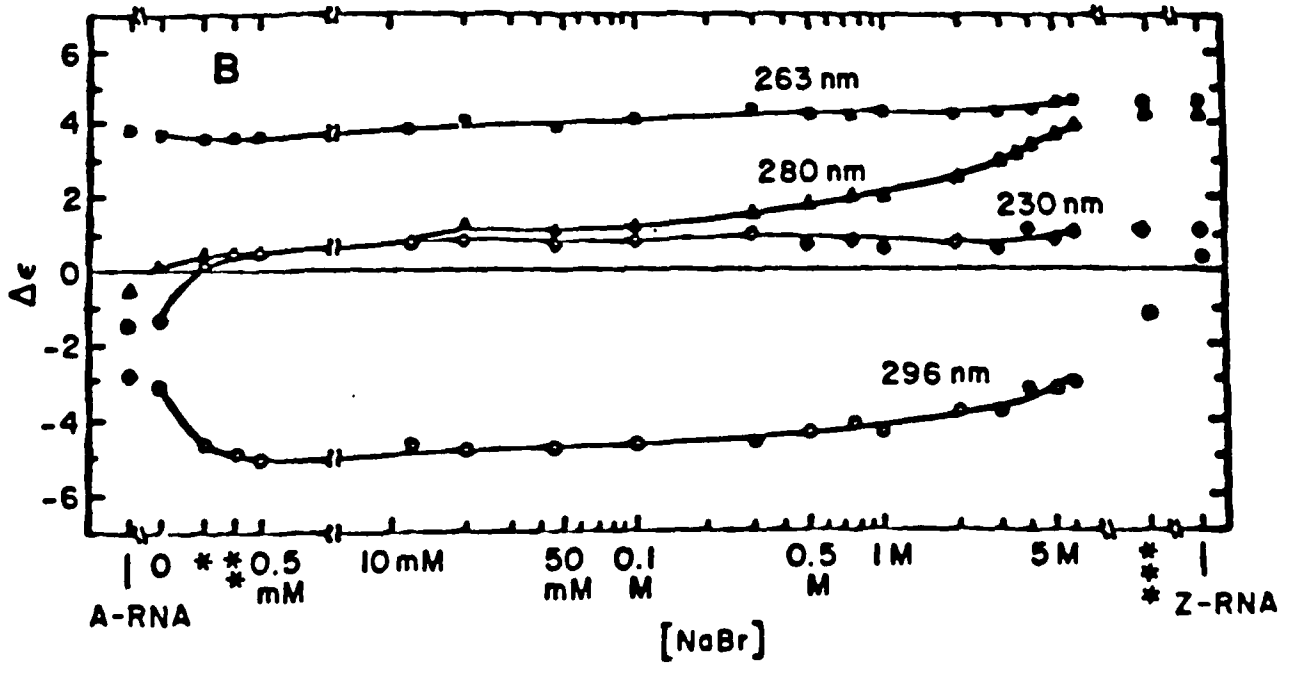
[b]Proposed NOE-contacted atom.

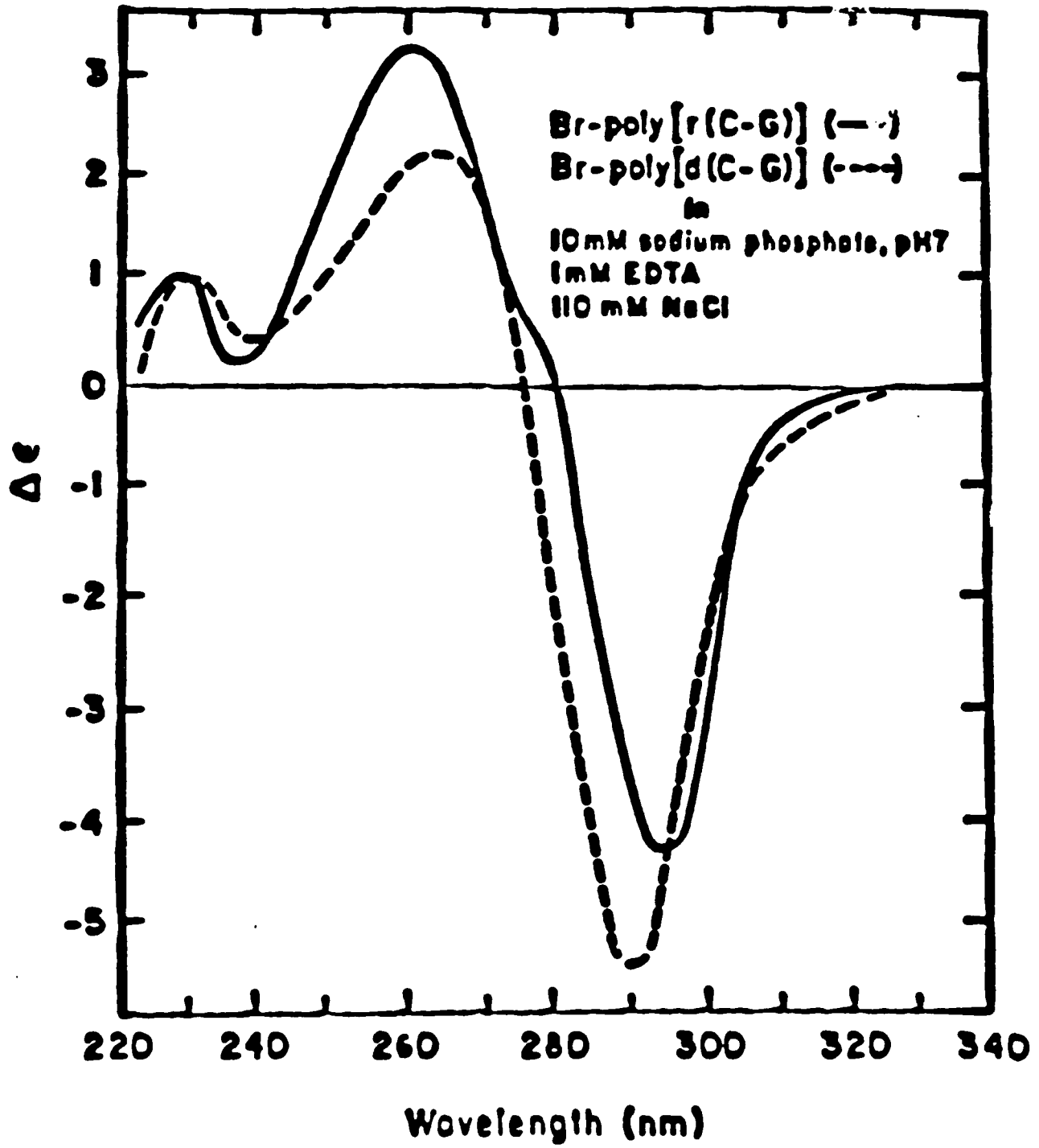
[c]NOEs that were inconsistent with the other assigned NOE contacts are denoted by asterisk. It is not known if these effects are physically significant (ie. exchange NOEs) or are due to subtraction errors (all are small % NOEs).










Br-poly [r(C-G)] (—)
Br-poly[d(C-G)] (- - -)
in
10mM sodium phosphate, pH7
1mM EDTA
110 mM NaCl
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3
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0
-1
-2
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220
240
260
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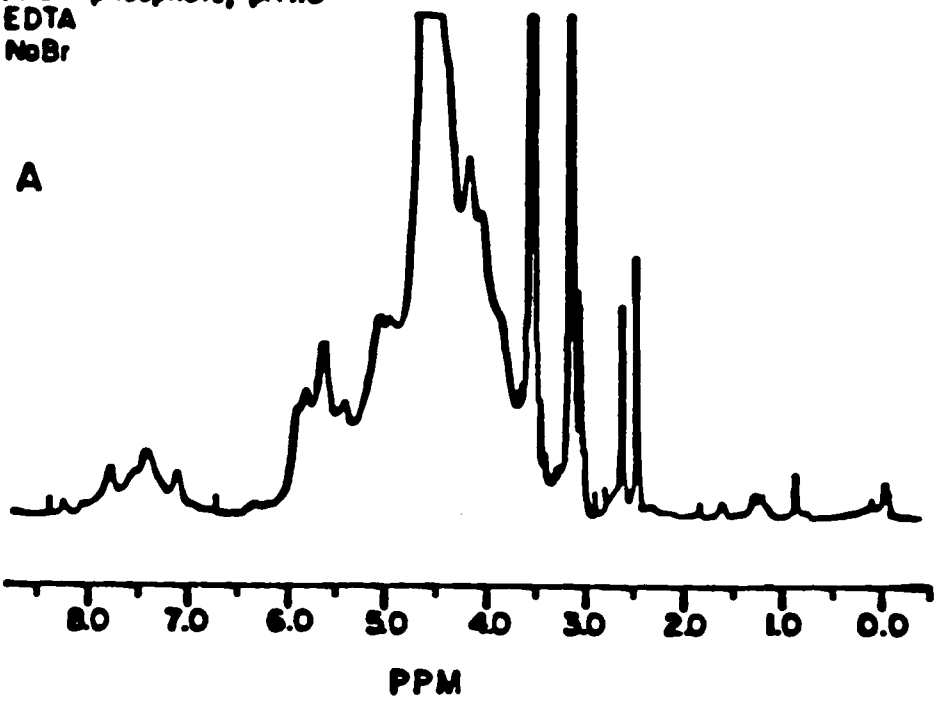
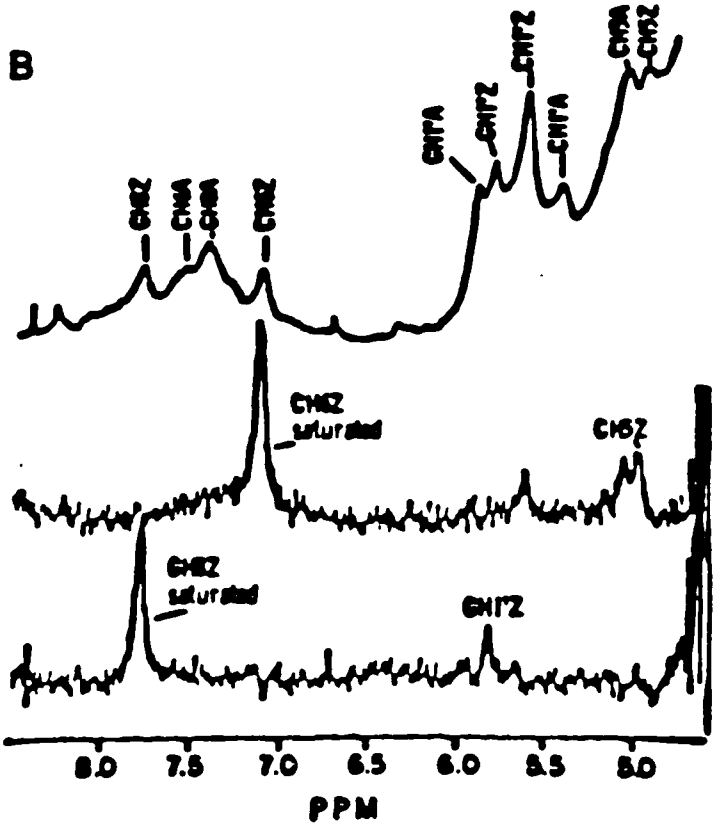
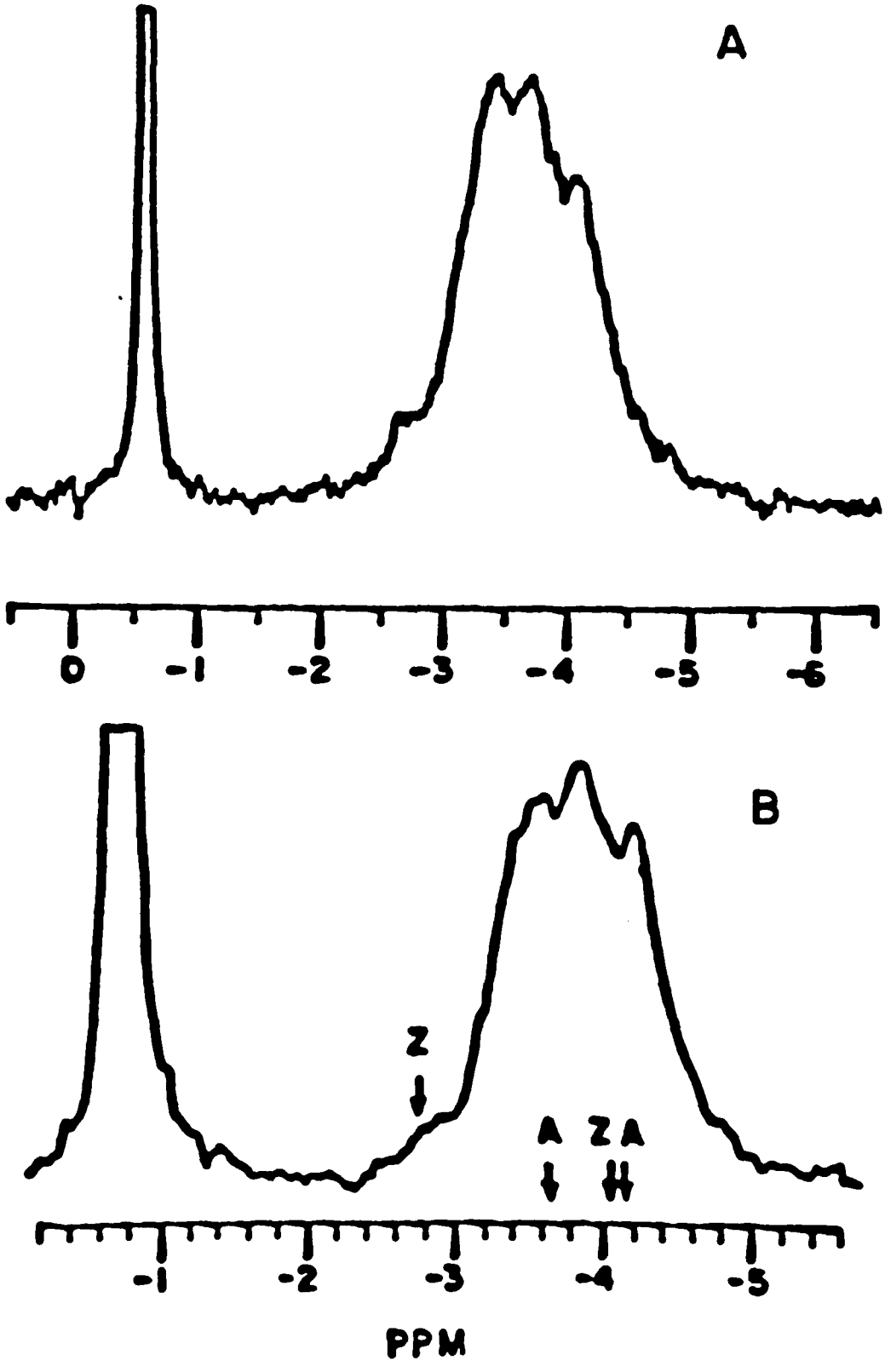
300
320
340
Wavelength (nm)

Br-poly [r(C-G)] in
10mM sodium phosphate, pH7.0
0.1 mM EDTA
110 mM NaBr



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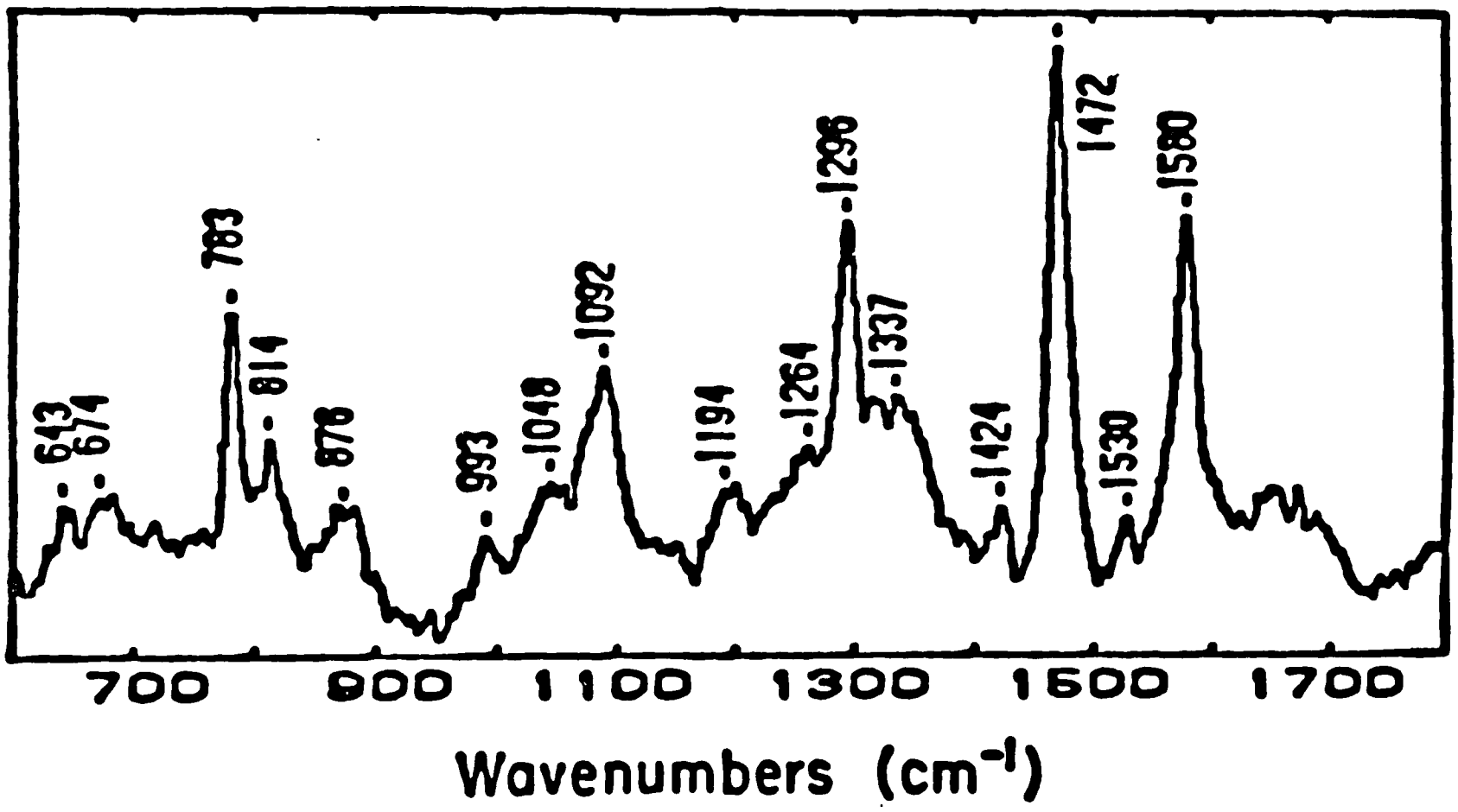
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1600
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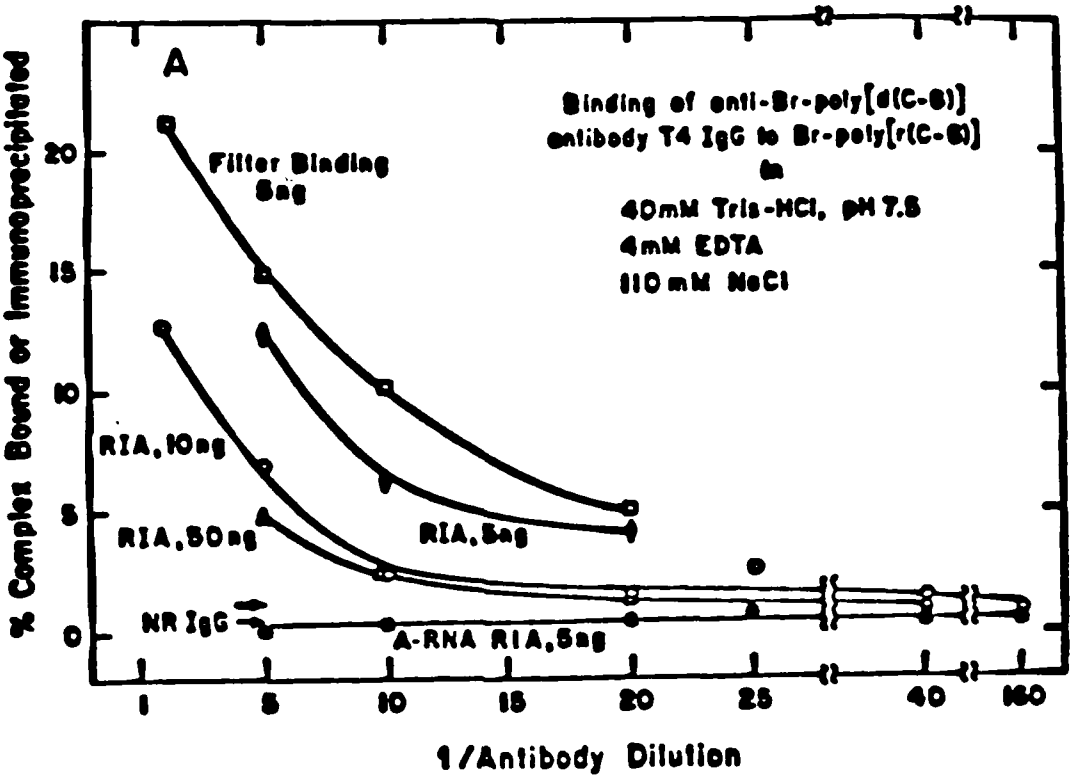
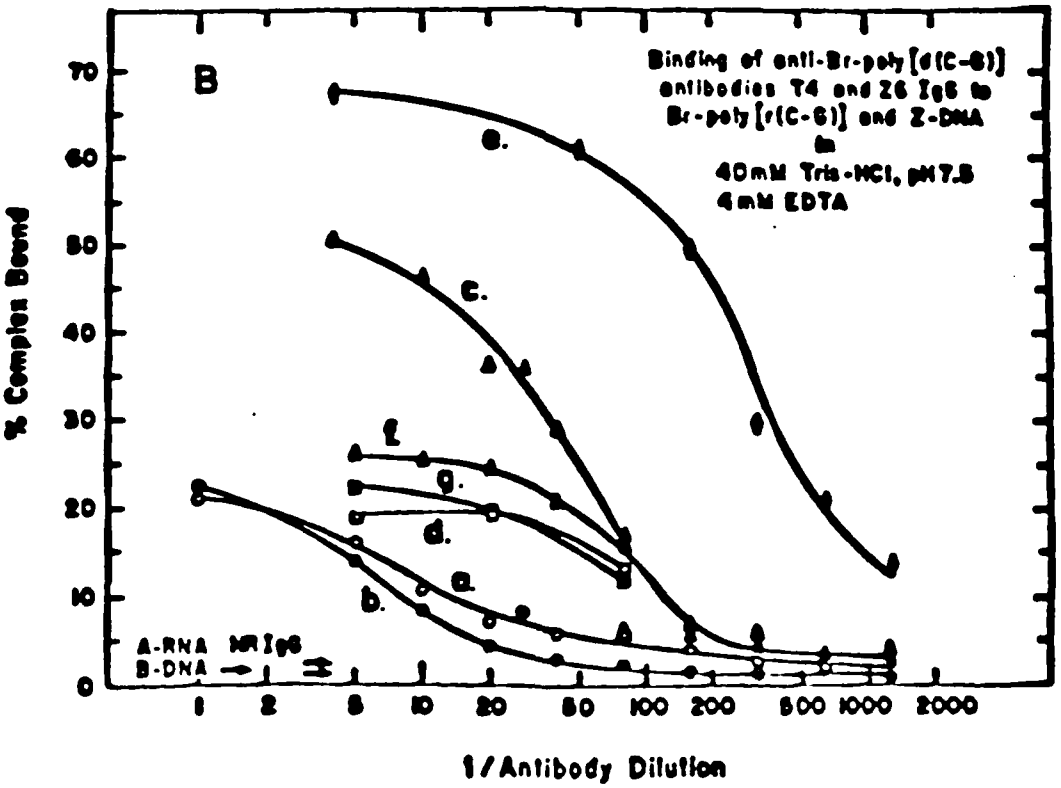
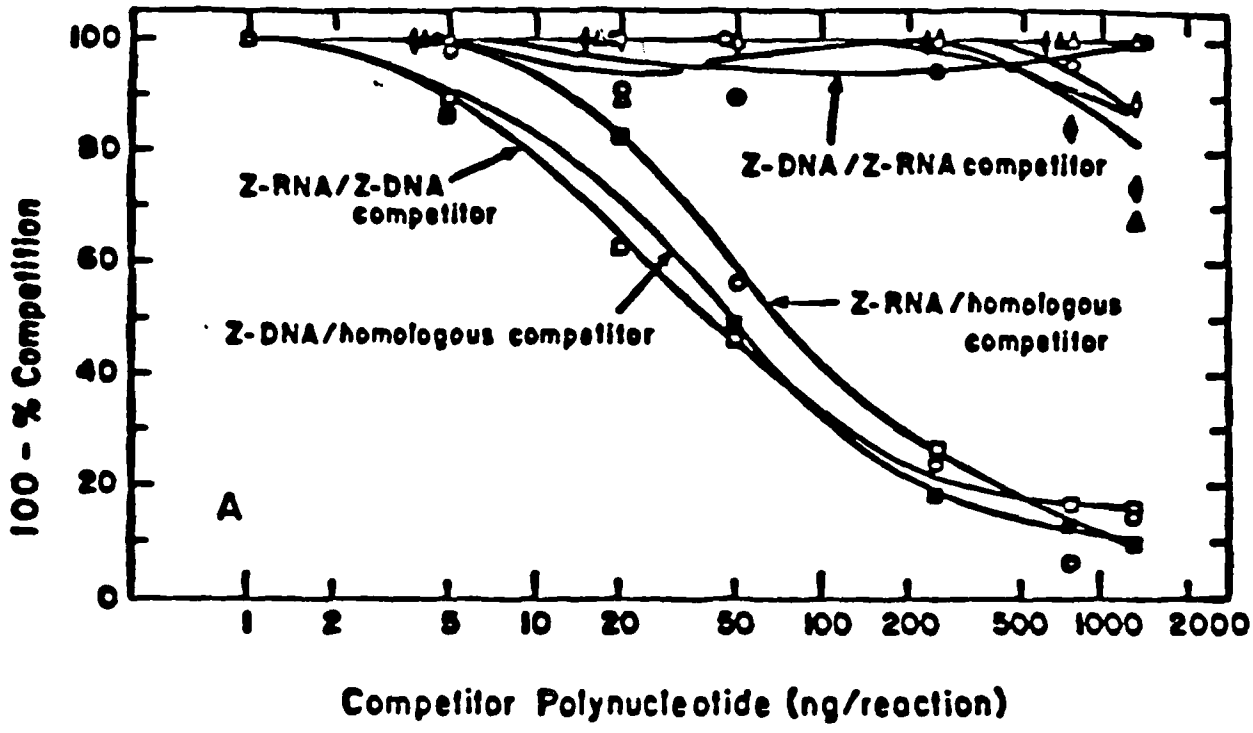
Wavenumbers (cm⁻¹)



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80
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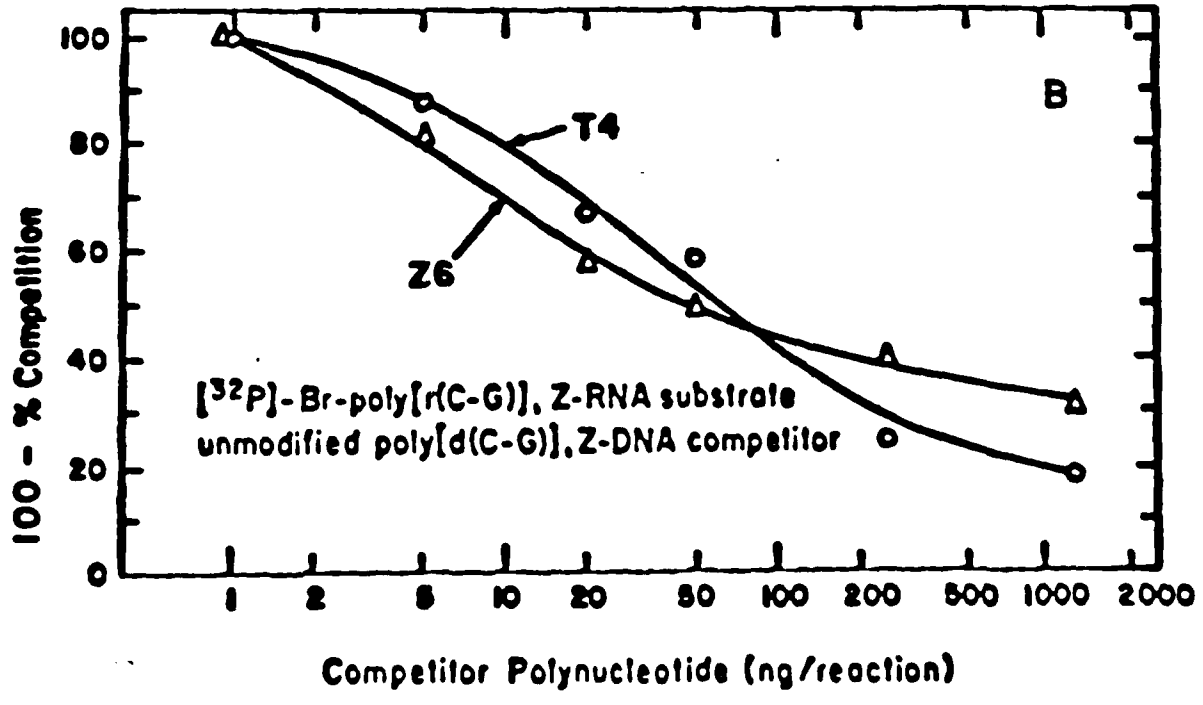
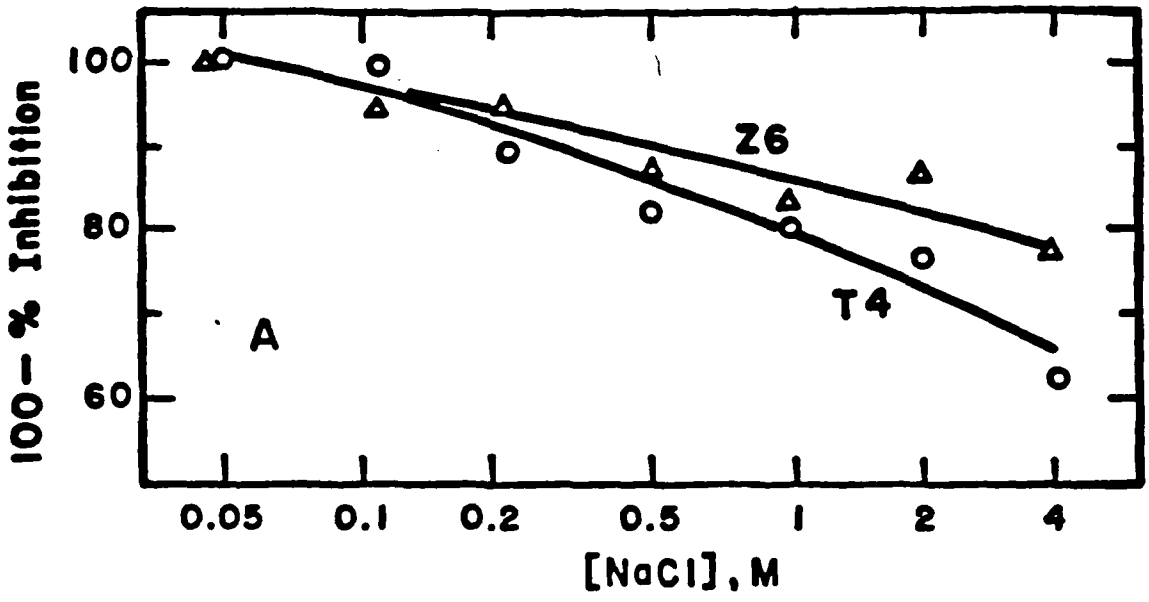
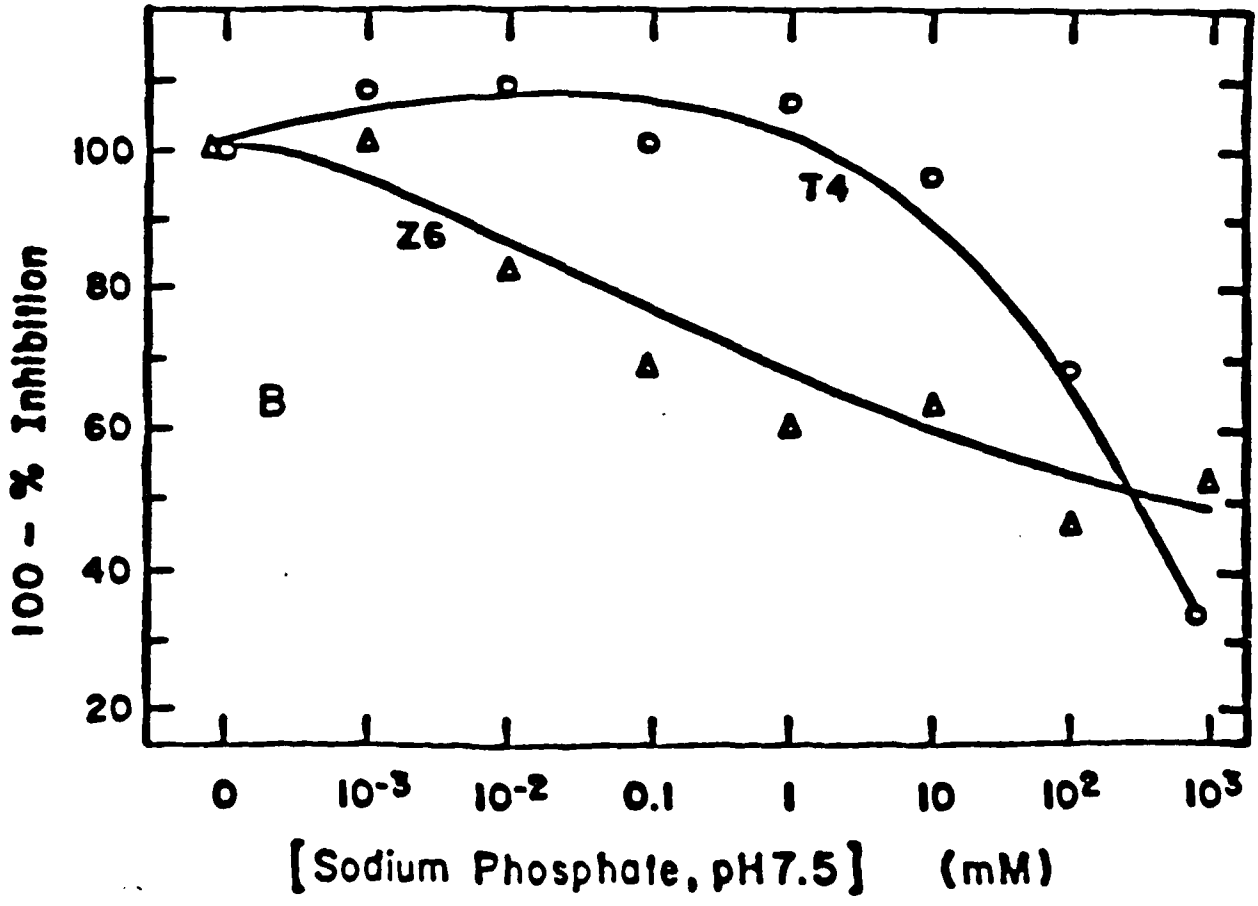
40
20
0
100 - % Competition
Z-RNA/Z-DNA competitor
Z-DNA/Z-RNA competitor
Z-RNA/homologous competitor
Z-DNA/homologous competitor
A
1
2
5
10
20
50
100
200
500
1000
2000
Competitor Polynucleotide (ng/reaction)


100
80
60
40
20
0
100 - % Competition
B
T4
Z6
[32P]-Br-poly[r(C-G)], Z-RNA substrate
unmodified poly[d(C-G)], Z-DNA competitor
1
2
5
10
20
50
100
200
500
1000
2000
Competitor Polynucleotide (ng/reaction)


100
80
60
100 - % Inhibition
Z6
T4
A
0.05
0.1
0.2
0.5
1
2
4
[NaCl], M


100
80
60
40
20
100 - % Inhibition
T4
Z6
B
0
10^{-3}
10^{-2}
0.1
1
10
10^{2}
10^{3}
[Sodium Phosphate, pH7.5] (mM)

END

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DTIC