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Right-handed and left-handed DNA: Studies of B- and Z-DNA by using proton nuclear Overhauser effect and P NMR

[poly(dG-dC)/poly(dG-m^{5}dC)/glycosidic torsion angles/phosphodiester backbone/thermal stability]

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ABSTRACT **We have differentiated between *syn* and *anti* glycosidic torsion angles in nucleic acid duplexes by measuring the transient nuclear Overhauser effect (NOE) between the sugar H-1′ protons and the purine H-8 and pyrimidine H-6 base protons. The transient NOE measurements demonstrate a *syn* glycosidic torsion angle at guanosine and an *anti* glycosidic torsion angle at cytidine in poly(dG-dC) in 4 M NaCl and in poly(dG-m^{5}dC) in 1.5 M NaCl solution. These features have been observed previously in the left-handed Z-DNA conformation of (dC-dG)$_3$ in the crystalline state. By contrast, transient NOE studies demonstrate that both guanosine and cytidine residues adopt the *anti* conformation about the glycosidic bond for the right-handed poly(dG-dC) and poly(dG-m^{5}dC) conformation in a low-salt solution. We have used P NMR to monitor the equilibrium between B- and Z-DNA forms of poly(dG-dC) in LiCl solutions; at high temperatures, the equilibrium shifts from B- to Z-DNA.**

Using circular dichroism studies, Pohl and Jovin (1) demonstrated that poly(dG-dC) undergoes a salt-dependent conformational change characterized by a spectral inversion in high-salt solution; the midpoint for the conformational change occurs at 2.5 M NaCl or 0.7 M $MgCl_2$. H and P NMR studies demonstrated that the (dG-dC)$_8$ duplex in high-salt solution contained two different types of nucleotide and P conformations, including a set of glycosidic torsion angles and phosphodiester linkages that were different from B-DNA (2, 3). The x-ray structure of (dC-dG)$_3$ has been solved to atomic resolution in the crystalline state (4, 5). The self-complementary hexamer forms a six-base-pair left-handed duplex with a dinucleotide repeat (4). The base pairing is of the Watson–Crick type, and the guanosines and cytidines have *syn* and *anti* glycosidic torsion angles, respectively (Fig. 1) (4). The dC-dG and dG-dC phosphodiester linkages have different torsion angles resulting in a zigzag arrangement of the backbone (4) called Z-DNA. The same left-handed duplex has been observed in crystals of (dC-dG)$_2$ (6, 7) and in polynucleotide fibers (8). In solution, the Raman spectrum of the high- and low-salt forms of poly(dG-dC) differ from each other (9). Recent Raman studies show that the spectrum of crystalline (dC-dG)$_3$ is the same as that of poly(dC-dG) in high-salt solution (10); thus, the conformations are the same.

In this paper we demonstrate the application of proton nuclear Overhauser effects (NOEs) in elucidating specific features of the conformation of DNA in solution. The NOE corresponds to a change in the intensity of a given spin on saturation of a nearby dipolar coupled spin. The magnitude and sign of NOE signals between proton spins depends on the inverse sixth power of the interproton distance and the frequency of motions of the spins (11–14). When the saturated and observed proton spins are in the vicinity of other proton spins, the transfer of saturation can disperse throughout the system giving rise to spin diffusion and loss of selectivity. This problem can be partially overcome by limiting the time duration of the saturation pulse. Such transient NOE measurements have been applied successfully to elucidation of structural features in solution of the proteins trypsin inhibitor and lysozyme (15, 16). These studies showed that NOEs were ≈2% at an interproton distance of 3.5 Å and increased to >15% at <2.0 Å. Redfield *et al.* (17) were the first to use NOE to measure base–base proton distances in secondary and tertiary base pairs of tRNA.

Proton NOE measurements have been made on nucleosides and nucleotides in organic and aqueous solvents (18, 19). These

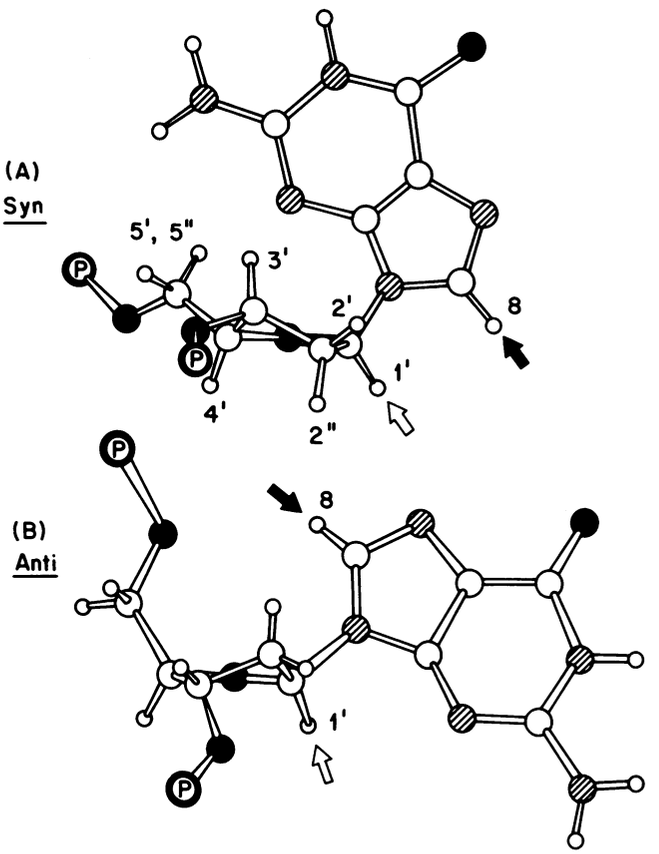

FIG. 1. Schematic representation of the *syn* and *anti* orientations about the guanosine glycosidic bond. The internuclear distance between the H-8 and H-1′ protons is 2.2 Å in the *syn* conformation and 3.75 Å in the *anti* conformation. The sugar pucker is C-3′ *endo* in *A* and C-2′ *endo* in *B*.

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Abbreviations: NOE, nuclear Overhauser effect; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate.

efforts focused on the distribution of the glycosidic torsion angles relating the base and sugar rings (Fig. 1) by making NOE measurements between protons on these residues. This demonstrated that guanosine phosphates are flexible in solution and adopt the *syn* conformation a large part of the time (19). In the present work, we have made transient proton NOE measurements on poly(dG-dC) and its methylated derivative poly(dG-m^5dC) (20) in low- and high-salt solution to deduce the *syn* and *anti* nature of the glycosidic torsion angles in both right-handed and left-handed DNAs in solution. The salt-dependent B-DNA-to-Z-DNA transition can also be measured by P NMR. We have measured the temperature-dependent equilibrium between these forms in 4 M LiCl. This yielded the novel result that the Z form is more stable at the higher temperature.

EXPERIMENTAL

NMR Spectroscopy. Transient proton NOE measurements were made at 498 MHz in the interleave mode with a 25-msec presaturation pulse. Difference spectra were recorded by subtracting the free induction decays prior to Fourier transformation. The 81-MHz phosphorus spectra were recorded with proton broad band decoupling on a Varian XL-200 spectrometer and are referenced relative to an internal standard of trimethyl phosphate.

Materials. Poly(dG-dC) was purchased from P-L Biochemicals; poly(dG-m^5dC) was prepared enzymatically as described (20). The polymers were sheared by using a Heat Systems model W225R Ultrasonics sonicator (21) with a 1/2-inch (1.25 cm) tip attached to the coupler section. Up to 300 A_{260} units of the DNA was dissolved in 7.5 ml of 10 mM Tris·HCl/1 mM EDTA, pH 8.0, and sonicated for 1 hr (50% duty cycle pulse control and position 6 output control setting) between 0 and 10°C. The sonication yielded DNA fragments with an average length of 150 ± 100 base pairs as measured by acrylamide gel electrophoresis.

Poly(dG-m^5dC) was further purified from unmethylated template DNA by DNase I digestion. After polymerization of poly(dG-m^5dC), the mixture was heated to 60°C for 10 min in 10 mM Tris·HCl, pH 8.0/50 mM NaCl/10 mM $MgCl_2$. Under these conditions, poly(dG-m^5dC) forms Z-DNA (20), but the unmethylated template DNA does not and is preferentially digested by DNase I (20 μg of enzyme, 60 min at 37°C for 50 A_{200} units of the polymerization mixture). The digestion products were phenol extracted and then separated by column chromatography.

RESULTS AND DISCUSSION

Proton Spectra. The 498-MHz H NMR spectra of the poly(dG-dC) duplex in the presence and absence of 4 M NaCl at 45°C are presented in Figs. 2*A* and 3*A*, respectively. We had previously identified the base protons (2) but were unable to distinguish among the sugar protons corresponding to the guanosine and cytidine residues in the low- and high-salt spectra. Resolution of this assignment ambiguity becomes more interesting because one of the sugar H-1′ resonances shifted dramatically on proceeding from low-salt (Fig. 3*A*) to high-salt (Fig. 2*A*). We used transient NOE measurements to probe spin–spin interactions between adjacent protons on the base and sugar residues of poly(dG-dC) in 4 M NaCl at 45°C (Fig. 2). The difference spectrum after saturation of the cytidine H-6 resonance at 7.25 ppm exhibited an ≈48% negative NOE for the resonance at 5.14 ppm (Fig. 2*C*). The cytidine H-5 is the closest proton to cytidine H-6. This permits an unambiguous assignment of the 5.14-ppm resonance to cytidine H-5 in the high-salt poly(dG-dC) solution. Chemical shifts are listed in Table 1. This assignment is verified by the observation of a negative NOE at the cytidine H-6 resonance on saturation of the cytidine H-5 resonance under the same experimental conditions.

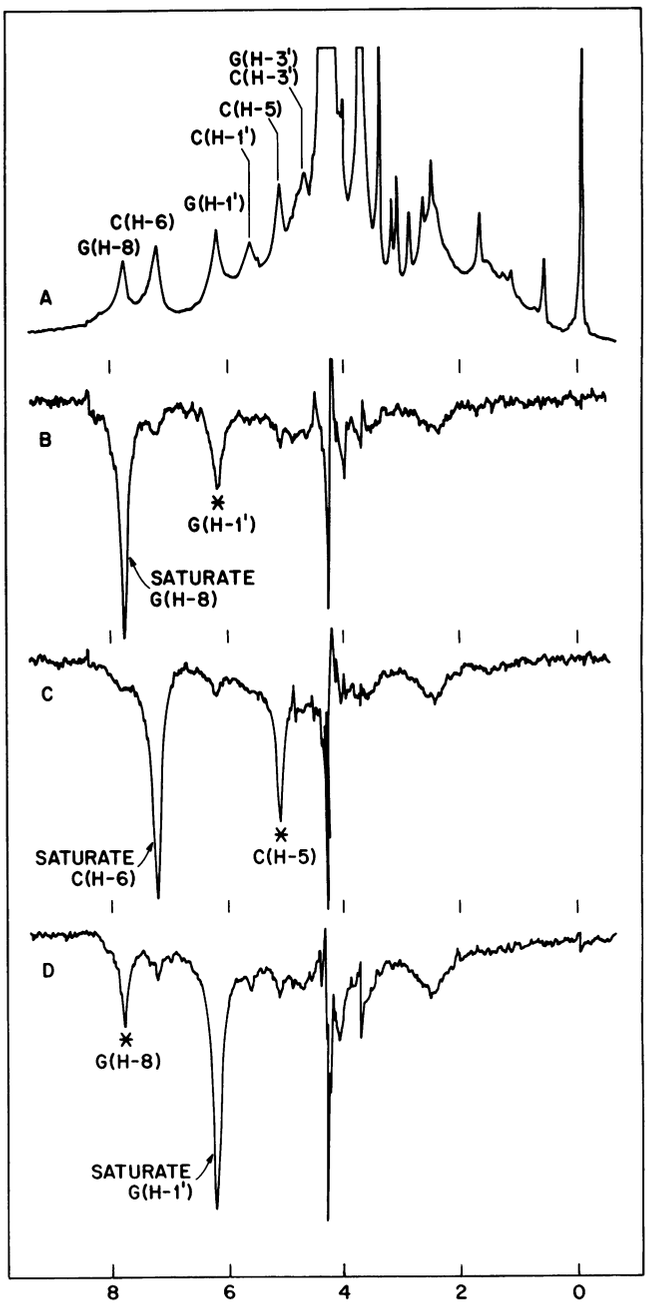

FIG. 2. Transient NOE measurements on poly(dG-dC) in 4 M NaCl/10 mM cacodylate/1 mM EDTA, pH 6.52, in ^2H_2O at 45°C. (*A*) The 498 MHz H spectrum between 0 and 9 ppm. The narrow resonances between 0 and 4 ppm correspond to EDTA and standard 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The strong resonance at ≈4.2 ppm corresponds to residual HO^2H from solvent. (*B*) Difference spectrum after saturation of the guanosine H-8 resonance at 7.81 ppm. A negative NOE enhancement is observed at the 6.21-ppm sugar H-1′ proton designated by an asterisk. (*C*) Difference spectrum after saturation of the cytidine H-6 resonance at 7.25 ppm. A negative NOE enhancement is observed at the 5.14-ppm cytidine H-5 resonance designated by an asterisk. (*D*) Difference spectrum after saturation of the 6.21-ppm sugar H-1′ resonance. A negative NOE enhancement is observed at the 7.81-ppm guanosine H-8 resonance designated by an asterisk. The signal-to-noise ratios of all spectra were improved by applying a 5-Hz exponential line-broadening contribution.

The difference spectrum after saturation of the 7.81-ppm guanosine H-8 proton exhibited an ≈37% negative NOE for the

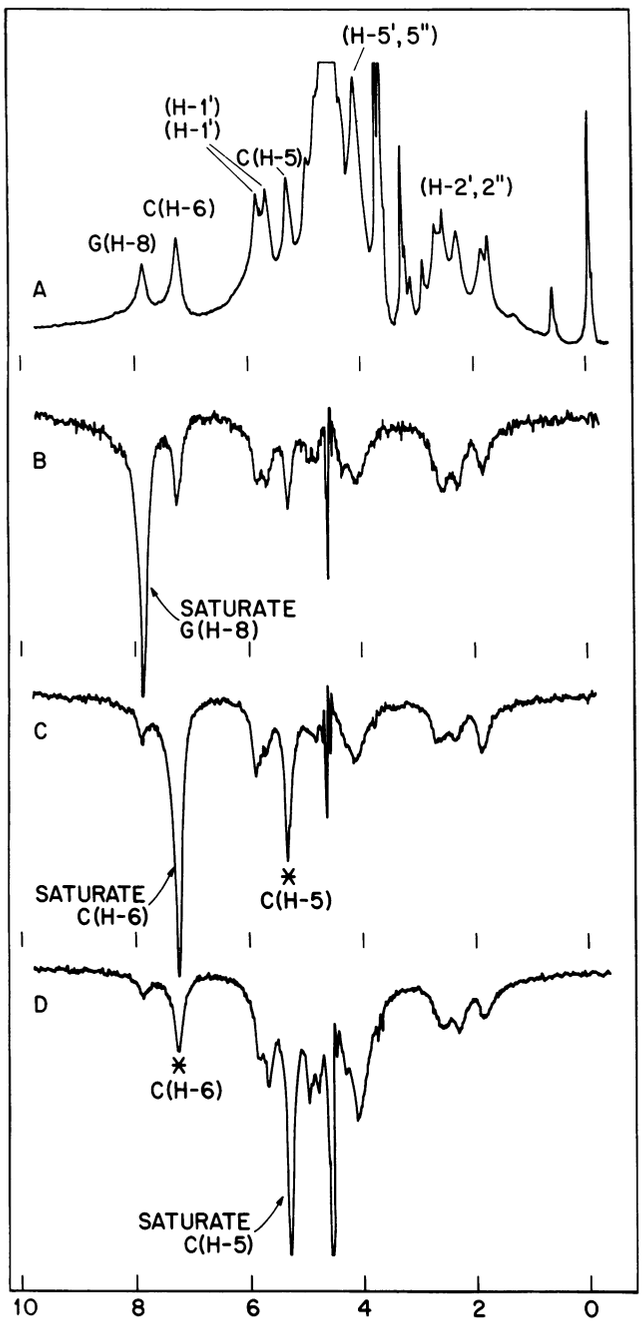

FIG. 3. Transient NOE measurements on poly(dG-dC) in 10 mM cacodylate/1 mM EDTA, pH 7.24, in ^2H_2O at 45°C. (*A*) The 498 MHz H spectrum between 0 and 9 ppm. The narrow resonances between 0 and 4 ppm correspond to EDTA and standard DSS. Difference spectra are after saturation of the guanosine H-8 (*B*), the cytidine H-6 (*C*), and the cytidine H-5 (*D*) resonances. The signal-to-noise ratios were improved by applying a 5-Hz exponential line-broadening contribution.

sugar H-1′ resonance at 6.21 ppm (Fig. 2*B*). This result is confirmed by the observation of an ≈31% negative NOE at the 7.81-ppm guanosine H-8 proton on saturation of the 6.21-ppm sugar H-1′ proton (Fig. 2*D*). The atomic resolution analysis of $(dC\text{-}dG)_3$ (4) revealed that the guanosine H-8 is 2.2 Å from its own sugar H-1′ in the Z-DNA *syn* conformation. However, they are separated by 3.75 Å in the *anti* conformation about the glycosidic bond (Fig. 1). The NOE decreases as the sixth power of the proton–proton distance (12–14). Thus, a significant signal is predicted between the guanosine H-8 and H-1′ in the *syn* conformation but no NOE is predicted in *anti*. The data in Fig.

Table 1. Proton chemical shifts of poly(dG-dC) at 45°C and of poly(dG-m^5dC) at 50°C

	Shift, ppm	
	No added salt	4 M NaCl*
	Poly(dG-dC)	
G(H-8)	7.82	7.81
C(H-6)	7.32	7.25
C(H-5)	5.28	5.14
G(H-1′)	5.64, 5.82	6.21
C(H-1′)		5.65
G(H-3′)	4.79, 4.92	4.75
C(H-3′)		4.75
	Poly(dG-m^5dC)	
G(H-8)	7.70	7.80
C(H-6)	6.95	7.04
$C(CH_3$-5)	1.44	1.06
G(H-1′)	5.56, 5.83	6.22
C(H-1′)		5.57
G(H-3′)	4.72, 4.88	4.75
C(H-3′)		4.75

Poly(dG-dC) was dissolved in 10 mM Na cacodylate/1 mM EDTA; poly(dG-m^5dC) was dissolved in 10 mM Na phosphate/1 mM EDTA. The chemical shifts are relative to DSS.
* The poly(dG-m^5dC) study was with 1.5 M NaCl instead of 4 M NaCl.

2 *B* and *D* conclusively demonstrate that the 6.21-ppm resonance is to be assigned to the guanosine H-1′ proton, and the guanosine residue is in the *syn* conformation in high-salt poly(dG-dC). It should be noted that irradiation of the cytidine H-6 resonance does not result in a significant NOE effect at either sugar H-1′ proton position for poly(dG-dC) in high-salt solution (Fig. 2*C*). These results are consistent with an *anti* conformation about the glycosidic bond of the cytidine residue in the high-salt form of poly(dG-dC) in solution.

We extended these proton NOE measurements to deduce the conformation about the glycosidic bond for the guanosine and cytidine residues in poly(dG-dC) in low-salt solution. The low-salt difference spectra after saturation of guanosine H-8, cytidine H-6, and cytidine H-5 resonances are presented in Fig. 3 *B*, *C*, and *D*, respectively. All the nucleic acid resonances were observed in these transient NOE difference spectra, indicating the existence of spin diffusion in the synthetic DNA in low-salt solution (22). Thus the transfer of saturation spreads throughout the system much more than is evident in high-salt solution (Fig. 2). The reason for this difference is not clear at this time.

The transient NOE difference spectrum after saturation of the low-salt guanosine H-8 resonance is presented in Fig. 3*B*. It is readily apparent that we did not observe a NOE between the guanosine H-8 and either sugar H-1′ proton, although the effects of spin diffusion are seen in the low-salt solution. This observation is consistent with the guanosine residue's adopting the *anti* conformation in low-salt solution. The transient NOE difference spectrum after saturation of the cytidine H-6 resonance (Fig. 3*C*) produced a negative NOE on the cytidine H-5 proton. The magnitude of this signal was much larger than the background effect of spin diffusion at all the other proton positions. By contrast, the two sugar H-1′ protons did not exhibit any NOE beyond the contributions of spin diffusion (Fig. 3*C*). These results are consistent with the cytidine residue's also adopting an *anti* conformation in poly(dG-dC) in low-salt solution.

Behe and Felsenfeld (20) have demonstrated that poly(dG-m^5dC) undergoes a B-DNA-to-Z-DNA cooperative salt-dependent circular dichroism transition with the midpoint occurring

Table 2. Phosphorus chemical shifts of poly(dG-dC) and poly(dG-m^{5}dC)

Added salt	Temp, °C	Shift (δ), ppm	$\Delta\delta$, ppm
	Poly(dG-dC)		
None	62	4.20, 4.20	0.0
4.0 M NaCl	67.5	3.01, 4.42	1.41
	Poly(dG-m^{5}dC)		
None	65	4.14, 4.39	0.25
1.5 M NaCl	69	2.90, 4.14	1.24

Chemical shifts are listed relative to internal trimethyl phosphate. The shifts are corrected for the temperature and salt dependence of trimethyl phosphate and are relative to the standard in "no-added-salt" at 27°C.

at 0.7 M NaCl. We made H NMR measurements on poly(dG-m^{5}dC) in the absence and presence of 1.5 M NaCl and obtained the proton chemical shifts listed in Table 1. The chemical shift of the guanosine H-1′ proton is a sensitive marker of Z-DNA formation (2) and the value of 6.22 ppm obtained for poly(dG-m^{5}dC) in 1.5 M NaCl is similar to the value of 6.21 ppm obtained for poly(dG-dC) in 4 M NaCl solution. In addition, the cytidine C-5 CH_3 group of poly(dG-m^{5}dC) underwent a large upfield shift from 1.44 to 1.06 ppm on addition of NaCl to 1.5 M. Specific NOEs were observed between the guanosine H-8 and H-1′ protons for poly(dG-m^{5}dC) in 1.5 M NaCl, consistent with a *syn* glycosidic torsion angle at the guanosine residue.

Chemical shifts for the P NMR are listed in Table 2 for poly(dG-dC) and poly(dG-m^{5}dC) in high- and low-salt solutions. In low-salt solution, there was either a single peak [poly(dG-dC)] or two closely positioned peaks [poly(dG-m^{5}dC)] (Fig. 4, upper curve), indicating identical or closely related phosphodiester conformations. However, in high-salt solutions, two widely separated phosphorus peaks were observed with similar separations for the two polymers (Fig. 4, lower curve). This is in agreement with the Z conformation for both of these in high-salt solutions.

The NOE measurements cited above together with H and P NMR chemical shifts (2) and proton ring current calculations (23), laser Raman measurements (10), and nuclease digestion studies (24) conclusively demonstrate that poly(dG-dC) and poly(dG-m^{5}dC) adopt the left-handed Z-DNA structure (4–7) in high-salt solution.

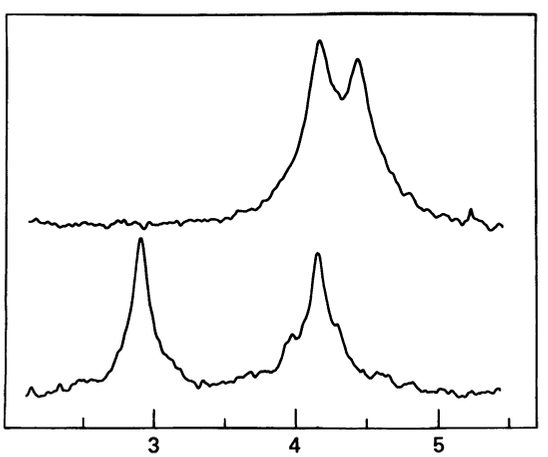

FIG. 4. Proton noise-decoupled P NMR spectra of poly(dG-m^{5}dC) at 44°C in 0.1 M Tris·HCl, pH 8.15/1 mM EDTA/^2H_2O (upper curve) and with additional NaCl to a final concentration of 1.5 M (lower curve).

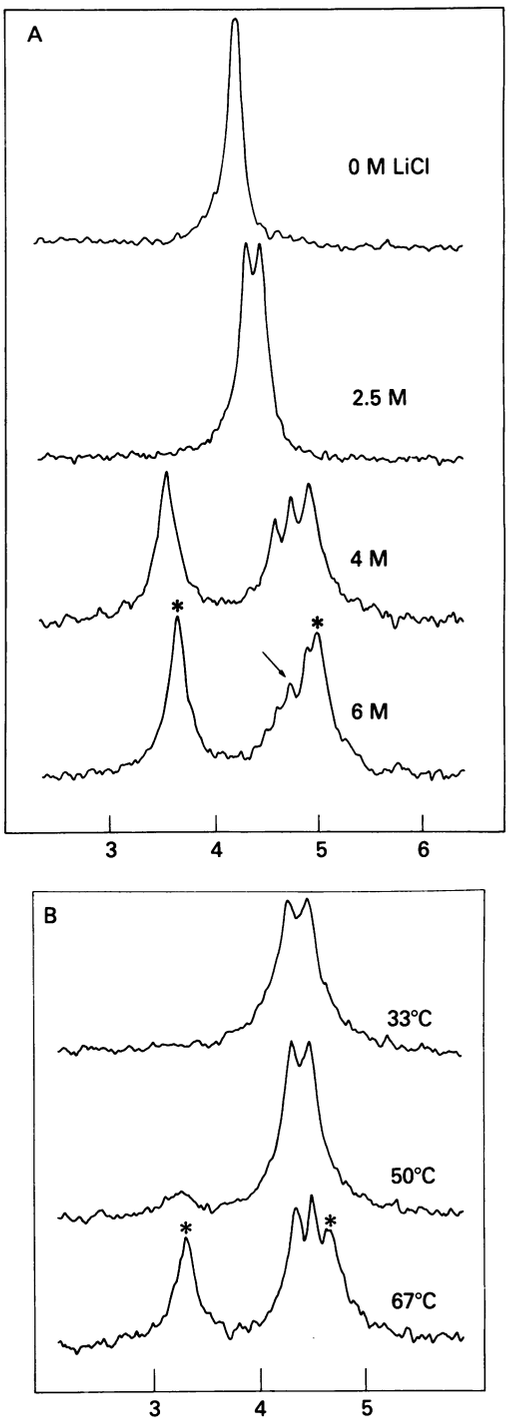

FIG. 5. Proton noise-decoupled 81-MHz P NMR spectra of poly(dG-dC) as a function of LiCl concentration and temperature in 10 mM Na cacodylate/1 mM EDTA, pH 7.24 in ^2H_2O. (*A*) The spectra are for 0 M LiCl (62.2°C), 2.5 M LiCl (66.4°C), 4 M LiCl (66.8°C), and 6 M LiCl (67.4°C). The asterisks represent P resonances characteristic of left-handed Z-DNA; the arrow represents alternative conformations of Z-DNA or alternating B-DNA components. (*B*) The spectra in 4 M LiCl at 33°C, 50°C, and 67°C. The observed spectral changes were reversible with temperature. The signal-to-noise ratios of all spectra were improved by applying a 2-Hz exponential line-broadening contribution. The P chemical shifts are corrected for the temperature and LiCl dependence of internal trimethyl phosphate and are relative to the standard in "no-added-salt" at 27°C.

Thermal Stability of B- and Z-DNA. We have recorded NMR spectra of poly(dG-dC) as a function of LiCl concentration at various temperatures in order to determine the B-to-Z equi-

librium as a function of temperature. Fig. 5*A* shows the proton-decoupled 81-MHz P NMR spectrum of poly(dG-dC) in various concentrations of LiCl at 65 ± 3°C. Without the addition of LiCl (0 M LiCl), there was a single peak; in 2.5 M LiCl, two closely spaced peaks were seen. This suggests that the molecule may be adopting some type of alternating B-DNA structure with two slightly different phosphodiester conformations. A similar situation was found in the methylated polymer in a low salt solution (Fig. 4*A*). In 4 M and especially 6 M LiCl, two new widely spaced peaks were seen (marked with an asterisk) with a chemical shift separation of 1.35 ppm. These are similar to the two Z-DNA peaks seen in 4 M NaCl (2, 3). A number of less-intense peaks were seen for poly(dG-dC) in 6 M LiCl (marked with an arrow), and these may represent alternative conformations of Z-DNA (6) or alternating B-DNA (25) components.

The spectrum in 4 M LiCl is shown in Fig. 5*B* as a function of temperature. At 67°C, four partially resolved resonances were present. A pair of resonances at 4.35 and 4.50 ppm are assigned to the right-handed poly(dG-dC) conformation because their intensities increased when the LiCl concentration was decreased. The nonequivalent dG-dC and dC-dG phosphodiester linkages exhibit a small chemical shift difference, 0.15 ppm, for the right-handed B-DNA conformation. A second pair of resonances at 3.31 and 4.66 ppm (designated by asterisks) are assigned to the left-handed poly(dG-dC) conformation because their intensities increased when the LiCl concentration was increased. The nonequivalent dG-dC and dC-dG phosphodiesters exhibited a large chemical shift difference, 1.35 ppm, for the zigzag left-handed Z-DNA conformation. The simultaneous observation of the two pairs of phosphodiester resonances for poly(dG-dC) in 4 M LiCl at 68°C is indicative of a slow exchange between the alternating right-handed B-DNA duplex and the left-handed Z-DNA duplex. This result is consistent with the large barrier (20 kcal) for the salt-dependent poly(dG-dC) transition (1).

The temperature-dependent P spectrum in 4 M LiCl is shown in Fig. 4*B*. These spectra demonstrate that the slow conformational equilibrium between right-handed B-DNA and left-handed Z-DNA conformations in 4 M LiCl shifts dramatically toward the latter with increasing temperature. This behavior is in agreement with the results reported by Narasimhan and Bryan (26) who measured the circular dichroism of poly(dG-dC) as a function of temperature in 0.2 mM NaCl. At 70°C, there was a simultaneous change in absorbance and an inversion of the circular dichroism which suggests that the molecule may have converted to Z-DNA at the elevated temperature.

The greater stability of the Z-DNA conformation relative to the B-DNA conformation at high temperature may reflect differences in the relative flexibility of these two helical structures. The dCpdG base pairs are sheared relative to each other in Z-DNA (5) which stiffens the left-handed duplex relative to the right-handed B-DNA duplex. Furthermore, Z-DNA does not have the flexibility to intercalate ethidium (27). The relative stiffness of the Z-DNA duplex may also be seen in the observation of two slowly exchanging protons for poly(dG-dC) in high-salt solution which were not observed in low-salt solution (28). At elevated temperature, a vibratory mode of B-DNA may involve unstacking of the bases, and this would destabilize the structure considerably. However, because of sheared bases and an extended polynucleotide backbone in Z-DNA, they probably remain stacked at elevated temperature and are thus stabilized relative to B-DNA.

We are extremely grateful to Dr. David Reuben of the National Magnet Laboratory for setting up the computer software to undertake the NOE measurements. We thank Dr. Gary Quigley for providing us with all the proton–proton distances of <4 Å in B-DNA and Z-DNA. The high-field NMR experiments were performed at the NMR Facility for Biomolecular Research located at the Francis Bitter National Magnet Laboratory (Massachusetts Institute of Technology). The NMR Facility is supported by Grant RR0095 from the Division of Research Resources (National Institutes of Health) and by the National Science Foundation under Contract C-670. This research was also supported by grants to A.R. from the National Institutes of Health, the American Cancer Society, and the National Science Foundation. A.N. was supported by the Deutsche Forschungsgemeinschaft.

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