Conformation of Double-Stranded Polydeoxynucleotides in Solution by Proton Two-Dimensional Nuclear Overhauser Enhancement Spectroscopy

BABUL BORAH and JACK S. COHEN, *Biophysical Pharmacology Section, Clinical Pharmacology Branch, NCI*; and AD BAX, *Laboratory of Chemical Physics, NIADDK, National Institutes of Health, Bethesda, Maryland 20205*

Synopsis

Proton 2D-NOE spectroscopy has been used to investigate the three-dimensional conformations of several sonicated polydeoxynucleotides in solution. The observed pattern of cross peaks indicate that poly(dA-dT) · poly(dA-dT) in all salt concentrations studied (up to 6.6*M* CsF), and poly(dG-m^{5}dC) · poly(dG-m^{5}dC) in low salt (0.1*M* NaCl) are right-handed B-structures. Poly(dG-m^{5}dC) · poly(dG-m^{5}dC) in Mg^{2+} (3 m*M*) solution exhibits a pattern characteristic of the left-handed Z-form. These results for poly(dA-dT) · poly(dA-dT) are in contrast to suggestions that this copolymer exists as a left-handed form, either in low or high salt. We present pure absorption-mode 2D-NOE spectra that enable us to compare several distances and define the conformations of these polydeoxynucleotides in solution.

INTRODUCTION

The Watson-Crick double-helical B-model of DNA was derived mainly from x-ray fiber diffraction patterns.[1,2] For many years, this model was widely accepted as the structure of DNA in solution and in the cell.[3] The proposal of side-by-side structures[4] and the observation of a left-handed Z-form in oligonucleotide crystals with the sequence d(CG)^{5,6} has, to some extent, reopened the question of the solution/cell structure of DNA.

Since oligo- and polydeoxynucleotides have been shown spectroscopically to undergo transitions in solution, it is no longer possible to presume a single DNA structure in solution for one base sequence. The fact that poly(dGdC) is converted from the B- to the Z-form in high-salt solution[7,8] has provided a useful basis for comparison with conformational transitions of other sequences. However, in the case of other polydeoxynucleotides, the nature of the conformation above and below the transition point is not well defined. This is indeed the case with poly(dA-dT), which has been suggested to be either a left-handed B-form in low salt[9] or a left-handed Z-form in high salt.[10]

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Under these circumstances, a rigorous method is required to provide detailed conformational analysis of polydeoxynucleotides in solution. Such a method is proton two-dimensional nuclear Overhauser enhancement (2D-NOE) spectroscopy.[11-13] This technique has been applied to the conformational analysis of small proteins[14-16] and has recently been used to study several oligonucleotides in solution[17-25]; a preliminary analysis of 2D-NOE spectra of several polydeoxynucleotides has been reported by Kearns et al.[26] We present a comparison of 2D-NOE spectra of two polydeoxynucleotides of different base sequence under different solution conditions. While this work was nearing completion, a detailed analysis of the 2D-NOE spectrum of poly(dA-dT) was published by Assa-Munt and Kearns,[27] which demonstrated that the low salt form of poly(dA-dT) is indeed a Watson-Crick right-handed B-form. We entirely agree with their analysis, and the quite unambiguous nature of the qualitative conclusions, as well as the capability to obtain quantitative distance information, indicates the great value of the 2D-NOE method for polydeoxynucleotides conformational analysis in solution.

In this work we have utilized the 5-methyl cytidine analog, i.e., poly(dG-m^{5}dC) not only because of its proclivity to convert to the Z-form at much lower salt concentration than the unmethylated analog,[8,28] but also because the presence of the C-methyl group, which causes the instability of the B-form,[29] provides very strong cross-relaxation interactions. This facilitates comparisons with 2D spectra of poly(dA-dT) in order to draw unambiguous conclusions on the nature of the conformations of these polymers in solution.

MATERIALS AND METHODS

Double-stranded poly(dA-dT) and poly(dG-m^{5}dC) were obtained from P-L Biochemicals. About 80–100 OD_{260} units of DNA were dissolved in 0.1*M* NaCl solution in phosphate buffer and sonicated at 2–6°C for 3–4 h, using a tapered microprobe with a Heat Systems Ultrasonics W225R (20 kHz) sonicator. The power level was optimized just below cavitation, usually about 25 W. The sonicated products were filtered through a Milllipore Millex Filter (0.22 μm) to remove titanium particles eroded from the microtip. The samples were then dialyzed by a 0.1*M* solution of NaCl in phosphate buffer using an immersible CX-10 Millipore Ultrafiltration unit. The sonication yielded polymer fragments with a maximum of ca. 50 base pairs (bp) for poly(dA-dT) and ca. 85 bp for poly(dG-m^{5}dC), as determined by gel electrophoresis.[30]

The dialyzed samples were lyophilized from D_2O three times. For nmr measurements, the lyophilized products were dissolved in 0.5 mL 99.96% D_2O (Merck) and transferred to Wilmad 528PP 5-mm nmr tubes. Samples had the following compositions: poly(dA-dT) ca. 80 OD_{260}

units in 0.1*M* NaCl, 38 m*M* K_2HPO_4, 12 m*M* KH_2PO_4, pH = 7; poly(dG-m^5dC) ca. 75 OD_{260} units in 0.05*M* NaCl, 38 m*M* K_2HPO_4, 12 m*M* KH_2PO_4, pH = 7. In several cases, samples were lyophilized and redissolved to the required concentration, but in the case of $MgCl_2$, aliquots of a concentrated solution were added directly to the sample.

NMR Methods

Proton 2D-NOE experiments were performed at 270 MHz on a Nicolet spectrometer with a Bruker superconducting magnet, interfaced with a Nicolet 1280 computer and Nicolet-293C pulse programmer. Two-dimensional NOE spectra were collected using a sequence of three nonselective pulses,[14]

$$(\pi/2 - t_1 - \pi/2 - \tau_{mix} - \pi/2 - t_2 - \text{AQ})$$

Data were collected in the pure absorption mode. The FIDs consisted of 512 data points with a sweep width of 2500 Hz and 64 t_1 values. Quadrature detection was used in both t_2 and t_1 dimensions.[31] The FIDs were apodized by a Gaussian function, with line broadening (LB) of 30 Hz in both dimensions. In all spectra presented, the strong peak from HDO was removed using the homonuclear decoupling channel for presaturation.

Absolute Value vs Absorption Mode in 2D-NOE Spectra

Two-dimensional correlation spectra are often presented in the absolute value mode. Generally, there are several advantages connected to this approach of data representation. First, one can employ a phase-modulated version of the experiment,[32,33] which allows the spectrum to be recorded in two-dimensional quadrature.[34-37] This minimizes requirements for disk-storage space and data-processing time. A second advantage is that no interactive phasing[38] of the two-dimensional spectrum is needed, and a blind-folded way of processing will lead to a near-optimal presentation of the absolute value mode spectrum. The major disadvantage of the absolute value mode representation is the broad base of the absolute value mode line shape, which strongly decreases spectral resolution and, in 2D-NOE spectra, makes it often nearly impossible to measure accurate intensities of cross-peaks. The base of absolute value mode line shapes can be removed by the use of appropriate digital filtering. For example, use of the "sine bell" filtering function,[39] a "pseudo-echo" function[40] or a convolution difference filter[41] are well-suited for this purpose. However, all these filtering functions have in common that they have a differential effect on signal intensities of broad and narrow resonances; the broader spectral components are attenuated much more strongly than the

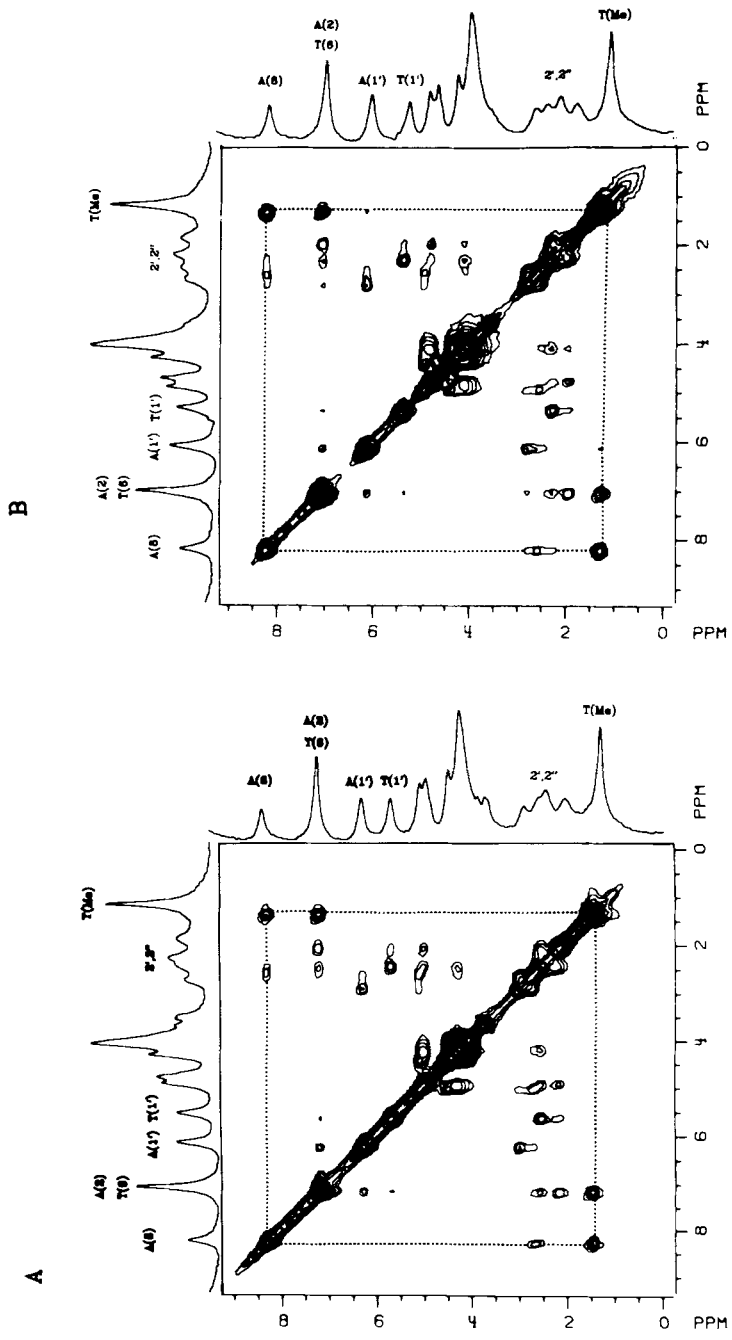

Fig. 1. Contour plots of the 2D-NOE spectra in absorption mode of double-stranded poly(dA-dT) in (A) 0.1 *M* NaCl and (B) 4 *M* CsF. A mixing time of 50 ms was used. Cross-peaks for pairs of interacting protons are identified by joining their resonances by horizontal and vertical lines on either side of the diagonal, as illustrated for AH8-T(Me) with a dashed line.

narrow spectral lines by these types of artificial resolution-enhancement filters. This makes it nearly impossible to quantify the real intensities of peaks in the 2D spectrum if those digital filters have been used.

While for some types of 2D experiments only peak positions are relevant, and signal-to-noise ratios for cross multiplets can even benefit from the use of those strong resolution-enhancement filters, this is not the case for 2D-NOE spectroscopy. This is due to the in-phase nature of the (usually nonresolved) individual components of the two-dimensional multiplets in this type of experiment. Therefore, both on the basis of sensitivity and on the basis of obtaining true intensities, it is important to record the 2D-NOE spectrum in the pure absorption mode.

RESULTS

2D-NOE Spectra of Poly(dA-dT) · Poly(dA-dT)

Figure 1 shows the contour plots of pure absorption mode 2D-NOE spectra of poly(dA-dT) using a mixing time of 50 ms for both low and high salt concentrations. Spectra on the two axes are symmetrical projections of diagonal peaks, which represent spin magnetization that does not cross-relax during the mixing time. Assignments of resonances relevant to this discussion are labeled. At short mixing time, cross-peaks appear between protons that are coupled by dipolar interaction and are separated by ca. 3.5 Å or less. Figure 2 is an expansion of part of Fig. 1, showing the interactions between base protons–sugar protons (H2′, H2″), base protons–T(Me), and H1′–sugar protons (H2′, H2″). Also included in Fig. 2 are expansions for the same interactions of poly(dA-dT) in ca. 40% ethanol-d_6. It can be observed that poly(dA-dT) has very similar cross-peak patterns in low and high salt and also in ethanol. The pattern of major cross-peaks in a 2D-NOE spectrum of poly(dA-dT) in 6.6*M* CsF was the same as in the spectra shown in Figs. 1 and 2, although the spectral quality was poorer due to extensive line broadening induced by the high salt concentration.

2D-NOE Spectra of Poly(dG-m^{5}dC) · Poly(dG-m^{5}dC)

Figure 3 shows cross-sections of spectra in pure absorption mode for double-stranded poly(dG-m^{5}dC) in 0.05*M* NaCl solution using 25- and 50-ms mixing times, and it illustrates the GH8–C(Me), GH8–sugar protons (H2′, H2″) interactions. Note that for GH8–CMe, the cross-peak intensities at 25 ms are reduced by a factor of ca. 2, as compared with those at 50 ms. This implies that the cross-peak intensities for these resonances are approximately linear in this range of mixing times. Although other cross-peak intensities may not be strictly linear

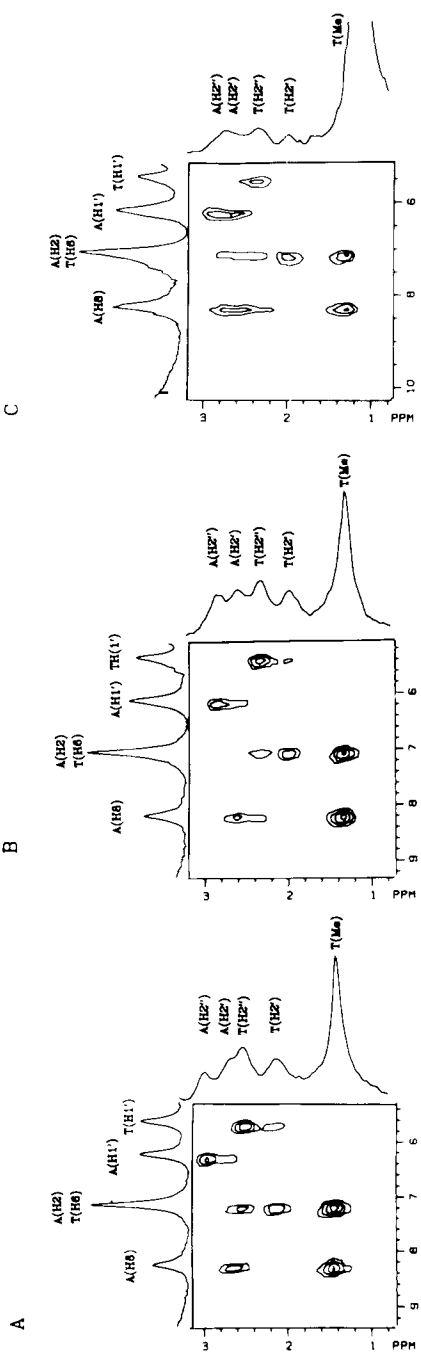

Fig. 2. Expansion of a portion of the 2D-NOE spectra of Fig. 1 showing cross-peaks for specific interactions of protons shown on one axis to the protons shown on the other axis. (A) Poly(dA-dT) · poly(dA-dT) in 0.1*M* NaCl, (B) poly(dA-dT) · poly(dA-dT) in 4*M* CsF, (C) poly(dA-dT) · poly(dA-dT) in 40% ethanol-d_6. A mixing time of 50 ms was used in all cases.

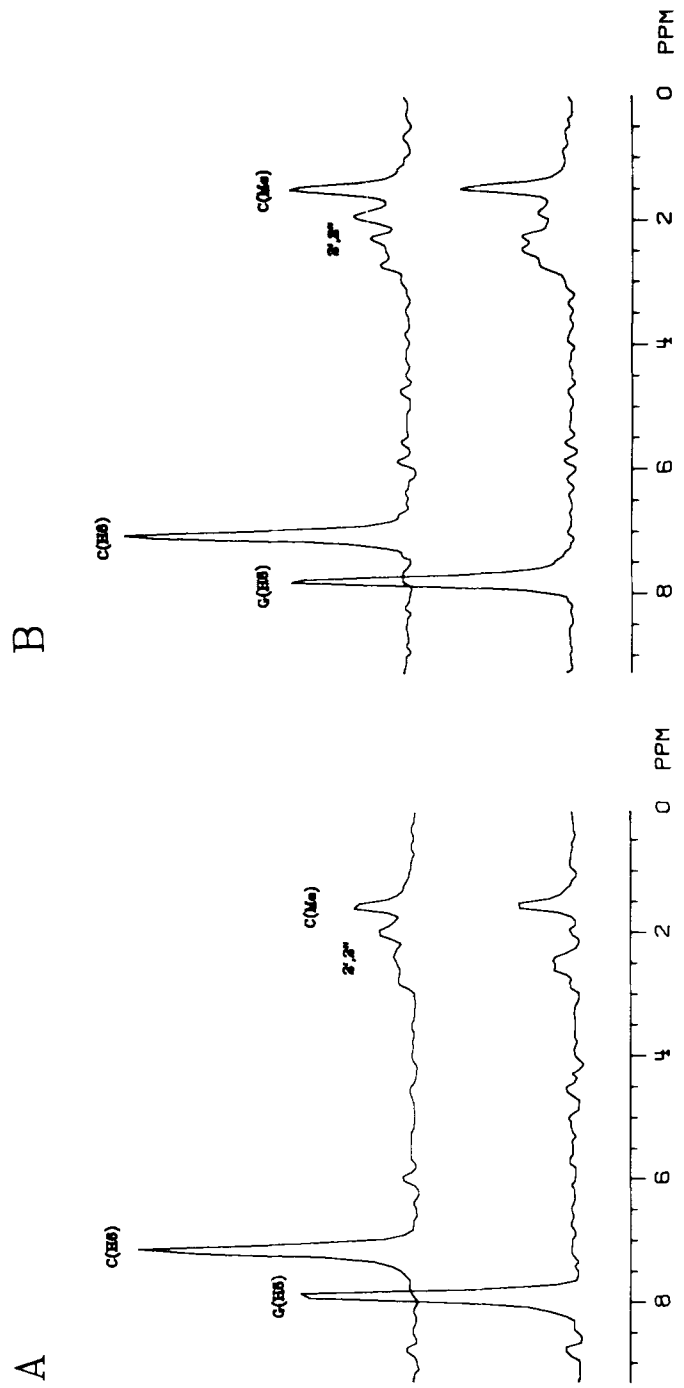

Fig. 3. Cross sections of the absorption mode 2D-NOE spectra of double-stranded poly(dG-m^{5}dC) in 50 mM NaCl solution with mixing times of (A) 25 ms and (B) 50 ms. Comparison of (A) and (B) illustrates the approximate linearity in the changes of cross-peak intensities in the range of mixing times used.

due to small second-order NOE effects, we chose 50 ms as a suitable value for the mixing time in order to retain high sensitivity.[42]

The contour plots of pure absorption mode spectra using 50 ms mixing time are shown in Fig. 4. For this polymer, while the 2D-NOE contour plot in NaCl solution [Fig. 4(A)] is very similar to that of poly(dA-dT) (Figs. 1 and 2), the contour plot in $MgCl_2$ solution is strikingly different [Fig. 4(B)]. In NaCl solution, cross-relaxations due to base protons (GH8 or CH6)–C(Me), base protons (GH8 or CH6)–sugar protons (H2′ or H2″), and H1′ (GH1′ or CH1′)–sugar protons (H2′ or H2″) interactions are clearly observed. Major differences in the poly(dG-m^{5}dC)/$MgCl_2$ spectra are the loss of cross-peaks due to GH8–C(Me) and GH8–sugar proton (H2′ or H2″) interactions and the appearance of a strong cross-peak for the GH8–GH1′ interaction. Figure 5 shows an expansion of part of Fig. 4 illustrating these cross-relaxation interactions.

DISCUSSION

The 2D-NOE spectra described above can be used to deduce several important features of the polymers under investigation. First, 2D-NOE spectra give better resolution compared with one-dimensional spectra, particularly in the crowded (H2′, H2″) spectral region, and, therefore, assignments may be made more easily. Second, structural information can be obtained on the basis of spatial connectivities between nuclei coupled by dipolar interactions. Consequently, several important structural aspects of DNA double helices can be investigated using 2D-NOE spectroscopy. Such features include determination of the handedness, and *syn* vs *anti* nucleotide conformation. Examples of the elucidation of such structural features of polydeoxynucleotides will be illustrated.

Assignments

This section will address some controversy regarding the assignments of H1′, H2′, and H2″ resonances in the proton spectra of poly(dA-dT) and poly(dG-m^{5}dC). We consider poly(dG-m^{5}dC) first, because in the high-salt form, the assignments of sugar protons using one-dimensional NOE can be considered to be firmly established on the basis of the Z-conformation of the duplex.[43] In Fig. 4(B), the 2D-NOE contour plot clearly shows the GH8-GH1′ interaction in the Z-form, and thus, the resonance at 6.33 ppm can be unequivocally assigned to GH1′. The resonance at 5.73 ppm is therefore assigned to CH1′.

In the low-salt conformation, the assignment of H1′ requires reexamination. For poly(dG-dC), Dhingra et al.[44] have reported a crossover of GH1′ and CH1′ proton chemical shifts in going from the low- to the high-salt form. The present work does not support this crossover of H1′ resonances, based on correlations between 2D-NOE spectra and model interproton distances for a right-handed B-conformation (Fig.

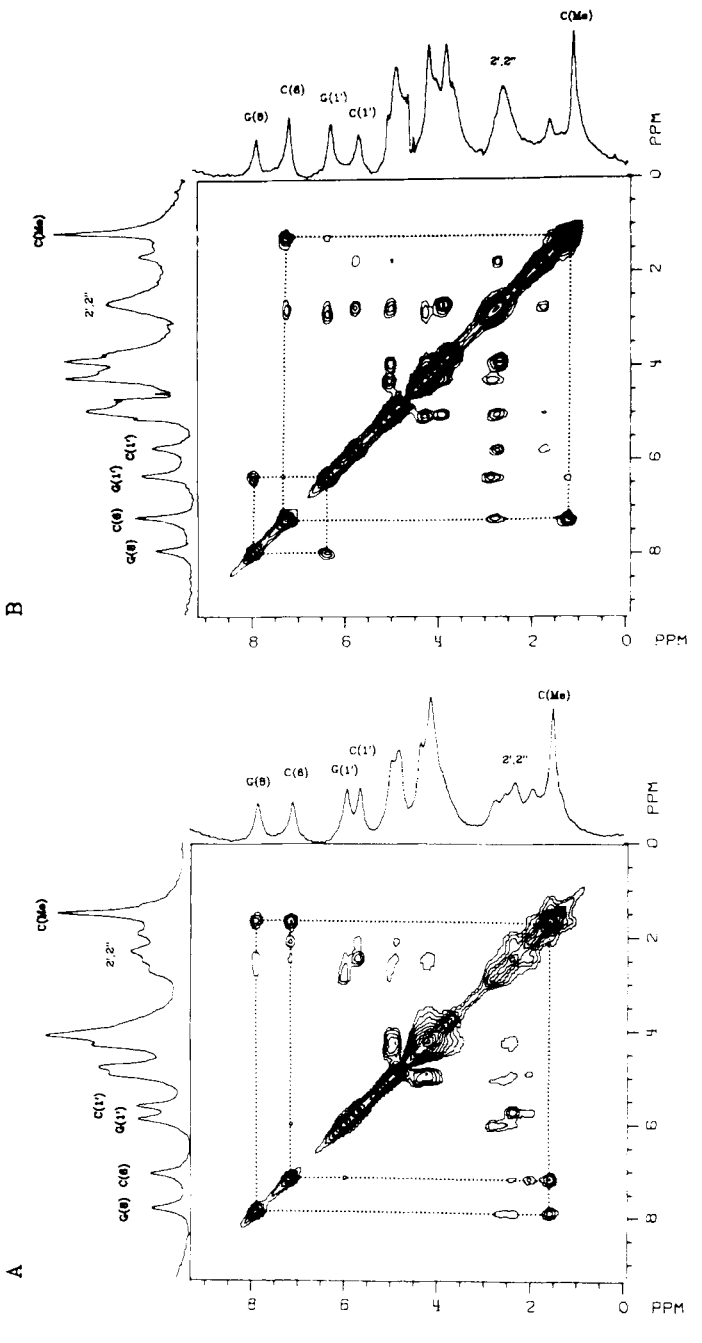

Fig. 4. Contour plots of absorption mode 2D-NOE spectra of double-stranded poly(dG-m^{5}dC) in (A) 50 m*M* NaCl and (B) 3 m*M* $MgCl_2$. Mixing time was 50 ms in both cases. Specific interactions are indicated by joining off-diagonal peaks for cross-relaxing protons.

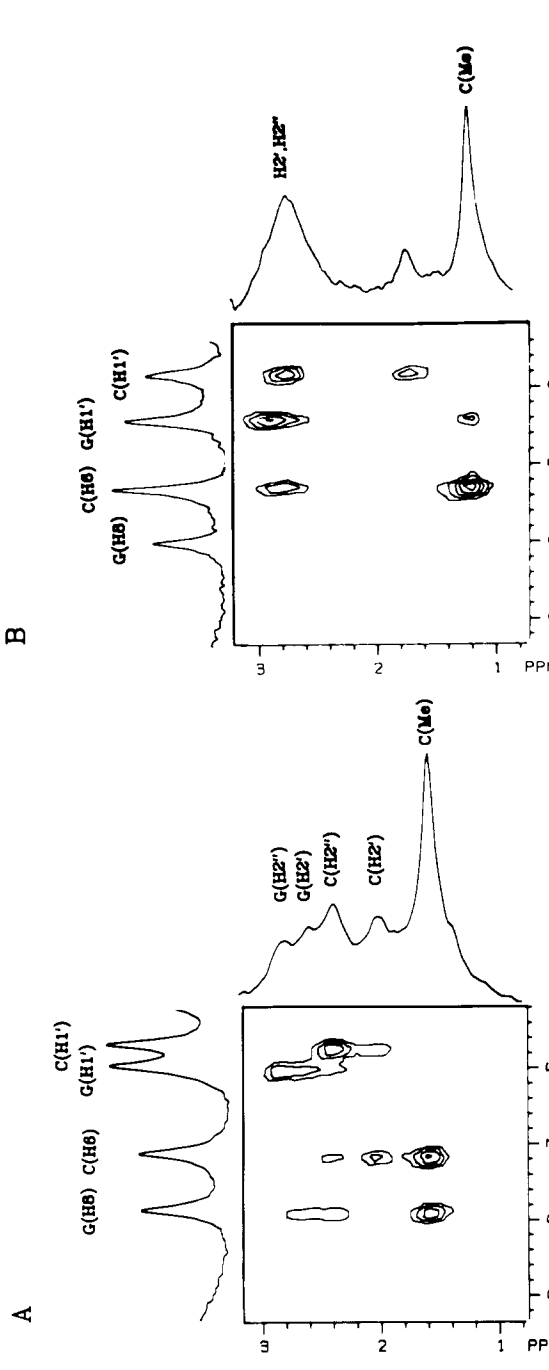

Fig. 5. Expansion of portions of the 2D-NOE spectra of Fig. 4. Cross-relaxations are indicated by cross-peaks for protons shown on one axis interacting with protons shown on the other axis. (A) Poly(dG-m^{5}dC) · poly(dG-m^{5}dC) in 50 m*M* NaCl and (B) poly(dG-m^{5}dC) · poly(dG-m^{5}dC) in 3 m*M* $MgCl_2$.

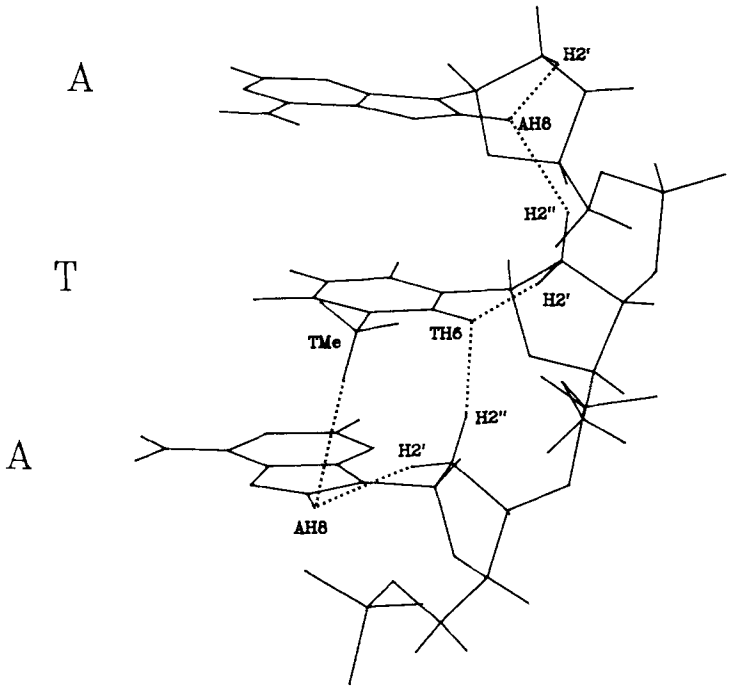

Fig. 6. Portion of a single-strand d(ATA) structure in a right-handed B-conformation. Specific intra- and internucleotide interactions that could give rise to cross-relaxation due to proton distances < 3.5 Å are joined by dashed lines. This figure was prepared using the XRAY program written by R. Feldmann on the DEC PDP10, DCRT, NIH.

6). Interactions that can be expected to be seen in the 2D-NOE spectra are indicated by joining the corresponding protons. Table I gives the interproton distances (in Å) for specific interactions of purine and pyrimidine protons for the right-handed B-conformation. It can be noted that, based on interproton distances, the H1′–H2″ interaction will be stronger than the H1′–H2′ interaction within the same nucleotide, independent of the sugar pucker. Thus, stronger cross-peaks are expected for the H1′–H2″ interaction. A proper assignment of H2′ and H2″ should therefore facilitate the assignments of H1′ resonances. Figure 6 also indicates that the base protons (H8 or H6) are within cross-relaxing distances to their own H2′ protons and to the H2″ protons of the adjacent 5′-nucleotide, although, based on models for the B-conformation, the second interaction is expected to be somewhat weaker. Figure 7 shows cross sections of 2D-NOE spectra showing these interactions, where corresponding to four diagonal peaks representing (H2′, H2″) of G and C, four cross-peaks can be clearly distinguished, the cross-peaks for H1′–H2″ being the strongest. In Fig. 7(A), the GH2′ and CH2′ assignments are based on their cross-relaxation peaks with GH8 and CH6 respectively. The assignments of GH2″ and CH2″ also

TABLE I
Interproton Distances (Å) Computed for a Right-Handed B-Form

Purine Proton Interactions	
Intranucleotide	Internucleotide (3′–5′)
H8–H2′ : 2.14	H8–H2′ : 3.71
H8–H2″ : 3.53	H8–H2″ : 2.40
H8–H1′ : 3.69	H8–Me : 2.62
H1′–H2′ : 2.83	
H1′–H2″: 2.22	

Pyrimidine Proton Interactions	
Intranucleotide	Internucleotide (3′–5′)
H6–H2′ : 1.92	H6–H2′ : 3.81
H6–H2″ : 3.35	H6–H2″ : 2.42
H6–H1′ : 3.58	
H1′–H2′ : 2.83	
H1′–H2″: 2.22	
H6–Me : 2.41	

follow from cross-peaks with GH8 and CH6. Therefore, the cross-peaks at 5.93 and 5.83 ppm must be assigned to the GH2″–GH1′ and CH2″–CH1′ interactions.

Figure 8 shows cross sections of 2D-NOE spectra for poly(dA-dT) in low and high salt. Assignments again follow from arguments similar to those given for the poly(dG-m^{5}dC)/NaCl system. The assignments of the 6.19- and 5.58-ppm resonances to AH1′ and TH1′, respectively, are consistent with previous work by Assa-Munt and Kearns.[27] Note that, unlike the poly(dG-m^{5}dC), the cross-relaxation interactions are very similar for poly(dA-dT) in both low and high salt. Also, for short mixing times, the cross-peak intensities for (AH8–AH2′), (AH1′–AH2″), (TH6–TH2′), and (TH1′–TH2″) interactions are comparable, indicating that interproton distances for the interacting proton pairs are also comparable and consistent with the distances derived for a right-handed B-conformation.

Conformation of Poly(dG-m^{5}dC)

From combined studies of shielding constants, chemical shifts, and one-dimensional NOEs, double-stranded poly(dG-dC) in low-salt solution was shown to exist as a right-handed B-DNA double helix with Watson-Crick base pairing.[44] 2D-NOE spectra of poly(dG-dC) in 0.1*M* NaCl also suggest a right-handed B-DNA structure.[26]

Poly(dG-m^{5}dC) has not been studied using 2D nmr methods. Using one-dimensional NOE measurements, Patel et al.[43] differentiated between *syn* and *anti* glycosidic torsion angles in poly(dG-dC) and

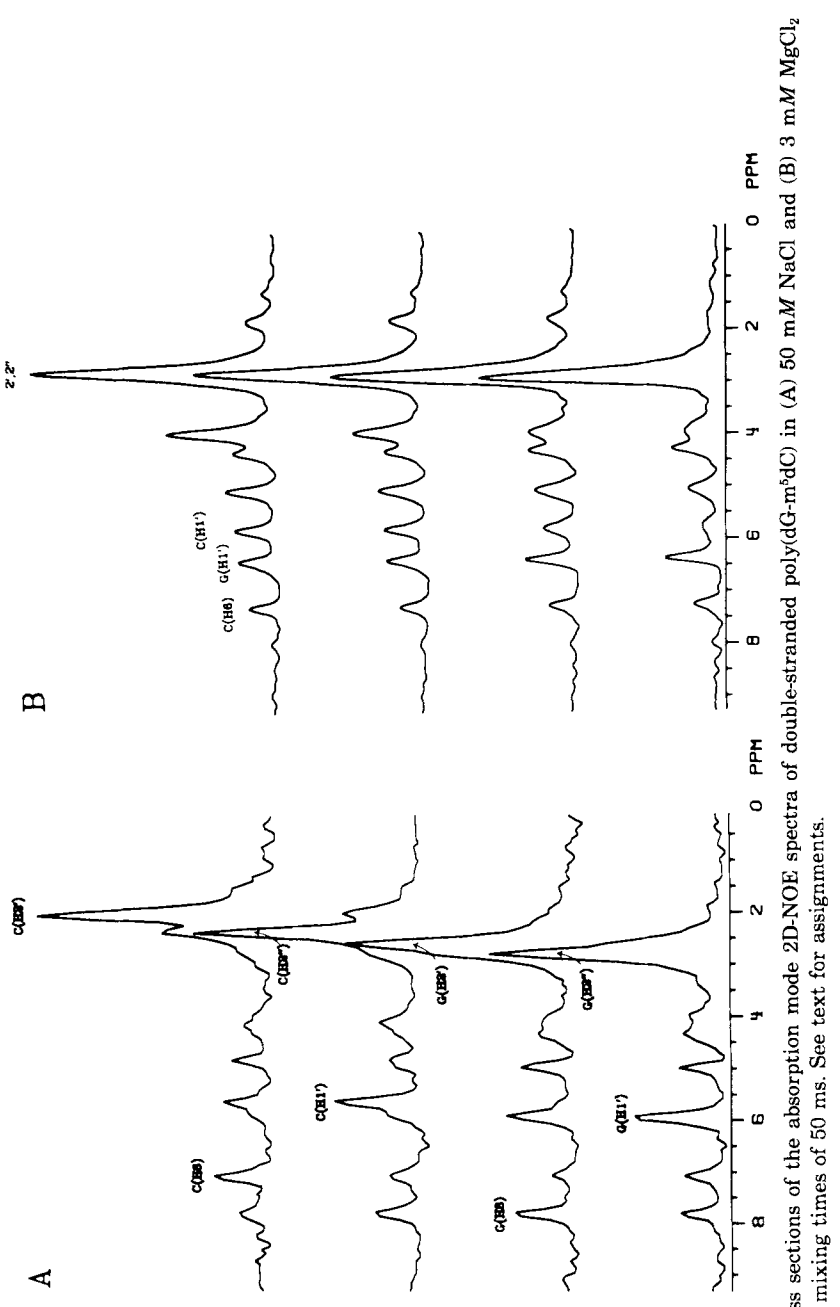

Fig. 7. Cross sections of the absorption mode 2D-NOE spectra of double-stranded poly(dG-m^{5}dC) in (A) 50 mM NaCl and (B) 3 mM $MgCl_2$ solutions with mixing times of 50 ms. See text for assignments.

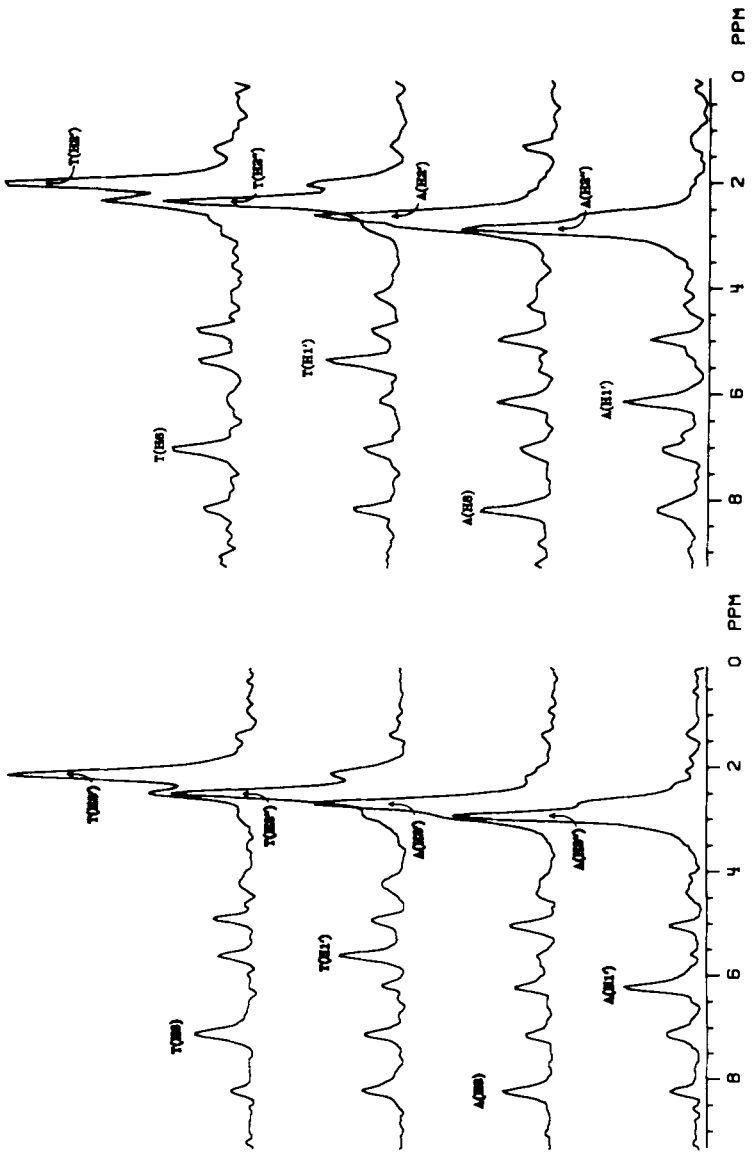

Fig. 8. Cross sections of the absorption mode 2D-NOE spectra of double-stranded poly(dA-dT) in (left) 0.1*M* NaCl and (right) 4*M* CsF with mixing times of 50 ms. See text for assignments.

poly(dG-m^{5}dC) duplexes. Feigon et al.[19] also demonstrated the B–Z transition in (m^{5}dC-dG)$_3$ using one- and two-dimensional nmr. However, it should be emphasized that the differences between the proton 1D spectra of the B- and Z-forms are not as clear-cut as they are in the 2D spectra.

To deduce structural information from the 2D-NOE spectra, we consider the strong interactions that are found between the base protons (H8 or H6) and the 5-methyl protons of cytidine. Within the cytidine moiety, the intrabase proton distance between CH6 and C(Me) is fixed at ca. 2.5 Å, assuming a distance of closest approach. The interbase proton distance between GH8 and the adjacent C(Me) is indicated to be ca. 2.6 Å based on the model for a right-handed B-conformation (Table I) and is comparable to the intrabase CH6–C(Me) distance. As seen in Fig. 9, the cross sections of 2D-NOE spectra for poly(dG-m^{5}dC)/NaCl show two cross-relaxation peaks of comparable intensities for both GH8–C(Me) (interbase) and CH6–C(Me) (intrabase) interactions. The methyl proton relaxation is mainly determined by internal motion, thereby making its dynamics different from those of other protons within the same molecule. Cross-relaxation between a proton interacting with a proton of a methyl group cannot, therefore, be used as an internal reference for other proton–proton interactions or distance calculations.

The arguments given above in assigning the sugar proton resonances also apply here in support of a right-handed B-form for poly(dG-m^{5}dC) in NaCl solution. In the B-form, both G and C have *anti* glycosidic torsional angles, whereas in the Z-form, G assumes a *syn* glycosidic conformation, and C retains an *anti* conformation (Fig. 10). In the *anti* form, the interaction distances for intranucleotide H8 (or H6)–H2′, intra nucleotide H1′–H2″, and internucleotide H8 (or H6)–H2″ are of comparable magnitude (Table I), and strong cross-relaxation peaks are expected for these interactions. In contrast, the intranucleotide H8 (or H6)–H1′ distances for the *anti* conformation are greater than ca. 3.5 Å, and are expected to give very weak or no cross-relaxation peaks. Figures 4, 5, and 7 confirm these features for poly(dG-m^{5}dC) in NaCl solution, confirming its B-form structure. For a left-handed B-form, calculated intranucleotide distances of ca. 4.2 Å between H8 (or H6) and H2′ predict that no direct magnetization transfer can occur between these protons.[44] On the contrary, the observation of strong cross-relaxation between H8 (or H6) and H2′ also eliminates the possibility of a left-handed B-form for poly(dG-m^{5}dC) in low salt.

In 3 m*M* $MgCl_2$ solution, several features of the 2D-NOE spectra conform to the Z-conformation. In the *syn* form, the glycosidic angle in G changes and the distance for the intranucleotide H8–H1′ interaction is ca. 2.32 Å and is associated with a significant cross-peak (Fig. 10). The intranucleotide H8–H2′ distance, however, increases to ca.

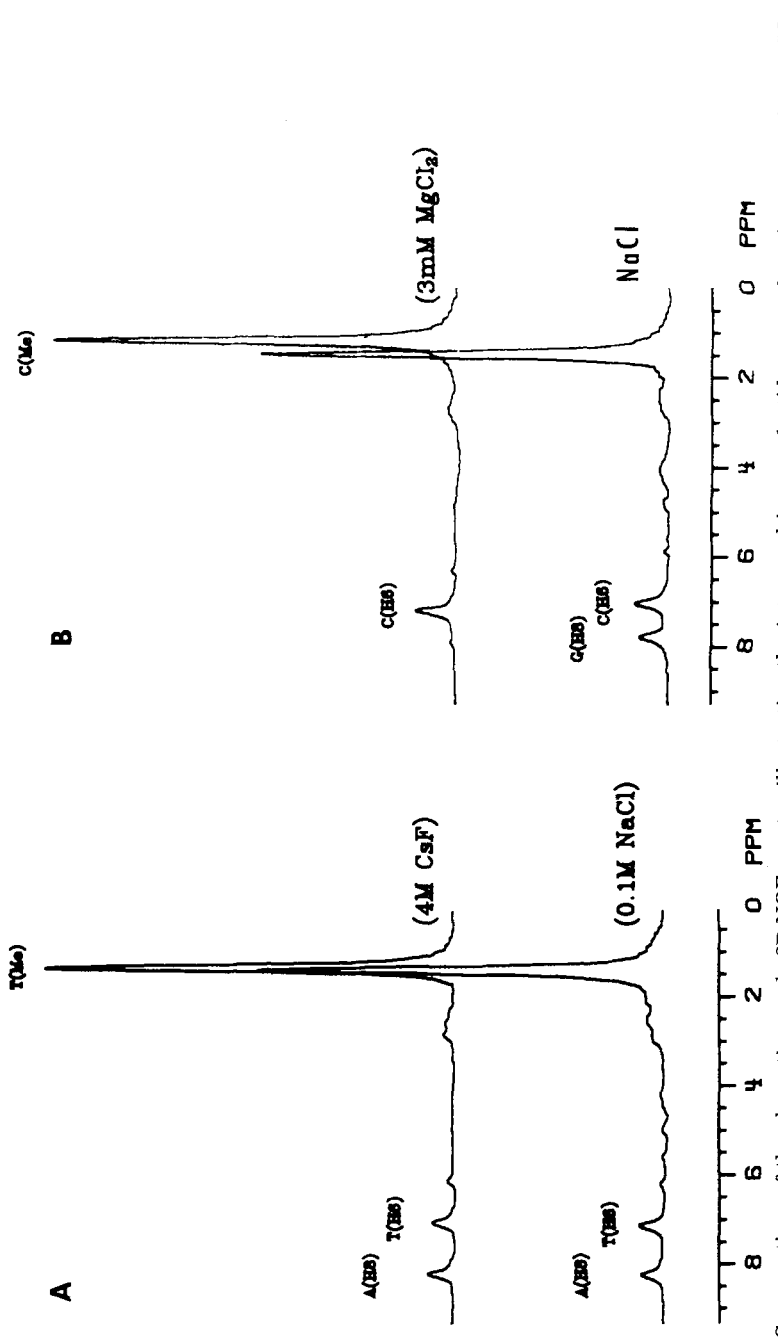

Fig. 9. Cross sections of the absorption mode 2D-NOE spectra illustrating the intra- and internucleotide cross-relaxation interactions of Me protons with base protons (A) double-stranded poly(dA-dT) in 0.1*M* NaCl and 4*M* CsF and (B) double-stranded poly(dG-m^{5}dC) in 50 m*M* NaCl and 3 m*M* $MgCl_2$ solutions. Mixing times used were 50 ms in all cases.

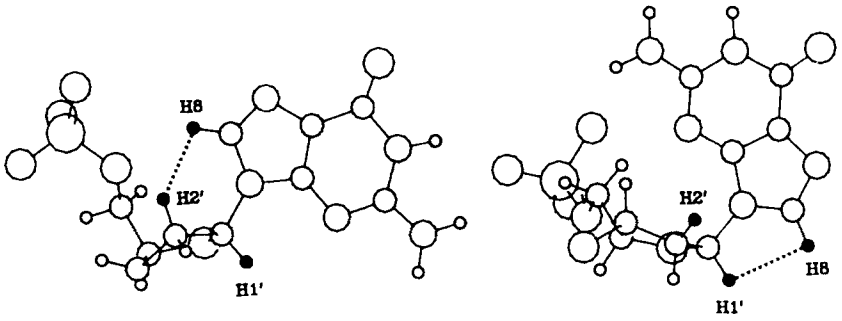

Fig. 10. The B-form *anti*-glycosidic (left) and Z-form *syn*-glycosidic conformations of a G nucleotide. The close specific interactions that are different in the two cases, i.e., H8–H2′ and H8–H1′, are indicated by a dashed line.

3.94 Å, with consequent loss of the cross-peak in the 2D-NOE spectra. In Fig. 9, note also that the cross-peak for the interbase H8–C(Me) interaction has disappeared, indicating a *syn* form for G. That C retains an *anti* conformation is evident from the observation of cross-peak for the intrabase H6–H2′ interaction. Consequently, we can conclude that the 2D-NOE spectra unambiguously support the transition to the Z-form.

Conformations of Poly(dA-dT)

In light of the above discussion, we now consider the 2D-NOE spectra of poly(dA-dT). A comparison of the 2D-NOE contour plots for poly(dA-dT) in both low and high salt (Figs. 1, 2 and 8) shows a very similar pattern to poly(dG-m^{5}dC) in NaCl solution. The spectral features demonstrate that poly(dA-dT) assumes a right-handed B-type conformation in low and high salt and also in 40% ethanol-d_6 solution. For instance, Fig. 9 shows that cross-peaks for the H8 (and H6)–T(Me) interaction are observed in both the low- and high-salt forms, and in both cases, the cross-peaks have comparable intensities relative to the diagonal peak.

The cross-relaxation peaks observed in Fig. 8 can be rationalized on the basis of a right-handed B-conformation, where both A and T have *anti*-glycosidic torsional angles. In both cases of low and high salt, the cross-peaks for intranucleotide interactions, e.g., AH8–AH2′, TH6–TH2′, AH1′–AH2″, and TH1′–TH2″ interactions have comparable intensities. Conforming to a B-structure, the internucleotide interactions, e.g., AH8–TH2″ and TH6–AH2″, have slightly weaker cross-peaks compared with other cross-peaks in this region, indicating greater interproton distances. The nature of the base pairing for poly(dA-dT) has been shown to be Watson-Crick type.[9,26] For a left-handed B-form, the intranucleotide distances between H8 (or H6) and H2′ were calculated to be ca. 4.1 Å,[9] and, therefore, we would not

expect cross-relaxation peaks for these interactions. The observed absence of such cross-peaks eliminates the possibility of a left-handed B-form for poly(dA-dT) in low-salt solution.

These results indicate that the apparent transition of poly(dA-dT) in high salt,[10] which affects the ^{31}P-nmr and the CD spectra,[45] does not significantly alter the overall conformation of this polymer. Consequently, the proposals that poly(dA-dT) exists as a left-handed B-[9] or Z-form[10] in solution can be decisively rejected. The consistent B-form pattern of poly(dA-dT) in the 2D-NOE contours was true even up to 6.6*M* CsF, perhaps indicating that the transition observed by these methods[10] could arise from tertiary structure effects due to the extremely high salt concentrations. The effects on the ^{31}P-spectra, while clearly indicating a conformational effect on the phosphodiester backbone,[10,45] may also have a salt/dehydration component on the ^{31}P chemical shifts at such high salt concentrations. In up to 40% ethanol, the conformation of poly(dA-dT) had not converted to the A-form, to which the 2D-NOE spectrum has been shown to be sensitive in oligoribonucleotides.[24]

CONCLUSION

Poly(dA-dT) exists as a right-handed B-form in solution over a wide range of salt concentrations. The 2D-NOE spectra confirm the transition of poly(dG-dm^{5}dC) from a right-handed B-form to a left-handed Z-form in $MgCl_2$ solution. The conformations of polydeoxynucleotides in solution can be unambiguously determined using 2D-NOE spectroscopy. It should now be possible to study, in appropriate molecular detail, the interactions of drugs and proteins with polydeoxynucleotides in solution.

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