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Comparison of the Conformation of Poly(dI-dC) with Poly(dI-dbr^{5}C) and the B and Z Forms of Poly(dG-dC). One- and Two-Dimensional NMR Studies[†]

Peter A. Mirau and David R. Kearns*

ABSTRACT: One- and two-dimensional nuclear Overhauser effects (2D NOE) have been used to compare the conformational properties of 60–80 base pair long duplexes of the synthetic DNA polymer poly(dI-dC) with those of poly(dI-dbr^{5}C) and poly(dG-dC) in the B and Z conformations. Cross peaks in the 2D NOE spectra arising from proton–proton dipolar interactions which are more or less independent of the DNA conformation are used to assign the spectra of these molecules. Other cross peaks are sensitive to the conformational details, and these are used to make deductions about the average conformation in solution. The proton–proton interactions that give rise to the cross peaks in the 2D NOE spectrum of poly(dI-dC) are indicative of a B family conformation and rule out the possibility of some alternative conformations, including A, Z, alternating B, and left-handed B-DNA. The spectra are similar to those obtained from B-form poly(dI-dbr^{5}C) and poly(dG-dC) but different from Z-form poly(dG-dC). Taken together, these results indicate that the solution conformation of poly(dI-dC) is not unusual but more closely resembles that of other B-form DNAs.

The sequence-dependent properties of DNA play a key role in the expression of information contained in DNA sequences (Wells et al., 1980), and the determination of the physical basis for the recognition of specific sequences is one of the fundamental unsolved problems in molecular biology. Among DNA features that might be recognized by proteins and drugs are differences in conformation, chemical structure, topology, and dynamics. The recent discovery of Z-DNA dramatically demonstrates that DNA may exist in a variety of conformational states that depend on the solvent conditions and the duplex sequence (Pohl & Jovin, 1972; Wang et al., 1979). Although a variety of techniques have been used to demonstrate that poly(dG-dC) adopts a left-handed helix under the appropriate conditions (Pohl & Jovin, 1972; Nickol et al., 1982; Thamann et al., 1981; van de Sande & Jovin, 1982), the situation is much less clear regarding the probable conformational states of other synthetic alternating purine–pyrimidine DNAs. It has been observed, for example, that under the appropriate solvent conditions the conformations of poly(dA-dT) and poly(dA-dC)·poly(dG-dT) are spectroscopically different from B-form DNA (Zimmer et al., 1982).

The conformation of the alternating DNA duplex poly(dI-dC) has long been the subject of controversy. In solution, the long wavelength band in the circular dichroic (CD) spectrum of poly(dI-dC) is inverted compared to that of normal B-form DNA, and a "bizarre" X-ray diffraction pattern for the sodium salt of the fibers at 75% relative humidity has been reported (Mitsui et al., 1970; Grant et al., 1972). Such data have been interpreted in terms of a left-handed helix by one group (Mitsui et al., 1970) and a right-handed D-form helix by another (Ramaswamy et al., 1982). More recently the data have been interpreted in terms of a left-handed double-helical structure in which the bases retain their usual anti orientation about the glycosidic bond but form Hoogsteen-type base pairs (Drew & Dickerson, 1982) (Figure 1). In contrast with the unusual structures which have been proposed to account for the X-ray diffraction data, some spectroscopic and enzymatic studies suggest that the conformation of poly(dI-dC) resembles "normal" B-DNA. DNA polymerase, ligase, and nucleases utilize poly(dI-dC) as well as other synthetic DNA polymers (Grant et al., 1972), and the ^{31}P spectrum of poly(dI-dC) is similar to that of poly(dA-dT) (Cohen et al., 1981). In addition, the vacuum UV CD spectrum of poly(dI-dC) resembles other B-form DNA polymers (Sutherland & Griffin, 1983).

In this study we have used one- and two-dimensional (2D) nuclear Overhauser effects (NOE) to examine the solution conformation of poly(dI-dC). These studies are easily performed on 60–80 base pair long fragments, and we report the comparison of the conformational features of poly(dI-dC) with

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[†] From the Department of Chemistry, University of California, San Diego, La Jolla, California 92093. *Received March 14, 1984*. This work was supported by NSF Grant PCM 83-03374.

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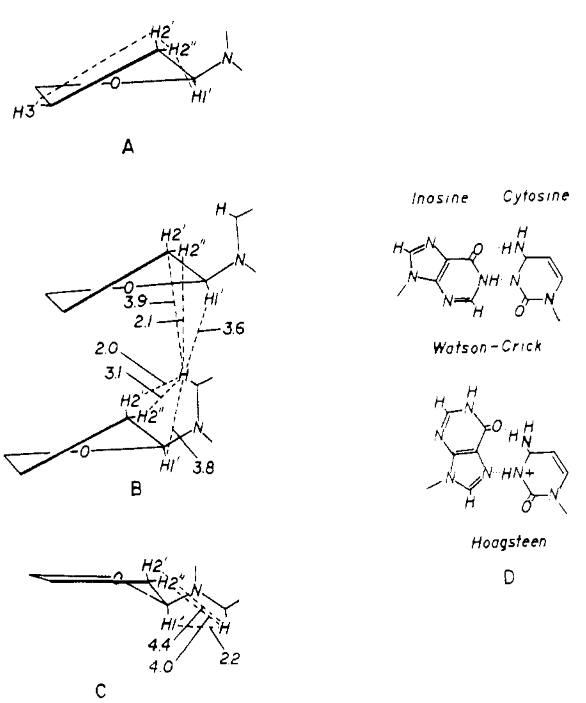

FIGURE 1: Structures showing the nuclear Overhauser effects expected (A) within a sugar residue, (B) between an aromatic base proton and the sugar protons in B-form DNA, (C) between the base and sugar protons in Z-form DNA where the purine base is in the syn conformation, and (D) for a Watson–Crick and Hoogsteen base pair.

those of poly(dI-dbr^{5}C) and the B and Z forms of poly(dG-dC). Observation of an NOE between the inosine imino and IH2 protons eliminates the possibility of Hoogsteen-type base pairs for poly(dI-dC) in solution. The results indicate that the conformation of poly(dI-dC) is not unusual but closely resembles B-form DNA. A preliminary account of this work has been presented (Kearns et al., 1983).

Materials and Methods

Poly(dI-dC) (lot no. 668-115), poly(dG-dC) (lot no. 676-7), and poly(dI-dbr^{5}C) (lot no. 709-73) were obtained from P-L Biochemicals and buffer reagents from Sigma Chemical Co. The size of the DNA polymers was reduced by ~5–6 h of sonication on a W-375 Ultrasonics sonicator using a 50% duty cycle at 0 °C in 0.25 M NaCl, 0.01 M cacodylate, and 0.01 M ethylenediaminetetraacetic acid (EDTA) at pH 7.0 (Granot et al., 1982). The samples, typically containing 25 A_{260} units of the DNA polymer, were ethanol precipitated twice, dried, dissolved in 0.125 mL of buffer containing 0.1 M NaCl–0.01 M sodium cacodylate at pH 7, and transferred to a Wilmad 508-CP microcell. For the Z-form poly(dG-dC), the buffer contained 4.5 M NaCl. The size of the polymers was determined by electrophoresis on 7% polyacrylamide gels vs. DNA restriction fragments.

^{1}H NMR spectra were obtained at 360 MHz on a home-built Fourier transform spectrometer equipped with an Nicolet 1180-E computer. Pure absorption phase 2D NOE spectra were acquired by using the $(90°-t_1-90°-t_m-90°-t_2)_n$ pulse sequence with alternate block accumulation (States et al., 1982), and the time-shared Redfield 214 pulse sequence was used to obtain spectra in H_2O solution (Redfield & Kuntz, 1979; Wright et al., 1981). Truncated-driven NOE spectra (Wagner & Wuthrich, 1979) were obtained by irradiating the low-field resonance for various lengths of time immediately followed by a time-shared Redfield 214 observe pulse. A control spectrum, obtained by using the same irradiation time, but off-resonance, was subtracted from the on-resonance irradiation spectrum to give the difference spectra.

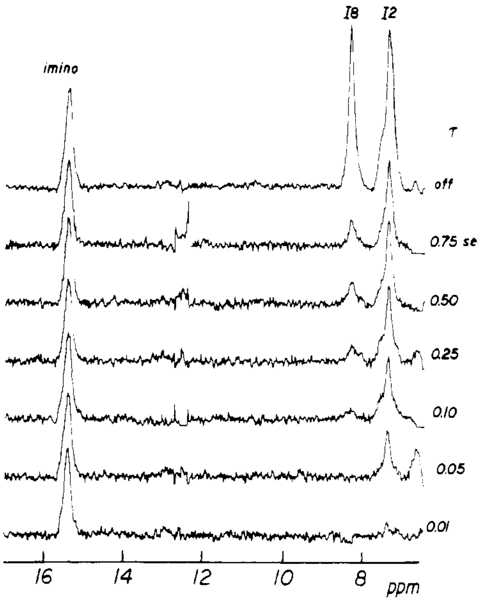

FIGURE 2: Truncated-driven nuclear Overhauser difference spectra for poly(dI-dC) in H_2O solution. The values to the right of the spectra denote the irradiation time of the imino protons at 15.3 ppm.

Results

Truncated-Driven NOE. It was recently suggested that the unusual fiber diffraction pattern observed for poly(dI-dC) may be accounted for by a left-handed DNA in which the bases retain their anti conformation but the base pairing is Hoogsteen rather than Watson–Crick (Drew & Dickerson, 1982). The possibility of Hoogsteen base pairing in solution may be examined by observing truncated-driven nuclear Overhauser effects (TOE) (Wagner & Wuthrich, 1979) between the low-field resonances and the base protons, as has been done for the base pairs in tRNA (Johnson & Redfield, 1978). In such an experiment the low-field resonance of poly(dI-dC) at 15.3 ppm is irradiated for varying lengths of time, and the transfer of magnetization to the other spins is observed. The initial rate of spin transfer is proportional to the cross-relaxation rate and the interproton separation. Figure 2 shows the TOE difference spectra of poly(dI-dC) at 13 °C obtained by irradiating the imino resonance for the length of time shown at the right edge of the spectra. We may distinguish between Watson–Crick and Hoogsteen-type base pairs by observing which inosine base protons show a strong NOE to the low-field resonance. As illustrated in Figure 1D, in the Watson–Crick base pair an NOE is expected between the imino proton and IH2, while in the Hoogsteen base pair the expected NOE is between the imino and the IH8. In the Hoogsteen base pair, a low-field resonance due to the protonated cytosine may be observed, but the free inosine imino proton would be broadened beyond detection due to rapid exchange. The TOE difference spectra in Figure 2 clearly show a strong NOE between the imino and IH2 protons at 7.3 ppm. At longer irradiation times (≥0.5 s), second-order peaks due to nonselective spin diffusion may be observed. These results demonstrate that the base pairing of poly(dI-dC) is Watson–Crick.

2D NOE. One virtue of the 2D NOE method is that it permits all of the major proton–proton interactions of the double helix to be probed in a single experiment (Jeener et al., 1979; Kumar et al., 1981). Figures 3–6 show contour plots of the 2D NOE spectra of 60–80 base pair fragments of poly(dI-dC), poly(dI-dbr^{5}C), and the B and Z forms of poly(dG-dC). The normal one-dimensional spectrum appears along the diagonal while the off-diagonal peaks arise from through-space dipolar interactions. If the mixing time is properly chosen, the intensity of the off-diagonal peak at ω_{AB} is proportional to the cross-relaxation rate between the two spins at ω_{AA} and ω_{BB} (Jeener et al., 1979). The cross-relaxation rate depends on the sixth power of the internuclear distance and the motional properties of the protons. In the case where the protons undergo motions of similar amplitude and frequency, the intensities of the cross peaks are proportional to the separation. However, in view of the numerous factors that may differentially affect the intensity of the cross peaks (differences in line width, relaxation rates, distortions resulting from data processing, etc.), we have only used this information to make qualitative deductions regarding the conformational features of DNA polymers.

Assignment of the Nonexchangeable Protons. The assignment of the spectra of high molecular weight simple sequence DNA is complicated because there are no unique base pairs that can be used as the starting point for a sequential assignment, as has been possible with short DNA helices (Feigon et al., 1983; Haasnoot et al., 1983). Consequently, we have had to use a slightly modified assignment strategy, which is based on the observation that certain cross peaks in the 2D NOE spectra arise from *intranucleotide* proton–proton interactions that are more or less independent of DNA conformation. These are used to identify protons within a nucleotide unit. Other cross peaks are then used to determine the nucleotide glycosidic torsional angle, and cross peaks arising from intranucleotide as well as internucleotide interactions are used to complete the assignment of resonances. Figure 1A shows some of the short interproton separations which are expected to give rise to NOEs and which may be used to correlate the protons on one type of sugar. The resonance may be assigned by following the connectivity from the H1′ → H2″ → H2′ → H3′ → H4′, H5′, H5″. The next step is to establish the orientation of the base (syn or anti) with respect to the sugar. This is accomplished by noting that in the syn conformation (Figure 1C) a strong NOE is expected between the base and the H1′ resonance, while in the anti conformation there are a number of the base–sugar proton distance shorter than the base–H1′ distance (Hart & Davis, 1971; Patel et al., 1982).

Assignment of the sugar resonances to nucleotide type is then based on the observation that, in the anti conformation, the aromatic base protons (IH8, GH8, and CH6) are relatively distant from the H2″ protons on the same nucleotide unit. Therefore, if an NOE is observed between the base protons and H2″ resonance (which may be unambiguously assigned from the H1′–H2″ interaction), then the H2″ protons must belong to the next nucleotide in the chain (5′–3′).

The 2D NOE spectrum of poly(dG-dC) at 50 °C is the most clearly resolved (Figure 5) and may be used to briefly illustrate this assignment procedure. From model studies (Tran-Dinh et al., 1982), it is known that the GH8, IH8, IH2, and CH6 resonances appear in the spectral region between 7 and 8 ppm, the H1′ and CH5 appear between 5 and 6.5 ppm, the H3′, H4′, H5′, and H5″ resonate between 3 and 5 ppm, and the highest field region, 1–3 ppm, contains the H2′ and H2″ resonances. A strong cross peak connects the CH5 and CH6 resonances at 5.5 and 7.8 ppm, and the H1′ protons at 6.0 and 6.2 ppm are connected by strong cross peaks to the H2″ protons in the same nucleotide at 2.5 and 2.9 ppm, respectively. A cross peak connects the H2″ resonance at 2.5 ppm with a peak at 2.2 ppm, which must be the H2′ protons on the same sugar residue. For the other residue, the H2″ and H2′ protons are not clearly resolved at 2.9 and 2.8 ppm, so the diagonal peak in the 2D NOE spectrum appears broadened. The H2′ resonances at 2.8 and 2.2 ppm also show cross peaks with the H3′ resonances located at 5.3 and 5.1 ppm, and the H3′ resonances show cross peaks to the broad envelope at 4.4 ppm, which contains the H4′, H5′, and H5″ proton resonances.

Table I: Chemical Shifts of the Base and Sugar Protons in Cytosine-Containing DNA Polymers

	chemical shift (ppm)			
proton	poly(dI-dC)	poly(dI-dbr^{5}C)	poly(dG-dC) B form	poly(dG-dC) Z form
IH8 or GH8	8.2	8.3	7.8	7.8
IH2	7.3	7.2		
CH6	7.2	7.6	7.2	7.2
CH5	5.1		5.2	5.0
GH1′ or IH1′	6.1	6.2	5.7	6.2
CH1′	5.6	5.6	5.4	5.3
GH2″ or IH2″	2.8	2.9	2.8	2.5
CH2″	2.3	2.4	2.3	2.5
GH2′ or IH2′	2.7	2.8	2.7	2.5
CH2′	1.8	1.9	1.8	2.5

Examination of the base–sugar interactions reveals that the GH8 interacts strongly with the overlapping H2′/H2″ resonances at 2.8 ppm and less strongly with the H2′ peak at 2.2 ppm. The presence of cross peaks between the base and H2′/H2″ protons and the absence of base–H1′ cross peaks demonstrates that both the guanine and cytosine residues have the anti orientation about the glycosidic bond. Since the GH8 is relatively far from its own H2″ protons (3.1 Å), the H2″ resonance with which the GH8 interacts must belong to the neighboring cytosine residue. From the correlation between the H2′ and H2″ protons, the peak at 2.2 ppm must be due to the CH2′. This is consistent with the observation of a cross peak between the CH6 and CH2′; the CH6–CH2′ separation is expected to be about ∼2.1 Å for the anti conformation nucleotides. Once we have assigned one of the sugar protons to a guanine or cytosine residue, we may use the intrasugar connectivities to assign all the resolved resonances to a particular nucleotide unit. A similar procedure has been used to make the resonance assignments in the other DNA polymers, and the assignments for poly(dI-dC), poly(dI-dbr^{5}C), and the B and Z forms of poly(dG-dC) are compiled in Table I. This procedure leads to the assignment of the highest field H1′, H2′, and H2″ protons to the cytosine residue in all three DNA polymers. This assignment is expected both on the basis of previous NMR studies on DNA oligomers (Tran-Dinh et al., 1982) and from the larger ring current shifts of the purine bases. In these alternating duplexes, the resonances from the pyrimidine bases are shifted furthest upfield because they are located between two purines. These assignments are consistent with the observation that the CH1′ resonances shift furthest downfield upon duplex melting in poly(dI-dC) (Patel, 1978), but they differ from those previously suggested for the H1′ protons in poly(dI-dC) (Patel, 1978) and in poly(dG-dC) (Dhingra et al., 1983).

Poly(dI-dC). Figure 3 shows the 2D NOE spectrum of poly(dI-dC) obtained at 20 °C with a 100-ms mixing time. As illustrated in Figure 1, the base–sugar NOEs are indicative of the conformational state of the DNA duplex. The intensities of the off-diagonal peaks, which are related to the interproton separation, appear in the following order:

(IH8–IH2′/IH2″) > (CH6–CH2′) >(CH6–CH5) > (IH8–CH2″)

A number of smaller cross peaks were observed in the 2D NOE spectrum between IH8 and IH1′, CH1′, and CH5 and

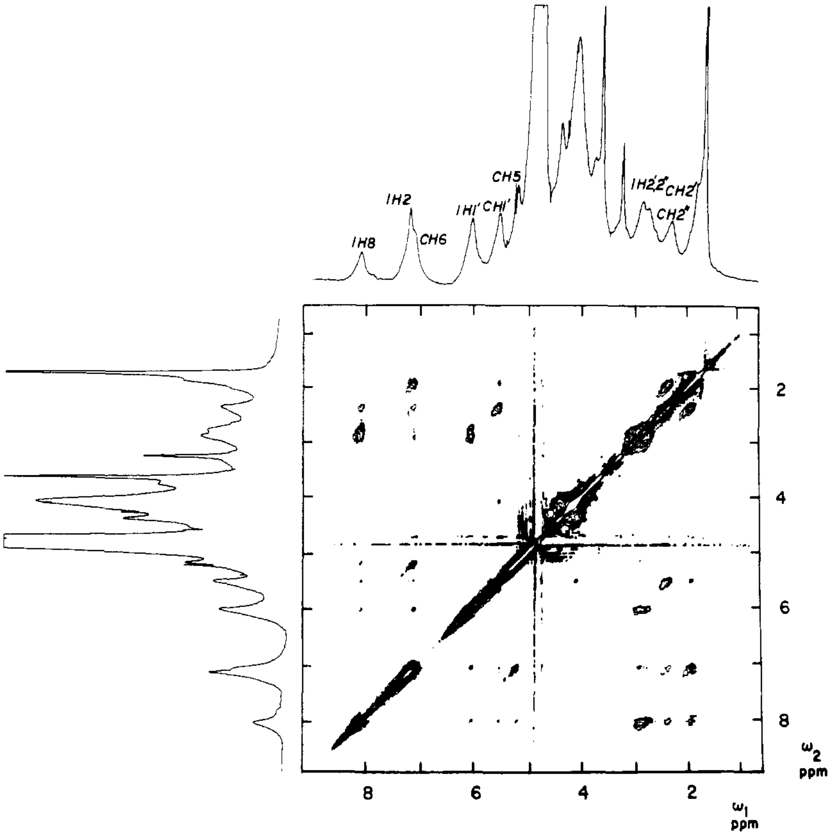

FIGURE 3: 360-MHz 2D NOE spectrum of B-form poly(dI-dC) at 20 °C obtained with a 100-ms mixing time. A total of 128 spectra each consisting of 1K data points with 32 acquisitions was obtained with a sweep width of ±1.6 KHz and was incremented by 192 μs. A 5-Hz line broadening was applied in both dimensions, and the spectra were zero filled from 128 to 256 data points prior to the second Fourier transformation. The sample contained 1.5 mg of poly(dI-dC) in 0.125 mL of buffer containing 0.1 M NaCl.

between CH6 and IH1′. These peaks may arise from protons that are situated further from each other than those that give rise to the large cross peaks, or they may be due to secondary NOEs mediated through the sugar protons. In our analysis, we have assumed that the cross peaks to the overlapping IH2/CH6 resonances at 7.4 ppm are due solely to interactions with the CH6. From examination of molecular models and previous NMR studies (Assa-Munt et al., 1984), it appears that the IH2 protons are relatively distant from the other base and sugar protons. The 2D NOE spectrum of poly(dI-dbr^{5}C) supports this assumption (see below).

To determine whether the conformation of poly(dI-dC) is similar to B-form DNA, we compared the relative intensities of the cross peaks with those expected from the idealized form of B-DNA shown in Figure 1B (Reid et al., 1983). For B-DNA, the predicted ordering of the cross peak intensities and the proton–proton separations are the following:

(CH6-CH2′) > (IH8-CH2″) > (CH6-IH2″) > (IH8-CH2″) >
2.0 Å 2.1 Å 2.2 Å 2.3 Å
(CH6-CH5), ∼(H1′-H2″)
2.4 Å 2.4 Å

Comparison of the relative intensities of the cross peaks observed for poly(dI-dC) with those predicted for an idealized B-form DNA reveals a remarkable similarity. While the model derived from X-ray diffraction data is probably not accurate to 0.2 Å, it suggests that there are a number of base–sugar distances shorter than 2.4 Å in B-DNA. Furthermore, the distance between CH5 and CH6 and H1′–H2″ protons is fixed at 2.4 Å. Using the intensities of the corresponding cross peaks as a qualitative measure of the interproton separation, we conclude there are a number of base–sugar interactions which are shorter than ∼2.5 Å. In addition to these short proton–proton contacts, there are a number of smaller cross peaks expected from interactions in the 3–4-Å range, including the interaction of the IH8 and CH6 protons with their own and neighboring H1′ protons (3.6–3.8 Å) and the interaction between the IH8 and CH5 (∼3.6 Å). The peaks resulting from the longer range interactions are observed in the 2D NOE spectrum of poly(dI-dC). While we cannot exclude possible contributions from spin diffusion to the base–H1′ interactions at such long mixing times, the IH8–CH5 interactions probably results from a direct, through-space interaction, as the secondary NOE would have to be transferred through the sugar via IH8 → CH2″ → CH2′ → CH6 → CH5. For a normal right-handed helix with a twist of 36°, the CH5 is just (∼3.6 Å) above the IH8 in the 5′–3′ direction along the chain.

To illustrate the sensitivity of the base–sugar distances and the NOEs to the exact nature of the DNA conformation (sugar conformation, glycosidic angle, twist, and rise of the helix), it is instructive to compare the NOE pattern expected for a B-form helix with that expected for an idealized A-form helix. In the A form, the sugar pucker is C3′-endo and the distance between the intranucleotide base and H2′ proton is 3.8 Å, instead of the 2.0 Å in B-DNA. The only contacts shorter than 2.5 Å are the internucleotide interaction between the base and the neighboring H2′ proton (1.5 Å) and the intranucleotide base–H3′ interaction (∼2.0 Å). Clearly, the pattern of cross peak intensities rules out the possibility of an A-type conformation for poly(dI-dC) in solution. The data also show that the IH8 and the CH6 interact with their own and neighboring sugar protons to a similar extent, indicating that both the inosine and cytosine nucleotides have the same conformation. This observation rules out the possibility of an alternating

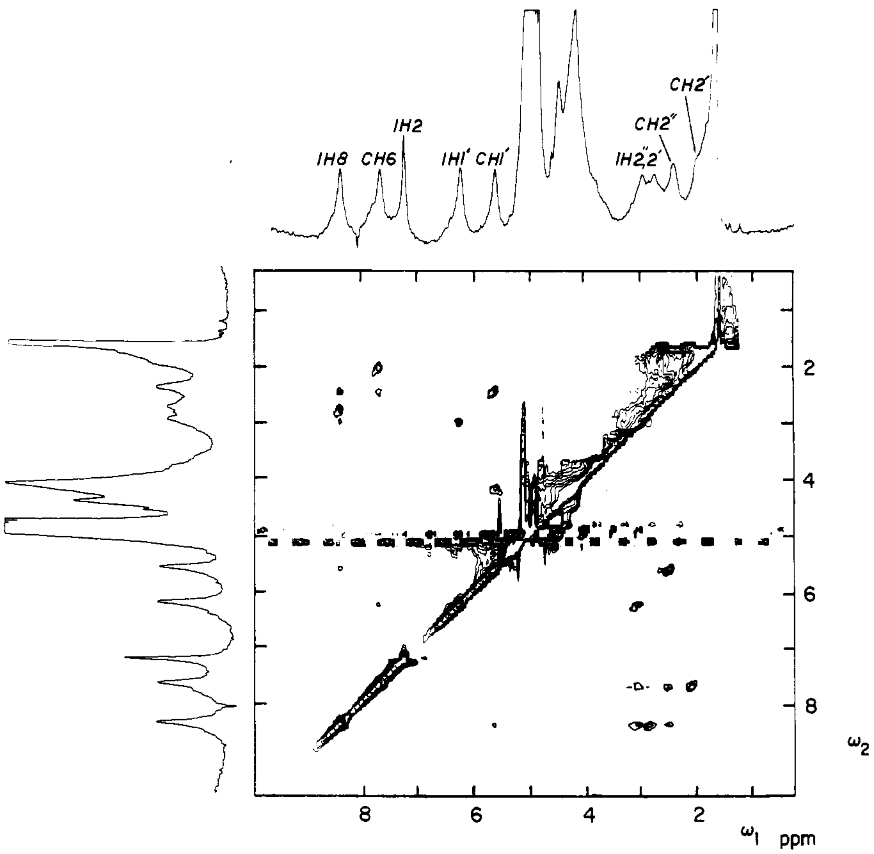

FIGURE 4: 2D NOE spectrum of poly(dI-dbr^{5}C) obtained at 20 °C with a 100-ms mixing time. See Figure 3 for details.

B-type conformation for poly(dI-dC). In this model, proposed by Klug et al. (1979) to account for the properties of poly(dA-dT), the purine and pyrimidine bases have alternating sugar puckers (C2′-endo and C3′-endo). The 2D NOE data also rule out the possibility of a left-handed B-type double helix, since it is not possible to build a left-handed helix which maintains the close interaction between the base and the neighboring H2″ protons with the bases in the anti conformation. The presence of cross peaks between the IH8 and CH6 and the H2′/H2″ protons demonstrates that both the guanine and cytosine are in the anti conformation and rules out the possibility of a Z-like conformation for poly(dI-dC) in solution. The D-DNA structure of Arnott et al. (1974) resembles B-DNA in that the sugar conformation is C2′-endo, the glycosidic torsional angle is in the anti regime, and the double helix is right-handed. Thus, many of the proton–proton distances are expected to be the same in B- and D-DNA. One difference between B-DNA and D-DNA is the separation between the base and the H1′ proton on the neighboring nucleotide; in B-DNA the separation is 3.8 Å, and in D-DNA it is 2.9 Å. The internucleotide separation in D-DNA is inconsistent with the observation of two weak, equal intensity cross peaks between the IH8 and IH1′ and the CH1′ in the 2D NOE spectrum of poly(dI-dC). On the other hand, the CH6 interacts more strongly with the IH1′ than with the CH1′. While the 2D NOE spectrum is not that expected for D-DNA, this possibility cannot be rigorously excluded because of the possible complications due to spin diffusion for such long-range interactions.

In summary, the 2D NOE spectrum of poly(dI-dC) shows a number of features that are expected for a B-form DNA helix. The short interproton contacts are indicative of a C2′-endo sugar pucker for both nucleotides and the rise and twist expected for classical B-form DNA. The data eliminate the possibility of an A-DNA alternating B-DNA of Klug et al. (1979), Z-DNA, or left-handed B-DNA. In order to be more specific about the precise geometry of poly(dI-dC), we will need to analyze the results of selective spin–lattice and spin–spin relaxation rate measurements and the effect of mixing time on the intensities of the cross peaks.

Poly(dI-dbr^{5}C). It is interesting to compare the 2D NOE spectrum of poly(dI-dC) with that of poly(dI-dbr^{5}C) (Figure 4). This modified polymer has a normal (positive) long wavelength CD band in low-salt solutions which inverts in the presence of high salt to form Z-DNA (Patel et al., 1979; Hartman et al., 1982). While the CD spectra of low salt solutions of poly(dI-dC) and poly(dI-dbr^{5}C) are substantially different, comparison of Figures 3 and 4 reveals that the proton–proton separations in the two duplexes are similar. The two major differences between the 2D NOE spectrum of poly(dI-dC) and poly(dI-dbr^{5}C) are the absence of the peaks due to CH5 and the shift of the CH6 to lower field, so that the CH6 and IH2 no longer overlap in the latter molecule. The relative intensities of the cross peaks appear in an order similar to that observed for poly(dI-dC), namely

$$(\mathrm{IH8{-}IH2'/IH2''}) > (\mathrm{CH6{-}CH2'}) > (\mathrm{CH6{-}IH2''}) > (\mathrm{IH8{-}CH2''})$$

The CH6 shows a relatively more intense cross peaks to the H2′ and H2″ protons than did poly(dI-dC), presumably because one of the major pathways for relaxation of CH6 (cross relaxation to CH5) has been eliminated by the presence of the bromine at the 5-position. No cross peaks are observed to the relatively isolated IH2 protons at 6.8 ppm, supporting the assumption made in the case of poly(dI-dC) that the cross peaks to the overlapping IH2–CH6 peak were due solely to CH6 interactions. From models, it appears that IH2 protons are isolated from the other protons, although propelling of the base pairs may bring IH2 protons on neighboring base pairs close enough together from them to interact (Assa-Munt et

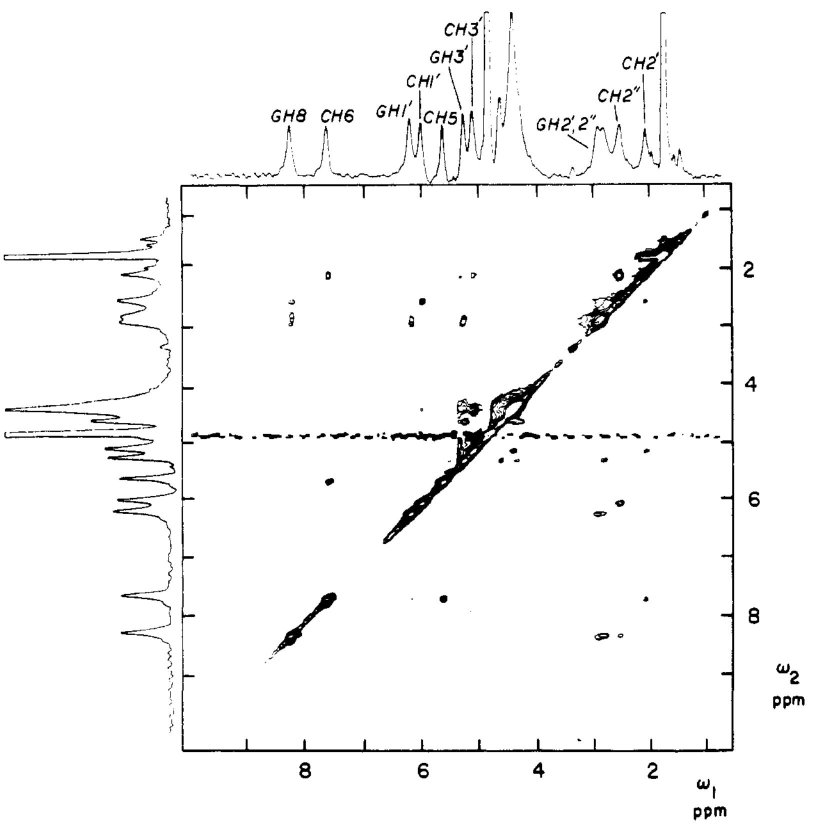

FIGURE 5: 2D NOE spectrum of B-form poly(dG-dC) obtained at 50 °C with a 50-ms mixing time.

al., 1984). However, since all of the IH2 protons have the same chemical shift, no cross peaks will be observed in the 2D spectra, but their interaction may be detected by a combination of spin–spin and spin–lattice relaxation measurements (R. W. Behling and D. R. Kearns, unpublished results). In summary, the 2D NOE analysis of the conformational properties of poly(dI-dbr^{5}C) indicates that, like poly(dI-dC), it is of the B family.

B-Form Poly(dG-dC). Figure 5 shows the 2D NOE spectrum of the low-salt form of poly(dG-dC) obtained with a 50-ms mixing time. At 50 °C the spectrum is well resolved, and the residual HOD peak no longer obscures the H3′ resonances. While this shorter mixing time emphasizes only the strongest interactions, the cross peaks in the 2D NOE spectrum of poly(dG-dC) show the features expected for B-form DNA. Namely, the GH8 interacts with the GH2′/GH2″ resonances and the CH6 interacts with the CH5 and CH2′ resonances. Resolution of the H3′ resonances is important because base–H3′ interactions are expected for certain DNA conformations. In A-form polymers, for example, the sugar adopts a C3′-endo conformation which places the H3′ protons in close proximity (~2.0 Å) to the base protons, so cross peaks with intensity similar to that observed for the CH5–CH6 interaction are expected. In poly(dG-dC), the lack of cross peaks between the GH8 or CH6 and the GH3′ or CH3′ and the presence of the peaks between the GH8 or CH6 and their respective H2′ protons are indicative of the C2′-endo conformation. These studies support the conclusions from previous X-ray (Leslie et al., 1980) and NMR studies (Patel et al., 1982) which suggest that under low-salt conditions poly(dG-dC) exists in the B form.

Z-Form Poly(dG-dC). The 2D NOE spectrum of Z-form poly(dG-dC) (Figure 6) is notably different from that obtained for the B form. In the Z form, the peaks due to the H2′ and H2″ protons overlap so it is not possible to assign them to a guanine or cytosine residue. Again, the strong peak connecting the CH5 and CH6 identifies these resonances. Evidently this interaction dominates the CH6 relaxation, as only very small cross peaks to the H2′ and H2″ protons are observed. In addition, there is a strong cross peak connecting the GH8 with the H1′ resonance at 6.2 ppm, demonstrating that the guanine nucleotide is in the syn conformation in Z-DNA (Patel et al., 1979; Wang et al., 1979). As shown in Figure 1C, a strong NOE is expected between GH8 and GH1′ since the conversion from the anti to the syn form decreases the distance between these protons from 3.8 to 2.4 Å (Hart & Davis, 1971: Reid et al., 1983). The intensity of the GH8–GH1′ cross peak is close to that observed between CH5 and CH6 and between the H1′ and the H2″ protons. Comparison of the 2D NOE spectra of the B and Z forms of poly(dG-dC) graphically illustrates the sensitivity of this technique to the details of the DNA conformation.

Discussion

In these studies, we have used one- and two-dimensional NMR studies to examine the possible sequence-dependent conformation properties of synthetic DNA polymers. These repeating sequence DNA polymers are useful for such studies because they represent extremes of the sequences which may occur in nature and their proton NMR spectra are considerably simpler than those of random sequence DNA. The 60–80 base pair fragments studied here may be a much better model for high molecular weight DNA since their solution behavior is not complicated by early melting and fraying at the ends.

The 2D NOE approach is an effective way to assign the DNA proton resonances and to make qualitative deductions about the average conformational state of the double helix in solution. The base–sugar NOEs, within the nucleotide unit, are sensitive to the sugar conformation and the glycosidic torsional angle. The internucleotide NOEs are sensitive to these same features and also to the helical parameters (rise and twist). The 2D NOE spectrum of poly(dI-dC) shows

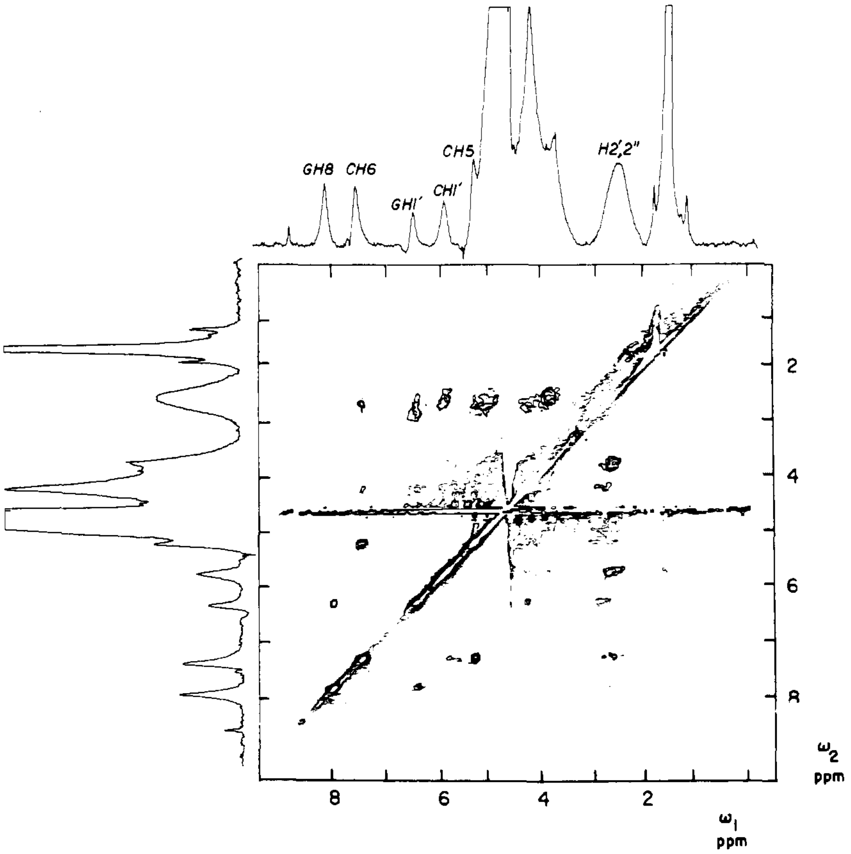

FIGURE 6: 2D NOE spectrum of Z-form poly(dG-dC) obtained in 4.5 M NaCl at 20 °C with a 50-ms mixing time. See Figure 3 for details.

many proton–proton interactions expected for a B-form DNA. By use of the CH5–CH6 and the H1′–H2″ cross peak intensities as qualitative indicators of the interproton spacing, it appears that there are a number of proton–proton contacts in poly(dI-dC) less than 2.5 Å. The interaction between the base protons, their own H2′ protons, and their neighboring H2″ protons are of the intensity expected for a DNA with a C2′-endo sugar conformation, a 3.4-Å axial rise per residue, and a 10 base pair helix helical repeat. In addition, cross peaks due to interactions in the 3–4-Å range are also observed. The pattern of cross peaks in poly(dI-dC) shows that the glycosidic angle is in the anti regime for both I and C, and the NOE between the imino and the IH2 protons in H_2O solution proves the base pairing is Watson–Crick rather than Hoogsteen. The 2D NOE data also eliminate the possibility of an A, Z, alternating B, or left-handed B-type conformation for poly(dI-dC). Taken together, these data provide experimental evidence that the solution state conformation of poly(dI-dC) is not unusual but closely resembles B-DNA. This conclusion is supported by the similarity of the 2D NOE spectra of poly(dI-dC) and of the low-salt form of poly(dI-dbr^{5}C) and poly(dG-dC), both of which are believed to exist in the B conformation.

Comparison of Figures 5 and 6 demonstrates that the 2D NOE is quite sensitive to the differences between B-DNA and Z-DNA. In Z-DNA, the interaction between the GH8 and GH1′ protons is diagnostic for syn conformation nucleotides; apparently this interaction dominates the relaxation of GH8 because only small cross peaks to H2″ and H2′ are observed. This interaction may be quite useful in the search for other Z-forming sequences such as poly(dA-dbr^{5}C)·poly(dG-dT), where the spectral region between 6.9 and 8.2 ppm contains the resonances from AH8, GH8, AH2, CH6, and TH6 (P. A. Mirau, D. R. Kearns, T. Jovin, and L. McIntosh, unpublished results). These studies are part of our continuing properties of DNA sequences.

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Registry No. Poly(dI-dC), 34639-43-5; poly(dG-dC), 36786-90-0; poly(dI-dbr^{5}C), 51853-68-0.

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Kinetic Mechanism in the Direction of Oxidative Decarboxylation for NAD–Malic Enzyme from *Ascaris suum*[†]

Sang-Hoon Park, Dennis M. Kiick, Ben G. Harris, and Paul F. Cook*

ABSTRACT: Measurement of the initial rate of the malic enzyme reaction varying the concentration of NAD at several different fixed levels of Mg^{2+} (0.25–1.0 mM) and a single malate concentration gave a pattern which intersects to the left of the ordinate. Repetition of this initial velocity pattern at several additional malate concentrations and treatment in terms of a terreactant mechanism suggests an ordered mechanism in which NAD adds prior to Mg^{2+} which must add prior to malate. On the other hand, when a broader concentration range of Mg^{2+} (0.25–50 mM) is used, data are consistent with a random mechanism in which Mg^{2+} must add prior to malate. By use of product inhibition studies, pyruvate is competitive vs. malate and noncompetitive vs. NAD, while NADH is competitive vs. NAD and noncompetitive vs. malate. These results are consistent with the random addition of substrates and further suggest rapid equilibrium random release of products. Tartronate, a dead-end analogue of malate, is competitive vs. malate and noncompetitive vs. NAD. Thio-NAD is a slow substrate which is used at 2.4% the maximum rate of NAD. When used as a dead-end analogue of NAD, thio-NAD is competitive vs. NAD and gives a complex inhibition pattern vs. malate in which competitive inhibition is apparent at low concentrations of malate (<12.5 mM), and this changes to uncompetitive inhibition at high concentrations of malate (>12.5 mM). These data are consistent with a steady-state random mechanism in the direction of oxidative decarboxylation in which Mg^{2+} adds in rapid equilibrium prior to malate. However, calculations based on intracellular levels of substrates suggest that the ordered mechanism discussed above probably operates under physiologic conditions.

The NAD–malic enzyme from *Ascaris suum* catalyzes the oxidative decarboxylation of L-malate. Initial velocity and isotope partitioning studies suggest that, with Mn^{2+} as the divalent metal ion, the enzyme has a random kinetic mechanism in the forward direction in which either malate or NAD can bind to the enzyme–Mn^{2+} complex (Landsperger et al., 1978). Thus, the kinetic mechansim of this enzyme appears to be quite distinct from the ordered sequential mechanism of the pigeon liver NADP–malic enzyme (Hsu & Lardy, 1967; Schimerlik & Cleland, 1977).

Very little is known of the extended mechanism, however (including the metal as a pseudoreactant), for either of the above enzymes. All previous studies on the *Ascaris* malic

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[†] From the Department of Biochemistry and Chemistry, North Texas State University/Texas College of Osteopathic Medicine, Denton, Texas 76203. *Received March 9, 1984.* This research was supported by National Institutes of Health Grants AI-12331 (B.G.H.) and GM 31686 (P.F.C.), an Organized Faculty Research Grant (P.F.C.) from the Texas College of Osteopathic Medicine, and BRSG S07 RR07195-03 (P.F.C.) awarded by the Biomedical Research Grant Program, Division of Research Resources, National Institutes of Health. P.F.C. is the recipient of a NIH Research Career Development Award (AM 01155).

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