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Poly(dA-dT) Has a Right-Handed B Conformation in Solution: A Two-Dimensional NMR Study†

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ABSTRACT: The structure of poly(dA-dT) molecules in solution has been probed by using two-dimensional nuclear Overhauser effect spectroscopy. Cross-relaxation patterns arising from *inter*nucleotide and *intra*nucleotide interactions are used to assign the sugar proton resonances and to deduce various structural features. The numerous proton–proton interactions that are observed indicate that poly(dA-dT) is a right-handed helix with a B-type conformation. Both the adenine and thymine nucleotides are in an anti conformation. Although slight differences in the purine–sugar and pyrimidine–sugar *intra*nucleotide interactions are observed, the large differences in the sugar pucker of the adenine vs. thymine nucleotide suggested by some models [Klug, A., Jack, A., Viswamitra, M. A., Kennard, O., Shakked, Z., & Steitz, T. A. (1979) *J. Mol. Biol. 131*, 669–680] are not evident in the solution structure of poly(dA-dT). In the low-temperature spectra there is unexpected evidence for cross-strand AH2–AH2 interactions.

Studies of simple sequence synthetic DNAs have contributed much to our current understanding of the physical properties of DNAs. Since poly(dA-dT) is a synthetic DNA that has been the subject of extensive investigation (Scheffler et al., 1968; Patel & Canuel, 1976; Viswamitra et al., 1978; Klug et al., 1979; Shindo et al., 1979; Shindo, 1981; Mahendrasingam et al., 1983; Vorlikova et al., 1983), it is surprising there is still a divergence of opinion regarding the probable structure of this molecule either in solution or in fibers. Fiber X-ray diffraction studies demonstrate that, depending upon the state of hydration and method of preparation, poly(dA-dT) adopts different forms, and a number of different models have been proposed to interpret the X-ray data. In most models it is assumed that the base pairing in poly(dA-dT) is Watson–Crick and that the helix is right-handed, but the models differ with respect to the sugar pucker and specific helical parameters (Arnott & Huckins, 1972; Arnott & Selsing, 1974; Klug et al., 1979). However, Drew & Dickerson (1982) have proposed a left-handed helix with Hoogsteen base pairs to interpret the fiber data for the D form of poly(dA-dT) [and poly(dI-dC)], and Gupta et al. (1983) suggest that poly(dA-dT) adopts a left-handed helix with Watson–Crick pairing.

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The first two-dimensional NMR experiments on double-helical DNA were reported in 1982 by Feigon et al. (1982) and independently by other groups (Frechet et al., 1983; Hare et al., 1983; Kaptein et al., 1983; Pardi et al., 1983a,b; Scheek et al., 1983). In this paper we have used two-dimensional nuclear Overhauser effect (2D NOE) spectroscopy to examine the structure of poly(dA-dT). Most of the structural features deduced from the 2D NOE measurements are consistent with a right-handed B-type conformation but not with other structures (A-DNA, Klug alternating B-DNA, left-handed helix with Watson–Crick or Hoogsteen pairing) that have been proposed. A preliminary account of this work, as well as studies on other alternating homopolymers, has been presented elsewhere (Kearns et al., 1983).

Materials and Methods

Samples. Poly(dA-dT) (P-L Biochemicals Lot 658-92) was reduced in size by $6^1/_2$ h of sonication at 0 °C under nitrogen. The sample size (54 ± 10 base pairs) was determined by ^{32}P labeling and acrylamide gel electrophoresis under nondenaturing conditions, by comparison with sized pBR322 restriction fragments. The sample was dialyzed and processed for the NMR experiments as described elsewhere (Granot et al., 1982; Assa-Munt et al., 1984). Poly(dA-dT) (120 μL) at a concentration of 42 mM in base pairs and 0.1 M NaCl, 0.010 M sodium phosphate, pH 7, and a trace of azide were deposited inside a 508-cP Wilmad microcell. The sample was dried

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Table I: Chemical Shifts Reported for the AH1′ and TH1′ Sugar Protons in Several Deoxyribonucleotides in the Melted State

	T (°C)	DNA sequence	ppm	ref		T (°C)	DNA sequence	ppm	ref
AH1′	40	5′CTAG3′	6.16	*a*	TH1′	40	5′CTAG3′	5.97	*a*
	65	TTA-	6.43	*b*		65	TTA	~6.16	*b*
	43	AAA-	6.28	*c*		72	ACATGT-	6.06	*d*
	43	AAA	6.03	*c*		65	ATGT	6.137	*e*
	43	-AAA	6.06	*c*		80	CACGTG	6.136	*f*
	72	-ACAGT	6.306	*d*		80	GTGCAC	6.132	*f*
	72	-ACAGT	6.348	*d*		81	ACATGT	6.01	*g*
	81	CCATGG	6.003	*h*					
	6	-ATGT	6.378	*e*					
	80	CACGTG	6.36	*f*					
	80	GTGCAC	6.39	*f*					
	81	CCATGG	6.348	*h*					
	81	ACATGT	6.348	*g*					

[a] Pardi et al., 1983a,b. [b] Altona et al., 1976. [c] Olsthoorn et al., 1980. [d] Tran-Dinh et al., 1982a. [e] Neumann et al., 1982. [f] Tran-Dinh et al., 1983. [g] Tran-Dinh et al., 1982c. [h] Tran-Dinh et al., 1982b.

under nitrogen and redissolved in 99% D_2O to minimize the HOD peak.

NMR Measurements. Experiments were performed on a 360-MHz Fourier transform spectrometer equipped with an Oxford Instruments magnet and a Nicolet 1180E computer. Two-dimensional NOE spectra were acquired by using the $90°-t_1-90°-\tau_{mix}-90°-t_2$ pulse sequence (Kumar et al., 1980; Nagayama et al., 1980) with alternate block accumulation and by using the pure absorption phase 2D NOE method devised by States et al. (1982).

Results and Discussion

General Assignments. The spectra of nonexchangeable protons of poly(dA-dT) at 1, 22, and 80 °C at 360 MHz are shown in Figure 1. The resonances from the AH8, AH2, TH6, and TCH_3 protons have been previously assigned (Patel, 1978). The adenine AH2 proton has much longer selective T_1 and T_2 lifetimes than the H8 proton (Early et al., 1980; Schmidt & Edelheit, 1981), and the AH8 proton has been identified by its chemical exchange with D_2O (Schweizer et al., 1964). The AH2 and TH6 resonances overlap in the 22 and 43 °C spectra, but at 1 °C they are resolved. The AH2 resonance was distinguished from the TH6 resonance in the 1 °C spectrum by truncated NOE difference spectra (Wagner & Wüthrich, 1979) in which the imino resonances were saturated for different amounts of time (data not shown; Assa-Munt et al., 1984; Kearns et al., 1983).

A rigorous assignment of the H1′ and H2′, H2″ protons of poly(dA-dT) has not previously been made, although Patel (1978) has proposed assignments based on comparison of the spectra of poly(dA-dT), poly(dA), and poly(dT) all in the melted, single-stranded state. The key element in his proposed assignments was the observation that the two H1′ resonances (~6.26 and 5.95 ppm) in single-stranded poly(dA-dT) correspond almost exactly to the H1′ resonances observed in poly(dT) and in poly(dA). We believe this crucial assignment is incorrect because the chemical shifts of the AH1′ and the TH1′ resonances are sensitive to the nature of the adjacent bases in the sequence. The H1′ sugar proton resonances can be assigned, however, by first comparing the chemical shifts of these resonances in single-stranded poly(dA-dT) with those in *appropriate* single-stranded oligonucleotides that have been assigned previously, and these data are collected in Table I. The chemical shift of an AH1′ resonance in a pyr-A-pyr sequence is typically ~6.3 ppm whereas for a pur-T-pur sequence, the TH1′ resonance is located close to 6.0 ppm. These coincide with the resonances observed in the spectra of single-stranded poly(dA-dT), and this permits the lower field resonance at ~6.3 ppm to be assigned to AH1′ and the higher field resonance at ~6.0 ppm to be assigned to TH1′. By following the positions of these two resonances through the melting point into the double-helical state (see Figure 1), we find that the 6.18 ppm resonance is from AH1′ and the 5.66 ppm resonance is from TH1′. These assignments also agree

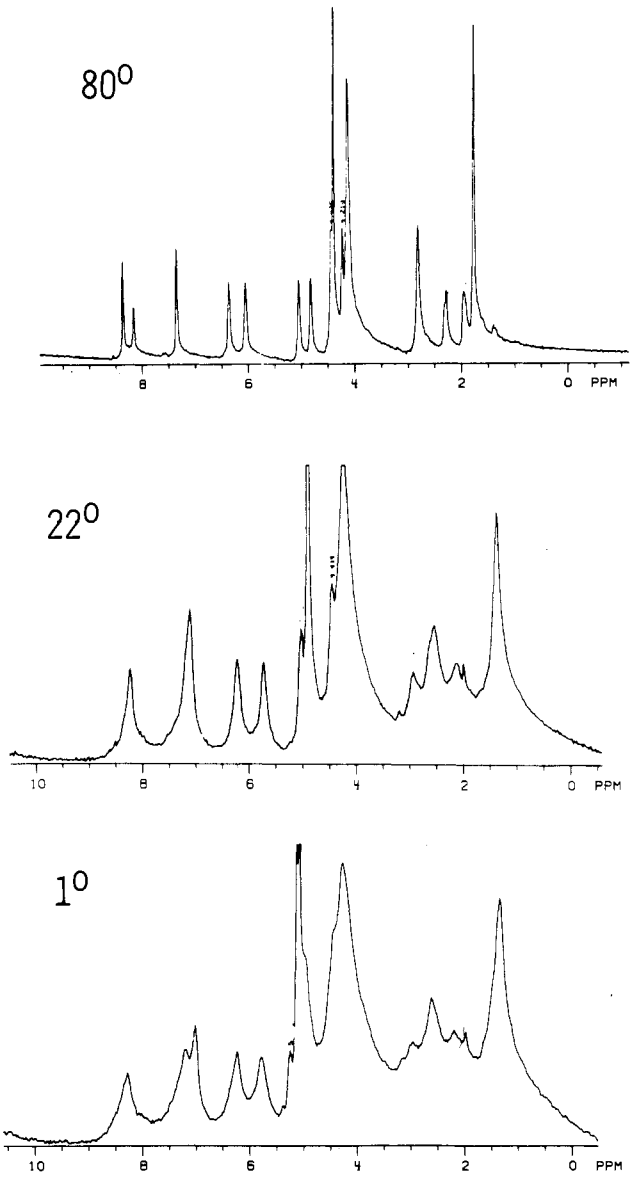

FIGURE 1: The 360-MHz spectra of poly(dA-dT) at 1, 22, and 80 °C in D_2O, pH 7.0. Note that the TH6 and AH2 resonances are resolved in the 1 °C spectrum.

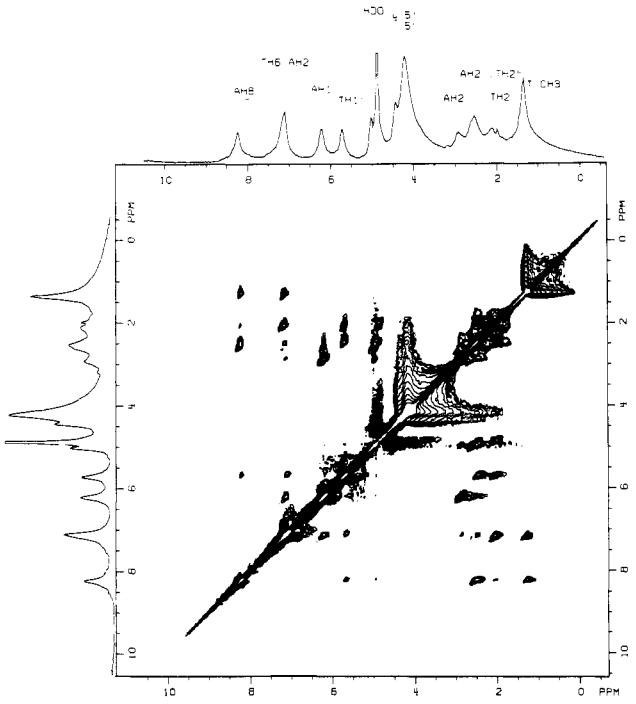

FIGURE 2: A 360-MHz pure absorption phase 2D NOE spectrum of poly(dA-dT) at 21 °C obtained with a 20-ms mixing time. A contour plot is shown, and the assignments of the various resonances are indicated on the spectra.

with the empirical assignment rules proposed by Tran-Dinh et al. (1982a,b,c) and assignments proposed by Pardi et al. (1983a,b).

Two-Dimensional NOE Studies. A 2D NOE contour plot of poly(dA-dT) obtained at 20 °C by using a 20-ms mixing time is shown in Figure 2. The intense "diagonal" peaks arise from protons that did not relax during the mixing time (Nagayama et al., 1980; Feigon et al., 1983). The smaller cross peaks that appear symmetrically off the diagonal at frequencies f_1, f_2 arise from dipole–dipole-induced cross-relaxation during the mixing time, τ_{mix}. Since cross-relaxation rates vary with the inverse sixth power of the interproton separation, a strong cross peak located at frequencies f_1, f_2 implies a short (>3.5-Å) internuclear separation between two interacting protons resonating at f_1 and f_2.

We now show how the 2D NOE spectra can be used to evaluate various structural features in poly(dA-dT) without making any assumption about the solution-state conformation of the DNA. First, sugar proton–proton interactions (e.g., H1′–H2″) are used to identify protons within a specific sugar residue. Interactions between the base and sugar protons are then used to establish the orientation of the bases relative to the sugar (i.e., syn or anti). Finally, *inter*nucleotide interactions are identified and, on the basis of the emerging patterns of interactions, a general solution conformation is deduced. Most of the experiments discussed were carried out at 43 °C because the resolution is generally better at this temperature. Qualitatively similar results were obtained at 21 °C, so the conclusions derived from the 43 °C data should also apply at 21 °C.

Assignment of Sugar Protons within a Nucleotide Unit. Sugar protons *within* a nucleotide unit can be identified by examining the 2D NOE connectivities that arise from the interaction of the protons within the *same* nucleotide unit (see Figure 3). The analysis starts with the H1′ protons because they are *always* closer to the H2″ protons than to the H2′ protons in the *same* nucleotide independent of the sugar pucker

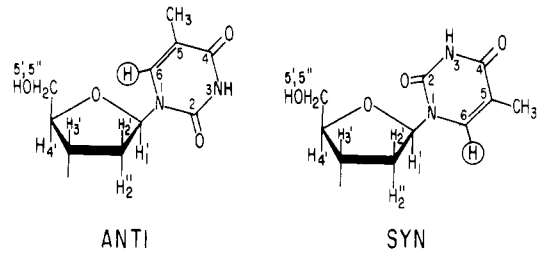

FIGURE 3: A schematic diagram illustrating the syn and anti nucleoside conformations.

(2′-endo, 3′-endo, other). Consequently, in the 2D NOE experiment the H1′–H2″ cross peaks are larger than the H1′–H2′ cross peaks, particularly at short mixing times. As evident in the cross section shown in Figure 4, the AH1′ proton at 6.18 ppm cross-relaxes mainly with a proton resonating at 2.86 ppm and to a lesser extent with a proton at 2.58 ppm. The 2.86 ppm resonance is, therefore, assigned to the H2″ proton in the A nucleotide, whereas the 2.53 ppm resonance is from the H2′ proton of sugar A. Similar reasoning leads to the assignment of a resonance at 2.50 ppm to the TH2″ sugar proton located next to the TH1′ proton resonating at 5.66 ppm. The highest field resonance in this group (2.06 ppm) is assigned to the TH2′ proton. These assignments can be corroborated by observing the corresponding *intra*nucleotide cross-relaxation patterns arising from excitation of either of the 2″ resonances at 2.9 and 2.44 ppm (Figure 4C).

The H2′ → H3′ interaction can be used to assign the H3′ sugar resonances. For example, in the cross section shown in Figure 4C the AH2′ proton at 2.58 ppm interacts with the proton at 4.98 ppm, which is thus assigned to the H3′ proton of sugar A. The resonance at ~4.86 ppm is assigned to the TH3′ proton on the basis of its interaction with the TH2′ resonance at ~2.06 ppm. Assignment of the H4′, H5′, and H5″ resonances at ~4.2 and ~4.4 ppm was not feasible because they overlap at all the temperatures studied.

Anti vs. Syn Conformation. Models show that in the syn conformation, the AH8 and TH6 base protons are positioned close (~2.4 Å) to their own H1′ sugar proton (AH1′ or TH1′) but distant (~4.0 Å) from the H2′ sugar proton, whereas in an anti conformation, these base protons are very close (~2.2 Å) to their own 2′ sugar proton and distant (~4.0 Å) from the H1′ proton (see Figure 3). Cross sections of the 2D NOE spectra, obtained at 43 °C by using a 20-ms mixing time (see Figure 4), demonstrate that the base protons (AH8 or TH6) interact most strongly with the H2′ proton on their *own* nucleotide unit. Interactions with the H1′ sugar proton of the same nucleotide are absent, or extremely weak, as are the interactions with the H2″ protons on the *same* nucleotide. This pattern of interaction indicates that both A and T have anti orientations with respect to their own sugar groups. Since the magnitudes of the H2″–H1′ and the AH8–AH2′ and TH6–TH2′ cross peaks are all comparable, we further conclude that the relevant interproton distances are comparable (~2.4 Å). The fact that cross peaks between the H3′ and the base (AH8 or TH6) resonances are very weak or absent indicates that both the A and T sugars have a 2′-endo (3′-exo) conformation.

Helical Structure of Poly(dA-dT). A summary of the relevant interproton distances [*intra-* and *inter-* (3′–5′) nucleotide] predicted for the alternating B-DNA (Klug et al., 1979), D-DNA (Arnott & Selsing, 1974), B-DNA (Arnott et al., 1983), and A-DNA (Arnott & Huckins, 1972) structures is given in Table II. While conclusions drawn from comparison of relative intensities of cross peaks have to be taken with caution, the fact that both AH8 and TH6 show

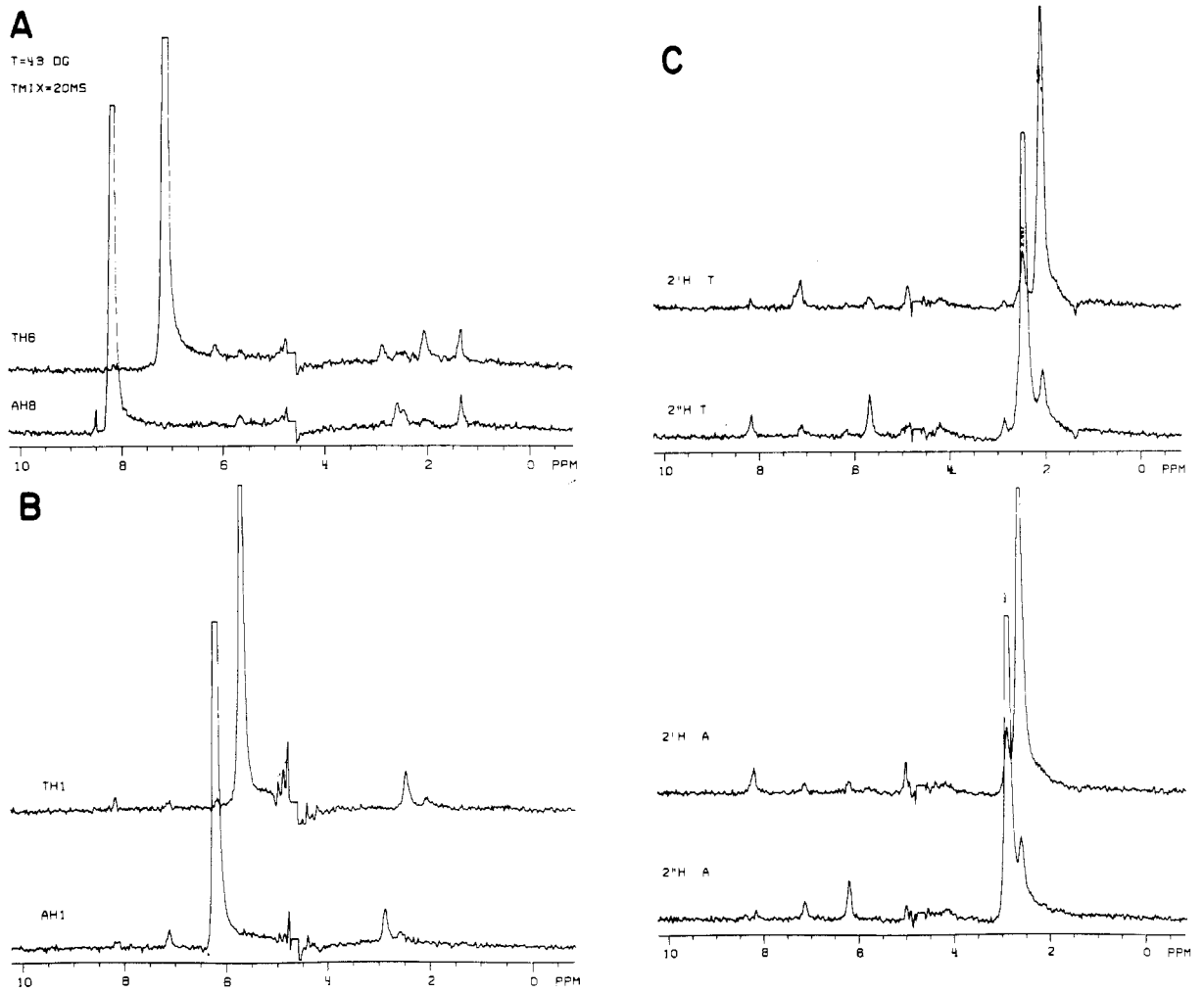

FIGURE 4: Cross sections of the 360-MHz 2D NOE spectrum of poly(dA-dT) at 43 °C illustrating interactions between the base protons (AH8 or TH6) and sugar protons (A), interactions between the H1′ sugar protons and other protons (B), and interactions between 2′,2″ sugar protons and other protons in poly(dA-dT) (C). The mixing time was 20 ms.

strong interactions with their own H2′ protons, slightly weaker internucleotide interactions with the H2″ protons of neighbor nucleotides, and virtually nonexistent interactions with any other sugar protons (except at long mixing times) requires a right-handed helix. Moreover, these are all features expected for a B-DNA conformation but not for other conformations. In particular, it is not possible to construct a left-handed helix in which the appropriate *inter*nucleotide interactions between the base (AH8 or TH6) protons and the H2″ sugar proton could be generated. For an A conformation (3′-endo sugar pucker), the *inter*nucleotide interaction between AH8 (or TH6) and H2′ is predicted to be much stronger than the AH8–TH2″ or the TH6–AH2″ internucleotide interaction (Table II). Since the reverse pattern is observed, the A conformation is eliminated. The D conformation also appears inconsistent with the 2D NOE results, since interactions between AH8 or TH6 of one nucleotide and the H1′ and H2″ sugar protons of the neighboring nucleotide are predicted to be comparable in strength, whereas the observed *inter*nucleotide base–H1′ sugar proton interactions are weak. Moreover, Arnott's D structure (Arnott et al., 1983) predicts that the *inter*nucleotide TH6–AH2′ interaction (4.36-Å interproton distance) is weaker than the H2″–H1′ *intra*nucleotide interactions (3.01 Å), whereas cross peaks of comparable intensity are observed (Figure 4).

In addition to the lack of evidence for a 3′-endo conformation for the adenine sugar, two other features of the Klug model (Klug et al., 1979) conflict with the experimental observations. First, the AH8 proton is predicted to be relatively far (3.1 Å) from its own AH2′ proton but close (2.21 Å) to the thymine TH2″ sugar proton, whereas the reverse pattern of interactions is observed. Second, the TH6 proton is predicted to be only 1.78 Å from the TH2′ proton and rather distant (2.9 Å) from the AH2″ proton. Experimentally, the cross peaks associated with these two interactions are quite

Table II: Interproton Distances (Å) Computed from Proposed Poly(dA-dT) Structures

	TH6 interactions					
	intranucleotide			internucleotide (3 → 5′)		
model	TH2′	TH2″	TH1′	AH2′	AH2″	AH1′
B-DNA[a]	1.78	3.19	3.66	2.90	3.30	5.32
B-DNA[b]	1.97	3.27	3.67	3.99	2.21	3.53
D-DNA[c]	1.81	3.41	3.69	4.36	2.63	2.90
A-DNA[d]	3.8	4.8	3.8	1.5	3.0	4.0
B-DNA[d]	2.0	3.5	3.8	3.9	2.5	2.9
	AH8 interactions					
	intranucleotide			internucleotide (3 → 5′)		
model	AH2′	AH2″	AH1′	TH2′	TH2″	TH1′
B-DNA[a]	3.10	4.45	3.83	3.82	2.21	3.15
B-DNA[b]	2.27	3.5	3.84	3.86	2.14	3.55
D-DNA[c]	2.13	3.69	3.89	4.13	2.44	2.85

[a] Klug et al., 1979. [b] Arnott et al., 1983. [c] Arnott & Selsing, 1974. [d] Arnott & Huckins, 1972. Averaged values for purines and pyrimidines.

comparable in intensity, and not different by an order of magnitude as predicted by the mode. We note also that large differences between the AH8–TH1′ and the TH6–AH1′ *inter*base interactions (3.15 vs. 5.32 Å, respectively) are predicted for the Klug model (see Table II), whereas the observed cross peaks corresponding to these interactions are of comparable intensity, indicating there is little alternation in the A and T conformations.

Taken collectively, the pattern of interactions detected by the 2D NOE experiments is most consistent with those expected for a right-handed B-DNA structure, although modifications of the B structure might lead to even better agreement with experiment. Some possibilities are noted below.

TCH_3–AH8 Interaction. A strong cross peak between the thymine methyl protons and the AH8 protons is observed in the 2D NOE spectrum (Figures 2 and 4A). This *inter*nucleotide interaction indicates that the AH8 protons are quite close ($\langle r \rangle \sim 2.8$ Å) to the neighboring thymine methyl group on the same strand. We first observed AH8–TCH_3 cross peaks in the NOESY spectra of the decamer $d(ATATCGATAT)_2$ and noted that the AH8–TCH_3 internucleotide interaction is a feature consistent with a general B-type structure (Feigon et al., 1982, 1983).

AH2–Sugar Interactions. The above discussion applies to experiments carried out at 43 °C. In order to obtain spectra in which the TH6 and AH2 resonances were resolved, it was necessary to carry out experiments at 1 °C. In 2D NOE experiments performed at 1 °C with a long mixing time (20 ms), a weak interaction between the AH2 proton and the AH1′ proton (6.2 ppm) and an even weaker interaction with the TH1′ proton (5.7 ppm) are observed. These interactions are not due to secondary NOEs, since no other strong cross peak is visible in the pertinent region of the contour plot (Figure 5). Since protons on the *same* nucleotide are located at a distance larger than 4.8 Å, the observed AH2–AH1′ interaction probably arises from adenine nucleotides on opposite strands (vide infra), as suggested by the Klug et al. (1979) model. The weak AH2–TH1′ interaction presumably arises from a neighboring thymine nucleotide on the same strand. These AH2 interactions with sugar protons could help explain the unexpectedly fast selective relaxation rates observed for AH2 [T_1(selective) = ~286 ms at 1 °C] (unpublished results; Kearns et al., 1983).

AH2–AH2 Interactions. One curious feature observed in the 1 °C contour plot and also in 21 °C contour plot is a cross peak appearing very close to the diagonal arising from interaction of AH2 protons resonating at 6.99 ppm with AH2 protons resonating at slightly higher field (~6.85 ppm), but still within the envelope of AH2 resonances. This cross peak might represent a cross-strand AH2–AH2 interaction between adenine nucleotides that have been brought to close proximity of each other at special sites along the DNA. Levitt (1978) predicted that the base pairs in DNA should be propeller twisted to achieve better stacking interactions of bases in the same strand. This propellering can also bring AH2 protons in opposite strands into close contact. Concrete experimental evidence for propellering of base pairs has now been obtained from the analysis of X-ray diffraction data of the dodecanucleotide d(CGCGAATTCGCG) (Drew et al., 1981). Moreover, Patel et al. (1983) have observed an NOE between AH2 protons in the -TATA- section of d(CGATTATAAT) (Patel et al., 1983). The observation of an AH2–AH2 cross peak in poly(dA-dT) is surprising, however, because it implies that slightly different conformations coexist within the poly(dA-dT) helix and give rise to a dispersion of chemical shifts. The exact origin of such altered conformations is not understood, although manifestations of its existence have been observed (Assa-Munt et al., 1984; Mirau & Kearns, 1983). The line widths observed in poly(dA-dT), poly(dG-dC), and other DNA

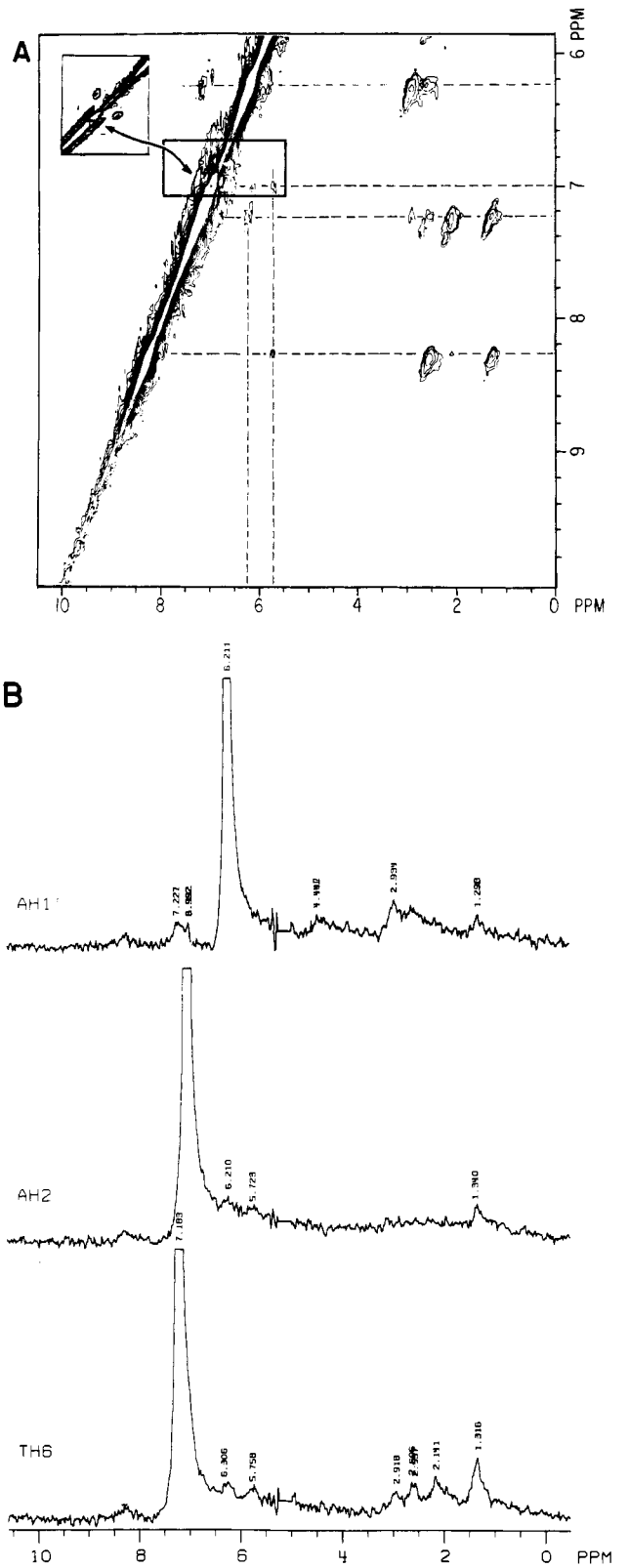

FIGURE 5: (A) A contour plot of the pure absorption phase 2D NOE spectrum of poly(dA-dT) obtained at 1 °C with a 20-ms mixing time. The insert shown at the upper left was obtained by using a set of contours that emphasize the AH2–AH2 cross peaks. (B) Cross section of the 1 °C 2D NOE spectrum illustrating interaction between the AH2, TH6, and AH1′ protons and sugar proteins. Note that the section passing through the peak of the AH2 resonance does not contain the AH2–AH2 cross peaks.

molecules are larger than expected from T_2 measurements by a factor of ~2–3, suggesting that a variety of slowly interconverting, discrete conformational states coexist in these molecules. Further experiments on this point are in progress.

In closing, we note that Gupta et al. (1983) suggested, on the basis of truncated driven NOE measurements, that poly(dA-dT) adopts a *left*-handed B conformation with $\chi \simeq 0°$ (as compared with $\chi \simeq 60°$ for a typical anti-nucleotide conformation). Since their conclusions are at variance with ours, we need to comment on the factors that might be responsible for the discrepancy. First, we note that their poly(dA-dT) was 400 ± 150 base pairs in length, whereas our samples were 53 ± 10 base pairs. Because of this difference (factor of 8) in size, we expect that spin diffusion effects will be much more serious in their experiments than ours. In our 20 °C measurements, where we used mixing times of 2, 8, and 20 ms, second-order NOEs definitely appear at the later times. Therefore, the use of preirradiation times of 30 ms, or larger, with higher molecular weight DNA will undoubtedly lead to extensive spin diffusion and nonspecific NOEs. Thus, the fact that Gupta et al. (1983) did not observe specific NOEs involving base–sugar proton interactions, a key feature leading to their conclusions regarding the structure of the molecule, may be understood.

Acknowledgments

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