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Sequence dependence of base-pair stacking in right-handed DNA in solution: Proton nuclear Overhauser effect NMR measurements

(dodecanucleotide/minor-groove proton/inter-base-pair nuclear Overhauser effect/propeller-twisted base pairs)

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ABSTRACT Single-crystal x-ray studies of d(C-G-C-G-A-A-T-T-C-G-C-G) exhibit base-pair propeller twisting [Dickerson, R. E. & Drew, H. R. (1981) *J. Mol. Biol.* 149, 761–786] that results in close contacts between adjacent purines in the minor groove in pyrimidine (3′-5′)-purine steps and in the major groove in purine (3′-5′)-pyrimidine steps [Calladine, C. R. (1982) *J. Mol. Biol.* 161, 343–362]. These observations require an ≈3.4 Å separation between the minor groove edges of adenosines on adjacent base pairs for the dA-dA step but predict a smaller separation for the dT-dA step and a larger separation for the dA-dT step in a d(A-T-T-A)·d(T-A-A-T) fragment. We have confirmed these predictions from steady-state nuclear Overhauser effect measurements between assigned minor groove adenosine H-2 protons on adjacent base pairs in the proton NMR spectrum of the d(C^1-G^2-A^3-T^4-T^5-A^6-T^6-A^5-A^4-T^3-C^2-G^1) self-complementary dodecanucleotide duplex (henceforth called the Pribnow 12-mer) in solution. The measured cross-relaxation rates (product of steady-state nuclear Overhauser effect and selective spin–lattice relaxation rates) translate to interproton separations between adjacent adenosine H-2 protons of 4.22 Å in the (dA^3-dT^4)·(dA^4-dT^3) step, of 3.56 Å in the (dT^4-dT^5)·(dA^5-dA^4) step, and of 3.17 Å in the (dT^5-dA^6)·(dT^6-dA^5) step for the Pribnow 12-mer duplex with an isotropic rotational correlation time of 9 ns at 5°C. These proton NMR results show that the sequence-dependent base-pair stacking resulting from base-pair propeller twisting of defined handedness for right-handed DNA in the solid state is maintained in aqueous solution.

The early fiber-diffraction analysis of the structure of natural DNA assumed a regular conformation with the double helix built up by the rotational and translational transformations of the same mononucleotide repeat (1). This view was modified when enzymatic digestion (2, 3) studies were extended to alternating purine-pyrimidine copolymers and it was proposed (4) and experimentally verified (5) that the sugar-phosphate backbone in the purine-pyrimidine and pyrimidine-purine steps were dramatically different. These insights into possible dinucleotide repeat conformations were put on a firmer basis after solution of the single-crystal x-ray structure of the left-handed Z-DNA conformation for d(C-G-C-G-C-G) with its unique zigzag phosphodiester backbone (6).

More subtle sequence-dependent effects came to light after the x-ray analysis of single crystals of d(C-G-C-G-A-A-T-T-C-G-C-G), in which Dickerson and Drew (7) noted propeller-twisted base pairs throughout the length of the dodecanucleotide duplex. This propeller twisting was initially proposed from theoretical studies by Levitt (8) to account for better stacking of bases on the same strand and independently observed experimentally by Crothers and co-workers (9) in their electric field dichroism measurements of DNA in solution. It soon became apparent that there would be steric clashes in the DNA grooves between purines on partner strands as a result of the propeller twisting and Calladine (10) has recently proposed several mechanisms for relief of these nonbonded close contacts. Thus, in a purine (3′-5′)-pyrimidine step, the side chain groups in the 6 position of purines on partner strands clash in the major groove (Fig. 1*A*), while in the pyrimidine (3′-5′)-purine step, the side chain groups in the 2 position of purines on partner strands clash in the minor groove (Fig. 1*C*). By contrast, there are no close steric contacts in pyrimidine (3′-5′) pyrimidine steps when the purines are on the same strand (Fig. 1*B*) (10, 11).

It is important to deduce whether the sequence-dependent observations observed for right-handed B-DNA fragments in crystals (7) also hold in solution. Progress in this area has been made by Klug and collaborators (12), who demonstrated that the relative rates of DNase I cleavage of the phosphodiester backbone of d(C-G-C-G-A-A-T-T-C-G-C-G) correlate closely with the extensive local variations in the helix twist angle in the heavily hydrated crystals (7).

The adenosine H-2 proton is located in the minor groove of dA·dT base pairs and it became apparent to us that this marker could help us differentiate between the three sequence-dependent geometries for the dinucleotide segments shown in Fig. 1. Specifically, the minor-groove base-pair edges of adjacent adenosines would be separated by the standard 3.4 Å separation in the pyrimidine (3′-5′)-pyrimidine segment (Fig. 1*B*), be >3.4 Å when the steric clash occurred in the major groove in the purine (3′-5′)-pyrimidine segment (Fig. 1*A*), and be <3.4 Å when the steric clash occurred in the minor groove in pyrimidine (3′-5′)-purine segments (Fig. 1*C*). These possibilities can in principle be differentiated because the separation between adenosine H-2 protons would differ in the three cases and can be approached through the distance dependence of the nuclear Overhauser effect (NOE).

The application of proton NOE to elucidate conformational features of nucleic acid structure was developed by Redfield *et al.* (13) in a series of investigations on tRNA. These workers realized the power of the method to correlate proton resonances on adjacent base pairs and followed up with elegant demonstrations of its potential in unravelling tRNA conformation. The method is quite general and has been applied in our laboratory to elucidate structural features of right-handed (14, 15) and left-handed (16) DNA and their base-pair mismatch and helix interruption analogs (14) and to monitor intermolecular interactions between antibiotics and DNA (17).

This paper describes proton NOE measurements to elucidate the sequence-dependent base-pair stacking in the dA·dT-rich octanucleotide core of the self-complementary d(C-G-A-T-T-A-T-A-A-T-C-G) dodecanucleotide duplex (henceforth called the Pribnow 12-mer) as shown below.

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Abbreviation: NOE, nuclear Overhauser effect.

C	G	A	T	T	A	T	A	A	T	C	G
1	2	3	4	5	6	6	5	4	3	2	1
G	C	T	A	A	T	A	T	T	A	G	C

There are four unique dA·dT base pairs, numbered 3, 4, 5, and 6, that provide us with all three sequence dinucleotides (dA^3-dT^4)·(dA^4-dT^3) (Fig. 1*A*), (dT^4-dT^5)·(dA^5-dA^4) (Fig. 1*B*) and (dT^5-dA^6)·(dT^6-dA^5) (Fig. 1*C*) necessary to check the consequences of steric clash between adjacent purines on partner strands resulting from base-pair propeller twisting of defined handedness in DNA fragments.

RESULTS AND DISCUSSION

Assignments. We have assigned the thymidine imino and adenosine H-2 protons in the Pribnow 12-mer duplex from intra- and inter-base pair NOEs recorded at low temperature. These assignments follow the procedure outlined by Redfield *et al.* (13) and a few examples are shown in Fig. 2 and discussed below.

The 498-MHz proton NMR spectrum (Redfield 214 pulse sequence) of the Pribnow 12-mer in 0.1 M phosphate (pH 6.90) at 5°C is presented in Fig. 2*A*. The imino exchangeable protons resonate between 12.5 and 14 ppm and the aromatic and amino exchangeable protons resonate between 6.5 and 8.5 ppm. The spectrum was not recorded upfield from 7 ppm because of the onset of the H_2O peak at ≈5 ppm. We observed five resolved imino protons between 12.5 and 14 ppm from the five non-terminal base pairs 2, 3, 4, 5, and 6 in the Pribnow 12-mer duplex. The imino proton of terminal base pair 1 has broadened out because of rapid exchange as a result of fraying at the ends of the duplex (14). The 12.83-ppm imino proton at highest field is next to broaden as the temperature is increased and is assigned to the dG·dC base pair 2. Saturation of the 12.83-ppm guanosine imino proton of base pair 2 results in a NOE to a broad cytidine amino resonance at 8.51 ppm in the same base pair and to the imino (13.65 ppm) and the narrow adenosine H-2 (7.80 ppm) of an adjacent dA·dT base pair (data not shown), which must be assigned to base pair 3 (Table 1).

Three of the dA·dT base pairs are flanked by different base pairs on either side while dA·dT base pair 6 is flanked by an identical base pair in one direction and by dA·dT base pair 5 on the other side. Thus, saturation of the imino proton of dA·dT base pair 6 should result in an inter-base-pair NOE only at the imino proton of base pair 5. We observe a single NOE at the 13.48 ppm imino proton on saturation of the 13.18-ppm imino proton (Fig. 2*C*), permitting assignment of the former to the thymidine imino proton of base pair 5 and the latter to the thy-

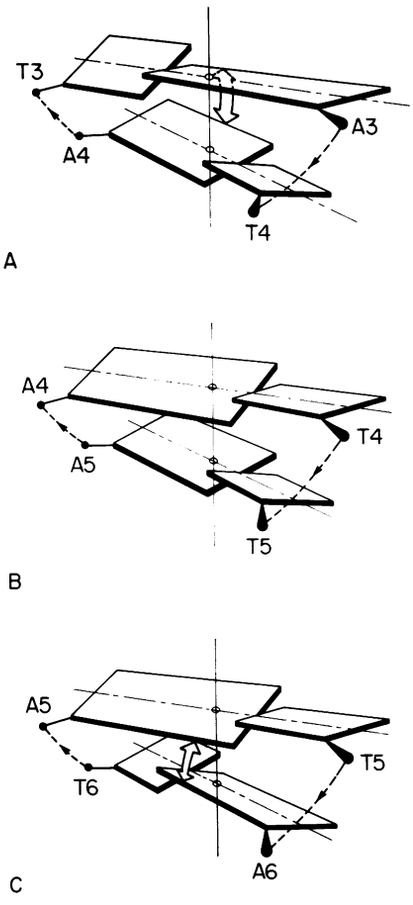

FIG. 1. Schematic drawings of successive propeller-twisted base pairs (dA^3-dT^4)·(dA^4-dT^3) (*A*), (dT^4-dT^5)·(dA^5-dA^4) (*B*), and (dT^5-dA^6)·(dT^6-dA^5) (*C*) in the d(C^1-G^2-A^3-T^4-T^5-A^6-T^6-A^5-A^4-T^3-C^2-G^1) Pribnow 12-mer duplex based on the single-crystal results on the d(C-G-C-G-A-A-T-T-C-G-C-G) 12-mer duplex. The propeller twists with indicated handedness introduce a clash of purine ring edges on adjacent strands in the major groove in the (dA^3-dT^4)·(dA^4-dT^3) dinucleotide step in *A* and in the minor groove in the (dT^5-dA^6)·(dT^6-dA^5) dinucleotide step in *C* (shown by curved double-headed arrows). This drawing is adapted with the author's permission from ref. 11.

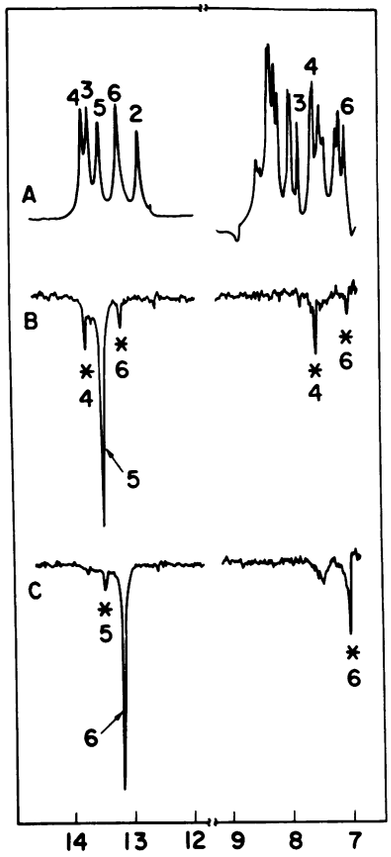

FIG. 2. (*A*) The 498-MHz proton Fourier transform (Redfield 214 pulse sequence) spectrum of the imino (12–14.5 ppm) and the aromatic (7–9 ppm) protons of the Pribnow 12-mer duplex in 0.1 M phosphate/H_2O, pH 6.90, at 5°C. (*B* and *C*) Difference spectra after 1-s saturation of the 13.48-ppm thymidine imino proton of dA·dT base pair 5 and the 13.18-ppm thymidine imino proton of dA·dT base pair 6, respectively. Arrows designate saturated resonances and asterisks indicate observed NOE effects.

Table 1. Proton chemical shifts of the thymidine H-3 imino and adenosine H-2 nonexchangeable protons of dA·dT base pairs in the Pribnow 12-mer

Base pair	Chemical shift, ppm	
	T (H-3)	A (H-2)
3	13.65	7.80
4	13.75	7.54
5	13.48	6.50
6	13.18	7.01

Shifts were measured in 0.1 M phosphate/H_2O, pH 6.90, at 5°C.

midine imino proton of base pair 6 (Table 1). Saturation of the 13.18-ppm imino proton of base pair 6 also results in a NOE at the 7.01-ppm adenosine H-2 of the same base pair (Fig. 2*C*) with the adenosine H-2 of adjacent base pair 5 resonating to higher field at 6.50 ppm (Table 1). These results permit assignments of the thymidine imino and adenosine H-2 protons of dA·dT base pairs 5 and 6.

Saturation of the 13.48-ppm thymidine imino proton of base pair 5 results in inter-base-pair NOEs to the 13.18-ppm thymidine imino proton assigned to base pair 6 and a thymidine imino proton at 13.75 ppm, which must be assigned to base pair 4 (Fig. 2*B* and Table 1). We cannot monitor the NOE to the 6.50-ppm adenosine H-2 of the same base pair because it resonates to high field of the spectra in Fig. 1 but can readily detect the NOEs to the adenosine H-2 protons of adjacent base pairs at 7.54 and 7.01 ppm (Fig. 1*B*). Since the 7.01-ppm resonance has been assigned to the adenosine H-2 of base pair 6, the 7.54-ppm adenosine H-2 must be assigned to base pair 4 (Table 1). This completes the assignment of the thymidine imino and adenosine H-2 protons of base pairs 3, 4, 5, and 6 (Table 1) and the conclusions were verified by observing the intra- and inter-base-pair NOEs on saturation of the 13.75-ppm thymidine imino proton of dA·dT base pair 4 (data not shown).

Minor Groove NOEs. The resolution-enhanced 498-MHz proton NMR spectrum (6–8.5 ppm) of the Pribnow 12-mer in 0.1 M phosphate/^2H_2O at 5°C is presented in Fig. 3*A*. The four adenosine H-2 singlets are well resolved from each other and resonate at 7.81 ppm (dA^3), 7.55 ppm (dA^4), 7.03 ppm (dA^6), and 6.50 ppm (dA^5) with the assignments based on results presented in the previous section.

The difference spectrum after 1-s saturation of the 7.55-ppm adenosine H-2 of base pair 4 exhibits small NOEs at the 7.81-ppm adenosine H-2 of base pair 3 and the 6.50-ppm adenosine H-2 of base pair 5 (Fig. 3*C*). By contrast, the difference spectrum after 1-s saturation of the 6.50-ppm adenosine H-2 of base pair 5 exhibits a large NOE at the 7.03-ppm adenosine H-2 of base pair 6 and a smaller NOE at the 7.55-ppm adenosine H-2 of base pair 4 (Fig. 3*B*).

The smallest NOEs are observed between the adenosine H-2 protons of base pairs 3 and 4 in the (dA^3-dT^4)·(dA^4-dT^3) dinucleotide segment. Somewhat larger NOEs are observed between the adenosine H-2 protons of base pairs 4 and 5 in the (dT^4-dT^5)·(dA^5-dA^4) dinucleotide segment while the largest NOEs are observed between the adenosine H-2 protons of base pairs 5 and 6 in the (dT^5-dA^6)·(dT^6-dA^5) dinucleotide segment (Fig. 3).

Relaxation Parameters. The adenosine H-2 protons of dA·dT base pairs are isolated from other nonexchangeable base and sugar protons, resulting in a two-spin system of saturated proton *i* and observed proton *j* interacting predominantly with each other.

The cross relaxation rate constant $\sigma_{ij} = \eta_{ij}\rho_j$ can be evaluated by measuring the steady-state NOE η_{ij} and the selective spin–lattice relaxation rate constant ρ_j. The cross-relaxation rate constant σ_{ij} can be expressed in terms of the isotropic rotation correlation time for molecular motion τ_c and the interproton separation r_{ij} (13)

$$\sigma_{ij} = \frac{-5.68 \times 10^{10}}{r_{ij}^6}\,\tau_c.$$

Thus, it is possible to evaluate the interproton distance r_{ij} between saturated spin *i* and observed spin *j* from experimentally measured values of σ_{ij} and an estimate of τ_c.

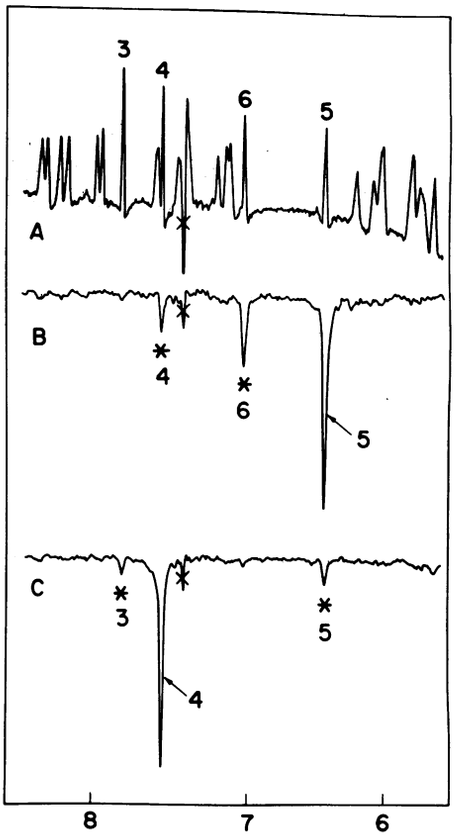

FIG. 3. (*A*) The resolution-enhanced 498-MHz proton Fourier transform spectrum of the aromatic (6–8.5 ppm) protons of the Pribnow 12-mer duplex in 0.1 M phosphate/^2H_2O, pH 7.17, at 5°C. (*B* and *C*) Difference spectra after 1-s saturation of the 6.50-ppm adenosine H-2 proton of dA·dT base pair 5 and the 7.55-ppm adenosine H-2 proton of dA·dT base pair 4, respectively. Arrows designate saturated resonances and asterisks indicate observed NOE effects. The NMR spectra contain a spike at 7.45 ppm designated by ×.

Cross-Relaxation Rate Constants. The time development of the NOEs at adjacent adenosine H-2 protons on saturation of the adenosine H-2 proton of dA·dT base pair 5 and of dA·dT base pair 4 in the Pribnow 12-mer in 0.1 M phosphate at 5°C are presented in Fig. 4 *A* and *B*. The steady-state condition is reached for saturation pulses of length above 2 s (Fig. 4) and the experimental steady state η_{ij} values are summarized in Table 2.

We have measured the selective spin–lattice relaxation rate constants at adenosine H-2 protons of dA·dT base pairs 3, 5, and 6 in the Pribnow 12-mer in 0.1 M phosphate at 5°C by the saturation recovery method. The adenosine H-2 protons recover their magnetization at different rates, with the selective spin–lattice relaxation rate constants ρ_j for the adenosine H-2 protons in the Pribnow 12-mer at 5°C decreasing in the order base pair 5 > 6 > 3 (Fig. 5). The experimental ρ_j values are summarized in Table 2.

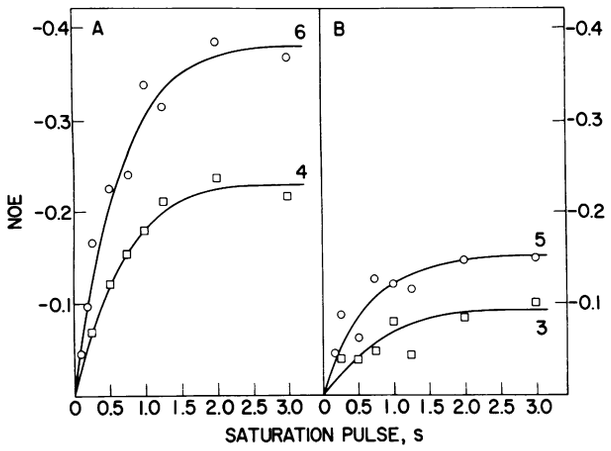

FIG. 4. Buildup of negative NOEs as a function of the length of the saturation pulse in the Pribnow 12-mer duplex in 0.1 M phosphate/^2H_2O, pH 7.17, at 5°C. NOEs were monitored at adjacent adenosine H-2 protons on saturation of the adenosine H-2 proton of dA·dT base pair 5 (*A*) and dA·dT base pair 4 (*B*). A recycle delay of 1 s was used between successive scans.

The cross-relaxation rate constants $\sigma_{ij} = \eta_{ij}\rho_j$ can be computed from a knowledge of η_{ij} and ρ_j and these are tabulated for the three possible pairs of interactions between adenosine H-2 protons on adjacent dA·dT base pairs in the Pribnow 12-mer at 5°C in Table 2.

Interproton Distances. Early *et al.* (18) have reported on a spin–lattice and spin–spin proton relaxation study of a 12-base-pair restriction fragment. They have analyzed their relaxation parameters to yield an isotropic rotational correlation time of 7 ns for the dodecanucleotide duplex at 21°C. We have used a somewhat longer value (9 ns) more appropriate to the temperature (5°C) at which our studies were undertaken in combination with the σ_{ij} values to determine the inter-proton distances between adenosine H-2 on adjacent base pairs in the Pribnow 12-mer at 5°C. These distances are summarized in Table 2.

We note that the interproton separation between adenosine H-2 positions is 4.22 Å in the (dA^3-dT^4)·(dA^4-dT^3) step, 3.56 Å in the (dT^4-dT^5)·(dA^5-dA^4) step, and 3.17 Å in the (dT^5-dA^6)·(dT^6-dA^5) step in the Pribnow 12-mer duplex at 5°C (Table 2).

Arnott and co-workers have deduced nucleic acid conformational parameters from an analysis of the x-ray diffraction patterns of natural DNA fibers. The early analysis by Arnott and Hukins (19) had no pronounced propeller twist for the base pairs. This results in small differences in the interproton separation between adenosine H-2 positions on adjacent dA·dT base pairs in the d(A^3-T^4-T^5-A^6)·d(T^6-A^5-A^4-T^3) segment (Table 3). More recent studies by Arnott *et al.* (20) indicate a much better fit between observed and calculated fiber diffraction patterns

Table 2. Cross-relaxation rate constants and interproton distances between adenosine H-2 protons on adjacent dA·dT base pairs in the Pribnow 12-mer

Pair	Spin *i*	Spin *j*	η_{ij}*	ρ_j†	σ_{ij}	r_{ij}‡
3, 4	4	3	−0.09	0.97	−0.09	4.22
4, 5	4	5	−0.15	1.63	−0.25	3.56
5, 6	5	6	−0.38	1.31	−0.50	3.17

Data were measured in 0.1 M phosphate at 5°C.
*Steady-state NOE value.
†Determined from saturation-recovery measurements.
‡For τ_c = 9 ns.

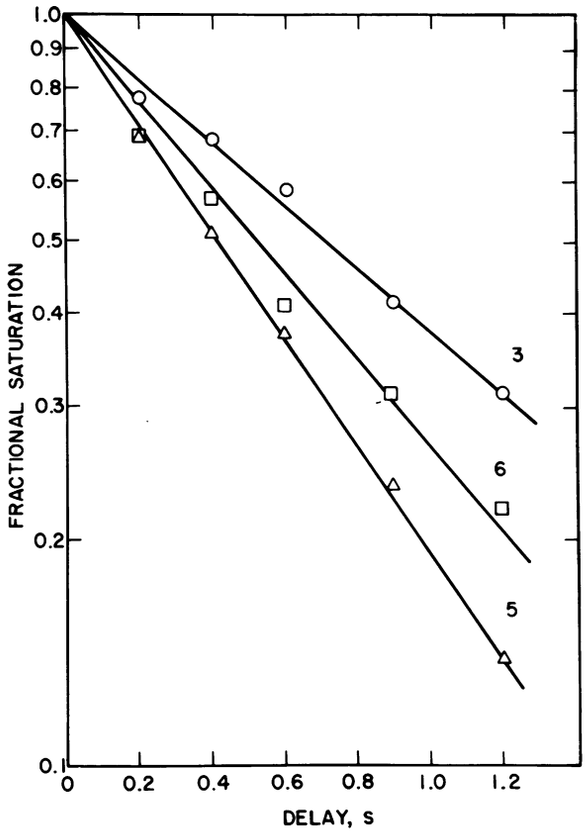

FIG. 5. Selective saturation-recovery measurements of individual adenosine H-2 protons of the Pribnow 12-mer duplex in 0.1 M phosphate/^2H_2O, pH 7.17, at 5°C. The plot represents the fractional saturation of the adenosine H-2 protons of dA·dT base pairs 3, 5, and 6 as a function of the delay between saturation and observation pulses. The saturation pulse was on for 100 ms and the power levels were adjusted to selectively saturate the adenosine H-2 proton of interest. A recycle delay of 5 s was used between successive scans.

on incorporation of a 13° propeller twist with the same handedness as in Fig. 1. This conformation results in large differences in the interproton separation between adenosine H-2 positions on adjacent dA·dT base pairs in the above segment (Table 3). Specifically, r_{34} is ≈0.7 Å longer than r_{45} while r_{56} is ≈0.7 Å shorter than r_{45} (Table 3) as would be expected from inspection of Fig. 1.

The distances obtained from NOE measurements are consistent with this latter model since r_{34} is ≈0.65 Å larger than r_{45} while r_{56} is ≈0.4 Å shorter than r_{45} independent of the exact

Table 3. Interproton distances between adenosine H-2 protons on adjacent dA·dT base pairs in the d(A^3-T^4-T^5-A^6)·d(T^6-A^5-A^4-T^3) segment on DNA conformations deduced from an analysis of x-ray fiber diffraction data

Pair	r_{ij}, Å: Arnott–Hukins	r_{ij}, Å: Arnott–Chandrasekaran
3, 4	4.12	4.36
4, 5	3.76	3.63
5, 6	3.57	2.94

Results are based on DNA conformations deduced from an analysis of x-ray fiber-diffraction data and were computed by R. Chandrasekaran. For the Arnott–Hukins results, the propeller twist is 4° and has an opposite sense of rotation from Fig. 1 (see ref. 19). For the Arnott–Chandrasekaran results, the propeller twist is 13° and has the same sense of rotation as in Fig. 1 (see ref. 20).

τ_c (9 ± 2 ns) used in the calculation (Table 2). Our NMR results thus show that the dA·dT base pairs are propeller twisted with the same handedness as observed in the solid state (7, 20).

Dr. R. Chandrasekaran kindly computed the interproton distances between adenosine H-2 protons on adjacent base pairs in the Pribnow 12-mer (Table 3) based on the Arnott–Hukins (19) and Arnott *et al.* (20) models of DNA. We are grateful to Michael Weiss and Dr. Horace Drew for discussions and to Prof. R. E. Dickerson for permission to use his drawings of propeller-twisted base pairs. The high-field NMR experiments were performed at the NMR Facility for Biomedical Research 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 of the National Institutes of Health and by the National Science Foundation under Contract C-670.

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