Proc. Nat. Acad. Sci. USA
Vol. 71, No. 5, pp. 1945–1948, May 1974

Proton Nuclear Magnetic Resonance Investigations and Ring Current Calculations of Guanine N-1 and Thymine N-3 Hydrogen-Bonded Protons in Double-Helical Deoxyribonucleotides in Aqueous Solution

(base pairs/actinomycin D)

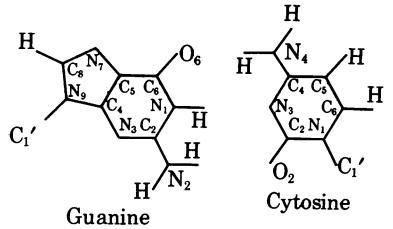
DINSHAW J. PATEL AND ALAN E. TONELLI

Bell Laboratories, Murray Hill, New Jersey 07974

Communicated by William D. Phillips, December 26, 1973

ABSTRACT **Methods are outlined for assigning the guanine-N_1H and thymine-N_3H protons to particular base pairs in the proton nuclear magnetic resonance spectra of double-stranded oligodeoxyribonucleotides of known sequence in aqueous solution. Ring current calculations have been used to evaluate the upfield shifts of the guanine-N_1H and thymine-N_3H protons from the pyrimidine and purine rings of nearest-neighbor base pairs in DNA B-type double-helical structures. Chemical shifts of 13.6 ± 0.1 ppm and 14.6 ± 0.2 ppm are assigned to the guanine-N_1H proton of an isolated G·C base pair and the thymine-N_3H proton of an isolated A·T base pair, respectively.**

Earlier high-resolution proton nuclear magnetic resonance (NMR) investigations of transfer RNA in H_2O solution established that one exchangeable Watson–Crick hydrogen-bonded proton per base pair, namely, the guanine N-1 proton (G-N_1H) and thymine N-3 proton (T-N_3H), are observed between 11 and 15 ppm downfield from standard 3-(trimethylsilyl) propanesulfonate (1). In this manuscript we report high-resolution proton NMR spectra and ring current calculations for a series of double-stranded deoxyribonucleic-acid sequences in H_2O solution.



EXPERIMENTAL

Oligodeoxyribonucleotides. The oligodeoxyribonucleotides were purchased from Collaborative Research, Inc. d(ApTpGpCpApT) was used as purchased while the other oligodeoxyribonucleotides were passed through a Chelex column and lyophilized before use. NMR sample concentrations were 25 mg/ml. Actinomycin D (Act-D) was purchased from Merck and added as a solid to the oligodeoxyribonucleotide solutions

Spectra. All spectra were run on a Varian HR 300 frequency/field swept spectrometer. The variable temperature probe maintained temperatures to ±1°. Spectra were time averaged to improve signal-to-noise ratio by using a Nicolet computer of average transients with 4096 channels. Chemical shifts are defined on a δ scale in ppm relative to 3-(trimethylsilyl)propane sulfonic-acid sodium salt, designated DSS, as standard. pH was measured with an Ingold combination electrode and a Radiometer pH meter.

RESULTS AND DISCUSSION

NMR studies

d(ApTpGpCpApT).

5′——→3′
A-T-G-C-A-T
. . . . . .
T-A-C-G-T-A
3′←——5′

Fig. 1 presents the high-resolution 300 MHz NMR spectrum of d(ApTpGpCpApT) in H_2O between 11.5 and 14.5 ppm as a function of temperature. Earlier studies predict (1) the observation of three resonances, each with an area of two protons per double-stranded helix, corresponding to the ring N protons of $(A\cdot T)_{terminal}$, $(A\cdot T)_{internal}$, and $(G\cdot C)_{internal}$ base pairs. At 3°, two distinct resonances at 13.77 ppm and 12.69 ppm and a very broad resonance at 13.15 ppm are observed. When the temperature is raised, the resonance at 13.77 ppm broadens out at 14°, while the resonance at 12.69 ppm is broadened out at 25°. The increase in line width with temperature is related to an increase in the exchange rate of the proton with solvent water, eventually resulting in broadening and coalescence with the solvent signal. It then follows that the T-N_3H resonance of the solvent-accessible $(A\cdot T)_{terminal}$ base pair should exchange out at lower temperatures than the G-N_1H resonance of the solvent-shielded $(G\cdot C)_{internal}$ base pair. The resonances at 13.77, about 13.15, and 12.69 ppm are assigned to the ring NH protons of $(A\cdot T)_{internal}$, $(A\cdot T)_{terminal}$, and $(G\cdot C)_{internal}$ base pairs, respectively, in the proton NMR spectrum of d(ApTpGpCpApT) in H_2O solution. The NMR spectrum of d(ApTpGp-

Abbreviations: NMR, nuclear magnetic resonance; G-N_1H, guanine N-1 proton; T-N_3H, thymine N-3 proton; Act-D, actinomycin D.

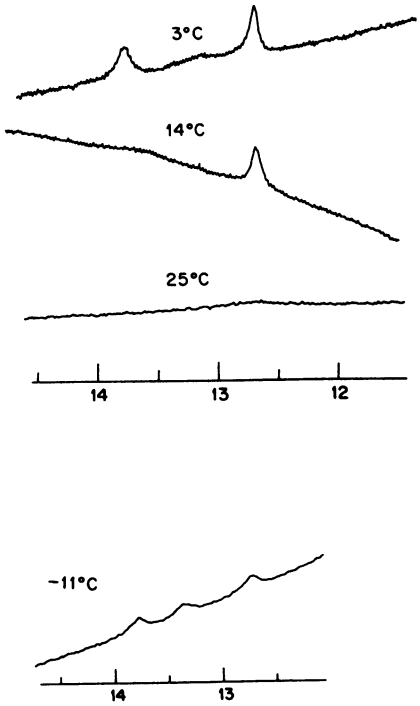


FIG. 1. The 300 MHz proton NMR spectra of double-stranded d(ApTpGpCpApT) in H_2O at pH 7 as a function of temperature (*top*) and H_2O:MeOH (3:2) at −11° (*bottom*).

CpApT) in H_2O:MeOH (3:2) (2) at −11° consists of three distinct resonances of equal area and line width, permitting a definite identification of the T-N_3H resonance of the $(A \cdot T)_{terminal}$ base pair.

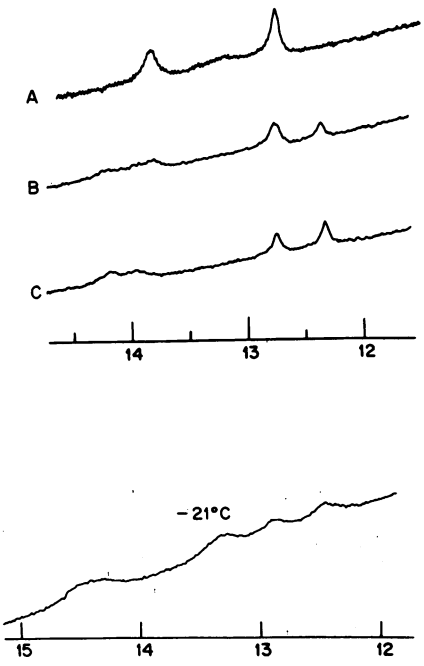


FIG. 2. The 300 MHz proton NMR spectra of double-stranded d(ApTpGpCpApT) in H_2O, pH 7, at 0° upon the gradual addition of Act-D (*top*). In (*B*) and (*C*) the ratios of Act-D to double-stranded d(ApTpGpCpApT) are 0.6:2 and 1:2. The spectrum of the 1:2 complex in H_2O:MeOH (3:2) at −21° is shown at the *bottom*.

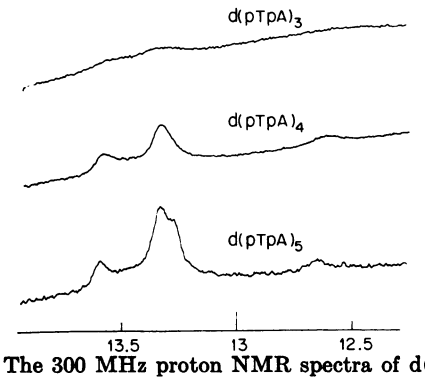


FIG. 3. The 300 MHz proton NMR spectra of $d(pTpA)_n$, n = 3, 4, 5, in 0.1 M NaCl–H_2O, pH 7 at 0°.

1:2 Act-D·d(ApTpGpCpApT) Complex. From an investigation of the 1:2 complex of actinomycin-D with deoxyguanosine (3), a detailed stereochemical model for the binding of the antibiotic to DNA has been presented (4). The phenoxazone ring of the antibiotic is predicted to intercalate between the sequence guanosine 3′ → 5′ cytosine in DNA, and the complex is stabilized by stacking, hydrogen bonding, and hydrophobic interactions (3).

Changes in the proton NMR spectrum of d(ApTpGpCpApT) in H_2O, at pH 7 and 0°, on gradual addition of Act-D are presented in Fig. 2. The spectrum of the complex at a ratio of Act-D:d(ApTpGpCpApT) of 1:2 (i.e., one molecule of Act-D per double-stranded oligonucleotide) exhibits chemically shifted resonances lacking the 2-fold symmetry of the uncomplexed oligonucleotide double helix. The phenoxazone ring of Act-D lacks an element of symmetry, and this is reflected in the spectrum of the tightly bound complex.

On complex formation at 0°, the resonance at 12.69 ppm is split by a 0.4 ppm chemical-shift difference and that at 13.77 ppm by a 0.2 ppm chemical-shift difference. The d(ApTpGpCpApT) resonance at 13.36 ppm in H_2O:MeOH (3:2) at −11° (Fig. 1) is observed as an unsplit resonance at 13.33 ppm in the 1:2 Act-D·d(ApTpGpCpApT) complex in H_2O:MeOH (3:2) at −21° (Fig. 2). Since Act-D is predicted to intercalate between G·C base pairs, the nonequivalence would be greatest for the G-N_1H protons of the (G·C) base pairs and smallest for the T-N_3H protons of the $(A \cdot T)_{terminal}$ base pairs. On this basis the d(ApTpGpCpApT) ring N protons at 12.69, 13.77, and 13.16 ppm in H_2O, pH 7, 3° are assigned to $(G \cdot C)_{internal}$, $(A \cdot T)_{internal}$, and $(A \cdot T)_{terminal}$ base pairs. This supports the conclusions reached from the temperature-dependent studies in the previous section.

It has been proposed that the specificity of Act-D complexation to DNA double helices and lack of complexation to RNA and DNA·RNA double helices indicate a requirement for the B rather than the A form of double-helical polyribonucleotides (4). Intercalation of Act-D into GpC sequences of double-helical d(ApTpGpCpApT) and d(pGpCpGpCpGpC)* in aqueous solution strongly suggests that these double-helical hexadeoxyribonucleotides exist in the DNA B form in solution.

$d(pTpA)_n$ and $d(pGpC)_n$. The x-ray analysis of the diribonucleotide ApU showed that two molecules of this dinucleotide exhibited a Watson–Crick antiparallel double-helix arrangement in the crystal (5). The small oligodeoxyribo-

* D. J. Patel, unpublished results.

nucleotides of sequence $d(pTpA)_n$ investigated in this study should also be capable of forming complementary Watson–Crick-type double helices in solution.

```
5'————————→3'
-T-A-T-A-T-A-
. . . . . .
-A-T-A-T-A-T-
3'←————————5'
```

The proton NMR spectra of $d(pTpA)_3$, $d(pTpA)_4$, and $d(pTpA)_5$ in 0.1 M NaCl–H_2O, pH 7, 0° between 12 and 14 ppm are presented in Fig. 3. There are three resonances in $d(pTpA)_4$ with chemical shifts at 13.58, 13.33, and 12.58 ppm. As one proceeds from the hexanucleotide to the decanucleotide, the ratio of internal to terminal A·T base pairs increases as does the resonance at 13.33 ppm. On this basis the T-N_3H resonance of the internal A·T base pair is assigned the chemical shift 13.33 ppm.

Similar NMR studies of $d(pGpC)_3$ and $d(pGpC)_4$ in 0.1 M NaCl–H_2O, pH 7, 0° suggest that the G-N_1H resonance of an internal G·C base pair exhibits a chemical shift at 13.1–13.2 ppm.

Ring current calculations

The least-squares refined structure of B-DNA given by Arnott *et al.* (6) is adopted. Two new coordinate systems are defined with origins in the planes of the bases guanine and thymine, each of which possesses a hydrogen-bonded ring N-H proton. After transforming the coordinates of all base atoms from the B-DNA reference frame to the more convenient coordinate system located in the guanine and thymine rings, the nuclear shielding values calculated by Giessner-Prettre and Pullman (7) at a distance of 3.4 Å from the base-pair plane are used to determine the ring current shifts expected from nearest neighbors on the G-N_1H and T-N_3H protons in each of the possible B-DNA base sequences.

TABLE 1. *Upfield shifts in ppm at the G-N_1H (G*·C base pair) and T-N_3H (T*·A base pair) due to ring currents (7) from neighboring base pairs 1 . . . 3 and 2 . . . 4 in a B-DNA helix*

1	2	3	4	Upfield ring current shifts, ppm G*·C	T*·A
C	T	G	A	0.75	0.40
C	C	G	G	0.50	0.50
T	T	A	A	1.05	0.50
A	A	T	T	1.15	1.45
T	A	A	T	1.35	1.05
C	A	G	T	1.05	0.95
A	T	T	A	0.75	0.90
T	C	A	G	0.80	0.45
A	C	T	G	0.50	0.90
G	C	C	G	0.50	0.50
G	A	C	T	1.00	1.10
G	T	C	A	0.75	0.55
A	G	T	C	0.55	0.10
G	G	C	C	0.50	0.70
C	G	G	C	0.50	0.60
T	G	A	C	0.85	0.65

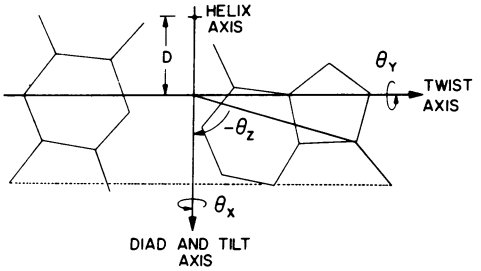


FIG. 4. A schematic diagram illustrating the tilt and twist axes relative to the helix axis and base pair plane (after Arnott *et al.*, ref. 6).

Calculated Shifts for Regular B-DNA Helices. The chemical shifts $\Delta\delta$ of the ring protons of N_1H (guanine) and N_3H (thymine) due to the ring currents of the neighboring base pairs 1 . . . 3 and 2 . . . 4 at a distance of 3.4 Å are summed to obtain the total ring current shift expected for each base sequence. The resulting calculated ring current shifts are presented in Table 1.

Calculated Shifts for Distorted B-DNA Helices. The effects of tilting and twisting (Fig. 4) a base pair adjacent to the ring NH under study have been evaluated for the sequence shown in Table 2. Tilting and twisting of the lower base pair were undertaken in steps of ±10° up to a total of ±30°. For cases where the T-N_3H proton distance to the A·T base pair deviated from 3.4 Å, the ring current shifts (7) were corrected according to the Johnson–Bovey tables (8). Table 2 summarizes a few values of the calculated upfield ring current shift of the T-N_3H belonging to the middle base pair due to the neighboring base pairs under different combinations of tilting and twisting of the lower base pair.

Changes of up to ±0.6 ppm are found in the calculated upfield ring current shifts of the ring NH protons due to tilt and twist distortions $\leq 30°$ in an adjacent base pair (Table 2).

Observed and calculated ring NH chemical shifts

Internal Base Pairs. The next step is to assign chemical shifts to the G-N_1H in an isolated G·C base pair and the

TABLE 2. *Upfield shifts in ppm at the T-N_3H (T*·A base pair) due to ring currents from neighboring base pairs for defined tilts and twists in the lower A . . . T base pair*

Twist	Tilt	Upfield ring current shift, ppm
0°	0°	1.45
0°	30°	1.27
30°	0°	0.95
−30°	0°	1.60
−10°	−10°	2.10

TABLE 3. *A comparison of the experimentally assigned and calculated ring current corrected chemical shifts in ppm for the internal G-N_1H ($G^*\cdot C$ base pair) and T-N_3H ($T^*\cdot A$ base pair) protons*

	$G^*\cdot C$		$T^*\cdot A$	
	Calculated	Observed	Calculated	Observed
A-T-G-C-A-T				
T-A-C-G-T-A	12.85 ± 0.1	12.7	13.5 ± 0.2	13.8
-T-A-T-A-T-A-				
-A-T-A-T-A-T-			13.15 ± 0.2	13.3
-G-C-G-C-G-C-				
-C-G-C-G-C-G-	13.1 ±0.1	13.1–13.2		

T-N_3H in an isolated A·T base pair, such that when corrected for ring current contributions from nearest-neighbor base pairs, the calculated chemical shifts are in agreement with those determined experimentally from rigorous independent assignments in oligodeoxyribonucleotides of known sequence.

The experimental and calculated values for the chemical shifts of the *internal* ring NH protons for the three double-stranded oligodeoxyribonucleotide sequences in Table 3 are in good agreement when the T-N_3H and G-N_1H protons in isolated base pairs have the following chemical shifts: G-N_1H, 13.6 ± 0.1 ppm; T-N_3N, 14.6 ± 0.2 ppm.

Shulman *et al.* (9) have undertaken an investigation of RNA structures and assumed that the chemical shifts of G-N_1H and U-N_3H in isolated base pairs are 13.7 and 14.8 ppm, respectively.

Terminal Base Pairs. A calculated chemical shift of 13.9 ppm is predicted for the T-N_3H proton of the terminal A.T base pair in d(ApTpGpCpApT). This is in sharp contrast to the experimental value of about 13.2 ppm in H_2O, pH 7 at 0°.

It is proposed that the terminal base pairs in double-stranded hexanucleotides are in rapid equilibrium between Watson–Crick hydrogen-bonded and open forms, with the observed ring NH chemical shifts reflecting an average of the weighted populations of these two forms. The chemical shift difference between the open form (10 ppm) (10) and Watson–Crick hydrogen-bonded form (14.6 ppm) is 4.6 ppm. The 0.7 ppm difference between the observed and calculated shifts for the T-N_3H of the terminal A·T base pair suggests that about 15% of the open form is in rapid equilibrium with the Watson–Crick hydrogen-bonded form for the terminal base pairs in double-stranded d(ApTpGpCpApT) in H_2O at 0°.

We thank Miss Lita Canuel for expert technical assistance.

1. Kearns, D. R., Patel, D. J. & Shulman, R. G. (1971) *Nature* **229**, 338–339.
2. Travers, F., Michelson, A. M. & Douzou, P. (1970) *Biochem. Biophys. Acta* **199**, 29–35.
3. Jain, S. C. & Sobell, H. M. (1972) *J. Mol. Biol.* **68**, 1–20.
4. Sobell, H. M. & Jain, S. C. (1972) *J. Mol. Biol.* **68**, 21–34.
5. Rosenburg, J. M., Seeman, N. C., Kim, J. J. P., Suddath, F. L., Nicholas, H. B. & Rich, A. (1973) *Nature* **243**, 150–154.
6. Arnott, S., Rover, S. D. & Wonacott, A. J. (1969) *Acta. Crystallogr. Sect. B* **25**, 2192–2206.
7. Giessner-Prettre, C. & Pullman, B. (1970) *J. Theor. Biol.* **27**, 87–95.
8. Johnson, C. E. & Bovey, F. A. (1958) *J. Chem. Phys.* **29**, 1012–1014.
9. Shulman, R. G., Hilbers, C. W., Wong, Y. P., Lim Wong, K., Lightfoot, D. R., Reid, B. R. & Kearns, D. R. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2042–2045.
10. Katz, L. & Penman, S. (1966) *J. Mol. Biol.* **15**, 220–231.