Nuclear Magnetic Resonance Investigations of the Structure of the Self-Complementary Duplex of d-ApTpGpCpApT in Aqueous Solution†

Dinshaw J. Patel* and Alan E. Tonelli

ABSTRACT: Several features of the structure of the d-ApTpGpCpApT self-complementary duplex in solution are proposed from a nuclear magnetic resonance (NMR) analysis of the proton relaxation times, selective sugar proton coupling constants, and a comparison between the experimental chemical shifts on conversion to strands with those predicted from ring current calculations. The experimental longitudinal proton relaxation times for the various protons in each base pair are consistent with a double helix comprised of antiparallel strands and an anti conformation about the glycosidic bonds for both purine and pyrimidine bases. The molecular rotational correlation time for d-ApTpGpCpApT was calculated to be 1.4×10^{-9} sec at 34°. The sums of the vicinal coupling constants, $J_{H_{1'}H_{2'}} + J_{H_{1'}H_{2''}}$, were found in the range 13–15 Hz, from the spectra of the six sugar $H_{1'}$ triplets of the hexanucleotide between 5.5 and 6.5 ppm. The magnitude of the coupling sum rules out a C3 endo sugar ring pucker and is consistent with a predominant C3 exo sugar ring pucker geometry at all six residues in the deoxyhexanucleotide. The overlap geometries (normal to the base planes) for a base pair above and below the base pair under investigation have been derived from published coordinates for the B-DNA, A-DNA, and D-DNA helices. The ring current contributions from all possible combinations of nearest neighbor base pairs on the Watson–Crick exchangeable protons, the nonexchangeable base protons, and base carbons of a given base pair have been computed and tabulated for each of the above double helices. A comparison between the limited experimental proton chemical shifts associated with the helix–coil transition with the ring current predictions for different DNA helices suggests base pair overlap geometries for the d-ApTpGpCpApT duplex similar to the B-DNA class of double helices.

The structures of nucleic acids in the crystalline state, of fibers, and in solution have been intensely investigated. These include, at the dinucleotide to oligonucleotide level, recent X-ray crystallographic (Sussman et al., 1972; Rosenberg et al., 1973; Suck et al., 1973; Camerman et al., 1973), nuclear magnetic resonance (NMR) (Chan and Nelson, 1969; Fang et al., 1971; Barry et al., 1972; Smith et al., 1973; Altona et al., 1974; Evans et al., 1975), and circular dichroism (CD) (Warshaw and Cantor, 1971; Borer et al., 1973) studies and theoretical calculations (Yathindra and Sundaralingam, 1974). At the DNA level, X-ray (Arnott et al., 1973, 1974; Arnott and Hukins, 1973; Arnott and Selsing, 1974) and infrared linear dichroism (Brahms et al., 1973) studies on fibers, as well as wide angle X-ray scattering (Bram, 1973; Bram and Baudy, 1974) in solution, have demonstrated the dependence of DNA structure on counterion, sequence, humidity, and alcohol content of solvent.

In the previous paper (Patel, 1975), the nonexchangeable base proton chemical shifts and the sugar $H_{1'}$ proton chemical shifts and coupling constants of d-ApTpGpCpApT in D_2O, low ionic strength, and pH 7 were monitored during the helix–coil transition in the temperature range 0 to 70°.

This paper probes three features of the structure of the d-ApTpGpCpApT self-complementary duplex in aqueous solution. (1) The observed base proton chemical shifts associated with the helix–coil transition are compared with those predicted from ring current effects for different DNA structures. This provides information on the overlap geometries of nearest neighbor base pairs. (2) The longitudinal relaxation times of base and sugar $H_{1'}$ protons have been determined and evaluated in terms of glycosidic torsion angles. This permits differentiation between syn and anti conformations at the glycosidic bond. In addition, the relaxation times permit an estimate for the rotational correlation time of d-ApTpGpCpApT in aqueous solution. (3) The sugar vicinal coupling constants between proton(s) attached to $C_{1'}$ and $C_{2'}$ have been measured and compared with those predicted for different sugar ring pucker geometries. The comparison permits differentiation between C3′ exo (C2′ endo) and C3′ endo pucker structures.

Experimental Section

I. Relaxation Studies. d-ApTpGpCpApT solutions (9 m*M* total strand concentration) were flushed for 1 hr with helium and then sealed prior to measurement of relaxation times. The data were accumulated on a Bruker HX-270 spectrometer interfaced with a Nicolet-80 computer. Inversion of magnetization by 180° required a 25-μsec pulse. A $(\pi, \tau, \pi/2)$ pulse sequence, where τ is the delay time between π and $\pi/2$ pulses, was utilized to evaluate the longitudinal relaxation time for observable protons. The pulse sequence was repeated after a delay of 10 sec.

Plots of $\ln 2(1 - (M/M_0))^{-1}$ against τ were utilized to determine T_1. The values of M and M_0, the magnetization at a given τ value and at $\tau = 9$ sec (i.e., $\geq 4\ T_1$ values), were determined from peak heights of the resonances. T_1 is the τ value at which $\ln 2(1 - (M/M_0))^{-1} = 2.72$.

II. Ring Current Calculations. The published coordinates for the A-RNA (Arnott et al., 1973), B-DNA (Arnott and Hukins, 1972, 1973), as well as for DNAs with alternating pyrimidine and purine sequences, designated D-DNA (Arnott et al., 1974), were utilized to determine

† From Bell Laboratories, Murray Hill, New Jersey 07974. *Received April 9, 1974.* Paper V in series.

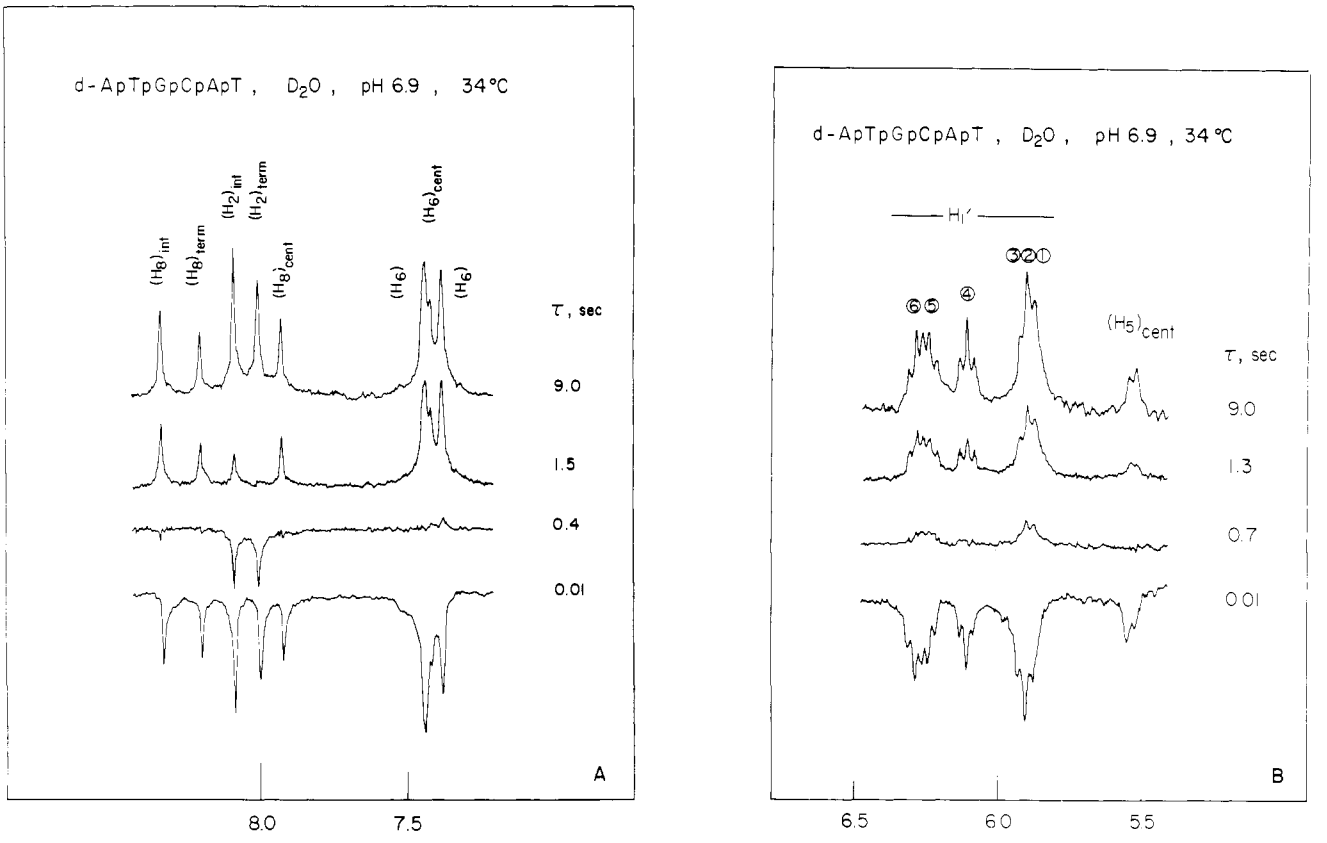


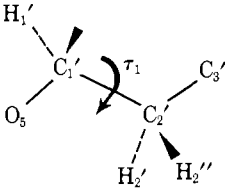
FIGURE 1: Representative high-resolution spectra recorded as a function of delay τ, between π and $\pi/2$ pulses in the Fourier transform mode for the spectral regions (A) 7–8.5 ppm and (B) 5.5–6.5 ppm, in degassed 9 m*M* d-ApTpGpCpApT, D_2O, low ionic strength, and pH 7.

base overlap geometries for a sequence of three base pairs as viewed normal to the base pairs.[1]

These coordinates were transfered to a reference plane located in the plane of the purine ring as described previously[2] (Patel and Tonelli, 1974). Nuclear shielding values calculated at 3.4 Å from the base planes (Giessner-Prettre and Pullman, 1970) were then used to determine the expected ring current shifts. For D-DNA, where adjacent base pairs are separated by 3.0 Å instead of 3.4 Å between base pairs in B-DNA and A-RNA, the calculated ring current shifts were scaled up by a factor of 25% following the Johnson–Bovey tables (Johnson and Bovey, 1958).

III. Dihedral Angles about $C_{1'}$–$C_{2'}$. The torsion angles about the $C_{1'}$–$C_{2'}$ sugar bond have been reported for the C3′ exo (B-DNA and D-DNA) and C3′ endo (A-DNA) pucker geometries of the sugar ring (Arnott and Hukins, 1972; Arnott et al., 1972).

For the starting geometry shown below, $C_{3'}$ and O_5 trans about $C_{1'}$–$C_{2'}$, the torsion angle τ_1 is 205° for the C3′ exo



sugar ring pucker and 155° for the C3′ endo sugar ring pucker. This corresponds to dihedral angles of 145° ($H_{1'}C_{1'}C_{2'}H_{2'}$) and 25° ($H_{1'}C_{1'}C_{2'}H_{2''}$) for the C3′ exo configuration and dihedral angles of 95° ($H_{1'}C_{1'}C_{2'}H_{2'}$) and 25° ($H_{1'}C_{1'}C_{2'}H_{2''}$) for the C3′ endo configuration.

Results

I. Relaxation Times. Typical spectra, for measurement of the longitudinal relaxation time, recorded as a function of delay τ, between π and $\pi/2$ pulses in the Fourier transform mode, are presented for the 7–8.5 ppm (Figure 1A) and 5.5–6.5 ppm (Figure 1B) spectral regions for d-ApTpGpCpApT, in D_2O, at low ionic strength and 34°. Relaxation times were measured at 7 and 34°, with each run involving an average of >10 τ values.[3] The experimental data for the adenine H_2 protons and thymine CH_3 protons at 7° and 34° are presented in Figures 2A and B, respectively.

The relaxation times (along with the chemical shifts) of all the resolvable resonances in d-ApTpGpCpApT at 7 and 34° are summarized in Table I. The hexanucleotide is predominantly double helical at 7° while the helix–coil transition is in the vicinity of $T_{1/2}$ at 34°.

Longer relaxation times are observed for the adenine H_2 resonance relative to the H_8 resonance and the pyrimidine H_5 resonance relative to the pyrimidine H_6 resonance when comparing data for a given base pair (Table I).

The relaxation time for the terminal adenine H_2 resonance is different from the internal adenine H_2 resonance

[1] Tetrahedral geometry, a C–H bond length of 1.0 Å and rapid rotation (on the NMR time scale) of the methyl group were assumed in locating the average pyrimidine CH_3 proton position.

[2] For D-DNA, translation of Δx = 1.400 Å, Δy = 1.260 Å, and Δz = −2.183 Å followed by rotation in order of 280° about the z axis, 2° about the x axis, and 16° about the y axis achieve the transformation of coordinates.

[3] It should be noted that the purine H_8 resonances were deuterated to the extent of ~50% in the d-ApTpGpCpApT sample used to evaluate relaxation times at 7 and 34°.

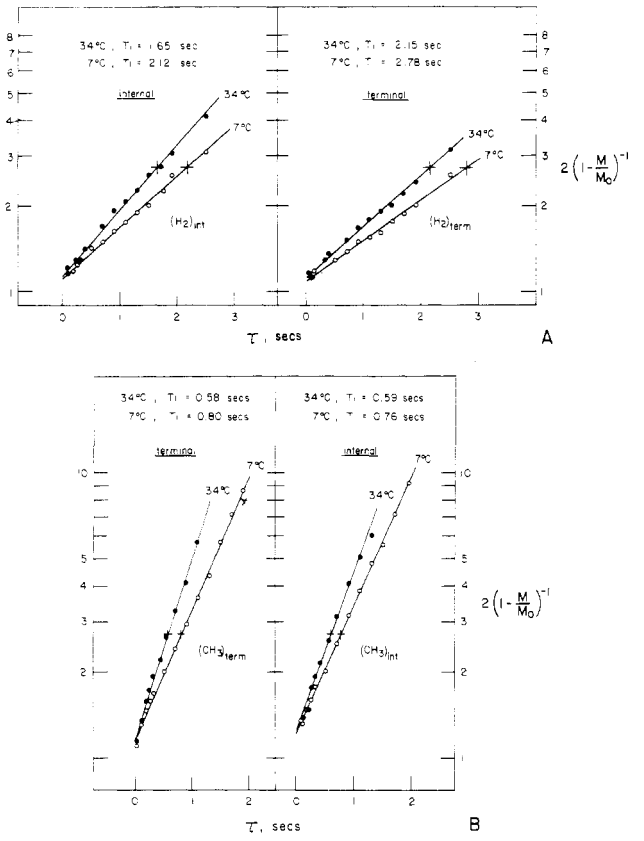


FIGURE 2: Plots of ln 2 $(1 - (M/M_0))^{-1}$ against delay τ for (A) the adenine H_2 and (B) the thymine CH_3 protons.

Table I: Chemical Shifts (7, 34, and 70°) and Relaxation Times (7 and 34°) for the Observable Nonexchangeable Protons of 9 m*M* d-ApTpGpCpApT in D_2O, Low Ionic Strength, pH 7.

	Chemical Shifts (ppm)			Relaxation Times (sec)[a]	
Assignment	7°	34°	70°	7°	34°
(H_8)int	8.345	8.315	8.335	0.71	0.73
(H_8)term	8.22	8.185	8.225	0.95	0.8
(H_8)cent	7.92	7.915	7.91	1.05	0.80
(H_2)int	8.005	8.055	8.185	2.12	1.65
(H_2)term	7.87	7.965	8.14	2.78	2.15
(H_6)cent	7.38	7.39	7.585	a	0.60
(H_6)term/int	7.38	7.40	7.425	a	0.65
	7.29	7.37	7.55	0.75	0.55
(H_5)cent		5.545	5.83		1.10
$(H_{1'})$term (1)	5.71	5.90	6.11	b	c
$(H_{1'})$term (2)	5.71	5.90	6.08	b	c
$(H_{1'})$int (4)	6.125	6.155	6.20	0.98	0.99
$(H_{1'})$int (5)	6.245	6.30	6.365	0.80	0.88
$(H_{1'})$cent (3)	5.93	5.95	6.11	0.91	c
$(H_{1'})$cent (6)	6.31	6.315	6.365	0.84	0.88
(CH_3)int	1.535	1.65	1.765	0.77	0.6
(CH_3)term	1.395	1.58	1.745	0.8	0.58

[a] For those resonances that could not be resolved, average T_1 values are reported: (a) 0.68 sec; (b) 0.92 sec; (c) 0.82 sec.

while the relaxation times are the same for the terminal and internal thymine methyl protons at a given temperature (Table I).

A decrease in relaxation times is observed for the purine H_8 and H_2 protons and the thymine CH_3 and H_6 protons on increasing the temperature from 7 to 34°, while little

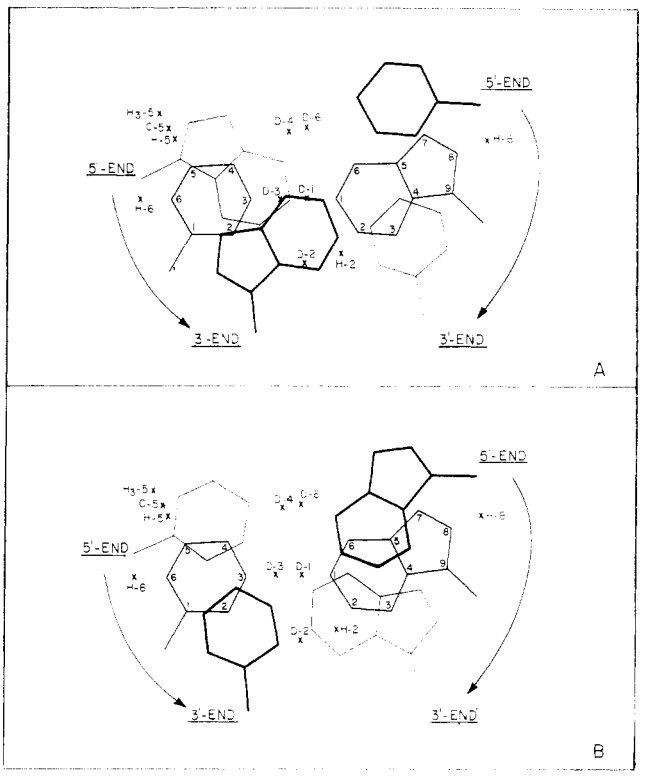


FIGURE 3: Overlap geometries of the bases for a three base pair sequence in the B-DNA helix. Base pairs are drawn in light, medium (shaded), and heavy to indicate bottom middle, and top positions, respectively. The stacking of the base pairs is viewed normal to the middle (shaded) base pair. Purine and pyrimidine alternate on each strand in (A) while (B) represents all pyrimidine and all purine strands. These overlaps represent all possible combinations of nearest neighbors relative to the shaded base pair. The positions of the nonexchangeable protons, designated H-, the exchangeable Watson-Crick protons, designated D-, and the numbered ring atoms (for the shaded base pair) are included in the figure.

change is observed for the sugar $H_{1'}$ resonances of the internal and central base pairs with temperature (Table I).

II. Ring Current Calculations. The overlap geometries (see Experimental Section) for a base pair above (dark lines) and a base pair below (light lines) the shaded base pair under consideration are presented in Figure 3 for the B-DNA double helix. Purine and pyrimidines alternate on each strand in A (top of each figure) while B (bottom of each figure) represents a double helix composed of all pyrimidine and all purine strands. Together, these figures depict all combinations of nearest neighbors relative to the shaded base pair. The figures include the positions of the nonexchangeable protons, designated H-, the exchangeable Watson-Crick protons, designated D-, side chain carbons, designated C-, and the numbered ring atoms; all for the shaded base pair. The ring current contours of Giessner-Prettre and Pullman (1970),[4] coupled with the overlap geometries (presented normal to the shaded base pair) in Figure 3, permit the evaluation of the ring current contribution to the proton and carbon resonances of a given base pair for all combinations of nearest neighbor base pairs.

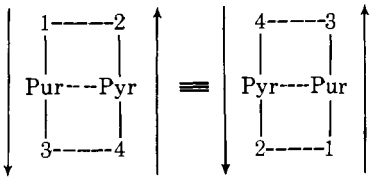
The upfield ring current contributions from base pairs 1-

[4] The purine and pyrimidine ring current contours at a distance of 3.4 Å were utilized for the B-DNA and A-RNA (A-DNA) calculations while the contours were scaled upwards by 25% for D-DNA (Johnson and Bovey, 1968), because the base pair separation is 3.0 Å (Arnott et al., 1974).

Table II: The Upfield Ring Current Contributions from Bases (1)----(2) and (3)----(4) on the Proton Resonances in the Middle Base Pair for the Sequence in Scheme I for B-DNA, A-RNA (A-DNA), and D-DNA helices.

	Base Position				Nearest Neighbor Proton Upfield Shifts (ppm)									
					Watson–Crick Exchangeable Protons					Nonexchangeable Protons				
	1	2	3	4	D-1	D-2	D-3	D-4	D-6	H-2	H-5	CH_3-5	H-6	H-8
B-DNA														
	C	G			0.3	0.5	0.35	0.1	0.1	0.25	0.0	0.0	0.0	0.05
	G	C			0.2	0.1	0.15	0.1	0.15	0.1	0.0	0.0	0.0	0.1
	T	A			0.8	1.0	0.8	0.1	0.1	0.7	0.0	0.0	0.0	0.0
	A	T			0.25	0.05	0.15	0.15	0.3	0.1	0.0	0.0	0.0	0.2
			C	G	0.25	0.15	0.35	0.2	0.15	0.1	0.45	0.2	0.15	0.0
			G	C	0.25	0.2	0.2	0.1	0.05	0.5	0.2	0.1	0.05	0.0
			T	A	0.5	0.15	0.9	0.5	0.3	0.15	0.5	0.25	0.15	0.0
			A	T	0.5	0.55	0.3	0.1	0.05	1.05	0.1	0.05	0.0	0.0
A-RNA (A-DNA)														
	C	G			0.7	0.3	0.7	0.2	0,2	0.2	0.0	0.0	0.0	0.1
	G	C			0.25	0.1	0.3	0.1	0.05	0.1	0.0	0.0	0.0	0.25
	T	A			1.3	0.5	1.1	0.4	0.4	0.5	0.0	0.0	0.0	0.05
	A	T			0.1	0.05	0.1	0.05	0.1	0.05	0.0	0.0	0.0	0.5
			C	G	0.15	0.1	0.2	0.1	0.05	0.2	0.75	0.55	0.2	0.0
			G	C	0.45	0.35	0.3	0.15	0.1	0.5	0.3	0.25	0.1	0.0
			T	A	0.2	0.05	0.35	0.2	0.1	0.1	1.05	0.7	0.45	0.0
			A	T	1.05	0.7	0.75	0.2	0.2	0.95	0.15	0.1	0.05	0.0
D-DNA														
	C	G			0.3	0.6	0.25	0.05	0.1	0.3	0.0	0.0	0.0	0.05
	G	C			0.3	0.2	0.2	0.2	0.25	0.2	0.0	0.0	0.0	0.1
	T	A			0.55	1.45	0.55	0.05	0.05	0.8	0.0	0.0	0.0	0.0
	A	T			0.6	0.2	0.3	0.25	0.5	0.25	0.0	0.0	0.0	0.1
			C	G	0.3	0.1	0.45	0.55	0.4	0.1	0.3	0.2	0.05	0.0
			G	C	0.25	0.25	0.25	0.25	0.1	0.45	0.1	0.1	0.0	0.0
			T	A	0.8	0.2	1.2	0.95	0.7	0.2	0.4	0.25	0.05	0.0
			A	T	0.5	0.6	0.3	0.1	0.1	1.25	0.05	0.05	0.0	0.0

- - -2 and 3- - - -4 on the resonances of the middle base pair in the sequence



for the B-DNA, A-RNA (A-DNA), and D-DNA helices have been computed. The exchangeable and nonexchangeable proton data are presented in Table II and the carbon data are presented in Table III.

III. Sugar Coupling Constants. The vicinal proton–proton coupling constants for the sugar ring have been related to the dihedral angle Φ according to the relationship (Altona and Sundaralingam, 1973)

$$J = 10.5 \cos^2 \Phi - 1.2 \cos \Phi \qquad (1)$$

For B-DNA and D-DNA, the sugar pucker is C3′ exo with $\Phi_{1'-2'} = 145°$ and $\Phi_{1'-2''} = 25°$ (see Experimental Section) which corresponds to $J_{H_{1'}H_{2'}} = 6.1$ Hz and $J_{H_{1'}H_{2''}} = 7.5$ Hz. For A-DNA, the sugar pucker is C2′ endo with $\Phi_{1'-2'} = 95°$ and $\Phi_{1'-2''} = 25°$ (see Experimental Section) which corresponds to $J_{H_{1'}H_{2'}} \simeq 0$ Hz and $J_{H_{1'}H_{2''}} = 7.5$ Hz.

The sum, $J_{H_{1'}H_{2'}} + J_{H_{1'}H_{2''}}$, is predicted to be 13.6 Hz for B-DNA and D-DNA and 7.5 Hz for A-DNA.

Experimentally, six sugar $H_{1'}$ triplets are observed in the proton NMR spectrum of the hexanucleotide d-ApTpGpC-pApT in D_2O, low ionic strength, and pH 7. The separation between the outer lines of the triplet correspond to the sum, $J_{H_{1'}H_{2'}} + J_{H_{1'}H_{2''}}$, and these values are presented in the temperature range 2–70° in Table IV.

Discussion

I. Glycosidic Torsion Angles. The longitudinal relaxation rate, $(T_1)^{-1}$, for a proton due to a dipole–dipole interaction with a neighboring proton at a distance r_{HH} in a molecule tumbling in solution with a correlation time τ_C is given by (Solomon, 1955)

$$\frac{1}{T_1} = \frac{6}{20} \frac{\gamma_H^4 \hbar^2}{r_{HH}^6} \left[\frac{1}{1 + \omega_H^2 \tau_C^2} + \frac{4}{1 + 4\omega_H^2 \tau_C^2} \right] \tau_C \qquad (2)$$

where ω_H is the Larmor precession frequency and γ_H the gyromagnetic ratio for protons (= 2.7×10^4 radians/(sec G)). The relaxation times, T_1, for the protons in d-ApTpGpCpApT at 7 and 34° are summarized in Table I.

For a right-handed antiparallel double helix with the anti conformation about the glycosidic bonds, the purine H_8 and pyrimidine H_6 resonances are proximal to the $H_{2''}$ and $H_{3''}$ protons of their own sugar ring and those of the neighboring sugar ring linked to the 5′ position on the same strand. For such an orientation, the purine H_2 proton is directed toward the interior of the helix and in D_2O, is distant from its proton neighbors. Since the relaxation rate depends on the inverse sixth power of the proton–proton distance, the purine H_8 protons should exhibit comparable relaxation times and in turn be shorter than the purine H_2 relaxation times (Ts'o et al., 1973). This is observed experimentally (Table I) and rules out a syn conformation about the glycosidic bond at the purine resonances. The anti conformation is also predicted for the pyrimidine bases, where the pyrimidine H_6 protons relax somewhat faster compared to the purine H_8 resonances due to the presence of additional proton(s) at position 5 of the pyrimidine ring.

The relaxation times vary for a given type of proton with its location in the helix (terminal, internal, and central) (Table I). Thus, the adenine H_2 protons exhibit relaxation

Table III: The Upfield Ring Current Contributions from Bases (1)----(2) and (3)----(4) on the Carbon Resonances in the Middle Base Pair for the Sequence in Scheme I for B-DNA, A-RNA (A-DNA), and D-DNA Helices.

	Base Position				Nearest Neighbor Upfield Carbon Shifts (ppm)									
					Purine Carbons					Pyrimidine Carbons				
Helix Type	1	2	3	4	C-2	C-4	C-5	C-6	C-8	C-2	C-4	C-5	CH_3-5	C-6
B-DNA														
	C	G			0.2	0.05	0.1	0.2	0.1	0.55	0.1	0.1	0.0	0.1
	G	C			0.15	0.2	0.5	0.35	0.2	0.25	0.05	0.05	0.0	0.05
	T	A			0.5	0.05	0.1	0.25	0.05	0.7	0.2	0.1	0.0	0.1
	A	T			0.2	0.5	1.1	1.0	0.45	0.1	0.05	0.0	0.0	0.05
			C	G	0.2	0.2	0.1	0.1	0.05	0.25	0.7	0.5	0.35	0.25
			G	C	0.55	0.35	0.2	0.2	0.1	0.05	0.25	0.2	0.2	0.1
			T	A	0.15	0.05	0.05	0.15	0.05	0.65	1.2	0.8	0.4	0.45
			A	T	1.2	0.55	0.3	0.35	0.05	0.1	0.1	0.1	0.05	0.05
A-RNA (A-DNA)														
	C	G			0.3	0.1	0.15	0.25	0.1	0.35	0.25	0.1	0.0	0.1
	G	C			0.1	0.15	0.25	0.2	0.35	0.2	0.15	0.05	0.0	0.05
	T	A			0.7	0.2	0.3	0.7	0.05	0.4	0.35	0.15	0.0	0.05
	A	T			0.1	0.35	0.65	0.35	0.7	0.05	0.05	0.05	0.0	0.0
			C	G	0.3	0.2	0.15	0.15	0.05	0.15	0.45	0.6	0.7	0.35
			G	C	0.7	0.35	0.25	0.3	0.05	0.1	0.15	0.2	0.3	0.1
			T	A	0.15	0.05	0.05	0.05	0.05	0.4	1.0	1.3	0.95	0.75
			A	T	1.05	0.4	0.3	0.5	0.05	0.2	0.2	0.1	0.15	0.05
D-DNA														
	C	G			0.25	0.1	0.25	0.25	0.1	0.4	0.05	0.0	0.0	0.05
	G	C			0.25	0.3	0.7	0.7	0.25	0.2	0.05	0.0	0.0	0.05
	T	A			0.45	0.05	0.1	0.2	0.05	0.6	0.05	0.0	0.0	0.05
	A	T			0.6	0.75	1.3	1.5	0.4	0.05	0.0	0.0	0.0	0.0
			C	G	0.2	0.25	0.05	0.2	0.05	0.25	0.75	0.45	0.25	0.2
			G	C	0.6	0.45	0.2	0.2	0.1	0.05	0.25	0.1	0.1	0.05
			T	A	0.2	0.05	0.05	0.25	0.0	0.45	1.3	0.6	0.4	0.25
			A	T	1.55	0.7	0.4	0.45	0.1	0.0	0.1	0.05	0.05	0.05

Table IV: The Sum, $J_{H_1'H_2'} + J_{H_1'H_2''}$, for the Six Sugar $H_{1'}$ Resonances in d-ApTpGpCpApT in D_2O, Low Ionic Strength, and pH 7 between 2 and 70°.

Temp	$(J_{H_1'H_2'} + J_{H_1'H_2''})^a$					
(°C)	6	5	4	3	2	1
70	12	13.5	13.5	14 ± 1		15
63	13	13	13.5	14.5 ± 1		14.5
55	13	14	13	14.5 ± 1		15 ± 1
36	13.5	15	13	*b*	*b*	*b*
27	15 ± 1	15 ± 1	13.5	15 ± 1	*b*	*b*
2	15 ± 1	15 ± 1	13.5 ± 1	*b*	*b*	*b*

[a] The couplings are reported to the closest 0.5 Hz. [b] The coupling could not be measured.

times of 2.12 and 2.78 sec for the internal and terminal base pairs at 7°. This probably reflects the distribution of nearest neighbor protons which varies with location of the observed proton in the helix. By contrast, similar relaxation times are observed for the terminal and internal thymine methyl resonances at a given temperature (Table I). This is attributed to the predominant relaxation of a CH_3 proton by the two remaining protons on the same methyl group and only negligible contributions from protons further away due to the inverse sixth power distance dependence.

The relaxation rate also depends on the correlation time, τ_C. The relaxation times decrease with increasing temperature (7–34°) for most of the protons in d-ApTpGpCpApT, indicative of $\omega_H\tau_C > 1$ in this temperature range. At 270 MHz, $\omega_H = 1.7 \times 10^9$ sec^{-1} and hence τ_C for double-stranded d-ApTpGpCpApT is longer than 0.6×10^{-9} sec. The decrease in T_1 values with increasing temperature reflect the motional freedom of the base residues in that fraction of the double helix converted to strands.

II. Rotational Correlation Time. The central cytosine H_5 resonance is approximately equidistant from its H_6 proton and the $H_{2'}$ proton of the neighboring sugar ring linked via the 5′ position on the same strand.[5] Since the H_5–H_6 distance is 2.45 Å, we assign a similar distance to H_5–$H_{2'}$; remaining protons are >3 Å away from the cytosine H_5 resonance and make negligible contributions to the dipolar term (2) which has an r^{-6} dependence. Equation 2 can be solved for the relaxation of the central cytosine H_5 resonance from dipolar interactions with the H_6 and $H_{2'}$ resonances. Since $T_1 = 1.10$ sec at 34° (Table I) for the cytosine H_5 proton, we estimate $\tau_C = 1.4 \times 10^{-9}$ sec for d-ApTpGpCpApT at 34°. We cannot evaluate τ_C at 7°, conditions under which the hexanucleotide is predominantly double helical, since the central cytosine H_5 resonance merges with the HOD side bands.

III. Sugar Ring Pucker. The six sugar $H_{1'}$ resonances in d-ApTpGpCpApT exhibit $J_{H_1'H_2'} + J_{H_1'H_2''}$ between 13 and 15 Hz (Table IV). These values are consistent with the coupling constant sum of ~13.5 Hz for the C-3′ exo (C-2′ endo) ring pucker but rule out an C-3′ endo ring pucker where the predicted sum of coupling constants is ~7.5 Hz.

The vicinal coupling constants $J_{H_1'H_2'} + J_{H_1'H_2''}$ are somewhat larger at low temperatures where the hexanucleotide is predominantly double helical as compared to high temperature where unstacked single-strand conformations predominate (Table IV). This decrease probably re-

[5] This applies to a right-handed double helix with an anti conformation about the glycosidic bonds.

flects the onset of conformational flexibility of the sugar ring in the single-strand state.

IV. Base Pair Overlaps. A contributing factor to the chemical shift variations of individual resonances as a function of temperature are the ring current contributions (Giessner-Prettre and Pullman, 1970) from nearest neighbor base pairs on the intrinsic chemical shift positions for double helical d-ApTpGpCpApT in solution.

The experimental base proton chemical shifts associated with the helix–coil transition of d-ApTpGpCpApT are compared with the calculated chemical shifts due to the ring current contributions from nearest neighbors for different DNA helical geometries (Table V).

The ring current contributions from the A-DNA helix overestimate by a factor of 2 the experimental chemical shifts at the terminal CH_3 and H_6 protons, the internal CH_3 and H_6 protons, and the central H_5 proton (Table V). By contrast, there is good correspondence between the experimental and B-DNA helix predicted upfield shifts at these positions.

Small ring current contributions at the purine H_8 position are predicted for both A- and B-DNA helices as is experimentally observed (Table V).

The calculated ring current upfield shift for the terminal adenine H_2 resonance in the B-DNA helix (0.15 ppm) and the A-DNA helix (0.1 ppm) underestimate the experimental value (0.275 ppm) (Table V). Second nearest neighbor effects which contribute upfield shifts of ~0.1 ppm (Kan et al., 1973) may play a role since the terminal adenine H_2 resonance is located directly over the middle of the central guanine, one base pair removed in the 3′ direction on the same strand, in the B-DNA helix, Figure 3B (but not in the A-DNA helix).

The ring current contributions from nearest neighbors at the internal adenine H_2 resonance for the different DNA helices overpredict the experimental upfield shift of 0.18 ppm (Table V).[6]

The D-DNA helix (C-3′ exo sugar pucker) belongs to the same class as the B-DNA helix (C-3′ exo sugar pucker) and is quite distinct from the A-DNA helix (C-3′ endo sugar pucker). The ring current predictions from the B and D helices are similar, except that the latter helix predicts smaller ring current shifts at the H-5 and H-6 protons than the former helix (Table V).

V. Ring Current Tables. The ring current Tables II and III should be used with caution. They are applicable to regular B-DNA, A-RNA (A-DNA), and D-DNA helices and are very sensitive to tilt and twist distortions in the regular helix geometry (Patel and Tonelli, 1974).

Much of the nuclear magnetic resonance structural studies of nucleic acids have focussed on the proton high-resolution spectra. This is unfortunate since the more numerous carbon resonances contain a great deal of structural information (Smith et al., 1973). The application of ring current chemical shifts to carbon resonances is open to question since steric effects such as ring strain and angle deformations contribute to the chemical shifts (Günther, et al., 1973). This limits the application to the sugar carbon resonances. Du Vernet and Boekelheide (1974) have investigated the proton and carbon chemical shifts in substituted tetrahydropyrenes and conclude that the absolute magnitude of the ring current effect is the same for ^{13}C as for ^{1}H when they occupy the same position in space relative to the aromatic π cloud. This suggests that the base carbon chemical shifts presented in Table III for all combinations of nearest neighbor base pairs should have wide applicability.

Table V: Comparison of the Upfield Experimental Chemical Shifts on Double Helix Formation (70–5°) with Those Predicted to Arise from the Ring Currents (Giessner-Prettre and Pullman, 1970) of Nearest Neighbor Base Pairs for the B-DNA (Figure 4), A-DNA (Figure 5), and D-DNA (Figure 6) Double Helices.

Assignment	Experimental (70–5°) Upfield Chemical Shifts (ppm)	Predicted Ring Current Upfield Chemical Shifts (ppm)		
		B-DNA	A-DNA	D-DNA
Terminal				
Adenine H-8	0.0	0.0	0.0	0.0
Adenine H-2	0.275	0.15	0.1	0.2
Thymine CH_3	0.36	0.25	0.7	0.25
Thymine H-6	*a*	0.15	0.45	0.05
Internal				
Adenine H-8	0.0	0.05	0.1	0.05
Adenine H-2	0.18	0.4	0.3	0.5
Thymine CH_3	0.23	0.25	0.7	0.25
Thymine H-6	*a*	0.15	0.45	0.05
Central				
Guanine H_8	0.0	0.0	0.05	0.0
Cytosine H_6	0.23	0.15	0.2	0.05
Cytosine H_5	$\gtrsim$0.355[b]	0.45	0.75	0.3

[a] The terminal and internal thymine resonances cannot be differentiated. The upfield shifts are either 0.28 and 0.03 ppm or 0.17 and 0.15 ppm. [b] Chemical shift difference between 25 and 70°.

Acknowledgment

Miss L. Canuel provided expert technical assistance.

References

Altona, C., and Sundaralingam, M. (1973), *J. Am. Chem. Soc. 95,* 2333.

Altona, C., Van Boom, J. H., Koeners, H. J., and Van Binst, G. (1974), *Nature (London) 247,* 558.

Arnott, S., Chandrasekaran, R., Hukins, D. W. L., Smith, P. J. C., and Watts, L. (1974), *J. Mol. Biol. 88,* 523.

Arnott, S., and Hukins, D. W. L. (1972a), *Biochem. Biophys. Res. Commun. 47,* 1504.

Arnott, S., and Hukins, D. W. L. (1972b), *Biochem. Biophys. Res. Commun. 48,* 1392.

Arnott, S., and Hukins, D. W. L. (1973), *J. Mol. Biol. 81,* 93.

Arnott, S., Hukins, D. W. L., Dover, S. D., Fuller, W., and Hodgson, A. R. (1973), *J. Mol. Biol. 81,* 107.

Arnott, S., and Selsing, E. (1974), *J. Mol. Biol. 88,* 551.

Barry, C. D., Glasel, J. A., North, A. C. T., Williams, R. J. P., and Xavier, A. V. (1972), *Biochim. Biophys. Acta 262,* 101.

Borer, P. N., Uhlenbeck, O. C., Dengler, B., and Tinoco, Jr., I. (1973), *J. Mol. Biol. 80,* 759.

Brahms, J., Pilet, J., Lan, T., and Hill, L. R. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 3352.

Bram, S. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 2127.

Bram, S., and Baudy, P. (1974), *Nature (London)* 250, 414.

Camerman, N., Fawcett, J. K., and Camerman, A. (1973), *Science 182,* 1142.

[6] The ring current calculations predict a larger upfield shift for the internal as compared to the terminal adenine H_2 resonance (independent of helix type), Table V, contrary to the experimental assignment based on $T_{1/2}$ values and temperature-dependent chemical shifts between 0 and 20° (Patel, 1975).

Chan, S.I., and Nelson, J. H. (1969), *J. Am. Chem. Soc. 91,* 168.
Du Vernet, R., and Boekelheide, V. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 2961.
Evans, F. E., Lee, C.-H., and Sarma, R. H. (1975), *Biochem. Biophys. Res. Commun. 63,* 106.
Fang, K. N., Kondo, N. S., Miller, P. S., and Ts'o P. O. P. (1971), *J. Am. Chem. Soc. 93,* 6647.
Giessner-Prettre, C., and Pullman, B. (1970), *J. Theor. Biol. 27,* 87.
Günther, H., Schmickler, H., Königshofen, H., Recker, K., and Vogel, E. (1973), *Angew. Chem., Int. Ed. Engl. 12,* 243.
Johnson, C. E., and Bovey, F. A. (1958), *J. Chem. Phys. 29,* 1012.
Kan, L. S., Barrett, J. C., and Ts'o, P. O. P. (1973), *Biopolymers 12,* 2409.
Patel, D. J. (1975), *Biochemistry,* preceding paper in this issue.
Patel, D. J., and Tonelli, A. E. (1974), *Biopolymers 13,* 1943.
Rosenberg, J. M., Seeman, N. C., Kim, J. J., Suddath, F. L., Nicholas, H. B., and Rich, A. (1973), *Nature (London) 243,* 150.
Smith, I. C. P., Mantsch, H. H., Lapper, R. D., Deslauriers, R., and Schleich, T. (1973), *Conform. Biol. Mol. Polym., 5,* 381.
Solomon, I. (1955), *Phys. Rev. 99,* 559.
Suck, D., Manor, P. C., Germain, G., and Saenger, W. (1973), *Nature (London), New Biol. 246,* 161.
Sussman, J. L., Seeman, N. C., Kim, S.-H., and Berman, H. M. (1972), *J. Mol. Biol. 66,* 403.
Ts'o, P. O. P., Barrett, J. C., Kan, L. S., and Miller, P. S. (1973), *Ann. N.Y. Acad. Sci. 222,* 290.
Warshaw, M. M., and Cantor, C. R. (1971), *Biopolymers 9,* 1079.
Yathindra, N., and Sundaralingam, M. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 3325.

Regulation of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Activity in Cultured Swine Aortic Smooth Muscle Cells by Plasma Lipoproteins†

Gerd Assmann,‡ B. Greg Brown, and Robert W. Mahley*

ABSTRACT: 3-Hydroxy-3-methylglutaryl coenzyme A reductase activity has been measured in cultured smooth muscle cells derived from swine aorta, and its regulation by serum lipoproteins from control and hypercholesterolemic swine has been determined. In confluent cells grown in the presence of 10% swine serum, 3-hydroxy-3-methylglutaryl coenzyme A reductase catalyzed the formation of 10 pmol of mevalonate/min per mg of protein (cell extract). Replacement of 10% swine serum in the growth medium at confluency for 18 hr by lipoprotein-deficient swine serum resulted in an eightfold increase of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Addition of very low density and low density lipoproteins, however, led to a lipoprotein-cholesterol concentration-dependent suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity which was comparable for control and hyperlipidemic swine lipoproteins. A unique lipoprotein characteristic of experimentally induced hypercholesterolemia in the swine, which has been classified as high density lipoprotein (HDL_c) based on its migration properties on electrophoresis and its lack of the B apoprotein, exerted an inhibitory effect on reductase activity comparable to that of very low density and low density lipoproteins. However, typical high density lipoproteins from control and hypercholesterolemic swine did not suppress reductase activity. To confirm these results, selected cultures were grown in the presence of [^{3}H]acetate and [^{14}C]mevalonate. Lipid analysis was carried out by radiogas chromatography and mass spectrometry, and cholesterol, desmosterol, dihydrolanosterol, and lanosterol were identified as radioactive sterols. Measurements of the $^3H/^{14}C$ ratio in isolated sterols indicated a positive correlation with direct measurements of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity.

Biosynthesis of cholesterol from acetate and mevalonate in arterial tissue has been demonstrated in a number of species (Siperstein et al., 1951; Werthessen et al., 1954; Azernoff, 1958; Feller and Huff, 1955; Rao and Rao, 1968; Chobanian, 1968; Daly, 1971; Avigan et al., 1972). Studies of rate and control of cholesterol synthesis in arterial smooth muscle cells, which is the predominant cell type in the intima and media of large vessels, have not yet been performed. The present study demonstrates that the rate-limiting enzyme in cholesterol biosynthesis from acetyl-CoA, 3-hydroxy-3-methylglutaryl coenzyme A reductase (reductase; EC 1.1.1.34), is detectable in cultured smooth muscle cells from swine aorta. Its regulation by isolated serum lipoprotein fractions from normolipidemic (control) and hyperlipidemic swine has been determined.

† From the Section on Experimental Atherosclerosis (R.W.M., B.G.B.) and the Molecular Disease Branch (G.A.), National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland 20014. *Received December 26, 1974.*

‡ Present address: Abteilung fuer Klinische Chemie und Zentrallaboratorium Kliniken der Universitaet Koeln, 5-Cologne, West Germany.