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Chemistry

Nuclear magnetic resonance studies of the helix–coil transition of poly(dA-dT) in aqueous solution

(nucleic acids/helix dissociation rate/DNA conformation)

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ABSTRACT **The well-resolved base and sugar proton resonances in the high resolution proton nuclear magnetic resonance (NMR) spectra of poly(dA-dT) can be monitored during the helix–coil transition. The observable resonances shift upfield on helix formation and the temperature-dependent chemical shifts exhibit a melting temperature $t_{1/2}$ = 69.9 ± 0.3° for 18.8 mM (with respect to phosphorus) poly(dA-dT) in 0.5 M Tris, 0.1 M cacodylate, D_2O, pH 7.05. The observable protons are in fast exchange throughout the poly(dA-dT) helix–coil transition. The adenine H_2 resonance that shifts upfield by about 1 ppm on helix formation exhibits uncertainty broadening in the fast exchange region. The line width changes are frequency dependent, as predicted, and yield a helix dissociation rate constant of 0.17×10^4 sec^{-1} at 61° and 0.55×10^4 sec^{-1} at 70°. The upfield proton chemical shifts for poly(dA-dT) on helix formation are compared with those predicted to arise from the ring current contributions of nearest neighbors for different DNA structures. The experimental values which monitor the cooperative duplex to strand transition rule out an A-DNA conformation for poly(dA-dT) in solution and are consistent with the B-DNA family of conformations. The ^{31}P internucleotide phosphate resonances of poly(dA-dT) shift upfield on helix formation. These shifts reflect a change about the ω, ω' phosphodiester bonds from *gauche, gauche* in the stacked helical structures to a distribution of *gauche, trans* in the unstacked coil structures.**

Arnott and coworkers (1) have recently extended the early studies of Davies and Baldwin (2) on the x-ray diffraction patterns from oriented, crystalline fibers of poly(dA-dT). They propose a novel, 8-fold helical structure, with an axial rise per residue of 3.03 Å and a C3 *exo* sugar pucker conformation. Baldwin and coworkers (3–6) have investigated the helix–coil transition of poly(dA-dT) and circular oligo(dA-dT). They have characterized the role of hairpin and straight chain helices by monitoring absorbance melting curves as a function of salt, temperature, and pH. The thermodynamic parameters for the helix–coil transition of poly(dA-dT) have been elucidated from differential heat calorimetry (7, 8).

High resolution nuclear magnetic resonance (NMR) spectroscopy is capable of monitoring many aspects of the helix–coil transition of alternating deoxypurine-deoxypyrimidine oligonucleotides in aqueous solution. These studies include investigations of the exchangeable and nonexchangeable protons in the duplex of the self-complementary hexanucleotide d(A-T-G-C-A-T) (9–12) and the d[C-G(-C-G)$_n$], n = 1,2 duplexes (13) in aqueous solution.

The Watson–Crick thymine imino protons in the oligomer duplexes d(pT-A)$_n$, n = 3,4,5, were monitored in our laboratory as a function of temperature by high resolution proton NMR spectroscopy in H_2O solution (14). The chemical shifts and line widths of these exchangeable resonances were observable when the double helix was predominantly intact but broadened with increasing coil concentration. An alternate NMR approach monitors the entire helix–coil transition as followed by the nonexchangeable protons and the phosphates in D_2O solution (11–13, 15–17).

Nuclear magnetic resonance spectral line widths yield kinetic information about the helix–coil transition when the exchange rate is on the order of the chemical shift difference between the helix and coil states. It will be demonstrated that the adenine H_2 proton in poly(dA-dT) exhibits an about 1 ppm chemical shift difference between the two states and provides a handle for monitoring the kinetic aspects of the helix–coil transition.

Abbreviation: NMR, nuclear magnetic resonance.

EXPERIMENTAL

Sample preparation

The alternating copolymer poly(dA-dT) samples for proton NMR studies were purchased from Long Island Biochemical Corp. and were shipped in Tris buffer (chain length: about 300 nucleotides).

270 MHz Proton NMR Studies. The purchased sample in Tris buffer was lyophilized and the spectra were recorded on 18.8 mM (with respect to phosphorus) poly(dA-dT) in 0.5 M Tris, 0.1 M cacodylate, 0.01 M ethylenediaminetetraacetic acid (EDTA), D_2O, pH 7.05.

360 MHz Proton NMR Studies. A sample of poly(dA-dT) was prepared as described above for a series of NMR experiments on a related problem. It was then passed twice through Sephadex G-25 columns to remove the Tris and cacodylate salts. The lyophilized salt-free poly(dA-dT) was redissolved in 0.1 M sodium phosphate, 1 mM EDTA, D_2O, pH 6.3, to make a 30 mM (P) solution.

The poly(dA-dT) samples for phosphorus NMR studies were purchased from Collaborative Research and were shipped in lyophilized form. The polymer was dissolved in 0.01 M cacodylate, 0.001 M EDTA, H_2O, pH 6.8, and dialyzed against the same buffer (four changes of buffer over a 24 hr period). The solution was concentrated tenfold and ^{31}P spectra were recorded on 26.3 mM (P) poly(dA-dT) in 0.1 M cacodylate, 0.01 M EDTA, D_2O, pH 7.08.

The reported pH values in D_2O solution are uncorrected pH meter readings.

Instrumentation

High resolution proton NMR spectra were recorded in the continuous wave mode on Bruker HX 270 and HX 360 spectrometers. Spectra were time averaged on Nicolet computers to improve the signal-to-noise ratio. The chemical shifts are referenced relative to standard sodium 2,2-dimethyl-2-silapentane-5-sulfonate.

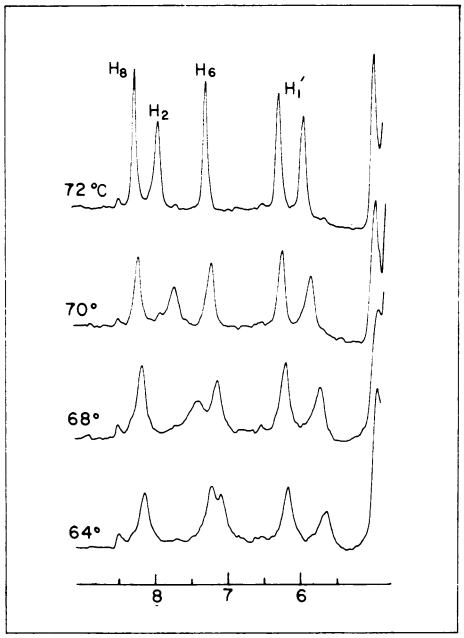

FIG. 1. The high resolution 270 MHz NMR spectra of 18.8 mM (P) poly(dA-dT) in 0.5 M Tris, 0.1 M cacodylate, D_2O, pH 7.05, between 5.5 and 8.5 ppm in the temperature range 64°–72°.

High resolution 145.7 MHz phosphorus NMR spectra were recorded in the Fourier transform mode on a Bruker HX-360 spectrometer interfaced with a Nicolet BNC-12 computer system. The spectrometer was locked to the deuterium of solvent and the chemical shifts were recorded relative to internal trimethylphosphate.

RESULTS

Proton NMR studies

Helix–Coil Transition. The high resolution 270 MHz proton NMR spectra (5.5–8.5 ppm) of poly(dA-dT) in Tris, cacodylate, D_2O, pH 7.05, between 64 and 72° are presented in Fig. 1.

The two upfield resonances between 5.7 and 6.2 ppm can be readily assigned to the sugar $H_{1'}$ resonances, though we cannot differentiate between the adenine and thymine sugar rings. The resonance farthest downfield is assigned to the adenine H_8 resonance, since it is deuterated in D_2O at elevated temperatures. The thymine H_6 is predicted to resonate upfield from the adenine H_2 resonance in model mononucleotides (17) and hence assignment of these two resonances can be made in the high temperature (72°) spectrum in Fig. 1 which characterizes the disordered strand structure.

Table 1. The melting temperature, $t_{1/2}$, °C, of the adenine H_8, H_2, and $H_{1'}$ and thymine H_6 and $H_{1'}$ resonances for 18.8 mM poly(dA-dT) in 0.5 M Tris, 0.1 M cacodylate, D_2O, pH 7.05

Resonance	$t_{1/2}$, °C
Adenine H_8	69.9
Adenine H_2	69.6
Thymine H_6	70.2
Upfield $H_{1'}$	69.8
Downfield $H_{1'}$	70.1

The experimental chemical shifts are plotted between 64 and 76° in Fig. 2 for the adenine H_2, H_8, and $H_{1'}$ resonances and the thymine H_6 and $H_{1'}$ resonances. All five observable protons shift upfield on helix formation as the temperature is lowered from 76° to 64°. The magnitude of the shifts (between 64° and 76°) which reflect the helix–coil transition are adenine H_8 = 0.16 ppm, adenine H_2 = 0.93 ppm, thymine H_6 = 0.27 ppm and the $H_{1'}$ protons = 0.17 and 0.37 ppm. The five observable resonances exhibit melting temperature $t_{1/2}$ values of 69.9 ± 0.3° in Tris–cacodylate, pH 7.05, D_2O (Table 1).

Helix Dissociation. The three base and two sugar protons (5.5–8.5 ppm) of poly(dA-dT) in the Tris–cacodylate shift downfield as average resonances on conversion from helix to coil in the temperature range 64°–76° (Figs. 1 and 2). This suggests that exchange between helix and coil is rapid on the NMR time scale compared to the chemical shift separation at 270 MHz between the states. Closer inspection indicates that the adenine H_2 resonance, which undergoes the largest chemical shift change during the transition, exhibits line width contributions due to uncertainty broadening in the fast exchange region.

Comparison of the 270 MHz spectra of poly(dA-dT) in 0.5 M Tris, 0.1 M cacodylate ($t_{1/2}$ = 70°) at 70° and the 360 MHz spectra of poly(dA-dT) in 0.1 M phosphate ($t_{1/2}$ =

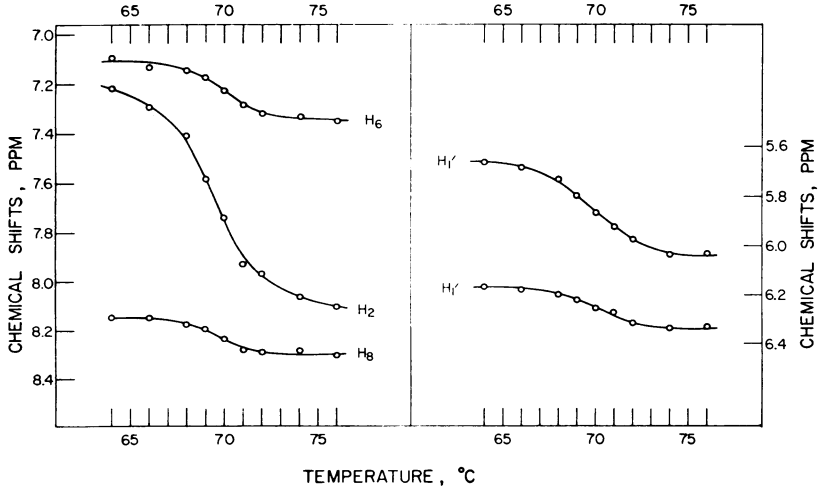

FIG. 2. The temperature dependence (64°–76°) of the chemical shifts of the adenine H_8, H_2, and $H_{1'}$ and thymine H_6 and $H_{1'}$ resonances of 18.8 mM (P) poly(dA-dT) in Tris–cacodylate, D_2O, pH 7.05.

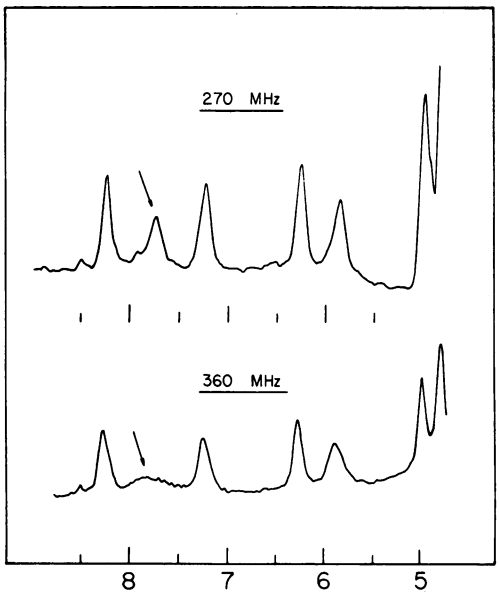

FIG. 3. Comparison of (top) the 270 MHz spectrum of 18.8 mM (P) poly(dA-dT) in 0.5 M Tris, 0.1 M cacodylate D_2O, pH 7.05 at 70° ($t_{1/2}$ = 70°) and (bottom) the 360 MHz spectrum of 30 mM (P) poly(dA-dT) in 0.1 M phosphate, D_2O, pH 6.3 at 61° ($t_{1/2}$ = 61°). The arrow points at the adenine H_2 resonance that shifts upfield by about 1 ppm on helix formation.

61°) at 61° confirms this conclusion. The greater chemical shift separation at the higher fields results in further broadening of the adenine H_2 resonance at the midpoint of the helix–coil transition, characteristic of uncertainty broadening contributions to the line width in the fast exchange region.

Eq. 1 describes the exchange of a proton between two sites for the condition of fast exchange with contributions from uncertainty broadening, where the helical state is designated site H and the coil state site C, with populations P_H and P_C and lifetimes τ_H and τ_C.

$$\frac{1}{\pi T_2} = \frac{P_H}{\pi T_{2H}} + \frac{P_C}{\pi T_{2C}} + 4\pi P_H^2 P_C^2 (\nu_H - \nu_C)^2 (\tau_H + \tau_C)$$

The calculation was undertaken on the 270 MHz proton NMR spectrum of poly(dA-dT) in 0.5 M Tris, 0.1 M cacodylate at the midpoint of the helix–coil transition, ($P_H = P_C = 0.5$), 70° (see Fig. 3). The line width at 76° is 15 Hz, and is assigned to $(\pi T_{2C})^{-1}$, the line width in the coil state. The line width at 66°, is 35 Hz, and is assigned to $(\pi T_{2H})^{-1}$, the line width in the helical state. The line width of the adenine H_2 resonance at $t_{1/2}$ = 70°, $(\pi T_2)^{-1}$ equals 43 Hz. The chemical shift difference between the helix and coil states at 270 MHz is 251 Hz.

Solving Eq. 1 with the above parameters yields $(\tau_H + \tau_C) = 3.64 \times 10^{-4}$ sec. Since $\tau_H = \tau_C$ when $P_H = P_C = 0.5$, one obtains the lifetime in the helical state, $\tau_H = 1.82 \times 10^{-4}$ sec. The unimolecular dissociation of the poly(dA-dT) double helix to strands in 0.5 M Tris, 0.1 M cacodylate, at 70° is given by the rate constant $k_{HC} = (\tau_H)^{-1} = 0.55 \times 10^4$ sec^{-1}.

The calculation was also undertaken on the 360 MHz NMR spectrum of poly(dA-dT) in 0.1 M phosphate, 61° ($t_{1/2}$ = 61° in this buffer). The following parameters were used: $(\pi T_2)^{-1}$ = 145 Hz, $(\pi T_{2H})^{-1}$ = 64 Hz, $(\pi T_{2C})^{-1}$ = 17 Hz, $P_H = P_C = 0.5$ and $(\nu_H - \nu_C)$ = 335 Hz. The unimolecular dissociation of the poly(dA-dT) double helix to strands in 0.1 M phosphate at 61° is given by the rate constant $k_{HC} = 0.17 \times 10^4$ sec^{-1}.

Table 2. A comparison of the upfield experimental base proton chemical shifts in poly(dA-dT) on helix formation with those predicted (12) from ring current calculations for the A-DNA, B-DNA, and D-DNA double helices*

Resonance	Experimental upfield shifts, ppm	Calculated upfield shifts, ppm		
		B-DNA	A-DNA	D-DNA
Adenine H_8	0.17	0.0	0.05	0.0
Adenine H_2	0.93	0.85	0.6	1.0
Thymine H_6	0.27	0.15	0.45	0.05
Thymine CH_3	0.36	0.25	0.7	0.25

* The calculated values are accurate to ± 0.05 ppm.

The rate constant calculations were verified using the DNMR Program of Binsch and Kleir, which simulates exchange-broadened NMR spectra from chemical shifts, relaxation times, rate constants, and populations (18).

Helical Conformation of Poly(dA-dT). A contributing factor to the temperature-dependent chemical shift variations of individual resonances through the helix–coil transition is provided by the ring current contributions (19) from nearest neighbor base pairs in the helical state on the intrinsic chemical shift positions of the resonances (12, 17, 20, 21).

The experimental base proton chemical shifts associated with the helix–coil transition are compared in Table 2 with the calculated chemical shifts due to ring current contributions from nearest neighbors for different DNA helical geometries (12).

Phosphorus NMR studies

The temperature dependence of the 145.7 MHz Fourier transform NMR spectra of poly(dA-dT) in 0.1 M cacodylate, D_2O, pH 7.08 is presented in Fig. 4.

The phosphorus resonances cover a wide temperature range (0.5–1.0 ppm) below 55°. They narrow and shift upfield with increasing temperature (Fig. 5). Separate phosphorus resonances (chemical shift difference 0.025 ppm) are observed above 65°, corresponding to the TpA and ApT phosphates in the poly(dA-dT) sequence.

DISCUSSION

Proton NMR studies

Spectra. The well-resolved high resolution proton spectra of poly(dA-dT) between 5.5 and 8.5 ppm (Fig. 1) indicate that the NMR methods applied previously to investigate the helix–coil transition of self-complementary oligomer duplexes (9–17) can be extended to the polymer level.

This magnetic resonance study reports on the helix–coil transition of poly(dA-dT) within ±6° of the melting temperature. We cannot differentiate at this time between two-stranded double helices and single-stranded hairpin double helices (6). This problem can be approached by an extension of these studies to lower temperatures.

The proton resonances are quite broad in the helical and coil states and this precludes measurement of coupling information from the sugar $H_{1'}$ resonances.

Melting Temperature. The proton NMR method monitors several base and sugar protons of the adenine and thymine residues of poly(dA-dT) during the helix–coil transition so that the information content is much greater than

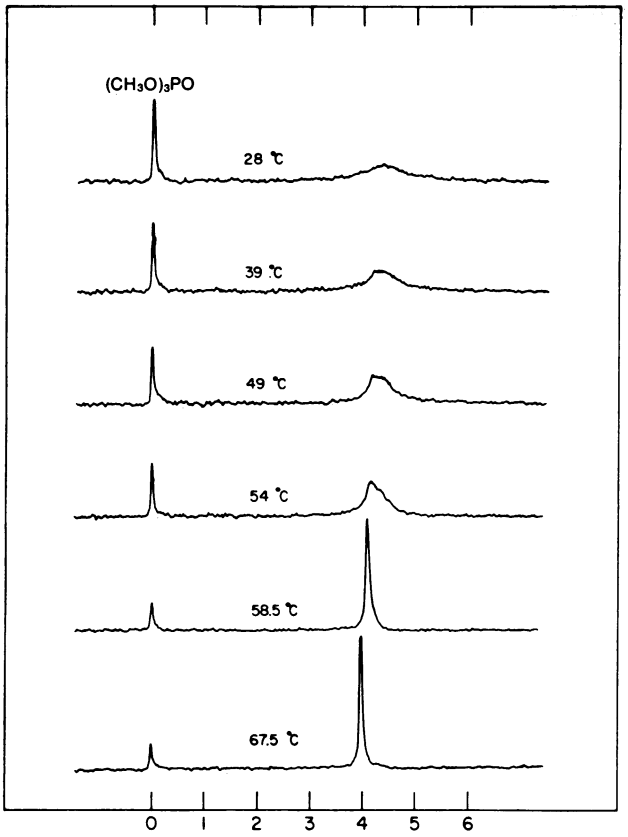

FIG. 4. The high resolution 145.7 MHz spectra of 26.3 mM (P) poly(dA-dT) in 0.1 M cacodylate, 0.01 M EDTA, D_2O, pH 7.08 between 0 and 6 ppm (relative to standard trimethyl phosphate) in the temperature range 28°–68°.

that derived by monitoring a single optical absorption band.

The observed average chemical shift for different resonances in poly(dA-dT) reflects both the population difference among conformations (stacked double helix, stacked single strand, and unstacked single strand) and the magnetic environment about each proton. Since the magnetic environment varies about each proton in a given conformation and is related to the magnetic anisotropy contributions of neighboring groups, the chemical shifts versus temperature plots yield different $t_{1/2}$ values for the protons in poly(dA-dT) (see Table 1).

The five observable resonances in the 270 MHz NMR spectra of poly(dA-dT) in 0.5 M Tris, 0.1 M cacodylate yield $t_{1/2}$ values of 69.9 ± 0.3°, in good agreement with that computed by Baldwin for the melting of the hairpin helix of poly(dA-dT) at 0.6 M monovalent counter ion (6).

Kinetics of the Helix-Coil Transition. Porschke and Eigen have reported temperature jump studies on the kinetics of the helix–coil transition of the oligo(ribouridylic acid)·oligo(riboadenylic acid) system (22). They observed that the rates of dissociation at a given temperature become smaller with increasing chain length and that at constant chain length the rates increase with temperature.

The NMR method is an alternate approach to elucidate the kinetics of dissociation of the helix–coil transition of nucleic acid polymers in solution. The adenine H_2 resonance in poly(dA-dT) undergoes a large chemical shift change associated with the transition and the line widths can be analyzed to yield the dissociation rate constant for conversion of the double helix to strands.

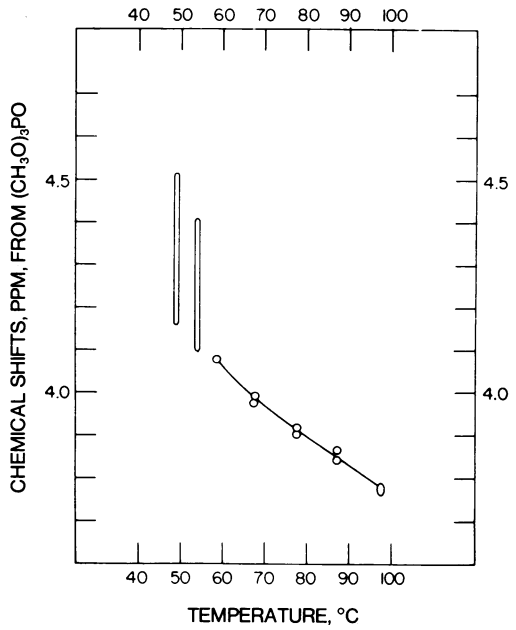

FIG. 5. The temperature dependence (45°–95°) of the phosphate chemical shifts of poly(dA-dT) in 0.1 M cacodylate, D_2O, pH 7.08.

The analysis has been carried out at the midpoint of the helix–coil transition of poly(dA-dT) in 0.1 M phosphate ($t_{1/2}$ = 61°) and 0.5 M Tris, 0.1 M cacodylate, ($t_{1/2}$ = 70°). The rate constant increases from 0.17×10^4 sec^{-1} at 61° to 0.55×10^4 sec^{-1} at 70°.

Base Overlap Geometries. It has been demonstrated by others that the nonexchangeable protons of complementary mononucleotides experience negligible shifts on Watson–Crick hydrogen bond formation (23, 24). The observed experimental upfield shifts for poly(dA-dT) on helix formation are compared with those predicted to arise from ring current contributions from nearest neighbors for different helical geometries (Table 2).

The predicted A-DNA ring current contributions (12) overestimate the experimental thymine H_6 and CH_3 shifts and underestimate the experimental adenine H_2 shift (Table 2). By contrast, there is reasonable agreement between the experimental shifts at these positions and those predicted on the basis of ring current calculations based on the B- and D-DNA helical conformations (12) (Table 2).

The experimental upfield shift at the adenine H_8 and thymine H_6 position is greater by about 0.15 ppm than the value predicted from ring current calculations for the B-DNA helix. These two positions are proximal to the backbone phosphates in the *anti* conformation about the glycosidic bond. The 0.15 ppm chemical shift which is not accounted for by the ring current calculations may reflect contributions from the phosphate backbone during the helix–coil transition.

Phosphorus NMR studies

^{31}P NMR studies on nucleic acids (at the oligonucleotide level) have been limited to investigations on transfer RNA (25, 26) and drug–nucleic acid complexes (13, 27). These studies have demonstrated the wide range of ^{31}P chemical shifts in nucleic acids and their sensitivity to O-P-O bond strain and phosphodiester torsion angles (13, 26–28).

Phosphodiester ω,ω' Angles. There is considerable experimental and theoretical information which suggests that the

stacked (helical) and unstacked (coil) polynucleotide structures differ primarily at the rotation angles ω and ω' about the phosphodiester bonds in both fibers and solution states (29–33).

We assign the downfield shifts of the ^{31}P resonances of poly(dA-dT) with increasing temperature (Figs. 4 and 5) to a change in the ω,ω' angles from *gauche, gauche* states in the helical stacked structures to a distribution of *gauche, trans* states in the coil structure.

The temperature dependence of the phosphorus resonances of poly(dA-dT) in 0.1 M cacodylate exhibit a large transition width (Figs. 4 and 5). These downfield phosphorus shifts with increasing temperature predominantly reflect the conversion of stacked strands to unstacked strands (60°–100°).

By contrast, the temperature dependence of the proton resonances of poly(dA-dT) in 0.1 M phosphate and 0.5 M Tris, 0.1 M cacodylate exhibits narrow transition widths (Figs. 1 and 2) indicative of a cooperative process. These downfield proton shifts with increasing temperature predominantly reflect the conversion of stacked duplex to strands.

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