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# Proton Nuclear Magnetic Resonance Investigations of Fraying in Double-Stranded d-ApTpGpCpApT in $H_2O$ Solution†

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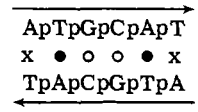
ABSTRACT: The chemical shifts and line widths of the Watson–Crick ring NH resonances of the self-complementary duplex of d-ApTpGpCpApT have been monitored at low ionic strength and in the presence of Mg ions at neutral pH in aqueous solution to determine the thermodynamic parameters associated with *fraying* (D. J. Patel (1974), *Biochemistry 13,* 2396) at the terminal and internal base pairs as a function of temperature and pH. From studies in $H_2O$–MeOH (3:2), the fraying process persists down to ~−20° for the internal TA base pair and down to and probably beyond −30° for the terminal AT base pair. The observed average chemical shift at each of these base pairs as a function of temperature suggests rapid exchange on the nuclear magnetic resonance (NMR) time scale between helix and coil (chemical shift separation of 3.2 ppm) and have been utilized to determine the dissociation constant at the terminal and internal base pairs. Comparison of the reaction enthalpies elucidated from the chemical shift parameters with those reported from optical studies suggests that the symmetry related internal TA base pairs break in a coupled manner at low ionic strength, with the coupling removed in the presence of Mg ions and high salt. By contrast, the symmetry related terminal AT base pairs break independently of each other in the absence and presence of Mg ions and high salt. The terminal base pair exhibits a $T_m$ of 10–15° lower than that of the internal base pair in the hexanucleotide, with divalent Mg ions and high salt stabilizing the double helix as reflected in the $T_m$ values of these base pairs. The observed line width changes as a function of temperature provide an estimate of the exchange rate of the proton from the coil form with water. The exchange reaction from the coil state is base catalyzed with rate constants in the diffusion limit.

The availability of short double-stranded nucleic acids of defined sequence has resulted in the evaluation of the thermodynamic and kinetic parameters associated with their helix–coil transition (Bloomfield et al., 1974). These results followed from optical changes monitored as a function of temperature in equilibrium studies and temperature jump experiments. They were first undertaken on self-complementary riboadenylic acid–uridylic acid block copolymers (Martin et al., 1971; Craig et al., 1971; Pörschke, 1971; Pörschke and Eigen, 1971) and later extended to GC containing ribonucleic acid sequences $(Ap)_nCpG(pU)_n$, where $n$ = 2,3,4 (Uhlenbeck et al., 1971) and $(Ap)_nGpC(pU)_n$, where $n$ = 2,3,4 (Pörschke et al., 1973; Ravetch et al., 1975). In parallel investigations, thermodynamic parameters have been determined for the helix–coil interconversion in self-complementary duplexes of $(dA\text{-}dT)_n$ (Scheffler et al., 1968, 1970; Scheffler and Sturtevant, 1969) and $(dG\text{-}dC)_n$ (Pohl, 1974).

It has been demonstrated that the Watson–Crick hydrogen-bonded ring imino protons (guanine $N_1H$ and thymine $N_3H$) resonate between 11 and 15 ppm downfield of sodium 2,2-dimethyl-2-silapentane-5-sulfonate in the high-resolution nuclear magnetic resonance (NMR) spectrum of double-stranded nucleic acids in $H_2O$ solution (Kearns et al., 1971). The observed chemical shifts of these resonances reflect the ring current contributions from stacked nearest neighbor base pairs to the intrinsic chemical shifts of the ring imino protons (Shulman et al., 1973; Patel and Tonelli, 1974).

Self-complementary single strands of the hexanucleotide d-ApTpGpCpApT form a double helix which exhibits twofold symmetry so that the six base pairs are pairwise equivalent.




This study reports on the helix–coil transition of double-stranded d-ApTpGpCpApT in $H_2O$ solution as monitored by the chemical shift and line-width changes of the guanine $N_1H$ and thymine $N_3H$ resonances as a function of temperature and pH. It will be demonstrated that the thermodynamic parameters associated with the fraying of the termi-

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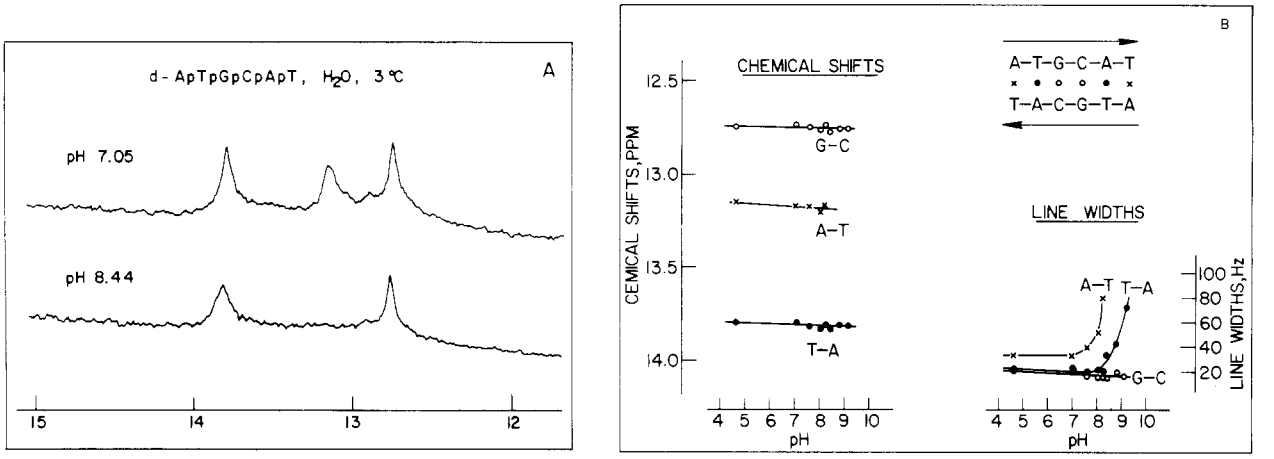



FIGURE 1: (A) The 300-MHz proton NMR spectra of d-ApTpGpCpApT as a function of pH in $H_2O$ (low ionic strength) at 3° between 12 and 14 ppm. (B) Plots of chemical shifts and line widths as a function of pH.

nal and internal base pairs and the sequential melting of the double helix can be directly determined by NMR.

## Experimental Section

*Materials.* d-ApTpGpCpApT was purchased from Collaborative Research. The hexanucleotide was passed through a Chelex column to remove paramagnetic ions prior to use. Samples were made up at concentrations of 25 mg/ml (14 m*M*). Samples containing salt were desalted, when necessary, by two passages through Bio-Gel P2 columns.

d-ApTpGpCpApT samples containing no added salt are designated by "low ionic strength."

*Spectral Methods.* Proton NMR spectra were recorded on a Varian HA-300 MHz spectrometer for all samples other than those containing $Mg^{2+}$ in which case they were recorded on a Bruker HX-270 spectrometer. Spectra were time averaged on Nicolet computers to improve the signal-to-noise ratio.

## Results

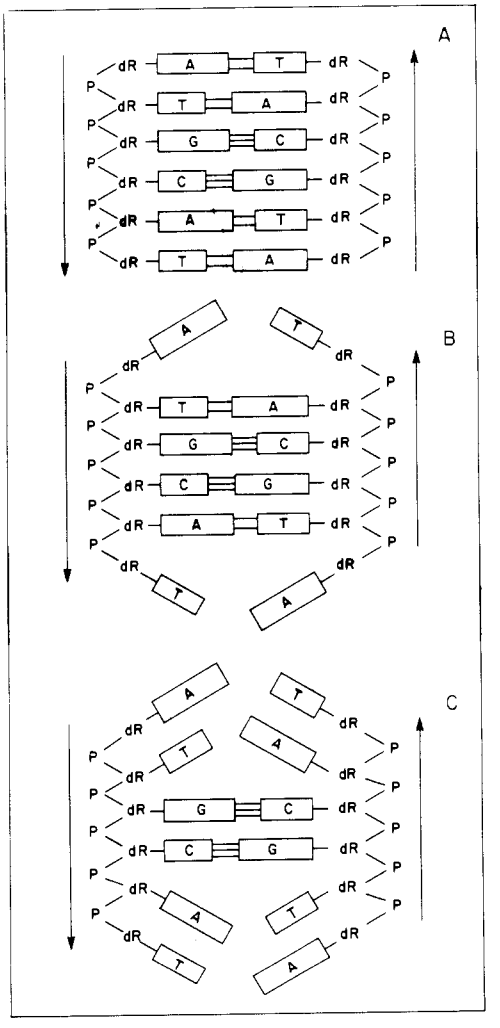
*I. Spectra.* High-resolution proton NMR studies in $H_2O$ solution have demonstrated the presence of three exchangeable resonances between 12 and 14 ppm. They have been assigned to specific ring NH Watson-Crick hydrogen-bonded protons from the sequential line-width changes with increasing temperature (Patel, 1974) (see Scheme I).

| | | |
|---|---|---|
| $T\text{-}N_3H$ | $(AT)_{terminal}$ | 13.15 ppm |
| $T\text{-}N_3H$ | $(TA)_{internal}$ | 13.80 ppm |
| $G\text{-}N_1H$ | $(GC)_{central}$ | 12.75 ppm |

A. pH DEPENDENCE (4.5–9.2) IN $H_2O$ (LOW IONIC STRENGTH) AT 3°. The high-resolution 300 MHz proton NMR spectra of d-ApTpGpCpApT in $H_2O$ at 3° have been recorded as a function of pH (4.5–9.2). Typical spectra are presented in Figure 1A and the chemical shifts and line widths plotted in Figure 1B.

All three resonances show pH independent chemical shifts under conditions where the $(AT)_{terminal}$ and $(TA)_{internal}$ ring NH resonance line-widths change by 50 Hz. There are no line-width changes detectable between pH 4.5 and 7 for the three resonances in double-stranded d-ApTpGpCpApT in $H_2O$ at 3°. The $T\text{-}N_3H$ resonance of the $(AT)_{terminal}$ base pair broadens out between pH 7 and 8 while the $T\text{-}N_3H$ resonance of the $(TA)_{internal}$ base pair broadens out between pH 8 and 9. The line width of the $G\text{-}N_1H$ of the $(GC)_{central}$ base pair remains unchanged between pH 4.5 and 9.2.

Scheme I.




B. TEMPERATURE DEPENDENCE (0–30°) IN THE ABSENCE AND PRESENCE OF $Mg^{2+}$. The temperature dependence of the chemical shifts and line widths of the double-stranded hexanucleotide have been determined in $H_2O$ (low ionic strength), pH 7 (Figure 2A), and in 0.1 *M* NaCl–0.025 *M* $Mg^{2+}$–$H_2O$ (pH 7.34) (Figure 2B).

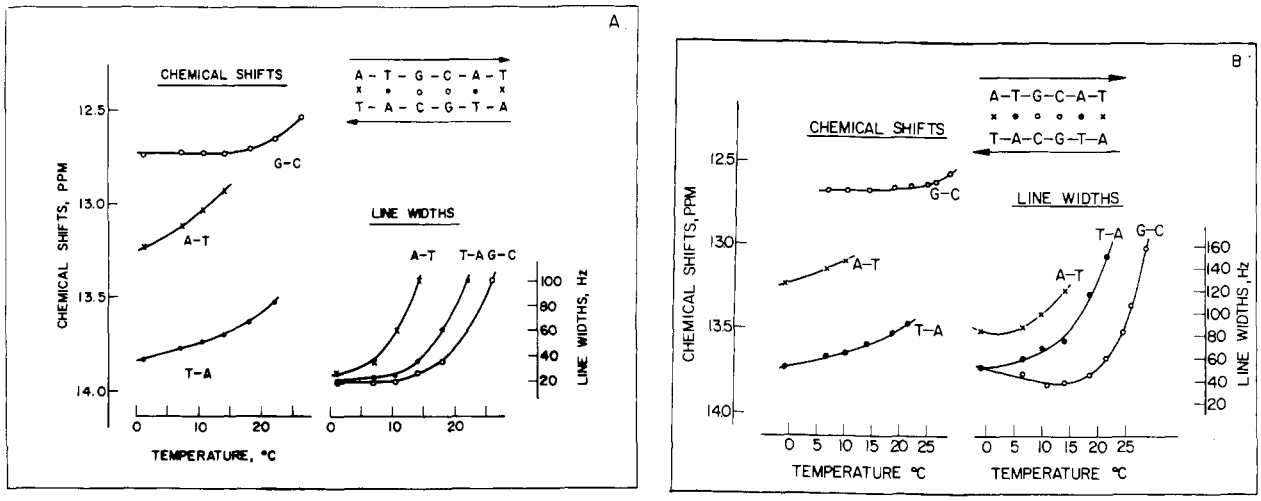



FIGURE 2: Plots of chemical shifts and line widths of d-ApTpGpCpApT as a function of temperature in (A) $H_2O$, low ionic strength, pH 7, and (B) 0.1 *M* NaCl-0.025 *M* $Mg^{2+}$-$H_2O$ (pH 7.34).

The observed line-width changes with temperature parallel the corresponding upfield chemical shift changes with temperature. Thus, between 0 and 26°, the ring NH resonances broaden and shift in the sequential order $(AT)_{terminal}$, $(TA)_{internal}$, and $(GC)_{central}$, respectively.

C. TEMPERATURE DEPENDENCE (−25° to +2°) IN $H_2O$-MeOH (3:2). In the experiments presented above, the chemical shifts of the T-$N_3$H resonances of the $(AT)_{terminal}$ and $(TA)_{internal}$ base pairs were temperature dependent down to 1°, making it necessary to investigate the hexanucleotide at temperatures below the freezing point of water.

Typical spectra in $H_2O$-MeOH (3:2) are presented in Figure 3. Over the temperature range +2.5° to −27.5°, the T-$N_3$H chemical shifts of the $(TA)_{internal}$ base pair shifts downfield by 0.13 ppm (down to ~−20°) while the T-$N_3$H chemical shift of $(AT)_{terminal}$ base pair shifts downfield by 0.34 ppm (down to and probably beyond −28°).

D. EFFECT OF ADDED SALT AND $Mg^{2+}$. Chemical shifts and line widths of the ring NH resonances have been recorded in the presence of salt, phosphate, and Mg ions in aqueous solution as a function of temperature (−5–30°). Line-width contributions from viscosity and/or aggregation effects are observed below 10° at high salt (~0.5 *M*) or low Mg (~0.025 *M*) concentrations for 15 m*M* (single-strand concentration) hexanucleotide solutions.

*II. Analysis.* A. DISSOCIATION CONSTANTS. Expressions have been derived that permit the evaluation of the dissociation constants at each base pair from their respective chemical shift data. The relationships[1] (Table I) are valid for fast exchange on the NMR time scale between hydrogen-bonded (helix) and open (coil) structures. The formation of terminal and internal AT base pairs are represented as unimolecular (propagation) reactions while the formation of the central GC base pairs are represented as a bimolecular (nucleation) reaction. The derivations are valid for the conversion of a stacked Watson-Crick hydrogen-bonded base pair to a stacked open base pair.

The chemical shifts in the helical ($\omega_H$) and coil ($\omega_C$) states at each base pair are summarized in Table II. They were determined from the experimental data in Figures 2 and 3, a knowledge of the ring NH chemical shifts for isolated base pairs (Shulman et al., 1973; Patel and Tonelli, 1974) and bases and the ring current contributions from nearest neighbor base pairs for the B-DNA double helix (Patel and Tonelli, 1974).

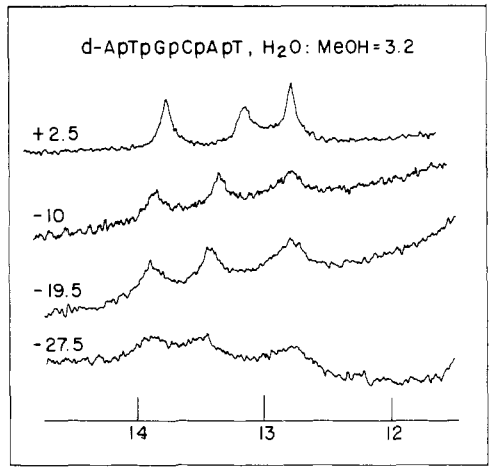



FIGURE 3: The 300-MHz proton NMR spectra of d-ApTpGpCpApT as a function of temperature in $H_2O$-MeOH (3:2) (low ionic strength) between 12 and 14 ppm. The spectra were aligned assuming that the chemical shift of the ring NH of the $(GC)_{central}$ base pair remains unchanged on lowering the temperature from +2.5 to −27.5°.

The equilibrium constant for the dissociation of the terminal and internal base pairs in d-ApTpGpCpApT in aqueous solution at neutral pH can then be derived from the observed average chemical shifts ($\bar{\omega}$) at these base pairs. Evaluation of dissociation constants as a function of temperature include studies at low ionic strength (Figure 4, I), at 0.3 *M* NaCl, 0.1 *M* phosphate (Figure 4, II) and at 0.1 *M* NaCl, 0.025 *M* $Mg^{2+}$ (Figure 4, III). The plots yield the dissociation enthalpies, entropies, and $T_m$ values at the terminal and internal base pairs for each solvent condition and are summarized in Table III.

The chemical shift and line-width data of the $(GC)_{central}$ base pairs in d-ApTpGpCpApT in the absence and presence of $Mg^{2+}$ cannot be analyzed in terms of fast helix-coil equilibrium to evaluate $K_d$ at this base pair. It will be demonstrated later (Hilbers and Patel, 1975) that the formation rate of the $(GC)_{central}$ base pairs, $\tau_{0.2}^{-1} \approx 10^3$ sec$^{-1}$. This value may be compared with the 2.5-ppm chemical shift

[1] The derivation of these relationships are available on request to the authors.

Table I: Relationships between the Dissociation Constants, $K_d$, at Each Base Pair (Terminal, T, Internal, I, and Central, N) and the Observed Chemical Shifts ($\bar{\omega}$) at a Given Temperature and the Chemical Shifts in the Helical ($\omega_H$) and Coil ($\omega_C$) States.

| | |
|---|---|
| A–T A T<br>T–A T–A<br>G–C → G–C<br>C–G C–G<br>A–T A–T<br>T–A T A | $K_{dT} = \dfrac{\omega_{TH} - \bar{\omega}_T}{(\omega_{TH} - \omega_{TC}) - (\omega_{TH} - \bar{\omega}_T)}$ |
| A T A T<br>T–A T A<br>G–C → G–C<br>C–G C–G<br>A–T A T<br>T A T A | $K_{dI} = \dfrac{(\omega_{TC} - \omega_{TH})}{(\omega_{IC} - \omega_{IH})} \dfrac{(\bar{\omega}_I - \omega_{IH})}{(\bar{\omega}_T - \omega_{TH})}$ |
| A T A T<br>T A T A<br>G–C → G C<br>C–G C G<br>A T A T<br>T A T A | $K_{dN} = 2 \dfrac{(\omega_{IC} - \omega_{IH})}{(\omega_{NC} - \omega_{NH})^2} \dfrac{(\bar{\omega}_N - \omega_{NH})^2}{(\bar{\omega}_I - \omega_{IH})} [N_0]$ |

separation between the helical and coil states at the $(GC)_{central}$ base pairs, i.e., $\Delta\omega_N = 4.7 \times 10^3$ sec$^{-1}$. Thus, the fast exchange condition, $\tau_{0.2}^{-1} \gg \Delta\omega_N$, does not hold at the $(GC)_{central}$ base pairs. The relationship for dissociation of $(GC)_{central}$ base pairs derived in Table I is valid only under conditions of fast exchange.

B. EXCHANGE WITH WATER. A ring NH in the hydrogen-bonded or helix form (H) can be transferred to the non-hydrogen-bonded or coil form (C). From the latter state it can exchange with water (W). The exchange reaction may

$$H \underset{k_{CH}}{\overset{k_{HC}}{\rightleftharpoons}} C \xrightarrow{k_{CW}} W \qquad (1)$$

proceed by transfer to hydroxyl or buffer. Expressions have been derived describing the relationship between the line width, the line position, and the lifetime of the proton prior to exchange with water (Crothers et al., 1975).

The situation where fast exchange between helix and coil occurs several times before exchange of the ring N proton with water takes place is considered below. The observed line position, $\bar{\omega}$, is the weighted average of the chemical shifts of the helical and coil states. The excess line width is a measure of $f_C/\tau_{CW}$. Since the fraction of the coil form, $f_C$, for a particular base pair at a given temperature can be evaluated from the observed average chemical shift, the experimental line-width data can be used to determine $\tau_{CW}^{-1}$, the transition probability for the transfer of ring N proton from the coil form to water (Table IV).

## Discussion

*I. Fraying.* Since the chemical shifts and line widths of the individual base pairs change in a sequential order for d-ApTpGpCpApT in the absence (Figure 2A) and presence (Figure 2B) of $Mg^{2+}$ with increasing temperature, one can rule out an "all or none" model to interpret the melting behavior of short double helical oligonucleic acid sequences.

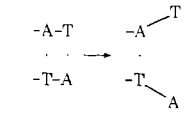
The above NMR data suggest that for short oligonucleotide sequences there is a finite probability that the terminal base pairs are non-hydrogen bonded in the Watson–Crick double helix. This process, termed *fraying*, occurs down to and below −30° for double-stranded d-ApTpGpCpApT since the chemical shift of the $(AT)_{terminal}$ base pair continues to shift downfield as the temperature is lowered from 0 to −30° in $H_2O$–MeOH (3:2) (Figure 3).

*II. Thermodynamic Parameters for Dissociation of AT Base Pairs.* Table V summarizes the available literature data on the formation thermodynamic parameters for addition of a base pair to a preexisting double-stranded helix in the ribo series (Borer et al., 1974). The $\Delta H$ value of 6.5 kcal (Table V) for AU,UA stacking in the ribo series compares favorably with the 6.1 kcal (Table III) observed for breakage of the terminal base pairs in d-ApTpGpCpApT at low ionic strength and hence suggests that the two terminal base pairs open independently of each other. The $\Delta H$ value of 11.8 kcal (Table III) for breakage of the internal base pairs in d-ApTpGpCpApT at low ionic strength is twice the corresponding value of 5.9 kcal (Table V) for CG,AU

Table II: Chemical Shifts of the Ring NH Resonances in the Helix and Coil States for the $(AT)_{terminal}$, $(TA)_{internal}$, and $(GC)_{central}$ Base Pairs.

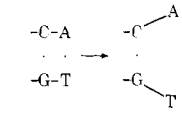
| | Chemical Shifts (ppm) |
|---|---|
| Helical state | |
| $(AT)_{terminal}$ $\omega_{TH}$ | 13.9 |
| $(TA)_{internal}$ $\omega_{IH}$ | 13.85 |
| $(GC)_{central}$ $\omega_{NH}$ | 12.73 |
| Coil state | |
| $(AT)_{terminal}$ $\omega_{TC}$ | 10.7 |
| $(TA)_{internal}$ $\omega_{IC}$ | 10.65 |
| $(GC)_{central}$ $\omega_{NC}$ | 10.23 |

Table III: Dissociation Parameters (Enthalpies, Entropies, and $T_m$ Values) for d-ApTpGpCpApT in Aqueous Solution in the Absence and Presence of 0.025 *M* $Mg^{2+}$ and 0.1 *M* Phosphate.




Terminal base pair

| | $\Delta H$ (kcal) | $\Delta S$ (eu) | $\Delta G^{25}$ (kcal) | $T_m$ (°C) |
|---|---|---|---|---|
| Low ionic strength[a] | +6.1 | +19.7 | +0.2 | 34.5 |
| $Mg^{2+}$ [b] | +4.2 | +12.7 | +0.4 | 54 |
| High ionic strength[c] | +4.7 | +15.2 | +0.2 | 40 |




internal base pair

| | $\Delta H$ (kcal) | $\Delta S$ (eu) | $\Delta G^{25}$ (kcal) | $T_m$ (°C) |
|---|---|---|---|---|
| Low ionic strength[a] | +11.8 | +3.7 | +0.7 | 44.5 |
| $Mg^{2+}$ [b] | +4.7 | +13.7 | +0.6 | 70 |
| High ionic strength[c] | +5.1 | +14.7 | +0.7 | ~75 |

[a] No added salt, $H_2O$, pH 7. [b] 0.1 *M* NaCl, 0.025 *M* $Mg^{2+}$, $H_2O$, pH 7.34. [c] 0.3 *M* NaCl, 0.1 *M* phosphate, $H_2O$, pH 7.

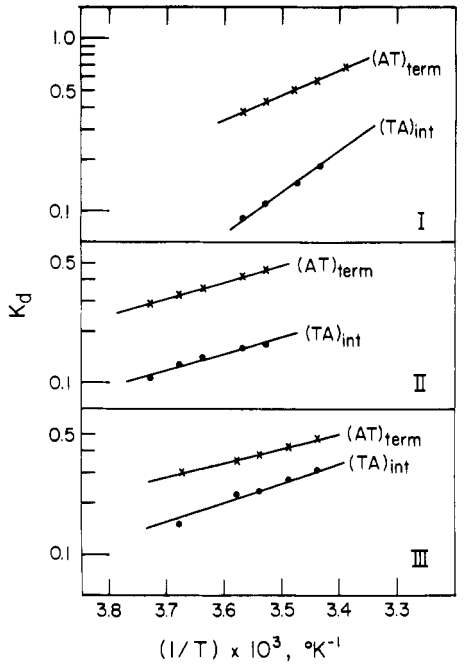



FIGURE 4: Plots of dissociation constants $K_d$ at the terminal and internal base pairs in d-ApTpGpCpApT against $(1/T)$ as a function of solvent. (I) $H_2O$, low ionic strength, pH 7; (II) 0.3 *M* NaCl–0.1 *M* phosphate–$H_2O$ (pH 7); (III) 0.1 *M* NaCl–0.025 *M* $Mg^{2+}$–$H_2O$ (pH 7.34).

stacking in the ribo series and hence suggests that the two internal base pairs in d-ApTpGpCpApT open in a coupled manner.

In the presence of divalent ions (0.1 *M* NaCl–0.025 *M* $Mg^{2+}$) or high salt (0.3 *M* NaCl–0.1 *M* phosphate), the $\Delta H$ values for dissociation of the internal base pairs are 4.7 and 5.1 kcal, respectively (Table III). The data suggest that the opening of the internal base pairs in d-ApTpGpCpApT is coupled at low ionic strength, but the coupling disappears in the presence of Mg or high salt. The uncoupling in the presence of $Mg^{2+}$ ions which complex the phosphates suggests a mechanism involving the oligonucleotide sugar–phosphate backbone. The effect of high salt is not currently understood.

The thermodynamic data are further support for a model which incorporates fraying at the ends of the helix since the $T_m$ of the terminal base pair is lower than that of the internal base pair at low ionic strength, in the presence of $Mg^{2+}$ and at high ionic strength (Table III). The $T_m$ values at each base pair as a function of solvent suggests that $Mg^{2+}$ and high salt stabilize the double helix against conversion to the coil form (Table III).

*III. Base Catalysis.* The pH dependent chemical shift and line-width data in Figure 1 support the model for exchange which incorporates intermittent opening of the double helix.

At 3°, the fraction of coil at the terminal, internal, and central residues in double-stranded d-ApTpGpCpApT (based on the observed chemical shifts) are 0.225, 0.011, and 0.0, respectively. Thus, even though the helical state predominates (99%) at the internal base pairs at 3°, transient opening of the helix broadens out the internal ring NH resonance in the pH range 8–9 due to base catalysis.

An analysis of the base-catalyzed exchange data yields $k_{OH^-} = 0.55 \pm 0.2 \times 10^{10}$ l. mol$^{-1}$ sec$^{-1}$ for exchange of the ring N proton of the internal and terminal thymines

Table IV: Rate Constants $k_{OH^-}$ as a Function of pH in $H_2O$ (Low Ionic Strength) at 3°.

| pH | Excess Width (Hz) | $(\tau_{CW})^{-1}$ | $k_{CW}$ (l. mol$^{-1}$ sec$^{-1}$) |
|---|---|---|---|
| $(AT)_{terminal}$ | $f_{coil}^{3°} = 0.225$ | | |
| 7.6 | 22 | 307 | $0.58 \times 10^{10}$ |
| 7.8 | 27 | 377 | $0.38 \times 10^{10}$ |
| 8.0 | 34 | 475 | $0.3 \times 10^{10}$ |
| 8.25 | 62 | 865 | $0.3 \times 10^{10}$ |
| $(TA)_{internal}$ | $f_{coil}^{3°} = 0.011$ | | |
| 8.5 | 14 | 4000 | $0.89 \times 10^{10}$ |
| 8.8 | 26 | 7420 | $0.74 \times 10^{10}$ |
| 9.2 | 52 | 14850 | $0.65 \times 10^{10}$ |

Table V: Formation Thermodynamic Parameters for Addition of a Base Pair to a Preexisting Ribonucleotide Double Helix (Borer et al., 1974).

| | $\Delta H$ (kcal) | $\Delta S$ (eu) | $\Delta G$ (kcal) |
|---|---|---|---|
| -A U -U A → -A-U · · -U-A | −6.5 | −16.4 | −1.6 |
| -C A -G U → -C-A · · -G-U | −5.9 | −12.7 | −2.1 |
| -G C -C G → -G-C · · -C-G | −14.7 | −34.9 | −4.3 |

from the coil form with water at 3°. The exchange is therefore diffusion limited and compares favorably with rate constants of $3 \times 10^{10}$ l. mol$^{-1}$ sec$^{-1}$ for exchange of the ring N proton of purine at 24° (Marshall and Grunwald, 1961) and $1 \times 10^{10}$ l. mol$^{-1}$ sec$^{-1}$ for exchange of the ring N proton of uracil at 25° (Eigen, 1964).

The $k_{OH^-}$ for exchange of the ring N proton from the coil form with water appears to be faster for the internal base pairs compared to the terminal base pairs (Table IV). The backbone phosphates may contribute to this exchange process. Since d-ApTpGpCpApT lacks terminal phosphates, the contribution from catalysis by the backbone phosphates will be greater at the internal than the terminal base pair.

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# Proton Nuclear Magnetic Resonance Investigations of the Nucleation and Propagation Reactions Associated with the Helix–Coil Transition of d-ApTpGpCpApT in $H_2O$ Solution†

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ABSTRACT: The chemical shifts and line widths of the Watson–Crick ring NH resonances of the self-complementary duplex of d-ApTpGpCpApT have been monitored in the presence of 0.1 *M* phosphate at neutral pH in aqueous solution. While the resonance positions of the terminal and internal AT base pairs shift upfield and broaden as average resonances with increasing temperature (helix and coil exchange several times prior to exchange with water from the coil form), those of the central GC base pairs broaden in the absence of upfield shifts (exchange with water occurs each time helix converts to coil). The line-width changes at the AT base pairs monitor the lifetime of the coil state at these positions prior to exchange with water while the line-width changes at the GC base pairs monitor the lifetime of the helix prior to dissociation to strands. This permits the separation of the propagation reaction at the AT base pairs from the nucleation reaction at the GC base pairs during helix formation. The experimental data have been quantitatively analyzed to yield (at 20°) a nucleation formation rate of $\sim 10^3$ $sec^{-1}$ for the GC base pairs (bimolecular rate constant of $\sim 6 \times 10^6$ l. $mol^{-1}$ $sec^{-1}$) and a dissociation rate of $6 \times 10^2$ $sec^{-1}$ at these same base pairs (unimolecular dissociation to strands). The unimolecular propagation reactions at the internal and terminal base pairs are associated with reaction rates $\gg 10^4$ $sec^{-1}$. These values are consistent with a slow formation of a stable nucleus at the GC base pairs followed by a rapid propagation reaction at the AT base pairs. The line width of the $(GC)_{central}$ base pairs in the presence of phosphate is a direct measure of the lifetime of the total helix and yields an activation energy of 45 kcal for helix to coil conversion measured over a narrow temperature range. The exchange from the coil form with water is catalyzed by 0.1 *M* phosphate with a rate constant $k_{HPO_4^{2-}} = 0.2 \times 10^6$ l. $mol^{-1}$ $sec^{-1}$.

Equilibrium and temperature jump optical experiments on short, well-defined RNA and DNA helices have recently provided considerable insight into the stability of nucleic acid double helices and into the mechanism of helix formation (for review see Riesner and Römer, 1973; Bloomfield et al., 1974). Such data have been analyzed in terms of a model which describes the generation of a nucleic acid double helix from its component single strands by a concentration dependent formation of the first few base pair(s) (the nucleus) followed by a fast formation of the subsequent base pairs (propagation) (Pörschke and Eigen, 1971; Craig et al., 1971).

Nuclear magnetic resonance is capable under conditions of high spectral resolution of unravelling many of the finer details of the dynamics and mechanisms involved in the helix–coil transition of nucleic acids. In the preceding papers of this series, proton nuclear magnetic resonance (NMR) spectroscopy was utilized to demonstrate that the double helix formed by the hexanucleotide d-ApTpGpCpApT opens in a sequential manner from its ends (Patel, 1974), and the thermodynamic parameters associated with the fraying of the individual base pairs were determined and interpreted (Patel and Hilbers, 1975). These studies are

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