Conformation and Interaction of Short Nucleic Acid Double-Stranded Helices. II. Proton Magnetic Resonance Studies on the Hydrogen-Bonded NH–N Protons of Ribosyl ApApGpCpUpU Helix†

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ABSTRACT: A self-complementary ribohexanucleotide, ApApGpCpUpU, was synthesized and its NH–N hydrogen-bonded protons were studied by proton magnetic resonance. At 1°C, 0.17 *M* Na^+, pH 7.6 with 10 m*M* phosphate–0.1 m*M* EDTA in H_2O, three proton resonances are found in the low-field region with the following chemical shifts and line widths at half-height: 13.2 ppm (80 Hz), 13.5 ppm (30 Hz), and 14.2 ppm (44 Hz). The existence of these resonances indicates the formation of a self-complementary, hydrogen-bonded duplex under these conditions. Upon elevation of temperature, these three resonances sequentially broaden and finally all disappear near 35°C. Unambiguous assignments of these three resonances can be made to the terminal A(1)·U(6) pairs, interior A(2)·U(5) pairs, and to the middle G(3)·C(4) pairs. The assignments were based on (i) the differential sensitivities of the line widths of these resonances to thermal variation, as well as on (ii) a comparison of the computed chemical shifts with the observed chemical shifts. The quantitative aspects of the NH proton transfer between helix, coil, and water are discussed in relationship to the line widths of these resonances and the lifetime of the helix state. The computed chemical shifts of the NH–N resonances based on the A-RNA (or A′-RNA) model agree more closely with the observed chemical shifts than the computed values based on the B-DNA model. These results suggest that the helical duplex of A_2GCU_2 assumes a conformation similar to A-RNA (or A′-RNA) in aqueous solution. The results on both the NH–N resonances and the C–H resonances are summarized and discussed in terms of the helical conformation of $(A_2GCU_2)_2$.

The A·U or G·C base pair in the Watson–Crick scheme (Figure 1) involves one NH–N hydrogen-bonded proton from U or G in the pair. The nuclear magnetic resonance (NMR) of these base-paired protons was first investigated by Kearns, Shulman, and their coworkers in intact tRNAs in aqueous (H_2O) solution (Kearns et al., 1971; Wong et al., 1972; Kearns and Shulman, 1974). These resonances which appear at low temperature in the region between 11 and 15 ppm downfield from DSS[1] provide direct evidence of the formation of hydrogen-bonded base pairs; they disappear upon helix melting at elevated temperature.

As for short oligonucleotide helices, Crothers et al. (1973) reported the NMR properties of the NH–N proton resonances of the pentadeoxynucleotide duplex d(ApApCpApA)·d(TpTpGpTpT) (Cross and Crothers, 1971). More recently, while the preparation of this manuscript was in progress, Patel and Tonelli (1974) and Patel (1974) reported studies of the NH–N resonances of several deoxyoligonucleotide duplexes. They concluded that the observed chemical shifts of NH–N resonances of the nonterminal residues of these deoxyoligomer duplexes are in accord with the computed values based on ring current effects and on the B-DNA model. Heller et al. (1974) reported the presence of a broad signal at 13.6 ppm from DSS in H_2O upon formation of oligo(rA)·oligo(rU) helix of about 20–25 nucleotides in length. Most recently, Arter et al. (1974) observed two NH–N resonances (12.45 and 13.18 ppm) in their spectrum of a ribosyl tetranucleotide complex, r(CpCpGpG), at low temperature. They reported that these chemical shift values are consistent with predictions based on ring current effects expected for an A-RNA helix.

In this communication, we report a study on the NH–N resonances of the self-complementary ribosyl duplex, A_2GCU_2, and the quantitative aspects of the NH proton transfer between the helix–coil states and water in relationship to the line widths of the resonances and the lifetime of the helix. The results strongly support the conclusion reached in the preceding paper that the helical duplex of A_2GCU_2 assumes an A-RNA (or A′-RNA) conformation in aqueous solution.

Preliminary reports of this work have been presented previously (Kan et al., 1974, Ts'o et al., 1975).

Materials and Methods

The synthesis and sequence identification of A_2GCU_2 were described in the preceding paper (Borer et al., 1975); 4 μmol (240 A_{260} units) of the hexanucleotide, $Na_5A_2GCU_2$, was dissolved in distilled water which was first deionized by passing through columns of IRA-400 anion and IR-20 cation mixed bed resin (Rohm and Hass, Co.) in a system established by Hydro Service and Supplies, Inc., Durham, N.C. The water was also filtered twice through a 0.2-μ Millipore filter. Concentrated NaCl and sodium phosphate (pH 7.6) solutions were added, and the sample was lyophilized and dissolved in 0.4 ml of H_2O. The final concentrations

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[1] Abbreviation used is: DSS, 2,2-dimethyl-2-silapentane-5-sulfonate.

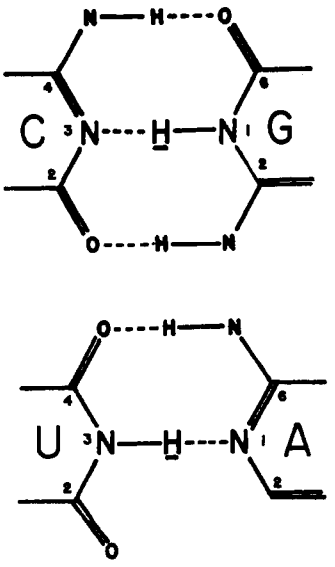

FIGURE 1: A schematic presentation of hydrogen bonding in the Watson-Crick base pairs of A·U and G·C.

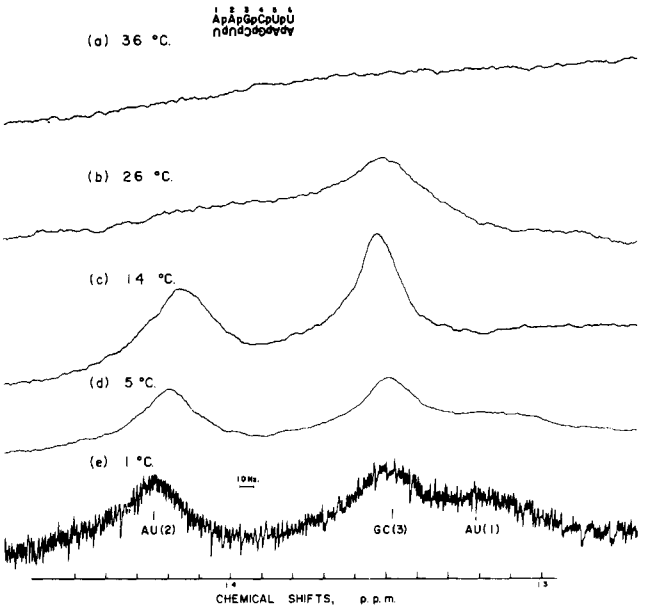

FIGURE 2: The 220-MHz ^1H NMR spectra of the hydrogen-bonded NH-N protons of the A_2GCU_2 duplex at various temperatures in H_2O solution containing 0.1 *M* NaCl-0.01 *M* sodium phosphate (pH 7.6, total sodium ion concentration = 0.17 *M*).

were 0.10 *M* Cl^-, 0.01 *M* phosphate, 0.17 *M* Na^+, and 10^{-4} *M* EDTA.

The ^1H NMR spectra were recorded on a Varian HR-220 spectrometer operating in the frequency mode. Probe temperatures were regulated by a Varian 4257 variable temperature accessory and monitored by observing the splitting in methanol and ethylene glycol. In order to enhance the signal-to-noise ratio, a continuous wave spectral averaging process was carried out by a Varian 620i computer. At least 100 scans were taken for each spectrum. A trace amount of *t*-butyl alcohol was added to the sample for an internal standard. However, in this paper all the chemical shift data were converted to DSS as reported in the preceding paper (Borer et al., 1975).

Results

Figure 2 displays the low-field proton resonances of the A_2GCU_2 duplex in H_2O. At 1°C, three resonances are seen at 13.2, 13.5, and 14.2 ppm downfield from DSS and are assigned to the NH resonances in NH-N bonds (Kearns and Shulman, 1974). These resonances are all designated in the figures with their proper assignments which will be verified in a later section. Because of the symmetry in the A_2GCU_2 self-complementary duplex, the six hydrogen-bonded NH resonances are represented by three sets of signals; each set consists of two identical NH resonances. The resonance at 13.2 ppm is noticeably broader than the two others. As the temperature is raised, these three signals progressively broaden, and finally disappear in the following order: first the signal at 13.2 ppm, then the signal at 14.2 ppm, and finally the signal at 13.5 ppm. This phenomenon is illustrated more quantitatively in Figure 3, in which the line width at half-height ($\omega_{1/2}$) of each resonance is plotted against the temperature. The line widths of all these resonances increase with temperature elevation, reaching a value of about 140 Hz where the signals become too broad to be measured. Upon lowering the temperature, the line widths of these signals decrease, the line width of the NH resonance from U(5) (A(2)·U(5) pairs) clearly reaching a plateau value of ~45 Hz at 10°. The line width of the NH resonance from G(3) (G(3)·C(4) pairs) may also reach a plateau value of ~30 Hz at 20°; at temperatures below 15°,

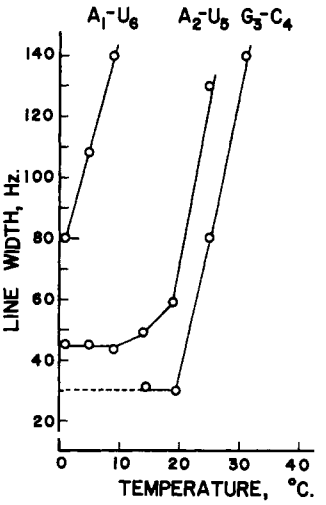

FIGURE 3: The plot of the line width at half-height of the NH-N resonances of the A_2GCU_2 duplex vs. temperature.

however, the NH from U(6) resonates at about the same frequency and interferes with line width measurement of the NH resonance from G(3) (see Figures 2 and 4). The line width of the NH resonances from U(6) (A(1)·U(6) pairs) is about 80 Hz at 1°C and clearly has not yet reached a plateau value. The data in Figure 2 and the plot in Figure 3 show that upon temperature elevation starting from 0°, $\omega_{1/2}$ of the NH resonance from U(6) increases immediately and disappears at ~10°; then the NH resonance from U(5) begins to broaden and disappears at ~25°C; finally, the NH signal from G(3) begins to broaden at ~20°C and disappears at ~30°C.

The temperature dependence of the chemical shifts of these three NH resonances is shown in Figure 4. The rate of change is greatest for the NH from U(6), less for the NH from U(5), and the least for the NH from G(3). Except possibly for the NH from G(3), the NH resonances from U(5) and from U(6) do not reach their limiting values of

Table I: The Chemical Shifts (in ppm) and the Line Width at Half-Height (in Hz) of the Hydrogen-Bonded NH–N Resonances of Guanine and Uracil in $(A_2GCU_2)_2$ at 1°C.[a]

	Obsd	δcalcd[b] (Kearns and Shulman)	δcalcd[c] (A′-RNA)	δcalcd[d] (B-DNA)	δobsd − δcalcd		Line Width ($\omega_{1/2}$)
					A′-RNA	B-DNA	
G(3)NH	13.5[e]	13.2	13.3	12.8	0.2	0.7	30
U(5)NH	14.2	14.0	14.1	14.1	0.1	0.0	44
U(6)NH	13.2	13.4	13.5	14.3	−0.3	−1.1	80

[a] The intrinsic values of the chemical shifts of the NH resonance in the A·U pair and in the G·C pair are taken to be 14.7 and 13.6 ppm, respectively (Kearns and Shulman, 1973), with the A·U pair in the B-DNA geometry given the same value, 14.6 ppm, as derived for the A·T pair (Patel and Tonelli, 1974). [b] Calculated values based on shielding effects recommended by Kearns and Shulman (1973). [c] Calculated values based on the geometry of the A′-RNA helix (Borer et al., 1975). [d] Calculated values based on the geometry of the B-DNA helix (Borer et al., 1975). [e] All negative signs are omitted.

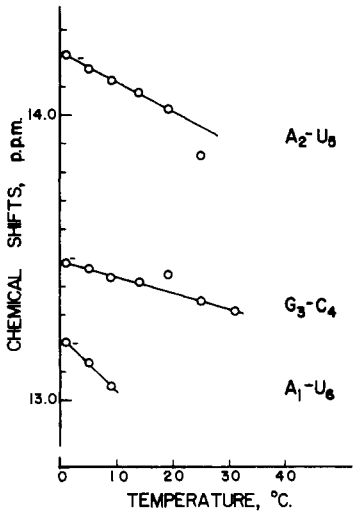

FIGURE 4: The plot of the chemical shifts of the NH–N resonances vs. temperature.

chemical shifts at 0°C under the present experimental conditions.

Discussion

Chemical Shifts of the NH Resonances. Definitive assignments of these NH resonances cannot be made solely on the basis of their chemical shifts. This is because the chemical shifts of the two resonances at 13.2 and 13.5 ppm are too close to be distinguished from each other by comparison with the computed values to be discussed below. The assignments shown in Table I come from a consideration of resonance line widths and of temperature effects in chemical shifts. This assignment will be confirmed in a later section when the line-broadening mechanism is considered.

The computation of the chemical shifts of the NH resonances is based on the knowledge and consideration of three factors: (i) the intrinsic spectral position of the NH–N resonance from G in an isolated G·C pair and from U in an A·U pair; (ii) the geometry of the helical duplex; (iii) the distance dependence of the shielding effects due to the neighboring bases. Currently, there are no experimental data to provide precise values for the intrinsic chemical shifts of the two NH–N resonances in base pairs in aqueous solution. The NH–N resonance from the G·C pair has been observed in Me_2SO and demethylformamide (Shoup et al., 1966; Newmark and Cantor, 1968; Katz and Penman, 1966) and the NH–N resonance from the A·U pair has been observed in $CHCl_3$ (Katz and Penman, 1966; Katz, 1969). Although the changes upon association were shown to be large and their directions established, these studies were not useful for quantitative estimation of the intrinsic NH–N chemical shifts. In the absence of an accurate experimental value for the intrinsic chemical shifts of the NH–N resonances in H_2O, various investigators adopted values based on self-consistent arguments which adequately described the ^1H NMR of tRNA fragments and other double-stranded oligonucleotides. In a series of publications, Kearns, Shulman, and coworkers used values from −14.5 to −14.8 ppm for the intrinsic U–NH resonance in an A·U pair and −13.6 to −13.7 ppm for the G–NH in a G·C pair (Lightfoot et al., 1973; Kearns and Shulman, 1974; Wong and Kearns, 1974). Based on the spectra of several deoxyoligonucleotides, Patel and Tonelli (1974) adopted values of −13.6 ± 0.1 ppm for the G·C pair and of −14.6 ± 0.2 ppm for the A·T pair. In Table I, the value of −14.7 ppm for the A·U pair and the value of −13.6 ppm for the G·C pair (Kearns and Shulman, 1974) were tentatively adopted for the present calculations based on RNA geometry. As for the geometry of the short helix, a Kendrew model of A_2GCU_2 has been built in the A′-RNA geometry according to the coordinates of Arnott et al. (1972) and part of the A_2GCU_2 helix has also been built in the B-DNA geometry (Arnott and Hukins, 1972). These models and the procedures for calculating the shielding effects on the NH–N resonances from nearest and next-nearest neighboring bases in the helix were described in the preceding paper (Borer et al., 1975). The positions of the NH–N protons were found to be typically 3.2 Å from the neighboring bases along a normal to the base plane. This deviation from the standard 3.4-Å distance arises from the tilt and twist of the bases in the A′-RNA geometry.

In Table I, the measured chemical shifts of the three NH–N resonances are compared with three sets of computed values. The first set came from the table provided by Kearns and Shulman (1974). Their table was constructed from the A′-RNA geometry and the 3.4-Å isoshielding contours computed by Giessner-Prettre and Pullman (1970). They increased the predicted shielding values by 20% to account for the ^1H NMR spectra observed for tRNA fragments and whole molecules. Although they did not attempt to rationalize the source of this increase in shielding, the contours constructed at 3.2-Å distance predict shielding values about 20% larger than those at 3.4-Å distance. The other two sets are computed by us based on the A′-RNA model and the B-DNA model described above and in the preceding paper. The two calculations based on the A′-RNA model agree very well, as expected. Two conclusions

Table II: The Ring Current Shielding Effects (in ppm) from the Neighboring Bases on the Hydrogen-Bonded NH Resonances (NH–N) in the Short Helix A_2GCU_2.[a]

	A(1)	A(2)	G(3)	C(4)	U(5)	U(6)	Total
			A′-RNA Model[b]				
G(3)·C(4)			0.02	0.19	0.11 (0.01)	(0.01)	0.34
A(2)·U(5)			0.46	(0.04)		0.08	0.58
A(1)·U(6)		1.15	(0.09)[c]				1.24
			B-DNA Model[b]				
G(3)·C(4)	(0.10)	0.35 (0.13)	0.15	0.05	0.03		0.81
A(2)·U(5)	0.12		0.12 (0.06)	0.07 (0.02)		0.12	0.51
A(1)·U(6)		0.22	(0.08)		0.04		0.34

[a] The ring current shielding contours of the four bases are based on Giessner-Prettre and Pullman (1970). [b] Borer et al., (1975). [c] The numbers in parentheses are the values derived from the shielding effects due to the next-nearest neighboring bases.

can be derived from the comparison of the values in Table I. (i) The chemical shift of the NH–N resonance at −14.2 ppm is sufficiently different from the other two, that this resonance can be unambiguously assigned to the U(5) residue in the A(2)·U(5) pairs. On the other hand, the resonances at −13.5 and at −13.2 ppm can*not* be assigned from the calculated values. In fact, the calculated values suggest that the resonance at −13.5 ppm should be assigned to U(6) in the A(1)·U(6) pairs and the resonance at −13.2 ppm to G(3) in the G(3)·C(4) pairs, contrary to the assignments cited in Table I. This problem will be further discussed in the succeeding section. (ii) The ring current shielding effects based on the A′-RNA geometry differ substantially from those based on the B-DNA geometry. In Table II, the shielding effects received by each NH–N proton in the helix are compared for both models. These computed values, especially those for the A(1)·U(6) pairs and the G(3)·C(4) pairs, clearly indicate that the chemical shifts of NH–N resonances in the helix can provide valuable information on the geometry of the helix. The values in Table I show that the δ values computed on the basis of A′-RNA geometry agree much better with the observed δ values than those computed on the basis of B-DNA geometry. This result is in accord with that from the ^{1}H NMR study on the C–H protons reported in the preceding paper (Borer et al., 1975), i.e., the base-stacking arrangement in the A_2GCU_2 duplex appears to be much closer to the geometry of the A′-RNA than the B-DNA model.

Line Widths of the Hydrogen-Bonded NH–N Resonances. The line widths of the NH–N resonances of the helical duplex are related to the rates of the following exchange reactions (Crothers et al., 1974)

$$NH_{helix} \underset{k_{ch}}{\overset{k_{hc}}{\rightleftarrows}} NH_{coil} \quad (1)$$

$$NH_{coil} \underset{k_{wc}}{\overset{k_{cw}}{\rightleftarrows}} H_2O \quad (2)$$

The Bloch equations relating the properties of the NMR signals to the various exchange rates for the helix–coil transition in water have been presented by Crothers et al. (1974). From the work of Pörschke et al. (1973) on A_2GCU_2 and the work of Ravetch et al. (1974) on $A_{3-4}GCU_{3-4}$, the rate constants k_{hc} and k_{ch} of A_2GCU_2 in 0.01 *M* phosphate–0.17 *M* Na^+ (pH 7.0) from 0 to 15°C can be estimated to be approximately 30 ± 20 sec^{-1} and (3 ± 2) × 10^6 M^{-1} sec^{-1}, respectively. The overall rate of duplex formation, $k_{ch}C_s$, in the present experiment with total strand concentration, C_s = 0.01 *M*, would be (3 ± 2) × 10^4 sec^{-1}. The average lifetime, $\tau = \tau_{hc}\tau_{ch}/(\tau_{hc} + \tau_{ch})$, is dominated by τ_{ch}, thus both are ~3 × 10^{-5} sec. For exchange process 1, the ^{1}H NMR frequency separation of the NH_{helix} resonances is $\Delta\omega = 2\pi(\nu_h - \nu_c)$. ν_c cannot be obtained at neutral pH in H_2O because of the very rapid exchange rate. However, the exchange is greatly suppressed at pH 4.1 where the U–NH resonates 11.3 ppm downfield from DSS (we have confirmed this private communication of Dr. S. W. Englander). The value of $\Delta\omega$ = ~4.7 × 10^3 sec^{-1} can then be estimated using δ = 14.7 ppm for ν_h. Thus, $1/\tau$ (~3 × 10^4 sec^{-1}) is significantly larger than $\Delta\omega$ (~4.7 × 10^3 sec^{-1}), therefore process 1, i.e., helix ⇌ coil, is fast in terms of the NMR time scale. The major question then is about the rate in process 2; is this exchange rate (τ_{cw}^{-1}) between the NH in the coil state and H in water sufficiently fast to permit a simple interpretation of the line width? As shown by Crothers et al. (1974), when τ_{cw}^{-1} is much larger than $1/\tau$, the lifetime of the helix determines the line broadening, i.e., $1/T_2 = 1/T_{2h} + 1/\tau_{hc}$, and the spectral position is given by the resonance of the helix, ω_h. In this situation, the NH exchanges virtually every time the helical duplex dissociates. Currently, there are no experimental data on the exchange rate of G–N_1H and U–N_3H with water in aqueous solution. An estimation of the overall rate can be made by the following equation (Englander et al., 1972):

$$\tau_{cw}^{-1}(A) = k_d \frac{10^{pK_A - pK_D}}{1 + 10^{pK_A - pK_D}} C_A$$

where $\tau_{cw}^{-1}(A)$ is the exchange rate with the acceptor A, pK_A refers to the pK of the acceptor, pK_D to the pK of the donor (uracil or guanine), C_A is the concentration of the acceptor, and k_d is the predicted collision rate constant which has a value of ~10^{10} M^{-1} sec^{-1} for small molecules in an acid–base reaction. At 1°C, 0.01 *M* phosphate concentration, the pK of HPO_4^{2-} is taken as 7.2 and the pK values of uracil and guanine residues are estimated to be 9.5 (Litan, 1966). Thus, with OH^- as the acceptor in water at pH 7.6 and 1°C (C_{OH^-} is 5 × 10^{-7} *M*), the τ_{cw}^{-1} is estimated to be 5 × 10^3 sec^{-1}; with HPO_4^{2-} as the acceptor at 0.01 *M* concentration (pH 7.6), the τ_{cw}^{-1} (HPO_4^{2-}) is estimated to be about 4 × 10^5 sec^{-1}; the overall τ_{cw}^{-1} including all other possible acceptors (such as EDTA, etc.) is estimated still to be ~4 × 10^5 sec^{-1}. Thus, τ_{cw}^{-1} (~4 × 10^5 sec^{-1}) is indeed larger than τ^{-1} (~3 × 10^4 sec^{-1}), or $\tau_{cw}^{-1}\tau \simeq 12$; however,

since the computation of both τ_{cw}^{-1} and τ^{-1} include a fair amount of assumption and extrapolation, the conclusion that $\tau\tau_{cw}^{-1} \gg 1$ must be regarded as tentative. Nevertheless, we shall proceed with this conclusion that the line widths of the NH resonances in the current experiment are primarily determined by the lifetime of the helix. This calculation also clearly indicates the phosphate ions are the predominant acceptor in the proton transfer process and the rate of exchange can be increased by addition of more phosphate ions to the solution.

The above conclusions readily lead to the assignment of the NH resonances based on the line-width data presented in Figure 3. The NH resonance which has the largest line width and the highest sensitivity to thermal effects on the line broadening and line shifting is assigned to the NH-N of the two terminal A(1)·U(6) pairs; the NH resonance which has the smallest line width and the lowest sensitivity to thermal effects is assigned to the NH-N of the two middle G(3)·C(4) pairs; and the NH resonance which has the intermediate line width and sensitivity is therefore assigned to the two interior A(2)·U(5) pairs; the last assignment is supported by the chemical shift computations shown in Table I.

It is believed that the ends of the short helices, such as the A_2GCU_2 helix, may exhibit a certain degree of "fraying". This phenomenon of fraying has been used to explain the very fast relaxation times ($\tau_f < 10^{-6}$ sec) observed in temperature-jump studies of oligonucleotides (e.g., Gralla and Crothers, 1973). This means that the relaxation process of the NH-N resonances of the two terminal A·U pairs may have a situation of $\tau_f\tau_{cw}^{-1} \ll 1$, a situation in which the ends open (coil) and close (helix) many times before the exchange of protons between NH and acceptors occurs. In this situation, Crothers et al. (1974) predicted that the line width is determined by the expression $1/T_2 = 1/T_{2h} + f_c/\tau_{cw}$, where $(1/T_{2h})(1/\pi)$ is the line width in the helix and f_c is the fraction of unpaired bases; and the resonance line position, $\bar{\omega}$, is determined by the expression $\bar{\omega} = (1 - f_c)\omega_h + f_c\omega_c$ where ω_c and ω_h are the resonance frequencies of the coil (unpaired) form and helix (paired) form, respectively. In this case, the resonances are expected to shift upfield, more toward their position in the coil form as the temperature is increased. Also, f_c increases with temperature so this fraying process should further broaden the lines. However, the value of τ_{cw}^{-1} has been estimated above to be 4×10^5 sec^{-1}; thus the $(f_c/\tau_{cw})(1/\pi)$ value should be ~70 Hz as defined by the observed line width of about 80 Hz (Table I) together with an estimation of $(1/T_{2h})(1/\pi) \simeq 10$ Hz. This implies that f_c would be less than 0.06%, an unreasonably small number in this consideration. Furthermore, f_c has to be about 5–10% before the fraying process can be detected by uv absorption measurement in the T-jump experiments. An f_c value of 5% would imply a line width of 7×10^3 Hz, which is unmeasurable in ^1H NMR studies. Either the fraying process is much less in magnitude than expected, or this dilemma suggests that τ_{cw}^{-1} value calculated for the exchange of the NH of the coil form to the acceptors is not suited for the calculation for the fraying process. The τ_{cw}^{-1} for the NH exchange in the fraying process may be much less (possibly 10^2-fold less) than the τ_{cw}^{-1} in the helix $\rightleftharpoons$ coil transition. This situation can be rationalized because of the high frequency of the opening and closing process in fraying and the relative inaccessibility of acceptors to the frayed ends.

In spite of the above discussion concerning the complexities of the exchange–relaxation phenomenon, if we assume that most of the line width of these NH resonances at 1°C is due to the lifetime of the base pair in the helix, i.e., $\tau_{hc} \simeq \pi\omega_{1/2}^{-1}$, then τ_{hc} for A(1)·U(6), A(2)·U(5), and G(3)·C(4) can be computed to be 4.1, 7.2, and 10.6 msec, respectively. These values are similar to the τ_{hc} of the entire helix (~30 msec) evaluated from the T-jump method. The lifetime of the entire helix should be slightly longer than the lifetimes of the individual base pairs, especially these located at the termini.

Temperature Effect on the Chemical Shifts. There are two plausible interpretations for the temperature effects on chemical shifts shown in Figure 4. The choice between these two interpretations depends on the choice of τ_{cw}^{-1} for these NH protons in the helix → coil transition of the short helix.

One explanation would be that the τ_{cw}^{-1} is sufficiently fast for the NH protons of all three sets of base pairs (i.e., $\tau_{cw}^{-1}\tau \gg 1$), that their spectral positions are given by the resonance of the helix, ω_h, as described in the above section. This explanation would suggest that the change of chemical shifts upon varying the temperature would be due to a change in conformation within the helix state. Thus, an accurate description of the system might require a series of ω_h values corresponding to various distinct helix microstates. This is particularly plausible for the end pairs which may assume slightly different geometry at varying temperature. This explanation is supported by the observation that the line width of A(2)·U(5) pairs is constant (Figure 3) over the temperature region in which the chemical shift value of A(2)·U(5) pairs is still sensitive to temperature. The differences in the temperature dependence of these three sets of base pairs would be assumed to originate from the different degrees of freedom in conformation allowed in the helix for these base pairs. Under this condition, the line widths of these pairs are related to their values of f_c/τ_{cw}, the broadest lines for A(1)·U(6) and the narrowest for G(3)·C(4). This variation would also support the current assignment of the resonances, since the f_c value (fraction of coil form) would be the largest for the end pair, A(1)·U(6), and the smallest for the middle pair, G(3)·C(4).

Another explanation assumes that the differences in temperature effect on chemical shifts are due to differences in the rates, τ_{cw}^{-1}, between these three sets of base pairs. This explanation suggests that the τ_{cw}^{-1} of the A(1)·U(6) pair and τ_{cw}^{-1} of the A(2)·U(5) pair are sufficiently slow that the spectral positions of these protons are influenced by the quantity $(1 - f_c)\omega_h + f_c\omega_c$, as discussed in the preceding section. The temperature effect would originate from the change of (f_c) and $(1 - f_c)$ as influenced by the temperature. This explanation requires that either τ_{cw}^{-1} or τ^{-1}, or both, are different for each of three sets of base pairs since the magnitude of each temperature effect on chemical shift is different.

The Conformation of the A_2GCU_2 Helix in Solution. In summarizing all the ^1H NMR data presented in the preceding paper and this communication, conformational details of the A_2GCU_2 helix in solution can now be ascertained on the basis of the following self-consistent information. (i) The $J_{1'-2'}$ values of all the residues in the helix indicate that each furanose is in a 3′-endo conformation. Model building based on the X-ray diffraction data of nucleic acid fibers reveals that the furanose conformation in the A′-RNA (or A-RNA) is 3′-endo, while the furanose in B-DNA is 3′-exo or 2′-endo. (ii) The chemical shifts of 17 C–H resonances of the A_2GCU_2 helix in solution agree with the computed

values based on the geometry of the A′-RNA much better than those based on the geometry of B-DNA. (iii) The chemical shifts of three sets of NH–N resonances representing six base pairs of the A_2GCU_2 helix also agree with the computed values based on the geometry of A′-RNA significantly better than those based on the geometry of B-DNA. Thus, these data strongly support the conclusion that the A_2GCU_2 helix in solution must assume a conformation closer to that of the A′-RNA than to that of B-DNA. It would be of greater interest to synthesize a short DNA helix containing the A_2GCU_2 sequence and to study the conformation of this short DNA helix in solution by ^1H NMR following the approach outlined in these two papers. Such a project is currently in progress in our laboratory.

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