Communications to the Editor

^{15}N NMR of a Specifically Labeled RNA Fragment Containing Intrahelical GU Wobble Pairs

Xiaohu Zhang, Barbara L. Gaffney, and Roger A. Jones*

Department of Chemistry
Rutgers, The State University of New Jersey
Piscataway, New Jersey 08855

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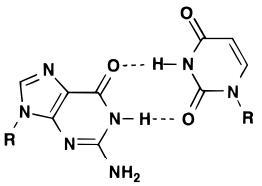
The guanine-uracil (GU) base pair is a phylogenetically highly conserved motif in many forms of RNA, including tRNA,[1] rRNA,[2−4] ribozymes,[5,6] spliceosomes,[7,8] and signal recognition particles.[9] The GU "wobble" geometry first proposed by Crick[10]



was later found in the X-ray structures of yeast tRNA^{phe}, in which it was found to cause only a slight disruption of the helical regularity of the acceptor stem.[11,12] Later work showed that there was in addition a pronounced change in the stacking pattern for the GU pair on adjacent bases, with the guanine overlapping extensively with an adenine to the 3′ side, but not with a guanine to the 5′ side.[13]

The unique properties of GU pairing may play a key role in binding of RNA to proteins. Accurate recognition of tRNA^{ala} by alanyl-tRNA synthetase has been shown to be highly dependent on a GU pair in the acceptor stem.[14,15] The guanine amino group in the minor groove contributes significantly to the specificity and efficiency of this interaction.[16,17] Furthermore, the significant consequence of a GU pair within the splice site helix (P1) of the *Tetrahymena* group I self-splicing intron recently has been shown to be more than the local helical distortion created by the wobble geometry.[18−20] In this case, the guanine amino group protruding into the minor groove helps fold the ribozyme into its correct tertiary structure by binding to the active site. A high-resolution NMR structure of a model of this group I splice site helix showed the major groove to be closed, while the minor groove was sufficiently open to expose the guanine amino group of the GU pair.[21] Again, the wobble geometry was found to alter the base stacking, with extensive overlap between the guanine and its 3′ base, but not with its 5′ base. A similar stacking pattern also had been found by NMR for an intrahelical GU pair in Helix I from *Escherichia coli* 5S RNA.[22] This altered stacking pattern appears to be a general consequence of the local distortion within a helix caused by the GU wobble geometry.[23,24]

Uniform ^{13}C/^{15}N labeling techniques have proved enormously useful for RNA structure determination,[25,26] including that of molecules with GU pairs.[21,27] Selective ^{15}N labeling of DNA fragments has been used to provide model-independent insight into localized hydrogen bonding,[28−32] protonation,[31,33] hydration,[34] and protection from hydration.[30] In view of the importance of GU pairing in RNA structure and function, we have begun a program of selective labeling focused on this pair, in increasingly complex systems. We now report the first of our results, on intrahelical GU wobble pairing in the self-complementary molecule 5′-G<u>A</u>U<u>G</u>C<u>G</u>UCp-3′. The two underlined guanines (GU and G<u>C</u> pairs) were labeled at the N1 and N2 positions. A ^{13}C "tag" at the C2 atom in the GU pair was used to differentiate them.[35] The only adenine present in this molecule was labeled at the N6 position. Oligonucleotide synthesis was done on a 30 μmol scale by an hydrogen phosphonate method[36] using an allyl linker to the solid support.[37] The presence of the GU wobble pair was confirmed by ^{1}H NMR (not shown). The chemical shifts of the labeled atoms were monitored through the melting transition of {5′-G<u>A</u>U<u>G</u>C<u>G</u>U-Cp-3′}$_2$ (Figures 1 and 2). The chemical shifts of the guanine amino group in the GC pair and of the adenine amino group behave as expected, with cooperative upfield shifts of 2 and 4 ppm, respectively, upon disruption of Watson−Crick hydrogen bonding during melting. We have observed similar deshielding of base-paired amino groups in three other systems with

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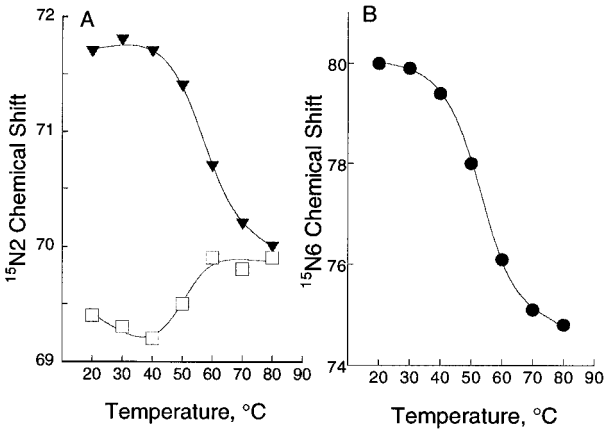

Figure 1. Plots of ^{15}N chemical shifts vs temperature for (A) guanine amino groups and (B) adenine amino groups in GAUGCGUCp, where ▼ represents the guanine N2 in the GC pair, □ represents the guanine N2 in the GU pair, and ● represents the adenine N6 in the AU pair. Spectra were acquired at 40.5 MHz on a Varian XL400 using 1D experiments with a delay of 1 s, and chemical shifts are reported relative to NH_3 using external 1 M [^{15}N]urea in DMSO at 25 °C at 77.0 ppm as a reference.[41] The total strand concentration was 10 mM in 100% D_2O, 0.1 M NaCl, 10 mM phosphate, and 0.1 mM EDTA at pH 6.7. A nonlinear least-squares fit gives the curves shown.

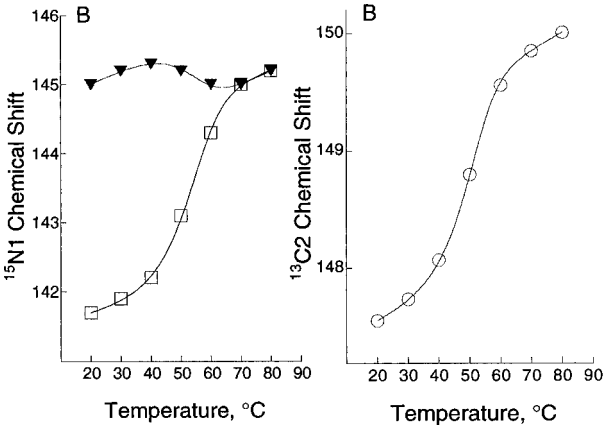

Figure 2. Plots of (A) ^{15}N chemical shifts and (B) ^{13}C chemical shifts vs temperature for GAUGCGUCp, where ▼ represents the guanine N1 in the GC pair, □ represents the guanine N1 in the GU pair, and ○ represents the C2 in the GU pair. Conditions are the same as for Figure 1.

Watson−Crick geometry: 1 ppm for a dA·dT pair,[28] 1 ppm for a dO^{6}MeG·dT pair,[29] and 6 ppm for a protonated dO^{6}MeG·dC pair.[31]

The chemical shift behavior for the guanine amino group in the GU pair is significantly different. In the intact pair at low temperature, the N2 chemical shift is about 2.5 ppm further *upfield* (shielded) than in the intact GC pair. As the temperature is increased, it continues to move upfield at first, until the melting transition begins, whereupon it changes direction and moves downfield to the same values associated with the melted GC pair. The chemical shift difference between duplex and single-strand for each of these ^{15}N resonances is less than 100 Hz, and none showed evidence of the line broadening at intermediate temperatures that would be indicative of intermediate exchange.[38] The amino group is not base paired in the GU wobble pair but is undoubtedly solvated.[39] If thermal disruption of hydration were the dominant factor affecting the chemical shift behavior throughout the transition, we would have expected a continuation of the upfield shift as this hydration was disrupted, most likely of a noncooperative nature.[29] Instead, the more upfield values that we observe in the intact pair at low temperature and the downfield shift during melting are likely to be due to shielding effects caused by the altered stacking associated with the wobble geometry of the GU pair. We have not previously observed such stacking-induced shielding for amino groups.

The guanine ^{15}N1 chemical shift of the GC pair is deshielded by about 1 ppm, relative to the single-strand, consistent with H-bond donation in this Watson−Crick pair (Figure 2).[40] In contrast, the chemical shift of the N1 in the GU pair is *shielded* by 2.5 ppm, so that it differs from the GC N1 by 3.5 ppm. The similar shielding of the ^{13}C2 atom in this GU pair in the duplex (Figure 2B) and the known upfield shift of guanine N1 protons in GU pairs support the idea that the origin of this effect is in the distinctive stacking of the GU wobble pair. In sum, the data presented above demonstrate that the N1, C2, and N2 atoms of guanine in the intact GU pair reported here are all strongly shielded relative to those in a Watson−Crick GC pair. This strong shielding effect presumably is caused by the fully stacked 3′ base and may well be diagnostic for an intrahelical GU wobble pair. Experiments are currently under way with other specifically labeled RNA fragments that should extend our understanding of the GU pair.

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