^{15}N NMR of RNA Fragments Containing Specifically Labeled Tandem GA Pairs

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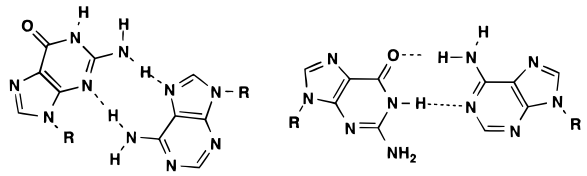
Specific ^{15}N and ^{13}C labeling of DNA and RNA can provide key information on local interactions such as hydrogen bonding,[1−5] protonation,[4,6] hydration,[7] ligand interactions,[3] and stacking.[8,9] We are interested in characterizing non-Watson−Crick base pairs in RNA by ^{15}N NMR because they play prominent structural and energetic roles that facilitate formation of secondary and tertiary interactions and binding to proteins.[10−16] We have recently reported ^{15}N NMR results for a variety of GU pairs[8,9] and now describe two types of GA pairing. Tandem GA pairs in the order 5′-GA-3′•3′-AG-5′ have been shown to adopt either a "sheared" conformation or a "face-to-face" conformation, depending entirely on the flanking sequences.[17−20] Thus, ^{1}H NMR structures show



sheared face-to-face

that GGC**GA**GCC contains a sheared arrangement,[17] while GCG**GA**CGC has face-to-face pairing.[18] Both arrangements have two base−base hydrogen bonds, and the duplexes have about the same thermal stability, but in the sheared pairing, additional base−backbone hydrogen bonds and unusual *inter*strand stacking of adenine on adenine and guanine on guanine help compensate for backbone distortion. In the face-to-face pairing, more typical *intra*strand base stacking occurs.[17,18] The striking differences in the overall shapes of these molecules as well as in their pattern of exposed functional groups undoubtedly have biological significance.[17,18,21]

A 5′ flanking pyrimidine seems to be a prerequisite for the sheared pairing with *inter*strand stacking. It has also been found in another fragment with 5′ flanking cytosines,[22] in a fragment with 5′ flanking uracils,[19] and in the hammerhead ribozyme where it is flanked on the unsymmetrical 5′ sides by a uracil and a cytosine.[23−25] This motif also occurs in DNA fragments.[26−30] In addition, related *inter*strand stacking in some UG and TG tandem pairs occurs in both RNA and DNA.[31−33] ^{15}N NMR may serve as a useful probe for this *inter*strand stacking.

To compare the sheared and face-to-face conformations in tandem GA pairs by ^{15}N NMR, we have synthesized ^{15}N-labeled versions of the molecules described by Turner,[17,18] 5′-GGC-GAGCCp-3′ (sheared) and 5′-GCGGACGCp-3′ (face-to-face), using [2-^{13}C-1,NH_2-$^{15}N_2$]guanosine and [7,NH_2-$^{15}N_2$]adenosine at the underlined sites.[34−36] The presence of the sheared GA conformation in the former was confirmed by proton NMR in which the guanine ^{15}N1H appeared as a doublet near 10 ppm (data not shown).[17] Similarly, proton NMR demonstrated the presence of the face-to-face GA conformation in the latter since the guanine ^{15}N1H appeared as a doublet near 12.5 ppm (data not shown).[18] We find that the guanine amino groups show strikingly different chemical shift behavior for these two conformations (Figure 1A). Upon melting, the guanine amino in the sheared conformation (▲) shows a 4 ppm upfield cooperative transition, while that in the face-to-face conformation (○), shows only a small irregular downfield drift. The cooperative transition for the former is in accord with expectations for hydrogen bonding to the adenine N7 and the adenine phosphate oxygen.[17] The small non-cooperative changes seen with the latter are consistent with the absence of either structural hydrogen bonding or significant stacking in the face-to-face conformation.[18] The^{15}N6 chemical shifts of the adenine amino groups, on the other hand, both show upfield cooperative transitions upon melting (Figure 1B), although the face-to-face conformation displays an unusually large 10 ppm change (○), about twice the magnitude as that of the sheared conformation (▲). In the former case, the behavior signifies loss of significant *deshielding* effects both from stacking and from hydrogen bonding. This result is consistent with the ^{1}H NMR structure in which the adenine amino nitrogen atom was shown

(1) Gao, X.; Jones, R. A. *J. Am. Chem. Soc*. **1987**, *109*, 3169−3171.
(2) Goswami, B.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc*. **1993**, *115*, 3832−3833.
(3) Rhee, Y.; Wang, C.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc*. **1993**, *115*, 8742−8746.
(4) Gaffney, B. L.; Goswami, B.; Jones, R. A. *J. Am. Chem. Soc*. **1993**, *115*, 12607−12608.
(5) Gaffney, B. L.; Kung, P.-P.; Wang, C.; Jones, R. A. *J. Am. Chem. Soc*. **1995**, *117*, 12281−12283.
(6) Wang, C.; Gao, H.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc*. **1991**, *113*, 5486−5488.
(7) Gaffney, B. L.; Wang, C.; Jones, R. A. *J. Am. Chem. Soc*. **1992**, *114*, 4047−4050.
(8) Zhang, X.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc*. **1997**, *119*, 6432−6433.
(9) Zhang, X.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc*. **1998**, *120*, 615−618.
(10) Chou, S.-H.; Zhu, L.; Reid, B. R. *J. Mol. Biol*. **1997**, *267*, 1055−1067.
(11) Gautheret, D.; Konings, D.; Gutell, R. R. *J. Mol. Biol*. **1994**, *242*, 1−8.
(12) Strobel, S. A.; Cech, T. R. *Science* **1995**, *267*, 675−679.
(13) Michel, F.; Westhof, E. *J. Mol. Biol*. **1990**, *216*, 585−610.
(14) Musier-Forsyth, K.; Usman, N.; Scaringe, S.; Doudna, J.; Green, R.; Schimmel, P. *Science* **1991**, *253*, 784−786.
(15) Gabriel, K.; Schneider, J.; McClain, W. *Science* **1996**, *271*, 195−197.
(16) Biou, V.; Yaremchuk, A.; Tukalo, M.; Cusack, S. *Science* **1994**, *263*, 1404−1410.
(17) SantaLucia, J.; Turner, D. H. *Biochemistry* **1993**, *32*, 12612−12623.
(18) Wu, M.; Turner, D. H. *Biochemistry* **1996**, *35*, 9677−9689.
(19) Heus, H. A.; Wijmenga, S. S.; Hoppe, H.; Hilbers, C. W. *J. Mol. Biol*. **1997**, *271*, 147−158.
(20) Walter, A. E.; Wu, M.; Turner, D. H. *Biochemistry* **1994**, *33*, 11349−11354.
(21) Wu, M.; SantaLucia, J.; Turner, D. H. *Biochemistry* **1997**, *36*, 4449−4460.
(22) Katahira, M.; Kanagawa, M.; Sato, H.; Uesugi, S.; Fujii, S.; Kohno, T.; Maeda, T. *Nucleic Acids Res*. **1994**, *22*, 2752−2759.
(23) Pley, H. W.; Flaherty, K. M.; McKay, D. B. *Nature* (*London*) **1994**, *372*, 68−74.
(24) Scott, W. G.; Finch, J. T.; Klug, A. *Cell* **1995**, *81*, 991−1002.
(25) Scott, W. G.; Murray, J. B.; Arnold, J. R. P.; Stoddard, B. L.; Klug, A. *Science* **1996**, *274*, 2065−2069.
(26) Lane, A.; Martin, S. R.; Ebel, S.; Brown, T. *Biochemistry* **1992**, *31*, 12087−12095.
(27) Greene, K. L.; Jones, R. L.; Li, Y.; Robinson, H.; Wang, A. H.-J.; Zon, G.; Wilson, W. D. *Biochemistry* **1994**, *33*, 1053−1062.
(28) Chou, S.-H.; Cheng, J.-W.; Fedoroff, O.; Reid, B. R. *J. Mol. Biol*. **1994**, *241*, 467−479.
(29) Li, Y.; Agrawal, S. *Biochemistry* **1995**, *34*, 10056−10062.
(30) Katahira, M.; Sato, H.; Mishima, K.; Uesugi, S.; Fujii, S. *Nucleic Acids Res*. **1993**, *21*, 5418−5424.
(31) McDowell, J. A.; Turner, D. H. *Biochemistry* **1996**, *35*, 14077−14089.
(32) Rabinovich, D.; Haran, T.; Eisenstein, M.; Shakked, Z. *J. Mol. Biol*. **1988**, *200*, 151−161.
(33) Biswas, R.; Wahl, M. C.; Ban, C.; Sundaralingam, M. *J. Mol. Biol*. **1997**, *267*, 1149−1156.
(34) Zhang, X.; Gaffney, B. L.; Jones, R. A. *Nucleic Acids Res*. **1997**, *25*, 3980−3983.
(35) Zhang, X.; Abad, J.-L.; Huang, Q.; Zeng, F.; Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett*. **1997**, *38*, 7135−7138.
(36) Zhao, H.; Pagano, A. R.; Wang, W.; Shallop, A.; Gaffney, B. L.; Jones, R. A. *J. Org. Chem*. **1997**, *62*, 7832−7835.

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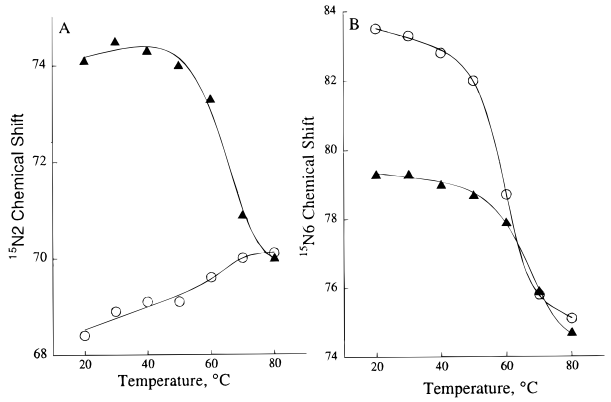

Figure 1. Plots of ^{15}N chemical shifts vs temperature for (A) guanine amino groups, and (B) adenine amino groups. In each case, ▲ represents the sheared pairing and ○ represents the face-to-face pairing. Spectra were acquired at 40.5 MHz on a Varian XL400 using 1D experiments with a delay of 1 s, and chemical shifts are reported relative to NH_3 using external 1M [^{15}N]urea in DMSO at 25 °C at 77.0 ppm as a reference.[40] The total strand concentration of the sheared molecule was 11 mM and that of the face-to-face molecule was 7 mM, both 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. Intermediate exchange, which can complicate the interpretation of some NMR data,[41] does not appear to be a problem with these examples. None of the resonances described here show evidence of line broadening during melting. The chemical shift differences between the high and low temperature forms of the ^{15}N atoms generally are less than 200 Hz. Furthermore, melting temperatures calculated from curve fitting of the ^{15}N NMR data agree well with the corresponding values calculated from UV melting studies (data not shown).

to be stacked at some distance from the adjacent guanine aromatic ring, very likely in a deshielding region of its ring currents.[18]

The chemical shifts of the guanine N1's, like those of the guanine aminos, show quite different behavior upon melting (Figure 2A). Although each shows a cooperative transition that is similar in magnitude, it is opposite in direction. The upfield change (○) is appropriate for the hydrogen bonding to the adenine N1 expected in the face-to-face conformation.[8,9] In the sheared conformation (▲), the guanine N1 does not participate in a structural hydrogen bond, so that the downfield change observed must be due to a loss of stacking induced shielding. The main source of this shielding is likely to be the strong *inter*strand stacking with the opposite guanine since *intra*strand stacking to its 5′ flanking cytosine has been shown to be quite weak.[17] Since a 5′-flanking pyrimidine appears to be a requirement for the sheared conformation,[19,20,22] the ∼2 ppm shielding that we have observed here may be diagnostic for sheared tandem GA pairs in general. We have previously seen a related ∼4 ppm shielding effect on guanine ^{15}N1 chemical shifts caused in part by *inter*strand stacking that is associated with tandem UG pairs.[9] In that case, however, strong *intra*strand stacking on an adjacent guanine also contributed to the overall shielding effect. In both the sheared and face-to-face GA pairs reported here, the guanine

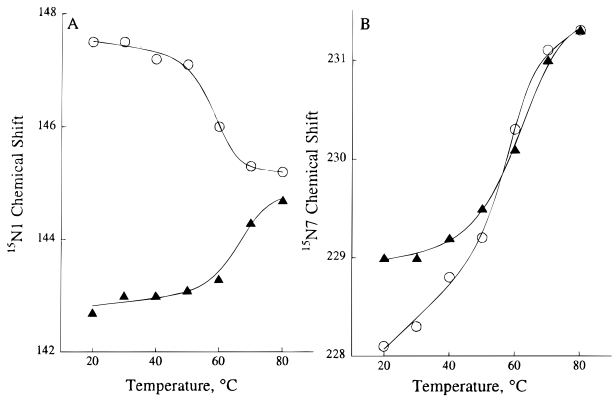

Figure 2. Plots of ^{15}N chemical shifts vs temperature for (A) guanine N1 atoms and (B) adenine N7 atoms. In each case, ▲ represents sheared pairing and ○ represents the face-to-face pairing. Conditions are the same as those in Figure 1.

^{13}C2 atoms show small downfield drifts with increasing temperature (0.5 and 0.2 ppm, respectively, data not shown).

The adenine ^{15}N7 chemical shifts in both cases show cooperative downfield transitions (Figure 2B). This behavior is consistent with base pair hydrogen bonding in the sheared conformation, in which the adenine N7 accepts a hydrogen bond from the guanine amino.[17] In contrast, in the face-to-face conformation, the adenine N7 does not participate in base pairing.[18] Our data indicate that either this nitrogen is in fact participating in some form of structural hydrogen bonding or experiences a strong *shielding* effect from stacking, neither of which was apparent from the ^{1}H NMR data.[18]

The *inter*strand stacking that is characteristic of the sheared tandem 5′-GA-3′•3′-AG-5′ pairs described here is also found in the similar sheared 5′-AA-3′•3′-AG-5′ pairs found in DNA[37] as well as in related 5′-UA-3′•3′-AG-5′ pairs with a reverse Hoogsteen UA flanking a sheared GA.[38,39] These sequences may form a unique functional motif with important biological significance.[11] The results described here demonstrate that ^{15}N NMR of a specifically labeled member of this group can identify its diagnostic *inter*strand stacking as well as help define hydrogen bonding and other interactions. Specific labeling should be particularly valuable in recognizing this motif in larger RNA fragments for which full structures are not known.

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(37) Maskos, K.; Gunn, B. M.; LeBlanc, D. A.; Morden, K. M. *Biochemistry* **1993**, *32*, 3583−3595.
(38) Wimberly, B.; Varani, G.; Tinoco, I. Jr. *Biochemistry* **1993**, *32*, 1078−1087.
(39) Szewczak, A. A.; Moore, P. B.; Chan, Y.-L.; Wool, I. G. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9581−9585.
(40) Wishart, D. S.; Bigam, C. G.; Yao, J.; Abildgaard, F.; Dyson, H. J.; Oldfield, E.; Markley, J. L.; Sykes, B. D. *J. Biomol. NMR* **1995**, *6*, 135−140.
(41) Pardi, A.; Martin, F. H.; Tinoco, I., Jr. *Biochemistry* **1981**, *20*, 3986−3996.