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DETECTION OF THE IMINO HYDROGEN BOND IN G-C PAIRS BY ^{1}H AND ^{15}N NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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Abstract: "Limiting" ^{1}H and ^{15}N chemical shifts for the imino moiety of G in the G-C base pair were evaluated in model studies using chloroform soluble 2',3'-O-isopropylidene-5'-O-t-butyldimethylsilyl derivatives and were found to be significantly downfield from previously reported shifts measured in dimethylsulfoxide.

The imino moieties in U and G are important probes for detecting hydrogen bonds and for studying the conformations of polynucleotides in solution by NMR.[1,2] Hydrogen bond formation involving U is accompanied by substantial downfield shifts for the protons and nitrogens of the imino unit, and the magnitudes of the shifts provide information about the environment of the imino unit. Model studies to determine the "intrinsic" chemical shifts of the proton and nitrogen for the G imino moiety of a G-C pair are difficult because of insolubility of the monomers. Attempts to overcome this problem by using dimethylsulfoxide as a solvent gave "limiting" values for the G imino proton well upfield of chemical shifts found for G-C base pairs in RNAs.[3-5] In related studies, dimethylsulfoxide is known to eliminate base pairing between U and A because solvent-monomer interactions adversely alter the monomer-base pair equilibrum.[4] In chloroform, however, where solvent-monomer interactions are reduced, A-U pairs form readily with concommittant large downfield ^{1}H and ^{15}N shifts for the imino unit to values similar to those found in RNAs.[6,7] We now report a chloroform-soluble model for the G-C pair which gives ^{1}H chemical shifts comparable to those seen in RNAs. ^{15}N chemical shifts were also obtained using a new procedure which gives a 2D chemical shift map for ^{1}H-^{15}N units in only 2 hours with a dilute solution of nucleosides.

After examining several derivatives of guanosine and cytidine, we discovered that the 2',3'-O-isopropylidene-5'-O-t-butyldimethylsilyl-blocked nucleosides[8] were soluble at 0.3 M concentrations in chloroform at -20°C. ^{1}H chemical shifts were obtained in deuterochloroform as a function of the mole fractions of 2',3'-O-isopropylidene-5'-O-t-butyldimethylsilyl-guanosine (**1**) and 2',3'-O-isopropylidene-5'-O-t-butyldimethylsilylcytidine (**2**). The data shown in Figure 1 was acquired at 26°C with a total, constant nucleoside concentration of 0.2 M in order to minimize effects of changing concentration. The imino proton at N1 of **1** moved downfield from 12.10 ppm to a limiting value of 13.78 ppm as the mole fraction of **2** was increased from zero to 0.9. At -18°, where base pairing is further enhanced, an additional downfield shift to 13.93 ppm was observed.[9]

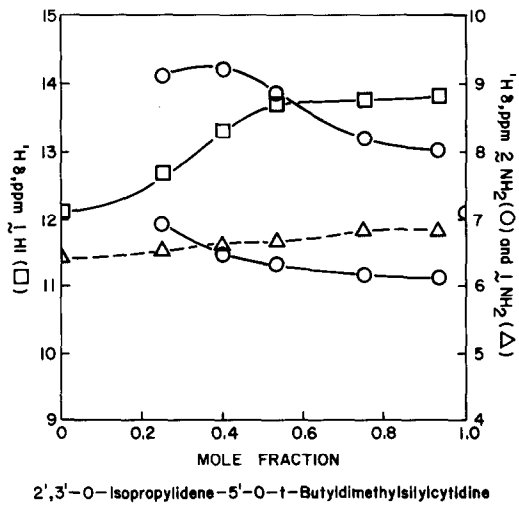

Figure 1. A plot of the mole fraction of **2** in a solution of **1** in chloroform versus ^1H chemical shifts for H1 (□) and amino (Δ) protons in **1** and the amino protons (O) in **2**. Total concentration of nucleosides was maintained at 0.2 M.

The rather low field chemical shift for the imino proton of a 0.2 M solution of **1** in chloroform and the relatively small additional downfield shift upon addition of **2** indicate that **1** pairs with itself. Supporting evidence was obtained by addition of 9 equivalents of dimethylsulfoxide to the solution of **1**; whereupon, the imino proton moved *upfield* by 1.4 ppm. As mentioned above, dimethylsulfoxide is known to disrupt base pairing. The chemical shifts shown in Figure 1, therefore, represent a transition from G-G base pairs for 0.2 M **1** to more stable G-C pairs as the mole fraction of **2** increases.

Shifts were also seen for the amino protons in **1** and **2**. The most dramatic changes were observed for **2**. Upon addition of **1** (mole fraction = 0.1) to a chloroform solution of **2**, the single broad two-proton resonance at 7.13 ppm split into two broad one-proton peaks at 6.10 and 8.00 ppm.[3] The lower field resonance, assigned to the hydrogen bound amino proton, moved downfield to a maximum value of 9.35 ppm at -18°C as the mole fraction of **1** was increased. Small downfield shifts were observed for the free amino proton in **2** and both amino protons in

1. The single amino peak seen for **1** indicates that the bound and unbound amino protons in the $G(NH_2)$-C(O2) hydrogen bond exchange more rapidly than those in the $C(NH_2)$-G(O6) bond of G-C pairs.

The ^{15}N chemical shifts for the imino nitrogen of **1** and the amino nitrogen of **2** were determined at natural abundance with a 1:1 mixture (total nucleoside concentration = 0.25 M) in only two hours using multiquantum ^{1}H-^{15}N 2D chemical shift correlation NMR spectroscopy.[10,11] The pulse sequence $^{1}H(90)$ -1/2J - $^{15}N(90)$ - t_1 - $^{15}N(90)$ - ^{1}H(acquire), where J is the ^{1}H-^{15}N one-bond coupling constant for protonated nitrogens, gave a 2D ^{1}H-^{15}N chemical shift map with ^{1}H-^{15}N coupling in the ^{1}H dimension. The N1 nitrogen of **1** appeared at 149.4 ppm with $^{1}J_{^{1}H-^{15}N}$ = 94 Hz. This chemical shift is downfield from the value reported for a 0.8 M solution of guanosine and cytidine in dimethylsulfoxide by 2.1 ppm. The amino nitrogen of **2** in the 1:1 mixture has a chemical shift of 95.4 ppm; however, the ^{1}H lines were too broad (60-75 Hz) to measure the ^{1}H-^{15}N coupling constant.

The lowfield chemical shift for the imino proton in **1** in the presence of high mole fractions of **2** demonstrates that the imino protons in A-U and G-C pairs have similar "intrinsic" chemical shifts. Thus, the considerable overlap of proton imino resonances for A-U and G-C base pairs seen in RNAs is consistent with the "intrinsic" values determined from model studies. Fortunately, this overlap does not extend to the imino nitrogens in A-U and G-C pairs. The "limiting" low field ^{15}N chemical shift for N1 in **1** of 149.4 ppm when base paired with **2** is slightly more than 8 ppm above the value of 157.5 ppm observed for N3 in "free" uridine and 13 ppm upfield from the U value in an A-U pair.[6] Differences of this magnitude should permit one to clearly differentiate between A-U and G-C imino units in polynucleotides and underscores the utility of ^{1}H-^{15}N chemical shift correlation spectroscopy.

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References and Notes:

1. Schimmel, P. R.; Redfield, A.G. *Ann. Rev. Biophys. Bioeng.* **1980**, 9, 181-221.
2. Reid, B. R. *Ann Rev. Biochem.* **1981**, 50, 969-996.

3. Shoup, R. R.; Miles, H. T.; Becker, E. D. *Biochem. Biophys. Res. Commun.* **1966**, 23, 194-201.
4. Katz, L.; Penman, S. *J. Mol. Biol.* **1966**, 15, 220-231.
5. Dyllick-Brenzinger, C.; Sullivan, G. R.; Pang, P. P.; Roberts, J. D. *Proc. Natl. Acad. Sci. USA* **1980**, 77, 5580-5582.
6. Poulter, C. D.; Livingston, C. L. *Tetrahedron Lett.* **1979**, 755-758.
7. Griffey, R. H.; Poulter, C. D.; Yamaizumi, Z.; Nishimura, S.; Hawkins, B. L. *J. Am. Chem. Soc.* **1983**, 105, 143-145.
8. Blocked nucleosides **1** and **2** were prepared by the method of Ogilvie and coworkers. Ogilvie, K. K.; Schifman, A. L.; Penny, C. L. *Can. J. Chem.* **1979**, 57, 2230-2238.
9. Kruger, U.; Breuer, H.; Abdel Kerim, F. M.; Perkampus, H.-H.; Scheit, K. H. *Z. Naturforschs.* **1968**, 23b, 1360-1366.
10. Griffey, R. H.; Poulter, C. D.; Bax, A.; Hawkins, B. L.; Yamaizumi, Z.; Nishimura, S. *Proc. Natl Acad. Sci. USA* **1983**, in press.
11. Bax, A.; Griffey, R. H.; Hawkins, B. L. Unpublished results.

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