Communications to the Editor

NMR Detection of Intermolecular N−H···N Hydrogen Bonds in the Human T Cell Leukemia Virus-1 Rex Peptide−RNA Aptamer Complex

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Hydrogen-bonding interactions are fundamentally important in the stabilization of biomolecular secondary structure elements and in defining the globular folds of polypeptide and RNA chains. In particular, they very often play a key role in enzymatic and ribozyme activities and are a fundamental element in biomolecular recognition events.[1] The recent direct detection of scalar couplings between hydrogen donor and acceptor moiety spins in both nucleic acids[2−6] and proteins[7−10] by NMR, has opened a new approach for monitoring intramolecular hydrogen bonds and provided invaluable parameters for the characterization of structure and dynamics of biological macromolecules in solution by NMR. Of significant interest is the possibility of detecting intermolecular hydrogen bonds at macromolecular interfaces such as in protein−protein and protein−nucleic acid complexes. In this contribution, we report the direct NMR observation of intermolecular hydrogen bonds between the guanidinium groups of arginines in the 16-mer arginine-rich HTLV-1 Rex peptide (Figure S1a, Supporting Information) and the major groove edges of guanine bases of a 33-mer RNA aptamer (Figure S1b), whose solution structure was solved recently by NMR in our laboratory.[12] This NOE-based solution structure established that the side-chain guanidinium groups of all the six arginine residues are involved in either electrostatic or hydrogen bonding interactions with the RNA target. The guanidinium groups of arginines R6, R9, and R10,

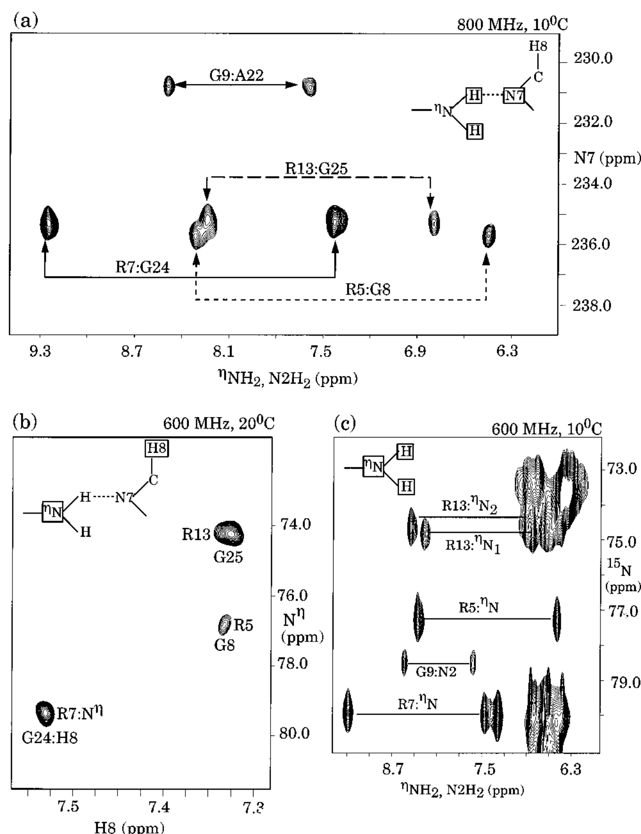

Figure 1. (a) Soft 2D HNN-COSY, (b) 2D H(CN)N(H), and (c) $^{1}H-^{15}N$ HSQC spectra of a 2.0 mM NMR sample of the $^{13}C/^{15}N$-labeled Rex peptide−$^{13}C/^{15}N$-labeled RNA aptamer complex dissolved in 250 mL of 95% H_2O/7% D_2O. The sample was prepared within a Shigemi microcell with 10 mM sodium phosphate, 0.2 mM EDTA and buffered at pH 6.4. In each spectrum, the correlated nuclei are shown. The spectrum in (a) was recorded at 10 °C on a Varian Inova spectrometer operating at 800 MHz (^{1}H frequency). Three pairs of cross-peaks correlate the guanidinium $^{15}N^{\eta}H_2$ groups of arginines 5, 7, and 13 with the $^{15}N7$ spins of guanine bases 8, 24, and 25, respectively, demonstrating the existence of $^{2h}J_{N\eta N7}$ couplings across hydrogen-bonds linking the corresponding atoms at the peptide-RNA interface. In addition, intramolecular hydrogen bonds within the RNA aptamer are manifested as cross-peaks between the $^{15}N2H_2$ protons of G9 and $^{15}N7$ nitrogen of A22 (see text). Data acquisition: ^{1}H and ^{15}N carriers were set to 4.9 and 237 ppm, respectively. A total of 816 transients/fid, with 704 (t_2) × 45 (t_1) complex data points were acquired, resulting in acquisition times of $t_{2max}(^{1}H)$ = 72 ms and $t_{1max}(^{15}N)$ = 18 ms. A recycle delay of 1.4 s was used, resulting in a total experimental time of 36 h. However, all of the expected signals could be recognized within 20 h data collection. The spectrum in (b) was recorded at 20 °C, on a Varian Inova spectrometer operating at 600 MHz (^{1}H frequency). Three cross-peaks correlate the H8 protons of the hydrogen bonded guanine residues with the $^{\eta}N$ nitrogens of the corresponding arginines via the $^{2h}J_{N\eta N7}$ couplings. The intramolecular A22:H8-G9:N7 cross-peak is not shown. Data acquisition: ^{1}H and ^{15}N carriers: 4.8 and 75 ppm, respectively, 816 transients/fid, 576 (t_2) × 45 (t_1) complex points, $t_{2max}(^{1}H)$ = 72 ms and $t_{1max}(^{15}N)$ = 24 ms, 1.6 s recycle delay, total time ∼40 h. The $^{1}H-^{15}N$ correlation spectrum in (c) was recorded at 10 °C, 600 MHz. $^{\eta}NH2(\omega_2)-^{\eta}N(\omega_1)$ and G9:NH2(ω_2)−G9:N2(ω_1) cross-peaks are indicated in the figure.

are within 4 Å from one or more backbone phosphates on the RNA aptamer. The guanidinium groups of R5, R7, and R13 form

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intermolecular hydrogen bonds with the major groove edges of adjacent guanine bases in the central stem of the RNA aptamer. For R5, one pair of $^{\eta}NH_2$ protons is nondegenerate in proton chemical shifts and forms a hydrogen bond with N7 of G8. The other pair of $^{\eta}NH_2$ protons, however, cannot be defined unambiguously. For R7, one pair of $^{\eta}NH_2$ protons forms a hydrogen bond with N7 of G24. The other pair does not participate in base-specific hydrogen bonding. For R13, both pairs of $^{\eta}NH_2$ protons are nondegenerate and form two hydrogen bonds with N7 and carbonyl oxygen, respectively, of G25.[12]

In this work, we establish correlations between $^{15}N^{\eta}H_2$ moieties of R5, R7, and R13 and the ^{15}N7 spins of G8, G24 and G25, respectively, through the observation of intermolecular *trans*-hydrogen-bond $^{2h}J_{N\eta N7}$ couplings, in a sample of the peptide−RNA complex in which both RNA and peptide are uniformly ^{13}C,^{15}N-labeled.[13] The appropriate region of the ^{1}H−^{15}N correlation (HSQC) spectrum (10 °C, 600 MHz) is shown in Figure 1c. The guanidinium protons of the arginine side chains are evidently hydrogen-bonded, as seen from their distinct, albeit broad, proton resonances. To observe the *trans*-hydrogen bond couplings, two previously established, complementary experiments were used: (i) the soft HNN-COSY technique,[4a,11] which correlates the arginine $^{\eta}NH_2$ protons of the peptide with the N7 nitrogens of guanines in the RNA and (ii) the H(CN)N(H) experiment,[4b,6] which correlates the H8 protons of guanines with $^{\eta}$N nitrogens of arginines. Figure 1a shows a soft-HNN COSY spectrum of the Rex peptide−RNA aptamer complex recorded at 10 °C, at 800 MHz. The three pairs of ($^{\eta}NH_2$,N7) cross-peaks clearly demonstrate the existence of $^{2h}J_{N\eta N7}$ couplings across hydrogen bonds linking the $^{15}N^{\eta}$ atoms of arginines 5, 7, and 13 with the ^{15}N7 atoms of guanine bases 8, 24, and 25, respectively. N7 assignments were obtained independently via long-range H8−N7 HSQC experiments (Figure S2, Supporting Information). In addition to intermolecular hydrogen bonds, cross-peaks are also seen between the amino protons of G9 and N7 of A22. These data provide additional support for the formation of a sheared G9•A22 pair within a A22•(G9•C20) base triple, as proposed by the NOE-based NMR structure of the complex.[12] Acquiring data at 800 MHz proved to be highly beneficial for this experiment by providing the sensitivity needed for observing signals from the broad NH_2 resonances, as well as the added dispersion needed for resolving the partially overlapped R13:$^{\eta}NH_2$−G25:N7 and R5: $^{\eta}NH_2$−G8:N7 cross-peaks.

Figure 1b shows a region of the H(CN)N(H) spectrum of the Rex peptide−RNA aptamer complex recorded at 20 °C, at 600 MHz (^{1}H). The cross-peaks between guanine H8 protons and arginine $^{\eta}$N nitrogens not only augment the results of Figure 1a by yielding the hydrogen-bond connectivity in a complementary manner, but also help remove the near degeneracy of the R13: $^{\eta}NH_2$−G25:N7 and R5: $^{\eta}NH_2$−G8:N7 cross-peaks in the soft-HNN COSY spectrum. It also demonstrates the possibility of obtaining $^{2h}J_{NN}$ couplings even in situations where the NH_2 protons are unobservable due to intermediate exchange broadening due to rotation about the exocyclic C−N bond. Although the H8 resonances were also observed to be somewhat broad, the use of nonexchangeable protons permitted the data to be acquired at higher temperatures under more favorable relaxation conditions, and could be performed even at 600 MHz with adequate sensitivity.

The intermolecular $^{2h}J_{NN}$ couplings—R7:G24 and R5:G8—were measured using spin−echo difference methods described previously[4a] and were found to be around 6.0 ± 0.5 Hz (Figure S3, Supporting Information). The R13:G25 coupling could not be measured accurately because of poor signal-noise and overlap problems associated with the R13:$^{\eta}NH_2$ protons. The reasonably large values of these couplings are not in conflict with the generally low signal-noise observed in the soft HNN-COSY and H(CN)N(H) spectra (both required >400 transients for adequate S/N), since the relevant NH_2 and H8 protons were of low intensity at the very outset. The intermolecular coupling constants in the peptide−RNA complex are comparable to their intramolecular counterparts observed in most of the nucleic acids reported so far, and this is indicative of tight binding and restricted local dynamics at the peptide−RNA interface.

In summary, we have presented the first direct NMR detection of hydrogen bonds between arginine side-chain guanidinium moieties and the major groove edge of guanine bases formed in an extended HTLV-1 Rex peptide complexed with its RNA aptamer target. The results are in agreement with our previous NOE-based solution structure of the complex.[12] Recently, successful detection of intermolecular ^{31}P−^{15}N and ^{31}P−^{1}H scalar couplings across hydrogen bonds between the protein backbone amide and the backbone phosphate of a nucleotide have been reported in the Ras(Q61L)•GDP complex[14] and flavodoxin.[15] Together, these new methodologies provide the necessary impetus for probing more complex systems to add to our current understanding of molecular recognition at macromolecular interfaces.

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Supporting Information Available: The sequences of the 16-mer Rex peptide and 33-mer RNA aptamer, a H8−N7 COSY spectrum showing guanine N7 resonance assignments, and plots showing estimation of $^{2h}J_{\eta NN7}$ couplings from spin−echo difference experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Uniformly ^{13}C/^{15}N-labeled RNA aptamer was prepared by in vitro transcription from a synthetic DNA template and purified by gel electrophoresis. Uniformly ^{13}C/^{15}N -labeled Rex peptide was HPLC purified after cyanogen bromide cleavage from a His-tagged TRP fusion protein which had been overexpressed in *Escherichia coli* growing on ^{13}C-glucose and $^{15}NH_4Cl$ as sole carbon and nitrogen sources, respectively.

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