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Application of Four-Dimensional Heteronuclear NMR to the Structure Determination of a Uniformly ^{13}C Labeled RNA

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The ability to resolve individual cross peaks in 2D nuclear Overhauser effect (NOE) spectra has limited NMR structure determinations of biopolymers to relatively small molecules. However, the upper limit for the solution structure determination of proteins recently has been increased to >20 kDa by application of three-dimensional (3D) and four-dimensional (4D) heteronuclear NMR.[1-3] These experiments spread the ^{1}H-^{1}H NOE data into additional dimensions, thus greatly simplifying the NMR analysis. However, these techniques have yet to be employed in structure determinations of nucleic acids. Here we report the first application of a four-dimensional heteronuclear (HMQC-NOE-HMQC) NMR experiment to the assignment and structure determination of a uniformly ^{13}C labeled RNA duplex, r-(GGCGCUUGCGUC)$_2$.

Many RNAs form tertiary structures that allow them to carry out a variety of biologically important functions including chemical catalysis.[4-6] Although detailed NMR structures have been determined for a number of RNAs,[7-9] these studies are restricted to short oligomers because of the limited dispersion of proton chemical shifts for the base and ribose protons in RNA.[10] One method for simplifying the proton spectra of RNAs is to increase the dimensionality of the NMR experiment.[1-3] A powerful application of this concept is the four-dimensional (^{13}C, ^{1}H, ^{13}C, ^{1}H) HMQC-NOE-HMQC experiment where both protons involved in the NOE are labeled with the frequency of their directly bonded carbon.[2,3] For this experiment the f_1 and f_3 dimensions contain the ^{13}C chemical shift information and the f_2 and f_4 dimensions contain the ^{1}H chemical shift data. Thus each f_2-f_4 plane in the 4D experiment represents a NOE spectrum that is edited in both f_1 and f_3 by the ^{13}C frequencies of the carbons directly attached to the protons involved in the NOE cross peak. Figure 1a shows such a ^{1}H-^{1}H (f_2-f_4) NOE plane of the H2′-H5′ region of the 4D spectrum for the ^{13}C-enriched RNA duplex. The corresponding region of a conventional 2D NOE spectrum is shown in Figure 1b. Few cross peaks are well enough resolved to be accurately analyzed in the 2D spectrum, but the 4D experiment provides NOE data for even the most crowded spectral regions. For example, intraresidue and interresidue distances involving the H2′ and H3′ to H5′/H5″ protons can define helical geometry but are never used due to the extreme overlap of this region (see Figure 1b). However, Figure 1a shows a completely resolved intraresidue H3′ to H5′/H5″ cross peak in the 4D experiment, and this sugar-sugar NOE can now be used in the structure determination. In addition, the sequential H1′(i)-H5′/H5″(i + 1) distance is 4.7 Å in an A-form helix, whereas it is only 1.8 Å in a B-form helix. Thus this distance is an important indicator of helical conformation, but it is presently not employed because it is impossible to assign these NOEs. A 2D plane of the 4D spectrum containing the interresidue H1′ to H5′/H5″ NOE between C9 and G10 is included in the supplementary material and illustrates how these NOEs are readily assigned in the 4D spectrum and therefore can also be included in structure calculations.

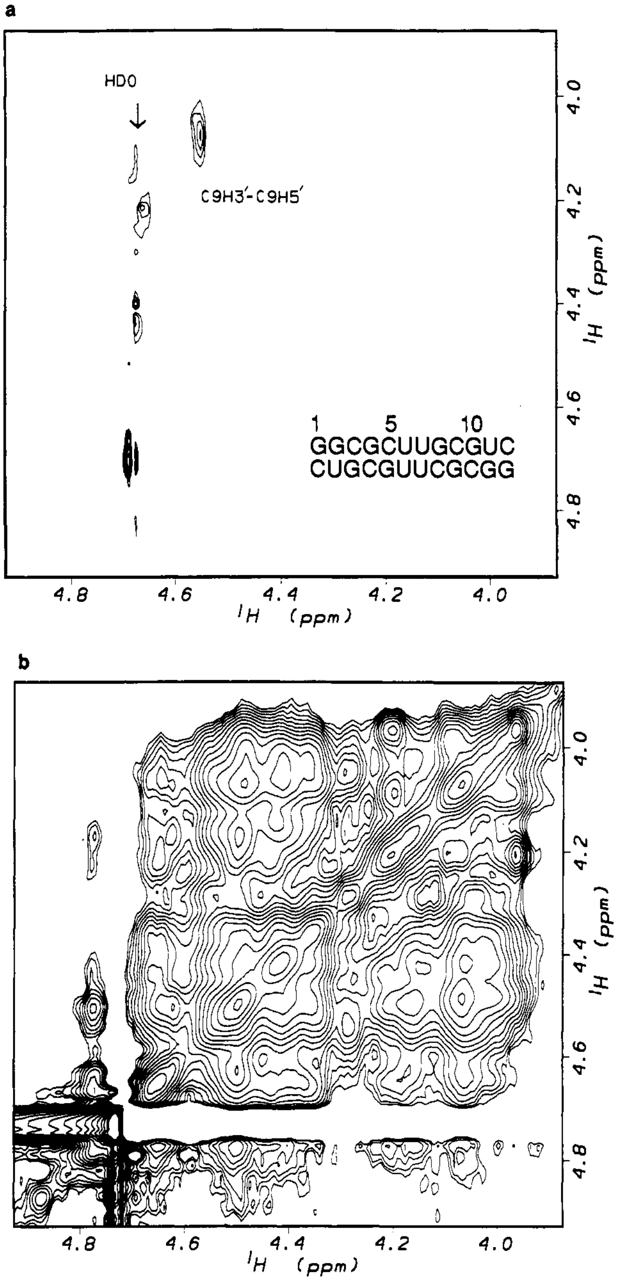

Figure 1. (a) A plot of the H2′-H5′ sugar region of the 4D HMQC-NOE-HMQC spectrum of the ^{13}C-labeled r(GGCGCUUGCGUC)$_2$ duplex. This ^{1}H-^{1}H NOE (f_2-f_4) plane of the 4D spectrum corresponds to f_1 = 66.3 ppm and f_3 = 74.3 ppm carbon frequencies. The experiment was carried out on a Varian VXR-500S spectrometer at 30 °C with a pulse sequence similar to that previously described[2] and employed a 400-ms NOE mixing time and a 0.9-s recycle time. The first and last HMQC portions of the 4D experiment were optimized for $^1J_{CH}$s of 205 and 185 Hz, respectively; 8, 32, 8, and 512 complex points were collected over sweep widths of 2500, 3200, 2500, and 3200 Hz for the t_1, t_2, t_3, and t_4 evolution times, respectively. Additional details of the pulse sequence, acquisition, and processing parameters employed in the 4D experiment are given in the supplementary material. (b) A plot of the same region of a 400-ms 2D NOE spectrum of the unlabeled RNA duplex acquired under similar experimental conditions.

Although the 4D heteronuclear NMR experiments require isotopically enriched RNA, the methods employed here for syn-

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thesis of uniformly ^{13}C labeled RNAs of defined sequence are quite general. The RNA duplex was generated by in vitro transcription with T7 RNA polymerase[11] using isotopically labeled nucleotide triphosphates (NTPs). The labeled NTPs were prepared by extracting ribosomal RNA from *Escherichia coli* grown on 99% ^{13}C enriched media, degrading the RNA to NMPs and enzymatically synthesizing the NTPs.[12,13] These techniques allow production of milligram quantities of isotopically labeled RNA oligomers of defined sequence.

In conclusion, we have shown how a 4D heteronuclear NMR experiment can enormously facilitate the resonance assignment and structural analysis of RNA. The tremendous increase in resolution in the 4D NMR experiment will allow detailed structure determination of much larger RNAs than previously has been possible.

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Supplementary Material Available: Details on the pulse sequence and processing parameters used in the 4D HMQC–NOE–HMQC experiment along with additional figures illustrating the improved resolution of the H1′/H5′ region of the RNA duplex in this 4D experiment (4 pages). Ordering information is given on any current masthead page.

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Reactions of State-Selected Co^+ with C_3H_8

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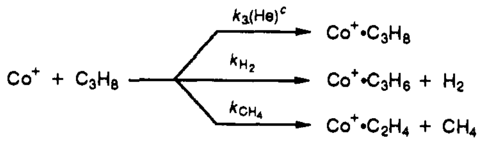
The growing use of mass spectrometric techniques for probing the kinetics, dynamics, and thermochemistry of transition-metal reactions has shown these reactions to be complex and usually extremely dependent on the metal ion electronic state.[1-10] Consequently, knowledge of state-specific reactions for these ions is especially important. Recently, we have developed a "chromatographic" technique to determine the electronic-state distribution present in transition-metal ions.[1] This allows us to study state-selected reactions. In this communication, we report the quantitative determination of rate constants and branching ratios for state-selected Co^+ reacting with propane. Observed rates for adduct formation as well as H_2 and CH_4 elimination channels were found to depend strongly on the electronic configuration of the metal ion.

Table I. Rate Constants for the Reactions[a,b]



	$k_3{}^d$	$k_{H_2}{}^d$	$k_{CH_4}{}^d$	k_{H_2}/k_{CH_4}
Co^+ (^3F, $3d^8$)	48	0.69	0.15	4.7
$(Co^+)^*$ (^3F, $4s3d^7$)	1.3	2.5	2.8	0.9
$k_{Co^+}/k_{(Co^+)^*}$	37	0.28	0.05	

[a] The accuracy of the absolute rate coefficient measurements is estimated to be within ±30%.[11] The relative rate coefficient measurements, however, are much more accurate. [b] The pressure of propane was varied from 1×10^{-5} to 4×10^{-5} Torr, yielding rate constants within ±15%. [c] Only He stabilization is important due to the low propane pressure. [d] 10^{-11} cm^3/s.

Details of the experimental apparatus have been published.[11] In experiments reported here, cobalt ions are formed by electron impact on $Co(CO)_3NO$ and $CoCp(CO)_2$ in the ion source of a double-focusing mass spectrometer. The Co^+ ions are mass selected and injected (with 2–3 eV of translational energy) into a high-pressure drift cell. The ions are quickly thermalized by collisions with He (P_{He} = 2 Torr) and react with a trace of C_3H_8 (4×10^{-5} Torr). A small electric field drifts the ions through the cell ($E/N < 4$ Td). Ions exit the cell, are passed through a quadrupole mass filter, and are detected.

Ion arrival time distributions (ATDs) are measured by pulsing the mass-selected ions into the drift cell (pulse width ≈ 1–3 μs). The pulse simultaneously triggers a time-to-pulse-height converter ramp. Ions that exit the cell are then collected as a function of time, yielding the ATD. Ions that have different mobilities have different drift times through the cell and appear as different peaks in the ATD. The ATD for Co^+ is bimodal, corresponding to ground ($3d^8$) and excited ($4s3d^7$) electronic state configurations.[1] The 4s-containing state of Co^+ is larger and more repulsive than the state containing only 3d electrons. This reduced attraction gives the excited-state Co^+ a 50% greater mobility than the ground state. The electronic-state populations are a function of both the electron energy and the neutral precursor used. For example, at 50 eV, electron impact on $Co(CO)_3NO$ produces 36% ground-state Co^+, whereas $CoCp(CO)_2$ produces 83% ground-state Co^+.

Once the electronic-state population of Co^+ is known, relative rates of ground- and excited-state Co^+ reacting with propane are obtained as follows. Propane is added to the drift cell, and the fractional decrease $[Co^+]/[Co^+]_0$ is measured as a function of time to obtain the total rate constant, k. Under the low conversion conditions in our experiment, the exponential decays of both ground- and excited-state Co^+ are well-described by linear functions. Thus, the sum of these two exponentials effectively reduces to a single exponential decay:

$$\frac{Co^+}{(Co^+)_0} = e^{-kt} \qquad (1)$$

where $k = fk_{gs} + (1 - f)k_{ex}$. Co^+ corresponds to the sum of ground- and excited-state Co^+, f is the fraction of ground-state

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