although greater solvent participation is seen on the AGGA side.[19] On the basis of the limited data and methods available earlier, no kinetic isotope effect appeared associated with removal of substrate deuterium by the activated drug.[5] However, careful phosphorimager evaluation of the several labeled oligonucleotides available in these and other experiments[20] have shown that small but reproducible isotope effects can be measured for the TCCT ($k_H/k_D = 1.2 \pm 0.2$) and the AGGA strands ($k_H/k_D = 1.4 \pm 0.1$). A full description of these experiments will be reported in due course.[21]

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Simple Procedure for Resonance Assignment of the Sugar Protons in ^{13}C-Labeled RNAs

Arthur Pardi* and Edward P. Nikonowicz

Department of Chemistry and Biochemistry
Campus Box 215, University of Colorado, Boulder
Boulder, Colorado 80309-0215
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Scalar homonuclear 2D NMR experiments (such as DQF-COSY, RELAY, or TOCSY) are generally used to make resonance assignments of the sugar protons in nucleic acids.[1,2] However, the 2′ through 5′/5″ sugar protons in RNAs resonate over a very narrow chemical shift range (<1.0 ppm), making it difficult to resolve and assign these protons.[3] The utility of these homonuclear experiments is further limited in RNAs because the very small (≈1 Hz) H1′ to H2′ J coupling constant in A-form RNA makes it difficult to transfer magnetization from the crowded 2′ through 5′/5″ protons, to the well-resolved 1′ protons. To overcome this problem, we have designed a novel strategy for the unambiguous assignment of all protons in a ribose spin system involving application of 2D HCCH-COSY, HCCH-RELAY, and HCCH-TOCSY experiments.[4-6] This method is easily applied to any ^{13}C-labeled RNA and is illustrated here on the uniformly ^{13}C labeled RNA duplex, r(GGCGCUUGCGUC)$_2$.

In an HCCH experiment, connectivities between two protons in an individual ribose ring are made by transferring magnetization through their intervening carbon atoms.[4-6] For example, an HCCH-TOCSY experiment on an RNA duplex identifies all protons in the same ribose spin system (Figure S1, supplementary material). However, the proton type cannot be assigned from the TOCSY experiment alone; therefore, a series of HCCH experiments (Figure 1) are employed to assign the 2′, 3′, 4′, and 5′/5″

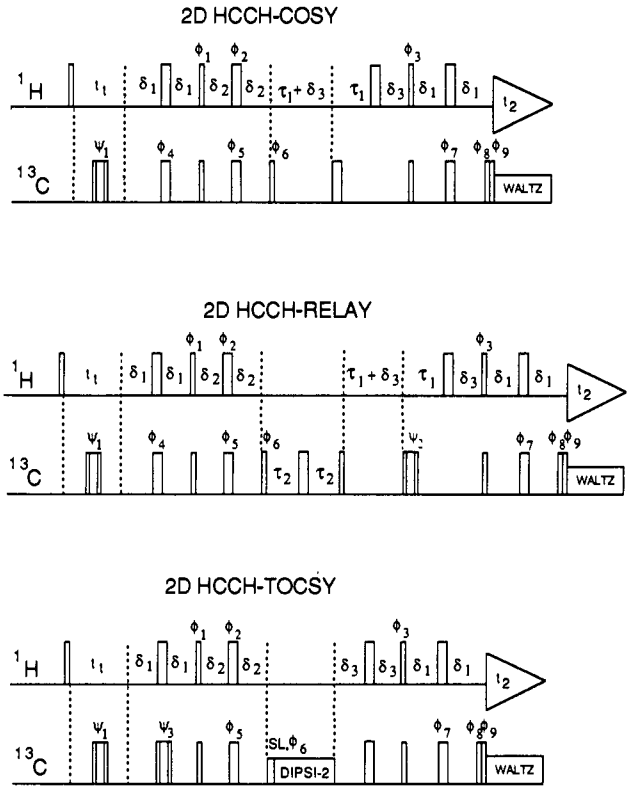

Figure 1. Pulse sequences used to collect the 2D HCCH experiments.[4,5] Narrow bars represent $\pi/2$ pulses, and wide bars represent π pulses. ψ denotes a composite π pulse of the form x-y-x. Unless otherwise stated, all pulses were of phase x. The phases of the pulses, are $\phi_1 = y, -y$; $\phi_2 = 4(x), 4(-x), 4(y), 4(-y)$; $\phi_3 = 4(x), 4(-x)$; $\phi_4 = 2(y), 2(-y)$; $\phi_5 = 2(x), 2(y), 2(-x), 2(-y)$; $\phi_6 = 4(x), 4(-x)$; $\phi_7 = 4(x), 4(-x)$, $\phi_8 = 16(x)$; $\phi_9 = 4(x), 4(-x)$; $\psi_1 = 8(x), 8(-x)$; $\psi_2 = 16(x)$; $\psi_3 = 2(x), 2(-x)$; and receiver = $(x, -x, -x, x, -x, x, x, -x)$. In the HCCH-TOCSY experiments, SL is a trim pulse of 1.2-ms duration. The delays in each of the experiments were $\delta_1 = 1.7$ ms, $\delta_2 = 1.1$ ms, $\delta_3 = 1.1$ ms, $\tau_1 = 1.6$ ms, and $\tau_2 = 4.6$ ms, except for one of the HCCH-TOCSY experiments, where $\delta_3 = 2.3$ ms, so that the 5′/5″ proton resonances are opposite in sign to the other ribose proton resonances. The HCCH-TOCSY experiments employed a 23-ms spin lock period using the DIPSI-2 sequence. In all the experiments the ^{13}C carrier was positioned in the center of the ribose region (~80 ppm). Sweep widths of 1600 and 3200 Hz were used in t_1 and t_2, respectively, 80 scans were collected for each FID, 120 complex points were collected in t_1, and 1024 complex points were collected in t_2. The NMR data were processed using FELIX (Hare, Inc.). The data were zero filled in t_1 and t_2 before Fourier transformation to give final real matrix sizes of 1024 × 2048 points.

protons as illustrated in Figure 2. Figure 2a shows part of the H1′ (ω_1) to the H2′ through H5′/H5″ (ω_2) region of a 2D HCCH-COSY experiment on the RNA duplex. The HCCH-COSY experiment only transfers magnetization through a single carbon–carbon bond,[4,5] and therefore the H1′ to H2′ is the only cross peak in this region. Figure 2b shows the same region of an HCCH-RELAY spectrum where magnetization is transferred up to two carbon–carbon bonds. Thus the new cross peak in this spectrum can only arise from a H1′ to H3′ connectivity. To assign the 4′ and 5′/5″ protons we employ refocused INEPT/reverse INEPT proton–carbon polarization transfers[7,8] in the HCCH-TOCSY experiment to differentiate between methylene and methyne protons (Figure 1). The HCCH-TOCSY experiment is used to filter out, or to select for, 5′/5″ methylene protons as illustrated in parts c and d, respectively, of Figure 2. In the first experiment (F_1), the reverse INEPT delay was set so that the 5′/5″ (methylene) protons are opposite in sign of all the other ribose protons. In the second experiment (F_2), the delay was set so that all the ribose protons have the same sign and similar intensities.

* Author to whom correspondence should be addressed.
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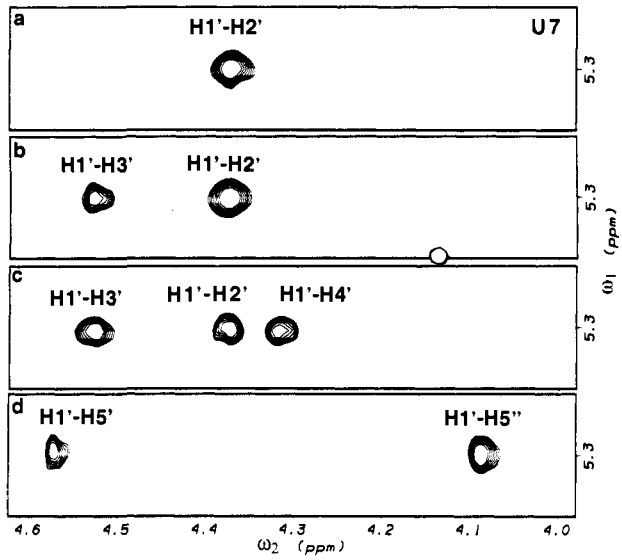

Figure 2. The H1′ (ω_1) to H2′ through H5′/H5″ (ω_2) region of the (a) HCCH-COSY, (b) HCCH-RELAY, (c) HCCH-TOCSY methylene filtered, and (d) HCCH-TOCSY methylene selected spectra on the uniformly ^{13}C labeled RNA duplex, r(GGCGCUUGCGUC)$_2$. Only the H1′ region for U7 is shown. The sample conditions were 1.8 mM RNA single strand, 150 mM NaCl, 10 mM potassium phosphate (pH = 6.8), and 0.1 mM EDTA. The labeled RNA was synthesized as previously described.[9,10,12] All the spectra were collected on a Varian VXR-500S NMR spectrometer at 30 °C.

The spectrum shown in Figure 2c is a linear combination of these two experiments, $F_1 + \alpha F_2$, where α is adjusted to cancel the 5′/5″ protons. Figure 2d is the difference of the two experiments, $F_1 - \beta F_2$, where β was adjusted to cancel the 2′ through 4′ protons. The optimal values for α and β were empirically determined by analyzing linear combinations of the first FIDs in the two experiments. An important advantage of this strategy is that, by directly selecting for only the 5′/5″ protons, it is possible to unambiguously assign the 5′/5″ protons even if these protons overlap with another proton in the same ribose ring. For larger RNAs this region of the spectrum will be more crowded, and therefore one can extend the strategy presented here to 3D heteronuclear HCCH experiments.[4-6] We have previously shown that the resolution of the RNA spectrum is substantially increased in 3D and 4D NMR experiments.[9,10]

The methods described here allow unambiguous identification of all the protons in an individual ribose ring, but each ribose ring must also be assigned to a specific residue in the RNA sequence. This assignment can be carried out by the standard sequential assignment techniques that rely on NOE connectivities between protons on neighboring residues.[1-3] However, tertiary interactions or unusual conformations in loops, bulges, or single-stranded regions of RNAs could lead to misassignments. Thus a superior method for making sequential assignments is to observe through-bond connectivities between neighboring residues by employing techniques such as the recently described hetero-TOCSY experiment.[11] We are presently testing a variety of triple-resonance (^1H, ^{31}P, ^{13}C) experiments to find optimal methods for through-bond sequential resonance assignment of uniformly ^{13}C labeled RNAs.

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Registry No. r(GGCGCUUGCGUC), 138153-87-4; ribose, 50-69-1; hydrogen ion, 12408-02-5.

Supplementary Material Available: Figure showing the H1′ to H2′-H5′/H5″ region of an HCCH-TOCSY spectrum acquired for the RNA duplex (1 page). Ordering information is given on any current masthead page.

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Non-Ionic Water-Soluble Dextran-Coupled Tetraphenylporphyrin Derivatives

Osamu Nakajima, Hidetoshi Mizoguchi, Yuichi Hashimoto,* and Shigeo Iwasaki

Institute of Applied Microbiology
The University of Tokyo
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

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Tetraphenylporphyrin (TPP) is an important classical hemin model for a variety of biological processes.[1] However, few mechanistic studies of the reactions of TPP and its ferric derivative under biological conditions have been carried out, primarily because of the insolubility of these compounds in aqueous solutions. In attempts to overcome this problem, water-soluble ionic porphyrins have been developed.[2] However, their tendency to aggregate, as well as the tendency of their ferric derivatives irreversibly to form an inactive μ-oxo dimer, restricts their usage as a tool for physicobiochemical research.[3] Though porphyrins which cannot form a μ-oxo dimer such as picket fence porphyrins[4] have been developed, they are water-insoluble. To overcome these problems, we have designed and prepared novel non-ionic water-soluble TPP analogs, i.e., Dex-TPP and Dex-TPPFeCl (Figure 1). As we had anticipated, coupling of TPP analogs to dextran polymer made them water-soluble as well as unable to aggregate or to form a μ-oxo dimer. In this paper, preparation of Dex-TPP and Dex-TPPFeCl, their behavior in aqueous solution, and their function as represented by DNA-cleavage ability are described.

The ligand, 5-(*p*-aminophenyl)-10,15,20-tri-*p*-tolylporphyrin, and its ferric derivative (TPP-NH_2 and TPPFeCl-NH_2, Figure 1) were prepared as described previously.[5] TPP-NH_2 or TPP-FeCl-NH_2 was coupled with dextran (MW > 2 000 000) by the method described by Norman et al.[6] with minor modifications. Briefly, dextran was partially oxidatively cleaved by $NaIO_4$ (0.01–0.2 equiv) in acetate buffer (pH 5), and then TPP-NH_2/TPPFeCl-NH_2 was coupled to it by reductive amination in DMSO containing an excess of $NaBH_3CN$. The adducts were precipitated by addition of EtOH, then redissolved in water, and purified by Sephadex G-50 gel chromatography (eluted with H_2O) to give Dex-TPP/Dex-TPPFeCl. The content of covalently bound TPP chromophores in dextran polymer could be controlled by varying the reaction conditions and was estimated to be 1–50 μmol/g by measuring the Soret band absorption (410–420 nm). For the experiments described below, Dex-TPP and Dex-TPPFeCl with porphyrin contents of 2.38 and 30.9 μmol/g, respectively,

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