the rate of the hydrolysis reaction. The alcohol dehydrogenase, coupled spectrophotometric assay may be used with little difficulty and with confidence at very small concentrations of substrate (<5 mM), whereas higher concentrations of substrate must be used in ^{13}C NMR in order to obtain reliable data. Thus, ^{13}C NMR may best be utilized in experimental situations where specific, clearly defined problems are conveniently and directly resolved such as the position of bond cleavage illustrated in the present study. At this time, ^{13}C NMR is probably not suitable for routine analyses of epoxide hydratase activity.

The ^{18}O isotope effect in ^{13}C NMR spectroscopy provides a continuous, direct method to evaluate simultaneously the rate of hydrolysis, the position of bond cleavage, and the extent of accompanying oxygen exchange in acid- and microsomal epoxide hydratase-catalyzed hydrolysis of 2,2-dimethyloxirane. This example further illustrates the applicability of this phenomenon in the analysis of a variety of research problems.

Acknowledgment. This investigation was supported by USPHS Research Grant GM 27003-10 from the National Institute of General Medical Sciences and by NIH Grant RR 01077 from the division of Research Resources. We thank Donna Radzik and Susan Hommel of Purdue University for a gift of mouse liver microsomes and Dr. Anthony Y. H. Lu of Merck Sharp & Dohme for the generous gift of homogeneous rat liver microsomal epoxide hydratase.

Registry No. ^{18}O, 14797-71-8; 2,2-dimethyloxirane, 558-30-5; epoxide hydratase, 9048-63-9; 2,2-dimethyl[3-^{13}C]oxirane, 84624-88-4; 2,2-dimethyl[3-^{13}C,^{18}O]oxirane, 84624-89-5; [^{13}C]methyldiphenylsulfonium perchlorate, 84624-91-9; acetone, 67-64-1; [^{18}O]acetone, 7217-26-7.

Communications to the Editor

Sequential Assignments for the ^1H and ^{31}P Atoms in the Backbone of Oligonucleotides by Two-Dimensional Nuclear Magnetic Resonance

Arthur Pardi,*‡ Roger Walker,† Henry Rapoport,† Gerhard Wider,§ and Kurt Wüthrich‡

Institut für Molekularbiologie und Biophysik
Eidgenössische Technische Hochschule
Hönggerberg, CH-8093 Zürich, Switzerland
Department of Chemistry and Lawrence Berkeley Laboratory
Univeristy of California, Berkeley
Berkeley, California 94720
and Spectrospin AG, CH-8117 Fällanden, Switzerland

Received November 10, 1982

A novel application of two-dimensional nuclear magnetic resonance (2-D NMR) for assignment of hydrogen and phosphorus nuclei in the sugar phosphate backbone of oligonucleotides is described and illustrated by the assignment of the tetranucleotide d-CpTpApG. The assignments are made by observation of homonuclear (^1H-^1H) and heteronuclear (^1H-^{31}P) scalar spin-spin couplings.

Proton NMR has been extensively used to study the conformation and dynamics of oligonucleotides in solution.[1] Although the coupling constants for the protons in the sugar rings provide information on the sugar and phosphate backbone conformation,[2] the difficulties involved in assigning these protons have limited the applications. 2-D NMR experiments overcome many of the problems of selective decoupling and extensive overlap of resonances observed in conventional one-dimensional studies. The approach of sequential assignments outlined here allows the complete assignment of the sugar phosphate backbone solely from the 2-D NMR experiments and knowledge of the covalent structure of the backbone.

The first step in the assignment procedure involves the identification of the proton spin systems of the individual sugar rings.

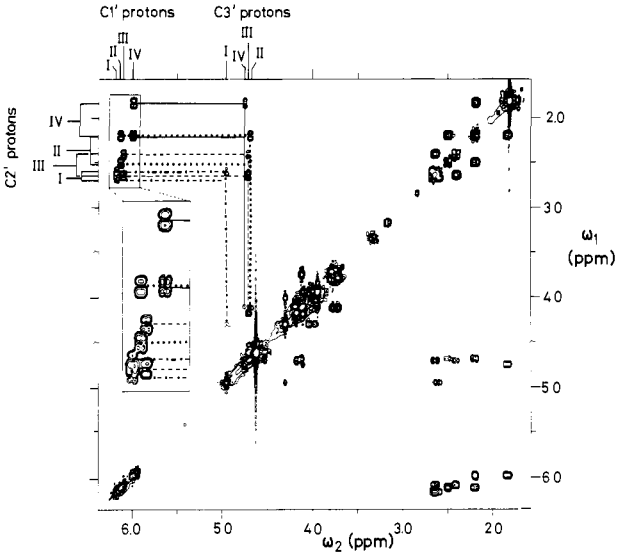

Figure 1. Contour plot of an absolute value 500-MHz ^1H COSY spectrum of 0.02 M d-CpTpApG in ^2H_2O, p^2H 8.0, T = 40 °C. The C1′C2′ proton cross peaks are also shown on an expanded scale in the inset. The chemical shifts of the C1′, C2′, and C3′ protons are indicated on the margins, where the four deoxyribose spin systems are arbitrarily labeled I (---), II (···), III (- - -), and IV (—).

This information was obtained with homonuclear correlated spectroscopy (COSY).[3,4] COSY spectra for d-CpTpApG[5] are shown in Figures 1 and 2. J connectivities between individual protons are manifested by cross peaks which appear symmetrically with respect to the diagonal. The deoxyribose spin system, which includes the lowest field C1′ proton at 6.16 ppm, was arbitrarily labeled "sugar I". It shows cross peaks to C2′ protons at 2.60 and 2.68 ppm (Figure 1). The C2′ protons then show coupling to the C3′ proton at 4.95 ppm (Figure 1), and this C3′ proton has

‡ETH Zürich.
†University of California, Berkeley.
§Spectrospin AG.

(1) (a) For a recent review see: Sarma, R. H. "Nucleic Acid Geometry and Dynamics"; Pergamon Press: New York, 1980. (b) Cheng, D. M.; Kan, L.-S.; Leutzinger, E. E.; Jayaraman, K.; Miller, P. S.; Ts'o, P. O. P. *Biochemistry* **1982**, *21*, 621-630. (c) Altona, C.; van Boom, J. H.; Haasnoot, C. A. G. *Eur. J. Biochem.* **1982**, *71*, 557-562. (d) Melleman, J. R.; Haasnoot, C. A. G.; van Boom, J.; Altona, C. *Biochim. Biophys. Acta* **1982**, *665*, 256-264. (e) Neumann, J.-M., Huynh-Dinh, T.; Kan, S. K.; Genissel, B.; Igolen, J.; Tran-Dinh, S. *Eur. J. Biochem.* **1982**, *121*, 317-323.

(2) Cheng, D. M.; Sarma, R. H. *J. Am. Chem. Soc.* **1977**, *99*, 7333-7348.

(3) (a) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2220-2246. (b) Nagayama, K.; Anil Kumar; Wüthrich, K.; Ernst, R. R. *J. Magn. Reson.* **1980**, *40*, 321-334. (c) Bax, A.; Freeman, R.; Morris, G. *J. Magn. Reson.* **1981**, *42*, 164-168.

(4) Detailed descriptions of the experimental parameters for the 2-D experiments are given in the supplementary material.

(5) (a) This tetramer was synthesized by the phospho triester method using *N*-benzyloxycarbonyl-blocked nucleosides in solution (see ref 6b and 6c). (b) Watkins, B. E.; Kiely, J. S.; Rapoport, H. *J. Am. Chem. Soc.* **1982**, *104*, 5702-5708. (c) Watkins, B. E., Rapoport, H. *J. Org. Chem.* **1982**, *47*, 4471.

0002-7863/83/1505-1652$01.50/0

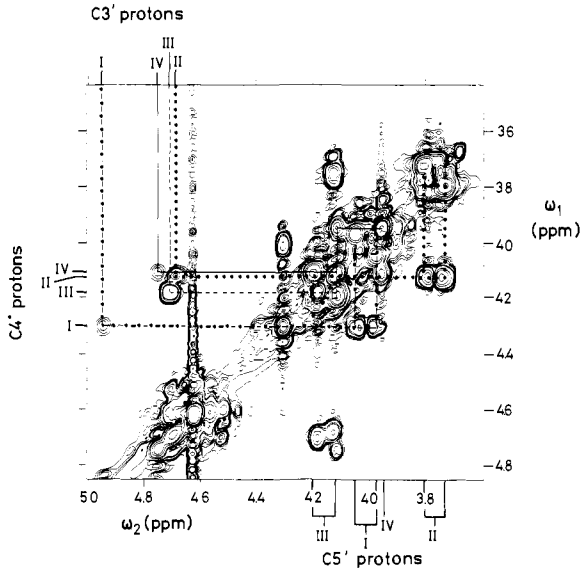

Figure 2. Expansion of the region of Figure 1 needed for analysis of the H3′–H4′ and H4′–H5′ connectivities. Same presentation as in Figure 1.

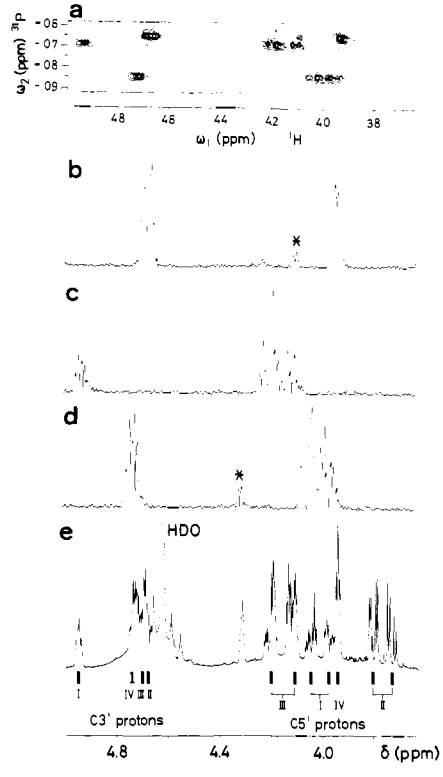

Figure 3. (a) Contour plot of an absolute value 121-MHz ^{31}P (300 MHz ^{1}H) ^{31}P–^{1}H chemical shift correlation spectrum of d-CpTpApG under the same conditions as in Figure 1 except that the concentration was 0.009 M. Cross sections of the ^{31}P signals at (b) –0.65, (c) –0.69, and (d) –0.85 ppm (relative to 15% phosphoric acid) are also shown. The one-dimensional proton spectrum is shown in e, where the chemical shifts of the C3′ and C5′ protons are also indicated. The asterisks in b and d indicate cross peaks arising from long-range couplings between C4′H and ^{31}P.

a cross peak with the C4′ proton at 4.30 ppm (Figure 2). Figure 2 also shows that there are cross peaks linking the C4′ proton with two C5′ protons at 3.97 and 4.05 ppm; the geminal coupling of the two C5′ protons is also manifest. As is illustrated in Figures 1 and 2 the three other sugar spin systems (labeled II–IV) can be traced in a manner similar to sugar I. For sugar III one of the cross peaks linking H4′ and the two C5′ protons overlaps with

Table I. Chemical Shifts (ppm) of the Sugar Protons in d-C^{1}pT^{2}pA^{3}pG^{4} (p^{2}H 8.0, T = 40 °C, Shifts Are Relative to Internal Sodium 3-(Trimethylsilyl)[2,2,3,3-$^{2}H_4$]propionate; "TSP")

residue (spin system)	chemical shift, ppm				
	C1′H	C2′H_2[a]	C3′H	C4′H	C5′H_2[a]
dC^{1} (II)	6.11	2.21, 2.52	4.69	4.12	3.72, 3.80
dT^{2} (IV)	5.97	1.85, 2.21	4.74	4.11	3.94, 3.94
dA^{3} (I)	6.16	2.60, 2.68	4.95	4.30	3.97, 4.05
dG^{4} (III)	6.08	2.42, 2.66	4.71	4.18	4.11, 4.20

[a] The stereospecifity of the two protons at these positions was not assigned.

the H5′H5″ cross peaks, and the other H4′–C5′ proton cross peak is too close to the diagonal to observe. In sugar IV the two C5′ protons have equivalent chemical shifts and thus no H5′H5″ cross peak is observed. The four sugar spin systems in d-CpTpApG have thus been completely identified, and the chemical shifts are listed in Table I.

The second step in the assignment procedure is to observe the ^{31}P resonances of the phosphate groups and to identify, by heteronuclear chemical shift correlation spectroscopy,[6,7] the deoxyribose spin systems bound to each phosphate group from the scalar ^{31}P–^{1}H couplings. Figure 3a shows a contour plot of this experiment for d-CpTpApG, where cross peaks are observed at positions (ω_1^A, ω_2^B) when there is scalar coupling between the proton at ω^A and the ^{31}P nucleus at ω^B. The pulse sequence employed[4] eliminates the proton coupling in the phosphorus signals along ω_2 and the phosphorus coupling in the proton signals along ω_1.

The ^{31}P resonance at –0.69 ppm shows coupling to the C3′ proton of sugar spin system I at 4.95 ppm (Figure 3c). The C5′ protons at 4.11 and 4.20 ppm, which are coupled to this phosphorus, are from sugar III. Therefore this ^{31}P atom connects the sugar spin systems I and III in a I(3′p5′)III linkage.

The ^{31}P resonance at –0.85 ppm (Figure 3d) shows a H3′ signal from sugar IV at 4.74 ppm and C5′ proton peaks at 3.97 and 4.05 ppm from sugar I, indicating a IV(3′p5′)I linkage. The third ^{31}P signal at –0.65 ppm (Figure 3b) has coupling to the C5′ protons from sugar IV at 3.94 ppm and also shows coupling to a C3′ proton at ca. 4.70 ppm. The C3′ protons of sugars II and III have very similar chemical shifts (Table I), so it is difficult to a priori assign this cross peak; however, sugar III can be ruled out since this would require a cyclic nucleotide. Thus the ^{31}P resonance at –0.65 forms a II(3′p5′)IV sugar linkage. These results show that the sequence of the sugars is IIpIVpIpIII, and the sugars I–IV are assigned, respectively, to the A, C, G, and T residues in d-CpTpApG.

We have outlined a novel method to sequentially assign the sugar phosphate backbone in oligonucleotides by application of 2-D NMR techniques. Complete assignments for the backbone of a tetranucleotide were obtained without reference to smaller fragments of the oligonucleotide. Although this sequential assignment method could in principle be used with one-dimensional NMR techniques, it is the inherently better resolution and the greater efficiency of the 2-D NMR experiments that promise to make this a generally practicable approach.

Acknowledgment. Financial support by Schweizerischer Nationalfonds (project 3.528.79), a NATO Postdoctoral Fellowship (to A.P.), and the Division of Biomedical and Environmental Research, Department of Energy, U.S.

Registry No. d-CpTpApG, 84602-75-5.

Supplementary Material Available: Experimental parameters for the 2-D NMR experiments are described (1 page). Ordering information is given on any current masthead page.

(6) Bodenhausen, G.; Freeman, R. *J. Magn. Reson.* **1977**, *25*, 471–476.
(7) Bax, A.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 501–505.