Acknowledgment. The capable technical assistance of D. Rothfuss is greatly appreciated.

Registry No. 1, 87101-78-8; **2a**, 87101-79-9; **2a**·$NaPF_6$, 87101-81-3; **2b**, 87114-18-9; **2b**·$NaPF_6$, 87101-83-5.

Supplementary Material Available: NMR (^1H, ^{13}C, and ^{31}P) characterization of **1**, **2a**, **2a**·$NaPF_6$, **2b**, and **2b**·$NaPF_6$ (3 pages). Ordering information is given on any current masthead page.

^{17}O NMR as a Probe of Nucleic Acid Dynamics

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Received April 21, 1983

Given the importance of structural flexibility of polynucleotides, considerable effort has been directed toward providing a detailed characterization of nucleic acid dynamics. NMR spin-relaxation techniques provide a convenient measure of molecular motion, and a variety of nuclei (^1H, ^{13}C, ^{15}N, and ^{31}P) have been used in studies of polynucleotides.[2-9] The relaxation times for various nuclei are sensitive to different types of internal motion by virtue of the different processes dominating the relaxation; in principle, the analysis of results obtained with several probe nuclei should lead to a detailed description of nucleic acid dynamics. A complete analysis of this type would be facilitated by a nuclear probe sensitive only to the reorientation of the phosphate groups and uncomplicated by spin interactions with sites on adjacent ribose rings. Specific enrichment of the nonesterified phosphoryl oxygens with ^{17}O would provide the desired spin probe. ^{17}O NMR spin relaxation is dominated by the interaction of the nuclear quadrupole moment with the electric field gradient defined by the bonding electrons; this relaxation mechanism provides a well-defined monitor of P–O bond reorientation. In this communication we demonstrate that useful ^{17}O NMR spin-relaxation data can be obtained for a simple polynucleotide, polyadenylic acid (poly A). While a complete analysis of the spin relaxation in this spin $^5/_2$ system is not presented, a qualitative analysis of the data leads to useful conclusions regarding internal motions.

The ^{17}O-labeled poly A was the generous gift of Professor Philip H. Bolton of Wesleyan University. This material was prepared by the action of polynucleotide phosphorylase on [α-$^{17}O_2$]ADP synthesized in this laboratory and having a ^{17}O enrichment of 50% in each labeled position.[10] Spectra were obtained on a Bruker CXP-200 spectrometer with a probe of our own design.

The spectra in Figure 1 demonstrate the temperature dependence of the poly A ^{17}O line shape. The sharp resonance defining 0 ppm in each spectrum is from the residual ^{17}O in ^{17}O-depleted H_2O (0.008%). The broad resonance centered at ~80 ppm is from the labeled nonesterified phosphoryl oxygens in the poly A. At 90 °C the poly A resonance is well represented as a single Lorentzian peak with a full width at half-height of 1400 Hz. The relative areas of the poly A and H_2O resonances are consistent with the relative concentrations of ^{17}O in each, indicating the single

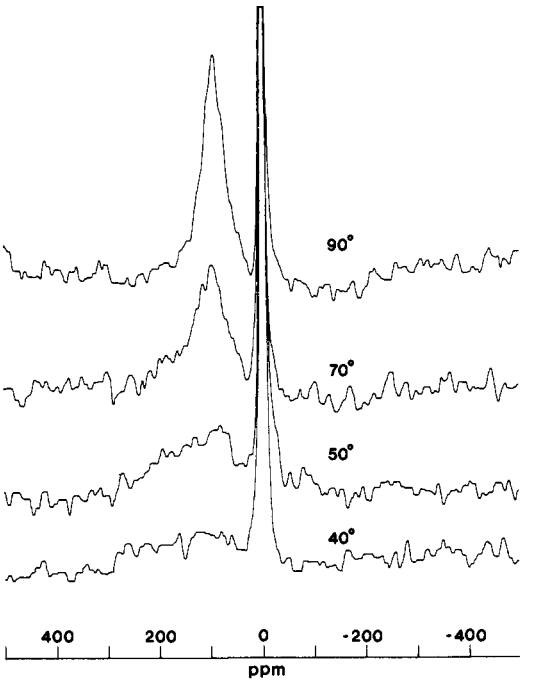

Figure 1. ^{17}O NMR spectra at 27.1 MHz of [^{17}O]poly A as a function of temperature. Labeled poly A (4 mM in monomeric units) was dissolved in 0.1 M sodium cacodylate buffer containing 0.1 M NaCl and 2 mM EDTA; ^{17}O-depleted water (0.008% ^{17}O) was used as the solvent.

Table I. ^{17}O Spin Relaxation Parameters for Poly A

temp, °C	line width, Hz	T_2,[a] ms	T_1,[b] ms	$\bar{l}$,[c] (bases)	α[c]
90	1400	0.23		1.6	0.12
80	1500 ± 100	0.21 ± 0.01	0.45	1.8	0.17
70	1750	0.18 ± 0.02		2.0	0.24
65	2130 ± 300	0.15 ± 0.02	0.25	2.1	0.28
60	NL[d]			2.3	0.32
50	NL[d]		0.4 ± 0.1	2.8	0.43
40	NL[d]			3.7	0.54
30	NR[d]			5.2	0.67

[a] Calculated from the full width at half-maximum; $T_2 = (\pi \times$ line width)$^{-1}$. [b] Estimates from the least-squares fits of ln ($[A(\infty) - A(t)]/A(\infty)$) vs. delay. [c] $\bar{l}$ = average number of contiguously stacked bases; α = fraction of bases in the stacked state. Both calculated using $\Delta H^\circ = -9.6$ kcal/mol stack, $\Delta S^\circ = -30.3$ cal K^{-1}/mol stack, $\sigma = 1$.[14] [d] NL = non-Lorentzian; NR = no observed resonance.

Lorentzian represents all of the labeled sites in poly A. At lower temperatures it is apparent that the resonance is no longer a single Lorentzian. This is particularly true at 50 °C. Below 40 °C it was not possible to discern a poly A resonance, indicating the resonance width to be substantially in excess of the response width of the probe, 10 KHz.

Table I contains estimates of the transverse relaxation times (T_2) calculated from line widths at those temperatures where the resonance appears to be Lorentzian and undiminished in area. For spin $^5/_2$ nuclei deviation from a Lorentzian line shape occurs outside of the fast motion limit.[11-13] For ^{17}O at 27.1 MHz (1.7×10^8 s^{-1}), non-Lorentzian resonances should be observed for effective correlation times on the order of a few nanoseconds.[12] The deviation from Lorentzian line shape near 40 °C is then consistent with ^{31}P studies of poly A in which the isotropic tumbling responsible for relaxation was found to be on the order of nanoseconds at this temperature.[7] The temperature dependence of the ^{17}O line shape near 40 °C is sharp and parallels the degree of base stacking, α, or number of contiguously stacked bases, l

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(Table I).[14] The isotropic tumbling times defined by the ^{31}P experiments show the same sharp temperature dependence.[7]

Inversion recovery experiments were also performed at 80, 65, and 50 °C. The recovery curves were linear on a log plot to a good approximation except at 50 °C where a smaller signal-to-noise ratio leads to a higher uncertainty. The T_1's from the log plots vary little from 80 to 50 °C (Table I), whereas the difference in line shapes over this temperature range is very significant. The data suggest that the T_1's are dominated by a different motion from that which dominates line shapes and that this motion is less sensitive to the degree of base stacking. In the limit of relatively unrestricted and fast internal motion an isotropic model can be used to analyze T_1 data. From a value of 4.1×10^7 s^{-1} for the quadrupole coupling constant[15] and the T_1 data at 80 °C, a correlation time of 0.06 ns is predicted. While this is somewhat shorter than the time assigned to internal motions from ^{31}P data, both ^{31}P and ^{17}O data suggest that a motional model with two time scales is necessary. Quantitative differences in time scales may reflect departure of real motions from assumed models for these motions.

Even without further analysis, our experiments suggest that nucleic acid dynamics can be effectively studied with ^{17}O NMR spectroscopy: the spectra are easily obtained and the relaxation data are readily interpreted by virtue of the dominating quadrupolar relaxation mechanism for ^{17}O. We anticipate that more detailed analyses of relaxation data will be facilitated by both the examination of sequence-defined oligonucleotides and improvements in instrument design that allow more accurate measurements of line shapes.

Acknowledgment. This research was supported by grants (GM-30562 to J.A.G. and GM-19035 to J.H.P.) from the National Institutes of Health. The high-field NMR spectrometer used in this research is supported in part by a grant from the National Science Foundation (CHE-791620).

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Photofragmentation Dynamics of $Cr(CO)_6$ in the Gas Phase

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The photochemistry of $Cr(CO)_6$ has been extensively studied.[1-4] Nevertheless, important questions remain concerning the role of excited states and the timing of the various ligand-dissociation steps. We have used a method based on time-resolved CO laser absorption spectroscopy[5,6] to address these questions.

In our experiments, a 1-m absorption cell is filled with 1-70 mtorr of $Cr(CO)_6$ at 300 K. The sample is photoactivated with a KrF* laser (249 nm, 15 ns, <0.5 mJ/cm^2). A continuous-wave, grating-tuned CO laser[7] is directed through the cell coaxially with the KrF* beam and then onto an InSb detector (rise time $\lesssim$ 100 ns). Photogenerated CO produces a transient decrease in CO laser intensity reaching the InSb detector, and the resulting signal change is recorded. A typical transient absorption is shown in Figure 1a. These data provide information on the rates of CO formation, vibrational relaxation, and metal carbonyl-CO recombination. It is apparent that all of the photogenerated CO is *not* consumed by recombination on a millisecond time scale, i.e., some of the unsaturated metal carbonyls irreversibly recombine with one another or with $Cr(CO)_6$. This will be discussed subsequently.[8] Here we discuss the portion of Figure 1a where CO absorption grows in, which provides information on the dynamics of CO generation. See Figure 1b, where CO absorption at early times is displayed with higher resolution. These data were obtained by observing CO(v = 0) with the $P_{12}(1,0)$ CO laser line, but qualitatively similar results are obtained if CO(v = 1) is observed with the $P_{10}(2,1)$ CO laser line. A comparison of absorption amplitudes using these two laser lines indicates that [CO(v = 0)]/[CO($v \geq 1$)] $\simeq$ 10. The data shown in Figure 1b cannot be fit by a single exponential and, as shown in Figure 2 and subsequent pressure-dependence studies, consist of at least four components (I-IV): I, a region of rise time < 100 ns, independent of pressure, constituting ca. 20% of the total absorption amplitude; II, a region where the rise time is pressure dependent with a CO appearance rate[9] $k_{II} \simeq 7 \times 10^6$ s^{-1} at 26 mtorr, corresponding

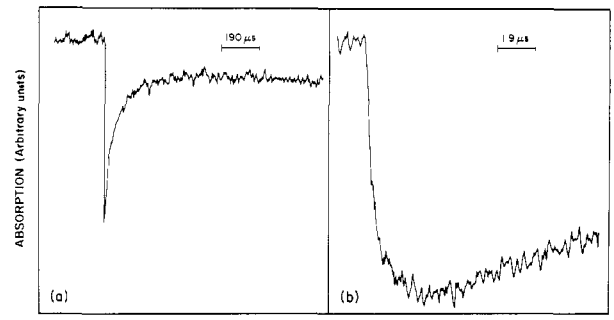

Figure 1. (a) Transient CO laser absorption [$P_{12}(1,0)$] produced by irradiating 49 mtorr of $Cr(CO)_6$ at 249 nm. (b) Rising portion of the CO laser absorption curve; $Cr(CO)_6$ pressure is 61 mtorr.

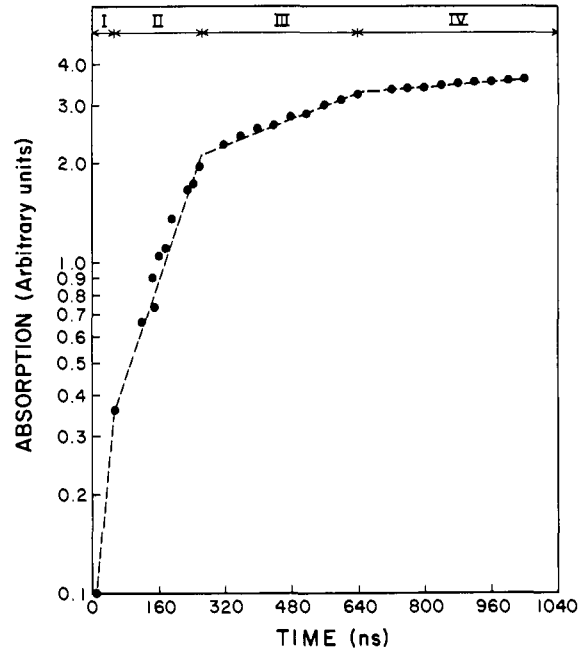

Figure 2. Semilog plot of data from Figure 1b. I-IV denote absorption regions described in text.

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