version. The situation may be different in other amines in which vicinal nonbonded repulsions are less severe, *e.g.*, methylamine.

Theoretical calculations for species electronically analogous to amines, *e.g.*, $^{-}CH_2SH$, $^{-}CH_2S(O)H$, $^{-}CH_2S(O_2)H$, indicate a common transition state for rotation and inversion.[3]

Thus, it is not surprising that separate changes at different temperatures in the dynamic nmr spectrum of trialkylamines corresponding to rotation and inversion have not been reported. It is apparent that the two rate processes share the same transition state.

We are continuing our investigations in this area, especially with respect to rotational phenomena in those amines in which inversion is significantly slowed by substitution of electronegative groups on nitrogen.[3]

Acknowledgment. We thank the referees for helpful suggestions and the National Science Foundation for support (GP-18197). J. W. O. thanks the National Aeronautics and Space Administration for a traineeship, 1969–1970.

C. Hackett Bushweller,* James W. O'Neil, Howard S. Bilofsky
Department of Chemistry, Worcester Polytechnic Institute
Worcester, Massachusetts 01609
Received September 28, 1970

Conformation and Segmental Motion of Native and Denatured Ribonuclease A in Solution. Application of Natural-Abundance Carbon-13 Partially Relaxed Fourier Transform Nuclear Magnetic Resonance[1]

Sir:

The high sensitivity of the Fourier transform nmr technique[3] makes it practical to study proton-decoupled natural abundance carbon-13 spectra of proteins.[2] Even at the relatively low magnetic field strength of 14.1 kG, a proton-decoupled carbon-13 spectrum of a protein is well separated into groups of resonances arising, in order of increasing shielding, from carbonyl groups, unsaturated side chains, α-carbons, and saturated side-chain residues.[2,4] By comparison, proton nmr spectra of proteins consist mainly of featureless envelopes resulting from a small range of chemical shifts and complicated spin–spin splittings.

We wish to demonstrate the value of carbon-13 spin–lattice relaxation time (T_1) measurements of individual resonances in protein spectra by means of partially relaxed Fourier transform (PRFT) nmr.[5,6] In this technique, the magnetization is first inverted by means of a 180° rf pulse. After an interval τ, a 90° pulse is applied and the digital signal averager is triggered. The sequence is repeated after a waiting period of several multiples of T_1. The resulting time-domain signal following an appropriate number of accumulations is Fourier transformed to yield a partially relaxed frequency-domain spectrum. If τ is much shorter than the T_1 of a particular resonance, a "negative" peak will appear, with an amplitude equal to that in the normal spectrum. As τ increases, this negative peak decreases in amplitude, goes through a null (at $\tau = T_1 \ln 2$), becomes positive, and for $\tau \gg T_1$ becomes equal to the normal resonance. Each amplitude A is given by[7]

$$A = A_0[1 - 2\exp(-\tau/T_1)] \quad (1)$$

where A_0 is the equilibrium amplitude.

The experiments were carried out on a high-resolution carbon-13 Fourier transform nmr spectrometer consisting mainly of a Varian high-resolution 14.1-kG electromagnet, a "home-built" nmr apparatus operating at 15.08 MHz, a Fabri-Tek 1074 signal averager, and a PDP-8/I computer. The apparatus included an external ^{19}F lock and noise-modulated proton decoupling.

A set of partially relaxed carbon-13 spectra of 0.019 *M* bovine pancreatic ribonuclease A at pH 6.51 (45°) is shown in Figures 1B–1F, with τ values ranging from 336.9 to 7.96 msec. Figure 1A shows the normal Fourier transform spectrum. Carbonyl signals are in the range 10–25 ppm "upfield" from CS_2. Unsaturated side chains are at 35–80 ppm. The β-carbon signal of the threonine residues is at 126 ppm. The α-carbon region is at 130–150 ppm. It excludes glycine but contains the β-carbon signals of the serine residues. The region above 150 ppm contains the α-carbon of the glycine residues and the remaining saturated side-chain carbons. More detailed assignments have been given by Allerhand, Cochran, and Doddrell.[2]

Least-squares analysis of the data in Figure 1 yields spin–lattice relaxation times for each resolved resonance. Representative results are given in Table I. We defer the interpretation of the T_1 values for nonprotonated carbons to a later publication.[8] If the ^{13}C

Table I. Some Carbon-13 Spin–Lattice Relaxation Times and Rotational Correlation Times in Aqueous Ribonuclease A[a,b]

Type of carbon	Native protein[c] T_1, msec	Native protein[c] τ_R, nsec	Denatured protein[d] T_1, msec	Denatured protein[d] τ_R, nsec
Carbonyls	416		539	
α-Carbons	42	30[e]	120	0.40
β-Carbons, Thr	~40	~30	99	0.48
Rigid side chains[f]	~30			
ϵ-Carbons, Lys	330	0.070	306	0.076

[a] Obtained for 0.019 *M* protein at 45° and 15.08 MHz. The T_1 and τ_R values have an estimated maximum error of ±30%. Bovine pancreatic ribonuclease A was obtained from Worthington Biochemical Corp., Freehold, N. J., and from Miles Laboratories, Inc., Elkhart, Ind. Samples from the two sources showed no measurable differences in behavior. [b] The temperature and pH dependence of the conformational transition are given by J. F. Brandts, *J. Amer. Chem. Soc.*, **87**, 2759 (1965). [c] pH 6.51. [d] pH 1.64. [e] Equation 2 yielded a second solution, 1.4 nsec, which gave α-carbon line widths of 8 Hz, in major disagreement with the observed envelope in Figure 1A, which was well simulated using τ_R = 30 nsec (45-Hz line width). [f] Broad signal component at 150–185 ppm in the spectrum of the native protein. Difficulties in separating these resonances from the narrow ones make their T_1 only an estimate of an average value.

(1) Carbon-13 Fourier Transform Nuclear Magnetic Resonance. III. Part II: ref 2.
(2) A. Allerhand, D. W. Cochran, and D. Doddrell, *Proc. Nat. Acad. Sci. U. S.*, 67, 1093 (1970).
(3) R. R. Ernst and W. A. Anderson, *Rev. Sci. Instrum.*, 37, 93 (1966).
(4) P. C Lauterbur, *Appl. Spectrosc.*, 24, 450 (1970).
(5) We propose the name partially relaxed Fourier transform (PRFT) nmr for the technique first described in ref 6.
(6) R. L Vold, J. S. Waugh, M. P. Klein, and D. E. Phelps, *J. Chem. Phys.*, 48, 3831 (1968).
(7) A. Abragam, "The Principles of Nuclear Magnetism," Oxford University Press, London, 1961, p 64.
(8) A. Allerhand, D. Doddrell, V. Glushko, D. W. Cochran, E. Wenkert, P. J. Lawson, and F. R. N. Gurd, manuscript in preparation.

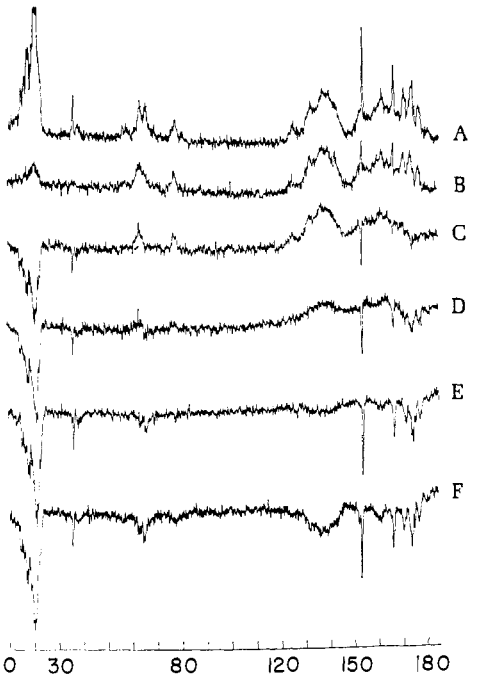

Figure 1. Proton-decoupled natural-abundance carbon-13 Fourier transform nmr spectra of 0.019 *M* ribonuclease A at 45°, pH 6.53, and 15.08 MHz. Each spectrum is the result of 16,384 scans, with a recycle time of 1.36 sec (about 6 hr total accumulation time per spectrum). Horizontal scale is in parts per million upfield from neat CS_2. (A) Normal Fourier transform spectrum, (B–F) PRFT spectra. The τ intervals (msec) are: (B) 336.9, (C) 82.2, (D) 39.8, (E) 18.6, (F) 7.96.

nuclei are proton decoupled, the spin–lattice relaxation of the protonated carbons is easy to interpret, because it is exponential and overwhelmingly dominated by the dipole–dipole interaction with the directly bonded protons.[9] In this case T_1 is given by

$$\frac{1}{T_1} = \frac{N\hbar^2\gamma_C^2\gamma_H^2}{10r_{CH}^6}\left[\frac{\tau_R}{1+(\omega_C-\omega_H)^2\tau_R^2} + \frac{3\tau_R}{1+\omega_C^2\tau_R^2} + \frac{6\tau_R}{1+(\omega_C+\omega_H)^2\tau_R^2}\right] \quad (2)$$

where γ_C and γ_H are the gyromagnetic ratios of ^{13}C and ^1H, N is the number of directly bonded hydrogens, r_{CH} is the C–H distance, ω_C and ω_H are the ^{13}C and ^1H resonance frequencies in radians/second, and τ_R is the correlation time for rotational reorientation. Equation 2 is easily derived using Solomon's treatment.[10] It is analogous to his eq 41 for paramagnetic solutions and it is strictly valid when the rotational reorientation is isotropic. Equation 2 is used here as a first approximation. A more rigorous treatment including anisotropic effects will be given elsewhere.[8]

Representative values of rotational correlation times for backbone and side-chain carbons are given in Table I. In general, two to six real solutions for τ_R can arise from eq 2, for *arbitrary* values of the ratio ω_C/ω_H. However, in our case ω_C/ω_H is 0.2514 at any magnetic field. It can be shown that in this case there are only two real solutions for τ_R. The frequency dependence of T_1 can be used to choose the correct solution. However, we were not equipped for measurements at frequencies other than 15.08 MHz. An alternative procedure is to measure the transverse relaxation rate $1/T_2$, which increases monotonically with τ_R.[10] Even if only a crude estimate of T_2 is made from line-wdith measurements, the correct choice of τ_R can be made when the two solutions are very different, because then the two corresponding values of T_2 are also very different. This procedure is not easily applied to the α-carbons of the native protein because the α-carbon lines are not resolved. The choice of τ_R was made in this case by computer simulation of the spectra, using known amino acid chemical shifts.[2,11] One of the solutions for τ_R, 30 nsec, yielded line widths which produced a good computer simulation of the experimental α-carbon envelope, while the other solution, 1.4 nsec, predicted a series of relatively sharp lines, in gross disagreement with the α-carbon spectrum.

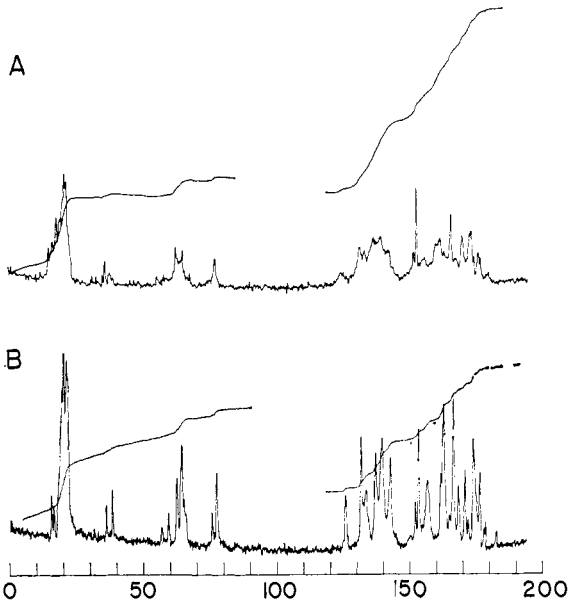

Figure 2. Proton-decoupled natural-abundance carbon-13 Fourier transform nmr spectra of ribonuclease A at 45° and 15.08 MHz. Horizontal scale is in parts per million upfield from neat CS_2. (A) Normal spectrum of 0.017 *M* native protein at pH 4.12, resulting from 32,768 scans in 6 hr. (B) Normal spectrum of 0.015 *M* denatured protein at pH 1.46, from 31,284 scans in 12 hr. See footnote *b* in Table I.

The correlation time of the α-carbons in native ribonuclease (Table I) is in good agreement with that expected for the protein backbone.[12] As for the side chains, some appear to have a small degree of segmental motion, as exemplified by the β-carbons of the threonine residues (at 126 ppm) and by the carbons giving rise to the broad envelope above 150 ppm. However, other side-chain resonances above 150 ppm are sharp, have relatively long T_1 values, and thus originate from carbons with a large degree of internal motion. An extreme example is provided by the ϵ-carbons of the ten lysine residues (at 153.7 ppm) which have a rotational correlation time approaching that of a small molecule.

The carbon-13 spectrum of acid-denatured ribonuclease (Figure 2B) has much sharper lines than that of the native protein (Figure 2A). We have also ob-

(9) K. F. Kuhlmann, D. M. Grant, and R. K. Harris, *J. Chem. Phys.*, 52, 3439 (1970).
(10) I. Solomon, *Phys. Rev.*, 99, 559 (1955).
(11) W. Horsley, H. Sternlicht, and J. S. Cohen, *J. Amer. Chem. Soc.*, 92, 680 (1970).
(12) J. Yguerabide, H. F. Epstein, and L. Stryer, *J. Mol. Biol.*, 51, 573 (1970).

tained T_1 values from partially relaxed spectra of the denatured protein (Table I). The correlation times of the α-carbons are much shorter than in the native protein (Table I), indicating an appreciable degree of segmental motion in the backbone of denatured ribonuclease. Significantly, τ_R of the ϵ-carbons of the lysine residues is not measurably affected by denaturation.

It is apparent that PRFT nmr spectra provide a powerful technique for studying proteins in solution. In the past, the most common way of using magnetic resonance to study biopolymer reorientation in solution has been by means of artificially attached electron[13] or nuclear spin labels.[14] Partially relaxed carbon-13 Fourier transform nmr is an alternative technique which provides a *multitude* of built-in probes in any *unmodified* biopolymer.

Acknowledgment. This research was supported by the National Science Foundation (Grant No. GP-17966, to A. A.), the donors of the Petroleum Research Fund, administered by the American Chemical Society (Grant No. 4559-AC5, to A. A.), and the National Institutes of Health (Grant No. HE-05556, to F. R. N. G.). We thank Mr. A. O. Clouse and Mr. T. Roseberry for their help.

(13) T. J. Stone, T. Buckman, P. L. Nordio, and H. M. McConnell, *Proc. Nat. Acad. Sci. U. S.*, **54**, 1010 (1965).

(14) T. R. Stengle and J. D. Baldeschwieler, *ibid.*, **55**, 1020 (1966); E. W. Bittner and J. T. Gerig, *J. Amer. Chem. Soc.*, 92, 5001 (1970).

(15) National Institutes of Health Predoctoral Fellow, 1967–1970.

Adam Allerhand,* David Doddrell
Victor Glushko, David W. Cochran[15]
Ernest Wenkert, Peter J. Lawson, Frank R. N. Gurd*
Contribution No. 1892, Department of Chemistry
Indiana University, Bloomington, Indiana 47401
Received October 15, 1970

Chemically Induced Dynamic Nuclear Polarization in Products from Radical Displacement Reactions

Sir:

Although it is generally accepted that chemically induced dynamic nuclear polarization (CIDNP) is only observed in the nmr spectra of molecules which have recently been formed from free radical precursors, our picture of the nature of the polarization process is still incomplete. The most convincing treatments have been those of Kaptein and Oosterhoff[1] and Closs,[2,3] which assume that nuclear polarization is a consequence of electron spin polarization accompanying the interaction of radical pairs within a solvent cage, and apply directly only to bimolecular radical processes of coupling and disproportionation.

A few cases of polarized spectra of products of radical displacement reactions have also been reported,[4] but here the details of the polarization process are obscure. We now report results with several displacement reactions involving benzyl radicals, where we believe for the first time both the displacement step and the reaction conditions have been systematically varied. We find significant changes in the polarized spectra observed, and propose an explanation.

When phenylacetyl peroxide is decomposed at 40° in CCl_4 containing CCl_3Br, the products shown in Table I are obtained. All but benzyl bromide (A) arising from the displacement process (eq 1) and the

Table I. Products from the Decomposition of Phenylacetyl Peroxide in CCl_4–CCl_3Br[a]

Product	δ_{TMS}, ppm[b]	% yield
$(PhCH_2)_2$	2.87	20–23
$PhCH_2COO$-CH_2Ph	3.55 5.04	12–20
$PhCH_2COO$-$COOCH_2Ph$	3.64 5.15	23–28
$PhCH_2CCl_3$	3.87	3–9
$PhCH_2Br$	4.38	15–27
Other benzyl deriv	4.56, 5.24, 5.28	6–7

[a] ~0.1 *M* peroxide, 0.1–3 *M* CCl_3Br, 40°. [b] Nmr peaks of *underlined* hydrogens. Analyses based on relative integrated intensities.

$$C_6H_5CH_2\cdot + CCl_3Br \longrightarrow C_6H_5CH_2Br + CCl_3\cdot \quad (1)$$

$C_6H_5CH_2CCl_3$ (B) and C_2Cl_6 (from radical coupling) are presumably products of cage recombination of initial radical or ion pairs.[5] Benzyl radicals are effectively trapped *via* eq 1 or by combination with $CCl_3\cdot$

$$C_6H_5CH_2\cdot + \cdot CCl_3 \longrightarrow C_6H_5CH_2CCl_3 \quad (2)$$

radicals since yields of bibenzyl do not rise until the CCl_3Br concentration is reduced below 0.1 *M*.[6]

When the decompositions are carried out at 40° in the spectrometer we observe strongly polarized spectra of the benzyl hydrogens of both benzyl bromide (emission, δ = 4.38) and $C_6H_5CH_2CCl_3$ (absorption, δ = 3.87). We find no polarization of any cage products in this system.[7] The time dependence of spectral intensity is shown in Figure 1, together with that of bibenzyl which monitors the peroxide decomposition rate. The actual enhancement factor α/β for the polarization may be calculated for the data of Figure 1 *via* the relation

$$(I_\infty - I)/I_\infty = (1 - \alpha k_1/\beta k_2)([P]/[P]_0) \quad (3)$$

where I and I_∞ are the integrated line intensities[8] at some point during reaction and at the end of reaction when all relaxation processes are complete, [P] is peroxide concentration, $k_1 = k_d$ for peroxide decomposition, k_2 is the rate constant for spin relaxation of the transition giving rise to the line, and α/β is a ratio of numbers proportional to the molar absorbances of polarized and unpolarized species (for emission, α is

(1) R. Kaptein and J. L. Oosterhoff, *Chem. Phys. Lett.*, **4**, 195, 214 (1969).

(2) G. L. Closs, *J. Amer. Chem. Soc.*, 91, 4552 (1969).

(3) G. L. Closs and A. D. Trifunac, *ibid.*, 92, 2183, 2186 (1970).

(4) (a) J. Bargon and H. Fischer, *Z. Naturforsch. A*, **22**, 1556 (1967); *ibid., A*, **23**, 2109 (1968); (b) R. Kaptein, *Chem. Phys. Lett.*, **2**, 261 (1968); (c) H. R. Ward, R. G. Lawler, and R. A. Cooper, *Tetrahedron Lett.*, 527 (1969); (d) S. V. Rykov, A. L. Buchachenko, and A. V. Kessenich, *Spectrosc. Lett.*, 3, 55 (1970), and references therein; (e) T. Koenig and W. R. Mabey, *J. Amer. Chem. Soc.*, 92, 3804 (1970).

(5) Phenylacetyl peroxide decomposes rapidly by concerted multibond scission: P. D. Bartlett and J. E. Leffler, *ibid.*, 72, 3030 (1950). We have recently suggested that both "polar" and "radical" cage products in such systems are formed subsequent to a single rate-determining transition state: C. Walling, H. P. Waits, J. Milovanovic, and C. G. Pappiaonnou, *ibid.*, 92, 4927 (1970).

(6) In CCl_4 alone, the bibenzyl yield is 43%, less than 1% benzyl chloride is formed, and cage products are unchanged.

(7) Polarization is also observed in the aromatic hydrogen region but cannot be resolved in detail.

(8) Use of integrated intensities is important, since our polarized spectra have abnormally narrow line widths ($\nu_{1/2}$ 0.6 cps) compared with the unpolarized species (1.1 cps).