Determination of Rotational Correlation Times of Proteins in Solution from Carbon-13 Spin–Lattice Relaxation Measurements. Effect of Magnetic Field Strength and Anisotropic Rotation

David J. Wilbur, Raymond S. Norton, Arthur O. Clouse, Robert Addleman, and Adam Allerhand*

Contribution No. 2882 from the Department of Chemistry, Indiana University, Bloomington, Indiana 47401. *Received May 10, 1976*

Abstract: We examine the effect of magnetic field strength and the use of the isotropic rigid rotor model (when rotational motion is anisotropic) on the accuracy of rotational correlation times (τ_R) of native proteins in solution determined from measurements of ^{13}C spin-lattice relaxation times (T_1) of α carbons. If τ_R is about 10^{-8} s, then small experimental errors in measured T_1 values at 14 kG can result in large errors in τ_R. We compare τ_R values obtained (with the use of the isotropic model) from T_1 measurements at 14.2 and at 63.4 kG. For horse myoglobin, the two magnetic field strengths yield essentially identical τ_R values. In the case of human hemoglobin, the difference between the two τ_R values is slightly outside the estimated experimental error. For the relatively nonspherical protein molecules hen egg-white lysozyme and bovine serum albumin, the τ_R values obtained at 14.2 kG are about twice as long as those obtained at 63.4 kG. The use of the axially symmetric ellipsoid model for the analysis of α-carbon relaxation data is examined. This model does not provide a significantly better agreement between the correlation times obtained at 14.2 and 63.4 kG (for lysozyme and albumin) than the isotropic model.

Measurements of spin–lattice relaxation times (T_1) of α-carbon envelopes[1-3] in proton-decoupled ^{13}C NMR spectra of native globular proteins in solution have been used to determine correlation times (τ_R) for the overall rotational motion of the protein molecules.[1-6] In some cases, this method has yielded τ_R values in good agreement with those obtained by other procedures. For example, in the case of muscle calcium binding protein at 20 °C, the τ_R value of 11–14 ns obtained from ^{13}C NMR measurements at 23.5 kG[4a] is in good agreement with a value determined from a depolarized light scattering experiment.[4b] In the case of hen egg-white lysozyme, depolarized light scattering measurements have yielded a correlation time of 10 ns at 20 °C,[7,8] in good agreement with the value of 8.5 ns (at 30 °C) obtained by ^{13}C NMR at 23.5 kG.[5] However, ^{13}C NMR measurements on lysozyme carried out at 14.2 kG yielded τ_R values of 22 ns at 40 °C[2] and 20 ns at 42 °C.[6] On the other hand, the rotational correlation time of myoglobin obtained by ^{13}C NMR at 14 kG[3,6] is in good agreement with the value obtained by ^{13}C NMR at 23.5 kG.[3] The reported α-carbon relaxation times[1-6] have been analyzed with the use of the isotropic rigid rotor model.[9] In this report we examine the effect of magnetic field strength and the use of the isotropic model (when rotational motion is anisotropic) on the accuracy of rotational correlation times of proteins determined from α-carbon T_1 values. Calculated α-carbon T_1 values at 14.2 and 63.4 kG, obtained with the use of the axially symmetric ellipsoid model,[10] are presented. The use of α-carbon T_1 measurements for studying the anisotropy of rotational motion of a protein is discussed. Experimental results at 14.2 and 63.4 kG are presented for two fairly spherical proteins (myoglobin[11] and hemoglobin[12]) and for two relatively nonspherical proteins (hen egg-white lysozyme[13] and bovine serum albumin[14]).

Experimental Section

Hen egg-white lysozyme from Sigma Chemical Co., St. Louis, Mo. (grade I), was purified by chromatography on DEAE-Sephadex.[15] Horse myoglobin (Type I) and bovine serum albumin (Type F) were obtained from Sigma Chemical Co. and were dialyzed against 0.1 M NaCl in H_2O. Human adult hemoglobin, prepared by the method of Williams and Tsay,[16] was kindly provided by J. B. Matthew. Preparation of samples for NMR was carried out as described previously.[17]

All T_1 values were obtained from partially relaxed Fourier transform (PRFT) ^{13}C NMR spectra.[1,18] Spectra at 15.2 MHz (14.2 kG) were obtained as described previously.[6] Nine PRFT spectra of bovine serum albumin were recorded at 14.2 kG, each with 4096 accumulations and a recycle time of $\tau + 0.505$ s (τ is the interval between each 180° radiofrequency pulse and the following 90° pulse). T_1 values at 14.2 kG for myoglobin, lysozyme, and hemoglobin are published values.[6] Spectra at 67.9 MHz (63.4 kG) were obtained on a spectrometer consisting of a Bruker high-resolution superconducting magnet, Bruker 10-mm probe, home-built radiofrequency electronics, and a Nicolet 1085 computer. The spectrometer was not equipped with a field-frequency lock. The width of the 90° radiofrequency pulse was 12–13 μs and the frequency was set at the ^{13}C resonance frequency of Me_4Si. The ^1H irradiation for proton decoupling (at 270 MHz) had a peak field strength of 0.8 G (3.4 kHz) and a random-noise modulation bandwidth of about 300 Hz and was centered 3.6 ppm downfield from the ^1H resonance of Me_4Si (unless otherwise stated). Each PRFT spectrum was obtained with the use of 2048 or 4096 time-domain addresses, a spectral width of 14 286 Hz, and 8192 accumulations with a recycle time of $\tau + kT_1$ (where $k \geq 3.4$). Fourier transformation was done on twice the number of addresses used for signal accumulation, by placing the appropriate number of addresses with a zero value at the end of each set of accumulated data points. At least five τ values were used for each T_1 measurement at 63.4 kG.

Results and Discussion

It is now well established that, at low magnetic field strengths (such as 14 kG), the T_1 values of protonated carbons in a large molecule are overwhelmingly determined by the ^{13}C–^1H dipolar relaxation mechanism.[19] At 63.4 kG, chemical shift anisotropy is the dominant relaxation mechanism for carbonyl carbons and nonprotonated aromatic carbons of a protein,[20] but the ^{13}C–^1H dipolar mechanism is still overwhelmingly dominant for the protonated carbons.[20] Therefore, we shall consider only ^{13}C–^1H dipolar relaxation in our analysis of the α-carbon T_1 values of proteins.

Figure 1 shows log–log plots of T_1 vs. τ_R (both in seconds) for a ^{13}C nucleus undergoing relaxation by a dipolar interaction with a single proton 1.09 Å away (typical C–H bond length), under conditions of proton decoupling, and when the rotational motion is isotropic. Results are shown for three magnetic field strengths currently in use (14.2, 23.5, and 63.4 kG) and for 117.4 kG (resonance frequencies of 500 MHz for ^1H and 125.7 MHz for ^{13}C), a magnetic field strength that may be used soon

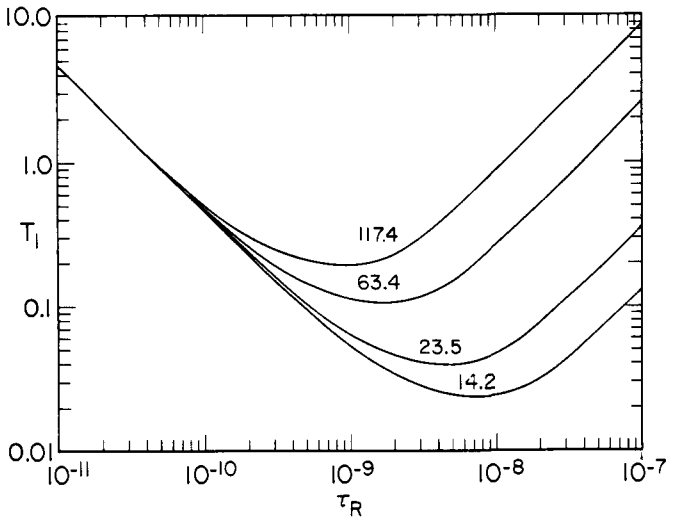

Figure 1. Log-log plots of T_1 vs. τ_R (both in seconds) for a ^{13}C spin relaxing by a dipolar interaction with a single proton 1.09 Å away, in the case of isotropic rotational reorientation and under conditions of proton decoupling. Plots are given for various magnetic field strengths, indicated in kilogauss.

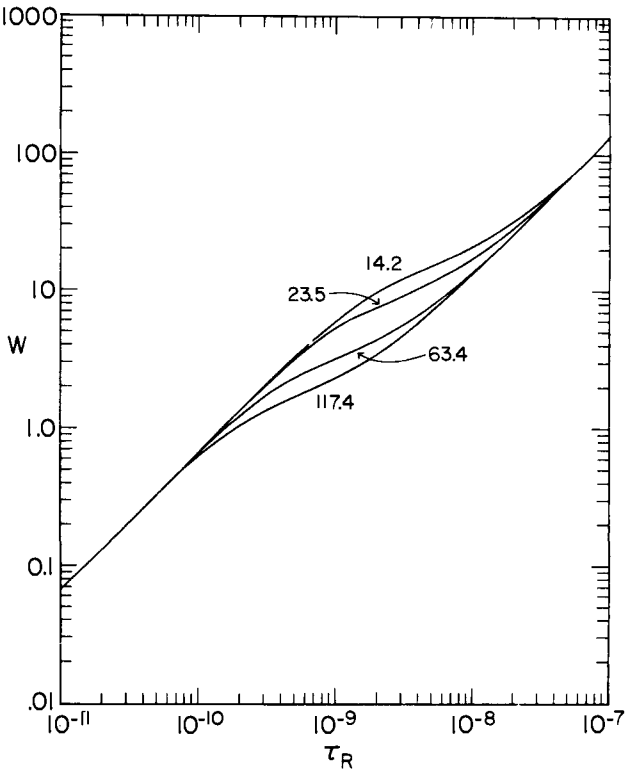

Figure 2. Log-log plots of line width (in hertz) vs. τ_R (in seconds) for various magnetic field strengths (in kilogauss). See caption of Figure 1 for other conditions.

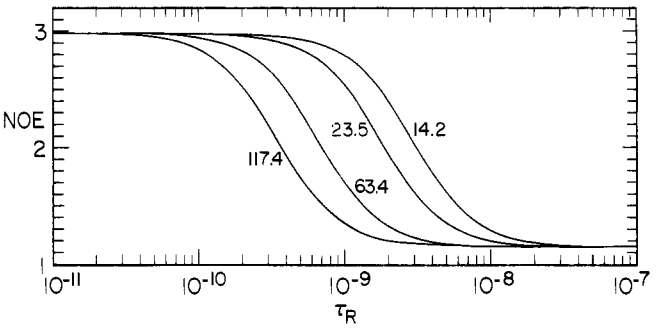

Figure 3. Semilog plots of NOE vs. τ_R (in seconds) for various magnetic field strengths (in kilogauss). See caption of Figure 1 for other conditions. Here we define the NOE as the ratio of the intensities of the ^{13}C resonance in the presence and in the absence of proton decoupling.

for studies of proteins. The theoretical results of Figure 1 were obtained with the use of equations presented elsewhere.[6,9] It follows from Figure 1 that if τ_R is about 10^{-8} s, then the effect of experimental errors in the measured T_1 values on the determination of τ_R will be much greater at 14.2 kG than at 23.5 kG and above. For example, if the true value of τ_R is 8 ns and the measured T_1 is in error by +10%, then the estimated value of τ_R is 14 ns at 14.2 kG and 10 ns at 23.5 kG.[21] If the measurement is carried out at 63.4 kG, a given percentage experimental error in T_1 will yield a similar percentage error in τ_R for any value of $\tau_R \gtrsim 6$ ns. A comparable situation is reached at 14.2 kG when $\tau_R \gtrsim 30$ ns. It follows that the use of high magnetic field strengths ($\gtrsim$30 kG) is desirable for determinations of rotational correlation times of small proteins such as lysozyme.

In principle, NMR line widths (W) and nuclear Overhauser enhancement (NOE) values can also be used to determine rotational correlation times of proteins.[6] Figures 2 and 3 show theoretical plots for W and the NOE, respectively, calculated for the same system as in Figure 1, with the use of equations given elsewhere.[9] It follows from Figure 3 that NOE measurements can yield accurate τ_R values for only a limited range of rotational correlation times, below typical τ_R values of most native proteins. In contrast, it should be possible to extract accurate τ_R values of proteins from α-carbon line width measurements at any of the magnetic field strengths under consideration (Figure 2). Such determinations will require the observation of resolved resonances of individual α carbons. At the present time, T_1 measurements on α-carbon envelopes provide the simplest approach for applying ^{13}C NMR to the determination of rotational correlation times of native proteins in solution.

Experimental α-carbon T_1 values at 14.2 and 63.4 kG and the resulting values of τ_R obtained with the use of the isotropic rigid rotor model (Figure 1) are shown in Table I. In the case of myoglobin, the experiment at 14.2 kG yields a τ_R value that is in good agreement with the value obtained at 63.4 kG. For hemoglobin, the difference between the two τ_R values is slightly outside the estimated experimental error. However, for the relatively nonspherical protein molecules lysozyme and albumin, the τ_R values obtained at 14.2 kG are about twice as long as those obtained at 63.4 kG. The differences are far outside the estimated experimental error. We examine now the possibility that these discrepancies are the result of neglecting the anisotropic nature of the rotational reorientation of lysozyme and albumin.

Consider the rotational Brownian motion of a molecule that is an axially symmetric ellipsoid with semiaxes of length a and $b = c$. We can compute the ^{13}C T_1 value of a protonated carbon in such a molecule with the use of general equations for ^{13}C-^1H dipolar relaxation[22] and Woessner's treatment of anisotropic rotation.[10] For a methine carbon under conditions of proton-decoupling we obtain[9]

$$1/T_1 = A/T_{1A} + B/T_{1B} + C/T_{1C} \quad (1)$$

where

$$A = \tfrac{1}{4}(3\cos^2\theta - 1)^2 \quad (2)$$

$$B = 3\sin^2\theta\cos^2\theta \quad (3)$$

$$C = \tfrac{3}{4}\sin^4\theta \quad (4)$$

and

$$1/T_{1K} = \tfrac{1}{10}\hbar^2\gamma_C^2\gamma_H^2 r_{CH}^{-6}\chi_K \quad (5)$$

$$K = A, B, C$$

Table I. α-Carbon Spin-Lattice Relaxation Times and Rotational Correlation Times of Horse Myoglobin, Human Hemoglobin, Hen Egg-White Lysozyme, and Bovine Serum Albumin

	α-carbon T_1, s[a]		τ_R, ns[b]	
Protein (°C)	14.2 kG	63.4 kG	14.2 kG	63.4 kG
Myoglobin (36)	0.030[c]	0.40[d]	18 ± 5[e,f]	16 ± 2[f]
Hemoglobin (36)	0.062[g]	0.75[h]	47 ± 7[i]	30 ± 5[i]
Lysozyme (31)		0.34[j]		13 ± 2[k]
Lysozyme (43)	0.031[l]	0.21[m]	19 ± 5[e]	8 ± 1
Albumin (37)	0.10[n]	0.88[o]	78 ± 12[p]	35 ± 5[p]

[a] T_1 values were measured from partially relaxed Fourier transform NMR spectra.[1,6] Estimated random error is ±15%. [b] Obtained from Figure 1.[19] Estimated errors are based on a ±15% error in the experimental T_1 values. [c] Measured for 8.6 mM horse carbon monoxide myoglobin in H_2O (0.1 M NaCl, 0.05 M phosphate buffer, pH 6.5); taken from Table I of ref 6. A T_1 value of 0.036 s (at 14.1 kG) has been reported[3] for sperm whale cyanoferrimyoglobin (16 mM, pH 7.5) at 32 °C. [d] Horse cyanoferrimyoglobin (17 mM) in H_2O (0.1 M NaCl, pH 7.6). [e] The measured T_1 actually yields a τ_R value that is 1 ns longer than is indicated in the table. The estimated error in τ_R is +4 ns and −6 ns. [f] A τ_R value of 10 ns has been obtained for horse myoglobin (1.3 mM, at 25 °C) from dielectric relaxation measurements by H. O. Marcy and J. Wyman, *J. Am. Chem. Soc.*, **64**, 638 (1942). [g] Measured for 3.7 mM (in tetramer) human adult carbon monoxide hemoglobin in H_2O (0.1 M NaCl, 0.05 M phosphate buffer, pH 6.9); taken from Table I of ref 6. [h] Human adult cyanoferrihemoglobin (3.8 mM in tetramer) in H_2O (0.1 M NaCl, pH 8.3). [i] A τ_R value of 44 ns has been obtained for horse hemoglobin (0.4 mM in tetramer, at 17 °C) from dielectric relaxation measurements by P. Schlecht, H. Vogel, and A. Mayer, *Biopolymers*, **6**, 1717 (1968). [j] Hen egg-white lysozyme (15.4 mM) in H_2O (0.1 M NaCl, pH 3.1). [k] A τ_R value of 10 ns has been obtained for lysozyme (10 mM, at 20 °C) from depolarized light scattering measurements.[7] [l] Measured at 42 °C for 14.6 mM hen egg-white lysozyme in H_2O (0.1 M NaCl, pH 3). [m] Measured at 44 °C for the sample described in footnote *j*. [n] Measured at 38 °C for 3.0 mM bovine serum albumin in H_2O (0.1 M NaCl, pH 6.5). [o] Measured at 35 °C for the sample described in footnote *n*. [p] See footnote *c* of Table III.

Here θ is the angle between the C-H vector and the symmetry axis of the ellipsoid, r_{CH} is the carbon-hydrogen bond length, γ_C and γ_H are the gyromagnetic ratios of ^{13}C and ^1H, respectively, and χ_K is given by eq 6.

$$\chi_K = \frac{\tau_K}{1 + (\omega_H - \omega_C)^2\tau_K^2} + \frac{3\tau_K}{1 + \omega_C^2\tau_K^2} + \frac{6\tau_K}{1 + (\omega_H + \omega_C)^2\tau_K^2} \quad (6)$$

Here ω_H and ω_C are the resonance frequencies, in radians per second, of ^1H and ^{13}C, respectively, and the values of τ_K ($K = A, B, C$) are given by eq 7-9.

$$\tau_A = \tau_\perp \quad (7)$$

$$\tau_B = 6\tau_\perp\tau_\parallel(\tau_\perp + 5\tau_\parallel)^{-1} \quad (8)$$

$$\tau_C = 3\tau_\perp\tau_\parallel(2\tau_\perp + \tau_\parallel)^{-1} \quad (9)$$

Here $\tau_\parallel$ and $\tau_\perp$ are the correlation times for rotation about the symmetry axis (a) and axis b, respectively.

Figure 4 shows the effect of anisotropy of rotational motion on the ^{13}C T_1 value of a methine carbon (in a prolate ellipsoid) at 14.2 and 63.4 kG. Each curve was computed for a fixed value of $\tau_\perp$ and a variable $\tau_\perp/\tau_\parallel$ ratio. However, the ratio of the lengths of the axes (a/b) is used as the independent variable in Figure 4. We have used Woessner's eq 58[10] as the relationship between $\tau_\perp/\tau_\parallel$ and a/b (Figure 5). The T_1 values of Figure 4 were calculated for $\theta = 80°$. The effect of varying θ is shown in Figure 6.

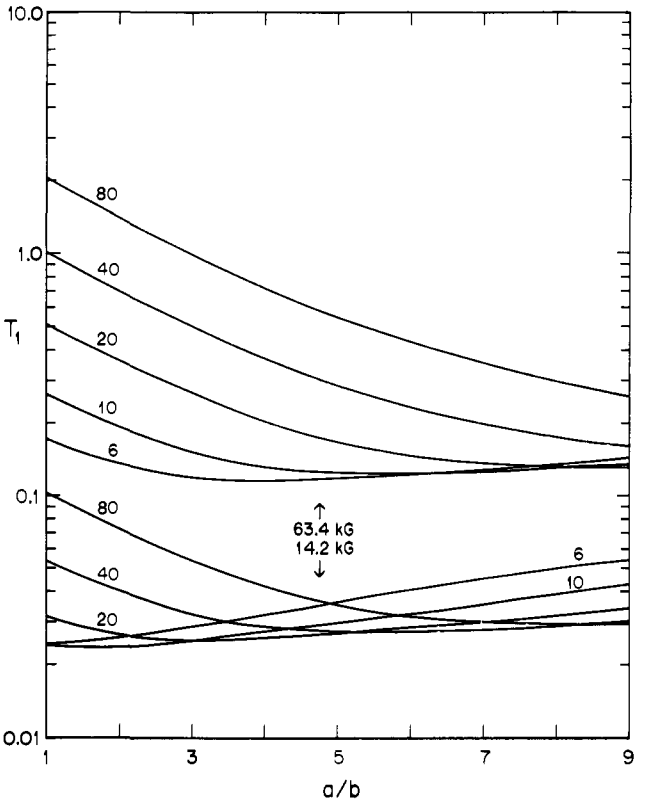

Figure 4. Semilog plots of T_1 (in seconds) vs. a/b for a ^{13}C spin relaxing by a dipolar interaction with a single proton 1.09 Å away (with $\theta = 80°$) in the case of an axially symmetric ellipsoid, under conditions of proton decoupling. Plots are given for various values of $\tau_\perp$ (in nanoseconds) at 14.2 kG (lower set of curves) and 63.4 kG (upper set of curves).

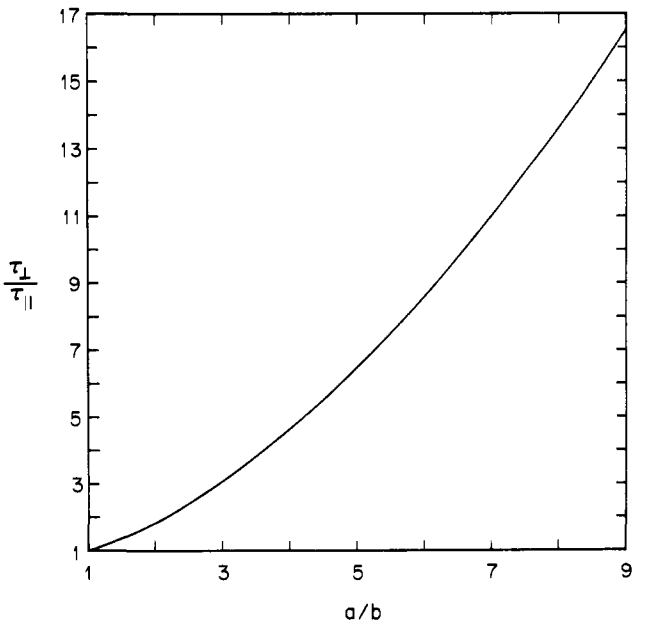

Figure 5. Plot of $\tau_\perp/\tau_\parallel$ vs. a/b, from eq 58 of ref 10.

The α carbons of native proteins have yielded broad envelopes in reported ^{13}C NMR spectra at low magnetic field strengths (14 and 23 kG).[1-3,5,6] Reported α-carbon T_1 values[1,3,6] are not those of individual carbons but of the α-carbon envelope. If the rotational motion is anisotropic, we must consider the possibility that the various contributing α carbons may not have identical T_1 values (Figure 6). It is obviously desirable to measure α-carbon T_1 values at a magnetic field strength high enough to yield resolved single α-carbon

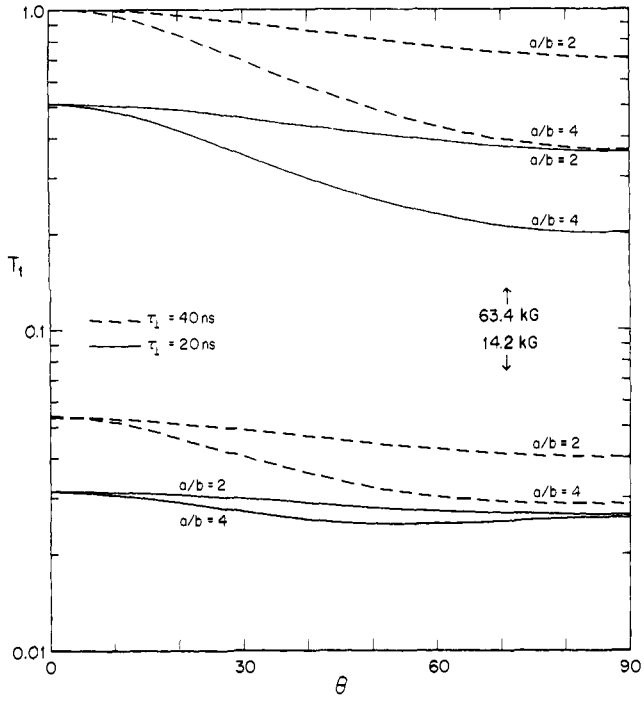

Figure 6. Semilog plots of T_1 (in seconds) vs. θ (in degrees) for a ^{13}C spin relaxing by a dipolar interaction with a single proton 1.09 Å away, in the case of an axially symmetric ellipsoid, under conditions of proton decoupling. Plots are given for $\tau_\perp = 20$ ns (solid curves) and $\tau_\perp = 40$ ns (dashed curves), with $a/b = 2$ and 4, at 14.2 kG (lower curves) and 63.4 kG (upper curves).

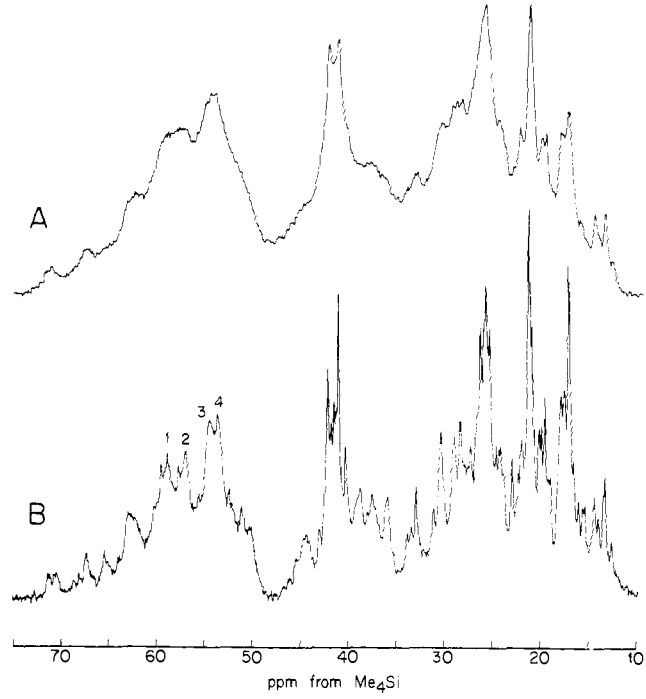

Figure 7. Region of aliphatic carbons in the fully proton-decoupled natural-abundance ^{13}C Fourier transform NMR spectra of hen egg-white lysozyme in H_2O and 0.1 M NaCl. (A) At 14.2 kG (15.2 MHz), using 14.1 mM lysozyme (pH 4.0, 36 °C), a spectral width of 3788 Hz, 16 384 time-domain addresses, 32 768 accumulations with a recycle time of 2.205 s (20 h total time), and 0.88-Hz digital broadening. (B) At 63.4 kG (67.9 MHz), using 15.4 mM lysozyme (pH 3.1, 30 °C), a spectral width of 14 286 Hz, 16 384 time-domain addresses, 32 768 accumulations with a recycle time of 3.016 s (28 h total time), and 2.78-Hz digital broadening. The noise modulated ^1H irradiation was centered 2.2 ppm downfield from Me_4Si.

resonances. Figure 7 shows the aliphatic regions of fully proton-decoupled ^{13}C NMR spectra of hen egg-white lysozyme at 14.2 and 63.4 kG.[20] The downfield portion of each spectrum (about 49–72 ppm) contains the resonances of C^α of all residues (except the 12 glycines) and the resonances of C^β of the seven threonines and ten serines.[23] However, these 17 C^β resonances probably contribute only to the downfield edge of the α-carbon region.[23] The range 49–60 ppm probably contains only the resonances of methine α carbons. The resolution in this region of the spectrum is considerably greater at 63.4 kG (Figure 7, B) than at 14.2 kG (Figure 7, A). However, even at 63.4 kG, none of the 117 methine α carbons of hen egg-white lysozyme yield resolved single-carbon resonances.[20] Each of the T_1 values for lysozyme at 63.4 kG given in Table I is an arithmetic average of the values for peaks 1–4 of Figure 7, B. The T_1 values of peaks 1–4 are all within ±10% of the given average value.

Application of the axially symmetric ellipsoid model to the analysis of α-carbon relaxation data requires known values of the angle (θ) between the pertinent C–H vectors and the symmetry axis. In the case of lysozyme, we obtained an approximate value of θ for each C^α–H^α vector from a molecular model built with the use of crystal coordinates.[13] The symmetry axis was located by visual inspection, roughly along a line between the NH hydrogen of Pro-70 and the β hydrogens of Ile-124. Table II lists the number of residues within a given range of θ values, in steps of 15°. Glycine residues have not been included because their α-carbon resonances fall outside the main α-carbon envelope (see above) and therefore do not contribute to our experimental T_1 value. We have assumed that the distribution of θ values for the α carbons that contribute to each of peaks 1–4 (Figure 7, B) is the same as that of Table II. Figure 6 indicates that if lysozyme has $\tau_\perp \approx 20$ ns and $a/b \approx 2$,[13] then the α-carbon region should yield an essentially exponential relaxation behavior at 14.2 kG, but a slightly nonexponential behavior (about 30% change in T_1 when going from $\theta = 0$ to 90°) at 63.4 kG. Our PRFT spectra of lysozyme

Table II. Estimated Values of θ for Hen Egg-White Lysozyme

θ^a	No. of residues[b]	θ^a	No. of residues[b]
0–15	4	46–60	24
16–30	8	61–75	28
31–45	21	76–90	32

[a] Angle (in degrees) between the C^α–H^α vector and the assumed symmetry axis of the ellipsoid. [b] Glycine residues are not included (see text).

(see Experimental Section) did not yield a detectable nonexponential behavior at either magnetic field strength. Very accurate relaxation measurements may yield information about the anisotropy of rotational reorientation, either from observation of nonexponential relaxation behavior of the α-carbon envelope or from determination of T_1 values of individual α carbons (perhaps specifically ^{13}C enriched) with different known values of θ.

It follows from eq 1 that, in general, the magnetic field dependence of T_1 will not yield information about rotational anisotropy. In the extreme narrowing limit ($\omega_H \tau_K \ll 1$, $K = A, B, C$) eq 1 becomes identical in form with the corresponding expression for isotropic motion,[22] but with $A\tau_A + B\tau_B + C\tau_C$ replacing τ_R. Therefore, application of the isotropic model to an axially symmetric ellipsoid will yield an "effective" $\tau_R = A\tau_A + B\tau_B + C\tau_C$ (a function of θ). In the limit of slow motion ($\omega_C \tau_K \gg 1$, $K = A, B, C$) eq 1 also becomes identical in form with the slow limit expression for isotropic motion, but with $(A/\tau_A + B/\tau_B + C/\tau_C)^{-1}$ replacing τ_R. Therefore, in the slow limit, application of the isotropic model to an axially symmetric

Table III. Application of the Axially Symmetric Ellipsoid Model to Hen Egg-White Lysozyme and Bovine Serum Albumin

	Lysozyme $\tau_\perp$, ns[b]		Albumin $\tau_\perp$, ns[b,c]	
a/b[a]	14.2 kG	63.4 kG	14.2 kG	63.4 kG
1	20	8	78	35
2	25	10	100	46
4	42	18	180	83
6	62	28	300	130

[a] The reported value of a/b is about 2 for lysozyme[13] and about 3.5 for albumin.[14] [b] Values of $\tau_\perp$ obtained from eq 1, with $\theta = 60°$ and the experimental T_1 values of Table I (at 43 °C for lysozyme). [c] Dielectric relaxation measurements [P. Moser, P. G. Squire, and C. T. O'Konski, *J. Phys. Chem.*, **70**, 744 (1966)] indicate that the rotational motion of aqueous bovine serum albumin is anisotropic, with $\tau_\perp$ = 77 ns and $\tau_\parallel$ = 25 ns (a/b = 3.0), at infinite dilution and 25 °C.

ellipsoid will yield an "effective" τ_R independent of magnetic field strength, even though T_1 is strongly field dependent.

As noted above, when the isotropic rigid rotor model (a/b = 1) was used to extract rotational correlation times from α-carbon T_1 values of lysozyme and albumin, measurements at 63.4 kG yielded different τ_R values from those at 14.2 kG (Table I). The use of the axially symmetric ellipsoid model does not provide a significantly better agreement between the rotational correlation times obtained at the two magnetic field strengths (Table III). We have considered the possibility that the discrepancies between the results at 14.2 and 63.4 kG are caused by systematic errors in measured T_1 values. However, there is satisfactory agreement between the low-field and high-field results for the relatively spherical myoglobin and hemoglobin molecules (Table I). We believe that our results for lysozyme and albumin reflect the inapplicability of the axially symmetric ellipsoid model. In the future, measurements of T_1 values of individual α carbons (probably ^{13}C enriched) of a protein may permit a determination of the components of the anisotropic diffusion tensor for overall rotation.[24] Such measurements may also yield information about possible contributions from internal motions[25] (of some portions of the polypeptide backbone) to the T_1 values of α-carbon envelopes.

Acknowledgment. This work was supported by the National Science Foundation (Grant MPS 73-04969) and the U.S. Public Health Service (Grant NS-10977-03). We thank Carol Williams and James Matthew for their help.

References and Notes

(1) A. Allerhand, D. Doddrell, V. Glushko, D. W. Cochran, E. Wenkert, P. J. Lawson, and F. R. N. Gurd, *J. Am. Chem. Soc.*, **93**, 544 (1971).
(2) A. Allerhand, R. F. Childers, and E. Oldfield, *Biochemistry*, **12**, 1335 (1973).
(3) R. B. Visscher and F. R. N. Gurd, *J. Biol. Chem.*, **250**, 2238 (1975).
(4) (a) S. J. Opella, D. J. Nelson, and O. Jardetzky, *J. Am. Chem. Soc.*, **96**, 7157 (1974); (b) D. R. Bauer, S. J. Opella, D. J. Nelson, and R. Pecora, *ibid.*, **97**, 2580 (1975).
(5) P. J. Cozzone, S. J. Opella, O. Jardetzky, J. Berthou, and P. Jollès, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 2095 (1975).
(6) E. Oldfield, R. S. Norton, and A. Allerhand, *J. Biol. Chem.*, **250**, 6368 (1975).
(7) S. B. Dubin, N. A. Clark, and G. B. Benedek, *J. Chem. Phys.*, **54**, 5158 (1971).
(8) Footnote 8 of ref 4b.
(9) D. Doddrell, V. Glushko, and A. Allerhand, *J. Chem. Phys.*, **56**, 3683 (1972).
(10) D. E. Woessner, *J. Chem. Phys.*, **37**, 647 (1962).
(11) J. C. Kendrew, G. Bodo, H. M. Dintzis, R. G. Parrish, H. Wyckoff, and D. C. Phillips, *Nature (London)*, **181**, 662 (1958); J. C. Kendrew, R. E. Dickerson, B. E. Strandberg, R. G. Hart, D. R. Davies, D. C. Phillips, and V. C. Shore, *ibid.*, **185**, 422 (1960); T. Tao, *Biopolymers*, **8**, 609 (1969).
(12) M. F. Perutz, M. G. Rossman, A. F. Cullis, H. Muirhead, G. Will, and A. C. T. North, *Nature (London)*, **185**, 416 (1960).
(13) T. Imoto, L. N. Johnson, A. C. T. North, D. C. Phillips, and J. A. Rupley in "The Enzymes", Volume VII, 3d ed. P. D. Boyer, Ed., Academic Press, New York, N.Y., 1972, Chapter 21.
(14) P. G. Squire, P. Moser, and C. T. O'Konski, *Biochemistry*, **7**, 4261 (1968).
(15) A. Allerhand, R. S. Norton, and R. F. Childers, *J. Biol. Chem.*, in press.
(16) R. C. Williams and K. Tsay, *Anal. Biochem.*, **54**, 137 (1973).
(17) E. Oldfield, R. S. Norton, and A. Allerhand, *J. Biol. Chem.*, **250**, 6381 (1975).
(18) R. L. Vold, J. S. Waugh, M. P. Klein, and D. E. Phelps, *J. Chem. Phys.*, **48**, 3831 (1968).
(19) A. Allerhand, D. Doddrell, and R. Komoroski, *J. Chem. Phys.*, **55**, 189 (1971).
(20) R. S. Norton, A. O. Clouse, R. Addleman, and A. Allerhand, *J. Am. Chem. Soc.*, in press.
(21) For any measured T_1 value, Figure 1 yields two solutions for τ_R. Here we have assumed that the shorter value can be rejected on the basis of physical arguments or measurements of line widths (Figure 2) and/or nuclear Overhauser enhancements (Figure 3).
(22) J. R. Lyerla and D. M. Grant, *MTP Int. Rev. Sci.: Phys. Chem., Ser. One*, **4**, 155 (1972).
(23) V. Glushko, P. J. Lawson, and F. R. N. Gurd, *J. Biol. Chem.*, **247**, 3176 (1972); P. Kelm, R. A. Vigna, J. S. Morrow, R. C. Marshall, and F. R. N. Gurd, *ibid.*, **248**, 7811 (1973).
(24) W. T. Huntress, *Adv. Magn. Reson.*, **4**, 1 (1970).
(25) R. Deslauriers and R. L. Somorjai, *J. Am. Chem. Soc.*, **98**, 1931 (1976).