Methyl Reorientation in Polycrystalline Amino Acids and Peptides: A ^2H NMR Spin-Lattice Relaxation Study

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Abstract: ^2H spin-lattice relaxation times have been measured at various temperatures for L-[3,3,3-$^2H_3$]alanine, L-[*methyl*-$^2H_3$]methionine, *cyclo*(L-Ala-L-[3,3,3-$^2H_3$]-Ala), and *t*-Boc-L-Ala-L-[3,3,3-$^2H_3$]Ala-OMe. In all these compounds the orientation dependence of T_1 calculated (for $\theta = 0°$ and $\theta = 90°$) by using a three-site jump model is in good agreement with the orientation dependence of T_1 measured over a large temperature range. Correlation times derived from the analysis of these data show that the activation energy is 8.5-11.4 kJ/mol for methyl reorientation in all cases except for L-[3,3,3-$^2H_3$]alanine, which has an activation energy of 22.6 kJ/mol. This abnormally large activation energy is correlated with the tight packing found in the crystal structure. This result suggests that measurement of methyl-group activation energies may be a useful way to locate tightly packed domains within proteins.

We have used ^2H NMR spectroscopy to investigate the effects of molecular packing on activation energy of methyl reorientation in polycrystalline amino acids and peptides. Activation energies can be obtained from rotational correlation times, τ_c, measured as a function of temperature. ^2H NMR spectroscopy is a useful method for measuring rotational correlation times in solids.[2-5] The values of correlation times in the range 10^{-4}-10^{-7} can be determined from analysis of line shapes while correlation times in the range 10^{-7}-10^{-12} s can be determined from analysis of spin-lattice relaxation times (T_1).

Since correlation times for methyl reorientation are normally less than 10^{-8} s at temperature above -150 °C,[6] we have derived the correlation times for methyl reorientation from T_1 measurements. In solids, the ^2H T_1 is orientation dependent and a measurement of the relaxation time at $\theta = 0°$ and $\theta = 90°$ uniquely defines the correlation time(s).[7] θ is the angle made by the methyl rotation axis and the external magnetic field. In addition, these T_1 measurements permit one to discriminate among various models for methyl reorientation.

We have measured ^2H T_1's over a wide temperature range for four polycrystalline compounds with deuterated methyl groups. The correlation times for methyl reorientation were obtained by using a three-site-jump model to analyze the T_1 data,[7] and the activation energies were determined from the temperature dependence of τ_c. The observed activation energies are discussed in terms of the reported crystal structures of the compounds and compared with the ^1H NMR results observed for alanine, other amino acids, peptides, and polypeptides by Andrew and coworkers.[8-10] We have also measured the averaged ^2H quadrupolar splitting, $\overline{\Delta\nu_q}$, as a function of temperature and have interpreted these measurements using a model in which the methyl rotation axis librates in a cone of semiangle Θ_c. The values of Θ_c determined in this manner are discussed with reference to the crystallographic temperature factors reported for these compounds.

Experimental Section

L-[3,3,3-$^2H_3$]Alanine and L-[*methyl*-$^2H_3$]methionine were purchased from Merck Isotopes. Elemental analysis showed that they were pure materials. X-ray diffraction showed that the space groups and cell dimensions of single crystals of these compounds were identical with values reported in the literature.[11-13] The linear and cyclic dipeptides were prepared as follows.

Synthesis of HCl-L-[3,3,3-$^2H_3$]Alanine Methyl Ester. The deuterated alanine (0.3 g) was suspended in 30 mL of freshly distilled 2,2-dimethoxypropane and then added to freshly prepared HCl-methanol solution (30 mL). The mixture was stirred at room temperature overnight and became dark brown. After the mixture was concentrated, the residue was dissolved in methanol. Addition of ether resulted in crystallization of the desired compound. This material was then recrystallized from methanol-ether. The yield was 0.46 g (99%). TLC showed that the product was not contaminated with any starting material and had the same R_f value as the authentic sample.

Synthesis of *t*-Boc-L-Ala-L-[3,3,3-$^2H_3$]-Ala-OMe. *t*-Boc-Alanine (0.62 g, 3.3 nmol) was dissolved in methylene chloride, and HCl-L-[3,3,3-$^2H_3$]alanine methyl ester neutralized with *N*-methylmorpholine (1 equiv) was also added to the methylene chloride solution. The solution was cooled to -15 °C with an ice-salt mixture for 15 min and treated with 1.2 equiv of 1-hydroxybenzotriazole and 1.1 equiv of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide. The reaction mixture was warmed slowly to 0° C, and stirring was continued overnight at 4 °C. After evaporation of the solvent, the residue was dissolved in ethyl acetate (300 mL) and successively extracted with 10-mL portions of 8% $NaHCO_3$, 8% $KHSO_4$, H_2O, and saturated NaCl (2 × each). After drying with Na_2SO_4, the solvent was evaporated. The crude product was crystallized from ethane-hexane to give the compound in 90% yield (0.81 g). The compound was pure by elemental analysis and ^1H NMR.

Synthesis of *cyclo*(L-Ala-L-[3,3,3-$^2H_3$]-Ala). The cyclic peptide was prepared by the method of Nitecki et al.[14] and was crystallized from the mother liquor after evaporation of the solvent. The crystals were collected, washed with ether, and dried in vacuo; yield 200 mg (68%). This material showed a single spot on thin-layer chromatography with butanol-acetic acid-water (4:1:1) and ethanol-water-aqueous ammonia (7:3:1). A single crystal of this compound was analyzed by X-ray diffraction and was determined to have the same space group and cell dimensions as reported for *cyclo*(L-Ala-L-Ala).[11,15]

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Table I. Equations Describing ^2H T_1 Behavior for Two Models of Methyl-Group Reorientation $\Theta = 70.5°$ [a]

Three-Site Jump Model

$$\theta = 0° \quad \frac{1}{T_1} = \frac{8\omega_Q^2}{81}\left(\frac{\tau_c}{1+\omega^2\tau_c^2} + \frac{8\tau_c}{1+4\omega^2\tau_c^2}\right)$$

$$\theta = 90° \quad \frac{1}{T_1} = \frac{12\omega_Q^2}{81}\left(\frac{\tau_c}{1+\omega^2\tau_c^2} + \frac{2\tau_c}{1+4\omega^2\tau_c^2}\right)$$

Free Diffusion Model

$$\theta = 0° \quad \frac{1}{T_1} = \frac{8\omega_Q^2}{81}\left(\frac{\tau_1}{1+\omega^2\tau_1^2} + \frac{8\tau_2}{1+\omega^2\tau_2^2}\right)$$

$$\theta = 90° \quad \frac{1}{T_1} = \frac{4\omega_Q^2}{81}\left(\frac{\tau_1}{1+\omega^2\tau_1^2} + \frac{2\tau_2}{1+\omega^2\tau_2^2} + \frac{4\tau_1}{1+4\omega^2\tau_1^2} + \frac{2\tau_2}{1+4\omega^2\tau_2^2}\right)$$

[a] $\omega_Q = (3e^2qQ)/(4\hbar)$; $\omega = \gamma B_0$; $\tau_c = 1/(3 \times \text{jump rate})$; $\tau_1 = 1/(\text{diffusion constant})$; $\tau_2 = \tau_1/4$.

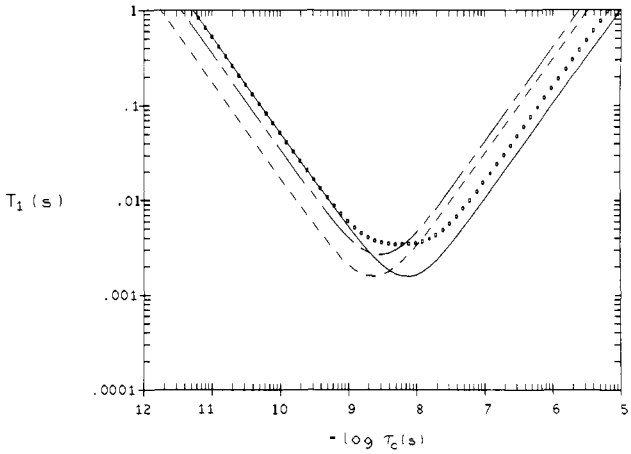

Figure 1. Theoretical behavior of ^2H T_1 at 38.45 MHz, plotted as a function of corelation time, $\omega_Q/2\pi = 128.2\ s^{-1}$. Three-site jumps: $\tau_c = {}^1/_3 \times$ jump rate; (– – –) $\theta = 0°$, (— — —) $\theta = 90°$. Free diffusion: $\tau_c = \tau_1 = 1/\text{diffusion constant}$; (—) $\theta = 0°$; (•••) $\theta = 90°$.

Solid-state ^2H NMR spectra were observed at 38.45 MHz on a home-built spectrometer using the solid echo pulse sequence.[16] A 6.4-μs 180° pulse inverted the ^2H magnetization. The return of the ^2H magnetization to equilibrium was observed by applying the echo sequence at a time T after the 180° pulse. The T_1 values were determined by a linear least-squares analysis of the equation

$$\ln (M_0 - M(T)) = -T/T_1 + \ln (M_0 - M(0)) \qquad (1)$$

where M_0 is the equilibrium magnetization, $M(T)$ is the magnetization at time T after the 180° pulse, and $M(0)$ is the magnetization at $T = 0$.

The ^2H T_1's at the parallel ($\theta = 0°$) and perpendicular ($\theta = 90°$) edges of the ^2H powder pattern were measured for each compound at each temperature. These two values of T_1 were sufficient to determine a unique correlation time for methyl reorientation using the equations in Table I.[7] See Figure 1.

Results and Discussion

The ^2H methyl T_1's of L-[3,3,3-$^2H_3$]alanine, L-[*methyl*-$^2H_3$]-methionine, *cyclo*(L-Ala-L-[3,3,3-$^2H_3$]-Ala), and *t*-Boc-L-Ala-L-[3,3,3-$^2H_3$]-Ala-OMe measured as a function of temperature from inversion–recovery spectra (Figure 2) are listed in Table II. At all temperatures, T_1 ($\theta = 0°$) < T_1 ($\theta = 90°$). The observed orientation dependence of T_1 is in quantitative agreement with the T_1 anisotropy predicted by the three-site-jump model of methyl reorientation (Table I) but not with the T_1 anisotropy predicted by the free diffusion model (Table I). Hence, the latter model of methyl reorientation is ruled out. The predicted dependence

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Table II. Observed ^2H Spin–Lattice Relaxation Times, T_1, as a Function of Temperature and Orientation[a,b]

	T_1, ms			T_1, ms	
T, °C	$\theta = 0°$	$\theta = 90°$	T, °C	$\theta = 0°$	$\theta = 90°$
L-[3,3,3-$^2H_3$]Alanine			L-[*Methyl*-$^2H_3$]methionine		
89	5.5	12.6	−29	270	360
69	3.8	8.2	−39	200	310
51	3.8	6.6	−58	130	240
23	1.9	3.3	−61	90	155
−9	2.0	3.0	−82	50	95
−29	2.2	3.7	−94	45	73
−39	6.6	9.0	−110	20	33
−96	120	*c*	−118	15	27
t-Boc-L-Ala-L-[3,3,3-$^2H_3$]Ala-OMe			*cyclo*-(L-Aia-L-[3,3,3-$^2H_3$]Ala)		
22	19	44	22	23	36
−13	12	28	−13	8.6	17
−38	6.7	14.5	−38	5.5	9
−60	3.5	8.2	−60	3.4	6.4
−119	1.9	3.3	−119	1.8	3.4

[a] θ is the angle made by the 3-fold axis of the methyl group and the external magnetic field. [b] The experimental error in determining the T_1's is 5% for $\theta = 90°$ and 15% for $\theta = 0°$. [c] Cannot be measured due to short homogeneous T_2 (see Figure 4 and text).

Table III. Correlation Times, τ_c, for Methyl Reorientation from Analysis of T_1 Data Using a Three-Site-Hop Model

	τ_c, ns			τ_c, ns	
T, °C	$\theta = 0°$	$\theta = 90°$	T, °C	$\theta = 0°$	$\theta = 90°$
L-[3,3,3-$^2H_3$]Alanine			L-[*Methyl*-$^2H_3$]methionine		
89	0.31	0.28	−29	0.0063	0.009
69	0.48	0.43	−39	0.008	0.011
51	0.48	0.55	−58	0.012	0.014
23	1.1	1.3	−61	0.018	0.022
−9	5.0	5.0	−82	0.034	0.035
−29	6.2	7.5	−94	0.037	0.045
−39	21	22	−110	0.08	0.1
−96	400		−118	0.12	0.12
t-Boc-L-Ala-L-[3,3,3-$^2H_3$]-Ala-OMe			*cyclo*-(L-Ala-L-[3,3,3-$^2H_3$]-Ala)		
22	0.09	0.08	22	0.075	0.095
−13	0.14	0.12	−13	0.19	0.19
−38	0.26	0.22	−38	0.31	0.38
−60	0.51	0.4	−60	0.52	0.56
−119	5.0	6.0	−119	4.5	6.2

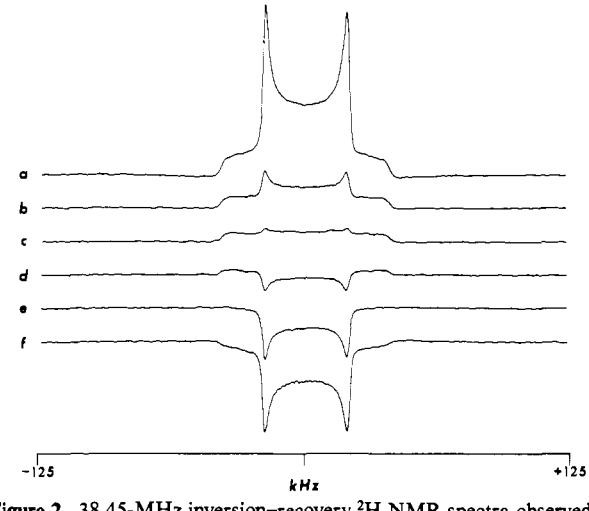

Figure 2. 38.45-MHz inversion–recovery ^2H NMR spectra observed for polycrystalline L-[3,3,3-$^2H_3$]alanine at 23 °C using the pulse sequence $180°_x$–T–$90°_{\pm x}$–t_1–$90°_y$–t_2 detect with: (a) $T = 50$ ms, (b) $T = 2.5$ ms, (c) $T = 2.0$ ms, (d) $T = 1.5$ ms, (e) $T = 1.0$ ms, (f) $T = 0.5$ ms. 6.4-μs 180° pulses were employed; $t_1 = 30$ μs, $t_2 = 34.5$ μs, and the sampling rate was 500 ns/pt.

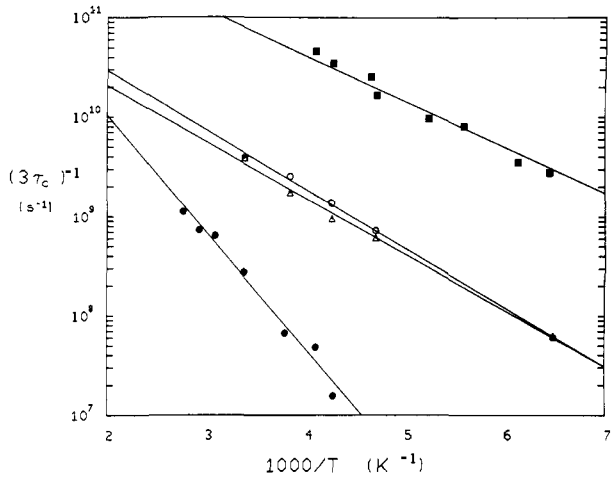

Figure 3. Methyl reorientation correlation times plotted as a function of inverse temperature: (●) L-[3,3,3-$^2H_3$]alanine; (○) *t*-Boc-L-Ala-L-[3,3,3-$^2H_3$]-Ala-OMe; (△) *cyclo*(L-Ala-L-[3,3,3-$^2H_3$]-Ala); (■) L-[*methyl*-$^2H_3$]methionine.

Table IV. Activation Energies, E_a, for Methyl Reorientation

compound	E_a, kJ/mol
L-[3,3,3-$^2H_3$]alanine	22.6
t-Boc-L-Ala-L-[3,3,3-$^2H_3$]Ala-OMe	11.4
cyclo-(L-Ala-L-[3,3,3-$^2H_3$]Ala)	10.8
L-[*methyl*-$^2H_3$]methionine	8.8

of T_1 (for $\theta = 0°$ and 90°) on the rotational correlation time for the three-site-jump model is plotted in Figure 1. The correlation times for the methyl reorientation listed in Table III were determined from the measured T_1 and the curves in Figure 1. It is clear from Table III that within experimental error the same correlation time is obtained from T_1 ($\theta = 0°$) and T_1 ($\theta = 90°$). This result is the basis for our previous statement that the anisotropy in T_1 is in accord with the predictions of the three-site-jump model. A more realistic model of methyl reorientation, diffusion in a 3-fold potential,[17] predicts T_1's that are experimentally indistinguishable from the three-site-jump model when the activation energy is greater than 10 kJ/mol.

Figure 3 shows a plot of $(3\tau_c)^{-1}$, the methyl-group reorientation rate, as a function of inverse temperature for the four compounds. With one exception the activation energy for reorientation of the methyl groups is in the expected range of 8.5–11.4 kJ/mol (Table IV). The activation energy for C^2H_3 reorientation in L-[3,3,3-$^2H_3$]alanine is 22.6 kJ/mol. This abnormally high activation energy can be rationalized in terms of the close packing of the methyl groups in the crystal structure.[12] The 3.6-Å methyl–methyl intermolecular distance in the crystal, 0.4 Å less than the van der Waals diameter of the methyl group, apparently imposes severe restrictions on methyl reorientation.

We can also rationalize the observed activation energies for the remaining compounds in terms of their crystal structures. In the crystalline cyclic dipeptide,[15] the nearest intermolecular neighbor to the methyl group is at a distance of greater than 4.0 Å. Therefore, no unusual hindrance to methyl reorientation is expected, which is in agreement with the 10.8 kJ/mol activation energy for this compound. The crystal structure of the linear dipeptide is not known, but it is inferred from the similar activation energies and correlation times observed in the linear and cyclic dipeptides (Tables III and IV) that packing does not hinder methyl reorientation in either crystal structure.

The low activation energy of L-[*methyl*-$^2H_3$]methionine, 8.8 kJ/mol, is consistent with the nearest-neighbor intermolecular $CH_3\cdots X$ distance (>4.0 Å, X = CH_3 or S) found in the crystal structure.[13] In addition, a methyl group bonded to sulfur experiences a lower potential energy barrier to rotation than a methyl group bonded to carbon. The activation energy for methyl reorientation in $(CH_3)_2SH$ is 8.9 kJ/mol[18] while that for CH_3CH_3 = 12.6 kJ/mol.[19]

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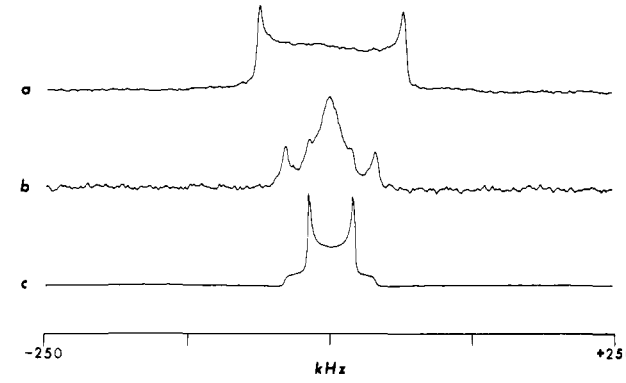

Figure 4. ^2H NMR spectra of polycrystalline L-[3,3,3-$^2H_3$]alanine at three temperatures: (a) −150 °C, (b) −96 °C, (c) 20 °C. All spectra were recorded at 38.45 MHz by using the solid echo pulse sequence and a sampling rate of 500 ns/pt.

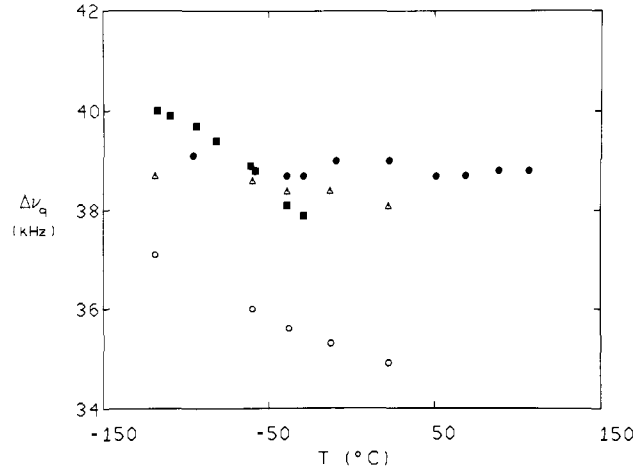

Figure 5. Observed averaged quadrupolar splittings, $\overline{\Delta\nu_q}$, plotted as a function of temperature: (●) L-[3,3,3-$^2H_3$]alanine; (○) *t*-Boc-L-Ala-L-[3,3,3-$^2H_3$]Ala-OMe; (△) *cyclo*(L-Ala-L-[3,3,3-$^2H_3$]-Ala); (■) L-[*methyl*-$^2H_3$]methionine.

Because of the high activation energy of the L-[3,3,3-$^2H_3$]alanine methyl reorientation, we observe a "static" axially symmetric ^2H powder pattern with $\Delta\nu_q$ = 128.2 kHz at the relatively high temperature of −150 °C (Figure 4). This result implies that $\tau_c^{-1} \ll \omega_Q$ at this temperature because we observe no averaging of the quadrupolar powder pattern expected for a static C–^2H bond. At −96 °C, $\tau_c^{-1} \sim \omega_Q$ and the static line shape is significantly altered by motional averaging. In addition, the line shape is severely distorted because of intensity losses. These occur in the quadrupolar echo experriment when $\tau_c^{-1} \sim \omega_Q$ because the homogeneous T_2 is very short and highly anisotropic.[20] At 20 °C, $\tau_c^{-1} \gg \omega_Q$ and an undistorted axially symmetric averaged powder pattern is observed. In this case the averaged quadrupolar splitting $\overline{\Delta\nu_q}$ is given by

$$\overline{\Delta\nu_q} = \Delta\nu_q S \tag{2}$$

where

$$S = (3\cos^2\Theta - 1)/2 \tag{3}$$

and Θ is the angle made by the C–^2H bond and the 3-fold axis. For tetrahedral geometry, $\Theta = 70.5°$ and $S = {}^1/_3$. Since $\Delta\nu_q$ = 128 kHz, we calculate $\overline{\Delta\nu_q}$ = 42.7 kHz whereas we observe $\overline{\Delta\nu_q}$

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Table V. Observed Averaged Quadrupole Splitting,[a] $\overline{\Delta\nu_q}$, as a Function of Temperature

T, °C	$\overline{\Delta\nu_q}$, kHz	T, °C	$\overline{\Delta\nu_q}$, kHz
L-[3,3,3-$^2H_3$]Alanine		L-[*Methyl*-$^2H_3$]methionine	
107	38.8	−29	37.9
89	38.8	−39	38.1
69	38.7	−58	38.8
51	38.7	−61	38.9
23	39.0	−82	39.4
−9	39.0	−94	39.7
−29	38.7	−110	39.9
−39	38.7	−118	40.0
−96	39.1		
−150	128.2		
t-Boc-Ala-L-[3,3,3-$^2H_3$]Ala-OMe		*cyclo*-(L-Ala-L-[3,3,3-$^2H_3$]Ala)	
22	34.9	22	38.1
−13	35.3	−13	38.4
−38	35.6	−38	38.4
−60	36.0	−60	38.6
−119	37.1	−119	38.7

[a] Uncertainty ±0.25 kHz.

= 38.8 kHz. One explanation for this discrepancy is that the methyl group departs slightly from tetrahedral geometry. If θ = 68.75°, S = 0.303 and we calculate $\overline{\Delta\nu_q}$ = 38.8 kHz in agreement with experiment. In an alternate explanation of the discrepancy between calculated and observed $\overline{\Delta\nu_q}$, we retain tetrahedral geometry but assume that in addition to three-site jumps the C–C$^2H_3$ bond axis librates rapidly in a cone of semiangle θ_c. The pattern remains axially symmetric and

$$\Delta\nu_q = \Delta\nu_q S \cos\theta_c\,(1 + \cos\theta_c)/2 \qquad (4)$$

Since θ_c is expected to increase with temperature, $\overline{\Delta\nu_q}$ should decrease with increasing temperature. The observed values of $\overline{\Delta\nu_q}$ (Table V, Figure 5) show the interesting feature that the L-[3,3,3-$^2H_3$]alanine $\overline{\Delta\nu_q}$ is constant from −100 °C to +107 °C. This result is strong evidence that the C–C$^2H_3$ bond axis in L-[3,3,3-$^2H_3$]alanine is not undergoing significant motion, and the reduced value of $\overline{\Delta\nu_q}$ is most likely due to a departure from tetrahedral geometry. Therefore, in the case, $\overline{\Delta\nu_q}$ is a sensitive probe of molecular geometry.

The behavior of $\overline{\Delta\nu_q}$ for *cyclo*(L-Ala-L-[3,3,3-$^2H_3$]-Ala) is similar to that observed for L-[3,3,3-$^2H_3$]alanine. If we set $\Delta\nu_q$ = 128 kHz and S = 0.303, the values obtained for L-[3,3,3-$^2H_3$]alanine, then according to eq 4 a θ_c = 10° accounts for the 38.1-kHz $\overline{\Delta\nu_q}$ observed for *cyclo*(L-Ala-L-[3,3,3-$^2H_3$]-Ala) at 22 °C. The temperature factors reported in the crystal structure of *cyclo*(L-Ala-L-Ala) do not indicate any large-scale motions of the methyl carbons in this compound.[15]

In contrast to the temperature insensitivity of $\overline{\Delta\nu_q}$ just discussed, $\overline{\Delta\nu_q}$ for *t*-Boc-L-Ala-L-[3,3,3-$^2H_3$]-Ala-OMe and L-[*methyl*-$^2H_3$]methionine shows significant reductions as temperature increases. Using eq 4 and $\overline{\Delta\nu_q}$ = 34.9 kHz at 22 °C for *t*-Boc-L-Ala-L-[3,3,3-$^2H_3$]-Ala-OMe, we calculate θ_c = 21.5°. Although the crystal structure for *t*-Boc-L-Ala-L-[3,3,3-$^2H_3$]-Ala-OMe is not known, the temperature factors for L-Ala-L-Ala–HCl[21] are compatible with libration of the methyl rotation axes. The large temperature factors reported for the methyl carbon of crystalline L-[*methyl*-$^2H_3$]methionine are in accord with significant reduction of $\overline{\Delta\nu_q}$ observed as the temperature increases. Using eq 4 with $\Delta\nu_q$ = 128 kHz, $\overline{\Delta\nu_q}$ = 37.9 kHz, and S = 0.333 (we assume tetrahedral geometry in absence of any counterevidence), we calculate θ_c = 22.5° at −29 °C. As temperature increases above −29 °C, the ^2H NMR powder pattern of L-[*methyl*-$^2H_3$]-methionine (not shown) becomes axially asymmetric due to large-amplitude asymmetric motions of the methyl rotation axis.

Both the ^1H NMR results[8-10] and the results presented here reveal that reorientation of the methyl group in L-alanine (E_a = 22.4–22.5 kJ/mol) or L-[3,3,3-$^2H_3$]alanine (E_a = 22.6 kJ/mol) exhibits the highest activation energy of all the compounds studied. This high activation energy is a result of the extremely close crystal packing found in alanine.[12] The activation energies obtained from ^1H NMR for methyl reorientation in alanylglycine, glycylalanine, and amino acids containing methyl groups[8-10] are in the 10–15 kJ/mol range as are the alanine dipeptides studied herein. These activation energies are all well below that found in alanine, the only compound for which unusually tight methyl group packing has been reported.

The energy barriers for methyl-group reorientation in L-[*methyl*-$^2H_3$]methionine (8.8 kJ/mol) and D,L-methionine (6.7 kJ/mol) are the smallest observed in each set of compounds from the two NMR studies. The difference between the ^1H and ^2H NMR results may be due to different crystal packings of the optically active and racemic forms of methionine.

Although both ^1H and ^2H NMR studies provide us with methyl-group reorientational energy barriers, ^2H NMR has the advantage of discriminating among various models of methyl reorientation. Since the dominant ^2H quadrupolar interaction is a single spin–relaxation mechanism, the correlation time is precisely defined $(3k)^{-1}$ for a three-site-jump model. This is not the case in ^1H NMR, where both inter- and intramolecular multispin interactions contribute to proton relaxation.[22]

The results presented here show that one can discriminate between models of methyl reorientation by measuring the orientation dependence of ^2H T_1's. In addition, the activation energies derived from these measurements qualitatively correlate with the packing of methyl groups in crystals. In this regard our results suggest that measurement of the activation energy for methyl reorientation may be a useful way to identify tightly packed domains in macromolecules. Finally, these measurements provide extensive data to test the predictions of molecular dynamics calculations[23] that are currently being developed to elucidate molecular dynamics in proteins.

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Registry No. L-[3,3,3-$^2H_3$]Alanine, 63546-27-0; L-[*methyl*-$^2H_3$]-methionine, 13010-53-2; *cyclo*(L-ala-L-[3,3,3-$^2H_3$]ala), 84787-44-0; *t*-Boc-L-ala-L-[3,3,3-$^2H_3$]ala-OMe, 84787-45-1; L-[3,3,3-$^2H_3$]alanine methyl ester·HCl, 84787-46-2; *t*-Boc-alanine, 15761-38-3.

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