Tyrosyl Motion in Peptides: ^2H NMR Line Shapes and Spin-Lattice Relaxation

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Abstract: ^2H quadrupole echo NMR line shapes and spin-lattice relaxation times have been measured for two crystalline tyrosine-containing peptides, *N*-acetyl-L-prolyl-L-tyrosine-d_2-*N'*-methylamide (PY-d_2) and *N*-acetyl-L-asparaginyl-L-prolyl-L-tyrosine-d_2-*N'*-methylamide (NPY-d_2). The temperature-dependent ^2H quadrupole echo line shapes for these two peptides, and their τ-dependence at fixed temperatures, can be simulated accurately with a model in which the tyrosyl rings undergo 180° rotational jumps with rates varying between 10^3 and 10^6 s^{-1}. The simulations also predict intensity losses which are experimentally documented for the first time. The spin-lattice relaxation times of NPY-d_2 and PY-d_2 also show a temperature dependence and anisotropy expected from a rotational jump model and can be employed to determine the twofold jump rate. In these two systems, the rates determined from the T_1 measurements are in close agreement with results from the line shape simulations.

Because of their twofold symmetry, the aromatic side chains of phenylalanine and tyrosine can execute 180° rotational jumps about the C^β–C^γ bond between two orientations of equal energy. In a densely packed structure such as the interior of a protein, or in a crystalline amino acid or peptide, the rotation of the ring between the two equilibrium orientations must displace neighboring side chains to some extent. Consequently, a rotational jump probably depends on a degree of cooperativity with motions of surrounding groups. Thus, the rate and mechanism of phenylalanyl or tyrosyl ring motion in a peptide or protein can provide information about the conformational flexibility of neighboring residues. Moreover, since the NMR spectra of ^2H-labeled phenylalanyl and tyrosyl rings change dramatically with this motion, the effects are easily measured. Specifically, the internal motion of the ring about the C^β–C^γ bond transforms the $\eta \simeq 0$ (axially symmetric) spectrum observed for δ and ϵ ^2H's in the absence of motion into an $\eta \simeq 0.6$ spectrum in the fast limit.[1-4] This particular shape results from the fact that the C–D bond vectors make an angle of 60° with respect to the C^β–C^γ axis, that the two ring orientations are equally probable, and that the rate of the motion exceeds the size of the rigid lattice quadrupole coupling by about two orders of magnitude ($>10^7$ s^{-1}).

If the rate of reorientation is not fast, then ^2H spectra exhibit additional interesting features. Specifically, if the spectra are recorded with a two-pulse quadrupole echo[5] and the jump rate lies in the range 10^4–10^6 s^{-1}, then spectra will appear to arise from a superposition of a rigid lattice and a fast-limit line shape.[1,6] However, it can be shown (vide infra) that these shapes are, in fact, due to a single population of jumping rings simply by measuring the temperature dependence of the integrated spectral intensity.[7] In the intermediate exchange region, the spectral intensity exhibits a characteristic decline which, for the two-site aromatic ring motion, reaches a minimum of about 20% of the rigid lattice or fast-limit intensity. This intensity dip and the line shapes are both a result of "echo distortions". Specifically, T_2 (the spin-spin relaxation time) becomes short and anisotropic, and, for certain regions of the powder pattern, is comparable to the τ value (the spacing between the pulses) in the echo experiment.[6] This physical effect is primarily responsible for determining the line shapes in quadrupole echo studies of exchanging systems. Thus, accurate recording of both the line shapes and spectral intensities is necessary to provide unambiguous information about the rate and mechanism of the jump process.

An alternative method to study motion in a solid involves the measurement of the spin-lattice relaxation time, T_1. For ^2H, the modulation of the quadrupole interaction provides the dominant relaxation mechanism, and therefore interpretation of T_1 data is straightforward. Moreover, since the quadrupole interaction is also responsible for the spectral line shape, then one might predict that the two methods could be used to measure the same quantity independently. We have found that, in the small peptides considered here, and in crystalline amino acids discussed elsewhere,[8] the experimental T_1's agree well with those predicted on the basis of the twofold jump rate measured from the line shapes. However, in other cases, such as proteins, there appear to be differences in the rates obtained by the two methods which can be explained by invoking additional librational motions which dominate T_1.[9]

In this paper, we present ^2H NMR results obtained from two tyrosine-containing peptides, *N*-acetyl-L-prolyl-L-tyrosine-*N'*-methylamide (PY-d_2) and *N*-acetyl-L-asparaginyl-L-prolyl-L-tyrosine-*N'*-methylamide (NPY-d_2) which illustrate the concepts outlined above. Crystalline samples of these two peptides present well-defined structures, where we have observed the twofold ring motion discussed above. Measurement of the spectral line shapes and intensities permit us to determine the rate of this process as a function of temperature. Using these data, we can calculate the contribution that this motion makes to T_1, and the experimentally determined T_1 is in good agreement with that predicted for twofold flips alone. We compare these results with those obtained for other crystalline peptides such as the amino acid phenylalanine,[8,10,11] the pentapeptide [Leu^5]enkephalin,[1] the protein bacteriorhodopsin,[2,3,9] and synthetic polymers.[11,12] These mole-

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cules show a variety of ^2H line shapes, and we observe both similarities and differences between these molecules and the crystalline peptides discussed here.

Experimental Section

Materials. The L isomer of [3,5-$^2H_2$]tyrosine was prepared as described by Rice et al.[1] It was used to synthesize PY-d_2 and NPY-d_2 by the procedure of Montelione et al.[13] who also determined their crystal structures. PY-d_2 was recrystallized from ethyl acetate, mp 218–219 °C (dec). NPY-d_2 was recrystallized from ethanol, mp 246–247 °C (dec). Samples for the NMR experiments consisted of 50 mg of polycrystalline powder sealed under vacuum (10^{-3} torr) in 6-mm o.d. glass tubes.

^2H NMR Experiments. The ^2H NMR spectra of the peptides were recorded at 48.8 MHz, using a quadrupole echo sequence, with quadrature detection and a typical dwell time of 500 ns. Fourier transformation was begun at the observed top of the echo, and no phase correction was required of these spectra. DC offset and quadrature phase errors were attenuated by phase cycling through the eight possible quadrupole echos resulting from four phases.[14] The 90° pulse width was 1.7 μs and the 180° pulse width was 4.0 μs, reflecting some pulse droop of the amplifier. At such power levels, normal quadrupole echo spectra require a minimal finite pulse width correction. However, the inversion recovery quadrupole echo spectra used for the measurement of T_1 are still considerably distorted. Approximately 80% inversion was obtained with a 4.0-μs 180° pulse. The frequency response of the probe was determined by observation of the intensity of a D_2O signal throughout the bandwidth, and the frequency response roll-off curve of the probe is of the same magnitude as the finite pulse correction.[15] Both effects have been accounted for in the spectral simulations. Recycle delays were determined by determining the equilibrium intensity quadrupole echo, and these and the number of scans are quoted in the figure captions.

Line Shape Simulations. Simulations of ^2H quadrupole echo line shapes which result from a 180° rotational jump have been carried out with a computer program to be described elsewhere.[7] The simulations predict both the shape and the relative intensities of spectra. Experimentally, the spectral intensities are determined by calibration of the probe response at various temperatures using a sample of valine-d_6. The ^2H quadrupole echo spectrum of this compound changes only slightly over the temperature range studied here, and so may be used as an intensity standard. At each temperature the integrated spectral intensity of the valine standard was determined from the $\tau = 0$ intercept of a plot of log I_{val} vs. τ. τ is the quadrupole echo pulse spacing and I_{val} is the quadrupole echo spectral intensity. Since the valine-d_6 spectrum is a fast limit line shape there was very little change of I_{val} with τ. The response function shows an approximate inverse temperature relationship described by $I(T) = 213/T + 0.217$. The intensity is normalized to 1.0 and 273 K. The uncertainty in experimental intensities due to probe response is ±3%.

T_1 Measurements. T_1 measurements were made with the inversion recovery quadrupole echo pulse sequence 180°–DE–90°$_x$–τ–90°$_y$–τ–echo (where DE is a T_1 delay). This sequence was employed with four-phase cycling of the latter two pulses which, in addition to removing DC offsets, also cancelled the signal from a second echo which results from the 90°$_x$–DE–90°$_x$–τ–90°$_y$–DE–echo sequence. In this case, it results from the finite 180° inversion pulse. T_1 data were obtained from the echo intensity and plotted according to the usual expression, log $(I - I_\infty)/2I_\infty$ vs. DE. I_∞ agreed with the intensity which results from a quadrupole echo obtained with an equilibrum recycle delay corresponding to roughly five T_1's. While we present experimental evidence for the presence of a T_1 anisotropy, we ignore it for the rate calculations shown here. Further studies of this phenomena will be presented elsewhere.[8]

Results and Discussion

^2H quadrupole echo line shapes and spin–lattice relaxation times were determined for the two peptides PY-d_2 and NPY-d_2, where each peptide was ^2H-labeled at the C$^\epsilon$ position of the tyrosine ring. Initially, ^2H line shapes of both peptides were observed up to a temperature of 160 °C in order to determine if structural phase transitions occur in the temperature range for these samples. Spectra of PY-d_2 are completely independent of sample history. Spectra of NPY-d_2 indicate that this compound undergoes a transition just above 100 °C from a species with a large activation

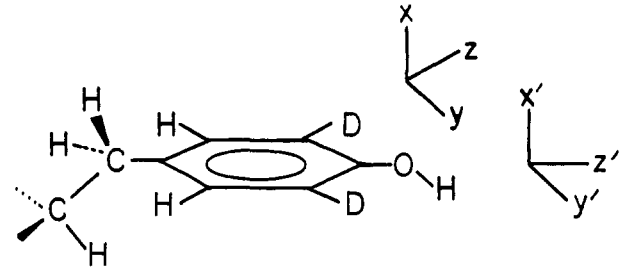

Figure 1. A tyrosyl side chain showing the principal coordinate axes of the quadrupole tensor (x, y, z) and the rotationally averaged coordinate system (x', y', z'). The ring was labeled with ^2H at the ϵ and ϵ' positions as shown. The twofold flips occur about the z' direction.

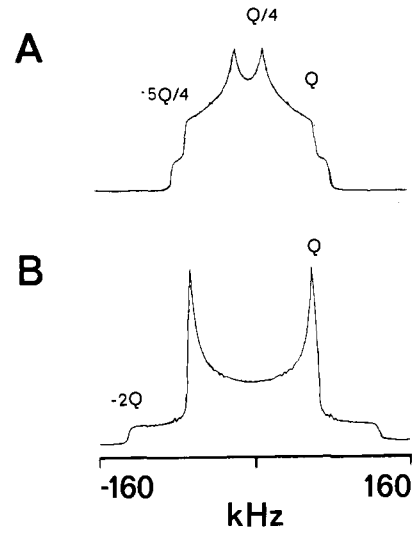

Figure 2. (A) Axially asymmetric ($\eta = 0.6$) line shape of ϵ-labeled Tyr-d_2 undergoing fast 180° rotational jumps about the C$^\beta$-C$^\gamma$ bond axis. The spectrum edges corresponding to the Q, $Q/4$, and $-5Q/4$ components are shown. (B) Axially symmetric rigid limit line shape of ϵ-labeled Tyr-d_2.

energy for rotational jumps to a second species with a low activation energy. The new form is completely stable during subsequent measurements up to 160 °C and down to -50 °C. We are currently investigating this new structure and report its NMR line shapes here. We observed a similar behavior for Tyr-d_2 in [Leu^5]enkephalin.[1]

^2H Line Shapes of PY-d_2 and NPY-d_2. Before considering the experimental results from PY-d_2 and NPY-d_2, we review the effects of twofold jumps on the ^2H NMR spectra of the aromatic rings, tyrosine and phenylalanine. In Figure 1 is illustrated a tyrosine ring, ^2H-labeled at the ϵ positions together with two (x, y, z) Cartesian axis systems. The unprimed system represents the molecular orientation of a ^2H quadrupole coupling tensor which leads to the nearly axially symmetric powder spectrum illustrated in Figure 2B, with principal values of Q and $-2Q$. The largest splitting ($\pm 2Q$) corresponds to an orientation of the field, H_0, lying parallel to the C–D bond. When the ring executes fast twofold flips, the axially symmetric spectrum is transformed to the axially asymmetric spectrum, $\eta \simeq 0.6$, illustrated in Figure 2A. In this case the $Q/4$ components correspond to orientations of H_0 along the C$^\beta$–C$^\gamma$ axis (the z' direction in Figure 2, which is also the twofold flip axis). For the Q components, H_0 is perpendicular to the ring plane (x'), and for the $-5Q/4$ components, H_0 is in the ring plane orthogonal to these two directions (y').

Figure 3A shows a set of temperature-dependent ^2H spectra of NPY-d_2 obtained by Fourier transformation of the quadrupole echo. These spectra required 10^3–10^4 scans each, and the recycle delays were about five times the measured spin–lattice relaxation time, which varied between 0.2 and 5 s. The individual spectra in Figure 3A have been scaled according to the number of scans in order to indicate their relative experimental intensities. The intensity of an individual spectrum depends upon its absolute intensity and also upon the response of the probe at the given

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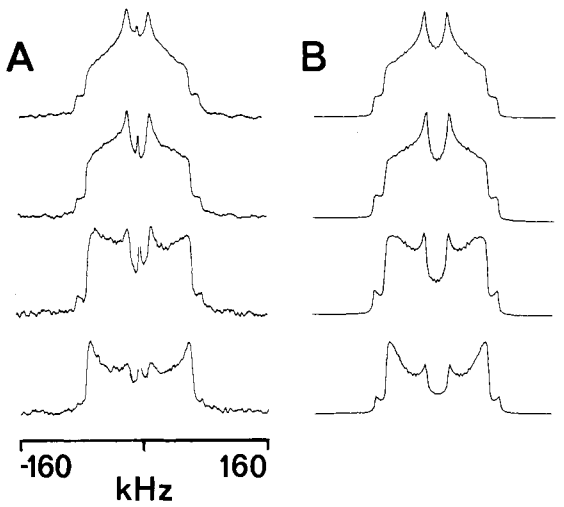

Figure 3. (A) Temperature-dependent ^2H quadrupole echo spectra of NPY-d_2, τ = 30 μs: (bottom to top) −4 °C, 1200 scans, recycle delay = 10 s; 27 °C, 5000 scans, recycle delay = 2.0 s; 61 °C, 10000 scans, recycle delay = 1.0 s; 104 °C, 10000 scans, recycle delay = 0.2 s. (B) Spectral simulations based upon a 180° rotational jump model. Spectra have been scaled to show their relative intensities, and simulations predict both line shape and integrated intensity.

Table I. Twofold Jump Rates (K_{LS})[a] Obtained from the ^2H Line Shapes of Figures 3 and 4 as a Function of Temperature[b]

T (°C)	K_{LS} (s^{-1})	QCC (kHz)
	PY-d_2	
126	1.0×10^5	171
142	4.0×10^5	168
163	1.3×10^6	166
	NPY-d_2	
−4	2.5×10^5	176
27	7.8×10^5	172
61	1.9×10^6	169
104	7.9×10^6	165

[a] LS = line shape. [b] QCC is the quadrupole coupling constant and η = 0.015 is the asymmetry parameter used in the calculations; 3-kHz line broadening was applied to the spectra.

temperature. The absolute intensity independent of probe response was obtained by scaling the spectra relative to the intensity of the standard valine-d_6 as described above. This procedure was also applied to the spectra in Figure 4A.

Inspection of Figure 3A shows that the spectra transform from an approximately axially symmetric line shape at the lower temperature (−4 °C) to a line shape at high temperature (104 °C) which approaches the η = 0.6 spectrum of Figure 2A. It should be noted that intermediate temperature spectra are of lower intensity, and their shapes are intermediate between the η = 0 and the η = 0.6 line shapes shown in Figure 2. As will become clearer below, the high-temperature spectrum does not show the full central intensity expected from a fast-limit η = 0.6 line shape. Figure 3B shows a set of simulations of the temperature dependence of the NPY-d_2 spectra which are based on a 180° rotational jump model. The simulations predict both the spectral intensity and the line shapes, and there is clearly excellent agreement with the experimental results. As will be discussed below, the observation of the correct temperature dependence of the spectral intensities is necessary to exclude the possibility that the line shapes arise from two separate populations of hopping rings, or perhaps a single set with a distribution of flip rates. The rates obtained from the simulations are compiled in Table I.

Figure 4 shows similar experimental spectra and simulations for PY-d_2. Again, there is good agreement between the two sets of spectra both with regard to the line shapes and the spectral intensities. It should be noted, for example, that the $5Q/4$ edges are present, and the shape of the central portion of the spectrum

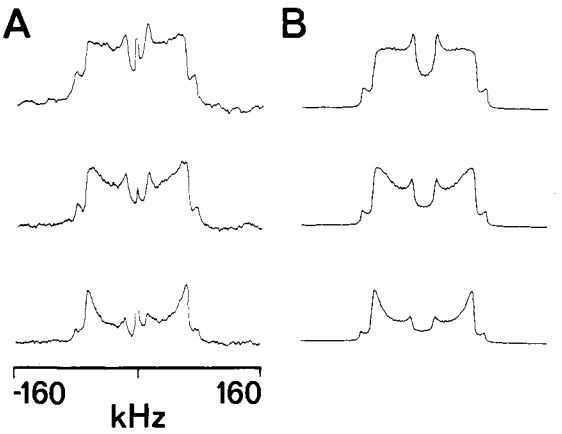

Figure 4. (A) Temperature-dependent ^2H quadrupole echo spectra of PY-d_2: (bottom to top) 126 °C, 5000 scans, recycle delay = 5.0 s; 142 °C, 10000 scans, recycle delay = 2.0 s; 163 °C, 19000 scans, recycle delay 1.0 s. (B) Spectral simulations based upon a rotational jump model.

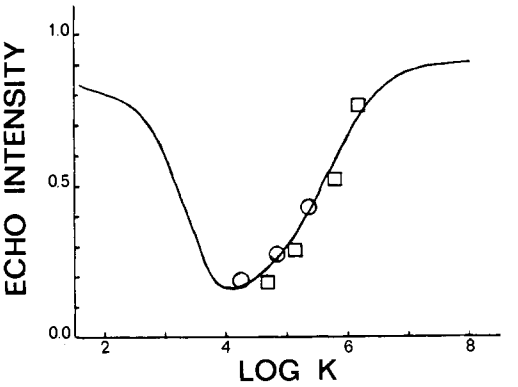

Figure 5. A plot of integrated intensity vs. jump rate for intermediate exchange ^2H line shapes based upon a rotational jump model. The solid line represents the theoretical intensity for a pulse spacing, τ = 30 μs: □ = PY-d_2 spectra, ○ = NPY-d_2 spectra. The jump rates were obtained from the experimental line shapes and plotted vs. the spectral intensity.

is correct at all temperatures. The rates obtained from the PY-d_2 simulations are also given in Table I, together with the quadrupole coupling constant and asymmetry parameter employed in the simulations. It was necessary to decrease the coupling constant slightly at higher temperatures, and this fact suggests that fast, small-amplitude librations are present in the lattice and increase in amplitude with higher temperature.

Above, we mentioned that it is also necessary to measure the spectral intensities in order to derive a motional model unambiguously from the spectra. In Figure 5 we have plotted the spectral intensities obtained from the PY-d_2 and NPY-d_2 spectra as a function of the twofold jump rate, K. The solid line represents the echo intensity predicted by the twofold jump model. It should be noted that the line does not approach unity (full intensity) in the slow and fast limits because of power roll-off effects.[15] In particular, the spectra are wider in the slow-limit regime, and therefore only about 85% of the full spectral intensity is observed with our finite rf pulses. In the fast limit this figure increases to about 90% for rates of $\sim 10^8$ s^{-1} because the spectral width narrows from ~250 to ~170 kHz. The experimental intensities for the PY-d_2 and NPY-d_2 fall very close to the calculated line and confirm the essential correctness of this feature of the lineshape calculation. Furthermore, the fact that the experimental intensity data fall on this line suggests that these spectral line shapes do not arise from a superposition of slow- and fast-hopping rings but rather from a single population of Tyr rings which exhibits a single correlation time.

In the case of twofold ring flips considered here, the intensity falls to about 20% of the full value. This is less than the value of 50% originally calculated by Spiess and Sillescu.[6] The origin

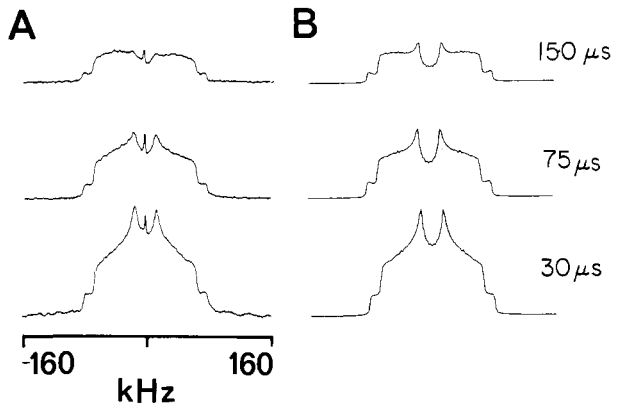

Figure 6. (A) ^2H quadrupole echo spectra of NPY-d_2 obtained at the indicated pulse spacings (in μs) at 104 °C. (B) Simulations based upon a 180° jump model, showing the effect of anisotropic quadrupole echo decay. Intensities of spectra with $\tau > 30$ μs are scaled with an additional intensity factor corresponding to an isotropic T_2 of 300 μs.

of this difference is that spectral intensities quoted by these workers were derived from the peak values in "stick" spectra and were meant primarily to serve as an indication that intensity losses should be expected. The more accurate values reported here and elsewhere[7] were derived by integrating the full-powder line shapes for various hopping rates. It should also be mentioned that the expected intensity losses for higher symmetry motions are even greater than those for twofold flips. For three- and fourfold hops only about 5% of the signal is observed in the intermediate exchange region.[7]

In addition to studying the temperature dependence of the Tyr-d_2 line shapes, we have also examined the τ dependence at several temperatures. In principle, both the shape and intensity of these spectra can provide additional data to determine the rates of various motions. However, for typical experimental parameters employed in ^2H quadrupole echo spectroscopy, the most dramatic changes in the spectra are predicted to occur for values of $\tau \leq 20$ μs. Since most receiver and probe recovery times do not permit observation of the signal at $\tau \leq 30$ μs, it is not possible to extract the full information from these spectra. Nevertheless, as illustrated in Figure 6, some information can be gleaned from the τ dependence. Here we show spectra of NPY-d_2 at 104 °C for three τ values (Figure 6A) together with simulations calculated assuming a twofold jump rate of 7.9×10^6 s^{-1}. There is clearly good agreement between the experimental and calculated results both in terms of the spectral line shapes and the spectral intensities. When signal to noise is high, for instance with perdeuterated samples, then τ can be increased to a few hundred microseconds which permits extension of the fast-limit regime to $>10^8$ s^{-1}.

The jump rate data obtained from the PY-d_2 and NPY-d_2 simulations are summarized in Table I together with the quadrupole coupling constant (QCC) used for the simulations at each temperature. It should be noted that it was necessary to decrease the QCC by about 2% for PY-d_2 over the 150 °C and 10% for NPY-d_2 over the ~100 °C range. We believe these decreases are due to fast, small amplitude motions which are present in the lattice and reduce the overall width of the spectra slightly. The activation energies obtained from the temperature dependence of the rate are 21.0 and 6.4 kcal/mol for PY-d_2 and NPY-d_2, respectively.

Spin-Lattice Relaxation. Traditionally, one of the most common approaches to determining correlation times with NMR involves measurement of spin-lattice relaxation times. In the case of ^2H, this method is particularly convenient since the relaxation is dominated by the quadrupole coupling, and contributions from other sources (dipolar relaxation) can be ignored. Generally, the interpretation of T_1 data involves postulating a motional model and then calculating the correlation time from a formula derived from that model. However, in liquid samples it is often very difficult to test models for T_1 critically, because all of the motional information is distilled into the relaxation behavior of a single line.

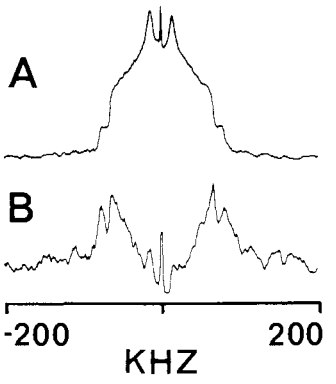

Figure 7. (A) ^2H quadrupole echo spectrum of NPY-d_2 at 104 °C. (B) Partially relaxed inversion recovery ^2H quadrupole echo spectrum at the null point: 32 000 scans, DE = 30 ms. The anisotropy in T_1 is evident close to the null point.

In such cases it is not possible to differentiate between models based on discrete hopping motions vs. continuous diffusion. However, the solid-state ^2H NMR line shapes discussed above permit us to improve on this situation. In particular, since we observe a powder line shape we can study the anisotropy of T_1 to determine the rate and the mechanism of the motion.[16,17] Simulations of these partially relaxed line shapes provide information about T_1 anisotropies and permit a more stringent test of a proposed dynamic model. Furthermore, since a line shape is being observed, its relaxation behavior permits a determination of a unique value of T_1 from a single (rather than two) experimental measurement(s).

An example illustrating this point is shown in Figure 7 which is a spectrum of NPY-d_2 taken at 104 °C. In Figure 7A we show a fully relaxed spectrum obtained with a quadrupole echo, whereas Figure 7B shows a partially relaxed line shape obtained with the sequence 180–DE–90_x–τ–90_y–τ–echo (an inverting pulse plus a quadrupole echo). Spectral distortions due to finite pulse widths are severe in this experiment and will be discussed further elsewhere. Nevertheless, the trace shown in Figure 7B clearly indicates the presence of a T_1 anisotropy. In particular, the $\pm 5Q/4$ and $\pm Q$ edges of the spectrum are in absorption, whereas the $\pm Q/4$ edges are still inverted. As will be shown elsewhere,[8] this spectral behavior is characteristic of motions where the correlation time satisfies the condition $\omega_0 \tau_c > 1$. Furthermore, the T_1 obtained from this spectrum yields a jump rate of 1.0×10^7 s^{-1} which is in good agreement with that obtained from the line-shape simulations. The agreement between the direct measurement of echo intensities and simulation of a partially relaxed spectrum indicates that T_1 is dominated by twofold ring flips. As a consequence of these observations, we have not performed a complete study of the T_1 line shapes at each temperature. Instead, we have determined T_1 from the intensity of the quadrupole echo following an inverting pulse. In particular, eq 2 of ref 16 gives the formula for the explicit angular dependence of T_1. Performing a powder average yields

$$\left\langle \frac{1}{T_1} \right\rangle = \frac{2k}{5}\left(\frac{\omega_Q}{\omega_0}\right)^2 \sin^2 2\theta$$

where $\theta = 60°$, $\omega_0 = 2\pi(48.8$ MHz) is the Larmor frequency, $\omega_Q = 2\pi(132$ kHz) is the quadrupole coupling constant, and $k = {}^1/_{2\tau}$ is the jump rate. This procedure yields hopping rates which are quite close to those obtained from a full line-shape analysis. The results are shown in Table II together with a column indicating the ratio of the rates obtained from the T_1 measurements and those obtained from the line shapes. It should be noted that the T_1 data always yield a slightly faster rate by a factor of about 1 – 2.5 (excluding the PY-d_2 data at 126 °C). With increasing temperature, the T_1 becomes shorter and would presumably pass

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Table II. Experimental ^2H T_1's for PY-d_2 and NPY-d_2 Measured as a Function of Temperature at 48.8 MHz[a]

T (°C)	T_1 (s)	K_{T1} (s^{-1})	K_{T1}/K_{LS}
		PY-d_2	
76	5.0	9.1×10^4	
126	0.90	5.1×10^5	5.1
142	0.39	1.2×10^6	3.0
165	0.16	2.9×10^6	2.2
		NPY-d_2	
−4	2.0	2.8×10^5	1.1
27	0.40	1.1×10^6	1.4
61	0.15	3.0×10^6	1.6
104	0.04	1.1×10^7	1.4

[a] Also included are the ratios of the jump rates obtained from the T_1 experiments (K_{T1}) and the line-shape simulations (K_{LS})

through a minimum at higher temperatures. The activation energies obtained from the T_1 data are 18.9 and 6.7 kcal/mol for PY-d_2 and NPY-d_2, respectively, and are in good agreement with the values of 21.0 and 6.4 kcal/mol obtained from the line-shape data.

Motion of the Tyrosine Aromatic Rings. Although there have been several studies of aromatic ring motion with ^2H NMR, in none of these studies has there been an investigation of the line shapes in the detail reported here. In the cases studied previously the line shapes fit qualitatively, but a careful comparison of the experimental and theoretical spectra reveals differences which are not attributable to experimental error. For example, in our previous work on [Leu^5]enkephalin,[1] the $\pm 5Q/4$ edges of the spectra were not well resolved, and the temperature dependence predicted for the spectra could not be simulated exactly. Similar comments are applicable to studies of aromatic ring motion in liquid crystalline polymers,[12] in bacteriorhodopsin,[9] and to a lesser extent in *p*-fluoro-D,L-phenylalanine.[18] In contrast, the temperature dependence of the NPY-d_2 and PY-d_2 line shapes (and the Phe-d_4·HCl line shapes to be reported elsewhere)[8] can be reproduced with much greater accuracy. In addition, in all of the previous studies mentioned above, no effort was made to simulate the τ dependence of the spectra. Finally, even though Spiess and Sillescu[6] discussed intensity losses in the intermediate exchange region some time ago, there have been no previous experimental data with which to confirm their prediction. The results described here are the first quantitative confirmation of these theoretical predictions.

These results are important for several reasons. First, they confirm the essential correctness of the line-shape theories in two relatively simple crystalline molecules. Second, the intensity losses, which have been predicted and observed, provide a means to differentiate between a single component and multiple component spectrum.[3] In the former case an intensity dip is observed as a function of temperature, while if the spectra arose from a superposition of fast- and slow-limit hopping rings, then the spectral intensity would be invariant with temperature. The observation of the correct temperature and τ-dependent line shapes and spectral intensities provides a firm basis for the application of these line-shape calculations to other systems. Thirdly, the results demonstrate that the Tyr-d_2 ring motion of NPY-d_2 and PY-d_2 is well represented by a model involving a twofold flip with a single correlation time. This behavior is evidently not observed in other systems and is probably the reason for the discrepancy between many of the calculated and experimental line shapes.

The activation energies for the twofold hopping motion differ by a factor of about 3 for PY-d_2 and NPY-d_2. PY-d_2 is similar to Phe-d_4·HCl[9] in that both are small molecular systems and exhibit activation energies, E_a, of about 20 kcal/mol. So far as we know, the Tyr in ferricytochrome *c* is the only other ring system that exhibits such a high value of E_a.[19] In the protein bovine pancreatic trypsin inhibitor the activation energies are in the neighborhood of 15 kcal/mol,[20] although some rings are immobile over the entire temperature range studied (up to 80 °C). NPY-d_2 with E_a about 6.5 kcal/mol possesses a lower activation energy than has previously been measured for ring flips. For Tyr in [Leu^5]enkephalin and Phe in bacteriorhodopsin, we found E_a = 9.8 and 12 kcal/mol, respectively. Perhaps this low E_a exhibited by NPY-d_2 is related to disruption of the crystal structure by the phase transition observed on the initial heating of the samples. A more complete understanding of these data and a meaningful comparison of the activation energies will probably not be possible until additional experimental data about structures and twofold hopping rates become available.

As mentioned above, the ^2H line shapes indicate that the dominant motional mode is twofold flips. The T_1 measurements produce rate constants which are very similar in magnitude to the rate constants obtained from the line shapes and indicate that T_1 is also determined primarily by the twofold flips. Nevertheless, there are probably other motions present since the quadrupole coupling constant used in the line-shape simulations must be decreased slightly in order to simulate the spectra at higher temperatures. We suggest that small angle librations (10–15°) are present. Because they are fast compared to the QCC these librations average the coupling constant and they may be included in the calculations by simply adjusting the size of the coupling. In other systems such as bacteriorhodopsin, librational motions are larger and nearer the Larmor frequency and therefore contribute strongly to T_1 and to the line shapes. For instance, in bacteriorhodopsin-*Phe*-d_4 the line shapes exhibit rounded $5Q/4$ edges and T_1 is $\sim 10^2$ times shorter than expected if it were calculated on the basis of rate data obtained from the line shape.

Conclusions

^2H quadrupole echo spectroscopy has been used for some time to examine molecular dynamics in a variety of molecular solids. However, for most of these systems the dynamic behavior is quite complex, and therefore it has been difficult to provide a critical assessment of the line shape theories and motional models which are employed to simulate the spectra. As a result, there has been no careful comparison between temperature-dependent experimental spectra and theoretical line shapes. In addition, there has been no careful study of the τ dependence of the spectra as a function of temperature, the theoretically predicted intensity losses have not been carefully documented, and only in one other case has a T_1 anisotropy been observed.[18] The experimental results presented here improve this situation. In particular, they demonstrate that a motional model of twofold ring flips based on a single correlation time is adequate to describe molecular motion in crystalline NPY-d_2 and PY-d_2. With this model it is possible to calculate the correct temperature dependence of the line shapes, their τ dependence, and the intensity losses which occur in the intermediate exchange regime. Furthermore, the spin–lattice relaxation times and their anisotropies lead to rate constants in good agreement with those obtained from line-shape calculations and indicate that T_1 is determined primarily by a twofold flip. The successful application of these line-shape calculations provides a firm basis for the interpretation of the more complex spectra which can arise from systems with many components or from systems with a distribution of motional correlation times.

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