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Spin–Lattice Relaxation Rates of Coupled Spins from 2D Accordion Spectroscopy

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Nuclear magnetic resonance spectroscopy is widely recognized as a useful technique for probing dynamic and structural properties of macromolecules (*1*). The use of nuclear Overhauser effects (NOEs) in one- and two-dimensional spectra has become the most common means of assessing structure (*1, 2*). However, the same $1/r^6$ distance dependence that makes NOE spectra useful enters into the spin–lattice relaxation time, T_1. A particularly useful approach combines T_1 relaxation and cross relaxation as a practical route to a more thorough analysis of both 1D (*2, 3*) and 2D cross-relaxation data (*4*). The effects of paramagnetic agents on T_1 can also be used to complement structural information (*5*).

Application of conventional 1D NMR techniques or use of information on the diagonal in 2D experiments for the measurement of spin–lattice relaxation rates of individual resonances in a macromolecule is often impossible due to the lack of spectral resolution in one dimension. One solution has been proposed by Bystrov and co-workers (*6*) in which they combine the inversion recovery technique for measurement of T_1 (*7*) with a two-dimensional COSY experiment (*8*) to generate an IR-COSY experiment,

$$180^\circ_x\text{—}\tau\text{—}90^\circ_{\phi 1}\text{—}t_1\text{—}90^\circ_{\phi 2}\text{—}t_2. \qquad [1]$$

In this experiment T_1 is characterized through measurements of cross-peak intensities in the 2D COSY map. If it were not for cross-relaxation effects during τ, a cross peak centered at (ω_A, ω_X) would have an intensity related to T_{1A} only, and a cross peak centered at (ω_X, ω_A) would have an intensity related to T_{1X} only, providing a very thorough picture of spin relaxation. Acquisition of N 2D data sets, each with a different τ value, generates an accurate profile of the evolution of magnetization associated with each resolvable resonance thereby enabling an accurate extraction of spin–lattice relaxation rates.

Equation [1] implies that the acquisition of N T_1 data points requires N COSY experiments which is a considerable burden on spectrometer time and data storage facilities. In this communication we illustrate a modification of the sequence of Eq. [1] which enables the determination of T_1 from a single 2D experiment. In favorable cases, extraction of the complete time course of the longitudinal magnetization is also possible.

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The elegant accordion experiment, proposed by Bodenhausen and Ernst (*9, 10*), provides a framework for the reduction of the time-consuming three-dimensional experiment of Eq. [1] to a more practical 2D experiment. The potential of the accordion experiment to measure rate constants of dynamic processes such as spin–lattice relaxation was first recognized by Bodenhausen and Ernst (*9, 10*). In this communication we report on several 2D accordion pulse sequences for measuring T_1.

Consider, for example, the sequence

$$180^\circ_x\text{—}\kappa t_1\text{—}90^\circ_{\phi 1}\text{—}t_1\text{—}90^\circ_{\phi 2}\text{—}t_2, \qquad [2]$$

where κ is a factor chosen to sample effectively the longitudinal relaxation time course of the spins and ϕ_1 and ϕ_2 are cycled as described in (*11*). The effects of this sequence can be best evaluated by a product–operator treatment (*12*). For an AX_3 spin system showing simple exponential relaxation, for example, it is easily shown that magnetization originating on spin A in t_1 and residing on spin X in t_2 evolves according to

$$[1 - 2\exp(-\kappa t_1/T_{1\mathrm{A}})]\exp(-t_1/T^*_{2\mathrm{A}})\sin(\pi J_{\mathrm{AX}}t_1)\cos^2(\pi J_{\mathrm{AX}}t_1)\exp(-i\omega_{\mathrm{A}}t_2) \qquad [3]$$

in t_1, while magnetization originating on spin X in t_1 and resonating at ω_A in t_2 evolves during t_1 according to

$$[1 - 2\exp(-\kappa t_1/T_{1\mathrm{X}})]\exp(-t_1/T^*_{2\mathrm{X}})\sin(\pi J_{\mathrm{AX}}t_1)\exp(-i\omega_{\mathrm{X}}t_1). \qquad [4]$$

In Eq. [4], T_{1i} and T^*_{2i} are the spin–lattice and spin–spin relaxation time constants, respectively, of spin i, and T^*_{2i} includes the effects of field inhomogeneities. In deriving Eqs. [3] and [4] we have assumed that spin diffusion effects are negligible. This assumption is rigorous for molecules in the extreme narrowing regime, or when relaxation interactions other than dipolar interactions, such as relaxation due to paramagnetic probes, dominate.

Equations [3] and [4] clearly show that T_1 information is present in the evolution of magnetization as a function of t_1. However, direct analysis of the evolution is complicated by several factors. First, a given cross section parallel to ω_1 at the frequency of a resonance in ω_2 will have modulations arising from the chemical shifts and coupling constants of the diagonal and all associated cross peaks. Second, both T_1 and T_2 decay processes contribute to the modulations in t_1. Fourier transformation in t_1 can restore resolution of various cross- and diagonal-peak components and eliminate some of the frequency modulation complexities. This places relaxation information in the ω_1 lineshapes. In principle, T_1 information can be obtained by fitting lineshapes of cross peaks in ω_1 (*9, 10*).

We find, however, that an analysis of the resolved data presented in the time domain is more straightforward than a lineshape analysis. Suppose we wish to obtain a T_1 associated with spin A, T_{1A}, where spin A is coupled to the X spins of an AX_3 spin system. After the phase-sensitive data set generated by Eq. [2] is processed in both dimensions a cross section parallel to ω_1 and at ω_X in ω_2 is chosen. This section shows cross peaks at ω_A and diagonal peaks at ω_X. If we wish to focus on resonances centered about (ω_A, ω_X), other signals in this cross section are eliminated by multiplication of the cross section by a suitable weighting function or by left and right shifting the data until a sufficiently narrow frequency band containing only multiplet components associated with this cross peak is obtained. The multiplet in this cross section is reverse

Fourier transformed and the complex frequency modulation of the time-domain data is removed by magnitude correction of the data. In this way the time evolution becomes

$$|[1 - 2\exp(-\kappa t_1/T_{1A})]\exp(-t_1/T_{2A}^*)\sin(\pi J_{AX}t_1)\cos^2(\pi J_{AX}t_1)|. \qquad [5]$$

This procedure has been discussed by Bodenhausen and Ernst for the extraction of rate constants from cross peaks in 2D exchange spectra (*9, 10*). A fit of the time profile obtained in this way to Eq. [5] should lead to a simple means of extracting T_1 data. However, insufficient resolution in ω_1 can cause partial cancellation of multiplet cross-peak intensities which can complicate extraction of T_1 values from a fit of the time evolution of the reverse Fourier-transformed data.

In poorly resolved spectra, approximate T_1 values could be obtained from the position of the T_1-dependent null in the time-domain data since according to Eq. [5]

$$T_{1A} = \kappa t_{\text{null}}/\ln 2. \qquad [6]$$

More quantitative T_1 values can be obtained by eliminating the complex scalar modulations that occur in t_1. Bax and Freeman (*13*) and later Ernst and co-workers (*14*) developed an elegant method for decoupling in ω_1, which we employ in the following sequence,

$$180^\circ_x\text{—}\kappa t_1\text{—}90^\circ_{\phi 1}\text{—}(\tau_e + t_1)/2\text{—}180^\circ_{\phi 2}\text{—}(\tau_e - t_1)/2\text{—}90^\circ_{\phi 3}\text{—}t_2, \qquad [7]$$

where ϕ_1, ϕ_2, and ϕ_3 are phase cycled to eliminate quadrature artifacts and to collect N type peaks. A product–operator calculation shows that for an AX_3 spin system the transfer of magnetization from A to X is modulated in t_1 according to

$$[1 - 2\exp(-\kappa t_1/T_{1A})]\exp(-t_1/T_2^h)\exp(-\tau_e/T_{2A})\sin(\pi J_{AX}\tau_e)\cos^2(\pi J_{AX}\tau_e)\exp(-i\omega_A t_1) \qquad [8]$$

while magnetization transferred from X to A is modulated in t_1 according to

$$[1 - 2\exp(-\kappa t_1/T_{1X})]\exp(-t_1/T_2^h)\exp(-\tau_e/T_{2X})\sin(\pi J_{AX}\tau_e)\exp(-i\omega_X t_1), \qquad [9]$$

where T_2^h describes the transverse relaxation due to field inhomogeneity effects only and T_{2i} describes the transverse relaxation due to molecular processes exclusively.

Inspection of Eqs. [8] and [9] shows that, in contrast to variable-time 2D spectroscopy, where scalar coupling modulates the evolution of magnetization during t_1, the effect of scalar coupling produced by the sequence of Eq. [7] is a simple τ_e-dependent attenuation of the signal. The collapse of multiplet components in ω_1 results in a considerable simplification of the spectrum and the complete spin relaxation time course can be obtained from a fit of the data.

Equations [3], [4], [8], and [9] show that the decay of magnetization during t_1 proceeds via both T_1 and T_2 relaxation. Note that for the sequence of Eq. [2] T_2 relaxation during t_1 is the result of both molecular processes and field inhomogeneity effects while for the sequence of Eq. [7] only the latter mechanism is operative (*13–15*). The dependence on T_2^h may be minimized by careful shimming of the magnet and by an appropriate choice of κ. For optimum sensitivity to T_1, κ should be chosen so that

$$T_2^h > T_1/\kappa. \qquad [10]$$

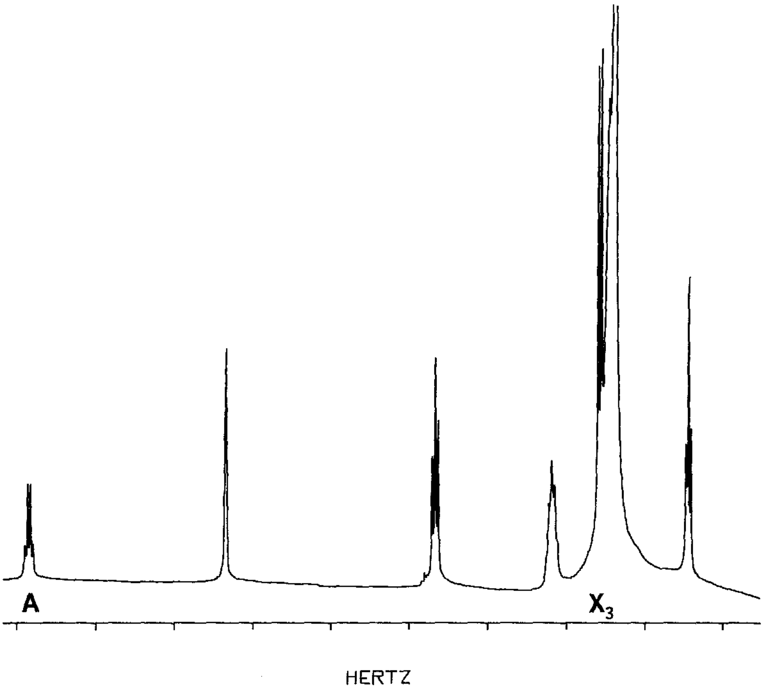

FIG. 1. One-dimensional spectrum of 50 m*M* *N*-myristyl-L-alanine dissolved in perdeuterated methanol recorded at 303 K. Eight scans of 8K data were obtained on a 490 MHz homebuilt spectrometer operating in the Fourier transform mode. The α-CH and β-CH_3 resonances of the alanine moiety are located at 4.30 and 1.34 ppm relative to TMS, respectively. Each tick mark corresponds to 200 Hz.

In addition, κt_1(max) should be greater than T_1 to obtain an accurate time course of the evolution of longitudinal magnetization. This requires, for most macromolecules, $\kappa \sim 10$.

The sequences described by Eqs. [2] and [7] can be modified to generate cross peaks and diagonal peaks with pure absorption lineshapes by inserting an additional 90° pulse after the t_1 period and by using suitable phase cycling so as to generate a double-quantum filter (*16*). In some of the applications considered in this communication we have employed the double-quantum-filtered sequences.

To illustrate these sequences we show a set of experiments on 50 m*M* *N*-myristyl-L-alanine dissolved in deuterated methanol at 303 K. *N*-Myristyl-L-alanine was synthesized by the method of Suyama *et al.* (*17*). A one-dimensional 490 MHz proton spectrum is shown in Fig. 1. We will focus on the AX_3 spin system of the alanine moiety of the compound. The X_3 part of the spin system at $\delta = 1.34$ ppm relative to TMS is partially obscured by the intense resonances of the methylene groups of the acyl chain. Thus, accurate measurements of the T_1 of this resonance by conventional 1D techniques would be difficult. In contrast, the A part of the spin system at $\delta = 4.30$ ppm is well resolved, and T_{1A} can be measured via 1D methods to test the reliability of the proposed technique.

Figures 2a and 2b show time profiles of the x magnetization in t_1 (cross section through ω_A in ω_2) obtained by using a double-quantum-filtered phase-sensitive COSY accordion sequence with $\kappa = 10$ and $\kappa = 20$, respectively. In Figs. 2a and 2b the positions of the nulls in the time profiles give $T_{1X} = 0.87 \pm 0.08$ s and 0.89 ± 0.08 s, respectively. Note that the null due to T_1 relaxation occurs sufficiently before the nulls due to J modulation to allow unambiguous evaluation of T_1. $T_{1A} = 1.3 \pm 0.1$ s, as measured from peaks at (ω_A, ω_X) with $\kappa = 10$ or 20, is in excellent agreement with $T_{1A} = 1.3 \pm 0.1$ s obtained by the 1D inversion recovery technique (7). One must, however, be aware that the use of nulls to determine T_1 is subject to systematic errors, particularly when incomplete inversion at the beginning of the sequence occurs.

Figure 3a shows a column cross section of a cross peak centered at (ω_X, ω_A) obtained from the ω_1-decoupled sequence indicated by Eq. [7] with $\tau_e = 3/(2J_{AX}) = 205$ ms. The peak shown is a sum of two Lorentzian lines with linewidths of $1/(\pi T_2^h)$ and $1/\pi \cdot (\kappa/T_{1X} + 1/T_2^h)$ and with opposite phases. Figure 3b shows the complete time profile of x magnetization in t_1 obtained from back transforming these data. The result is free of J modulation and a complete fit of the curve is possible. Both the position of the null and a fit of the time course of the data give $T_{1X} = 0.80 \pm 0.08$ s. An ω_1-decoupled spectrum was also recorded with $\tau_e = 5/(4J_{AX}) = 171$ ms and gave $T_{1X} = 0.83 \pm 0.08$ s.

It is clear that the sequences described can provide accurate T_1 measurements. Evaluation of the sensitivity in comparison to other means of T_1 measurement from data resolved in two dimensions is difficult. Experiments such as that proposed by Bystrov and co-workers (6) generate T_1 information from the time dependence of

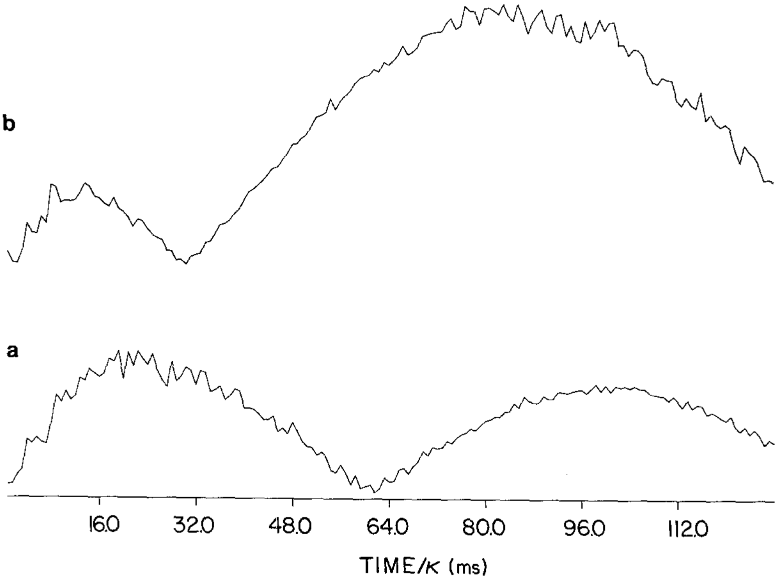

FIG. 2. Time profiles (t_1) of magnetization associated with the methyl part of the alanine spin system of 50 mM N-myristyl-L-alanine dissolved in CD_3OD at 303 K (cross sections through ω_A in ω_2). The data were generated from a double-quantum-filtered phase-sensitive COSY accordion sequence (see text) with $\kappa = 10$ (a) and $\kappa = 20$ (b). Three hundred twenty experiments, each containing 1K complex points, were acquired in the phase-sensitive mode using the method of States *et al.* (20). The data were processed on a Vax 11/750 computer equipped with a CSPI Minimap array processor to yield a 2K × 2K matrix without the use of any resolution enhancement weighting functions.

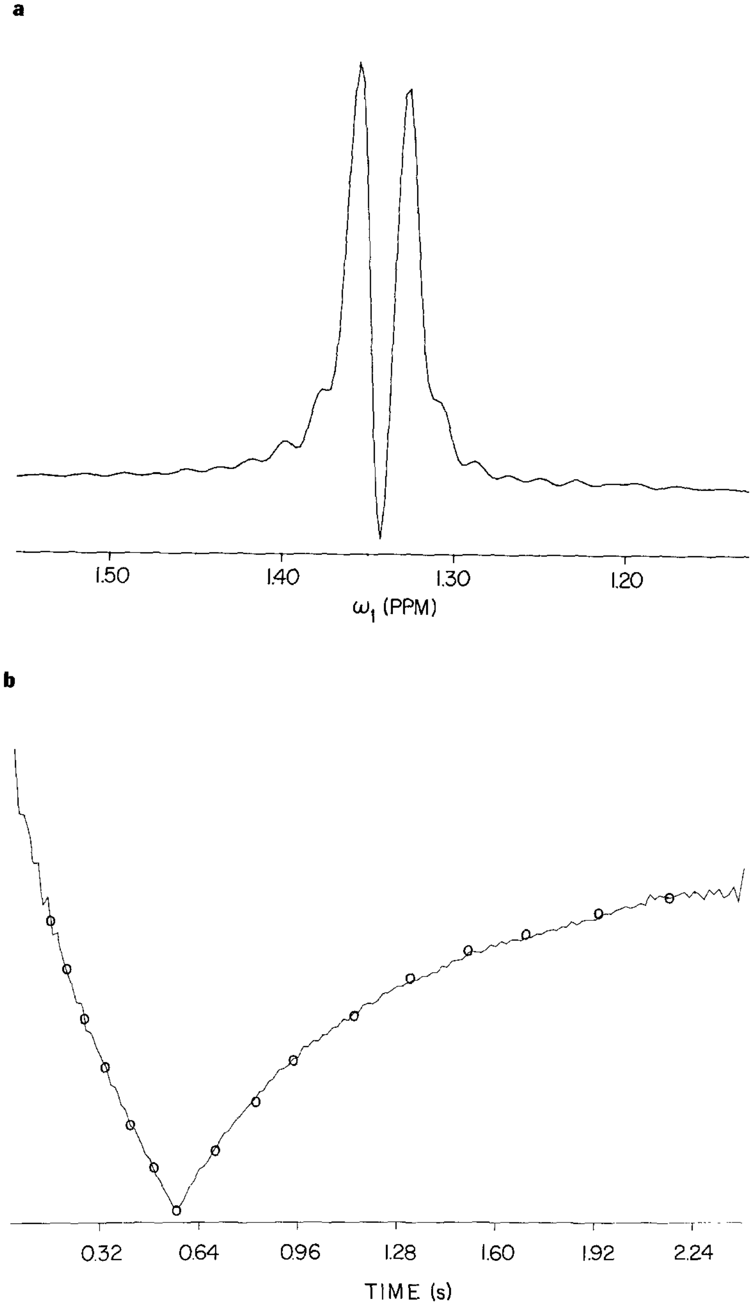

FIG. 3. (a) The column cross section centered at (ω_X, ω_A) from which the time course (b) was calculated. The peak displayed is a sum of two Lorentzian lines with linewidths of $1/(\pi T_2^h)$ and $1/\pi \cdot (\kappa/T_{1X} + 1/T_2^h)$ and with opposite phases (see text for further details). (b) The time profile (t_1) of x magnetization (cross section through ω_A in ω_2); $\kappa = 20$ and $\tau_e = 3/(2J_{AX}) = 205$ ms. All other parameters are the same as indicated in the legend to Fig. 2 with the exception that a 40 m*M* *N*-myristyl-L-alanine sample was used. The best fit of the data gave magnetization proportional to $|1 - 2\exp(-t/0.80)|\exp(-t/0.71)$ which corresponds to a T_1 of 0.80 s. Theoretical points generated from this expression are indicated by the circles in the figure.

intensities of cross peaks in a 2D experiment, while the experiments proposed here generate T_1 information from the linewidths of cross peaks. In the limit that $T_2^h > T_1 \sim T_2$, sensitivity of the sequences defined by Eqs. [2] and [7] would be very similar to a single point in an IR-COSY experiment resulting in a great saving in acquisition time. As T_1 becomes greater than T_2, spectra obtained from the sequences proposed in this communication suffer a proportional sensitivity loss reducing their efficiency as a means of measuring T_1. Nevertheless, the sequence of Eq. [2] has been successfully applied to acyl carrier protein (*18*), a protein of 8800 Da involved in the synthesis of fatty acids (*19*), using acquisition times which are not significantly longer than those normally used for more conventional 2D data sets. In addition, the methods described here retain advantages in providing a complete recovery curve and thereby eliminate the need for judicious choices of discrete points on the T_1 recovery curve.

In conclusion, we have presented pulse sequences which are useful for measuring T_1 relaxation rates of complex molecules where resolution is insufficient in 1D spectra. Only a single 2D experiment is required. The experiment should be of particular value for the measurement of T_1 values in molecules where spin diffusion is not limiting or where relaxation is dominated by a paramagnetic probe.

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