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Measurement of Carbon-13 Longitudinal Relaxation Using ^{1}H Detection

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^{13}C relaxation rates provide direct information concerning the dynamic behavior at specific sites in a molecule (*1–4*). Interpretation of the T_1 values can be straightforward because for protonated aliphatic carbons the longitudinal relaxation is dominated by the large ^{1}H–^{13}C dipolar interaction. Unfortunately, accurate measurement of ^{13}C T_1's is often very time consuming because ^{13}C has a low magnetogyric ratio and correspondingly low NMR sensitivity. Moreover, ^{13}C relaxation times are often quite long, requiring long delay times between scans. As will be demonstrated here, the sensitivity of the measurement can be increased significantly by using the more sensitive proton resonance to monitor changes in ^{13}C magnetization. This indirect ^{13}C T_1 measurement utilizes the sensitive hydrogen nucleus in a way similar to recently proposed ^{1}H-detected heteronuclear chemical-shift correlation experiments (*5–7*). Unfortunately, for the T_1 experiment *net* magnetization transfer between ^{1}H and ^{13}C nuclei is required, which makes the experimental schemes more complex than those used for heteronuclear chemical-shift correlation.

Two different schemes for ^{1}H-detected measurement of ^{13}C T_1's are depicted in Fig. 1. In the simplest scheme (Fig. 1a), the amount of ^{13}C z magnetization present is measured by transferring this magnetization to ^{1}H magnetization by using a reverse DEPT (*8, 9*) transfer. The experiment can be conducted with or without presaturation of the ^{1}H resonances, i.e., with or without an NOE effect on the ^{13}C. To minimize systematic errors and to make it possible to fit the experimental data by a two parameter fit a difference experiment is performed (*10*), with and without inversion of the ^{13}C magnetization at the beginning of the variable relaxation delay. If at the time of this first ^{13}C pulse the ^{13}C magnetization equals aM_0, where M_0 is the equilibrium ^{13}C z magnetization, and the (imperfect) ^{13}C 180° pulse changes this into bM_0, then the difference experiment will yield an amount of magnetization transfer proportional to $(a - b)e^{-Bt}$. For optimal sensitivity, a delay time between scans of about twice the ^{13}C T_1 value is needed (*11*). Since the relaxation time of the ^{13}C is unknown, the delay time needed may be difficult to estimate. Note, however, that a nonoptimal delay time

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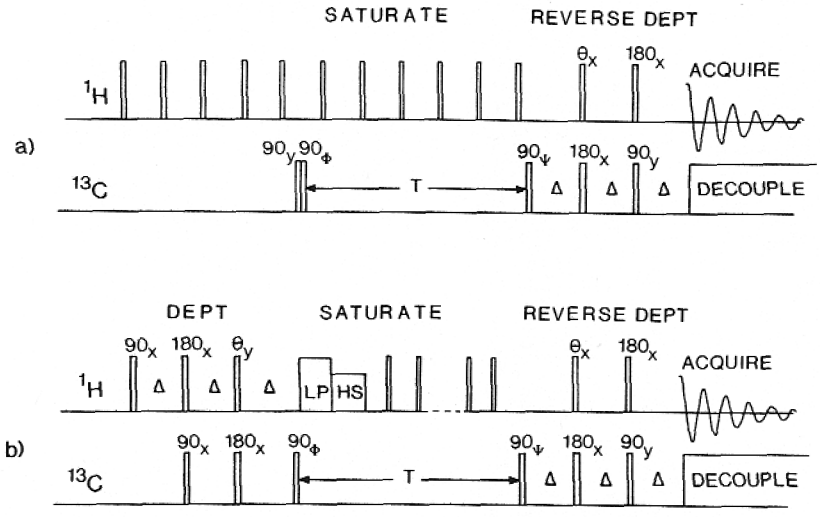

FIG. 1. Two schemes for ^{1}H-detected measurement of ^{13}C T_1 values. (a) A scheme that involves a single polarization transfer from ^{13}C to ^{1}H. A series of 90° pulses is used for saturation of the ^{1}H resonances prior to the reversed polarization transfer. This increases the sensitivity because of the NOE effect and simplifies suppression of signals from protons not bound to ^{13}C. (b) A scheme utilizing double polarization transfer. ^{1}H saturation is employed during the variable relaxation delay period, T, and is accomplished with a long pulse (LP), followed by a homospoil pulse (HS), followed by a series of 90° pulses spaced 10 ms apart. The delay time, Δ, is set to $1/(2^1J_{CH})$. Scheme (b) can yield erroneous T_1 values when applied to methyl and methylene sites. For both schemes, the phase cycling employed is: $\phi = y, -y, y, -y$; $\psi = x, x, -x, -x$; Acq. = +, −, −, +. The flip angle θ is adjusted to 90, 45, and 30° for methine, methylene, and methyl sites, respectively.

will merely reduce the sensitivity of the measurement without introducing systematic errors.

Typically, the ^{13}C T_1 value is significantly longer than the T_1 value of a proton directly attached to the ^{13}C. In addition, for macromolecules the heteronuclear NOE effect is often incomplete. Therefore, a scheme that generates ^{13}C longitudinal magnetization by transferring magnetization from its directly attached ^{1}H should be preferable to the scheme of Fig. 1a that relies on the ^{13}C longitudinal relaxation for building up its z magnetization. Figure 1b shows one of the possible methods, using a DEPT transfer, to generate ^{13}C longitudinal magnetization. To minimize dynamic range problems and the problem of suppressing signals from protons not attached to ^{13}C, ^{1}H saturation can be used during the variable ^{13}C recovery delay. In Fig. 1b this saturation is accomplished by a long (5 ms) nonselective pulse followed by a homogeneity spoiling pulse (5 ms) followed by a series of 90° pulses spaced at 10 ms intervals. At first sight one might expect that such a ^{1}H saturation procedure should be efficient at eliminating all ^{1}H coherences and longitudinal magnetization components. As will be shown below, for $^{13}CH_2$ and for $^{13}CH_3$ groups this is not the case.

Analogous to the operator formalism expressions presented by Sørensen and Ernst (*12*), the density matrix terms at the end of the first DEPT sequence can be derived. For an optimally adjusted DEPT sequence (including the final $90^\circ_{\pm y}$ ^{13}C pulse (Fig. 1)

for conversion into z magnetization) the density matrix components that involve an S_z term created for an I_2S system are given by

$$\sigma_f = \mp S_z \mp 4I_{1x}I_{2x}S_z, \quad [1]$$

where, for convenience, the I spins are assumed to be on-resonance. The second term at the right-hand side of expression [1] contains the product terms $I_{1x}I_{2x}S_z$, which represents the sum of zero- and double-quantum coherence. The homogeneity spoiling pulse and the ^1H irradiation applied during the variable relaxation delay period do not destroy the zero-quantum component and in part it will be transferred back into observable ^1H magnetization by the subsequent reverse-DEPT sequence. This can lead to errors in the indirect measurement of the S-spin T_1 because the relaxation behavior of the product will in general be quite different from the relaxation of S_z. In a similar fashion, it can be shown that S-spin T_1's measured for an I_3S system measured with the double-DEPT experiment of Fig. 1b also contain a contribution from the multiple product terms. Although, at least in principle, methods can be devised that eliminate transfer of the product terms into observable magnetization, in practice the sequences become very lengthy and critical to correct adjustment. Experimental results presented below show that the double-DEPT experiment can give relaxation rates significantly different from the rates measured directly or with the sequence of Fig. 1a. Similar results were found for an experiment that involves INEPT-type (*13–15*) instead of DEPT-type transfers.

The methods for measuring the ^{13}C values indirectly are demonstrated for two samples: (a) a 0.1 M solution of $^{13}CH_3$-labeled sodium acetate at 16°C and (b) a 1.5 mM solution of staphylococcal nuclease (S. Nase), prepared from genetically transformed *Escherichia coli* (*16*). The protein is ^{13}C-labeled in the C_ϵ (CH_3) position of all five methionine residues, p^2H 7.6, at 43°C. Experiments were carried out on a modified NT-500 spectrometer equipped with a Cryomagnet Systems probe.

The sodium acetate sample was used to verify whether the conventional inversion-recovery (IRFT) experiment (*17*) yields the same relaxation rate as the indirect schemes of Fig. 1. Using a three-parameter fit (*18*), the conventional experiment gives a T_1 value of 7.94 s ±0.5%, where 0.5% is the rms error calculated by the computer-fitting program. Repeating the experiment several times gives a value of 7.91 s ±2%. Measurement using the scheme of Fig. 1a yields a value of 7.96 s ±2%, with a reproducibility

TABLE 1

Relaxation Times Measured for a 0.1 M Solution of $^{13}CH_3$-Labeled Sodium Acetate at 16°C Measured with Different Methods

	Measured T_1 (s)	rms error	Reproducibility[a]
IRFT	7.94	0.04	0.30
Reverse DEPT	7.96	0.16	0.46
Double DEPT	6.35	0.09	0.14

[a] The reproducibility is the largest difference between T_1 values measured in three consecutive experiments.

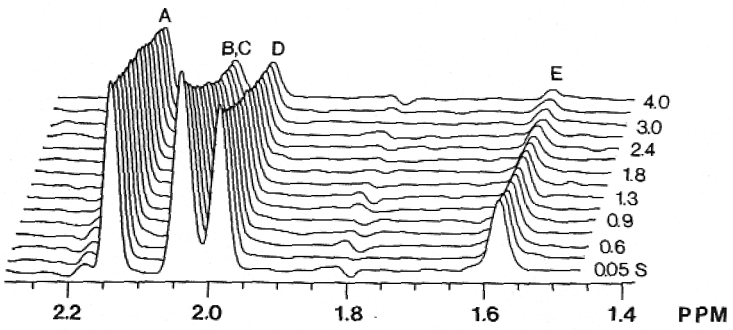

FIG. 2. Set of ^1H spectra of MET-$^{13}C_\epsilon$-labeled S. Nase, measured with the scheme of Fig. 1a for a series of T values. Only the resonances of ^{13}C-attached protons are present. Each spectrum is the result of 128 scans, with 8 s delays between scans.

better than 3% (Table 1). These values show excellent agreement between the two experiments. In contrast, the scheme of Fig. 1b yields a ^{13}C relaxation time that is significantly shorter: 6.35 s ±1.5% with a reproducibility better than 1.5%. This confirms that the product terms, present during the relaxation delay, can cause a significant error in the measured relaxation time. Because the product terms relax at a rate different from the ^{13}C longitudinal relaxation rate one would expect a multiexponential behavior for the amount of magnetization as a function of the relaxation delay time, T. In practice, however, the difference in relaxation rates is insufficient to be fit reliably by a multiexponential curve.

The schemes of Fig. 1 were also used to measure the ^{13}C T_1's of the $^{13}C\epsilon$-labeled methionine residues in S. Nase. Figure 2 shows a set of spectra obtained with the scheme of Fig. 1a. In the ^1H spectrum four of the five methyl groups, labeled A–E in the order of increased shielding, are well resolved; resonances B and C overlap. The

TABLE 2

^{13}C Longitudinal Relaxation Times for the $^{13}C\epsilon$-Labeled Methionine Carbons in S. Nase

Resonance	Reverse DEPT T_1 (s)	Double DEPT T_1 (s)
A	4.06[a] ± 0.03[c] (0.11)[d]	3.62[b] ± 0.03[c] (0.10)[e]
B, C	2.22 ± 0.02 (0.04)	1.96 ± 0.03 (0.01)
D	2.55 ± 0.02 (0.03)	2.28 ± 0.04 (0.11)
E	2.01 ± 0.03 (0.12)	1.89 ± 0.03 (0.18)

Note. The resonances are labeled A–E as indicated in Fig. 2.

[a] Median value from three experiments.

[b] Two experiments were performed. The value measured in the experiment that gave the smallest rms errors is quoted.

[c] Root mean square error.

[d] Difference between the extreme values from three experiments.

[e] Difference between the values measured in two experiments.

T_1 values, measured from the data shown in Fig. 2, are presented in Table 2. As seen from these data, the relaxation times vary significantly for the different positions in the protein. For comparison, Table 2 also shows the "T_1's" measured with the scheme of Fig. 1b. Although the data indicate a similar pattern, the values for the apparent T_1 are significantly shorter, well outside the experimental error.

We have demonstrated here the feasibility of measuring the ^{13}C T_1's indirectly via the more sensitive ^1H signal. This provides a significant gain in sensitivity, on the order of a factor $(\gamma_H/\gamma_C)^2$, where γ_H and γ_C are the ^1H and ^{13}C magnetogyric ratios, respectively. An even larger gain in sensitivity may be expected from an approach that involves a double polarization transfer, first from ^1H to ^{13}C and upon completion of the relaxation delay from ^{13}C to ^1H. Indeed, for methine sites this double transfer method is well suited. However, for methylene and methyl sites the double transfer approach can give rise to significant errors in the measured T_1 value. Attempts to develop double transfer methods that provide more accurate results for methylene and methyl sites are still in progress. Also, the applicability of the indirect T_1 measurement approach to natural abundance samples will be investigated.

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