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Measurement of ^{13}C Spin–Spin Relaxation Times by Two-Dimensional Heteronuclear ^{1}H–^{13}C Correlation Spectroscopy

N. R. NIRMALA AND GERHARD WAGNER*

Biophysics Research Division, Institute of Science and Technology, The University of Michigan, Ann Arbor, Michigan 48109

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We have developed a heteronuclear ^{1}H-detected 2D NMR experiment for measurements of ^{13}C T_2 values. This experiment is suited for measurements of T_2 values in macromolecules, such as proteins, where conventional measurements of ^{13}C T_2 values are not possible because of the low resolution of 1D spectra and the lack of sensitivity of ^{13}C detection. Samples of alanine ^{13}C-enriched either at the C^{α} or at the C^{β} position were used to test whether this technique and direct detection of ^{13}C in a Carr–Purcell experiment (*1*) yield the same results.

The experiment we propose is shown in Fig. 1. It consists of a 2D double DEPT (*2*) sequence which is related to a sequence we have used recently (*3*) to determine spin–lattice relaxation times in basic pancreatic trypsin inhibitor, and for reverse detection 1D NMR experiments proposed for T_1 measurements (*4, 5*). Longitudinal ^{1}H magnetization is transferred to transverse ^{13}C magnetization by means of a DEPT sequence. This is allowed to undergo spin–spin relaxation during a time τ_2. A carbon 180° pulse is applied in the middle of this period to refocus chemical-shift precession and dephasing due to magnetic field inhomogeneity. At the end of the τ_2 period, the carbon magnetization is converted to longitudinal magnetization during which time a homospoil pulse (typically of 1 ms duration) is applied along the z direction to randomize magnetization in the transverse plane. The ^{13}C z magnetization is now reconverted to transverse magnetization and labeled with carbon frequencies during the t_1 evolution period. Subsequently, it is converted to observable ^{1}H magnetization by means of an inverse DEPT sequence.

The phase cycles are given in the legend to Fig. 1. Phase cycle ϕ_1 eliminates contributions originating from carbon instead of proton polarization, while ϕ_2 takes care of magnetization not converted to I_zS_y by the θ pulse. It is not necessary if $\theta = 90°$. Phase cycles ψ_2 and ψ_3 constitute a z filter; ψ_3 and the receiver phase cycle also eliminate magnetization of protons not coupled to ^{13}C. Experiments with τ_2 delays from 1 μs to 2 s were recorded. The intensities of the resonances were fitted to an exponential curve and the values of the spin–spin relaxation times were determined to be 205 ms for the α-carbon and 457 ms for the methyl carbon (Figs. 2A and 2C). For comparison we have also measured the T_2 values with a Carr–Purcell type sequence with direct detection. The T_2 values measured were 205 and 513 ms for the C^{α} and

* To whom correspondence should be addressed.

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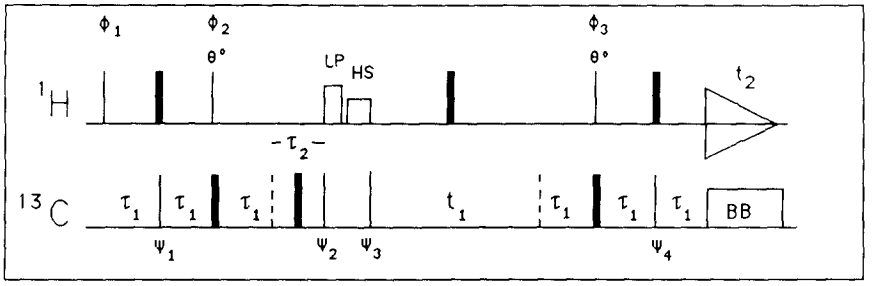

FIG. 1. Double DEPT pulse sequence for measurements of ^{13}C T_2 relaxation times in 2D heteronuclear ^{13}C–^1H correlated spectra. The delays τ_1 are tuned to $1/2J_{CH}$. The lengths of the long pulse (LP) and the homospoil pulse (HS) were 1 ms each. We have used the following phase cycles: $\phi_1 = x, -x$; $\phi_2 = y, y, -y, -y$; $\phi_3 = x, x, x, x, -x, -x, -x, -x$; $\psi_1 = x$; $\psi_2 = y, -y, -y, y$; $\psi_3 = y, y, y, y, y, y, y, y, -y, -y, -y, -y, -y, -y, -y, -y$; $\psi_4 = x, x, -x, -x$; receiver = $x, x, -x, -x, -x, -x, x, x, -x, -x, x, x, x, x, -x, -x$. The phases of the 180° pulses were cycled independently. Decoupling of the ^{13}C spins during acquisition was done using the WALTZ-16 decoupling sequence.

the C^β, respectively. The data are shown in Figs. 2B and 2D. The agreement between the measured spin–spin relaxation times using the sequence of Fig. 1 and the direct detection method is quite good.

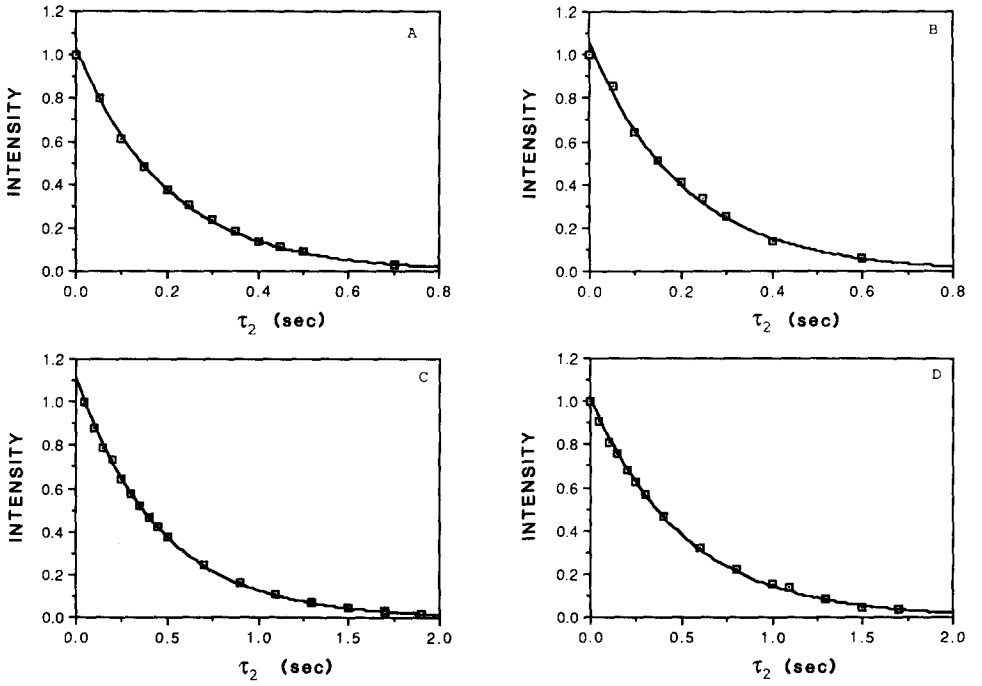

FIG. 2. Exponential decay of transverse magnetization as a function of τ_2 using the sequence shown in Fig. 1 for reverse detection and the Carr–Purcell sequence for direct detection. (A) α-carbon of alanine using reverse direction. (B) α-carbon of alanine using direct detection. (C) β-carbon of alanine using reverse detection. (D) β-carbon of alanine using direct detection.

The purpose of developing the technique described is to measure spin–spin relaxation times of backbone carbons in proteins in 2D heteronuclear correlation experiments. Two-dimensional techniques and reverse detection provide the only means to obtain this information in terms of resolution and sensitivity. Initial measurements of ^{13}C T_1 values (*3*) and temperature-dependent changes in ^{13}C–^1H COSY spectra (*6*) indicate a significant variation of the backbone mobility in the protein basic pancreatic trypsin inhibitor. The method for ^{13}C T_2 measurements described will provide an additional valuable tool to study these dynamic aspects in proteins.

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