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Measurement of T_1/T_2 relaxation times in overlapped regions from homodecoupled ^{1}H singlet signals

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ABSTRACT

The implementation of the HOmodecoupled Band-Selective (HOBS) technique in the conventional Inversion-Recovery and CPMG-based PROJECT experiments is described. The achievement of fully homodecoupled signals allows the distinction of overlapped ^{1}H resonances with small chemical shift differences. It is shown that the corresponding T_1 and T_2 relaxation times can be individually measured from the resulting singlet lines using conventional exponential curve-fitting methods.

1. Introduction

The measurement of relaxation rates by Nuclear Magnetic Resonance (NMR) spectroscopy can provide important insights into the dynamics of molecules in solution [1]. Longitudinal spin–lattice T_1 relaxation times are usually determined from the Inversion-Recovery (IR) experiments [2,3] whereas transverse spin–spin T_2 relaxation times are measured from Carr–Purcell–Meiboom–Gill (CPMG) sequences [4,5]. Recently, an improved compensated CPMG sequence that achieves Periodic Refocusing Of J Evolution by Coherence Transfer (PROJECT) has been proposed to minimize the effects of J evolution during the echo periods, allowing a more accurate extraction of T_2 values by fitting the experimental data to a clean exponential decay of pure-phase, non-J-modulated signals [6,7]. A common feature of all these experiments is that measurements are based on exponential signal decays that can be described by first-order differential equations. In spectral regions with well resolved peaks the corresponding time constants are easily determined from nonlinear least-squares fits of each decaying signal to a separate mono-exponential function. However, simple data analysis are hampered in spectral regions with significant peak overlap, where the observed signal decays may be the result of superposition of several individual decays which are difficult to distinguish and require the use of sophisticated fitting methods [8–10]. Several NMR approaches have been proposed to avoid signal overlapping in relaxation experiments, such as the initial use of selective coherence by TOCSY transfer from an isolated signal [11], although the improved signal dispersion achieved in 2D/3D NMR experiments has become the common technique to study the conformational and dynamics aspects of biomolecules in solution [12].

On the other hand, a number of broadband homodecoupled NMR methods have been reported to obtain simplified ^{1}H singlet signals without the typical fine J(HH) multiplet structure [13–24], and recently an excellent overview of the homodecoupling techniques and applications has been reviewed [18]. The most recent applications, that have been encompassed under the term "pure-shift NMR", are based on the original Zangger–Sterk (ZS) experiment [14]. Basically exists two different acquisition protocols: (i) a time-consuming pseudo-2D acquisition mode based on adding the first part of different interferograms [14,15], and (ii) a real-time one-shot mode that reduce the experimental time and do not need for sophisticated processing tools [17]. Most of them use spatial encoded techniques, and therefore pronounced sensitivity losses due to slice selection are unavoidable that requires long acquisition times. Other homodecoupling methods using the BIRD module [19] do not suffer of sensitivity penalties but their applications are limited to heteronuclear correlation experiments [24]. Alternatively, a novel HOmodecoupled Band-Selective (HOBS)

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approach [25,26], closely related to the instant ZS experiment [17] has been proposed. The HOBS technique is not a broadband homodecoupling method that covers all the spectral width, rather it is a frequency-selective inverse homodecoupled method. However, it has been shown to be a sensitive and valuable practical tool when focusing specifically on a narrow part of the whole spectrum and applications have been provided for enantiodifferentiation studies [27], discrimination of diastereoisomers [28] or the measurement of heteronuclear coupling constants [29]. The main drawback is that it is a frequency-selective experiment and only a particular part of the ^{1}H spectrum can be monitored in a single experiment. As a major advantage, the HOBS method omits the spatial encoding gradient applied simultaneously with the selective pulses in the original instant scheme, avoiding any sensitivity loss and allowing its performance with reasonable experimental times. This communication reports the straightforward implementation of the HOBS technique in standard IR and PROJECT experiments (Fig. 1) with the aim to resolve overlapped ^{1}H resonances with small chemical shift differences. Thus, T_1 and T_2 relaxation times can be accurately measured from the resulting singlet lines using conventional exponential curve-fitting methods, without need for additional data analysis based on deconvolution or line fitting techniques [30,31].

2. Results and discussion

The major novelty with respect to the original experiments is the incorporation of the homodecoupled element during the detection period that consists of a pair of hard/selective 180° ^{1}H pulses (represented as solid and shaded shapes) at the middle of $2\Delta = AQ/n$ periods, where AQ is the acquisition time and n the number of concatenated loops [25,26]. In addition, a ^{1}H-selective gradient echo has been inserted prior to acquisition to select the area of interest, where the involved selective 180° ^{1}H pulse is the same as used for homodecoupling. For a perfect broadband homodecoupling, these experiments should be applied to particular areas of the ^{1}H spectrum where appear overlapped protons that are not mutually J coupled.

HOBS experiments can use the same automated data acquisition, processing and fitting analysis subroutines as the original experiments. A series of 1D ^{1}H spectra are sequentially recorded as a function of the recovery delay (τ) or the total echo time ($\tau_e = 4m\tau'$) in IR (Fig. 1A) and PROJECT (Fig. 1B) experiments, respectively. Fig. 2 compares the experimental results obtained for the IR and HOBS-IR experiments applied to the H_α proton region in the peptide cyclosporine. Good agreement is observed between the T_1 measured for all isolated signals with both methods demonstrating that the incorporation of homodecoupling does not distort the measurement (Table 1). The excellence of the method is illustrated by distinguishing the individual decays of the overlapped H_7 and H_8 resonances at 4.78–4.80 ppm. Clearly, the successful analysis of the two resolved singlets (separated by 13 Hz) allows an accurate determination of each distinct T_1 value without resorting to more complex data analysis. The same strategy can be applied for T_2 measurements. The simplicity and the accuracy of the measurements is demonstrated when comparing the equivalent CPMG, PROJECT and HOBS-PROJECT spectra, all of which acquired with a total echo time of 156 ms (Fig. 3B–D). Whereas the standard CPMG spectrum shows strong multiplet distortions due to the unavoidable J_{HH} evolution, perfect in-phase multiplets are obtained from both PROJECT spectra.

Clearly, the in-phase properties are fully retained in the HOBS-PROJECT spectra (Fig. 3D), where improved sensitivity and resolution are obtained due to the efficient multiplet collapsing. The method works equally well for mutually J-coupled protons that experience the effect of the selective 180° pulse, and therefore they are not fully homodecoupled. T_2 measurements on the partially decoupled olefinic $H1_\varepsilon$ and $H1_\zeta$ protons (asterisks in Fig. 3D) can be also monitored efficiently from the simplified doublet patterns.

The HOBS methods can be very useful to simplify highly congested areas, such as those found in the aliphatic region of the steroid progesterone (Fig. 4). Three resonances with complex multiplet patterns appear completely overlapped at 2.0 ppm. The simplified HOBS spectrum shows clean singlets for each of these signals, with small chemical differences of 14–18 Hz. Note the equivalence between IR and HOBS-IR data by observing the same exact null point for the strong methyl signal (see experimental details and experimental T_1/T_2 values in the supporting information).

Experimentally, the HOBS technique requires a very simple and fast implementation. Only two parameters need to be defined in a

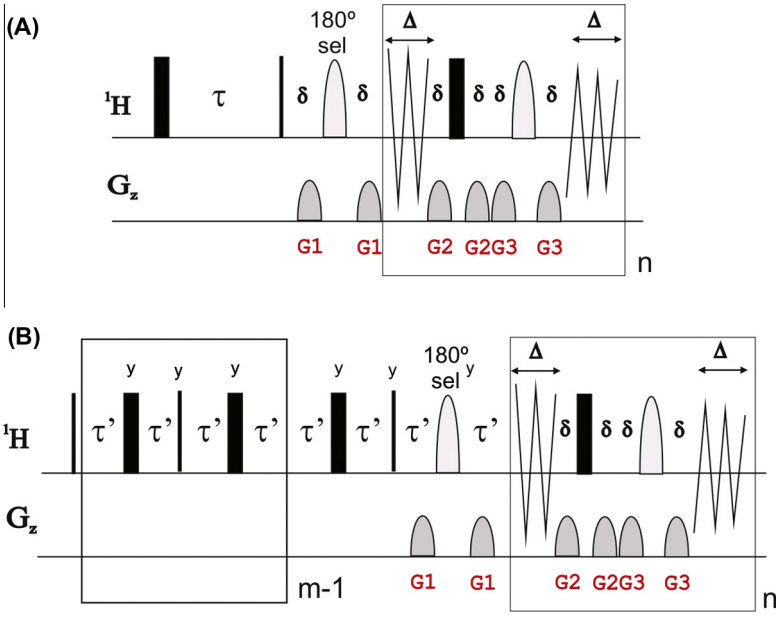

Fig. 1. NMR pulse schemes of the HOBS-IR and HOBS-PROJECT experiments used to measure T_1 and T_2 relaxation times, respectively, in overlapped proton signals.