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Application of Semi-Selective Excitation Sculpting for Homonuclear Decoupling During Evolution in Multi-Dimensional NMR

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A band-selective homonuclear decoupled TOCSY sequence is described and compared with the standard TOCSY experiment, using a sample of a DNA 15mer. Band selection and homonuclear decoupling (BASHD) was achieved along the evolution dimension using a band-selective double pulse field gradient spin echo (DPFGSE) technique. This semi-selective excitation sculpting technique substantially improves the resolution and sensitivity of a crowded region in the TOCSY spectrum of the DNA 15mer chosen. The methodology described is general and can be implemented in other 2D and 3D techniques to achieve band selection and homonuclear decoupling during proton evolution.

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INTRODUCTION

Band-selective pulses have been used in multi-dimensional NMR to select a desired spectral region in one or more dimensions.[1–7] The reduction of the spectral width in one or more dimensions improves the digital resolution achievable in the chosen dimension and helps to reduce ambiguities in the resonance assignment procedure in a crowded spectral region. The reduction in spectral width also shortens the measuring time of the experiment. This is particularly important in three- and higher dimensional experiments where the limited spectral width in one dimension results in significant savings in the total measuring time. Recently, it has been demonstrated that the combined use of selective pulses and pulse field gradients (PFGs) in one-dimensional selective experiments results in spectra devoid of artifacts.[8–11] This principle was further extended for recording homonuclear and heteronuclear semi-selective 2D NMR experiments.[12]

Another application of semi-selective excitation is for decoupling a limited spectral region. Use of homonuclear semi-selective shaped pulse decoupling in combination with the time-shared decoupling mode, where the receiver and the decoupling are alternately activated[13,14] during data acquisition, was recently demonstrated.[15] Application of a shaped semi-selective refocusing pulse in combination with a non-selective refocusing pulse in the middle of the evolution period achieves homonuclear decoupling in the indirectly detected dimension.[16] Semi-selective homonuclear decoupling during both the acquisition and the evolution dimensions substantially improves resolution.[15] We now report the application of excitation sculpting technique[11] for evolution domain band selection along with homonuclear decoupling. The use of this technique achieves both band selection and decoupling without any additional phase cycling and allows one to realise the achievable resolution and/or time reduction compared with the non-selective experiment.

RESULTS AND DISCUSSION

The key element in the excitation sculpting method[11] is the double pulse field gradient spin echo (DPFGSE), as shown in Fig. 1(A). The magnetization returns to its starting position at the end of the DPFGSE train, the excitation profile depending only on the inversion profile of the band-selective π pulses. The phase of the magnetization is unaffected whereas the amplitude is scaled by the inversion profile of the π pulses and by loss due to relaxation during the spin echo. Selective excitation using a DPFGSE train requires no phase cycling. In Fig. 2, a standard (non-selective) ^{1}H NMR spectrum of a DNA 15mer, d(GGTTGGTGTGGTTGG), is compared with the H-1′ band-selective ^{1}H spectrum. The band selection was done using the DPFGSE technique. The band-selective π pulses were Q3 shaped pulses.[19] Both spectra were obtained with eight scans each, but without any phase cycling. Both spectra were processed by adjusting only the frequency-independent phase-correction factor and presented with same absolute vertical scaling factor. The pure phase characteristics of the band-selective DPFGSE spectrum [Fig. 2(B)] makes this excitation sculpting method an attractive choice for evolution domain band selection and decoupling in multidimensional NMR. In this study, the TOCSY sequence is chosen as an example and the DPFGSE train is incorp-

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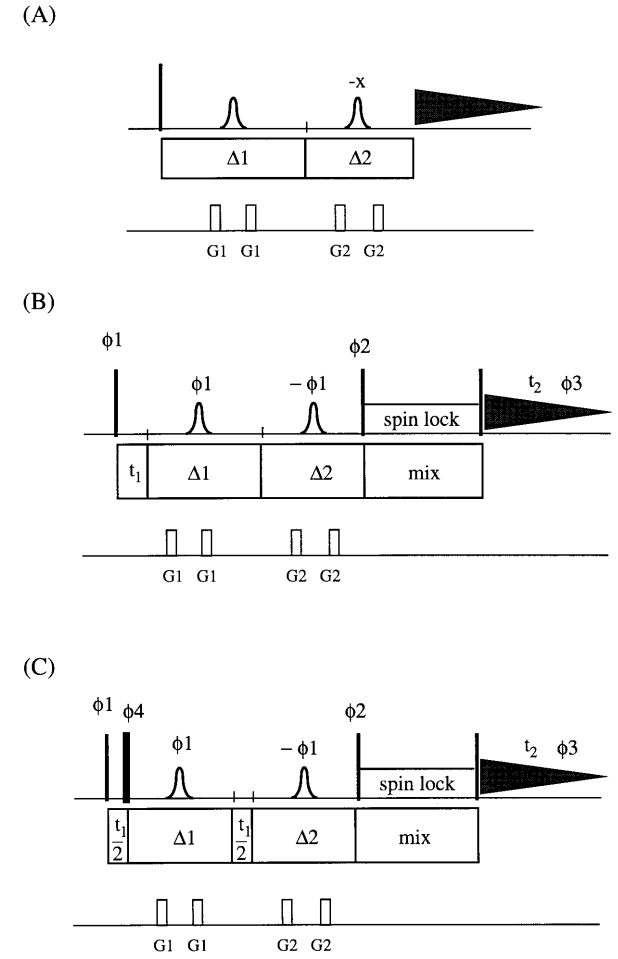

Figure 1. Pulse schemes for (A) the double PFG spin-echo; (B) band-selective (BASE) TOCSY; and (C) band-selective homonuclear decoupled (BASHD) TOCSY. Thin and thick vertical lines represent 90° and 180° pulses, respectively. The band-selective pulses during **Δ1** and **Δ2** are π pulses. The basic phase cycling is $\phi_1 = x, -x, \phi_2 = x, -x, \phi_4 = x, -x$ and $\phi_3 = x, -x$. Phases not shown are along the x-axis. In addition, ϕ_1 is phase-cycled to obtain States-TPPI[17] t_1 quadrature detection. For the 2D sequences in (B) and (C) two data sets were collected with ϕ_2 inverted with respect to each other and the data processed as discussed by Cavanaugh and Rance.[18]

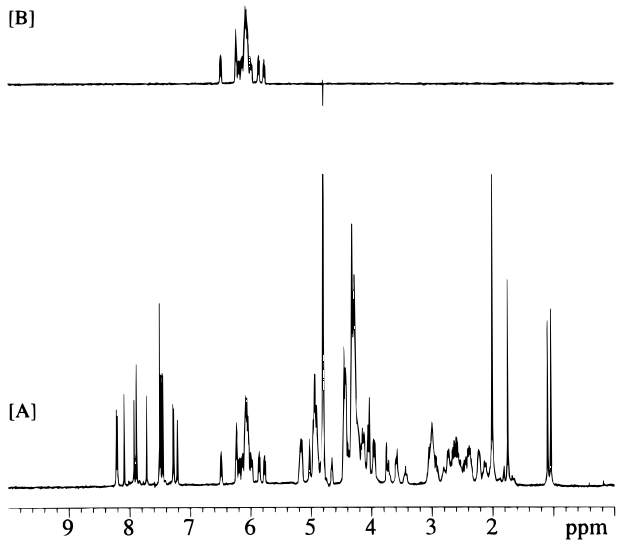

Figure 2. Comparison of (A) the standard ^{1}H NMR spectrum with (B) the band-selective DPFGSE spectrum of d(GGTTGGTGTGGTTGG) in D_2O. Eight scans (no phase cycling) each of 16 384 complex points were collected and transformed with no zero-filling or data manipulation. A 1 s inter-scan delay was used. Both spectra were plotted with the same absolute vertical scaling factor.

orated to achieve band selection and decoupling in the F_1 dimension.

In Fig. 1(B), the band-selective TOCSY (BASE-TOCSY) sequence is shown. This sequence is a modification of the sensitivity-enhanced TOCSY[18] experiment. A band-selective DPFGSE train is introduced immediately following the evolution delay (t_1). (Alternatively, the DPFGSE train can also be introduced between the first non-selective $\pi/2$ pulse and t_1 to achieve the same result.) The experiment can be run with no additional phase cycling over the conventional experiment. The only phase cycling used in this study was the phase alternation of the first non-selective $\pi/2$ pulse for axial peak suppression. For dilute samples requiring more than two scans per increment, an additional four-step cyclops phase cycling of all the pulses can be introduced. In Fig. 3 a BASE-TOCSY spectrum of the DNA 15mer d(GGTTGGTGTGGTTGG) is compared with the sensitivity-enhanced TOCSY[18] spectrum. The H-1′ proton region of the deoxyribose sugars is band selected and the F_2 region shown is the H-2′/H-2″ TOCSY correlations. The TOCSY spectrum in Fig. 3(A) was acquired over 48 min (two scans each for 256 t_1 increments with an F_1 spectral width of 5200 Hz) and the BASE-TOCSY spectrum in Fig. 3(B) was acquired over 6 min (two scans each for 32 t_1 increments with an F_1 spectral width of 650 Hz). The virtually identical spectra in Fig. 3(A) and (B) reflect the true saving in the total experiment time achievable by F_1 band selection. The spectrum in Fig. 3(C) is a BASE-TOCSY spectrum acquired with 128 t_1 increments for a total acquisition time of 25 min. By increasing the total number of t_1 increments one can thus achieve a substantial improvement in F_1 digital resolution. It must be noted that the spectra in Fig. 3 were processed without any data manipulation, such as baseline correction, frequency-dependent phase correction along F_1 and backward and/or forward linear prediction. The only phase correction applied was a frequency-independent corrections to generate pure absorption signals.

In Fig. 1(C), the band-selected homonuclear decoupled TOCSY (BASHD-TOCSY) sequence is shown. This sequence is a further modification of the BASE-TOCSY sequence shown in Fig. 1(B). In this modification, the evolution period (t_1) is equally split on either side of the first band-selective π pulse. In addition, a non-selective π pulse immediately follows the first half of the evolution. In effect, with this modification, the 'selected' (by the first band-selective π pulse) protons experience a 2π pulse and all others experience a π pulse midway through t_1. This achieves homonuclear decoupling during t_1. However, midway through the first echo period ($\Delta 1$) only the 'selected' protons experience a π pulse and are refocused at the end of $\Delta 1$. Again, the experiment can be run with no additional phase cycling over the conventional experiment. As in the case of

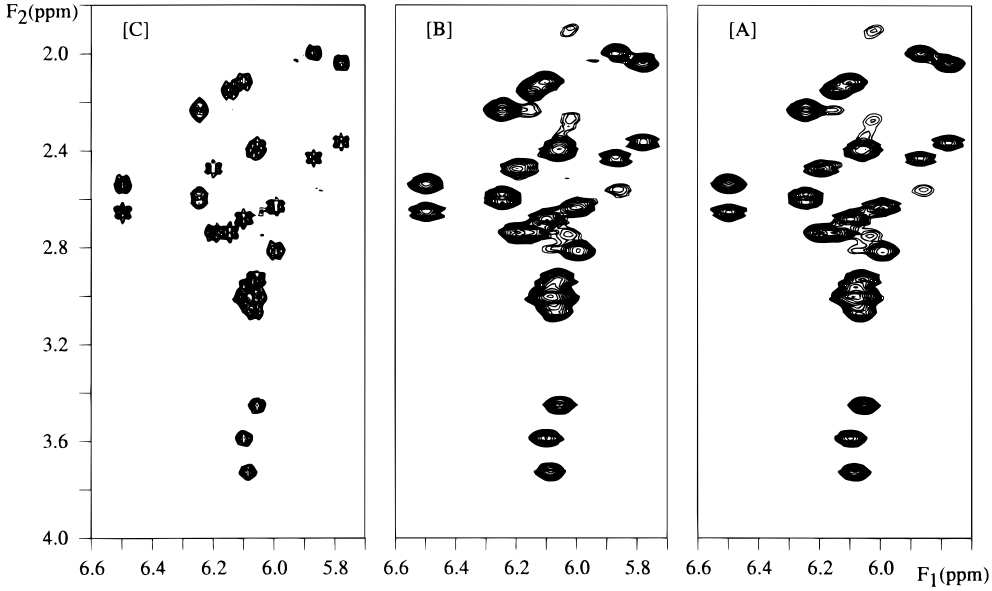

Figure 3. Comparison of (A) sensitivity-enhanced TOCSY spectrum with (B and C) BASE-TOCSY spectra of d(GGTTGGTGTGGTTGG) in D_2O. Only a selected (H-1′–H-2′/H-2″ cross peaks) region is shown. Two scans of 2048 complex points were collected over an observed spectral width of 5200 Hz for each of the t_1 values. The residual HDO resonance at 4.8 ppm (carrier frequency) was presaturated ($\gamma H_2 = 5$ Hz) during the 1 s relaxation time. A DIPSI-2 spin lock of 50 ms duration with $\gamma H_2 = 8$ kHz was used. The following additional parameters were used: F_1 spectral width, (A) 5200 and (B and C) 650 Hz; number of t_1 increments, (A) 256, (B) 32 and (C) 128; $F_1 \times F_2$ complex transform size, (A) 2048 × 2048, (B and C) 1024 × 2048. Total acquisition time: (A) 48, (B) 6 and (C) 25 min.

BASE-TOCSY experiment, the only phase cycling used in this study is the phase alternation of the first non-selective $\pi/2$ pulse for axial peak suppression. In Fig. 4, the BASHD-TOCSY spectrum of the DNA 15mer d(GGTTGGTGTGGTTGG) is compared with the BASE-TOCSY spectrum. The spectra in Fig. 4(A) and (B) were acquired over 25 min each (two scans each for 128 t_1 increments). Expansions of specific regions from these two spectra are given in Fig. 4(C) and (D). The spectral overlap in the BASE-TOCSY spectral expansion shown in Fig. 4(C) is due to homonuclear couplings. This ambiguity is removed in the BASHD-TOCSY spectrum [Fig. 4(D)] and all the five cross peaks are now clearly resolved. Selected F_1 traces from the BASE-TOCSY and BASHD-TOCSY spectra are shown in Fig. 4(E)–(H). These traces are plotted with identical absolute vertical scaling factors. As would be expected, the BASHD-TOCSY spectrum not only improves the resolution [as in Fig. 4(D)] but also improves the sensitivity [as in Fig. 4(F) and (H)] by collapsing the multiplet patterns to singlets.

EXPERIMENTAL

All spectra were recorded at 25 °C on a Varian Unity*Plus* 500 MHz NMR spectrometer equipped with a Programmable Pulse Modulator in the proton channel, a gradient accessory and using a $^1H\{^{13}C, ^{15}N\}$ triple resonance probe. A solution containing 300 OD_{260} of d(GGTTGGTGTGGTTGG) in 600 μl of D_2O (*ca.* 3.5 mM) containing 20 mM KCl was used to generate the spectra in Figs 2–4. The proton carrier frequency was positioned on the residual HDO resonance at 4.8 ppm. All non-selective proton 90° pulses were of 6.4 μs duration. All band-selective π pulses were Gaussian cascade Q3 pulses[19] of 7 ms duration with phase modulation[20] to shift its inversion center to + 650 Hz, which is the center of the H-1′ region. They were generated using the Pandora's Box[21] pulse shaping program available in Varian NMR software. A DIPSI-2 spin-lock[22] of *ca.* 8 kHz effective field strength was used for the TOCSY magnetization transfer. The strengths of the G1 and G2 gradients during the DPFGSE train were 8 and 5 G cm^{-1}, respectively, and their durations were 250 and 500 μs, respectively. A 100 μs delay followed all gradients for eddy current recovery. Minimum Δ1 and Δ2 delays were used to accommodate the gradients and the recovery delays. Data were processed using standard Varian software. All 2D spectra in Figs 3 and 4 were transformed after multiplying the time-domain data with an unshifted Gaussian window function along both the F_1 and F_2 dimensions. In addition, a +650 Hz frequency shift (difference between the carrier frequency and the center of the H-1′ region) was applied to the t_1 interferograms prior to transformation for the band-selective experiments [i.e. the spectra in Figs 3(B), 3(C) and 4]. One-dimensional spectra in Fig. 2 were transformed without any sensitivity or resolution enhancement.

CONCLUSION

Band selection and homonuclear decoupling using the DPFGSE technique is robust and achieves a substantial improvement in resolution and sensitivity and savings in total experiment time. This is particularly attractive in the study of oligonucleotides and oligosaccharides where the anomeric protons are well resolved from the

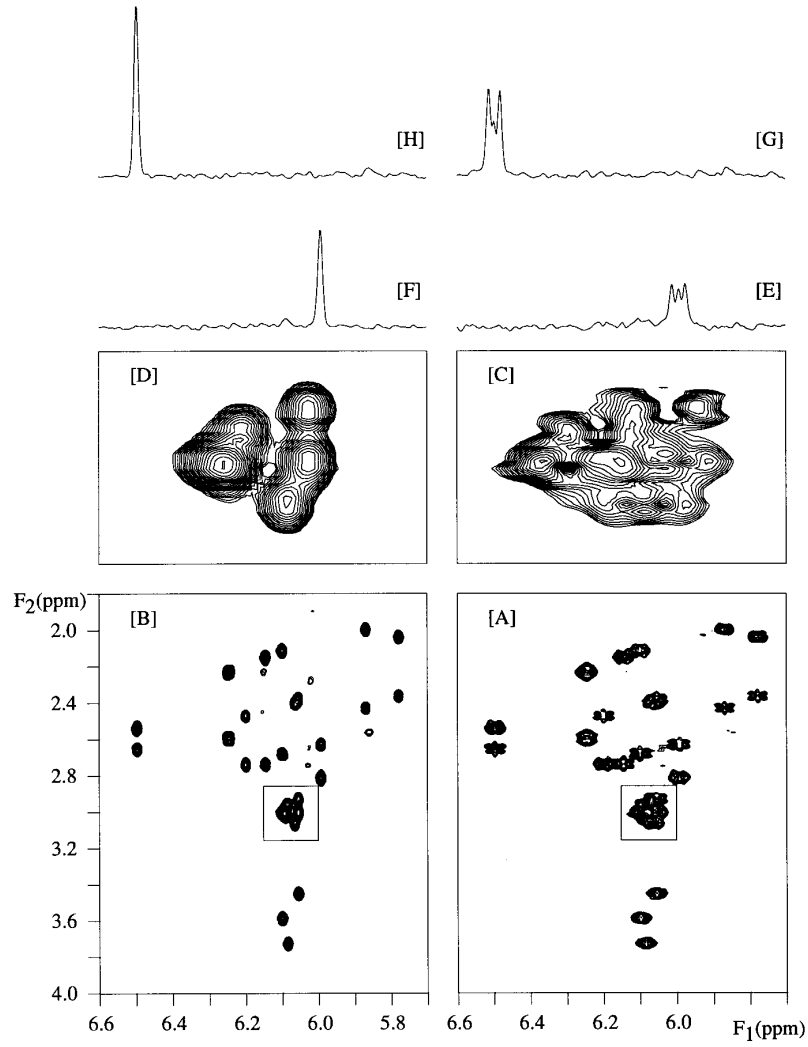

Figure 4. Comparison of (A) the BASE-TOCSY spectrum with (B) the BASHD-TOCSY spectrum of d(GGTTGGTGTGGTTGG) in D_2O. All acquisition, processing and display parameters were same as those used for the spectrum in Fig. 3(C). Expansions (C and D) shown are of the boxes indicated in (A) and (B). One-dimensional traces shown are F_1 traces at 2.82 ppm (E and F) and 2.53 ppm (G and H).

rest of the resonances. The BASHD technique using DPFGSE is not unique to the TOCSY sequence. We have successfully used it in other homonuclear 2D experiments such as NOESY and ROESY and in the homonuclear evolution dimension(s) in 3D experiments such as TOCSY–NOESY–3D and HSQC–TOCSY–3D.

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