Journal of Biomolecular NMR, 7 (1996) 169–172
ESCOM

J-Bio NMR 337

Suppression of spin diffusion in selected frequency bands of nuclear Overhauser spectra

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Received 26 September 1995
Accepted 26 January 1996

Keywords: Nuclear Overhauser effect; Spin diffusion; Selective pulses; Water suppression

Summary

A variant of two-dimensional nuclear Overhauser effect spectroscopy (NOESY) is described that yields information about cross-relaxation rates between pairs of spins, while the migration of magnetization through several consecutive steps (spin diffusion via neighboring spins) is largely suppressed. This can be achieved by inserting a doubly-selective inversion pulse in a conventional NOESY sequence.

The primary source of structural information on biomolecules in isotropic solution is the nuclear Overhauser effect (Neuhaus and Williamson, 1989). In simple cases, cross-peak intensities in two-dimensional nuclear Overhauser effect spectroscopy (NOESY) can be directly related to proton–proton distances (Anil Kumar et al., 1980). However, NOESY spectra can be misleading if spin-diffusion effects are not properly taken into account (Kalk and Berendsen, 1976; Anil Kumar et al., 1981; Lane, 1988; Massefski and Redfield, 1988; Zwahlen et al., 1994). Spin diffusion can often be recognized by recording cross-peak amplitudes as a function of the mixing time (Anil Kumar et al., 1981). It is possible to analyze NOESY spectra and buildup curves by considering the simultaneous effects of all cross-relaxation rates σ_{ij}, using the 'full relaxation matrix' method (Boelens et al., 1988; Borgias and James, 1988). However, the accuracy of the determination of internuclear distances can be greatly improved if spin diffusion is quenched, since in this case each buildup curve reflects a single internuclear distance. Several groups have tackled this problem in different ways (Massefski and Redfield, 1988; Fejzo et al., 1991, 1992; Boulat et al., 1992; Burghardt et al., 1993; Macura et al., 1994; Zwahlen et al., 1994; Schwager et al., 1996): spin-diffusion processes may be inhibited by suitable radio-frequency irradiation schemes, ranging from saturation of the undesirable 'clandestine' spins (Massefski and Redfield, 1988) to synchronous nutation (Boulat et al., 1992; Burghardt et al., 1993), and sophisticated combinations of laboratory and rotating-frame Overhauser experiments (Fejzo et al., 1991,1992; Macura et al., 1994). This communication presents a simpler alternative.

The pulse sequence shown in Fig. 1a is identical to that used for conventional two-dimensional NOESY, except that a doubly-selective inversion pulse has been inserted in the middle of the mixing time τ_m. Typically, a cosine-modulated Gaussian cascade Q^3 (Emsley et al., 1992; Zwahlen et al., 1994) allows the simultaneous inversion of the longitudinal magnetization in two frequency bands. The intersections of these two frequency bands define four squares ('quiet windows') in the two-dimensional spectrum (see Fig. 2).

Cross peaks at coordinates $(\omega_1, \omega_2) = (\Omega_A, \Omega_X)$ contained in these windows are due to direct cross-relaxation processes $A \rightarrow X$, while indirect pathways $A \rightarrow K \rightarrow X$ are eliminated, provided the chemical shifts of the clandestine spins K do not fall in either of the frequency bands. Parasitical pathways via spins K are suppressed because, to first order, a partial conversion of magnetization $A \rightarrow K$ in the first half of τ_m is cancelled in the second half of τ_m, due to the sign reversal of the Zeeman polarization (spin temperature of the thermal bath) of the A spins (Zwahlen

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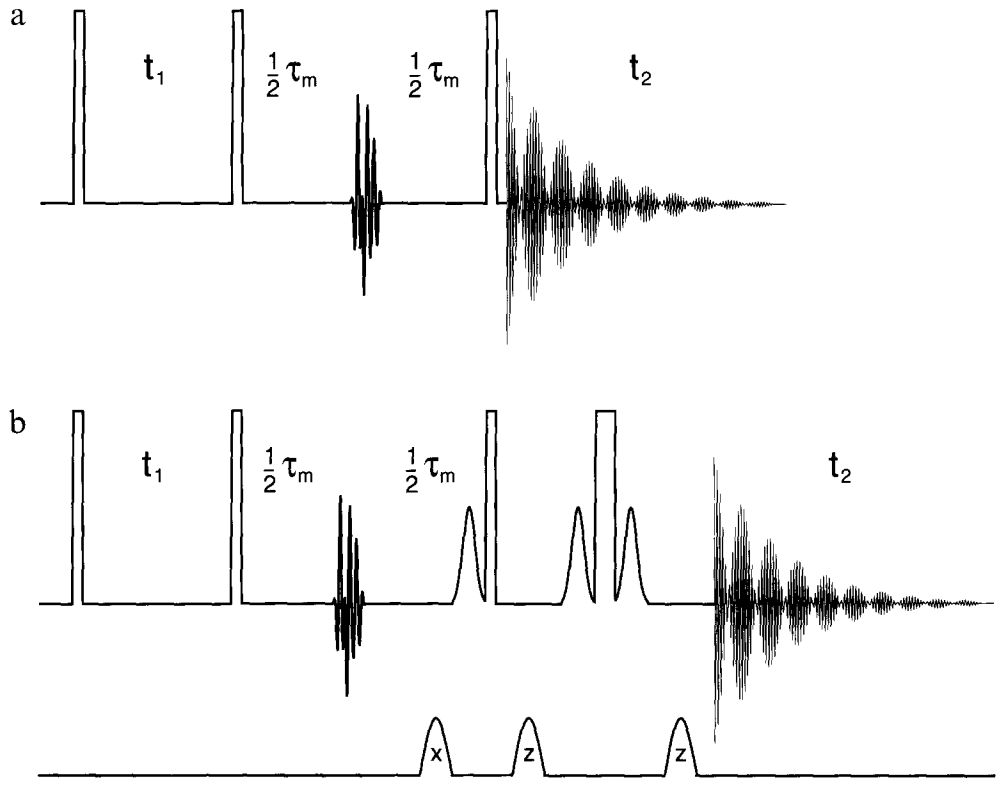

Fig. 1. Pulse sequences for (a) two-dimensional QUIET-BAND-NOESY and (b) a combination with the water-flip-back extension of WATERGATE. The experimental conditions were: mixing time $\tau_m = 200$ ms, doubly-selective Gaussian cascade Q^3 of 12 ms duration with 274 Hz peak radio-frequency amplitude for each side band. The Q^3 cascade was applied at $\tau_m/2$ to invert two frequency bands, each of 330 Hz width, centered at 7.50 and 2.70 ppm, respectively. Sineshaped pulsed field gradients were used with amplitudes $G_{x,z} \approx 25$ G/cm, the recovery time being 160 μs for all three 1-ms gradients. Selective 90° Gaussian pulses of 3 ms duration were used for selective water excitation in the water-flip-back and WATERGATE methods, and time-proportional phase increments were employed in combination with ±x alternation of the phase of the initial 90° pulse in conjunction with the receiver.

et al., 1994). Likewise, a process K → X is cancelled to first order as a result of the sign reversal of the longitudinal magnetization of the X spins.

For samples containing H_2O, the sequence can be improved by using the water-flip-back extension (Grzesiek and Bax, 1993) of WATERGATE (Piotto et al., 1992; Sklenář et al., 1993), as shown in Fig. 1b. Unlike the one-dimensional precursor of the experiment of Fig. 1 (Zwahlen et al., 1994), QUIET-BAND-NOESY (which stands for *q*uenching of *u*ndesirable *i*ndirect *e*xternal *t*rouble in *band*-selective NOESY) makes it possible to monitor several cross peaks simultaneously, although the likelihood of spurious spin-diffusion processes increases with the breadth of the selected frequency bands. Signal losses due to relaxation during pulses are negligible in band-selective experiments, in contrast to their one-dimensional multiplet-selective precursors (Schwager et al., 1996), because band-selective inversion pulses have a duration much shorter than T_2.

Although the mechanism is quite different, the motivation of our experiment is closely related to the objective of the BD and CBD methods (Fejzo et al., 1991,1992; Macura et al., 1994). The latter methods exploit the fact that cross-relaxation rates in the rotating and laboratory frames have opposite sign. Since the ideal ratio −2 : +1 may not be exactly fulfilled, particularly if there are local variations in correlation times, the intervals where cross relaxation is allowed to proceed in the rotating and laboratory frames have to be adjusted empirically to achieve a balance between cross-relaxation processes with opposite sign. Once a balance is achieved, cross relaxation is re-introduced in chosen regions of CBD-NOESY spectra by inserting selective inversion pulses. Spin-locking ROESY methods may suffer from Hartmann–Hahn effects, and signal losses may result if the magnetization is not completely spin-locked. However, the CBD-NOESY scheme has the advantage over QUIET-NOESY in providing a larger window with diminished spin diffusion. Typically, the useful area is a strip of 1 × 10 ppm in CBD-NOESY, while QUIET-NOESY merely provides a square window of 1 × 1 ppm. In both methods, the widths of the windows depend on the characteristics of the inversion pulses. The wider the window, the greater the risk that extraneous spin-diffusion processes sneak through (i.e., the greater the risk that the chemical shifts of the clandestine spins K accidentally fall within the window). In this respect the two schemes are similar. One may ask how many QUIET-BAND-NOESY spectra need to be col-

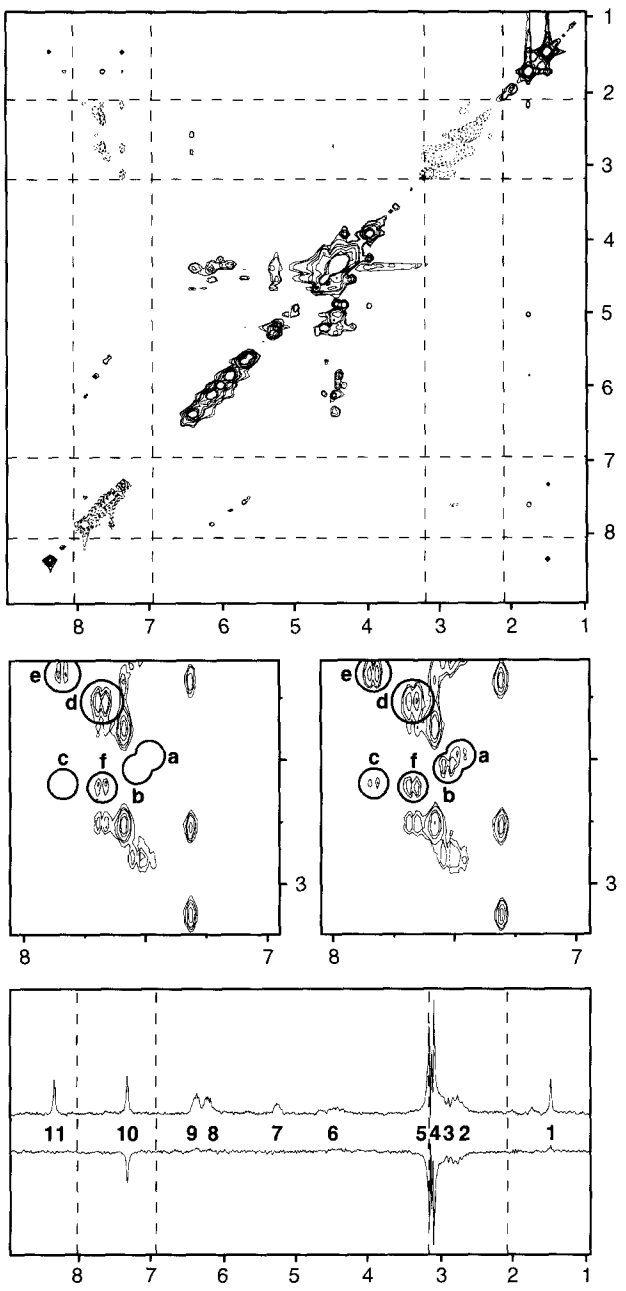

Fig. 2. Top: QUIET-BAND-NOESY spectrum of d(CGCGAATTC-GCG)$_2$ (Dickerson's dodecamer) obtained with simultaneous inversion of the magnetization in the middle of the mixing time of two frequency bands, each of 1.1 ppm width. The limits indicated correspond to an inversion efficiency of 50%. The spectrum was recorded at 303 K on a Bruker DMX 300 spectrometer, with 8 ppm spectral widths in both dimensions, 1K × 2K data points, no zero filling, and exponential multiplication with a 3 Hz line broadening in both dimensions. Middle left: quiet window enlarged from the QUIET-BAND-NOESY spectrum (see text for assignment of signals). Middle right: corresponding window extracted from a conventional NOESY spectrum recorded under identical conditions. All signals within the quiet windows are of opposite sign compared to the NOESY. Bottom: cross sections taken at the ω_1 frequency (3.13 ppm) of the two overlapping protons dA5 H2" and dA6 H2", which lie within the quiet window, from conventional NOESY (upper trace) and from QUIET-BAND-NOESY (bottom trace). A total of 11 NOESY peaks can be assigned (numbered from 1 to 11), six of which are suppressed in the QUIET-BAND-NOESY experiment (see text for details).

lected. An exhaustive approach would require as many as 45 complementary 2D experiments with quiet windows of 1 × 1 ppm. In practice, of course, one should focus attention on strategic regions (amide/amide, amide/α-protons, etc).

Our method may also be compared with the idea of saturating all regions containing clandestine spins that could contribute to spin diffusion (Massefski and Redfield, 1988). Indeed, if it were possible to saturate three regions simultaneously (e.g. 0–4, 5–7, and 8–10 ppm), while leaving two well-defined windows completely unaffected (e.g. 4–5 and 7–8 ppm), then a similar effect could be achieved with the scheme of Redfield and Massefski as we have obtained with QUIET-BAND-NOESY. In practice, instead of *saturating* three regions, it may be easier to *invert* three regions, repeatedly if necessary, using suitably tailored pulses. Perhaps the cleanest way to achieve this experimentally would be to invert two bands (e.g. 4–5 and 7–8 ppm) and then to invert the entire spectrum by a nonselective 180° pulse, so that the magnetization in the two bands is subjected to a 360° pulse and therefore not affected. Such a scheme, which can be regarded as a realization of Redfield's and Massefski's idea, would be essentially equivalent to QUIET-BAND-NOESY.

Figure 2 shows a QUIET-BAND-NOESY spectrum of Dickerson's dodecamer, a widely studied B-DNA fragment (Drew et al., 1981; Withka et al., 1991). The selected frequency bands cover part of the H2' and H2" region on the low-frequency side, and part of the aromatic H6 and H8 regions on the high-frequency side. Within the quiet windows, cross and diagonal peaks can be interpreted in the usual manner, except that misleading signals due to indirect two- or three-step processes are eliminated, provided no clandestine spins fall within the selected frequency bands. The rectangular strips that extend from one quiet window to another in Fig. 2, as well as the strips between the quiet windows and the margins of the spectrum, should ideally be completely empty. A few residual signals, clustered around 6 ppm in ω_1 and 7.5 ppm in ω_2, indicate that some parasitical transfer processses A → K have not been suppressed completely. These cross peaks arise from strong NOEs between the neighboring aromatic H5 and H6 protons of the four cytosine bases dC1, dC3, dC9, and dC11. In principle, suppression could be improved by inserting two modulated inversion pulses at $\tau_m/4$ and $3\tau_m/4$. The remainder of the 2D spectrum should be equivalent to a conventional NOESY.

The middle section of Fig. 2 shows enlargements of a quiet window taken from the upper spectrum, and of the corresponding window taken from a conventional NOESY spectrum. The cross peaks dC3 H2" → dC3 H6, labeled (a), dC11 H2" → dC11 H6, labeled (b), and dC1 H2" → dC1 H6, labeled (c), which are due predominantly to two-step processes dC3 H2" → dC3 H2' → dC3 H6,

dC11 H2" → dC11 H2' → dC11 H6, and dC1 H2" → dC1 H2' → dC1 H6, are suppressed in the QUIET-BAND-NOESY, since the clandestine spins dC3 H2', dC11 H2', and dC1 H2' fall outside the two selected frequency bands. The lines bracketing the selected bands have been drawn where the inversion is effective to 50%, i.e., where $M_z(\tau_p^+)/M_z(\tau_p^-) = -0.5$. The suppression of these cross peaks may be regarded as evidence that they are due to misleading two-step processes. On the other hand, the strong direct cross peak dC9 H2' → dC9 H6, labeled (d), which lies in the center of the window, is essentially unaffected in QUIET-BAND-NOESY, thus confirming that this cross peak is due to a genuine Overhauser effect.

Some of the signals that have been emphasized in the central windows of Fig. 2 illustrate borderline cases, which must be interpreted with care. The strong direct cross peak dC1 H2' → dC1 H6, labeled (e), which should ideally be preserved, is slightly attenuated because it lies close to the edge of the window. This problem can in principle be alleviated by using selective inversion pulses with a narrower transition region, and by a careful choice of the frequency bands. The misleading dC9 H2" → dC9 H6 cross peak, labeled (f), which is mostly due to a two-step process dC9 H2" → dC9 H2' → dC9 H6, is only slightly attenuated because the clandestine spin dC9 H2' accidentally falls within the quiet window. This problem cannot be avoided, except by using a very high-field spectrometer, so that careful interpretation using a reduced 'total relaxation matrix' (including only three spins in this case) remains advisable. In these two examples, the suppression of spin diffusion remains a challenge. By and large, these examples must be considered as special cases: the vast majority of spin-diffusion pathways are quenched to a very high extent.

The cross sections in Fig. 2, taken at the ω_1 frequency of the chemical shift of the overlapping dA5 H2" and dA6 H2" protons (3.13 ppm), demonstrate the extent of signal suppression. Signals that survive correspond to cross peaks and diagonal peaks within the quiet windows. Their negative intensities are due to the inversion of the magnetization in the middle of τ_m. Six out of 11 signals are suppressed in the band-selective experiment: (1) dA6 H2" → T7 CH_3, (6) dA5 H2" → dA5 H4' and dA6 H2" → dA6 H4', (7) dA5 H2" → dA5 H3' and dA6 H2" → dA6 H3', (8) dA5 H2" → dA5 H1', (9) dA6 H2" → dA6 H1', and (11) dA5 H2" → dA5 H8 and dA6 H2" → dA6 H8. In addition to the two diagonal peaks, (4) dA6 H2" → dA6 H2" and (5) dA5 H2" → dA5 H2", two signals remain in the quiet window near the diagonal, (2) dA6 H2" → dA6 H2' and (3) dA5 H2" → dA5 H2', and one cross peak in the off-diagonal quiet window, (10) dA6 H2" → T7 H6.

In conclusion, the two-dimensional QUIET-BAND-NOESY experiment allows monitoring of genuine Overhauser effects within selected frequency bands, while eliminating most misleading indirect pathways. Inspection of signals in the quiet areas gives a direct measure of the extent to which spin diffusion has been quenched. Two-dimensional QUIET-BAND-NOESY experiments are straightforward to implement and their analysis does not require any novel tools. These experiments should be useful to improve the accuracy of structural studies of biological macromolecules and supramolecular complexes, and to study the conformation of bound substrates and cofactors with transferred Overhauser techniques.

Acknowledgements

We are indebted to Dr. Philip Bolton (Wesleyan University, Middletown, CT) for a sample of d(CGCGAATTCGCG)$_2$. This research was supported by the National High Magnetic Field Laboratory, Tallahassee, FL.

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