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Improved lineshape and sensitivity in the HNCO-family of triple resonance experiments

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Abstract

A simple scheme is presented for the suppression of dispersive contributions to cross peaks in HNCO-type spectra where the ^{15}N chemical shift is recorded in a constant-time manner immediately prior to the transfer from ^{15}N to ^{1}HN at the end of the sequence. These dispersive contributions arise when the delay for refocusing the ^{15}N-^{13}CO one-bond coupling is set to less than $0.5/^{1}J_{N,CO}$ and when $^{2}J_{HN,CO} \neq 0$. Improvements in sensitivity in ^{1}HN detected experiments recorded on ^{15}N,^{13}C-labeled samples can be realized by application of $^{13}CO/^{13}C^{\alpha}$ decoupling during acquisition. Sensitivity gains on the order of 15% and 5% have been obtained for an SH3 domain (62 residues) and maltose binding protein (370 residues), respectively.

A major use of triple resonance NMR spectroscopy lies in the assignment of ^{15}N, ^{13}C, ^{1}H chemical shifts during the initial stages of a protein structure determination (Bax, 1994). In this role issues of resolution and sensitivity are critical, but because spectra are used in a qualitative sense, imperfections such as slight deviations from purely absorptive lineshapes, for example, are not likely to create serious problems for data interpretation. In contrast, when data sets are used in a more quantitative manner, such as might be the case in the measurement of couplings where peak positions or intensities must be quantitated more accurately (Bax et al., 1994), interpretation of data is less tolerant to deviations from ideality. We have recently observed that in HNCO-based experiments where the ^{15}N chemical shift is recorded in constant-time mode immediately prior to transfer back to ^{1}HN for acquisition, the lineshapes in the ^{15}N and the ^{1}HN dimensions are not purely absorptive. The degree of deviation from pure absorption depends on the length of the constant-time evolution period and the magnitude of the two-bond ^{13}CO, ^{1}HN coupling, $^{2}J_{HN,CO}$. In this communication we report a simple modification to HNCO-based pulse schemes which restores absorptive lineshapes. We also demonstrate that for applications to proteins with narrow ^{1}HN linewidths $^{13}CO/^{13}C^{\alpha}$ decoupling during acquisition results in measurable sensitivity gains.

The discussion that follows is relevant for the HNCO family of experiments including HNCO (Kay et al., 1994), HN(CO)CA (Bax and Ikura, 1991) and HN(COCA)CB (Shan et al., 1996). These sequences have been discussed in detail in the literature and will not be reviewed here. For simplicity we consider the basic HNCO sequence for which the magnetization pathway can be described as

$$^{1}HN \xrightarrow{^{1}J_{HN}} {}^{15}N \xrightarrow{^{1}J_{N,CO}} {}^{13}CO(t_1) \xrightarrow{^{1}J_{N,CO}} {}^{15}N(CT\text{-}t_2) \xrightarrow{^{1}J_{N,CO}} {}^{1}HN(t_3) \quad (1)$$

where the active couplings are indicated above each arrow. Several different implementations of CT-HNCO have been published, including versions with and without coherence transfer selection by pulsed field gradients (Kay et al., 1994) as well as TROSY-based experiments (Salzmann et al., 1998; Yang and Kay, 1999a). While the exact details will vary with the precise sequence, the origin of the dispersive contributions to the F_2/F_3 lineshapes can be readily under-

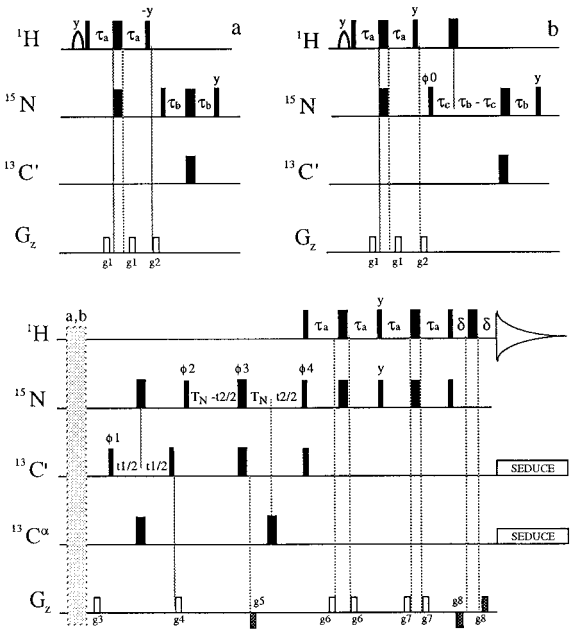

Figure 1. TROSY-HNCO without (a) and with (b) active suppression of the anti-TROSY component using the implementation of Yang and Kay (1999a). Scheme (a) or (b) is inserted into the pulse sequence in place of the light-shaded rectangle in the figure. Experimental details are given in Yang and Kay (1999a,b) [see supplementary material of Yang and Kay (1999b)]. $^{13}CO/^{13}C^{\alpha}$ decoupling is applied with a 59 ppm cosine modulated SEDUCE-1 decoupling field (McCoy and Mueller, 1992) (350 μs 90° SEDUCE pulses at 500 MHz) with the carrier positioned midway between the ^{13}CO and $^{13}C^{\alpha}$ chemical shifts (≈117 ppm).

stood without consideration of the exact form of the experiment.

Figure 1 illustrates a TROSY-based implementation of the CT-HNCO pulse sequence that has been developed for applications to large, highly deuterated proteins (Yang and Kay, 1999a). This experiment has been discussed in the literature previously and we focus here only on the terms that give rise to the dispersive character of the peakshape. At the start of the ^{15}N constant-time evolution period (CT-t_2) the magnetization of interest can be written as $2N_x(1-2HN_z)CO_z\cos(\omega_{CO}-\pi^2J_{HN,CO})t_1$, where all the multiplicative terms which describe transfer efficiency and relaxation losses have been neglected and X_x, X_z, denote the x and z components of X magnetization, respectively. Only the term which gives rise to the TROSY multiplet component is retained. The anti-TROSY cross peak is severely attenuated via relaxation during the substantial delays in the pulse sequence where ^{15}N transverse magnetization evolves. If necessary, this unwanted component can be actively suppressed using scheme b in Figure 1 (Pervushin et al., 1998a; Yang and Kay, 1999a). During the constant-time delay of duration $2T_N$, ^{15}N chemical shift evolves for t_2, while evolution from the one-bond ^{15}N-^{13}CO coupling, $^1J_{N,CO}$, proceeds for the complete period $2T_N$. Thus, at the end of $2T_N$ the signal of interest can be written as

$$\begin{aligned}
&A\{N_y(1-2HN_z)\cos(\omega_N-\pi^1J_{NH})t_2\\
&\quad -N_x(1-2HN_z)\sin(\omega_N-\pi^1J_{NH})t_2\}\\
&\quad +B\{2N_x(1-2HN_z)CO_z\cos(\omega_N-\pi^1J_{NH})t_2\\
&\quad +2N_y(1-2HN_z)CO_z\sin(\omega_N-\pi^1J_{NH})t_2\},\\
&A=\sin(2\pi^1J_{N,CO}T_N)\cos(\omega_{CO}-\pi^2J_{HN,CO})t_1,\\
&B=\cos(2\pi^1J_{N,CO}T_N)\cos(\omega_{CO}-\pi^2J_{HN,CO})t_1. \quad (2)
\end{aligned}$$

Focusing exclusively on the transfer of N_y terms, and omitting for the moment the ^{13}CO 90° purge pulse applied at the end of the constant-time period and the $^{13}CO/^{13}C^{\alpha}$ decoupling during t_3, the observable signal is given by:

$$\begin{aligned}
&-A\cos(\omega_N-\pi^1J_{NH})t_2\cos(\omega_{HN}+\pi^1J_{NH})t_3\\
&\quad \cos(\pi^2J_{HN,CO}t_3)\\
&+B\sin(\omega_N-\pi^1J_{NH})t_2\sin(\omega_{HN}+\pi^1J_{NH})t_3\\
&\quad \sin(\pi^2J_{HN,CO}t_3). \quad (3)
\end{aligned}$$

If $2T_N$ is set to $0.5/^1J_{N,CO}$, only the first term in Equation 3 is non-zero and purely absorptive lineshapes in all three dimensions are obtained. However, for applications to many proteins this large value of $2T_N$ may lead to unacceptable losses in sensitivity and values of $2T_N$ somewhat less than $0.5/^1J_{N,CO}$ are most often employed. This results in lineshapes that are not purely absorptive in either F_2 or F_3, as illustrated in Figure 2a where a portion of a 2D HN(CO) data set recorded with a non-TROSY, HNCO scheme (Figure 1 of Kay et al. (1994)) on the spectrin SH3 domain [62 residues (Blanco et al., 1997)] is shown ($2T_N = 24.8$ ms). It is noteworthy that in the limit that $^2J_{HN,CO} \longrightarrow 0$, purely absorptive lineshapes are restored. $^2J_{HN,CO}$ couplings of 4.4 ± 0.4 Hz have been measured in the case of maltose binding protein (MBP, 370 residues) and the asymmetry in peak shapes caused by the dispersive contributions can be quite noticeable, as Figure 2a indicates.

Equation 2 shows that the terms that give rise to the skewed peak shapes are proportional to CO_z at the end of the ^{15}N constant-time evolution period. Thus, application of a ^{13}CO 90° purge pulse at

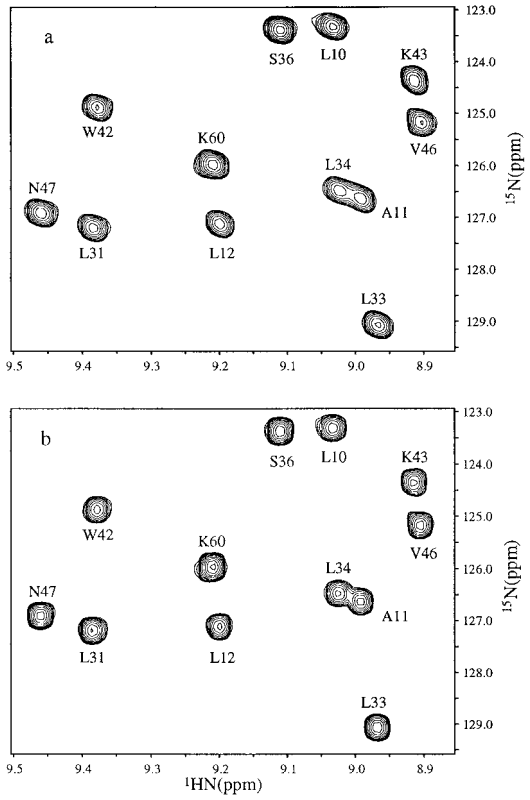

Figure 2. 2D HN(CO) slices recorded on a 2 mM sample of ^{15}N,^{13}C,^{2}H-labeled spectrin SH3, pH 3.5, 90% H_2O, 10% D_2O, 1 μM leupeptin, 1 μM pepstatin, 800 μM pefabloc, 100 μM DSS, 25 °C (Blanco et al., 1997). Spectra were recorded at 500 MHz on a UNITY+ spectrometer without ^{13}CO/^{13}C$^{\alpha}$ decoupling during acquisition (88 ms) (a) and with a ^{13}CO 90° purge and decoupling during acquisition (b). In (a) the decoupling element was inserted during the relaxation delay so that differential heating between (a) and (b) is minimized. The sequence of Figure 1 in Kay et al. (1994) was employed (with the modifications listed above). For applications to small proteins this experiment is approximately a factor of 2 more sensitive than TROSY sequences. Data sets were processed using NMRPipe software (Delaglio et al., 1995) and analyzed using the PIPP/CAPP suite of programs (Garrett et al., 1991).

any time after the $2T_N$ delay and prior to detection removes these contributions. This is illustrated in Figure 2b, where correlations corresponding to those in Figure 2a are shown from a data set where a ^{13}CO 90° pulse has been inserted immediately after the ^{15}N constant-time evolution period ($2T_N = 24.8$ ms). Alternatively, setting $2T_N = 0.5/^{1}J_{N,CO}$ also results in purely absorptive correlations (data not shown) but as discussed above, this delay may not be practical for all applications.

The presence of the skewed lineshapes in HNCO-type correlation spectra which result from finite $^{2}J_{HN,CO}$ coupling values suggests that in ^{1}HN-

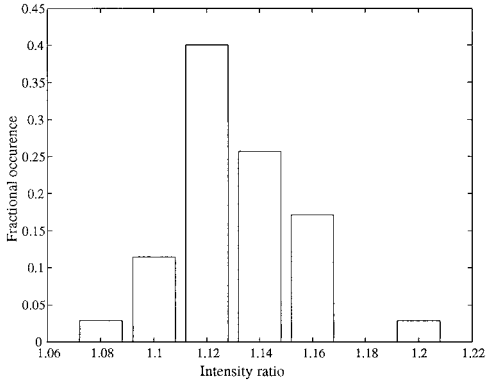

Figure 3. Fractional occurrence vs. intensity ratio of cross peaks in non-TROSY HN(CO) spectra of the spectrin SH3 domain at 25 °C (intensity ratio = intensity of cross peaks in spectra with ^{13}CO/^{13}C$^{\alpha}$ decoupling during acquisition and a ^{13}CO purge pulse/intensity of cross peaks in spectra without decoupling or a ^{13}CO purge). The intensity ratio is a function of the acquisition time as well as the window used in the acquisition dimension. An 81° shifted sinebell was employed for all spectra.

detected experiments involving ^{15}N,^{13}C-labeled proteins with narrow ^{1}HN linewidths it may be possible to improve sensitivity by applying ^{13}CO decoupling during acquisition. In addition, because of finite intraresidue $^{2}J_{HN,C\alpha}$ couplings [$^{2}J_{HN,C\alpha} \approx 2.5$ Hz (Bystrov, 1976)], the sensitivity can also be improved by ^{13}Cα decoupling. Figure 3 shows the intensity ratios measured for cross peaks from non-TROSY HNCO spectra recorded on a ^{15}N, ^{13}C, ^{2}H-labeled spectrin SH3 domain with and without ^{13}CO/^{13}C$^{\alpha}$ decoupling during an acquisition of 88 ms (ratio = with decoupling/without decoupling). In the case that decoupling was not applied during acquisition, the decoupling train was inserted during the relaxation delay to ensure that differences in intensity ratios are not the result of differential sample heating. Average sensitivity gains of 13 ± 2% have been measured on the SH3 domain sample at both 25 °C and 5 °C in the presence of ^{13}CO/^{13}C$^{\alpha}$ decoupling during acquisition. The gain in sensitivity depends significantly on the ^{1}HN transverse relaxation times (i.e., the size of the molecule) and the acquisition time. In the case of the SH3 domain correlation times, τ_C, of 4.4 ± 0.2 ns (25 °C) and 7.2 ± 0.4 ns (5 °C) have been estimated from ^{15}N relaxation data (Farrow et al., 1994), while ^{1}HN T_2 values of 92 ± 16 and 64 ± 15 ms have been measured at 25 °C and 5 °C, respectively. (Here, 16 and 15 refer to the standard deviation in the values, not the error in the measurements.) It is noteworthy that the sensitivity gains measured are in good agreement with calculations based on the relaxation times listed above

and acquisition and processing parameters used in the experiments.

Experiments have also been performed on a Val, Leu, Ile (δ1 only) methyl-protonated, ^{15}N,^{13}C,^{2}H-labeled sample of MBP in complex with β-cyclodextrin (Gardner et al., 1998). At 37 °C the correlation time of the complex is 17 ± 0.8 ns and an average ^{1}HN T_2 value of 23 ± 4 ms is measured. It is noteworthy that T_2 values were obtained by inserting a simple spin echo sequence of the form τ 180°(^{15}N) τ 180°(^{1}H) τ 180°(^{15}N) τ, in the beginning of an HSQC experiment. This effectively suppresses cross correlation between ^{1}H-^{15}N dipolar and ^{1}H CSA interactions and the value quoted is therefore somewhat less than the operative relaxation time in TROSY-based experiments. TROSY-HNCO experiments have been recorded using the sequence of Figure 1 (with active purging of the anti-TROSY component) with and without ^{13}CO/$^{13}C^{\alpha}$ decoupling during acquisition. Intensities of correlations from spectra recorded with decoupling are 5.2 ± 2.3% higher than the corresponding correlations in spectra recorded without decoupling during the 88 ms acquisition period (but with the decoupling train applied during the relaxation delay), in good agreement with the calculated enhancement. Thus, sensitivity gains, albeit modest, can be obtained for larger molecules as well.

Finally, it is important to mention that a ^{13}CO purging pulse is not necessary in the HNCO family of experiments where the ^{15}N chemical shift is recorded immediately after the transfer from ^{1}HN to ^{15}N at the outset of the experiment (Grzesiek and Bax, 1992). Even in this case, however, sensitivity gains can be obtained from ^{13}CO/$^{13}C^{\alpha}$ decoupling. With the development of enhanced sensitivity experiments (Palmer et al., 1991; Cavanagh and Rance, 1993) including coherence transfer selected schemes (Schleucher et al., 1993; Muhandiram and Kay, 1994), and the implementation of TROSY from our laboratory (Yang and Kay, 1999a,b) as well as TROSY-based experiments developed by Pervushin, Wüthrich and co-workers (Pervushin et al., 1997, 1998b; Salzmann et al., 1998), the majority of sequences record ^{15}N evolution prior to the final transfer. Purely absorptive lineshapes can be easily obtained in this case by ^{13}CO/$^{13}C^{\alpha}$ decoupling during acquisition or by application of a ^{13}CO purge pulse as illustrated in Figure 1.

In the present communication we have described a simple approach for the generation of purely absorptive lineshapes in HNCO-based experiments in a manner independent of delays chosen for the experiment. Sensitivity gains can be achieved with ^{13}CO/$^{13}C^{\alpha}$ decoupling during acquisition even for relatively large proteins and protein complexes.

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