Journal of Biomolecular NMR, 2 (1992) 301–305
ESCOM

J-Bio NMR 065

Gradient-enhanced 3D NOESY-HMQC spectroscopy

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Received 31 October 1991
Accepted 31 December 1991

Keywords: Gradient-enhanced NMR; Mnt repressor; 3D NMR; NOESY-HMQC

SUMMARY

A gradient-enhanced 3D NOESY-HMQC experiment is presented and applied to ^{15}N-labeled Mnt repressor (1–76) in $^{1}H_2O$. Both coherence selection and $^{1}H_2O$ suppression are achieved using gradients. A single-scan experiment can be recorded for each time increment, which greatly reduces recording time with respect to conventional methods using phase cycling. This can be of use for kinetic studies and for relatively unstable molecules.

Coherence selection using gradients provides a single-scan alternative over phase cycling (Maudsley et al., 1978; Bax et al., 1980; Counsell et al., 1985). The present availability of high-quality shielded gradients has stimulated publication of a number of new gradient-enhanced NMR experiments (Sotak et al., 1988; Hurd, 1990; Knüttel et al., 1990,1991; Swanson and Yeung, 1990; Brereton et al., 1991; Hurd and John, 1991a,b; von Kienlin et al., 1991). Recently, we reported gradient-enhanced heteronuclear multiple-quantum coherence (HMQC) and single-quantum coherence (HSQC) spectra of ^{15}N-labeled Mnt (1–76) in $^{1}H_2O$, obtained in only 150 s experiment time (Vuister et al., 1991). One of the most important 3D experiments for biomolecular structure determination is the NOESY-HMQC. Here, we show that the gradient-enhanced HMQC experiment can be readily combined with 2D NOE (Jeener et al., 1979; Kumar et al., 1980; Macura and Ernst, 1980) to yield gradient-enhanced 3D NOESY-HMQC. This experiment

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can be recorded in a single-scan mode (i.e. one scan per time increment) in only 6 h, which is a great time saving with respect to the conventional approach using phase cycling (Fesik and Zuiderweg, 1988; Marion et al., 1989a). Solvent suppression occurs during the HMQC and is achieved by gradient dephasing due to coherence selection (Swanson and Yeung, 1990; Hurd and John, 1991a,b; Knüttel et al., 1990,1991; Van Zijl and Moonen, 1992; Vuister et al., 1991). Compared to jump-return techniques the pulse sequence with suppression is extremely simple and does not result in any baseline-roll or other artefacts. C^{α} protons resonating close to the ^1H_2O resonances are not attenuated, as would be the case with presaturation. Furthermore, NOEs of amide protons can be studied in a simple way.

Figure 1 shows the pulse sequence. Coherence selection in t_2 is achieved by the combined gradients G1, G2, and G3 and depends on the gyromagnetic ratios of ^1H and ^{15}N ($\gamma_{1H}/\gamma_{15N} = 9.88$). We used the ratio G1:G2:G3 = 4.94:4.94: $\pm$ 1.0. A more detailed treatment of different ratios will be given elsewhere (Ruiz-Cabello et al., in press). Suppression of coherences $p \neq 0$ by gradient dephasing during the NOE mixing time τ_m was first suggested by Jeener et al. (1979). A new aspect is gradient cycling in successive scans to avoid gradient-recalled echoes from the coherently dephased water resonance.

Since both the p = 1 and p = -1 coherence pathways are retained during t_1, amplitude modulation is obtained in this domain and the spectrum can be displayed in a phase-sensitive mode (Ernst et al., 1987). In contrast, during t_2 the two coherence-transfer pathways ZQ→DQ and DQ→ZQ are phase-encoded with opposite signs and only one pathway can be refocused by G3 (Hurd and John, 1991a,b; Vuister et al., 1991). As a result a phase-modulated signal is obtained for this domain and the spectrum is displayed in absolute-value mode. As a consequence strong sine-bell filtering in t_3 had to be used to achieve the necessary resolution.

Figures 2a and b show the ω_1, ω_3 cross sections at ω_2 = 120 ppm for the single- and 8-scan gradient-enhanced NOESY-HMQC spectrum of 2 mM ^{15}N-enriched Mnt repressor (1–76), respec-

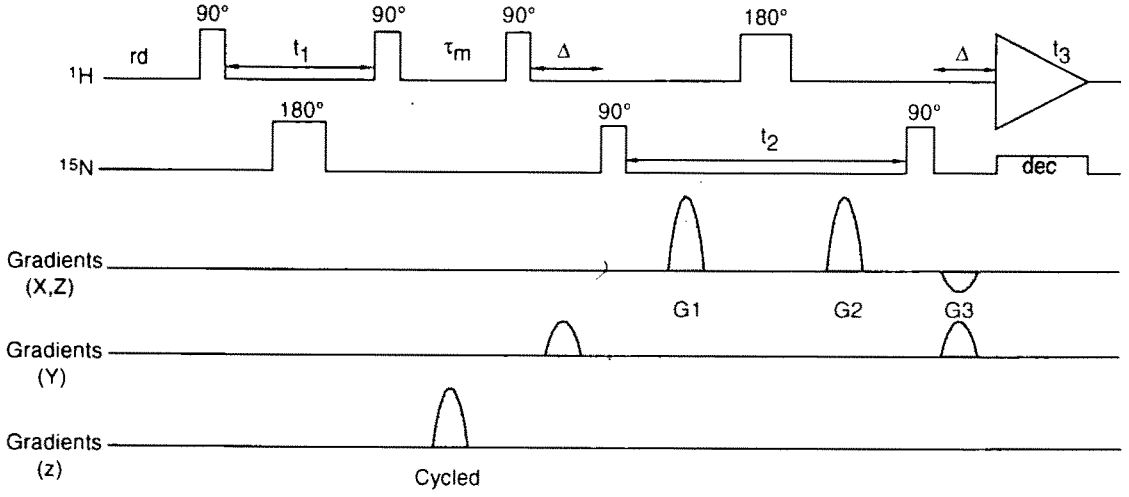

Fig. 1. Pulse sequence for gradient-enhanced 3D NOESY-HMQC. For coherence selection during t_2, x and z gradients were used. In addition, y gradients were employed during Δ to dephase unwanted coherences resulting from an imperfect 180° pulse. During τ_m all coherences $p \neq 0$ are destroyed by a cycled z-gradient pulse (homospoil). rd denotes the relaxation delay (1 s), $\Delta = [2J(^{15}N\text{-}^1H)]^{-1}$ (4.5 ms), and dec denotes low-power Garp decoupling (1.7 kHz).

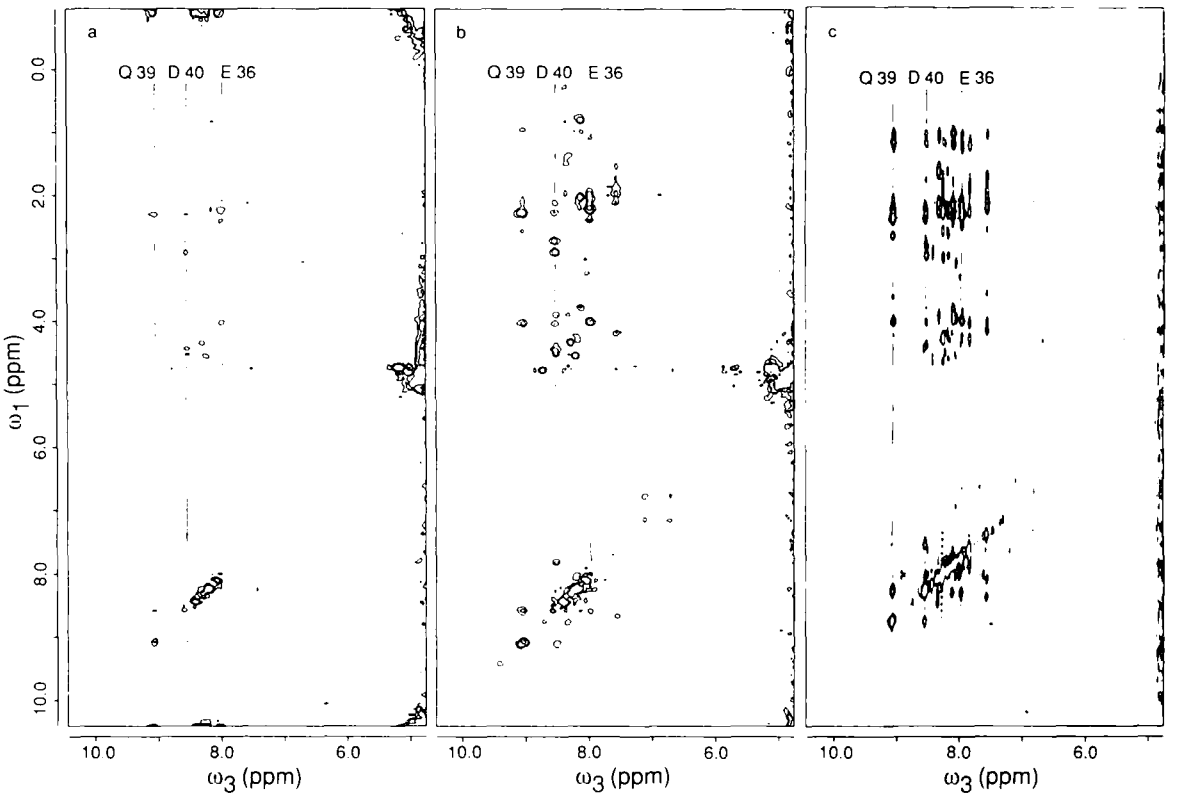

Fig. 2. Cross sections at ω_2 = 120 ppm of single-scan (a) and 8-scan (b) gradient-enhanced 3D NOESY-HMQC spectra of 2 mM ^{15}N-Mnt repressor (1–76) in 95:5 (v:v) H_2O:D_2O (room temperature, pH = 4.5). Experiments were recorded on a GE PSG wide-bore 400-MHz NMR machine, equipped with shielded gradients. Gradient pulses were 0.8 ms and sine-bell shaped, followed by a 50-μs delay. G1, G2, and G3 were 0.637, 0.637, and −0.129 Tm^{-1}, respectively for x and 0.205, 0.205, and −0.042 Tm^{-1}, respectively for z. The y-gradient pulses during Δ were 0.197 Tm^{-1}. The z-gradient pulse of 4 ms during τ_m (150 ms) was cycled through 0.07, 0.21, 0.35, and 0.49 Tm^{-1} in successive scans. 128 complex (t_1) × 64 (t_2) single-scan FIDs of 256 complex points, preceded by 4 dummy scans were recorded for the single-scan spectrum. Total experiment time was 6 h. Quadrature detection in t_1 was obtained by the States-TPPI method (Marion et al., 1989c). The only difference for the 8-scan experiment (48 h), as compared to the single scan, was the use of a cyclops phase cycle (Ernst et al., 1987). (c) Cross section at ω_2 = 120 ppm of a conventional 600-MHz 3D NOESY-HMQC spectrum of ^{15}N-Mnt repressor (1–76) in 95:5 (v:v) H_2O:D_2O (300 K), recorded on a Bruker AM600 spectrometer. Presaturation was employed during the recycle delay and during the NOESY mixing time of 100 ms for suppression of the H_2O resonance. During acquisition decoupling was achieved using a GARP pulse sequence with soft ^{15}N pulses. Δ was set to 4.0 ms, slightly less than [2J(^{15}N−^{1}H)]$^{-1}$. A spectral width of 6944 Hz was used in both the ω_3 and ω_1 domains, the spectral width in ω_2 was 3472 Hz. The phase-sensitive (TPPI) 3D dataset was recorded as 200 (t_1) × 122 (t_2) × 1024 (complex) (t_3) points. Each FID consisted of 8 scans, preceded by 2 dummy scans. The total instrument time was 116 h. All spectra were processed using the Triton processing software. The DC offset in the single-scan experiment was removed by fitting a linear function to the 76 last points of both real and imaginary parts of the FID. From there on, spectra shown in Figs. 2a and b were processed identically. Sine-bell windows shifted by 60° and 45° were used prior to Fourier transformation in t_1 and t_2, respectively. In t_3 squared sine-bell windows shifted by 15° were used. The data were zero-filled once in t_1 prior to Fourier transformation. An automatic third-order polynomial baseline correction was applied to each dimension of the spectra of Figs. 2a and b in order to compensate for small baseline offsets. Spectra are shown in absolute-value mode in ω_2 and ω_3 and in phase-sensitive mode in ω_1. The spectrum of Fig. 2c was zero-filled in t_1 and t_2 to yield 512 × 128 × 512 real spectral points and is shown in phase-sensitive mode.

tively. The 3 lines in the figure indicate the amide resonance frequencies of the residues Gln^{39}, Asp^{40}, and Glu^{36}. Several NOE cross peaks can be identified. Since the ^{15}N chemical shifts of Gln^{39} and Asp^{40} are identical within the digital resolution in ω_2, both d_{NN} cross peaks can be identified, as expected for the helical conformation in this part of the molecule. The other NOE cross peaks could also be assigned in agreement with those in a conventional 3D NOESY-HMQC (cf. Fig. 2c). NOEs to protons resonating close to the ^1H_2O resonance are easily observed. For example, the NOE for $C^{\alpha}H$ of Lys^{44} at 4.68 ppm (cross section not shown) is observed, which is not possible if presaturation is used for suppression. Clearly, the cross sections do not show artefacts, such as tails or baseline-roll, due to the strong ^1H_2O resonance.

In the 8-scan spectrum several weaker NOEs show up, which are below the noise level of the single-scan spectrum. However, this is due to the fact that the experiments were recorded on a 400-MHz NMR spectrometer. The experimental probe of our wide-bore 400-MHz spectrometer was not optimized for the recording of a conventional NOESY-HMQC experiment with presaturation. Therefore, a state-of-the-art 600-MHz NOESY-HMQC spectrum of ^{15}N-enriched Mnt, recorded in 116 h, is shown for comparison in Fig. 2c. Most cross peaks of the conventional spectrum are also observed in the 8-scan spectrum. Note that the experiment time of the 8-scan gradient-enhanced spectrum was less than half of that of the conventional 8-scan spectrum (48 h vs. 116 h, respectively). The sensitivity of a single-scan experiment should be sufficient at 600 MHz, which is more commonly used in NMR studies of large biomolecules. A disadvantage of the present experiment is a signal loss due to absolute-value display in combination with strong filtering. However, since the time for magnetization transfer between exchangeable protons and water (saturated by dephasing) is in the order of only 2–30 ms (depending on t_2), an actual gain may be attained for rapidly exchanging protons.

A single-scan gradient-enhanced 3D NOESY-HMQC experiment gives several new possibilities for the study of biomolecules, such as the recording of a 'build-up' series of 3D NOESY-HMQC spectra, kinetic studies, and 3D and 4D NMR experiments with increased digital resolution.

Field-gradients can be applied in a similar fashion in other 3D experiments such as the 3D HOHAHA-HMQC (Marion et al., 1989b; John et al., 1991) or the 3D ROESY-HMQC (Clore et al., 1990). The latter seems especially promising since, because of the efficient ^1H_2O suppression, it can be easily used to study NOEs of protein-bound H_2O molecules.

ACKNOWLEDGEMENTS

This research was financially supported by PharmaGenics, Inc., and the Netherlands Foundation for Chemical Research (SON) with aid of the Netherlands Organisation of Scientific Research (NWO). The shielded gradients that made this study possible were provided by GE NMR Fremont. We thank Drs. Boban John and Ralph Hurd (GE Fremont) for valuable advice and assistance. We are grateful to Dr. J. Cohen (Georgetown University) for stimulating this research.

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