Communication

Observing in-phase single-quantum $^{15}\text{N}$ multiplets for NH$_2$/NH$_3^+$ groups with two-dimensional heteronuclear correlation spectroscopy

Yuki Takayama, Debashish Sahu, Junji Iwahara *

Department of Biochemistry and Molecular Biology, Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch, 301 University Boulevard, 6.614A Basic Science Building, Galveston, TX 77555-0647, USA

**Article info**

**Abstract**

Two-dimensional (2D) F1-$^1$H-coupled HSQC experiments provide 3:1:1:3 and 1:0:1 multiplets for AX$_3$ spin systems, respectively. These multiplets occur because, in addition to the 2S$_g$H$_x^1$ to 2S$_p$H$_x^2$ process, the coherence transfers such as 2S$_g$H$_x^2$ to 2S$_p$H$_x^1$ occurring during the $t_2$ period provide detectable magnetization during the $t_1$ period. Here, we present a 2D F1-$^1$H-coupled $^1$H-$^{15}$N heteronuclear correlation experiment that provides a 1:3:1:3 quartet for AX$_3$ spin systems and a 1:2:1 triplet for AX$_2$. The experiment is a derivative of 2D HISQC experiment [J. Iwahara, Y.S. Jung, G.M. Clore, Heteronuclear NMR spectroscopy for lysine NH$_3$ groups in proteins: unique effect of water exchange on $^{15}$N transverse relaxation, J. Am. Chem. Soc. 129 (2007) 2971–2980] and contains a scheme that kills anti-phase single-quantum terms generated in the $t_1$ period. The purge scheme is essential to observe in-phase single-quantum multiplets. Applications to the NH$_2$ and NH$_3$ groups in proteins are demonstrated.

© 2008 Elsevier Inc. All rights reserved.

For heteronuclear AX$_3$ and AX$_2$ spin systems, one-dimensional NMR measurement on nucleus A that comprises a single excitation pulse immediately followed by detection without decoupling generally gives an in-phase 1:3:3:1 quartet and a 1:2:1 triplet, respectively, provided that relaxation rates for individual multiplet components are identical. It is because overall modulations of detected magnetizations due to $J$ and chemical shift evolutions are given by:

$$
\exp(i\Omega t) \cos^3 \pi J t = \frac{1}{8} \exp [i(\Omega - 3\pi J)t] + \frac{3}{8} \exp [i(\Omega - \pi J)t] + \frac{3}{8} \exp [i(\Omega + \pi J)t] + \frac{3}{8} \exp [i(\Omega + 3\pi J)t]$$

for an AX$_3$ spin system, and

$$
\exp(i\Omega t) \cos^2 \pi J t = \frac{1}{4} \exp [i(\Omega - 2\pi J)t] + \frac{1}{2} \exp(i\Omega t) + \frac{1}{4} \exp [i(\Omega + 2\pi J)t]$$

for AX$_2$. For simplicity sake, we use terms such as ‘1:3:3:1’ and ‘1:2:1’ hereafter, although actual intensity ratios of multiplet components can deviate due to cross-correlations [1].

In the case of a two-dimensional heteronuclear correlation experiment, it is not trivial to obtain the in-phase 1:3:3:1 quartet and 1:2:1 triplet. In an F1-$^1$H-coupled HSQC experiment (such as one shown in Fig. 1A), heteronuclear AX$_3$ and AX$_2$ spin systems exhibit 3:1:1:3 quartet and 1:0:1 triplet, respectively [2–4], because not only the 2S$_g$H$_x^1$ to 2S$_p$H$_x^2$ process but also the coherence transfers such as 2S$_g$H$_x^2$ to 2S$_p$H$_x^1$ occurring during the $t_1$-evolution period generates magnetizations detectable in the $t_2$-period. With the additional contributions, the real part of the overall modulation due to $J$ and chemical shift evolutions in the $t_1$-period for AX$_3$ is given by:

$$
(cos^3 \pi J t_1 - 2 \sin^2 \pi J t_1 \cos \pi J t_1) \cos \Omega t_1
$$

resulting a 3:1:1:3 quartet. Likewise, the corresponding modulation for AX$_2$ is:

$$
(cos^3 \pi J t_1 - \sin \pi J t_1 \cos \pi J t_1) \cos \Omega t_1
$$

which gives a 1:0:1 triplet. Since it appears to be a doublet, the multiplet itself does not indicate whether the spin system is of AX$_2$ or AX unless the true $J$-coupling is known.

In the present study, we have developed a new 2D $^1$H-$^{15}$N correlation experiment to observe an in-phase 1:3:3:1 quartet for a NH$_3^+$ group and a 1:2:1 triplet for a NH$_2$ group along F1 axis. Fig. 1B shows the 2D $^1$H-$^{15}$N F1-coupled $^1$H-$^{15}$N heteronuclear correlation experiment to observe 1:3:1:1 and 1:2:1 multiplets for NH$_3^+$ and NH$_2$, respectively. The experiment was derived from the water-flip-back 2D $^1$H-$^{15}$N HISQC (heteronuclear in-phase single
quantum coherence; Fig. 1C) experiment for NH₃ groups [2], and therefore we refer to it as F1-¹H-coupled HSQC. This pulse sequence starts with the ¹H excitation, and the coherence transfer form H₂ to NH₂ occurs before the t₁ period. The length of delay t₀ (=1.3 ms) is a compromise to simultaneously observe NH₂, NH₂, and NH, and overall J-modulations for these groups through four t₀ periods are given by 3cos²2πf₁t₀sin²2πf₁t₀ (=0.49 with f₁ = 74 Hz), 2cos²2πf₁t₀sin²2πf₁t₀ (=0.74 with f₁ = 89 Hz), and sin²2πf₁t₀ (=0.55 with f₁ = 93 Hz), respectively. Due to these attenuations along with relaxation loss during the additional schemes, the sensitivity of the F1-¹H-coupled HSQC experiment is roughly a half of that of the F1-¹H-coupled HSQC. A similar experiment that starts with the ¹⁵N excitation instead of the ¹H excitation could be more sensitive if the magnetization loss during the coherence transfer from H₂ to NH₂ in the scheme of Fig. 1B is over 90% (=1/3¹H/¹⁵N), which is not the case in the present study; however, such an experiment that starts on ¹³C with NOE enhancement via ¹H saturation should be with acceptable sensitivity for ¹H-¹³C systems [5]. At the beginning of the t₁ period, the observed magnetization is an in-phase single-quantum term N⁺ or N₋ on the phase φ₁. Since there is no ¹H-decoupling during the t₁ period, anti-phase single-quantum terms such as 2N⁺H₁, 4N⁺H₂, and 8N⁺H₃ are generated. The scheme right after the t₁-period (hereafter, referred to as the AP purge scheme; indicated with an arrow in Fig. 1B) kills the 2N⁺H₂ and 8N⁺H₃H₄ terms, so only N⁺ and 4N⁺H₄ terms can survive. The reason for the survival of 4N⁺H₄ is that 4N⁺H₄ generated by ¹H 90° pulses in the AP purge scheme cannot be killed with the pulse field gradient because it is a homonuclear zero-quantum term [6–8]. However, the following scheme for coherence transfers does not allow such zero-quantum terms to become observable magnetizations in the t₂ acquisition period. Therefore, only the in-phase single-quantum term N at the end of the t₁ period is detectable. Since the real part of the overall modulation for the N term in t₁ is given by cos²πf₁t₀cosΩ₁t₀ (n, number of hydrogens), the spectra obtained with this pulse sequence should show 1:3:3:1, 1:2:1, and 1:1 multiplets for NH₂, NH₂, and NH, respectively.

Using the pulse sequences shown in Fig. 1, we recorded 2D ¹H-¹⁵N heteronuclear correlation spectra for NH₂/NH₂ groups in proteins (Figs. 2 and 3). Data were collected with Varian 800- or 750-MHz NMR systems. Fig. 2 displays spectra recorded on the Lys57 NH₂ group of the HoxD9 homeodomain bound to 24-bp DNA. Owing to formation of an ion-pair with a DNA phosphate group, this NH₂ group exhibits relatively slow hydrogen-exchange with water molecules and the ¹H-¹⁵N cross peak from this group can clearly be observed [2]. Just as expected from considerations above, F1-¹H-coupled HSQC (Fig. 2A) and F1-¹H-coupled HSQC (Fig. 2B) exhibits in-phase quartets of 3:1:1:3 and 1:3:3:1 types, respectively. Actual intensity ratios deviate from these numbers.
because the relaxation rates for inner and outer components of the quartet are different due to cross-correlations [2,5,9].

Fig. 3 shows spectra recorded on side-chain NH$_2$ groups of glutamine (Gln) residues in proteins. Panels A, B, and C display spectra recorded on Gln20 NH$_2$ group in 15N-labeled HMGB1 A-domain. The rotational correlation time $\tau_r$ for this protein at 25 °C is 9 ns [10]. The NH$_2$ group exhibited 1:0:1 triplets in the F1-1H-coupled HSQC spectrum (Fig. 3A) and 1:2:1 triplets in the F1-1H-coupled HSQC spectrum (Fig. 3B). The J-coupling was measured to be 89 Hz. For a system with a long $\tau_r$, the relaxation rates of individual triplet components for an AX$_2$ spin system can be quite different because of cross-correlations between distinct relaxation mechanisms [11]. Such a case is clearly seen in the spectra measured on the Gln12 NH$_2$ groups in the $^{15}$N-labeled HoxD9 homeodomain bound to 24-bp DNA at 16 °C (Fig. 3D, E and F). The value of $\tau_r$ is 15 ns for this system. In this case, the downfield components are substantially shaper than the other components in triplets.

Although one may think that removal of $^{1}$H-decoupling from the original HISQC experiment [2] would simply result in 1:3:3:1 and 1:2:1 multiplets, such a pulse sequence (Fig. 1C) does not give the desired multiplets. This occurs because the anti-phase single-quantum terms generated in the $t_1$-period also become $^{1}$H magnetizations detectable in the $t_2$ acquisition period. In fact, the spectra measured with the simplistic pulse sequence on the same NH$_3$ and NH$_2$ groups (Figs. 2C and 3C, F) are very different from those measured with the AP purge scheme (Figs. 2B and 3B, E). Intensity ratios are far from 1:3:3:1 for NH$_3$ and 1:2:1 for NH$_2$; indeed, the multiplets in Fig. 3C and F are more similar to 1:0:1 triplets. In addition, some contributions from the anti-phase terms occur with 90°-shifted phases that cause dispersive distortion of the multiplets, which is evident especially in Fig. 2C. Thus, the AP purge scheme is essential to obtain 1:3:3:1 and 1:2:1 multiplets.

In conclusion, we have demonstrated the 2D F1-1H-coupled $^{1}$H-15N correlation experiment that permits observation of in-phase 1:3:3:1 quartets for NH$_3$ groups and 1:2:1 triplets for NH$_2$ groups along the F1 axis. This experiment provides a means to distinguish AX, AX$_2$, and AX$_3$ spin systems in a straightforward manner. It is particularly useful when $^{1}$H chemical shifts are degenerated. For example, the deprotonated state of an alkyl amine group (NH$_3$) shows a single $^{1}$H resonance because of rapid chiral inversion [12]. In such a case, it is hard to distinguish AX and AX$_2$ spin systems with F1-1H-coupled HISQC unless J-coupling is already known, because a 1:0:1 triplet appears to be a doublet. A 1:2:1 triplet is easier to interpret. It should be noted that a rapid hydrogen exchange with a rate greater than 2/$\tau_r$ can cause the self-decoupling effect that results in a 15N singlet even in absence of $^{1}$H-decoupling. Considering the range of $^{1}$H coupling constants, however, it is likely that such a rapid hydrogen exchange simply broadens the signal beyond the detection limit in the present case, because the hydrogen exchange also increases $^{1}$H transverse relaxation rates. Finally, it should be pointed out that the principle presented here can readily be applied to $^{1}$H-13C systems.

Acknowledgments

This work was supported by Grant H-1683 from Welch foundation (to J.L.) and Grant ES006676 from the National Institute of Environmental Health Sciences.

References