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BEST-HNN and 2D-(HN)**NH** experiments for rapid backbone assignment in proteins

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ABSTRACT

HNN has proven to be an extremely valuable experiment for rapid and unambiguous backbone (H^N, ^{15}N) assignment in (^{13}C, ^{15}N) labeled proteins. However, low sensitivity of the experiment is often a limiting factor, especially when the transverse relaxation times (T_2) are short. We show here that BEST modification Schanda et al. (2006) [2] increases the sensitivity per unit time by more than a factor of 2.0 and thus substantially increases the speed of data collection; good 3D data can be collected in 8–10 h. Next, we present a simple method for amino-acid type identification based on simple 2D versions of the HNN experiment, labeled here as 2D-(HN)**NH**. Each of these experiments which produce anchor points for Gly, Ala, Ser/Thr residues, can be recorded in less than an hour. These enable rapid data acquisition, rapid analysis, and consequently rapid assignment of backbone (H^N, ^{15}N) resonances. The 2D-(HN)**NH** experiment does not involve aliphatic/aromatic protons and hence can be applied to deuterated protein samples as well, which is an additional advantage. The experiments have been demonstrated with human ubiquitin (76 aa) and acetic-acid denatured HIV-1 protease (99 aa), as representatives of folded and unfolded protein systems, respectively.

1. Introduction

The first and the key step of NMR[1] study of a protein is 'backbone assignment'. Getting this information rapidly is a further demand in structural genomics research. With this view methodological developments are occurring continuously, and many new pulse sequences have been published to save on experimental time [1–8]. HNN and HN(C)N experiments [9] published few years ago have proven extremely useful in this regard [9–15]. Compared to the other methods routinely used for backbone assignment, the main strength HNN and HN(C)N experiments [9] lies in the fact that the various planes in the 3D HNN and HN(C)N spectra contain positive and negative peaks. The patterns of these positive and negative peaks, which connect triplets of residues, depend upon the nature of the residues in the triplet sequences [9,11–14]. These are called triplet fixed points along the polypeptide chain. There are variants of the basic experiments [9–11,14], which generate special patterns around glycines, alanines, and serines/threonines. This advantage of HNN and HN(C)N is in addition to the fact that they provide direct correlations in the ^{15}N plane of a particular residue to its neighboring residues (i.e. the amide group of residue i shows correlation with the amide nitrogen of residues $i+1$ and $i-1$). These unique features make the assignment process extremely fast and unambiguous. The utility of these experiments can be enhanced further, if the information contained in them can be extracted faster by (i) enhancing the sensitivity of the experiments, (ii) reducing the time taken to record these spectra and/or (iii) designing 2D variants which can be recorded much faster to achieve the same purpose. The present paper describes our efforts in that direction for the HNN experiment.

We first describe a simple modification incorporating the BEST NMR concept [2,16] which substantially increases the sensitivity per unit time and thus the speed of data collection; good 3D data can be collected in 8–10 h. Next, we present a simple method for amino-acid type identification based on 2D versions of the 3D HNN experiment, labeled here as 2D-(HN)**NH**; this is basically the projection down the F_1 axis of the 3D HNN spectrum. The three variants of the experiment [2D-(HN)**NH-G**, 2D-(HN)**NH-A**, and 2D-(HN)**NH-ST**)] produce sufficient start/anchor points for Gly, Ala, Ser/ Thr residues which in turn will aid the assignment process; each of these can be recorded in less than an hour. Several methods have also been introduced earlier for identifying amino acid types in the NMR spectra [17–24]. These include side chain based methods [17–20], 2D-(HC)**NH** [25], MUSIC sequences [23,24], HADAMAC method [22] and the method proposed by Barnwal et al. [21] which is based on using combination of chemical shifts [21].

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[1] *Abbreviations used:* NMR, nuclear magnetic resonance; HSQC, heteronuclear single quantum correlation; BEST, band-selective excitation short-transient; TE, transfer efficiency.

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However, the main advantage of the method proposed here lies in the fact that 2D-(HN)**NH** experiment is applicable to deuterated proteins as well. Also, its experimental implementation is much simpler.

We envisage that a combination of the two developments described above, namely, strategy based on variants of 2D-(HN)**NH** and BEST modifications thereof, will contribute to several fold enhancement in the speed of backbone assignment. The experimental benefits have been demonstrated with human ubiquitin (76 aa) and acetic-acid denatured HIV-1 protease (99 aa), as representative of folded and unfolded protein systems. The modifications with regard to the HN(C)N pulse sequence, which, in principle, would be very similar to those in HNN will, however, be presented separately for purposes of greater focus and clarity.

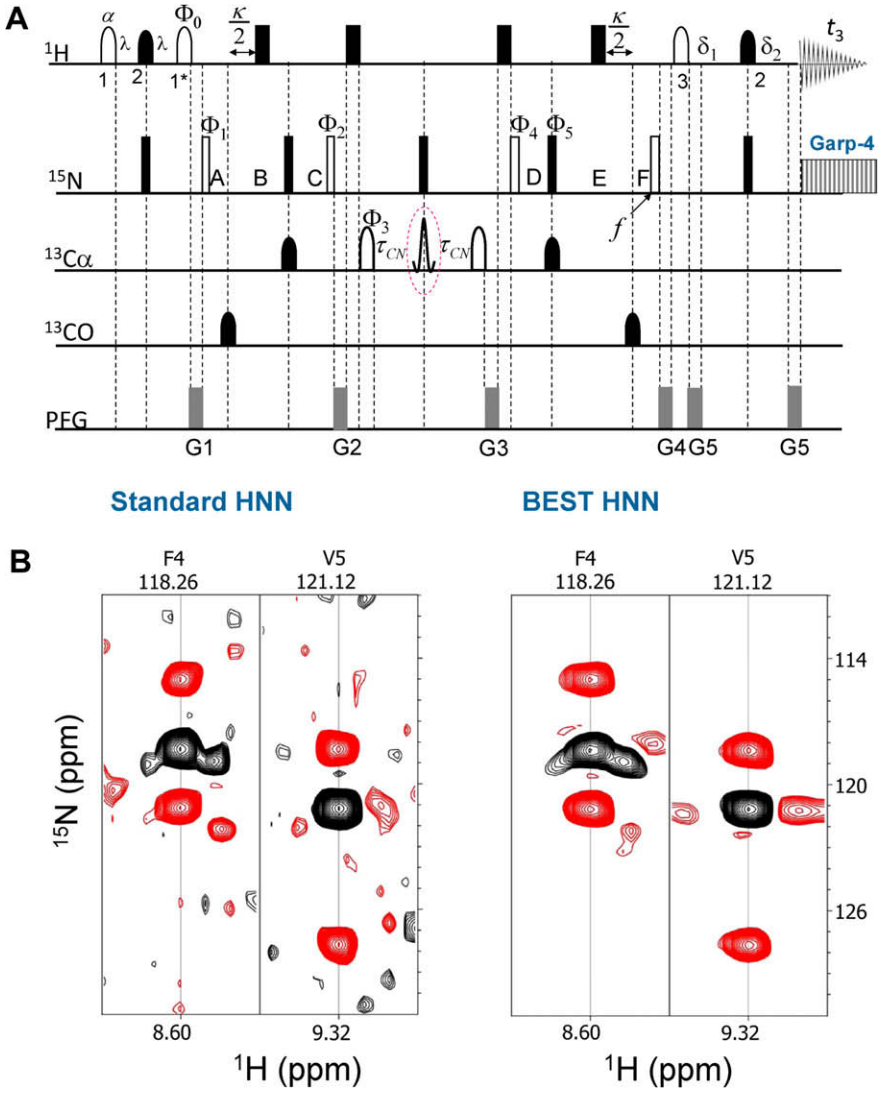

Fig. 1. (A) BEST_HNN pulse sequence. The details of the pulse sequence are the same as in reference [14], but for the following modifications: shaped pulses on proton channel indicate selective ^{1}H pulses, which are centered at 8.2 ppm, covering a bandwidth of 4.0 ppm (~6–10 ppm). The shaped pulses 1, 2, and 3 in the figure represent PC9 [30,34], REBURP [28], and EBURP2 [28], respectively. The pulse labeled 1^* is a flip back pulse obtained by time and phase reversal. The flip angle α of the first pulse has to be optimized and we have used a value of 100°. At 800 MHz, the duration of these pulses are respectively, 2.25, 1.5, and 1.44 ms. Filled rectangular pulses on ^{1}H channel indicate BIP-720-50-20 pulses (200 μs) [35] applied for ^{1}H decoupling during most of the t_1 and t_2 evolution periods, while ^{15}N decoupling during acquisition is obtained using the GARP-4 sequence with the field strength of 0.7 kHz. The ^{13}C carrier frequency for pulses, respectively, on ^{13}Cα and ^{13}CO channels are set at 56.0 and 176.0 ppm, except for the encircled pulse on carbon channel, where it is set as per the HNN variant (see Table 1). The strength of the ^{13}Cα pulses (standard Gaussian cascade Q3 (180°) and Q5 (90°) pulses) is adjusted so that they cause minimal excitation of carbonyl carbons. The band-selective 180° pulse – responsible for tunability of HNN – is encircled for emphasis. The 180° ^{13}CO shaped pulse (width 200 μs) had a standard Gaussian cascade Q3 pulse profile with minimal excitation of $^{13}C^{\alpha}$. For PC9, REBURP, and EBURP pulses, chemical shift and J_{NH}-coupling evolution remains active during ~50% of the pulse length. Thus, the delays used here are set to $\lambda = 2.5 - (2.25 \times 0.5) - (1.5 \times 0.5)$ ms = 625 μs, $\kappa = 5.4$ ms, $\delta_1 = 2.5 - (1.44 \times 0.5) - (1.5 \times 0.5)$ ms = 1.03 ms, and $\delta_1 = 2.5 - (1.5 \times 0.5)$ ms = 1.75 ms. The τ_{CN} must be optimized and is around 12–14 ms. The delays in the constant time periods are: $A = t_1/2$, $B = T_N$, $C = T_N - t_1/2$, $D = T_N - t_2/2$, $E = T_N$, and $F = t_2/2$. The value of T_N is 14 ms. The phase cycles are: $\Phi_1 = 2(x), 2(-x)$; $\Phi_2 = \Phi_3 = x, -x$; $\Phi_4 = 4(x), 4(-x)$; $\Phi_5 = x$, and $\Phi_{receiver} = 2(x), 4(-x), 2(x)$. Frequency discrimination in t_1 and t_2 has been achieved using States-TPPI phase cycling of Φ_1 and Φ_5, respectively, along with the receiver phase. The gradient (smoothed square shaped, SMSQ10.100) levels are as follows: $G_1 = 30\%$ (1 ms), $G_2 = 30\%$ (1 ms), $G_3 = 30\%$ (1 ms), $G_4 = 50\%$ (1 ms), and $G_5 = 80\%$ (500 μs) of the maximum strength of 53 G/cm along the z-direction. (B) $F_1(^{15}N) - F_3(H^N)$ strips of the standard and BEST_HNN spectra for residues F4–V5 in ubiquitin to depict a sensitivity comparison. Both the spectra were recorded on a Bruker 800 MHz spectrometer at 25 °C in the same amount of time (~9 h 1 min) using 512 complex points in the direct dimension, 32 complex points in the indirect dimensions and an inter-scan relaxation delay of 300 ms. The spectra plotted at the same contour levels clearly depict higher sensitivity in BEST_HNN compared to that in the normal HNN. The $F_2(^{15}N)$ values – which help to identify diagonal peaks – and the corresponding residues are indicated at the top for each strip. The black and red contours indicate positive and negative peaks, respectively. The residues corresponding to diagonal peaks in each strip have been shown at the top of each strip. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)