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Communication

DEPT spectral editing in HCCONH-type experiments. Application to fast protein backbone and side chain assignment

Bernhard Brutscher*

Institut de Biologie Structurale—Jean-Pierre Ebel, UMR5075 CNRS-CEA-UJF, 41, rue Jules Horowitz—38027, Grenoble Cedex 1, France

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Abstract

2D DEPT-$H^{\alpha,\beta}C^{\alpha,\beta}$(CO)NH and 2D CT-DEPT-HC(CO)NH-TOCSY experiments are presented which allow fast resonance assignment of aliphatic protein side chains. In these 2D reduced-dimensionality experiments, two or three nuclei are frequency labeled in the indirect dimension. DEPT spectral editing reduces the number of correlation peaks detected in each 2D spectrum, and helps in amino-acid-type determination during sequential backbone resonance assignment. Applications are shown for a small 68-residue, and a highly deuterated 167-residue protein. The new experiments complement the set of 2D HNX correlation experiments, previously proposed for fast protein resonance assignment [J. Biomol. NMR, 27 (2003) 57].

Keywords: NMR; Protein; Reduced dimensionality; GFT; DEPT; Spectral editing; Resonance assignment

Two-dimensional (2D) versions of 'higher-dimensional' correlation experiments have been proposed to reduce the overall acquisition time for recording a set of NMR data [1–8]. In 2D *reduced-dimensionality* [1] NMR experiments, n ($n \geqslant 2$) correlated nuclei are frequency labeled in the indirect dimension t_1. A total of $2^{(n-1)}$ subspectra are created during data processing by a G-matrix transformation [6], where the peak positions along ω_1 are linear combinations of the N chemical shifts. 2D reduced-dimensionality NMR has proven its usefulness for sequential backbone resonance assignment based on a set of uni-directional HNX (X=C^{α}, H^{α}, or C^{β}) correlation experiments [7]. These experiments have in common that the number of peaks detected in each spectrum does not exceed the number of residues in the peptide sequence, which facilitates data analysis. For larger molecules, additional band-selective Hadamard frequency sampling further decreases the number of peaks per spectrum [9], and allows application to a wide range of proteins. It has been shown previously [7,9], that the whole set of 2D experiments required for sequential assignment can be performed in typically less than 1 day using a high-field NMR spectrometer, possibly equipped with a cryogenic probe. This not only paves the way for high-throughput protein NMR investigations, it also makes it possible to study very unstable molecular systems.

Despite some recent advances in NMR structure determination based only on backbone residual dipolar couplings [10,11] or long-range amide ^1H–^1H NOEs [12–14], de novo protein fold determination generally requires at least partial assignment of side chain ^1H and ^{13}C resonances. It is therefore important to develop fast acquisition schemes to extend the backbone assignment to the side chains. Side chain resonance assignment is most easily achieved using NMR experiments correlating aliphatic (or aromatic) side chain ^1H and ^{13}C with the backbone amide ^1H and ^{15}N nuclear spins. The most prominent and sensitive examples of such experiments are $H^{\alpha,\beta}C^{\alpha,\beta}$(CO)NH [15,16], and HC(CO)NH-TOCSY [17,18]. In the first experiment, the C^{α} and C^{β} sites are correlated with the amide of the following residue, whereas in the latter, chemical shift information for the complete aliphatic side chain is obtained. Generally, a set of three-dimensional (3D) spectra is recorded with different frequency labeling, e.g., (H^C, N, H^N) or (C, N, H^N). The major problem for implementation of these

* Corresponding author. Fax: +33-4-38-78-54-94.
E-mail address: Bernhard.Brutscher@ibs.fr.

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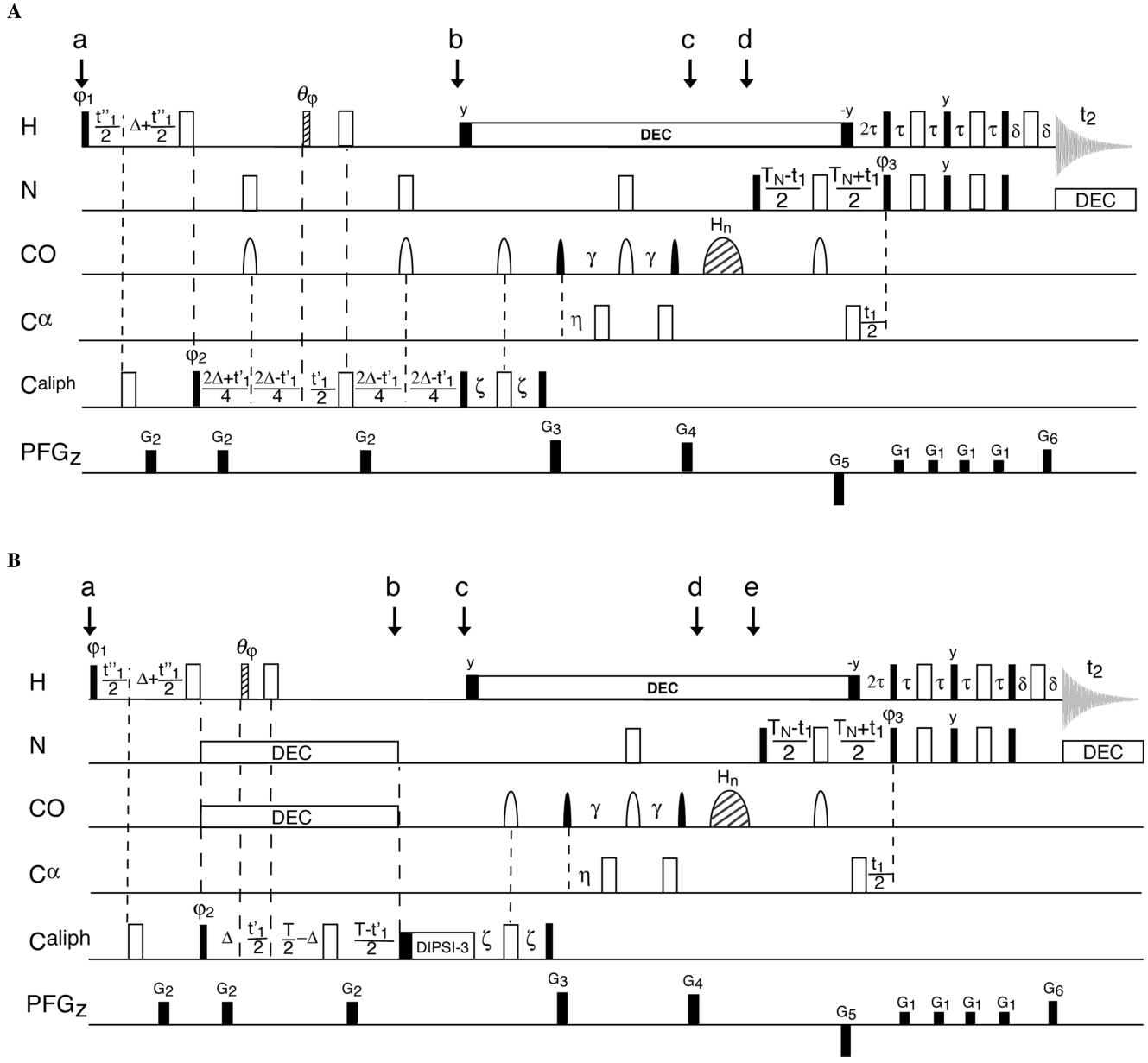

Fig. 1. Pulse sequences for (A) 2D DEPT-$H^{\alpha,\beta}C^{\alpha,\beta}$(CO)NH and (B) 2D CT-DEPT-HC(CO)NH-TOCSY correlation experiments. All radio-frequency (rf) pulses are applied along the x-axis unless indicated. 90° and 180° rf pulses are represented by filled and open pulse symbols, respectively. The dashed ^{1}H pulse corresponds to the DEPT pulse with variable flip angle θ and phase φ [19,20]. The dashed shaped CO pulse allows additional Hadamard-type CO frequency labeling by applying different band-selective CO inversion pulses according to an appropriate Hadamard matrix [9]. All other ^{13}C pulses applied to CO have the shape of the center lobe of a $\sin x/x$ function, whereas C^{α} and C^{aliph} pulses are applied with a rectangular shape and field strength of $\Delta/\sqrt{15}$ (90°) and $\Delta/\sqrt{3}$ (180°), where Δ is the separation in Hz between the centers of the C^{α} (56 ppm) or C^{aliph} (46 ppm) and CO (175 ppm) chemical shift regions. For the methyl-selective CT-DEPT-HC(CO)NH-TOCSY experiment the C^{aliph} carrier is set to 20 ppm, and the ^{1}H carrier to 0.7 ppm between time points (a) and (b). The transfer delays are adjusted to $\Delta = 1/(2J_{CH}) \cong 3.7$ ms, $\zeta = 1/(4J_{C^{\alpha}CO}) \cong 3.1$ ms ($\zeta \cong 4.5$ ms for highly deuterated proteins), $\tau = 1/(4J_{HN}) \cong 2.7$ ms, $\eta = 1/(4J_{C^{\alpha}CO}) \cong 4.5$ ms, $2\gamma = T_N = 1/(2J_{CON}) \cong 28.0$ ms, and $T = 1/(J_{CC}) \cong 28.0$ ms. Isotropic mixing in (B) is performed using 2 or 3 cycles of DIPSI-3 [34]. Pulsed field gradients, G_1, G_2, G_3, G_4, G_5, and G_6 are applied along the z-axis (PFG_z) with a gradient strength of approximately 20 G/cm and lengths ranging from 100 to 2000 μs, followed by a recovery delay of 100 μs. The relative durations of G_5 and G_6 are given by the gyromagnetic ratios of ^{1}H and ^{15}N as $G_5/G_6 = \gamma_H/\gamma_N$. A two-step phase cycle is used with $\varphi = y, -y$ and the receiver phase $\varphi_{rec} = x, -x$. The three nuclei ^{15}N, $^{13}C^{aliph}$, and $^1H^{aliph}$ are frequency labeled along the t_1 time-dimension by setting $t_1' = \lambda_C t_1$ and $t_1'' = \lambda_H t_1$. Two quadrature components are recorded per nucleus using the following phase settings: $\varphi_1 = x, y$ ($^1H^{aliph}$), $\varphi_2 = x, y$ ($^{13}C^{aliph}$), and $\varphi_3 = x, -x$ (^{15}N). In addition, the sign of G_5 is inverted for experiments with $\varphi_3 = -x$. For data processing, first a purely amplitude-modulated data set is created [35]. Then the real and imaginary components of the t_1 time evolution are reconstructed by a G-matrix transformation. The relevant G-matrices are shown as equations S13 and S14 in the Supporting Information of Kim and Szyperski [6]. Pulse sequence codes (Varian) and transformation protocols (Felix) can be obtained from the author upon request.

experiments as 2D reduced-dimensionality versions is the relatively large number of correlation peaks. Here, we propose modified pulse sequences which use additional DEPT spectral editing [19,20] to separate the correlation peaks from CH, CH_2, and CH_3 side chain resonances [21]. This significantly reduces spectral complexity and makes recording of fast 2D reduced-dimensionality spectra an attractive alternative to the standard 3D (or 4D) experiments. DEPT spectral editing also helps in amino-acid type determination, complementing information extracted from ^{13}C chemical shifts [16], which is crucial for sequential resonance assignment.

The pulse sequence for 2D DEPT-$H^{\alpha,\beta}C^{\alpha,\beta}$(CO)NH is shown in Fig. 1A. The major difference with respect to the experiments of Grzesiek and Bax [15,16] is the insertion of a DEPT filter into the initial $H^{\alpha,\beta} \rightarrow C^{\alpha,\beta}$ and $C^{\beta} \rightarrow C^{\alpha}$ transfer steps from time points (a) to (b). DEPT has been chosen because it allows simultaneous polarization transfer, frequency labeling, and spectral editing with a good tolerance of a spread in the J_{CH} coupling constants. The scalar J_{CH} couplings in aliphatic protein side chains cover a range of roughly 120–150 Hz. The DEPT sequence yields signal amplitudes for the different CH_n groups proportional to $\sin(\theta)\cos^{n-1}(\theta)$. This transfer function is identical to INEPT-based sequences with $\theta = \pi J_{CH}\varepsilon$, and ε the J_{CH}-evolution delay as defined by Grzesiek and Bax [15,16]. No additional delays are required for the DEPT filter, which ensures comparable sensitivity to the standard experiments. The only difference is the presence of a multiple-quantum coherence $2C_xH_x$ (DEPT) instead of a single-quantum coherence $2C_xH_z$ (INEPT) during the short transfer delay $\Delta = 3.7$ ms, which does not significantly affect the sensitivity of the experiment. The $^1H^{\alpha,\beta}$ and/or $^{13}C^{\alpha,\beta}$ nuclear spins are frequency labeled together with ^{15}N in t_1. Different scaling factors λ [3,22] can be chosen by setting $t_1' = \lambda_C t_1$ and $t_1'' = \lambda_H t_1$ which allows sensitivity- and resolution-optimized frequency labeling. Two quadrature components are recorded for each of the n (2 or 3) frequency-labeled nuclei (^{15}N, ^1H, ^{13}C) resulting in 2^n FIDs per t_1 increment [4,6–8]. Appropriate transformation of the raw data set results in $2^{(n-1)}$ 2D spectra where the ω_1 frequency positions are different linear combinations of the n chemical shifts: $\omega_1 = \omega_N \pm \lambda_C\omega_C \pm \lambda_H\omega_H$. Additional CO–$H_n$ Hadamard-type frequency editing [23], applying band-selective CO inversion pulses at time point c, can be used, as described elsewhere [9], to further reduce spectral complexity. If the Hadamard dimension is kept small ($n = 2$, 4, or 8), spectra can still be recorded in a short experimental time, as compared to 3D or 4D experiments.

To demonstrate the performance of the new experiment, 2D spectra were recorded on the small protein segment MerAa [24] consisting of the 68 N-terminal residues of the cytosolic mercuric reductase merA from *Ralstonia metallidurans*. All spectra were acquired at 600 MHz ^1H frequency and 25 °C on a 1.5 mM sample of Hg(II)-bound MerAa. We have recently obtained complete backbone resonance assignment for this protein fragment based on a set of 2D reduced-dimensionality spectra recorded in an overall experimental time of less than 1 day [7]. The spectra shown in Figs. 2A and B were recorded using the pulse sequence of Fig. 1A with $t_1' = 0.25\ t_1$ and $t_1'' = 0$. A 2-step DEPT filter was used with the ^1H pulse flip angle set to $\theta = 60°$ and $\theta = 120°$ to separate NMR signals from CH/CH_3 (sum spectrum) and CH_2 groups (difference spectrum). For simplicity, only the sub-spectra with correlation peaks detected at the difference of the two chemical shifts are plotted in Figs. 2A (CH/CH_3) and 2B (CH_2). Additional 2D HN(CO)CA and 2D HN(COCA)CB spectra, recorded on the same protein sample, are shown in Figs. 2C and D, respectively. Acquisition times were adjusted to account for the relative intrinsic sensitivity of the experiments, and set to 30 min for DEPT-($H^{\alpha,\beta}$)$C^{\alpha,\beta}$(CO)NH, 15 min for HN(CO)CA, and 2 h for HN(COCA)CB. The quoted experimental times refer to recording the complete set of 2D spectra. Overall the CH/CH_3 spectrum (Fig. 2A) is similar to the C^{α} spectrum (Fig. 2C), whereas the CH_2 spectrum (Fig. 2B) resembles the C^{β} spectrum (Fig. 2D). The major difference is that some C^{β} resonances are detected in the CH/CH_3 spectrum together with the C^{α} resonances, and that the C^{α} resonances of glycine residues are only detected in the CH_2 spectrum. The relatively small number of correlation peaks in these spectra makes it easy to assemble the peak pairs from the two sub-spectra, and to extract the required ^{13}C chemical shift information. The 'central peak' information is obtained from a standard ^1H–^{15}N HSQC spectrum. Central peak detection is crucial for reduced-dimensionality experiments as it allows to resolve chemical shift overlap in the amide ^1H spectrum, and to recover the sensitivity of the corresponding higher-dimensional experiment [25,26]. Because of its roughly twofold higher sensitivity, recording of a 2D DEPT-($H^{\alpha,\beta}$)$C^{\alpha,\beta}$(CO)NH instead of a 2D HN(COCA)CB experiment reduces the experimental time, required to obtain the sequential C^{β} chemical shift information for MerAa, by about a factor of 4. An interesting additional feature of the DEPT-($H^{\alpha,\beta}$)$C^{\alpha,\beta}$(CO)NH experiment is that it allows discrimination between residues with a CH or CH_3 at the C^{β} position (Val, Ile, Thr, and Ala) and residues with a CH_2 (all others). Together with the information contained in the C^{α} and C^{β} chemical shifts [16], this allows unambiguous residue-type determination at the (i-1) position for amino acids Ala, Gly, Thr, Ser, and the pair Val–Ile. The DEPT-($H^{\alpha,\beta}$)$C^{\alpha,\beta}$(CO)NH experiment thus provides important anchor points for sequential resonance assignment. Note that this feature will also prove useful when running a standard 3D DEPT-($H^{\alpha,\beta}$)$C^{\alpha,\beta}$(CO)NH experiment. The assignment

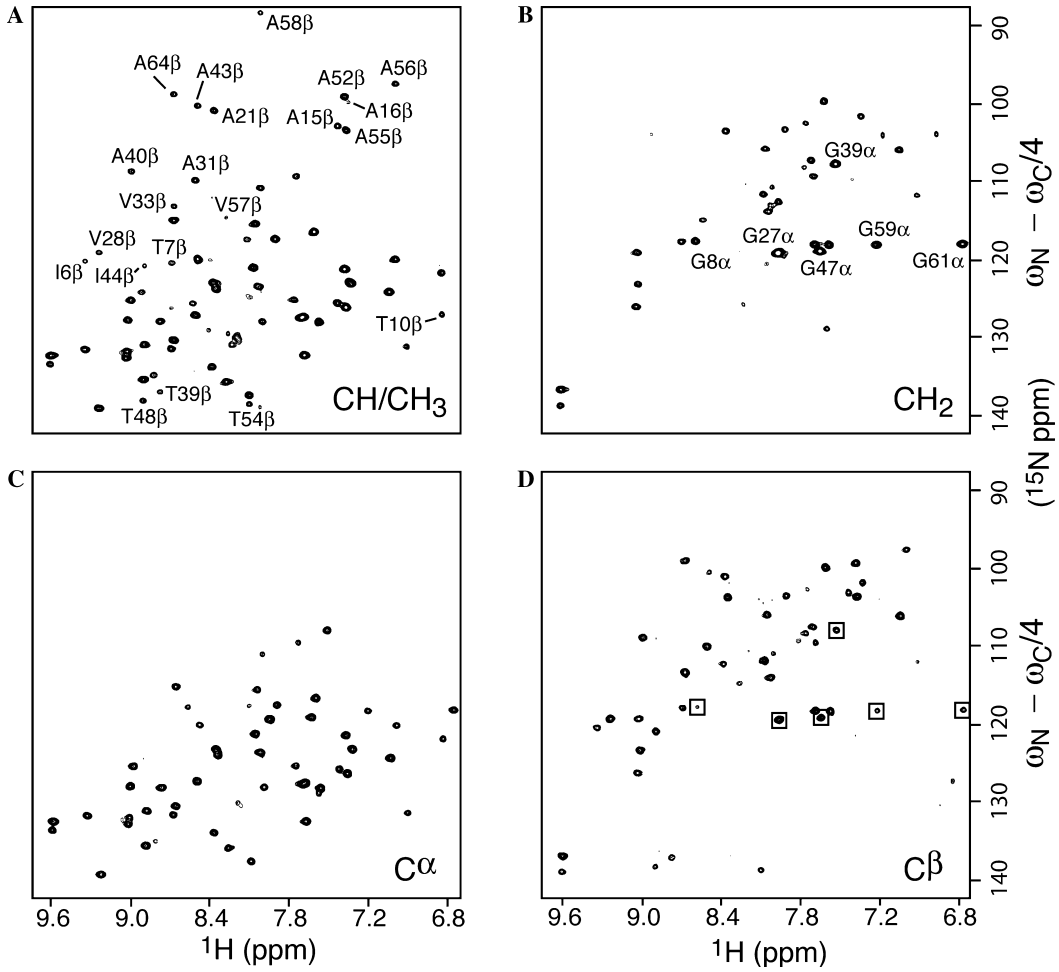

Fig. 2. 2D (C–N)–H^N correlation spectra recorded on a sample of ^{13}C, ^{15}N-labeled Hg(II)-bound MerAa: CH/CH_3 (A) and CH_2 (B) sub-spectra acquired using the 2D DEPT-$(H^{\alpha,\beta})C^{\alpha,\beta}$(CO)NH sequence of Fig. 1A, and 2D HN(CO)CA (C) and HN(COCA)CB spectra recorded with pulse sequences presented previously [7]. The spectra (A) and (B) were recorded with a 2-step DEPT filter ($\theta = 60°, 120°$) and the scaling factors set to $\lambda_H = 0$ and $\lambda_C = 0.25$. All spectra were obtained using identical acquisition and processing parameters, except the total acquisition time which was set to 30 min for DEPT-$(H^{\alpha,\beta})C^{\alpha,\beta}$(CO)NH, 15 min for HN(CO)CA, and 2 h for HN(COCA)CB. Data sets of 512×110 complex points were recorded for spectral widths of 7000 Hz (^1H) $\times$ 4000 Hz (ω_1). Note that the resolution in spectra (A) and (B) may be further increased by mirror-image linear prediction [36]. All correlation peaks arising from C^β sites are annotated in (A), and peaks from C^α are annotated in (B). A box in spectrum (D) indicates a negative cross peak detected for Gly residues.

can be further extended to the $H^{\alpha,\beta}$ protons by performing additional 2D DEPT-$H^{\alpha,\beta}(C^{\alpha,\beta}CO)NH$ or 2D DEPT-$H^{\alpha,\beta}C^{\alpha,\beta}$(CO)NH experiments to record $(H^{\alpha,\beta}–N)–H^N$ or $(H^{\alpha,\beta}–C^{\alpha,\beta}–N)–H^N$ correlation spectra (data not shown).

The HC(CO)NH-TOCSY experiment [17,18] can be modified in a similar way to allow for fast aliphatic side chain ^1H and ^{13}C resonance assignment. The pulse sequence for the new 2D CT-DEPT-HC(CO)NH-TOCSY experiment is shown in Fig. 1B. CT ^{13}C editing was chosen for the high spectral resolution obtained, which can be fully exploited in 2D reduced dimensionality experiments. Furthermore, DEPT filtering is performed during the CT delay. This allows separation of NMR signals from CH, CH_2, and CH_3 groups in different sub-spectra. Again, the ^1H and ^{13}C chemical shifts are labeled together with ^{15}N in the t_1 dimension using different scaling factors λ. In principle, the 2D CT-DEPT-HC(CO)NH-TOCSY experiment provides chemical shift information for the whole side chain. In practice, however, it is especially attractive for methyl resonance assignment. First, because of the favorable relaxation properties of ^{13}C in methyl groups, signal loss during the CT delay of 28 ms is limited, and high signal-to-noise can be obtained for methyl-amide correlations in a short experimental time. Second, only a maximum of two correlation peaks per residue is detected in the 2D reduced-dimensionality spectra, making spectral analysis particularly straightforward. Two peaks are expected for Val, Ile, and Leu, and one peak for Ala and Thr side chains. In addition, selective methyl protonation in a perdeuterated protein has been proposed as a tool for NMR fold determination of larger proteins [27,28]. Again, fast methyl resonance

assignment for such systems may be obtained using the 2D CT-DEPT-HC(CO)NH-TOCSY experiment proposed here. Methyl groups are extremely valuable probes for NMR structure elucidation as they are predominantly located in the hydrophobic protein core. A small set of long-range ^{1}H–^{1}H NOEs involving amide and methyl protons, combined with structural constraints on the protein backbone is often sufficient to accurately determine the three-dimensional fold of the protein [24,29]. Furthermore, methyls are also useful probes for NMR studies of protein side chain dynamics (see for example [30]).

2D CT-DEPT-(H)C(CO)NH-TOCSY and 2D CT-DEPT-HC(CO)NH-TOCSY spectra, recorded on the 68-residue Hg(II)-bound MerAa sample at 600 MHz ^{1}H frequency, are shown in Figs. 3A and B, respectively. DEPT spectral editing was used with the ^{1}H pulse flip angle set to (I) $\theta = 30°$, (II) $\theta = 150°$, and (III) $\theta = 90°$. This particular choice of flip angles allows methyl selection with optimal sensitivity by performing the linear combination (I) + (II) − (III). A part of the 2D (C^{met}–N)–H^N methyl correlation spectra is plotted in Fig. 3A. The scaling factor was set to $\lambda_C = 1.0$. The acquisition time of 2 h was sufficient to detect all of the possible methyl resonances (residues preceding prolines are not detected in these spectra). Complete methyl ^{1}H and ^{13}C resonance assignment was obtained by recording additional 2D (H^{met}–C^{met}–N)–H^N correlation spectra, where the three nuclei H^{met}, C^{met}, and N are frequency-labeled in t_1 with the scaling factors set to $\lambda_C = 1.0$ and $\lambda_H = 0.25$. A total acquisition time of 8 h was chosen to ensure a signal-to-noise ratio for the different sub-spectra comparable to the spectra in Fig. 3A. A small part of 2 (out of 4) sub-spectra is shown in Fig. 3B. A symmetric displacement along the ω_1 axis with respect to the corresponding 'central peak'

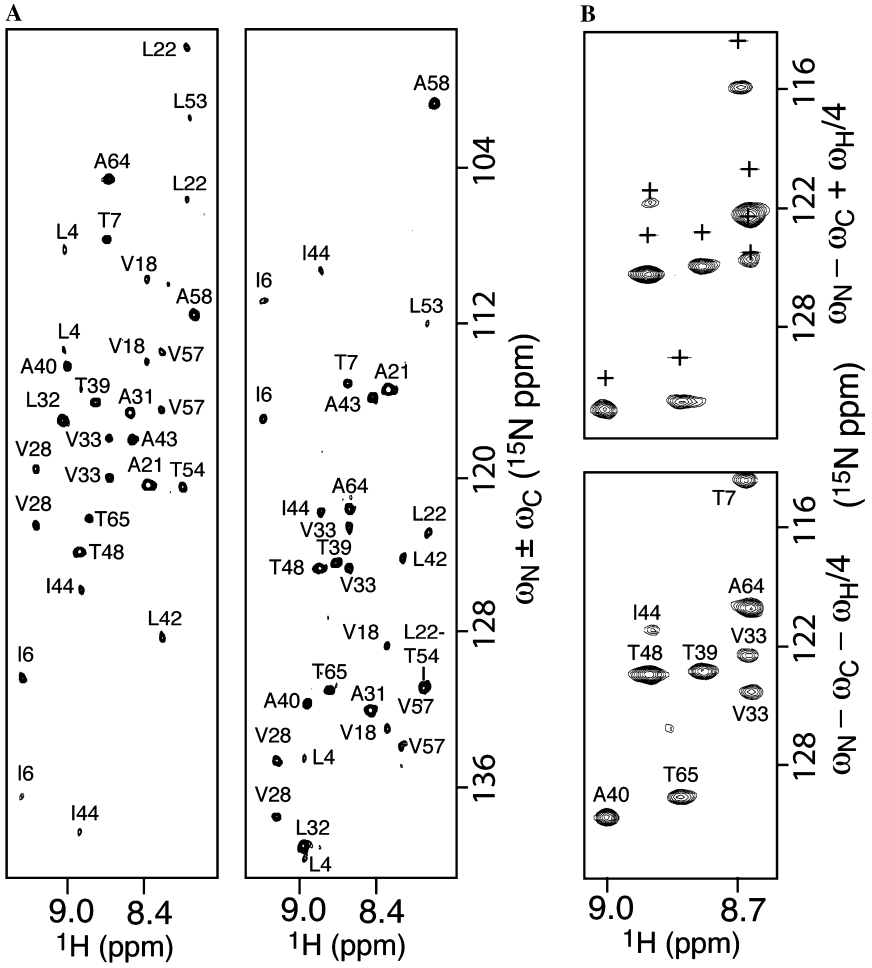

Fig. 3. 2D CT-DEPT-(H)C(CO)NH-TOCSY and 2D CT-DEPT-HC(CO)NH-TOCSY spectra recorded on a sample of ^{13}C, ^{15}N-labeled Hg(II)-bound MerAa using the pulse sequence of Fig. 1B. A 3-step DEPT filter ($\theta = 30°, 150°, 90°$) was used for methyl selection as explained in the text. A part of the (C^{met}–N)–H^N correlation spectra is shown in (A). The scaling factor was set to $\lambda_C = 1.0$. All peaks are annotated by the residue number of the methyl-containing side chain. Spectra acquired with additional H^{met} frequency labeling ($\lambda_H = 0.25$) are shown in (B). Only a small part of 2 out of the 4 sub spectra are shown. The 'central peak' information is obtained from the spectrum on the right in (A). Crosses in the upper spectrum indicate a peak detected in the lower spectrum. For all spectra, data sets of 512×80 complex points were recorded for spectral widths of 7000 Hz (^{1}H) × 3000 Hz (ω_1) in an experimental time of 2 h (A) and 8 h (B). Mirror-image linear prediction [36] was applied prior to Fourier transformation.

in the spectrum of Fig. 3A is observed in the two spectra. This provides the additional ^{1}H chemical shift information for the methyl groups. Note that this information is obtained twice (for the $\omega_N + \omega_C$ and $\omega_N - \omega_C$ 'central peaks') which increases the precision of the ^{1}H chemical shift measurement. The high spectral resolution (narrow lines in ω_1) allows accurate and unambiguous methyl ^{1}H and ^{13}C chemical shift assignment. Contrary to standard methods, this information can be obtained in a short experimental time, 10 h of data acquisition on a standard 600 MHz spectrometer for MerAa. It can be expected that CH_3-selective 2D CT-DEPT-HC(CO)NH-TOCSY will prove equally useful for fast methyl assignment in large perdeuterated proteins, selectively protonated at the methyl positions. For larger proteins, additional Hadamard CO-frequency labeling [9] will help assigning the 2D spectra.

2D CT-DEPT-HC(CO)NH-TOCSY is also important for methyl assignment in proteins where most of the protons are replaced by deuterons. Deuteration levels of 70–80% are usually obtained for protein expression in *Escherichia coli* using a minimal medium containing D_2O and protonated glucose. Such protein samples are commonly used for sequential resonance assignment and for the measurement of residual dipolar couplings in the protein backbone. It has been shown that a protonation level of 20–30% is still sufficient to provide useful structural information on the protein side chains, and especially on methyl groups [31,32]. A complication when dealing with methyls in highly deuterated proteins is the presence of different ^{1}H–^{13}C correlation peaks for each of the three isotopomers CH_3, CH_2D, and CHD_2, separated by a characteristic isotopomer shift [28]. Usually, only the CH_2D, and CHD_2 isotopomers are sufficiently populated to give NMR signals of significant intensity. Spectral multiplicity editing presents a way to increase the resolution and sensitivity of methyl correlation experiments of highly deuterated proteins. This is illustrated for a 1.5 mM ^{13}C, ^{15}N, and 77% ^{2}H-labeled sample of the flavodoxin-like domain of the *E. coli* sulfite reductase (SiR-FP18), a 167-residue protein [33]. 2D CT-DEPT-(H)C(CO)NH-TOCSY experiments were performed at 600 MHz ^{1}H frequency and a sample temperature of 30 °C. Two data sets were recorded (i) with the ^{1}H pulse flip angle of the DEPT filter set to a single value of $\theta = 45°$, and (ii) using a 2-step DEPT filter with $\theta = 45°$ and $\theta = 135°$. For both experiments the overall acquisition time was set to 10 h. The 2D spectrum obtained without spectral editing is shown in Fig. 4A. As expected two peaks are detected for each methyl-amide correlation, corresponding to the CH_2D, and CHD_2 isotopomers. They are separated by a uniform shift of 0.3 ppm along ω_1. The 2-step DEPT filter separates the two peak families in different sub spectra, thus significantly increasing spectral resolution. Shifting the CH_2 with respect to the CH/CH_3 spectrum by an amount of 0.3 ppm along ω_1, and adding them again, yields the spectrum of Fig. 4B. The frequency shift is realized most accurately by a first order phase correction of the time domain data along t_1. Addition of the two sub-spectra yields an increase in signal-to-noise for the methyl-amide correlation peaks, which is on average 30%. Note that resonances other than methyl correlations may be detected in the spectra of Fig. 4, but they are generally of much weaker intensity and easily identified by comparison with the CH_2 sub-spectrum. Only signals arising from methyls and the C^β of serine residues are observed in the CH_2 sub-spectrum (not shown).

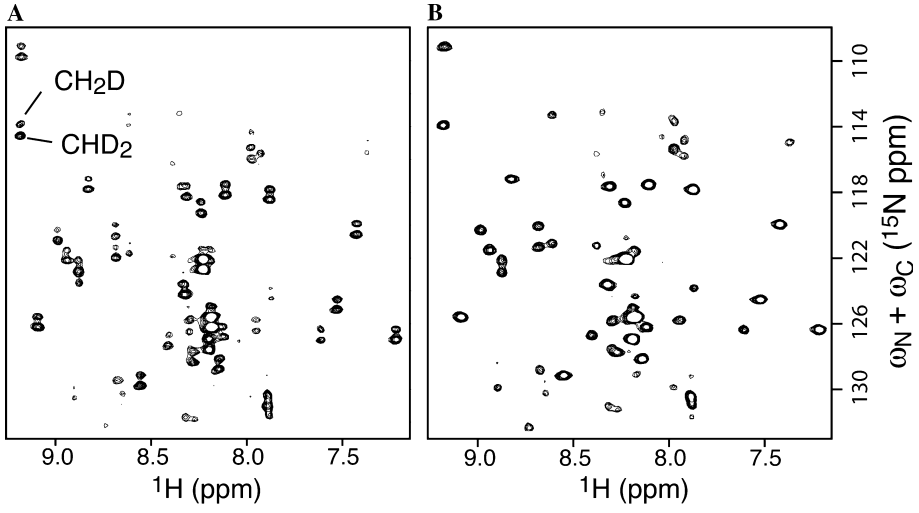

Fig. 4. 2D CT-DEPT-(H)C(CO)NH-TOCSY spectra recorded without (A) and with (B) a 2-step DEPT filter ($\theta = 45°, 135°$) on a sample of ^{13}C, ^{15}N, and 77% ^{2}H-labeled SiR-FP18. The spectra were acquired using the sequence of Fig. 1B with $\lambda_H = 0$ and $\lambda_C = 1.0$. Data sets of 512×80 complex points were recorded for spectral widths of 7000 Hz (^{1}H) $\times$ 3000 mHz (ω_1) in an experimental time of 10 h per experiment. Mirror-image linear prediction [36] was applied prior to Fourier transformation. Spectrum (B) was obtained by adding the CH/CH_3 and CH_2 sub-spectra after applying a relative frequency shift of 0.3 ppm along ω_1.

In conclusion, new DEPT-$H^{\alpha,\beta}C^{\alpha,\beta}$(CO)NH and CT-DEPT-HC(CO)NH-TOCSY experiments have been proposed which allow fast ^{1}H, ^{13}C resonance assignment of aliphatic protein side chains by correlating them with the backbone amide proton and nitrogen. The time advantage with respect to standard experiments is obtained by recording a set of 2D (instead of 3D or 4D) spectra where 2 or 3 nuclei are frequency labeled in the indirect dimension. DEPT spectral editing reduces the number of correlation peaks per spectrum, thus simplifying spectral analysis. It also provides valuable additional amino-acid type information, which is important for sequential resonance assignment. The 2D DEPT-$H^{\alpha,\beta}C^{\alpha,\beta}$(CO)NH and 2D CT-DEPT-HC(CO)NH-TOCSY complement the recently proposed set of uni-directional 2D H–N–X correlation experiments for fast protein resonance assignment [7,9]. It has been shown that for the small 68-residue protein MerAa complete backbone and a large amount of aliphatic side chain assignment (C^{β}, H^{β} and methyls) is obtained from a data set recorded in an overall acquisition time of about 1 day. It has also been demonstrated that the 2D CT-DEPT-HC(CO)NH-TOCSY experiment is useful for methyl side chain assignment in highly deuterated proteins. Because the sensitivity of the experiments can be further increased by the use of a cryogenic probe (or other future technical improvements), it is expected that these experiments will prove very useful for the study of many protein systems.

Acknowledgments

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