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Editing of multidimensional NMR spectra of partially deuterated proteins. Measurement of amide deuterium isotope effects on the chemical shifts of protein backbone nuclei

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Abstract

Novel multidimensional NMR pulse sequences are presented for determination of amide deuterium isotope effects on the ^{13}C chemical shifts of the backbone in proteins. The sequences edit heteronuclear triple resonance spectra into two subspectra according to whether a deuterium or a proton is attached to ^{15}N for the pertinent correlations. The new experiments are demonstrated using ^{13}C,^{15}N-labeled RAP 17-112 (*N*-terminal domain of α_2-macroglobulin receptor associated protein).

That the exchange of a proton with a deuterium has an effect on the chemical shifts of nearby NMR-active nuclei is well established and valuable in conformational and structural studies of small and large molecules (Feeney et al., 1974; Hawkes et al.,1978; Kainosho et al., 1987; Henry et al., 1987; Hansen, 1988; Tüchsen and Hansen, 1991; LeMaster et al. 1994). Although this effect has been known for many years, deuterium isotope effects have not yet been widely used in protein NMR. This situation is, however, starting to change, as there is another most important benefit of partial deuteration, namely that relaxation times can be prolonged to the benefit of sensitivity. Hence many proteins are nowadays prepared with partial deuteration and are available for the study of deuterium isotope effects. Fully deuterated proteins are not useful for the study of deuterium isotope effects, since individual effects cannot be discerned. Thus the requirement is for only partial deuteration, ideally of around 50%.

The problem that arises in this context is one of spectral dispersion. Partial deuteration leads to a large number of different isotopomers that in turn produce a large number of closely spaced and overlapping resonances. Thus it is impossible to measure and assign the small two- and three-bond isotope effects unless the corresponding resonances can be separated by some means. Ottiger and Bax (1997) have proposed to do this in a multidimensional way, taking advantage of large and well-resolved one-bond isotope effects spreading out two classes of overlapping peaks in a way that resembles E.COSY-type cross peak patterns (Griesinger et al. 1985, 1986, 1987). In the terminology of E.COSY, the two-dimensional displacement vectors have the coordinates of the one-bond isotope effect in one dimension and the longer-range isotope effect in the other. The other isotopomers may still overlap, but the resulting line broadening is not a problem for the individual measurement as the one-bond effect allows for unambiguous assignment and determination of the long-range effect.

This communication proposes a novel alternative approach for the separation of resonances from overlapping isotopomers. It maintains the benefits of the approach of Ottiger and Bax but also adds the flexibility of editing into two subspectra the two classes of peaks described above, which effectively doubles

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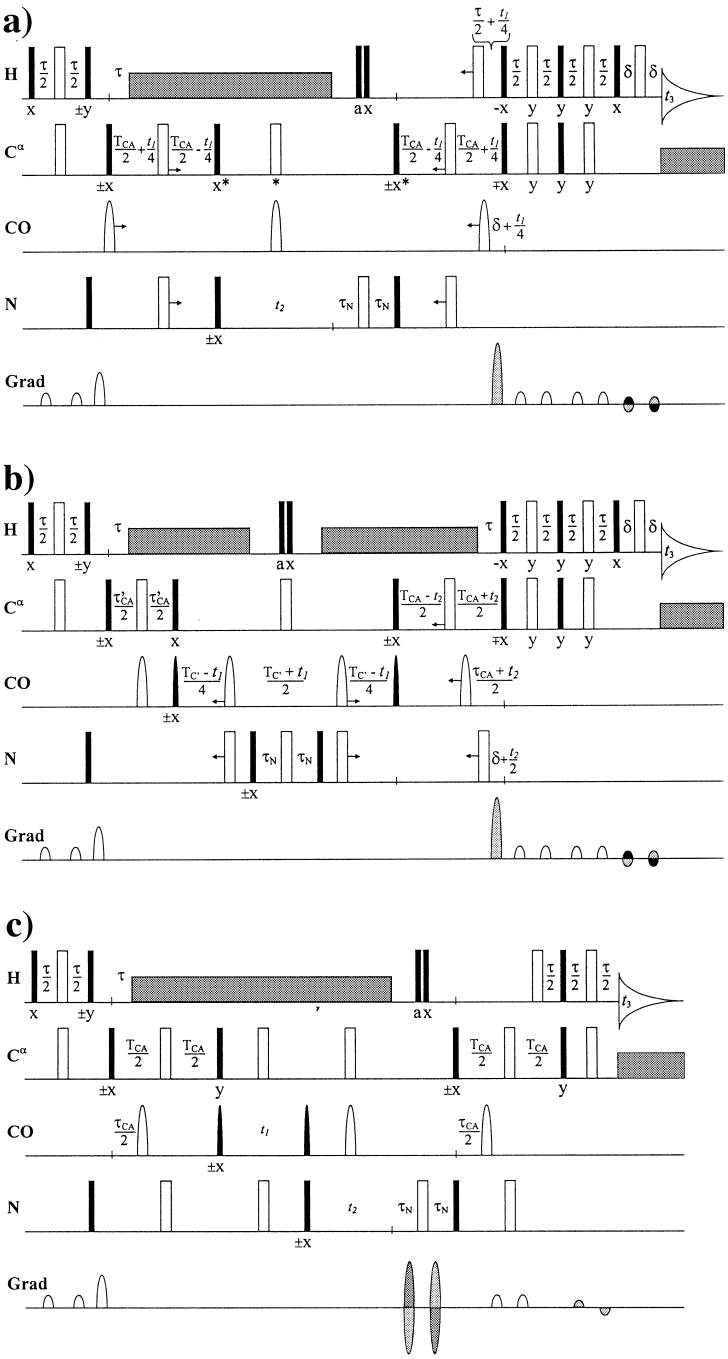

Figure 1. ISP H^N/D^N-editing pulse sequences for determination of the amide deuterium isotope effect on the chemical shifts of C^α and CO in protein backbones. $\tau = (2^1J_{C^\alpha H})^{-1}$, $\tau_N = (2^1J_{NH})^{-1}$, $\tau_{CA} = (2^1J_{C^\alpha CO})^{-1}$, δ gradient delay. Filled and open bars indicate $\pi/2$ and π pulses, respectively, and phases are included below the pulses. Pulse phases with the prefix $\pm$ indicate independent two-step phase cycles with alternating receiver phase, while the $\mp$x pulse is used for echo or antiecho selection in combination with the shaded pulsed field gradients (ratios 8:1 in (a) and (b); 10:1 in (c)). The editing cycle consists of two steps $a = -x$ and x where the resulting two data sets are stored separately and subsequently added and subtracted to yield the two subspectra. The experiments in (a) and (b) employ heteronuclear $ZQ/2Q$ π rotations (Cavanagh et al., 1991; Schulte-Herbrüggen et al., 1991; Kay et al., 1992) for the final magnetization transfer from C^α to H^α. (a) ISP-HCAN for measurement of two- and three-bond deuterium isotope effects on the C^α chemical shifts, $T_{CA} \approx 32$ ms. The three pulse phases marked with an asterisk must be phase cycled for selection of zero change in coherence order in a concerted manner (Sørensen and Ernst, 1983; Madsen and Sørensen, 1992), i.e. an *n*-step phase cycle of increments $2\pi/n$ with $n \geq 3$ and constant receiver phase. (b) ISP-HCACO(N) for measurement of two-bond isotope effects on CO and three-bond effects on C^α, $T_{CA} \approx 28$ or 50 ms, $\tau'_{CA} \approx 7$ ms, $T_{C'} \approx 78$ ms. (c) ISP-H(CA)CO,N for measurement of two- and three-bond isotope effects on the ^{13}CO of the backbone, $T_{CA} \approx 32$ ms. These pulse sequences are not optimized for glycine residues. For glycine the τ delay involved in the T_{CA} and τ'_{CA} periods should be $(4^1J_{C^\alpha H})^{-1}$.

the resolution. Thus it may be possible to 'save' one dimension since it is no longer necessary to have chemical shift evolution of the spin associated with the one-bond isotope effect in order to measure the longer-range effect. Moreover, the editing approach works even when the one-bond isotope effects are unresolved, which would cause the method of Ottiger and Bax to fail. Finally, there is a minor point of the editing approach unequivocally assigning the peaks to the isotopomers, i.e. establishing the sign of the longer-range isotope effect without having to relate it to the sign of the one-bond effect.

Spectral editing techniques are well established in ^{13}C NMR (Pegg et al. 1982; Bildsøe et al. 1983; Sørensen et al. 1983) and these pulse sequence elements are potential candidates for insertion into multidimensional NMR experiments in order to edit the spectra of the occurring isotopomers. In this communication we concentrate on new pulse sequences for measurement of the amide deuterium isotope effects on the chemical shifts of the backbone nuclei. For this purpose it is only necessary to distinguish effective one-spin (^{15}N–D) and two-spin (^{15}N–^{1}H) systems, which can be done in a number of ways. The most general approach for distinguishing these two spin systems is BIG-BIRD (Briand and Sørensen, 1997) which allows for independent selection of rotation angles and phases. Yet a simple spin echo with or without a π evolution of $^{1}J_{\mathrm{NH}}$ suffices for the pulse sequences outlined in Figure 1, which we refer to with the prefix ISP (Isotope Selective Polarization). Addition and subtraction of the data sets with and without J evolution achieve the editing into the two subspectra with ^{15}N–D and ^{15}N-^{1}H spin systems, respectively.

The sequence in Figure 1a is a modification of the HCAN experiment of Ottiger and Bax for measurement of primarily two- but also three-bond amide deuterium isotope effects on the C^{α} chemical shifts. The editing is performed right after the ^{1}H multipulse decoupling element and the t_2 evolution period and necessitates two additional delays of $\tau_{\mathrm{N}} = (2^{1}J_{\mathrm{NH}})^{-1}$. Figure 1b shows a more selective HCACO(N)-type sequence which measures two-bond isotope effects on the carbonyl carbon and three-bond effects on C^{α} and where a frequency dimension with ^{15}N chemical shifts is not required. This selectivity is due to vanishing $^{2}J_{\mathrm{N-CO}}$ coupling constants which prevent the associated two-bond coherence transfers from occurring. In the central element of the pulse sequence the entire delay necessary for isotope editing is exploited for simultaneous constant time evolution of CO chemical shifts with refocusing of J couplings to ^{15}N and $^{13}C^{\alpha}$. Finally, the sequence in Figure 1c primarily measures the three-bond isotope effects on the carbonyl carbons of the backbone. The three pulse sequences together yield all the amide deuterium isotope effects on the backbone nuclei. In the sequences of Figures 1a and 1c, it is advantageous to adjust the delay T_{CA} so as to emphasize magnetization transfer via $^{1}J_{C^{\alpha}N}$ at the expense of $^{2}J_{C^{\alpha}N}$. Assignment of either two- or three-bond effects is straightforward as the spectrum resulting from the sequence in Figure 1b is unambiguous in this respect.

Disregarding relaxation and assuming infinitely short pulses, the intensity functions to maximize during the periods involving transverse ^{13}C magnetization are:

ISP-HCAN (Figure 1a):

$\sin(\pi^{1}J_{C^{\alpha}-N}T_{\mathrm{CA}})\cos(\pi^{1}J_{C^{\alpha}-C^{\beta}}T_{\mathrm{CA}})\cos(\pi^{2}J_{C^{\alpha}-N}T_{\mathrm{CA}})$

ISP-HCACO(N) (Figure 1b):

$\sin(\pi^{1}J_{C^{\alpha}-CO}\tau'_{\mathrm{CA}})\cos(\pi^{1}J_{C^{\alpha}-C^{\beta}}\tau'_{\mathrm{CA}})$,

$\sin(\pi^{1}J_{C^{\alpha}-CO}\tau_{\mathrm{CA}})\cos(\pi^{1}J_{C^{\alpha}-C^{\beta}}T_{\mathrm{CA}})$,

$\sin(\pi^{1}J_{\mathrm{N-CO}}(T_{C'}/2-\tau_{\mathrm{N}}))$

ISP-H(CA)CO,N (Figure 1c):

$\sin(\pi^{1}J_{C^{\alpha}-CO}\tau_{\mathrm{CA}})\sin(\pi^{1}J_{C^{\alpha}-N}T_{\mathrm{CA}})\cos(\pi^{1}J_{C^{\alpha}-C^{\beta}}T_{\mathrm{CA}})\cos(\pi^{2}J_{C^{\alpha}-N}T_{\mathrm{CA}})$

The pulse sequence in Figure 1b is a compromise between two other related versions. If only the deuterium isotope effect on the carbonyl carbon is of interest one can replace the element with T_{CA} by one corresponding to the τ'_{CA} element earlier in the sequence. The opposite is also possible, namely to replace the τ'_{CA} element by a T_{CA} element (with $\delta = 0$), which provides higher resolution in the C^{α} dimension. In this case the C^{α} pulses between the elements must be phase cycled for selection of zero change in coherence order in a concerted manner (Sørensen and Ernst, 1983; Madsen and Sørensen, 1992).

The pulse sequence in Figure 1b was tested on a sample of ^{13}C,^{15}N-RAP 17-112 (N-terminal domain of α_2-macroglobulin receptor associated protein) (Nielsen et al., 1997) in a 50% H_2O/50% D_2O solvent mixture, and the results are shown in Figure 2. The spectra demonstrate that ISP editing offers a very convenient approach for measuring deuterium isotope effects on the chemical shifts of protein backbone nuclei. The isotope effects are easy to measure as the relative peak displacements in the edited subspectra and are amenable to automatic extraction by suitable computer algorithms. Following the work of Ottiger and Bax (1997) correlating amide deuterium isotope effects on

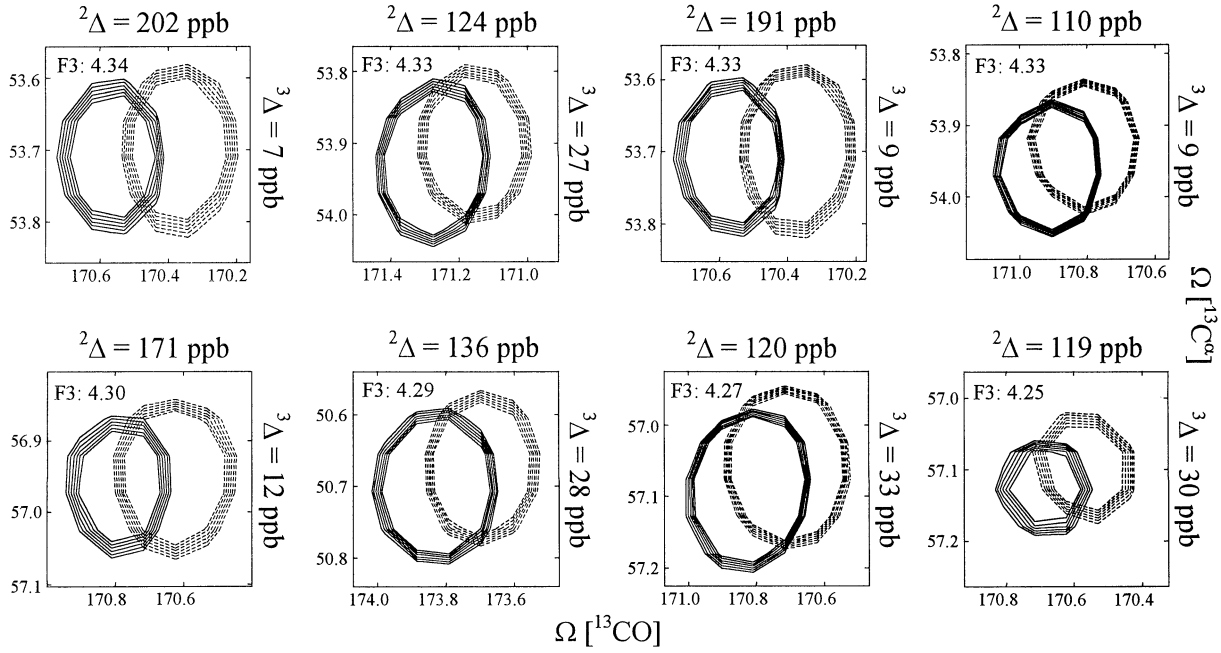

Figure 2. Representative cross peaks from H^N/D^N edited ISP-HCACO(N) spectra of the first domain of $^{13}C,^{15}N$-RAP 17–112 (50%H_2O/50%D_2O, pH 6.4) recorded with the sequence in Figure 1b on a Varian Unity Inova 750 MHz spectrometer. The two edited subspectra corresponding to protonated (full line contours) and deuterated (dashed contours) amide groups ($i + 1$), respectively, have been overlaid using the software package PRONTO (Kjær et al., 1994). Parameters: relaxation delay 1.5 s, t_1(max) = 17.3 ms; t_2(max) = 27.6 ms; 16 scans; $\tau = 3.57$ ms; $\tau'_{CA} = 7.58$ ms; $\tau_N = 5.56$ ms; $T_{C'} = 77.8$ ms; $T_{CA} = 28.6$ ms; $\delta = 1.0$ ms; WALTZ-16 (Shaka et al., 1983) was used for proton decoupling whilst GARP (Shaka et al., 1985) was used for ^{13}C decoupling in t_3. EBURP (Geen and Freeman, 1991) shape was applied for selective CO $\pi/2$ pulses with duration of 596.4 μs and GAUSS (Bauer et al., 1984) shape for selective CO π pulses with a duration of 118.8 μs. A data matrix of 80 × 140 × 2048 points covering 2250 × 2500 × 10000 Hz was zero-filled to 128 × 256 × 2048 prior to Fourier transformation. Cosine square in t_1 and t_2 and 7 Hz exponential line broadening in t_3 was applied. States-TPPI mode was employed in t_1 and echo-antiecho mode in t_2/t_3. The isotope effects were estimated from F_1/F_2 2D sections with a precision of about ± 3 ppb. The determined $^2\Delta$(CO) and $^3\Delta(C^{\alpha})$ isotope effects are indicated next to the boxes, while the chemical shifts of H^{α} in F_3 is given in the upper left corners. The NMR spectra of RAP 17–112 have not been assigned as yet.

$^{13}C^{\alpha}$ chemical shifts with the ϕ and ψ angles our new ISP pulse sequences are expected to stimulate further empirical studies of this type and the results should be useful in protein backbone structure refinements. ^{13}C- and ^{15}N-labeled material without partial deuteration normally prepared for protein structure determinations can be used for the ISP experiments, since the only requirement is to equilibrate the protein sample in a solution with about a 50/50 distribution of H_2O/D_2O.

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