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Multiplicity-Selective Coherence Transfer Steps for the Design of Amino Acid-Selective Experiments—A Triple-Resonance Experiment Selective for Asn and Gln

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A multiplicity-selective coherence transfer step is discussed, that can replace the normal INEPT transfer in triple-resonance experiments. Depending on the pulse sequence in which they are implemented, amino acid-selective experiments will be created. Two experiments selective for Asn and Gln are proposed. © 1998 Academic Press

Key Words: **triple-resonance, NMR assignment, amino acid-selective experiments**

The determination of the three-dimensional structures of small proteins by NMR has become a routine matter. This is largely due to the event of the so-called triple-resonance experiments (*1, 2*) which facilitate unbiased sequence-specific resonance assignments for biological systems with a molecular weight of less than 40 kDa. Due to the high sensitivity of these experiments and their intrinsic resolution they are supposed to be ideally suited as a basis for an automation of the assignment procedure. Accordingly, a number of attempts to achieve semiautomated assignment have been reported (*3*), but experience shows that a fully computerized process seems to require a high amount of specificity and redundancy in the data set used.

It is therefore desirable to supplement the data with additional information, e.g., on the resonance positions of certain types of amino acids. One way of generating such amino acid-selective information is to exploit specific coherence transfer pathways that are unique for certain amino acids. In this context, several schemes for amino acid-selective experiments have already been proposed. Proline and glycine can be distinguished by their unique backbone pattern (*4–6*). Arginine can be selected utilizing the fact that the arginine-N^{ϵ} is separated in frequency to selectively exite arginine side-chain resonances (*7–9*). Aspartic acid and glutamic acid can be selected based on the fact that the carbonyl does not couple to a nitrogen (*10*). Asparagine and glutamine have been selected by selecting NH_2 next to a carbonyl (*11*). Aromatic amino acids can be selected due to their special side chains (*12, 13*). Other sequences are based on the number of carbon–carbon couplings (*14–17*) or the multiplicity (i.e., the number of protons attached to a heteronucleus) of the carbon atom (*15, 16, 18*).

In this paper, we discuss multiplicity-selective coherence transfer steps that can replace the normal INEPT transfer in most triple-resonance experiments. The new transfer steps will accomplish an in-phase transfer of magnetization for either $X\,H_2$ or $X\,H_3$, while other multiplicities will be suppressed (X can be either nitrogen or carbon). Since all triple-resonance experiments begin and end with a coherence transfer from and to protons, the sequences can be implemented in a variety of techniques. Depending on the pulse sequence in which they are implemented, specific patterns will be selected, creating an amino acid-selective pulse sequence. An advantage of the new sequences is the superior selection that can be obtained by separating coherence orders rather than adjusting delays to the size of coupling constants. This will be an important consideration for systems with varying mobility.

The new coherence transfer sequences are shown in Fig. 1. They are implemented in a refocused HSQC described by Kay *et al.* (*19*) (Fig. 1a) as the simplest pulse sequence with proton detection where an in-phase coherence transfer can be incorporated. Two types of sequences can be used, depending on whether the filtered transfer is to or from the heteronucleus (Figs. 1b and 1c, respectively). Both consist of three delays Δ of $1/2(^1J_{HX})$ interrupted by two groups of pulses and are related to the POMMIE sequences (*20, 21*).

The ''down'' sequence (Fig. 1b) starts with the preparation of proton magnetization, which is converted into heteronuclear zero- and double-quantum coherence after a period of $1/2(^1J_{HX})$ by the heteropulse. During a second delay $1/2(^1J_{HX})$ the one-bond coupling between the remaining protons coupled to the same heteronucleus is active. The following 90° pulse creates heteronuclear triple-quantum coherence for $X\,H_2$ and heteronuclear quadruple-quantum coherence for $X\,H_3$, which can be selected by phase cycling of the next 90° pulse. The key

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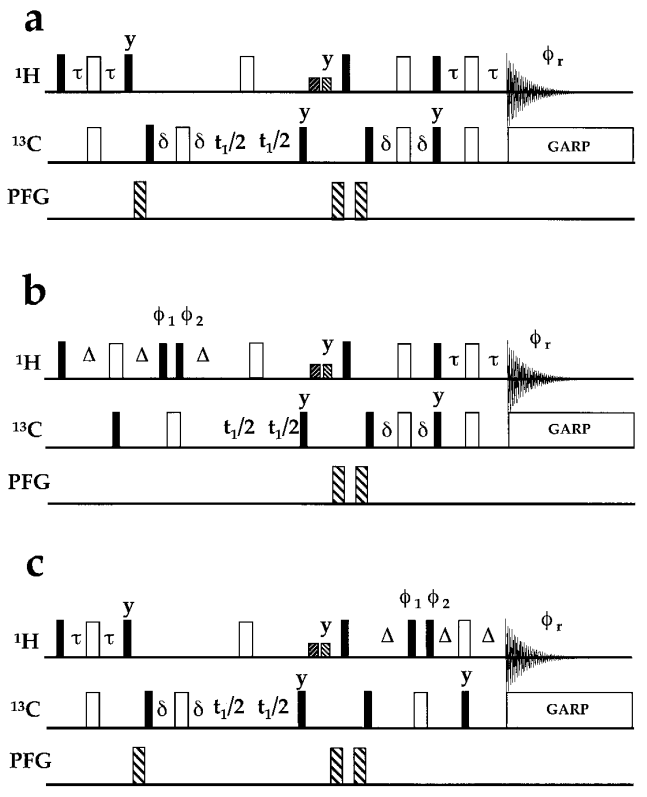

FIG. 1. Pulse sequences of the multiplicity-selective coherence transfer steps implemented in an HSQC sequence. (a) HSQC sequence with water suppression using trim pulses and gradients (*19*). (b) The ''down'' sequence for multiplicity-selective transfer from proton to the heteronucleus replaces the first INEPT step in sequence (a). For selection of $X\mathrm{H}_2$ the phase cycle is $\phi_1 = 0°$; $\phi_2 = 45°, 135°, 225°, 315°$; $\phi_r = 0°, 180°$; for selection of $X\mathrm{H}_3$ the phase cycle is $\phi_1 = 0°$; $\phi_2 = 30°, 90°, 150°, 210°, 270°, 330°$; $\phi_r = 0°, 180°$. (c) The ''up'' sequence for multiplicity-selective transfer from the heteronucleus to protons replaces the second INEPT step in sequence (a). For selection of $X\mathrm{H}_2$ the phase cycle is $\phi_1 = 45°$; $\phi_2 = 0°, 90°, 180°, 270°$; $\phi_r = 0°, 90°, 180°, 270°$; for selection of $X\mathrm{H}_3$ the phase cycle is $\phi_1 = 30°$; $\phi_2 = 0°, 60°, 120°, 180°, 240°, 300°$; $\phi_r = 0°, 120°, 240°$.

feature is that the phase difference between these two 90° pulses is not 0° or 90° but rather 45° for the selection of $X\mathrm{H}_2$ and 30° for the selection of $X\mathrm{H}_3$. This allows for the creation of magnetization with all protons in antiphase with respect to the heteronucleus, which can then be refocused into single-quantum coherence of the heteronucleus in the final delay $1/2({}^1J_{HX})$.

The ''up'' sequence starts out on the heteronucleus. During the first delay $1/2({}^1J_{HX})$ all protons coupled to the heteronucleus via a one-bond coupling will end up in antiphase. A proton 90° pulse creates different types of heteronuclear multiple-quantum coherence for $X\mathrm{H}_2$ and $X\mathrm{H}_3$. Again the phase of this pulse is not 0° or 90° with respect to the second. This ensures the creation of magnetization that can be converted in heteronuclear zero- and double-quantum coherence, which will be refocused to proton single-quantum coherence in the usual manner.

Note that contrary to homonuclear multiple-quantum coherences the creation of these heteronuclear multiple-quantum coherences can be achieved quite efficiently via the large proton–carbon one-bond coupling. A loss of sensitivity will occur, however, since several types of multiple-quantum coherences are created, of which only some are selected by phase cycling; e.g., the second proton 90° pulse in the down sequence creates proton double- and zero-quantum coherences, of which only the double-quantum coherences are selected.

The sequences shown in Fig. 1 were tested on a sample of the GFL peptide (*22*) with carbon as the heteronucleus. The result is shown in Fig. 2 as one-dimensional versions of the experiments. Figure 2a shows the spectrum of sequence 1a, exhibiting all resonances of the three labeled amino acids. Figures 2b and 2c show the two versions of CH_2 selection. The resonances of Phe and Leu H_α have disappeared as well as the Leu H_γ. The methyl groups of Leu are still present with reduced intensity, since they can form heteronuclear triple-quantum coherences as well. Their intensity can be further reduced by optimization of the delay Δ in order to minimize the amount of those coherences. A complete suppression of methyl groups, however, will be difficult to achieve. For many of the triple-resonance experiments this is not important, since methyl groups are not present along the backbone of proteins and their resonances will be removed by the conventional phase cycle. Figures 2d and 2e show the selection of CH_3; no leakage of other multiplicities is possible, since only CH_3 can form heteronuclear quadruple-quantum coherence.

As first examples for the implementation of the new sequences in a triple-resonance experiment we chose the selection of NH_2 groups at the end of the CBCA(CO)NNH (*23*) and the HBHA(CBCACO)NNH (*24*) experiments. Since only Asn and Gln have an NH_2 next to a carbonyl group, these modified experiments will be selective for those amino acids. Asn and Gln are then easily distinguished based on the different chemical shift patterns. These experiments will be extremely helpful in distinguishing Asn from other amino acids with AMXY spin systems, and Gln from Met and Glu.

The modified CBCA(CO)NNH and HBHA(CBCACO)NNH sequences were applied to a sample of the SH3 domain of spectrin. The spectrum recorded with the modified CBCA(CO)NNH sequence (Fig. 3a) contains the C_γ and C_β of Gln and the C_β and C_α of Asn. The spectrum recorded with the modified HBHA(CBCACO)NNH sequence (Fig. 3b) then contains the H_γ and H_β of Gln and the H_β and H_α of Asn. No other residues appear in the spectrum and the identification of the two Gln and the three Asn is straightforward.

The transfer steps presented here should be of use as a building block in many pulse sequences in order to select for certain spin topologies. The modification of HCACO-type experiments, for example, in a similar way as presented

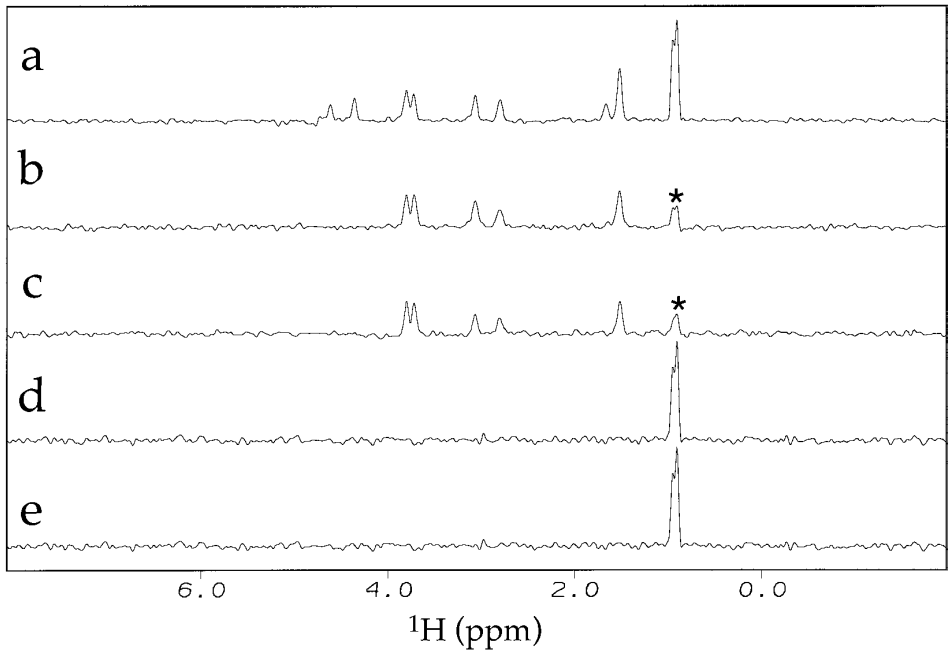

FIG. 2. Application of the pulse sequences shown in Fig. 1 to the GFL peptide. (a) Normal HSQC sequence (1a); all eight signals are visible. (b, c) Selection of CH_2 with the up and down sequence, respectively; the three CH signals have disappeared, and the two methyl group signals are weaker but not completely suppressed. (d, e) Selection of CH_3 with the up and down sequence, respectively; only the methyl groups are visible. The experiments were executed with the minimum number of scans necessary for the editing phase cycle combined with a two-step phase cycle on a heteronuclear pulse: 8 scans for (b) and (c), 12 scans for (d) and (e). The reference experiment in (a) was executed with 8 scans.

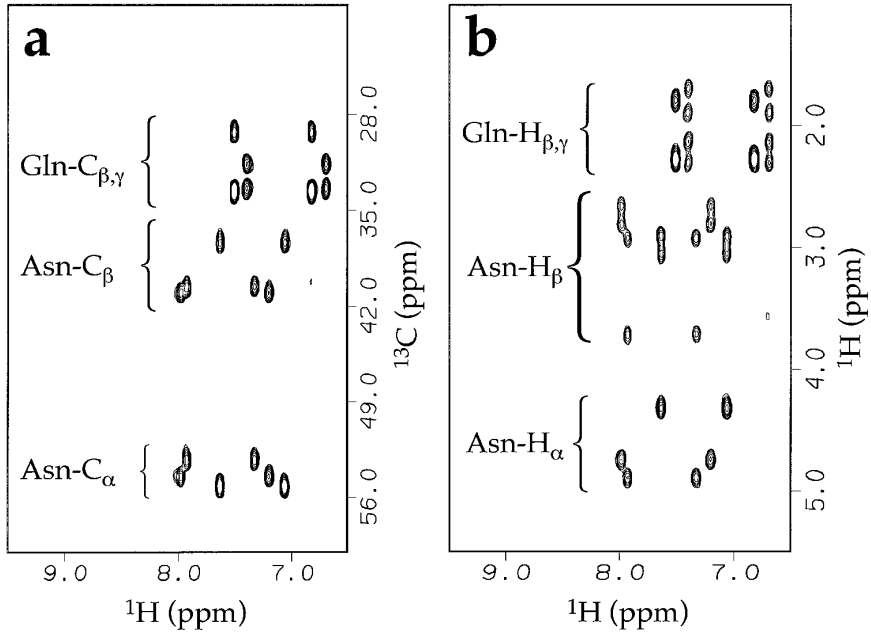

FIG. 3. Application of the selection of NH_2 at the end of CBCA(CO)NNH (a) and HBHA(CBCACO)NNH (b). The protein contains two Gln and three Asn residues; the β and γ resonances of Gln and the α and β resonances of Asn can be clearly identified from the spectrum. No other residues appear in the spectrum. The spectra were recorded on a Bruker DRX600 spectrometer using a 5-mm triple-resonance probe equipped with three-axis self-shielded gradient coils. The sample was a SH3 domain from spectrin, labeled with ^{15}N and ^{13}C, the concentration was 0.8 mmol, and the spectra were recorded at 300 K. The sequences were implemented as described in the literature, except that the final INEPT transfer was replaced by the up sequence as shown in Fig. 1. The same water-suppression scheme as in Fig. 1 was used for both sequences. HBHA(CBCACO)NNH was recorded with ''shared incrementation time'' for the indirect dimension. 128 FIDs were recorded in each experiment with 128 scans each. Standard processing was applied to the spectra.

here will yield the other Asx and Glx residues together with glycine. The new sequences can also be used to restrict the spectral width in indirect dimensions of multidimensional spectra, since the selection of multiplicity usually corresponds to a restriction of the spectral width. While we are focusing on the application to triple-resonance experiments of double-labeled proteins, it should be noted that the sequences can also be useful with samples at natural abundance.

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