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Article

Spectral editing: selection of methyl groups in multidimensional solid-state magic-angle spinning NMR

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Abstract

A simple spectroscopic filtering technique is presented that may aid the assignment of ^{13}C and ^{15}N resonances of methyl-containing amino-acids in solid-state magic-angle spinning (MAS) NMR. A filtering block that selects methyl resonances is introduced in two-dimensional (2D) ^{13}C-homonuclear and ^{15}N–^{13}C heteronuclear correlation experiments. The 2D ^{13}C–^{13}C correlation spectra are recorded with the methyl filter implemented prior to a ^{13}C–^{13}C mixing step. It is shown that these methyl-filtered ^{13}C-homonuclear correlation spectra are instrumental in the assignment of C_δ resonances of leucines by suppression of C_γ–C_δ cross peaks. Further, a methyl filter is implemented prior to a ^{15}N–^{13}C transferred-echo double resonance (TEDOR) exchange scheme to obtain 2D ^{15}N–^{13}C heteronuclear correlation spectra. These experiments provide correlations between methyl groups and backbone amides. Some of the observed sequential ^{15}N–^{13}C correlations form the basis for initial sequence-specific assignments of backbone signals of the outer-membrane protein G.

Abbreviations: 2D – two-dimensional; CP – cross-polarization; CPPI – cross-polarization with polarization inversion; DARR – dipolar-assisted rotational resonance; MAS – magic-angle spinning; NMR – nuclear magnetic resonance; OmpG – outer-membrane protein G; PI – polarization inversion; REDOR – rotational-echo double resonance; TEDOR – transferred-echo double resonance; TPPM – two-pulse phase modulation

Introduction

Solid-state MAS NMR has evolved into a method that allows structure investigations of biomacromolecules (Castellani et al., 2002, 2003; Ritter et al., 2005; Zech et al., 2005; Lange et al., 2006). The study of membrane proteins forms a challenging field in structural biology, and several approaches to obtain structural information using solid-state NMR have been presented during the last decades (Nicholson and Cross, 1989; Watts et al., 1995; Glaubitz and Watts, 1998; Marassi and Opella, 1998; Hong and Jakes, 1999; de Groot, 2000; Hughes and Middleton, 2003; Nielsen et al., 2004; Kamihira et al., 2005).

Membrane proteins are mostly of high molecular weight and show very crowded NMR spectra, which can make it difficult to achieve complete resonance assignments (Hiller et al., 2005). Moreover, membrane proteins may suffer from inherent

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structural inhomogeneity, resulting in reduced resolution. Therefore, it is desirable to develop methods that allow for simplification of NMR spectra. One way to alleviate spectral crowding and to achieve additional resolution enhancement can be accomplished by spectral editing, *i.e.*, by selecting the resonances of specific moieties by virtue of their unique chemical, spectral and/or conformational properties. Selective detection of methyl groups, for instance, may ease assignments and provide useful structural information, since contacts involving methyl groups form major parameters for structure calculations. Furthermore, methyl resonances are good monitors for ligand binding as demonstrated by Zech et al. (2004), who investigated changes in the chemical shifts of methyl resonances of Bcl-xL induced by ligand binding.

Opella and Frey (1979) demonstrated that basic spectral editing for solid-state NMR can be achieved using dipolar dephasing, in order to distinguish chemically-different carbons on the basis of the number of attached protons. Likewise, it was shown that cross-polarization with polarization inversion (CPPI) can be applied to select between carbons with different multiplicities. (Wu and Zilm, 1993a, b; Sangill et al., 1994; Wu et al., 1994; Burns et al., 2000). Other techniques using ^{1}H double-quantum filtering (Rossi et al., 1999) and chemical shift anisotropy dephasing (Peng and Frydman, 1995) have been suggested. A different approach for spectral editing has been proposed by Emsley and coworkers, that relies on scalar couplings and multiple-quantum filtering to distinguish carbons with different proton multiplicities (Lesage et al., 1998; Sakellariou et al., 2001). The advantage of the latter approach is that the filtering is independent of molecular dynamics. On the other hand, long delays for evolution of *J*-couplings and the use of unlabelled material may limit the sensitivity of the experiment.

In this paper, we continue along these lines, presenting a simple but robust method that combines CPPI and dipolar dephasing in a double filtering step, to allow filtering of methyl resonances. We demonstrate that this methyl-filtering technique can be applied to remove the overlap between C_δ–C_δ and C_γ–C_δ cross peaks of leucine residues. Moreover, we show that the double filter implemented into a ^{15}N–^{13}C TEDOR (Jaroniec et al., 2001, 2002) correlation experiment, enables straightforward detection of correlations between backbone nitrogens and CH_3 groups. The pulse sequences have been applied to the α-spectrin SH3 domain and outer-membrane protein G (OmpG), to achieve assignments of leucine resonances and sequential connectivities via methyl filtering.

Material and methods

Samples of the α-spectrin SH3 domain (Pauli et al., 2000) and OmpG (Hiller et al., 2005) were prepared as described previously. The α-spectrin SH3 domain sample was uniformly labelled with ^{13}C and ^{15}N, and is referred to as [U-^{13}C,^{15}N] SH3. The OmpG sample (in following termed as '1,3-OmpG') was expressed using [1,3-^{13}C]-glycerol (2 g/l culture) as the carbon source and ^{15}N-NH_4Cl (0.5 g/l culture) as the nitrogen source. For the solid-state cross-polarization (CP) MAS NMR correlation experiments, samples containing typically ~1.4μmol (10 mg) [U-^{13}C,^{15}N] SH3 domain and ~0.6μmol (20 mg) 1,3-OmpG were used.

All solid-state NMR spectra were recorded at a MAS frequency $\omega_R/2\pi = 8.0$ kHz. The 2D ^{13}C–^{13}C and ^{13}C–^{15}N dipolar correlation experiments (Figure 1) were acquired at 280 K, at a field of 9.4 T on a wide-bore Avance-400 spectrometer (Bruker, Karlsruhe, Germany). The spectrometer was equipped with a 4 mm triple-resonance CP/MAS probe (Bruker, Karlsruhe, Germany). For the sequences shown in Figure 1, a ramped CP (75–100%) from ^{1}H to ^{13}C was applied, followed by a squared phase-inversion CP at the $n = 1$ Hartmann–Hahn condition. This polarization inversion (PI) period cancelled the CH-signals, whereas CH_2 groups were inverted to approximately $-1/3$ of their initial intensity. We determined the PI time experimentally and found that 50μs lead to the desired signal inversion. Following PI, the CH_2 and residual CH signals were zeroed by applying a short (60μs) dipolar-dephasing period. The methyl (and non-protonated) carbons were weaker coupled to ^{1}H and were not dephased. The carbon chemical shift evolution was refocused by a π–pulse in the middle of the dephasing period.

For the filtered ^{13}C–^{13}C homonuclear correlation experiment on SH3, a 70 ms dipolar-assisted rotational resonance (DARR) (Takegoshi et al., 2001) mixing period was used. For the ^{13}C–^{15}N

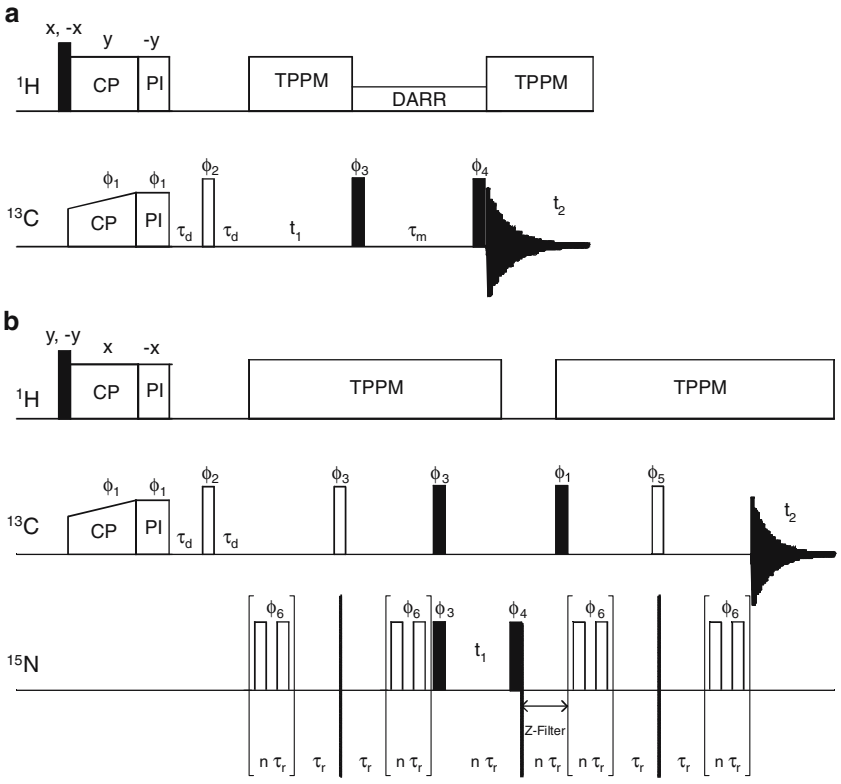

Figure 1. Pulse sequences for ^{13}C-homonuclear (a) and ^{15}N–^{13}C heteronuclear (b) dipolar correlation experiments used in this work. In both sequences, polarization was first transferred from protons to carbons using a ramped cross-polarization (CP). In a next step, cross-polarization with polarization inversion (CPPI) (Burns et al., 2000), in combination with dipolar dephasing (Opella et al., 1979; Opella and Frey, 1979) was used to select methyl groups. In (a), the filter was followed by ^{13}C evolution during t_1 and a dipolar-assisted rotational resonance (DARR) mixing scheme (Takegoshi et al., 2001). In (b), the filtering block was followed by a rotational-echo double resonance (REDOR) mixing scheme that sandwiched the evolution of ^{15}N in t_1. During t_1-evolution, REDOR mixing and data acquisition, a two-pulse phase modulation (TPPM) scheme was applied to decouple the protons (Bennett et al., 1995). The following phase cycle was applied (a): $\varphi1$ = x, − x, y, − y, − x, x, − y, y, $\varphi2$ = y, y, − x, − x, − y, − y, x, x, $\varphi3$ = y, y, x, x, − y, − y, − x, − x, $\varphi4$ = − y, − y, − x, − x, y, y, x, x, φrec = x, x, y, y, − x, − x, − y, − y, (b): $\varphi1$ = − x, − x, − y, − y, x, x, y, y, $\varphi2$ = y, − y, x, − x, $\varphi3$ = x, x, y, y, − x, − x, − y, − y, $\varphi4$ = − x, − x, − y, − y, x, x, y, y, x, x, y, y, − x, − x, − y, − y, $\varphi5$ = y, − y, − x, x, − y, y, x, − x, φrec = x, − x, y, − y, − x, x, − y, y, − x, x, − y, y, x, − x, y, − y, $\varphi6$ was cycled according to the XY16 sheme (Gullion et al., 1990).

exchange, a TEDOR (Jaroniec et al., 2001, 2002) transfer scheme was introduced after selection of methyl carbons by CPPI and dipolar dephasing. REDOR (Gullion and Schaefer, 1989) mixing times of 2, 3, 6 or 8 ms were applied in these studies, to achieve transfer over distances from ~2.5 Å (short mixing time) up to about 5 Å for the longest mixing times[1]. Typical ^{15}N π–pulse lengths during the REDOR mixing were 12μs for the SH3 domain and 13.3μs for OmpG. After t_1-evolution of ^{15}N, a *z*-filter was used to retain rotor synchronization by compensating for pulse durations (Hong and Griffin, 1998; Jaroniec et al., 2002). During t_1-evolution, acquisition and REDOR-mixing, two-pulse phase-modulation (TPPM) decoupling at ~ 75 kHz (SH3 domain) or ~90 kHz (OmpG) were used for heteronuclear decoupling (Bennett et al., 1995). The t_1-increment was chosen to $1/\omega_R$ = 125μs. The total indirect acquisition time amounted to 6 ms.

All spectra were processed using XWINNMR software (Bruker, Karlsruhe Germany) version 3.5 and further analyzed using the software Sparky,

[1] We use the acronym "REDOR" in cases where we refer to the mixing blocks and the mixing times, whilst we used the acronym "TEDOR" for the 2D-experiment where two REDOR mixing-blocks are sandwiched by an INEPT step.

version 3.110 (T.D. Goddard & D.G. Kneller, University of California, San Francisco).

Results and discussion

Pulse sequences were designed for the following two experiments: a CH_3-double-filtered DARR (Figure 1a) and a CH_3-double-filtered TEDOR (Figure 1b). For both experiments, we implemented a short 50μs polarization inversion after a long initial CP of 2 ms. The polarization-inversion time was chosen as the time needed for signals from rigid CH groups to cross zero (*i.e.*, going from positive to negative intensity). Under these conditions, strongly-coupled, rigid CH_2 moieties attained $-1/3$ of their initial intensity (Wu and Zilm, 1993a). For methyl groups, fast rotation lead to self-decoupling, reducing the effective ^1H-^{13}C dipolar couplings. Hence, CH_3 carbon spins exchanged more slowly during CP and retained a positive intensity. Likewise, non-protonated carbon spins experienced weaker heteronuclear dipolar interactions and remained positive.

The CPPI filter block was combined with a dipolar-dephasing period in the sequences shown in Figure 1a and b. During dipolar dephasing, proton decoupling was not applied. This lead to rapid transverse relaxation of methylene (CH_2) and methyne (CH) carbon signals due to strong heteronuclear interactions with their directly bonded protons. This efficiently dephased the negative CH_2 magnetization and any residual CH signals, whilst ~35% of the CH_3 signals remained.

This two-step filtering provided a better sensitivity for selection of methyl groups than a dipolar dephasing period alone. For CH groups, a relatively long dephasing period of ~200μs was required to zero the signals. On the other hand, to cancel out the CH signals using CPPI, a short 50μs PI-time was sufficient. This same CPPI period reduces the CH_2 signals to approximately (minus) 30% of their initial intensity; for dephasing the remaining CH_2 signal after PI, a short dephasing period of 60μs was adequate. In contrast, a straightforward dipolar-dephasing period to zero both CH and CH_2 coherences needed to be much longer than the combined filtering step and resulted in a sensitivity of only ~15%. Hence, the most effective filter combination was to initially zero out the CH signals using CPPI, and subsequently, to zero out the residual CH and negative CH_2 signals with dipolar dephasing.

CPPI and dipolar dephasing in 2D ^{13}C–^{13}C dipolar correlation spectroscopy of proteins

In the case of the 2D ^{13}C–^{13}C correlation experiment, the filter block was implemented prior to evolution of ^{13}C signals during t_1 (Figure 1a), that was followed by a homonuclear ^{13}C recoupling period using a DARR (Takegoshi et al., 2001) mixing scheme. During the mixing, CH and CH_2 resonances received magnetization from nearby methyl carbons and could be observed along F2. Hence, cross peaks involving CH and CH_2 signals appeared only at one side of the diagonal since they did not evolve during t_1, whilst correlations involving methyl carbons appeared at both sides. Figure 2 shows an example of the double filter performed on [U-^{13}C,^{15}N] α-spectrin SH3 domain. This spectrum shows a superposition of leucine $C_{\delta1}$–C_γ, $C_{\delta2}$–C_γ and $C_{\delta1}$–$C_{\delta2}$ cross peaks on the left side of the diagonal, that appears as a large conglomerate. On the right side of the diagonal, isolated $C_{\delta2}$–$C_{\delta1}$ cross peaks allows the extraction of the respective chemical shifts. The efficiency of the filtering scheme can be appreciated by disappearance of the strong isoleucine C_δ–$C_{\gamma1}$ cross peak at 11.2/26.6 ppm, respectively, on the right-hand side of the diagonal.

The effects of the double filter and of side chain mobility are illustrated in Figure 3. There, spectra of the [U-^{13}C,^{15}N] SH3 domain recorded with a CPPI filter only (a), and with CPPI in combination with an additional 60μs dipolar dephasing step (b), are compared. The spectral region around 42 ppm in F1 and 25–30 ppm in F2 is shown, that is dominated by C_β–C_γ cross peaks of leucine residues and C_ε–C_δ/C_ε–C_γ cross peaks of lysine residues. In the CPPI-DARR spectrum (Figure 3a), the CH_2 signals from non-flexible parts of the protein were not zeroed out due to the absence of dipolar dephasing, and instead evolved during t_1 with a negative intensity. Consequently, the rigid C_β spins of leucines produced cross peaks with negative intensity (indicated by dotted contour lines in Figure 3a). Since the selection of signals with the CPPI filter relied on the relative strengths of heteronuclear dipolar interactions, carbons that

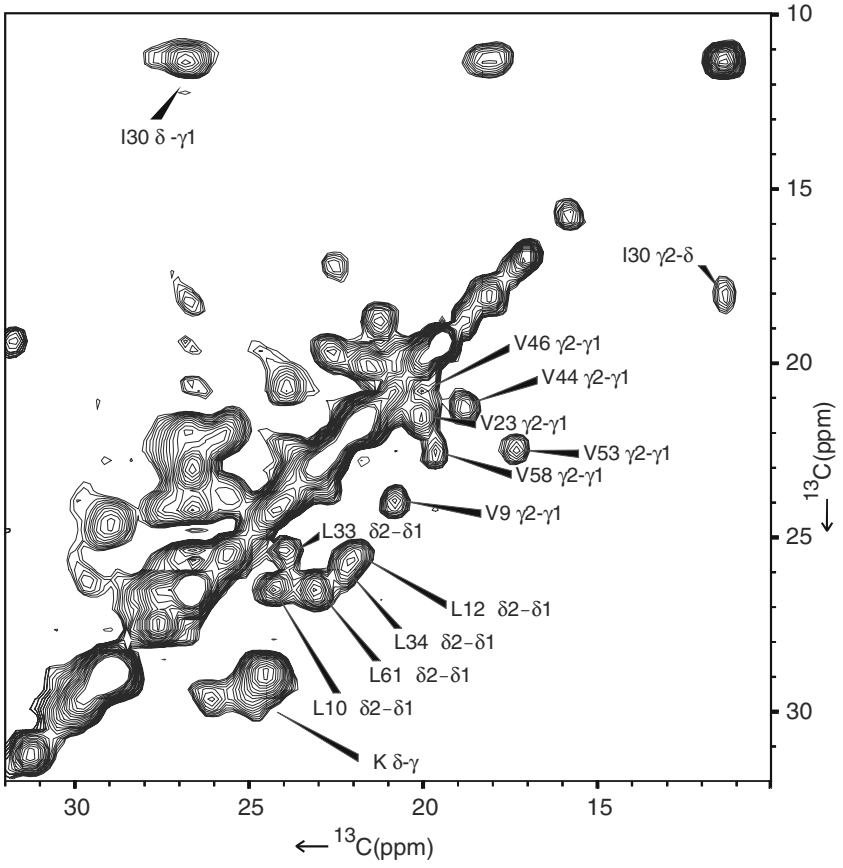

Figure 2. Contour plot of a two-dimensional ^{13}C–^{13}C homonuclear correlation experiment of uniformly-^{13}C, ^{15}N labelled α-spectrin SH3 domain. The spectrum was recorded using cross-polarization with polarization inversion (CPPI) filtering and dipolar dephasing. It demonstrates the resolution enhancement in the methyl region. The spectrum was obtained with 50μs phase inversion after CP and a 70 ms dipolar-assisted rotational resonance mixing time. Assignments of methyl–methyl cross peaks are indicated for the amino-acids that contain two methyl groups, like isoleucines, leucines and valines.

were weakly coupled to protons remained positive during CPPI. This was not only the case for methyl groups and non-protonated carbons, but also for CH and CH_2 signals of mobile side chains. We observed, for example, that C_ε signals of mobile lysine side-chains remained positive (Figure 3a). Likewise, due to the weaker couplings, mobile side-chains were also marginally affected by the dipolar dephasing. Hence, the combination of CPPI and dipolar dephasing only zeroed out negative signals arising from rigid C_β of leucine residues due to their multiplicity and strong carbon-proton dipolar couplings, whilst mobile C_ε resonances of lysine side-chains were hardly affected (Figure 3b). As a result, side-chain mobility may reduce the filter selectivity, and signals from non-methyl groups may ''leak through,'' also remaining positive. Hence, care must be taken when analyzing the spectra. In the spectra of the α-spectrin SH3 domain, such leaking appeared rather systematically, in that only CH_2 signals from the end of long hydrophilic side chains (and among those, mainly lysines) were not suppressed by the double filter.

CPPI and dipolar dephasing in 2D ^{15}N–^{13}C correlation spectroscopy of proteins

CPPI in combination with dipolar dephasing can also be used in connection with a TEDOR transfer scheme to recouple methyl carbon spins to those of nitrogens, using the pulse sequence depicted in Figure 1b.

A spectrum of [U-^{13}C,^{15}N] α-spectrin SH3 domain, recorded with a 2 ms REDOR mixing time is shown in Figure 4a. The spectrum shows pure methyl-carbon to backbone nitrogen

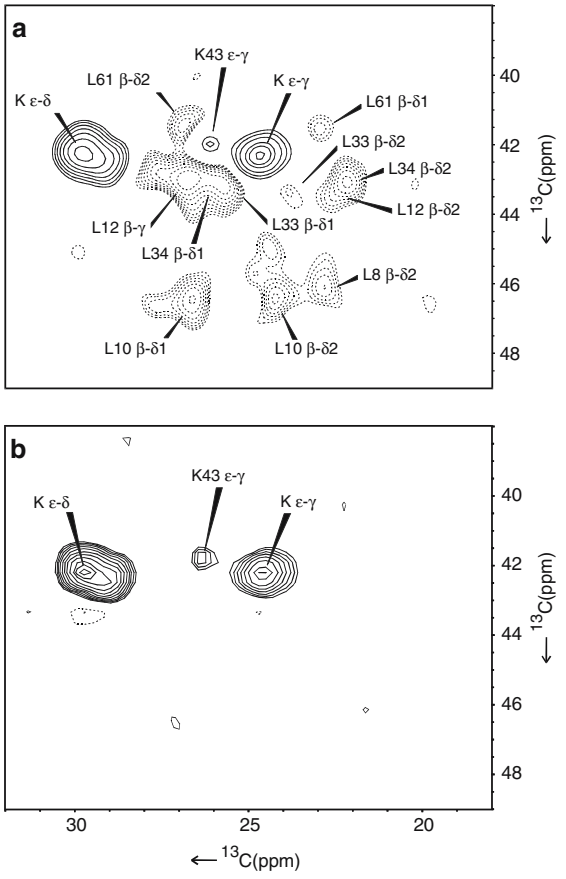

Figure 3. Contour plots of two-dimensional ^{13}C–^{13}C homonuclear correlation experiments recorded on uniformly-^{13}C,^{15}N labelled α-spectrin SH3 domain. The spectral region dominated by C_β–C_γ cross peaks of leucines and C_ε–C_δ/C_ε–C_γ cross peaks of lysines is shown. The ^{13}C–^{13}C correlation spectrum shown in (a) was recorded without dipolar dephasing. The spectrum shown in (b) was recorded using dipolar-dephasing after polarization inversion. Positive contour levels were drawn with solid lines, while negative contour levels were indicated by dotted lines.

correlations. At this short REDOR mixing time, cross peaks were visible for $^{13}CH_3$ and ^{15}N nuclei that showed distances in the range of ~2.5–3 Å. These were mostly intraresidual ^{15}N–^{13}C pairs of alanines. In addition to all three alanines, we observed intraresidual correlations for one of the six valines (V53) and for one of the four threonines (T37). At a longer REDOR mixing time of 6 ms, more methyl carbons could be observed, correlated with nitrogens in their environment (Figure 4b). For comparison, a TEDOR experiment without the double filter step is shown in Figure 4c. This spectrum is more crowded, due to overlap with non-methyl signals. In particular, arginine C_γ signals are found in the chemical shift (CS) range from 12 to 38 ppm (centred around 27 ppm), leucine C_γ signals in the range 15–42 ppm (centred around 26 ppm) and isoleucine $C_{\gamma1}$ signals in the range 9–38 ppm (centred around 27 ppm) (Seavey et al., 1991). Additional overlap may occur between methyl signals with lysine C_γ signals in the CS range from 17 to 40 ppm (centred at 25 ppm). In the methyl filtered experiment (Figure 4b), cross peaks of none of these resonances interfere with cross peaks from methyl carbons.

Most of the correlations in Figure 4b were again intraresidual. However, some correlations involved sequential residues. Ala 55 and 56, for example, showed a sequential cross peak at 113 ppm and 15.7 ppm in F1 and F2, respectively, that was weaker than the intraresidual cross peaks of Ala 55 (F1 = 129.1 ppm, F2 = 15.7 ppm) and Ala 56 (F1 = 113 ppm, F2 = 18.1 ppm). Likewise, we observed a weak correlation of a methyl carbon signal with a proline backbone nitrogen signal at 136.9 ppm (F1) and 17.2 ppm (F2). This cross peak involved transfer between the amide nitrogen of Pro 54 and the $C_{\gamma1}$ of Val 53.

Additionally, structural information could be extracted from the cross-peak pattern of branched amino acids. The distances between the valine methyl groups, for example, and the nitrogens in their neighborhood are conformation-dependent. Only one of the methyl groups of Val 53 showed a cross peak to the sequential proline. Similarly, we observed a weak cross peak at 11.2 ppm in F2 and 119.8 ppm in F1, involving C_δ of Ile 30 and its backbone nitrogen. The cross peak involving $C_{\gamma2}$ was weaker and could only be observed at a deeper contour level (not shown). Indeed, according to the recent X-ray structure (PDB code 1U06) (Chevelkov et al., 2005), $C_{\gamma2}$ of Ile 30 is more distant from the backbone nitrogen than the C_δ. As a final example, the two cross peaks involving the methyl groups of Leu 31 and the backbone amide differed slightly in their intensity. Again, this was consistent with the crystal structure, which showed that the individual C_δ are ~ 3.5 and ~ 4.3 Å away from the intraresidual nitrogen.

In a previous paragraph, we discussed that signals from mobile parts of the protein may leak through the filter. The CH_2 coherences from mobile side-chains that may pass the filter, however, will

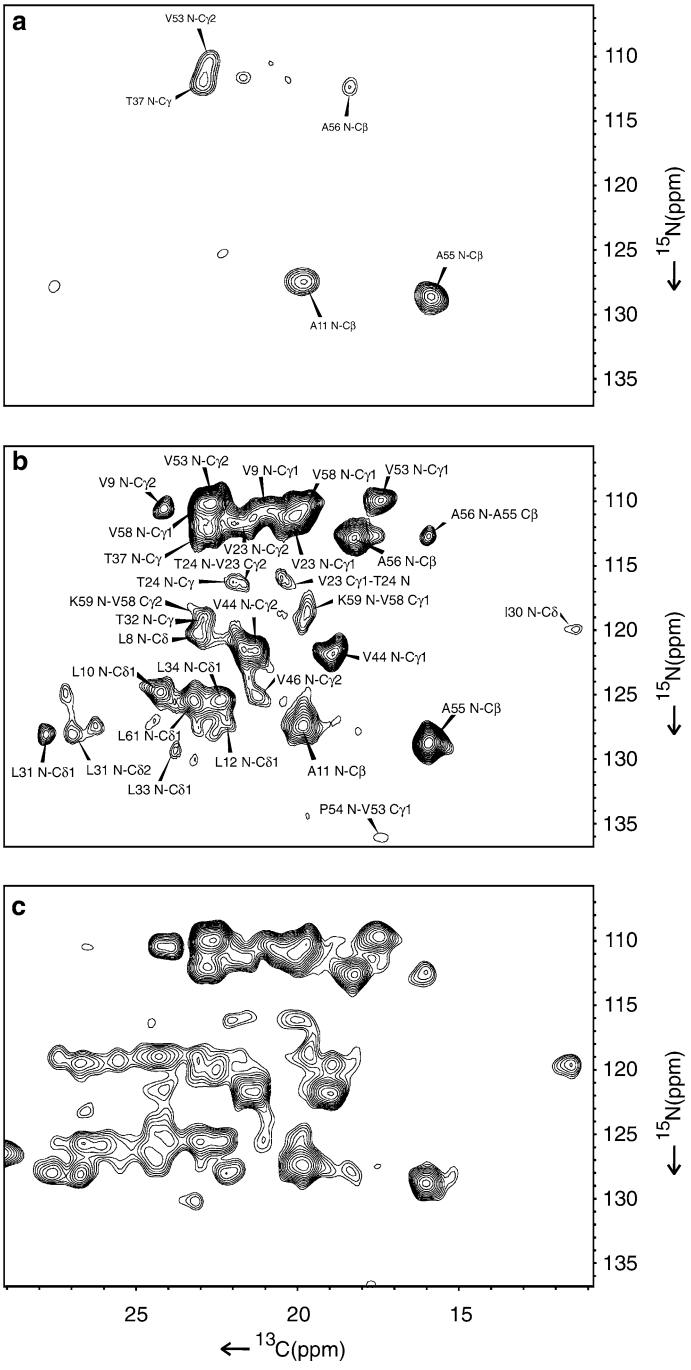

Figure 4. Contour plot of ^{15}N–^{13}C methyl-selective transferred-echo double resonance (TEDOR) correlation spectra of uniformly-^{13}C,^{15}N labelled α-spectrin SH3 domain, using the pulse sequence depicted in Figure 1b. For the spectrum shown in (a), a short rotational-echo double resonance mixing of 2 ms was applied. The contour plot in (b) shows a methyl-selective TEDOR spectrum recorded with a 6 ms mixing time. For comparison, a standard TEDOR experiment (i.e., without the double filter), obtained using a 6 ms mixing time, is shown in (c).

not show cross peaks involving nitrogens, since the mobility reduces the effective heteronuclear dipolar coupling and interferes with the REDOR transfer.

The methyl-filtered TEDOR experiment was applied to OmpG. OmpG is a membrane-integrated pore for non-selective transport of mono-, di- and

tri-saccharides (Fajardo et al., 1998). The protein consists of 281 amino acids and forms 2D crystals that show well resolved MAS NMR spectra (Hiller et al., 2005). Despite the favorable line width, resonance overlap complicates the sequence-specific assignment of resonances in crowded regions.

By using the CH_3-double-filtered TEDOR sequence at short mixing times (3 ms), we observed many correlations between the backbone nitrogen and the methyl carbons of alanines using a 1,3-^{13}C-glycerol labelled OmpG sample (Figure 5a). In this spectrum, intraresidual N–C_β correlations of alanines were expected to appear with the strongest signal intensity; valine, threonine and isoleucine methyl groups could also give rise to weak intraresidual cross-peaks. They, however, tended to be weaker since these methyls were more distant from the backbone nitrogens.

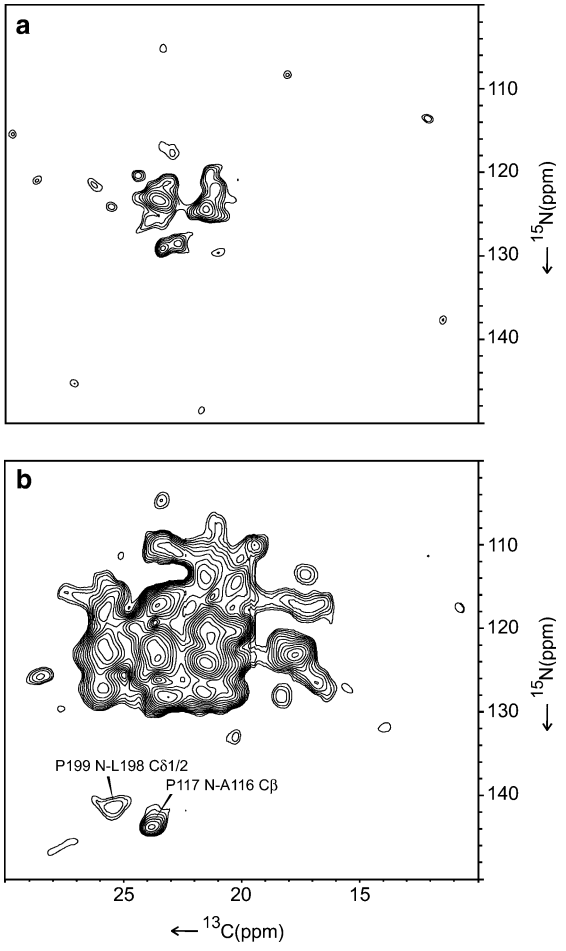

Figure 5. Contour plots of methyl-selective transferred-echo double resonance correlation spectra of [1,3-^{13}C]glycerol-grown outer-membrane protein G, recorded using rotational-echo double resonance mixing times of 3 ms (a) and 8 ms (b). The data were collected using the pulse sequence of Figure 1b. The sequential assignments are indicated.

In the spectrum recorded with an 8 ms mixing time (Figure 5b), we found many signals that were not present in Figure 5a. These signals predominantly arose due to transfer from methyl groups of isoleucines ($C_{\gamma 2}$–C_δ), valines ($C_{\gamma 1/2}$), leucines ($C_{\delta 1/2}$) and threonines (C_γ) to backbone nitrogen. Furthermore, we observed a strong signal at 23.8 ppm in F2 and 143.7 ppm in F1. The downfield shift in the ^{15}N dimension for this signal ensured that it could be attributed to a correlation involving a backbone amide of a proline. The methyl filtering excluded many assignment options for the correlated carbon. Hence, this correlation could be attributed to sequential transfer from a methyl group at residue *i*-1 to the nitrogen of a proline at position *i*. According to the protein sequence, there were only three potential residue pairs that could account for this transfer: A116-P117, L198-P199, T208-P209. Transfer from the C_γ of T208 could, however, be excluded since the chemical shifts detected for the threonine C_γ's did not match (Hiller et al., 2005). The correlation at 23.8 ppm/143.7 ppm (F2/F1) was most likely due to the A116–P117 pair. A second, weaker, sequential cross-peak was observed at 25.4 ppm in F2 and 141.5 ppm in F1. This peak was tentatively assigned to the other sequential candidate, involving a methyl group of Leu 198 and the backbone nitrogen of Pro 199, although we should keep in mind that a long range correlation could not be excluded. The weaker intensity was consistent with the larger distance between the methyl groups of leucine and the backbone nitrogen of the next amino acid. Sequential correlations of this type can provide a good starting point for the sequential assignment.

Conclusions

The combination of CPPI and dipolar dephasing provides a simple way to obtain efficient filtering of methyl groups. This double filter can be easily implemented as a building block into multidimensional MAS NMR correlation experiments, for instance by using it as preparative filtering step prior to homonuclear (^{13}C–^{13}C) or heteronuclear (^{15}N–^{13}C) transfer schemes. The filtering relies on

the relative strengths of the ^{1}H–^{13}C dipolar interactions. As a consequence, coherences from dynamic side chains may behave differently to those from rigid side chains, and some non-methyl signals can escape from the filter. On the other hand, we observed that this 'leaking-through' is limited to a small number of signals from mobile side chains, mainly lysines. In particular, in the heteronuclear variant of the experiment, mobile fractions do not pass the REDOR transfer step and do not interfere with the filtered signals. Suppression of CH and CH_2 signals can simplify significantly spectral regions containing methyl groups. The assignment of leucine signals, for example, may profit from the reduction of overlap between $C_{\delta 2}$–$C_{\delta 1}$ and C_{γ}–C_{δ} cross peaks. Moreover, it is shown that a methyl-selective ^{15}N–^{13}C correlation experiment can be particularly helpful for achieving starting points for the assignment. Using a short REDOR mixing time of 2–3 ms, it is possible to select intraresidual correlations between amide nitrogens and methyl groups that are separated by distances of $\sim$3 Å. Hence, when combined with a short REDOR transfer, this experiment selects predominantly methyl-nitrogen correlations for short amino-acid side-chains, like alanines (methyl group at C_{β}), and to a lesser extent residues with a methyl group at C_{γ} (*e.g.*, valines, threonines). For longer REDOR mixing times, additional sequential and conformational information can be extracted.

Acknowledgements

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