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Band-selective 3D NOESY-TOCSY: Measurement of through-space correlations between aliphatic protons of membrane peptides and proteins in non-deuterated detergents

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Summary

Recently the use of band-selective excitation to obtain ^{1}H 2D NMR spectra of membrane peptides and proteins in non-deuterated detergents has been demonstrated [Seigneuret, M. and Levy, D. (1995) *J. Biomol. NMR*, **5**, 345–352]. A limitation of the method was the inability to obtain through-space correlation between aliphatic protons. Here, a 3D F3-band-selective NOESY-TOCSY experiment is described that allows such correlations to be observed in the presence of an excess of non-deuterated detergent. Application to the measurement of proximities between aliphatic protons of the membrane peptide mastoparan X solubilized in non-deuterated *n*-octylglucoside is presented. With this additional experiment, it is now possible to obtain the same amount of structural constraints on membrane peptides and protein in non-deuterated detergent as in deuterated detergent and therefore to perform complete structural studies.

Multidimensional solution NMR has recently become an efficient technique for structure determination of membrane polypeptides solubilized in detergent (for reviews, see Henry and Sykes, 1994; Opella et al., 1994). In particular, membrane peptides, small membrane proteins and protein domains can be investigated by homonuclear ^{1}H NMR, i.e. without the use of stable-isotope labeling. Since a large excess of detergent is needed for solubilization, this latter approach initially mandatorily required the use of deuterated detergents in order to observe the protein ^{1}H resonances. Currently, only two deuterated detergents can be used: dodecylphosphocholine and sodium dodecylsulfate. This was a notable limitation, since, for many membrane polypeptides, maintenance of the native state (Møller et al., 1986), absence of aggregation (see McDonnell and Opella, 1993) or high resolution in NMR spectra (Seigneuret et al., 1992) may require specific detergents not available in deuterated form. Recently, we have proposed an NMR method that overcomes this limitation and allows the ^{1}H NMR investigation of membrane proteins and peptides in any non-deuterated detergent (Seigneuret and Levy, 1995). By using band-selective excitation of the protein amide-aromatic region in the acquisition dimension of 2D experiments, it is possible to decrease by two orders of magnitude the spectral contribution of the protonated detergent which occurs in the aliphatic region. The resulting F2-band-selective TOCSY, COSY and NOESY spectra display all scalar or though-space connectivities or scalar coupling values involving at least one amide or aromatic proton, i.e. 50% of the information available in classical 2D spectra. The applicability of the method was demonstrated by the determination of the complete sequence-specific assignments and the secondary structure of the membrane peptide mastoparan X solubilized in the non-deuterated detergent *n*-octylglucoside. One important limitation was however that through-space correlations between protein aliphatic protons could not be obtained on F2-band-selective NOESY spectra, a feature which precluded determination of proximities within non-aromatic side chains and α-protons. Such proximities are mandatory for the determination of side-chain conformation and tertiary structure and also useful for an accurate secondary structure evaluation.

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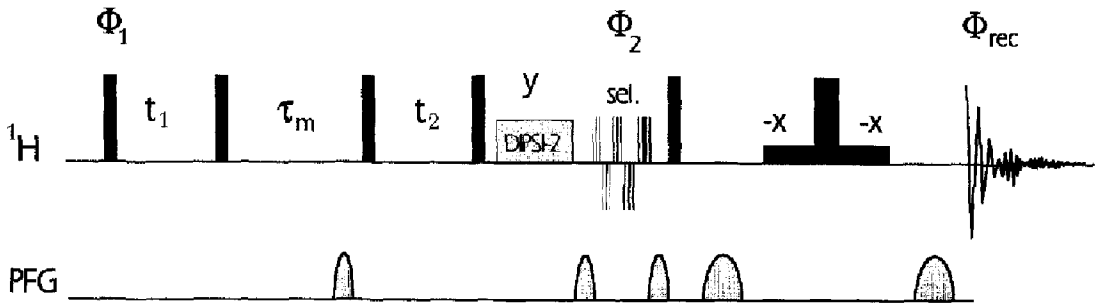

Fig. 1. Pulse sequence used for the F3-band-selective NOESY-TOCSY. Phases are $\Phi_1 = x,-x$, $\Phi_2 = 2x,2(-x)$, $\Phi_{rec} = x,-x,-x,x$. All other pulses have phase x except when stated otherwise. The TOCSY step uses a z-filtered scheme (Rance, 1987) and a DIPSI-2 composite pulse train (Shaka et al., 1988). Band-selective excitation in the F3 dimension is achieved using a DANTE-z/I-BURP-2 pulse train with a two-step subtraction (Roumestand et al., 1995). The carrier frequency is kept at the water resonance position, except during band-selective excitation where it is shifted to the center of the amide region. The three first pulsed field gradients are used for dephasing of transverse magnetization. The two last field gradients are used for coherence selection during the WATERGATE water suppression (Piotto et al., 1992).

In this communication, we propose the extension of the method to 3D homonuclear NMR. Specifically, we present an F3-band-selective version of the NOESY-TOCSY experiment (Vuister et al., 1988). We show that this experiment can be used with membrane peptides and proteins in non-deuterated detergent and provides an efficient suppression of detergent resonances. Moreover, besides promoting an increase in spectral resolution typical of 3D experiments, the F3-band-selective NOESY-TOCSY experiment allows one to obtain through-space correlations between protein aliphatic protons in spite of the protonated detergent. The above-mentioned limitation is therefore abolished. This opens the possibility of obtaining complete 3D structures of membrane peptides and proteins in non-deuterated detergents.

Figure 1 shows the pulse sequence for the F3-band-selective NOESY-TOCSY experiment. Band-selective excitation of the protein amide-aromatic region in the acquisition dimension is achieved by a DANTE-z/IBURP-2 scheme (i.e. the lengths of the individual pulses of the alternated 180° and 0° DANTE trains are modulated according to the IBURP-2 coefficients, see Roumestand et al. (1995) for details). The use of three purging z-gradient pulses (Roumestand and Canet, 1995) allows a limited phase cycle to be used. Note that this four-step cycle can be further reduced to two steps by omitting axial peak suppression (Simorre and Marion, 1991). A WATERGATE scheme (Piotto et al., 1992) is used for water suppression.

Figure 2 shows the F3-band-selective 3D NOESY-TOCSY spectrum of the membrane peptide mastoparan X (INWKGIAAMAKKLL-NH_2) solubilized in non-deuterated *n*-octylglucoside (mole ratio 1:75). A very high level of detergent suppression is obtained by the selective excitation of the amide-aromatic region of the peptide. Small remaining baseline distortions can be corrected digitally. This allows one to obtain 50% of the spectral data contained in a normal 3D cube. In principle, complete sequence-specific assignments and determination of interproton through-space proximities can be obtained from a single 3D NOESY-TOCSY experiment (Vuister et

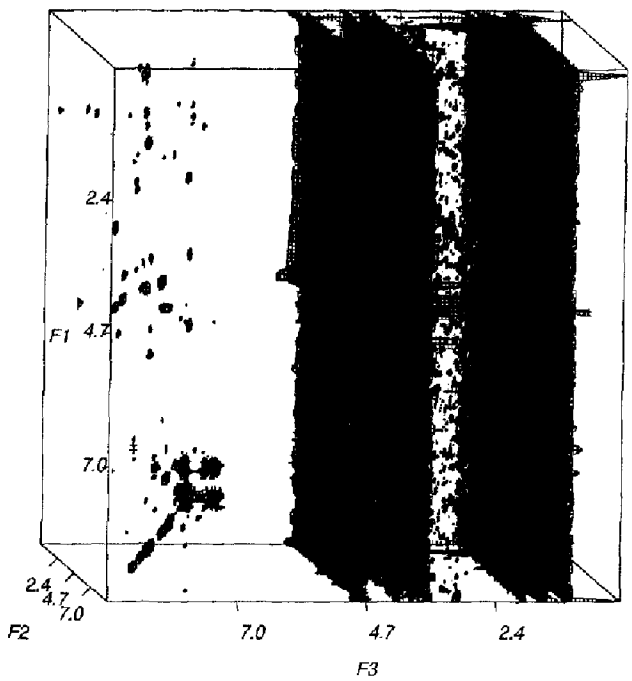

Fig. 2. F3-band-selective NOESY-TOCSY spectrum of mastoparan X solubilized in *n*-octylglucoside (peptide concentration 7.5 mM, detergent to peptide ratio 75:1). The spectrum was recorded at 50 °C on a Bruker AMX 400 spectrometer using a Bruker 8 mm triple-resonance probe equipped with a z-gradient coil. Mixing times were respectively 150 and 65 ms for the NOESY and TOCSY mixing steps. The three first gradient pulses had a 160 μs duration and the two last gradient pulses a 1 ms duration (gradient strength: 15 G/cm). The DANTE-z/IBURP-2 pulse train was set to excite a 2000 Hz bandwidth in the amide-aromatic region (rf power: 10 kHz, interpulse delay: 78 μs). Other parameters were: spectral widths of 5048 Hz in F3 and 3681 Hz in F1 and F2 (i.e. with folding of the indole resonance), time domains of 512 complex points in F3, 110 complex points in F1 and 80 complex points in F2, four transients per t_1/t_2 increment. Quadrature detection in both indirect dimensions was achieved using the States-TPPI method (Marion et al., 1989). Due to memory limitations, a threshold five times the noise level and only every fourth point were used for drawing so that not all cross peaks are displayed.

al., 1990). However, our current experience on several membrane peptides indicates that it is more efficient to analyze the band-selective NOESY-TOCSY experiments in conjunction with 2D NOESY and TOCSY experiments. This approach has in fact been used previously for non-selective experiments on soluble proteins (Simorre et al., 1991). In the particular case of mastoparan X in *n*-octylglucoside, complete sequence assignments were previously obtained from F2-band-selective TOCSY and NOESY experiments (Seigneuret and Levy, 1995).

Figure 3 shows the aliphatic region of the F1-F2 planes taken at the F3 amide frequencies of 12 successive residues of mastoparan X in *n*-octylglucoside. All cross peaks outside the NOESY, TOCSY and back-transfer lines originate from NOESY transfer from one aliphatic ^{1}H to another followed by TOCSY transfer from this second aliphatic ^{1}H to an amide ^{1}H. By this approach, proximities between aliphatic ^{1}H can be determined in spite of the protonated detergent. Although some overlap occurs between some amide resonances, all such aliphatic correlations could be unambiguously assigned. As an example, the delineation of a complete stretch of αβ(i,i+3) through-space connectivities from Trp^3 to Leu^{14} is illustrated in Fig. 3. Each of these cross peaks can be directly assigned with reference to the TOCSY line of the corresponding residues. The occurrence of such a stretch confirms the α-helical conformation of the peptide (Seigneuret and Levy, 1995). The fact that such αβ(i,i+3) correlations, which are usually of intermediate or weak intensity in α-helices, can be visualized in the F3-band-selective NOESY-TOCSY experiment indicates that the sensitivity of the experiment is acceptable. In our previous study of mastoparan X in

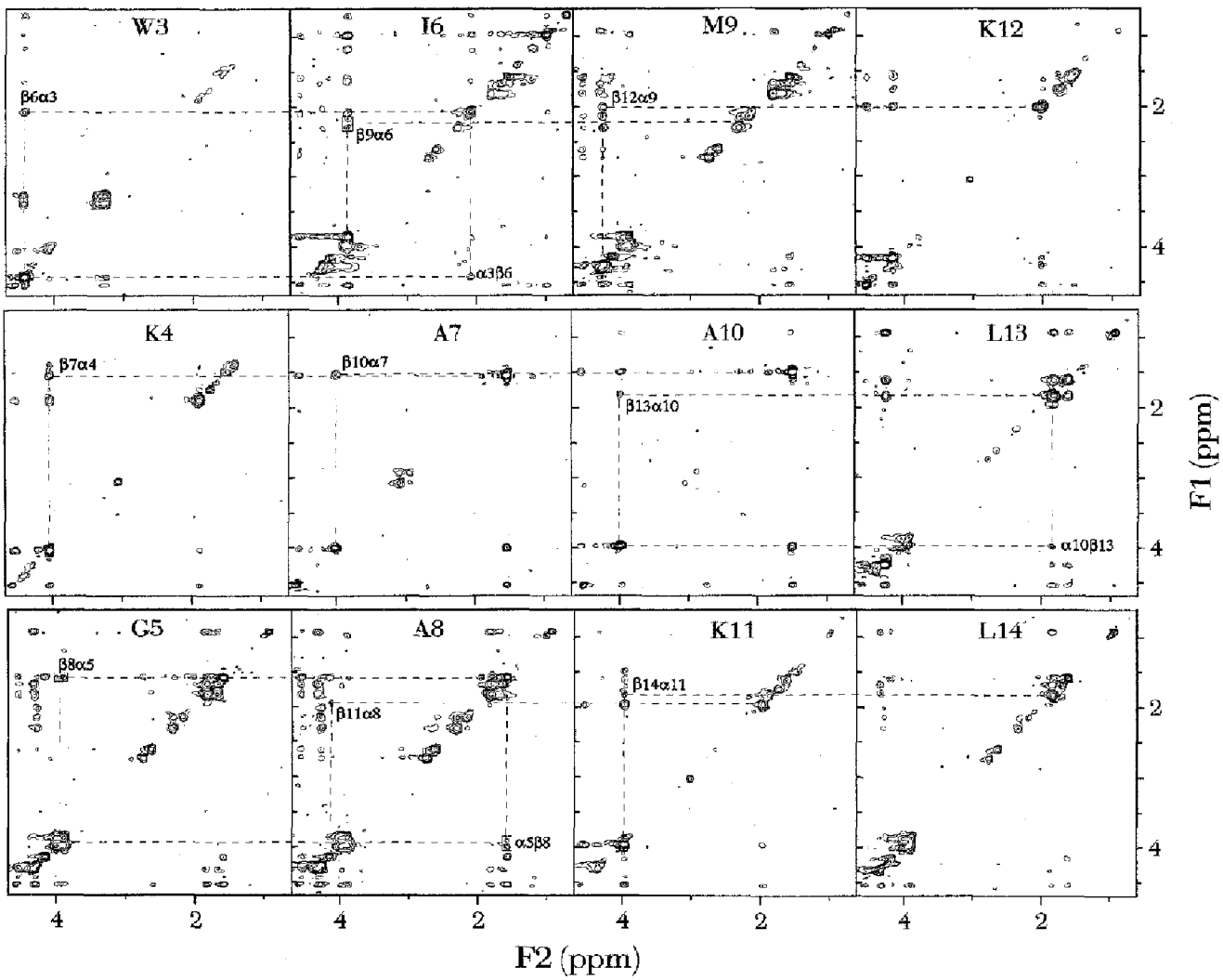

Fig. 3. F1-F2 planes from the F3-band-selective NOESY-TOCSY spectrum of mastoparan X in *n*-octylglucoside. The spectrum was processed with the NMRPipe software (Delaglio et al., 1995). Before Fourier transformation, the time domain data was extended to 180 complex points in F1 and 128 complex points in F2 using forward–backward linear prediction (Zhu and Bax, 1992), multiplied with cosine-bells and zero-filled once in all dimensions. A local baseline correction of the amide-aromatic region using a 4th order polynomial was performed in F3. Each F1-F2 plane corresponds to the F3 chemical shift of the indicated residue (see Seigneuret and Levy (1995) for the chemical shift values). The cross peaks corresponding to αβ(i,i+3) through-space connectivities are indicated and linked to the cross-diagonal peaks of the corresponding α and β protons by dotted lines.

n-octylglucoside, 32 intraresidue and 50 interresidue through-space correlations involving at least one amide or aromatic ^{1}H were obtained from F2-band-selective NOESY experiments. Here, 24 intraresidue and 18 interresidue new through-space correlations between aliphatic ^{1}H were measured on the F3-band-selective NOESY-TOCSY experiment. Therefore, the latter experiment significantly increases the amount of structural data available for structure determination. At a 400 MHz ^{1}H frequency and using a 8 mm probe, a F3-band-selective NOESY-TOCSY experiment with sufficient sensitivity can be recorded in a reasonable time of two days with a 7.5 mM membrane peptide sample. Obviously, the use of higher field strengths would allow the study of more diluted membrane peptide or protein samples. Apart from the ability to measure through-space proximities between aliphatic ^{1}H in the presence of a protonated detergent, the F3-band-selective NOESY-TOCSY experiment has the advantage of furnishing a significant increase in resolution which facilitates assignments.

In conclusion, the present work and our previous study (Seigneuret and Levy, 1995) indicate that the combined use of 2D and 3D band-selective NMR experiments for the study of membrane peptides and proteins in non-deuterated detergents allow one to obtain virtually the same amount of data that would be available with classical 2D experiments in deuterated detergents. In particular, proximities between aliphatic protons can now be measured. This is essential for the study of tertiary structures and particularly for helix–helix interactions, which are important determinants of the membrane protein structure. It now appears possible to study such features without the limitation of using the currently available deuterated detergents.

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