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A high-resolution ^{1}H NMR approach for structure determination of membrane peptides and proteins in non-deuterated detergent: Application to mastoparan X solubilized in *n*-octylglucoside

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Summary

Application of ^{1}H 2D NMR methods to solubilized membrane proteins and peptides has up to now required the use of selectively deuterated detergents. The unavailability of any of the common biochemical detergents in deuterated form has therefore limited to some extent the scope of this approach. Here a ^{1}H NMR method is described which allows structure determination of membrane peptides and small membrane proteins by ^{1}H 2D NMR in any type of non-deuterated detergent. The approach is based on regioselective excitation of protein resonances with DANTE-Z or spin-pinging pulse trains. It is shown that regioselective excitation of the amide–aromatic region of solubilized membrane proteins and peptides leads to an almost complete suppression of the two orders of magnitude higher contribution of the protonated detergent to the ^{1}H NMR spectrum. Consistently TOCSY, COSY and NOESY sequences incorporating such regioselective excitation in the F2 dimension yield protein ^{1}H 2D NMR spectra of quality comparable to those obtained in deuterated detergents. Regioselective TOCSY and NOESY spectra display all through-bond and through-space correlations within amide–aromatic protons and between these protons and aliphatic and α-protons. Regioselective COSY spectra provide scalar coupling constants between amide and α-protons. Application of the method to the membrane-active peptide mastoparan X, solubilized in *n*-octylglucoside, yields complete sequence-specific assignments and extensive secondary structure-related spatial proximities and coupling constants. It is shown that mastoparan adopts an α-helical conformation when bound to nonionic detergent micelles. The present method is expected to increase the applicability of ^{1}H solution NMR methods to membrane proteins and peptides.

Introduction

Detergent solubilization is a common procedure in investigations of membrane proteins and peptides through methods designed for soluble biopolymers. In particular, since the pioneering work of Brown and Wüthrich (Brown and Wüthrich, 1981; Brown et al., 1982), many studies have been performed on detergent-solubilized peptides and small proteins by ^{1}H 2D NMR (for a review see Opella and McDonnell (1993)). This has led to the structure determination of several membrane peptides (Arseniev et al., 1985; Inagaki et al., 1989), small membrane proteins (Maurer and Rüterjans, 1994) and fragments of larger membrane proteins (Pervushin et al., 1991; Lomize et al., 1992; Macquaire et al., 1993). All these studies have invariably used deuterated detergent in order to fully observe the protein ^{1}H resonances. While this approach is conceptually simple, its scope is limited by the current availability of only two detergents in deuterated form: sodium dodecylsulfate and dodecylphosphocholine. ^{1}H NMR could be made applicable to a wider range of membrane proteins and peptides if other detergents could be used. Indeed, from a biochemical point of view, efficient solubilization and structural integrity of a particular peptide or protein can often be obtained with only few selected detergents. Furthermore,

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Abbreviations: 2D NMR, two-dimensional NMR; COSY, correlated spectroscopy; DANTE, delays alternating nutations for tailored excitation; NOESY, nuclear Overhauser enhancement spectroscopy; TOCSY, total correlation spectroscopy.

from a spectroscopic point of view, the spectral resolution attainable with a particular membrane protein strongly depends on the detergent used for solubilization (Seigneuret et al., 1991a; Seigneuret and Kainosho, 1993). Since very high detergent-to-protein molar ratios are necessary to solubilize the protein (Seigneuret et al., 1992a,b) and to avoid aggregation (McDonnell and Opella, 1993), use of a protonated detergent in standard 2D NMR seems to be precluded by its two orders of magnitude higher ^1H signal, which is expected to obscure protein resonances. Of course, chemical synthesis of the necessary detergent in deuterated form is in principle feasible, but this is demanding and clearly impracticable in cases where an NMR screening of the most suitable detergent is necessary. Isotope labeling of the protein and heteronuclear editing of NMR experiments have been shown to efficiently filter out the contribution of a protonated detergent (Anglister et al., 1993; Seigneuret et al., 1992), but this approach is probably profitable only in cases where it is also justified by the size of the protein.

In the present study, a ^1H NMR approach is introduced which allows one to obtain the structure of membrane peptides and small proteins in any *non-deuterated* detergent. The method is based on TOCSY, COSY and NOESY experiments recorded with recently described regioselective excitation methods (Xu et al., 1992; Roumestand et al., 1994). Regioselective excitation of part of the protein spectrum is used in the acquisition dimension in order to remove the detergent contribution. Information concerning protein resonances that are overlapped with the detergent is recovered in the other dimension. This method is illustrated with the membrane-active peptide mastoparan X from wasp venom (Nakajima et al., 1986). Mastoparan X belongs to an important class of peptides which are supposed to form amphipathic helices when bound to membranes (Segrest et al., 1990). Using *n*-octylglucoside, a widely used detergent in membrane biochemistry, we show by ^1H NMR that mastoparan X adopts a helical secondary structure when bound to nonionic detergent micelles.

Materials and Methods

The peptide–detergent samples used in NMR experiments contained 10 mM mastoparan X (Bachem) and 750 mM *n*-octylglucoside (Sigma) in 20 mM sodium citrate, pH 3.5, and 10% (v/v) D_2O. Control circular dichroism experiments indicated that this detergent-to-peptide ratio induced a maximum intensity of the 222 nm negative band, characteristic of an α-helical conformation (Higashijima et al., 1983). This corresponds to a number of peptide per micelle ranging from 0.3 to 1, depending on the chosen value of the aggregation number of *n*-octylglucoside (see Lorber et al., 1990). ^1H NMR spectra were recorded at 50 °C on a Bruker AMX-400 spectrometer at 400 MHz in 5 mm sample tubes. Chemical shifts are reported relative to internal trimethylsilyl(2,2,3,3-$^2H_4$)propionate. All 2D spectra were recorded with water presaturation during the 1 s relaxation delay and using time-proportional phase incrementation (Marion and Wüthrich, 1983). F2-regioselective NOESY spectra were recorded by incorporation of a two-frequency concatenated shaped DANTE-Z pulse train before the last 90° pulse, termed BIDAZ by Roumestand et al. (1994). This pulse train was designed to excite a 2000 Hz band width matching the amide-aromatic region (pulse angles 12°, 6° and 3°; interpulse delay 50 μs). Other acquisition parameters were: mixing time 100 ms, spectral widths 6024 Hz in F2 and 5048 Hz in F1, number of data points 2048 in F2 and 384 in F1, number of transients 192. Doubly regioselective NOESY spectra incorporated an additional BIDAZ pulse train, matching a 600 Hz band width in the amide region (pulse angles 12°, 6° and 3°; interpulse delay 166 μs) before the initial 90° pulse. Acquisition parameters were: mixing time 100 ms, spectral widths 6024 Hz in F2 and 600 Hz in F1, number of data points 2048 in F2 and 128 in F1, number of transients 192. F2-regioselective TOCSY spectra were recorded using a z-filtered scheme (Rance, 1987) incorporating a DIPSI-2 spin-lock (Shaka et al., 1988) and with a BIDAZ pulse train similar to the F2-regioselective NOESY before the last 90° pulse. Acquisition parameters were: mixing times 50 and 100 ms, spectral widths 6024 Hz in F2 and 5048 Hz in F1, number of data points 2048 in F2 and 256 in F1, number of transients 256. F2-regioselective COSY spectra were recorded by insertion of a spin-pinging scheme (Xu et al., 1992) after the last 90° pulse, incorporating a 180° DANTE pulse train designed to match a 1960 Hz band width in the amide region (pulse angle 6°, delay 17 μs). Acquisition parameters were: spectral widths 3623 Hz in F2 and 3003 Hz in F1, number of data points 4096 in F2 and 512 in F1, number of transients 128. Since here detergent suppression was less efficient than with the DANTE-Z pulse train, unlike the other experiments, the carrier frequency was kept on the amide region for detection and

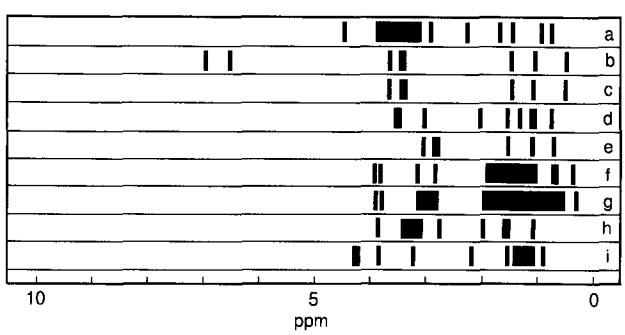

Fig. 1. ^1H chemical shifts of representative detergents in aqueous solution. (a) *n*-Octylglucoside; (b) Triton X100; (c) hydrogenated Triton X100; (d) Tween 20; (e) LDAO; (f) sodium cholate; (g) CHAPS; (h) zwittergent 3-10; (i) myristoyl lysophosphatidylcholine.

the F2 spectral width was reduced in order to allow for a limited amount of folding. Spectra were processed using the software packages UXNMR or FELIX. For all experiments, data were doubled in size by zero-filling and apodized with shifted sine bells in both dimensions before Fourier transformation. A local baseplane correction using the FLATT method (Güntert and Wüthrich, 1992) was applied between 6 and 11 ppm in the F2 dimension. Scalar coupling values were calculated from the antiphase COSY multiplets, using a routine of the FELIX package which involves Gaussian lineshape fitting of both the F1 and F2 projections, followed by averaging of the two values. Interproton distance constraints were obtained from integrated intensities of NOESY cross peaks and were classified as strong, medium or weak using cutoff distances of 2.7, 3.3 and 5.0 Å, respectively.

Results

Selective excitation of protein resonances in non-deuterated detergent

The starting point of the proposed method to obtain ^{1}H 2D NMR spectra of a membrane protein or peptide in non-deuterated detergent is to use selective excitation of part of the ^{1}H resonances. The method therefore requires that a significant spectral region exists in which the protein and detergent ^{1}H signals do not overlap. Figure 1 displays the ^{1}H NMR chemical shifts of the most common types of detergent. It appears that the 5–12 ppm region, which corresponds to the amide and aromatic resonances of peptides and proteins, is almost invariably free of detergent lines. The widely used detergent Triton X100 is a notable exception, but can be substituted by its hydrogenated analog. In several cases, it appears that a significant portion of the spectral region corresponding to peptide α-protons is also free of detergent lines. Thus, a large spectral region of the ^{1}H NMR spectra of membrane proteins is in principle available for regioselective excitation in the presence of non-deuterated detergent.

To illustrate the feasibility of this approach, the membrane peptide mastoparan X, solubilized in *n*-octylglucoside micelles, was chosen. A very high detergent-to-peptide ratio (75:1 mol/mol, i.e., 15:1 w/w) was used in order to test the efficiency of the method. Figure 2a shows the non-selective ^{1}H NMR spectrum of the *n*-octylglucoside–mastoparan mixture at 50 °C. As expected, the spectrum is dominated by the detergent contribution which obscures the aliphatic resonances and, unlike other detergents, also the α-proton resonances of the peptide. The amide-aromatic resonances can be observed but appear over a distorted baseline (Fig. 2b). Figure 2c shows the spectrum of the same sample, recorded with selective excitation of the peptide amide-aromatic region. A very high suppression of the detergent signal (by a factor of 100) is effected, with negligible intensity losses for the peptide resonances, which now appear without baseline distortion.

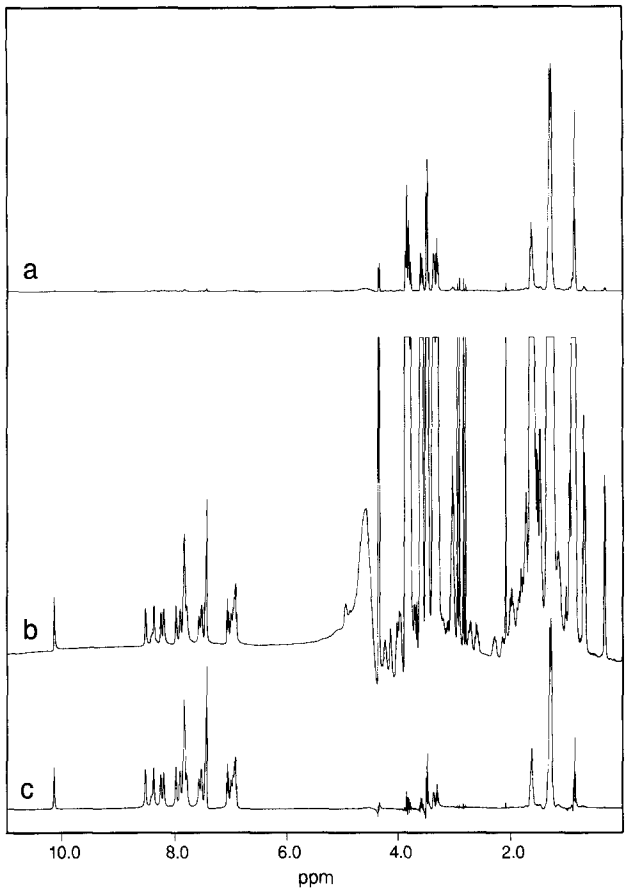

Fig. 2. ^{1}H NMR spectra of mastoparan X in the presence of *n*-octylglucoside. (a) Nonselective excitation; (b) same as (a), scaled ×100; (c) regioselective excitation using a BIDAZ pulse train, scaled ×100.

TOCSY, COSY and NOESY spectra of mastoparan X–*n*-octylglucoside samples obtained with conventional pulse sequences were almost illegible with regard to peptide correlations. Figure 3a shows a conventional NOESY spectrum as an example. As expected, the regions corresponding to correlations within aliphatic and α-protons are obscured by the detergent signals. Furthermore, regions corresponding to correlations of aliphatic and α-protons with amide and aromatic protons are obscured by baseline distortions and ridges arising from the edges of detergent diagonal peaks. These artefacts cannot be digitally corrected and preclude the observation of most peptide cross peaks. Incorporation of regioselective excitation of the peptide amide-aromatic region in the F2 dimension of 2D experiments was found to be a straightforward way to overcome this difficulty. As shown in Fig. 3b for an F2-regioselective NOESY spectrum, this approach afforded a strong reduction of the detergent diagonal and almost eliminated the corresponding baseline distortions and ridges. Small remaining artefacts could be removed by baseline correction. As a consequence, 2D NMR spectra of quality comparable to those recorded

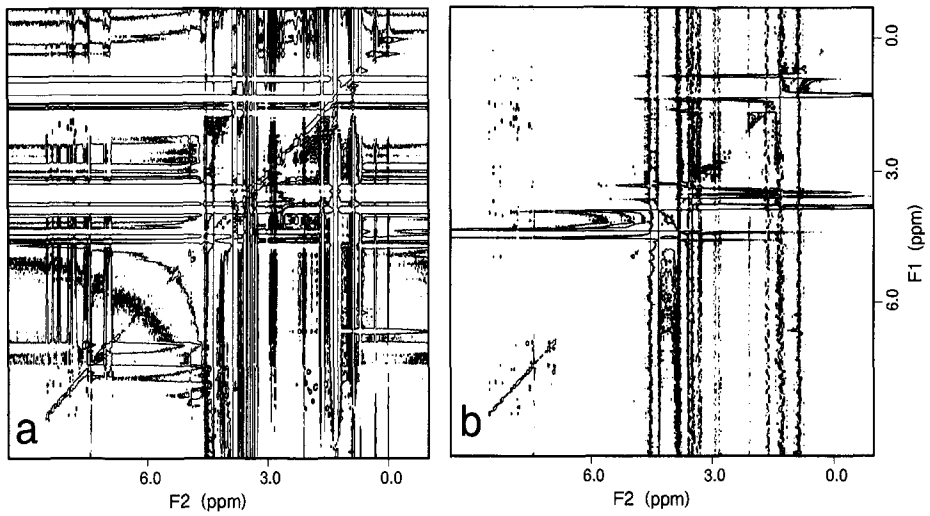

Fig. 3. Conventional (a) and F2-regioselective (b) NOESY spectra of mastoparan X in the presence of *n*-octylglucoside. No baseline correction was applied.

with deuterated detergents could be obtained that displayed correlations between amide and aromatic protons, as well as those of amide and aromatic protons with aliphatic and α-protons (see below). Control experiments performed with a soluble protein (hen egg white lysozyme) indicated that the cross peak intensity losses associated with selective excitation were 10–15% compared to standard experiments. Regioselective experiments were used to obtain assignments of ^{1}H resonances and to elucidate the proton spatial proximity pattern of mastoparan X in micelles.

Resonance assignments

All the observable ^{1}H NMR resonances of mastoparan X in *n*-octylglucoside at 50 °C could be assigned from F2-regioselective 2D experiments in H_2O by using the sequential assignment strategy (Wüthrich, 1986). The spin systems of 13 of the 14 amino acid residues were delineated from F2-regioselective TOCSY spectra, starting from the amide diagonal. Despite the high molecular weight of the detergent–peptide complex (see Discussion), efficient TOCSY transfers over each of the amino acid spin systems were obtained with the two mixing times of 50 and 100 ms (Fig. 4a). Among the long-chain amino acid residues, isoleucines could be unambiguously distinguished from leucines by their efficient transfer toward the γ methyl protons at the shortest mixing time. Lysine residues were identified by the chemical shift of their ε protons. The unresolved amino protons of lysine residues that exchanged slowly with the solvent were identified by their TOCSY transfers to other lysine protons. Tryptophan and asparagine spin systems were first distinguished by the characteristic NOESY coupling of their β-protons to indole protons for the former and to side-chain amide protons for the latter. Tryptophan cyclic proton resonances were then assigned on the basis of their characteristic TOCSY and NOESY mutual coupling network and their NOESY coupling to β-protons. Methionine terminal methyl protons were identified from their intraresidue

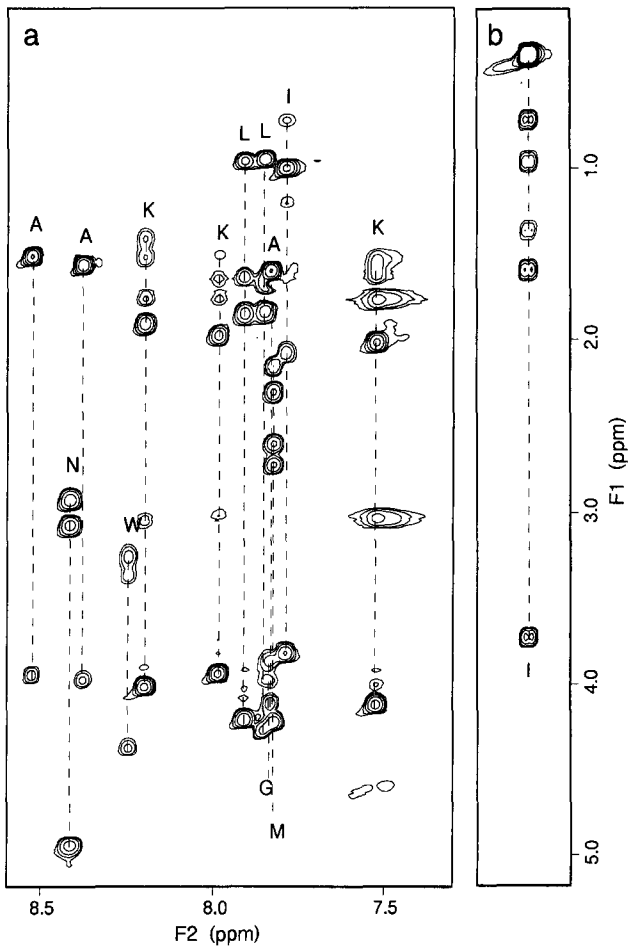

Fig. 4. F2-regioselective TOCSY spectra of mastoparan X in *n*-octylglucoside (mixing time 100 ms) in the cross-peak region correlating aliphatic and α-protons to (a) amide protons and (b) Ile^1 γ methyl protons. See text for details.

TABLE 1
CHEMICAL SHIFTS OF PROTON RESONANCES OF MASTOPARAN IN *n*-OCTYLGLUCOSIDE MICELLES

Residue	Chemical shift (ppm)			
	NH	$C^{\alpha}H$	$C^{\beta}H$	Others
Ile^{1}		3.68	1.55	$C^{\gamma}H$ 1.31, 0.93; $C'^{\gamma}H$ 0.29; $C^{\delta}H$ 0.67
Asn^{2}	8.42	4.95	3.05, 2.87	$N^{\varepsilon}H$ 7.88, 6.93
Trp^{3}	8.25	4.37	3.35, 3.21	N1H 10.14; C2H 7.45; C4H 7.47; C5H 6.93; C6H 7.03; C7H 7.43
Lys^{4}	8.20	4.01	1.89	$C^{\gamma}H$ 1.48, 1.38; $C^{\delta}H$ 1.71, 1.71; $C^{\varepsilon}H$ 3.04, 3.04; $N^{\zeta}H$ 7.48
Gly^{5}	7.84	3.97, 3.85		
Ile^{6}	7.79	3.80	2.04	$C^{\gamma}H$ 1.58, 1.15; $C'^{\gamma}H$ 0.95; $C^{\delta}H$ 0.66
Ala^{7}	8.38	3.97	1.51	
Ala^{8}	7.83	4.11	1.55	
Met^{9}	7.82	4.21	2.12, 2.28	$C^{\gamma}H$ 2.70, 2.57; $C^{\varepsilon}H$ 1.89
Ala^{10}	8.53	3.94	1.46	
Lys^{11}	7.98	3.93	1.94, 1.94	$C^{\gamma}H$ 1.60, 1.46; $C^{\delta}H$ 1.74, 1.74; $C^{\varepsilon}H$ 2.99, 2.99; $N^{\zeta}H$ 7.48
Lys^{12}	7.52	4.12	1.99, 1.99	$C^{\gamma}H$ 1.46, 1.56; $C^{\delta}H$ 1.71, 1.71; $C^{\varepsilon}H$ 3.02, 3.02; $N^{\zeta}H$ 7.48
Leu^{13}	7.91	4.21	1.81, 1.81	$C^{\gamma}H$ 1.59; $C^{\delta}H$ 0.89, 0.89
Leu^{14}	7.87	4.28	1.80, 1.80	$C^{\gamma}H$ 1.64; $C^{\delta}H$ 0.91, 0.91
$CONH_2$	7.00, 6.96			

Experimental conditions: 50 °C, pH 3.5.

NOESY coupling to the corresponding amide proton. In principle, the method does not allow the identification of the first residue, since terminal amino protons are usually not observable. However, here several Ile^{1} protons were identified from their NOESY cross peaks with other residues. Among these, the γ methyl protons, which yielded a doublet at 0.29 ppm, i.e. upfield with respect to the detergent spectrum, could be used as a starting point in the F2-regioselective TOCSY spectrum in order to assign the Ile^{1} spin system (Fig. 4b). Assignment of the spin systems of alanine and glycine was straightforward. Sequence-specific assignments were subsequently obtained by tracing $C^{\alpha}H_i$-NH_{i+1}, NH_i-NH_{i+1} and $C^{\beta}H_i$-NH_{i+1} sequential connectivities in the 100 ms NOESY spectrum (Figs. 5 and 6). The proton assignments are listed in Table 1.

Secondary structure

The 100 ms F2-regioselective NOESY spectrum was used to trace the distance connectivities of mastoparan X in the presence of *n*-octylglucoside micelles. A total of 32 intraresidue, 32 sequential and 18 medium-range through-space correlations was found. In particular, the NOESY spectrum displayed clear patterns of strong NH_i-NH_{i+1} connectivities (Fig. 5) between residues 3–14. Since the very close chemical shifts of some of the amide protons precluded the observation of some cross peaks that were merged with the diagonal (Fig. 5a), a doubly regioselective NOESY experiment was used to increase the resolution in a small part of the amide spectral region. This allowed three otherwise undetectable NH_i-NH_{i+1} connectivities to be displayed (Fig. 5b). The regular F2-

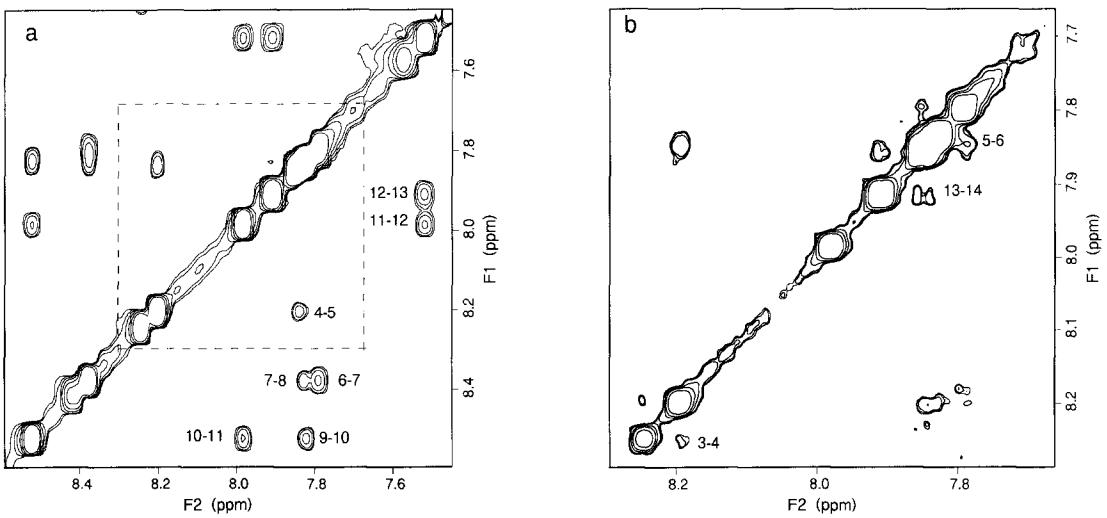

Fig. 5. Regioselective NOESY spectra (amide region) of mastoparan X in *n*-octylglucoside (mixing time 100 ms). (a) F2-regioselective spectrum. (b) Doubly regioselective spectrum of the boxed region in (a). Cross peaks corresponding to amide–amide connectivities are labeled with the residue numbers of the corresponding amino acids.

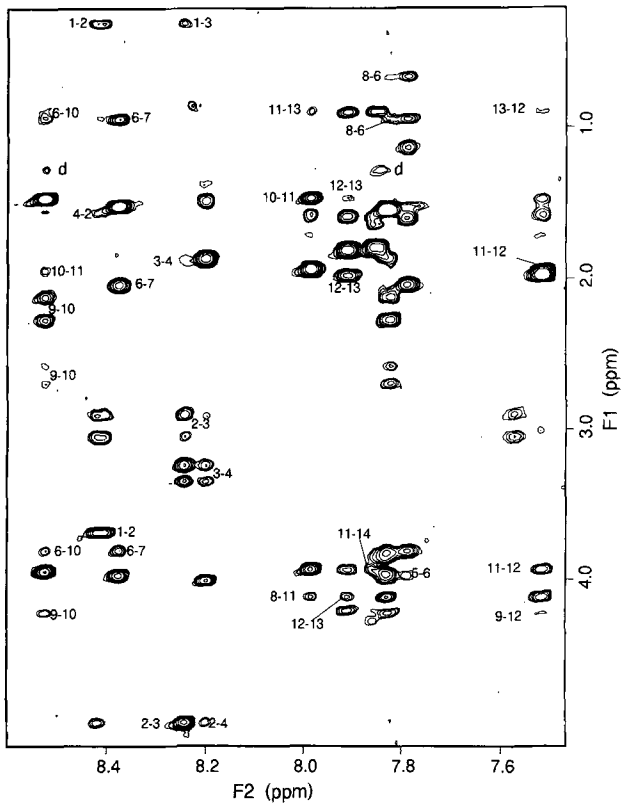

Fig. 6. F2-regioselective NOESY spectrum (mixing time 100 ms) of mastoparan X in *n*-octylglucoside in the region correlating amide to aliphatic and α-protons. Cross peaks corresponding to interresidue connectivities are labeled with the numbers of the corresponding amino acids. Cross peaks between the peptide and the detergent are labeled with d.

regioselective NOESY also displayed medium or weak $C^{\alpha}H_i$-NH_{i+1} connectivities, strong or medium $C^{\beta}H_i$-NH_{i+1} connectivities, as well as weak $C^{\alpha}H_i$-NH_{i+3} and $C^{\alpha}H_i$-NH_{i+4} connectivities (Fig. 6). All these patterns are characteristic of a helical conformation. The fact that some characteristic helix connectivities could not be observed was due to chemical shift degeneracy. Further insight into the backbone conformation was obtained from an F2-regioselective COSY spectrum (Fig. 7) which yielded NH-$C^{\alpha}H$ scalar coupling values of non-glycine residues. Small coupling values were found for most residues, confirming the occurrence of a helix. The main sequential and medium-range connectivities and the backbone scalar coupling values found for mastoparan X in *n*-octylglucoside are displayed in Fig. 8. The detergent-bound peptide appears to adopt a helical conformation, spanning residues 3 to 14.

Discussion and Conclusions

The present study demonstrates that it is possible to apply ^{1}H 2D NMR in the determination of the secondary structure of a membrane peptide solubilized in a non-deuterated detergent. The method is based on the use of regioselective excitation, which eliminates the detergent contribution to TOCSY and NOESY spectra and allows one to obtain all through-bond and through-space connectivities involving peptide amide and aromatic protons. The method appears to be applicable to any detergent that does not yield an NMR contribution in the amide-aromatic region. This is the case for almost all detergents currently in use in membrane research. In this regard, it must be noted that many detergents also do not yield any NMR contribution in part of or in the entire α-proton region. Regioselective excitation can then be extended to this region in order to obtain further connectivities. However, this was not possible with the detergent used here and in fact was found not to be necessary.

For the selection of the peptide region of interest, pulse sequences incorporating 'user-friendly' regioselective pulse trains (Xu et al., 1993; Roumestand et al., 1994) were used. Clean 2D spectra were obtained by this approach, in spite of the very high detergent-to-peptide ratio. The ratio used here is within the range necessary to solubilize most membrane proteins (Moller et al., 1986). We have recently observed that good detergent suppression can be obtained in the case of the 26 kDa membrane protein bacteriorhodopsin at submillimolar concentrations, solubilized with *n*-dodecylmaltoside at molar ratios up to 200. The only drawback of the DANTE-Z and spin-pinging approach, namely doubling of the number of phase steps in the 2D experiment, is unimportant in our application since large numbers of acquisitions are usually required for membrane protein samples. Up to now, regioselective excitation has mainly been used in the F1 dimension of 2D NMR experiments, in order to reduce experimental time or to increase resolution (for a review see Kessler et al. (1991)). Here, F2-regioselective excitation is used to suppress unwanted signals. In addition, doubly regioselec-

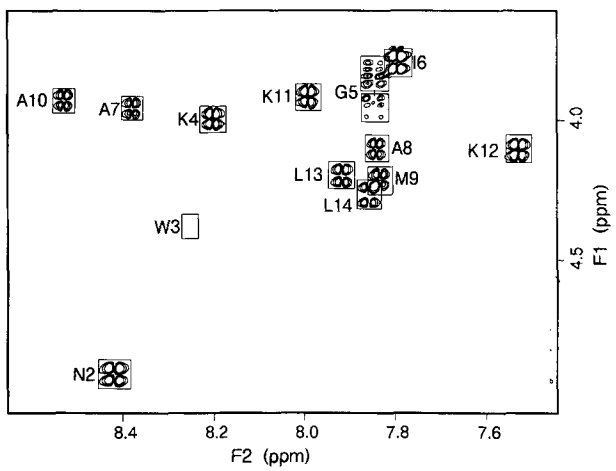

Fig. 7. F2-regioselective COSY spectrum of mastoparan X–*n*-octylglucoside in the fingerprint region. Cross peaks are labeled with the residue number of the corresponding amino acid.

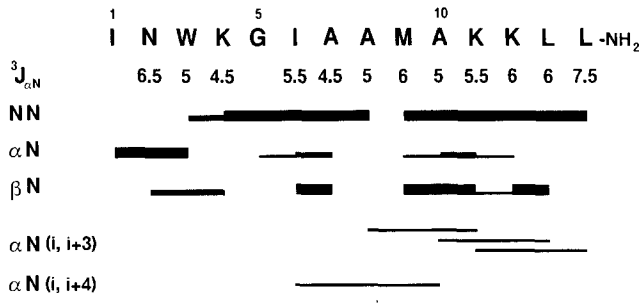

Fig. 8. Sequential and medium-range through-space connectivities and NH-C$^{\alpha}$H scalar coupling constants observed for mastoparan X bound to *n*-octylglucoside micelles.

tive 2D spectra were used to increase resolution in order to compensate for the low chemical shift dispersion that is characteristic of membrane peptides.

In the present approach, through-bond and through-space connectivities have to be traced out with the amide and aromatic (and occasionally α) protons as the only starting points. This might in principle make assignments more difficult, due to incomplete TOCSY transfer as a consequence of the large sizes of detergent–protein complexes. Indeed, for mastoparan bound to *n*-octylglucoside micelles, an effective molecular weight of 15–21 kDa is expected, depending upon the chosen detergent aggregation number (Lorber et al., 1990). However, complete delineation of spin systems from the TOCSY spectra was obtained, which led to complete sequence-specific assignments from NOESY spectra with the sequential methodology (Wüthrich, 1986). For solubilized membrane proteins that are within the scope of 2D ^{1}H NMR (i.e. less than 80 residues), the effective size is dominated by the bound detergent and should remain below 25 kDa provided that detergents forming small micelles are used. Therefore, given the possibilities with many detergents to extend selective excitation to α-protons and to use the less constraining main-chain approach (Wand and Nelson, 1991), it can be expected that extensive intraresidue and sequential assignments should be possible for any protein of this size range by using the regioselective NMR approach described here. In fact, a comparable assignment approach based on TOCSY and NOESY spectra, involving amide ^{1}H, has been used for the structural study by ^{1}H-^{15}N 3D NMR of soluble proteins up to 25 kDa (Archer et al., 1993). Additionally, with mastoparan X, very few NOESY cross peaks between the peptide and the detergent were found. These appear to be stronger in intrinsic transmembrane peptides such as gramicidin A, but do not significantly affect the quality of NOESY spectra (Lévy, D. and Seigneuret, M., unpublished results). A current limitation of the approach is that through-space correlations within most side-chain protons are not recovered in the selective NOESY experiment, even if the selection is extended to α-protons. These side-chain correlations are not essential for secondary structure determination. On the other hand, they are useful for the determination of tertiary structure, e.g. helix–helix interactions. We are currently exploring the use of semi-selective ^{1}H 3D and pseudo-3D TOCSY-NOESY experiments in order to recover these correlations.

The application of this method to the 14-residue peptide mastoparan X has allowed us to determine unambiguously its secondary structure in *n*-octylglucoside. The peptide appears to form a helix ranging from residue 3 to residue 14. The number of distance constraints that have been obtained would be sufficient to obtain a refined structure from restrained molecular dynamics (for a review see James (1994)). The secondary structure determined for mastoparan X is in complete agreement with previous transferred NOE results obtained in the presence of phospholipid vesicles, which evidenced formation of an amphipathic α-helix also spanning residues 3 to 14 (Wakamatsu et al., 1992). On the other hand, the observation of such a structure in *n*-octylglucoside has significant consequences. It has been suggested that interactions between peptide positive side chains and lipid negative groups are important for the interaction of amphipathic helices with membranes (Segrest et al., 1990). Indeed, previous NMR studies of amphipathic helix formation have used lipids or detergents with charged groups (Inagaki et al., 1989; Wakamatsu et al., 1992; Bechinger et al., 1993). The present results indicate that electrostatic interactions do not appear essential for the formation of an amphipathic helix per se, since such a conformation also occurs in an uncharged detergent environment. On the other hand, electrostatic interactions are likely to be important for the positioning of amphipathic helices at lipid membrane surfaces. This result also illustrates that the possibility to choose any detergent for NMR experiments, without the constraint of deuteration, may provide new information.

In conclusion, the method described here may extend the use of ^{1}H NMR for the study of membrane peptides and small proteins. Detergents for NMR might now be selected from other criteria than availability in deuterated form, namely (Seigneuret et al., 1991a): (1) ability to solubilize the protein at high concentration; (2) maintenance of the native state at the highest possible temperature; (3) small size of the detergent–protein complex; (4) low solution viscosity. This might increase the reliability, sensitivity and resolution of such NMR experiments.

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