*Journal of Biomolecular NMR*, **13:** 31–41, 1999.
*KLUWER/ESCOM*



# Mapping of the detergent-exposed surface of membrane proteins and peptides by $^1$H solution NMR in detergent: Application to the gramicidin A ion channel

Michel Seigneuret[a,*] & Christine Le Guernevé[b]
[a]*LPBC (URA 2056), Université Paris 6, 4 place Jussieu, F-75252 Paris cedex 05, France;* [b]*INRA-IPV, 2 place Viala, F-34060 Montpellier cedex 1, France*

Received 5 May 1998; Accepted 14 September 1998

*Key words:* detergents, gramicidin A, membrane protein, molecular surface, selective excitation, three-dimensional NMR

**Abstract**

The present work evaluates the use of intermolecular polypeptide–detergent $^1$H through-space connectivities to determine the bilayer exposed-surface and the bilayer topography of membrane polypeptides solubilized in non-deuterated detergents. For this purpose, the membrane peptide gramicidin A, solubilized in non-deuterated sodium dodecylsulfate as its dimeric $\beta^{6,3}$ helix channel conformation was used. For this peptide, a high-resolution 3D structure, as well as reasonable assumptions concerning its membrane arrangement, exist. Band-selective 2D NOESY, ROESY and 3D NOESY-NOESY experiments were used to detect detergent–polypeptide through-space correlations in the presence of an excess of the non-deuterated detergent. The observed intermolecular NOEs appear to be strongly temperature-dependent. Based on the known 3D structure of the gramicidin channel, the detergent–polypeptide through-space correlations appear to be selective for $^1$H located on the hydrophobic surface of gramicidin A with very few contributions from interior $^1$H or water-exposed $^1$H. It is suggested that this method can be of general use to evaluate the bilayer-exposed surface and topography of membrane peptides and small proteins.

## Introduction

Understanding the activity of membrane proteins and peptides at the molecular level requires knowledge of their detailed structural characteristics in the membrane associated state. Recently extensive efforts have been made to experimentally determine the three-dimensional (3D) structure of several membrane proteins and peptides using the same types of approach as for soluble proteins and peptides, among which NMR (for reviews, see Henry and Sykes, 1994; Opella et al., 1994). In the case of such membrane associated polypeptides, a structural feature as important as the detailed conformation is the arrangement of the protein relative to the lipid bilayer, i.e. knowledge of the lipid embedded regions and their topography. Although the protein arrangement in the bilayer can sometimes be inferred from the 3D structure, this approach is at best qualitative and can be misleading e.g. in cases where the protein or peptide forms oligomers. Direct experimental approaches are required to provide a quantitative determination of the polypeptide portions in contact with the lipid bilayer.

$^1$H solution NMR of solubilized membrane proteins and peptides can in principle identify lipid-exposed regions by measurement of NOESY correlations between detergent and polypeptide $^1$H. However, this method has not been often employed (Papavoine et al., 1994; Williams et al., 1996) and its potential is largely unknown. The method indeed requires the presence of a protonated detergent in excess, the NMR signal of which is likely to mask all polypeptide resonances in standard $^1$H NMR experiments. Attempts

*To whom correspondence should be addressed.

to solve this problem have used either dilution of the protonated detergent with a deuterated analogue (Papavoine et al., 1994) or heteronuclear experiments (Williams et al., 1996). Recently we have shown that band-selective excitation incorporated into 2D or 3D homonuclear $^1$H experiments alleviates the problem of a solubilizing protonated detergent present in excess. (Seigneuret and Levy, 1995; Le Guernevé and Seigneuret, 1996). Here we show that this approach can be applied to detect detergent–protein NOEs and therefore to measure the micelle-exposed surface of a membrane peptide.

For this purpose, we use the peptide antibiotic gramicidin A, a channel forming antibiotic often employed as a model for transmembrane proteins. The structure of its membrane-bound form was first determined by solution NMR in deuterated sodium dodecylsulfate as a right-handed $\beta^{6,3}$ helix head-to-head dimer (Arseniev et al., 1985; Lomize et al., 1992). This has been recently confirmed by solid-state NMR in real membrane environment (Ketchem et al., 1993). The dimer is assumed to span the membrane, thereby forming a channel. Here we use the peptide solubilized in non-deuterated sodium dodecylsulfate to measure the detergent-exposed surface of the channel peptide and show that it is compatible with its 3D structure and current assumptions on its membrane topology.

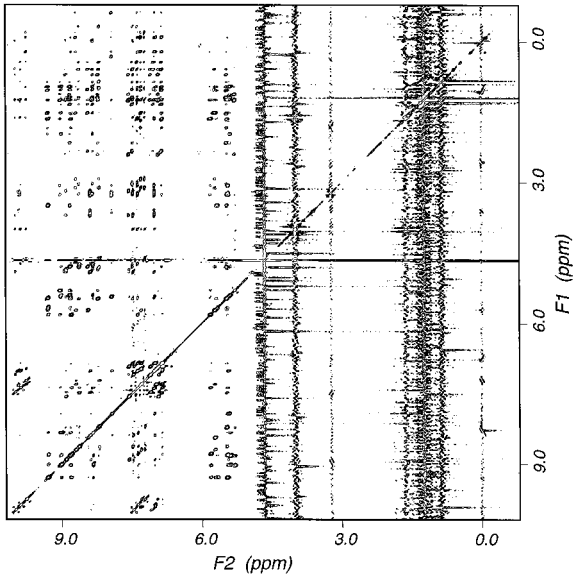


*Figure 1.* F2 band-selective NOESY spectrum of gramicidin A solubilized in non-deuterated sodium dodecylsulfate. A bandwidth from 5.1 to 10.2 ppm including the F2 frequencies of all amide, aromatic, amine and formyl $^1$H and of eight of the $\alpha$ $^1$H was used for selective excitation. An F2 baseline correction was applied in the low field region.

## Materials and methods

Gramicidin A (99% pure) was obtained from Fluka. Perdeuterated and protonated sodium dodecylsulfate were respectively from Eurisotop (France) and Biorad. The gramicidin A / sodium dodecylsulfate samples were prepared according to Arseniev et al. (1985) with the modifications suggested by Killian et al. (1994). Briefly, equal volumes of a 7.5 mM solution of gramicidin A in TFE and of a 275 mM solution of sodium dodecylsulfate in water were first mixed and then diluted 10 times with water. The resulting solution was lyophilized and resuspended at 6 mM peptide in $H_2O$-$D_2O$-TFE 85:5:10 (v:v:v) for NMR studies. $^1$H NMR spectra were recorded at 400 MHz with an AMX 400 spectrometer. NOESY and z-filtered TOCSY spectra were recorded as described (Rance, 1987; Neuhaus and Williamson, 1989). Band-selective 2D and 3D experiments were recorded as in our previous work (Seigneuret and Levy, 1995; Le Guernevé and Seigneuret, 1996). Briefly, band-selective NOESY and z-filtered TOCSY spectra were recorded by inserting a DANTE-z/IBURP pulse train (Roumestand et al., 1995) set to excite a 2050 Hz bandwidth centered at 7.8 ppm (interpulse delay 73 $\mu$s, RF power 10 kHz) before the read pulse. Band-selective ROESY spectra were recorded using a spin-pinging/REBURP pulse train (Roumestand et al., 1995) placed after the spin-lock and set to excite a similar region (interpulse delay 45 $\mu$s, RF power 10 kHz). Band-selective 3D NOESY-NOESY (see Boelens et al. (1989) for the original NOESY-NOESY experiment) spectra were recorded using a sequence similar to the previously described band-selective NOESY-(z-filtered) TOCSY (Le Guernevé and Seigneuret, 1996), except that the spin-lock was replaced with a second mixing time delay, and using identical phase cycling. Spectra were recorded in the States-TPPI mode (Marion et al., 1989) using F1/F2 time domains of 256/1024 complex points or F1/F2/F3 time domains of 128/64/512 complex points. Water suppression was achieved with a WATERGATE pulse sequence (Piotto et al., 1992) using 1 ms and 15 G/cm z-gradient pulses. All spectra were processed with the NMRPipe software (Delaglio et al., 1995). 3D spectra were extended to 184 and 92 complex points in F1 and F2 by forward-backward linear prediction (Zhu and Bax, 1992). All spectra were weighted with shifted sinebells and zero-filled

once in all dimensions before Fourier transformation. A local baseline correction from 5 to 11 ppm was effected in the acquisition dimension using a 5th order polynomial.

Molecular modeling was performed using the so-called molecular surface (Connolly, 1993), defined as the boundary of the volume defined by the periphery of a probe sphere rolling over the molecule. All calculations described here were performed with structure no. 1 from the PDB file 1grm of gramicidin A in sodium dodecylsulfate (Arseniev et al., 1983). Use of another structure did not significantly change the results. A probe radius of 1 Å corresponding to a detergent hydrogen was employed. The contribution of each atom to the molecular surface of gramicidin A was calculated with the program Naccess 2.1 (Hubbard and Thornton, 1993). The minimum distances of interior atoms to the molecular surface were calculated using the program GRASP (Nicholls et al., 1991) which was also used for all surface drawings. Local hydrophobicities were expressed using the atomic fractional hydrophobicity parameters of Ghose et al. (1988) which were summed over atoms for carbonyl and protonated carbons or nitrogens of gramicidin A.

## Results

### *Temperature dependence of $^1H$ NMR spectra and 3D structure of gramicidin A*

The present study is based upon the observation of NOESY connectivities between gramicidin A $^1$H and non-deuterated sodium dodecylsulfate $^1$H. The strong temperature dependence of the corresponding cross peaks required studies to be performed at three temperatures: 55, 35 and 25 °C. The previous $^1$H NMR study of the gramicidin A structure in deuterated sodium dodecylsulfate has provided complete assignments and the peptide 3D structure at 55 °C. It was therefore necessary as a preliminary step to perform assignments and examine the gramicidin structure in deuterated sodium dodecylsulfate at lower temperature. At 55 °C, the gramicidin A chemical shifts and NOESY constraints were identical to those of Arseniev et al. (1985) in spite of the slightly different sample preparation used. At lower temperatures, the increased rotational correlation time associated with the higher viscosity of the detergent solution led to larger linewidth and decreased efficiency of scalar coupling-based experiments. At 35 °C, TOCSY spectra were sufficiently informative to provide most intraresidue assignments. At 25 °C, such spectra were very poor and provided very few assignments. On the other hand, high-quality NOESY spectra were obtained at 35 and 25 °C that displayed cross peak patterns very similar in chemical shifts and intensities to those measured at 55 °C. This allowed us in particular to assign all gramicidin A resonances at 25 and 35 °C by simple comparison of the corresponding NOESY spectra with those obtained at 55 °C. Comparison of interproton distances calculated from interresidue cross peaks intensities also indicated that the 3D structure of gramicidin A in sodium dodecylsulfate is largely independent of temperature from 25 to 55 °C

### *Band-selective excitation of gramicidin A $^1H$ in the presence of non-deuterated detergent*

Measurement of through-space correlations between a membrane peptide and detergent $^1$H in micelles requires the use of a non-deuterated detergent at concentrations two orders of magnitude higher than that of the peptide. NMR experiments incorporating band-selective excitation of peptide resonances have therefore been developed in order to obtain homonuclear 2D and 3D spectra in which the interference from detergent resonances is removed (Seigneuret and Levy, 1995; Le Guernevé and Seigneuret, 1996).

Figure 1 shows an example of the application of band-selective NMR experiments to gramicidin A solubilized in non-deuterated sodium dodecylsulfate. Band-selective excitation of a spectral region encompassing all amide–aromatic resonances and of the low-field alpha resonances is effected in the acquisition dimension of a NOESY experiment. Since the detergent diagonal peaks (occurring in the aliphatic region) are reduced by two orders of magnitude, it is possible to observe all cross peaks involving the selected peptide resonances without interference from the edges of these diagonal peaks that would otherwise mask most cross peaks. Although correlations between aliphatic protons are absent in the 2D band-selective experiments, these can be recovered in 3D band-selective experiments.

### *Detection of through-space correlations between detergent protons and gramicidin A aromatic and formyl protons by band-selective NOESY*

An important aspect of such band-selective experiments is that, although the contribution from most aliphatic $^1$H is cancelled in the acquisition dimension, it is present during all previous phases of the

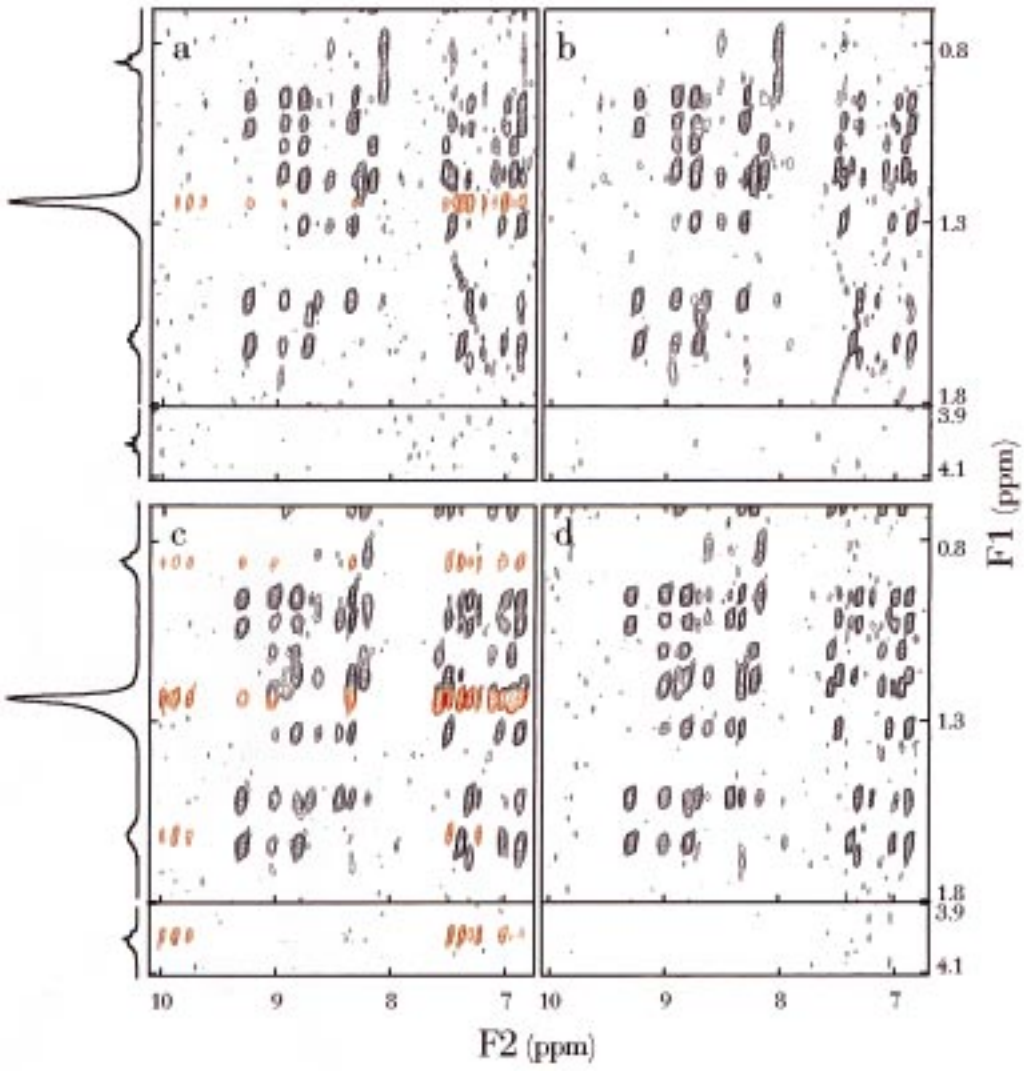


*Figure 2.* Regions correlating amide/aromatic and aliphatic $^{1}$H of the F2-band-selective NOESY spectrum (mixing time 100 ms) of gramicidin A in non-deuterated (left side: a, c) and deuterated (right side: b, d) sodium dodecylsulfate recorded at 55 (a, b) and 35 °C (c, d). Cross peaks corresponding to detergent–peptide intermolecular correlations are drawn in red. The 1D spectrum of the corresponding sample in the detergent aliphatic region is drawn on the side for each temperature. The resonances correspond respectively to the terminal methyl, the nine bulk methylenes, the β methylene and the α methylene of the detergent when going downfield.

experiments. This is true in particular for detergent $^{1}$H magnetisation and this feature allows through-space connectivities between detergent and peptide to be detected in NOESY experiments. At the various studied temperatures, most detergent resonances have frequencies that are distinct from those of aliphatic peptide resonances but occasional degeneracy occurs. In order to bypass this limitation as well as for illustrative purposes, we have therefore chosen to compare band-selective NOESY spectra obtained with non-deuterated and deuterated sodium dodecylsulfate. Figure 2 shows identical portions of the region correlating aliphatic $^{1}$H to amide/aromatic $^{1}$H for gramicidin A solubilized in both non-deuterated and deuterated sodium dodecylsulfate at two temperatures. 1D spectra showing the detergent resonances are displayed on the left. At both temperatures, for the protonated detergent sample, additional cross peaks (in red) are apparent, the F1 frequencies of which correspond to chemical shifts of detergent resonances. These features correspond to detergent–peptide through-space correlations. Sodium dodecylsulfate yields four re-

solved resonances corresponding respectively to terminal $CH_3$, the nine bulk methylenes, $\beta CH_2$ and $\alpha CH_2$ with increasing chemical shifts. All these protons from the detergent appear to be able to interact dipolarly with peptide protons and to yield visible intermolecular cross peaks. On the peptide side, these are mainly aromatic $^1H$ as well as the formyl and ethanolamine $^1H$ that are implicated. Very few cross peaks occur between detergent and amide $^1H$, as well as with the $\alpha$ $^1H$ (region not shown). The intensity of the detergent–peptide cross peaks depends strongly on temperature. At 55 °C, such cross peaks are relatively weak and occur only with the most intense methylene detergent resonance. On the other hand, at 35 °C, cross peaks corresponding to the other detergent $^1H$ are present and the corresponding intensities become stronger. Although at such low temperatures particular detergent resonances occasionally are nearly degenerated with peptide resonances, the detergent–peptide cross peaks can be identified unambiguously. Intermolecular cross peaks observed at 25 °C were similar to those at 35 °C, except for the disappearance of indol $^1H$-detergent $CH_3$ cross peaks (not shown).

A possible explanation for the intensity decrease or disappearance of detergent–peptide cross peaks at high temperature is that one of the correlation times that modulates intermolecular dipolar interactions becomes close to the resonance frequency and therefore leads to quenched NOEs. In order to check this hypothesis, band-selective ROESY experiments were performed. A limitation was that, due to the short $T_2$ of peptide resonances, as well as to the use of a less efficient sping-pinging/REBURP selective excitation, ROESY experiments suffered from an overall loss in intensity as compared to NOESY experiments. All peptide–peptide and peptide–detergent ROESY cross peaks are negative at all studied temperatures. As shown in Figure 3, several of the detergent–peptide cross peaks that are not present at 55 °C in the NOESY spectrum of gramicidin A are indeed apparent in the corresponding ROESY spectrum recorded at the same temperature (see Discussion).

Most of the detergent–peptide cross peaks could be directly assigned to specific gramicidin $^1H$ from the above 2D band-selective NOESY spectra. However, several aromatic $^1H$ have degenerate or nearly degenerate chemical shifts, especially at low temperatures. In these particular cases, the exact assignments were deduced from 3D band-selective NOESY-NOESY (see below) from the cross peaks resulting from detergent-$^1H$-aromatic $^1H$-aromatic $^1H$ transfers

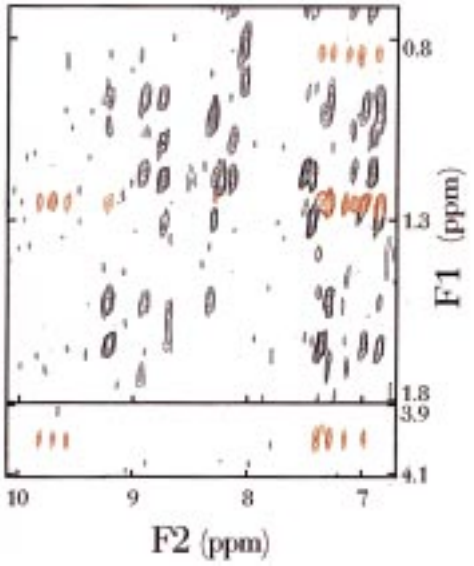


*Figure 3.* Region correlating amide/aromatic and aliphatic $^1H$ of the F2-band-selective ROESY spectrum recorded with a mixing time of 50 ms at 55 °C for gramicidin A solubilized in non-deuterated sodium dodecylsulfate. Cross peaks corresponding to detergent–peptide intermolecular correlations are drawn in red.

(such assignments are non-ambiguous since all strong through-space connectivities between aromatic $^1H$ are intraresidue in gramicidin A).

*Detection of through-space correlations between detergent protons and gramicidin A aliphatic protons by band-selective NOESY-NOESY*

Knowledge of through-space correlations in the aliphatic region is essential since these contain most of the information on proximities between the detergent hydrocarbon chain and the peptide hydrophobic surface side chains. In a previous work, we have shown that aliphatic–aliphatic through-space correlation can be recovered in 3D band-selective NOESY-TOCSY experiments (Le Guernevé and Seigneuret, 1996). In the case of gramicidin A in sodium dodecylsulfate, we have found that NOESY-NOESY experiments displayed similar information with a much higher sensitivity. Figure 4 compares the aliphatic portions of the same two F1-F2 planes for samples containing hydrogenated and deuterated sodium dodecylsulfate and corresponding respectively to one amide F3 frequency (Figures 4a and b) and one $\alpha$ $^1H$ frequency (Figures 4c and d) for gramicidin A. Again additional cross peaks (in red) that correspond to detergent F1 frequencies are found for the non-deuterated sample. These are due to NOESY transfer between detergent and peptide

*Table 1*. Gramicidin A protons yielding NOEs with sodium dodecylsulfate protons at 25 and 35 °C and their relations to the peptide molecular surface

| Residue | Proton | Detergent $^1$H involved in NOE | Contribution to molecular surface (Å$^2$)[a] | Distance to molecular surface (Å)[b] |
|---|---|---|---|---|
| Formyl | HCO | $CH_3$, $(CH_2)_9$ | 0.685 | |
| L-Val 1 | NH | $CH_3$, $(CH_2)_9$ | 0.000 | 1.23 |
| | $C^{\gamma}H$ | $(CH_2)_9$ | 5.095 | |
| Gly 2 | $C^{\alpha}H$ | $(CH_2)_9$ | 5.275 | |
| L-Ala 3 | $C^{\beta}H$ | $CH_3$, $(CH_2)_9$ | 2.421 | |
| D-Leu 4 | $C^{\beta}H$ | $CH_3$, $(CH_2)_9$ | 1.325 | |
| | $C^{\gamma}H$ | $(CH_2)_9$ | 0.378 | |
| L-Ala 5 | NH | $(CH_2)_9$ | 0.000 | 1.24 |
| | $C^{\alpha}H$ | $CH_3$, $(CH_2)_9$ | 0.000 | 1.05 |
| | $C^{\beta}H$ | $CH_3$, $(CH_2)_9$, $C^{\alpha}H_2$ | 1.165 | |
| D-Val 6 | $C^{\gamma}H$ | $CH_3$ | 6.315 | |
| L-Val 7 | $C^{\alpha}H$ | $CH_3$, $(CH_2)_9$ | 0.054 | |
| | $C^{\gamma}H$ | $CH_3$, | 5.376 | |
| D-Val 8 | $C^{\beta}H$ | $(CH_2)_9$ | 0.000 | 1.10 |
| | $C^{\gamma}H$ | $CH_3$, $C^{\beta}H_2$ | 4.121 | |
| L-Trp 9 | 2H | $CH_3$ | 0.823 | |
| | N1H | $CH_3$[c], $C^{\beta}H_2$, $C^{\alpha}H_2$ | 9.049 | |
| | 6H | $CH_3$, $(CH_2)_9$ | 10.937 | |
| D-Leu 10 | $C^{\delta}H$ | $CH_3$, $(CH_2)_9$, $C^{\beta}H_{2,}$, $C^{\alpha}H_2$ | 3.580 | |
| L-Trp 11 | $C^{\beta}H$ | $CH_3$, $(CH_2)_9$, $C^{\beta}H_2$ | 4.520 | |
| | N1H | $CH_3$[c], $(CH_2)_9$, $C^{\beta}H_{2,}$, $C^{\alpha}H_2$ | 12.559 | |
| | 4H | $CH_3$, $(CH_2)_9$ | 3.032 | |
| | 7H | $(CH_2)_9$, $C^{\alpha}H_2$ | 11.029 | |
| | 5H | $CH_3$, $(CH_2)_9$ | 7.895 | |
| | 6H | $CH_3$, $(CH_2)_9$ | 11.035 | |
| D-Leu 12 | $C^{\delta}H$ | $CH_3$, $(CH_2)_9$, $C^{\alpha}H_2$ | 6.938 | |
| L-Trp 13 | $C^{\beta}H$ | $CH_3$, $(CH_2)_9$ | 4.919 | |
| | 2H | $(CH_2)_9$, $C^{\alpha}H_2$ | 10.993 | |
| | N1H | $CH_3$[c], $(CH_2)_9$, $C^{\beta}H_2$, $C^{\alpha}H_2$ | 14.004 | |
| | 7H | $CH_3$, $(CH_2)_9$, $C^{\alpha}H_2$ | 10.854 | |
| | 6H | $CH_3$, $(CH_2)_9$, $C^{\beta}H_2$ | 11.050 | |
| D-Leu 14 | $C^{\beta}H1$ | $C^{\beta}H_2$, $C^{\alpha}H_2$ | 1.417 | |
| | $C^{\delta}H$ | $CH_3$, $(CH_2)_9$, $C^{\beta}H_2$, $C^{\alpha}H_2$ | 5.594 | |
| L-Trp 15 | N1H | $CH_3$[c],$(CH_2)_9$, $C^{\beta}H_2$, $C^{\alpha}H_2$ | 3.506 | |
| | 4H | $(CH_2)_9$ | 0.000 | 1.09 |
| | 7H | $CH_3$, $C^{\beta}H_2$, $C^{\alpha}H_2$ | 2.742 | |
| | 5H | $CH_{3,}$ $(CH_2)_9$ | 0.543 | |
| EA | NH | $C^{\alpha}H_2$ | 4.135 | |

[a]Calculated with Naccess 2.1 using a probe radius of 1 Å.
[b]The shortest distances of interior atoms to exterior molecular surface range from 1.05 to 2.87 Å.
[c]Only visible at 35 °C.

aliphatic $^1$H followed by a second NOESY transfer from the peptide aliphatic $^1$H to the amide or $\alpha$ $^1$H. In spite of occasional overlap with intrapeptide aliphatic cross peaks due to the limited resolution of 3D experiments, 32 such detergent–aliphatic $^1$H could be detected by examination of 3D spectra at 35 °C and 25 °C.

*Evaluation of gramicidin A bilayer exposed surface from detergent–peptide NOESY contacts*

In all, 82 through-space correlations between detergent and gramicidin A protons were derived from band-selective NOESY and NOESY-NOESY experiments. One of our goals was to determine whether such intermolecular connectivities can be used as a probe of the detergent-exposed molecular surface of the peptide. The molecular surface associated with the channel conformation of gramicidin A in micelles (Arseniev et al., 1985; Lomize et al., 1992) was calculated. Table 1 lists all peptide $^1$H for which through-space connectivities with the detergent were observed as well as all protons from the molecular surface. It is visible from Table 1 that the majority of the gramicidin $^1$H that yield NOE cross peaks with the detergent belong to the molecular surface of the peptide according to the structure of Arseniev et al. (1985). The only few exceptions to this rule are some interior $^1$H that are among the closest to the surface of the peptide. There are several $^1$H of the gramicidin A molecular surface that do not correspond to any of the established peptide–detergent through-space correlations. These belong to three categories. One trivial category consists of those peptide $^1$H for which no cross peak with any of the detergent $^1$H could be established due to the occurrence of intrapeptide cross peaks at the corresponding resonance positions (this occurs mainly for aliphatic $^1$H due to the limited resolution of 3D experiments). A second, much more significant category comprises all the surface $^1$H that are located around the water-exposed 'mouth' of the gramicidin channel based on the structure of Arseniev et al. (1985) and Lomize et al. (1992), i.e. out of the bilayer-exposed surface. A third category consists of $^1$H that should belong to the bilayer-exposed surface but do not display any cross peak with the detergent, presumably due to sensitivity reasons. Figure 5 shows a representation of the molecular surface of the channel conformation of gramicidin A in which the atom surface contributions are colored in blue for all $^1$H having detergent connectivities and in red for other $^1$H. Clearly, most of the peptide $^1$H sampled by the detergent appear to be located on that part of the surface that is expected to be micelle- or membrane-exposed. In spite of some missing $^1$H due to spectral overlap, there appears to be a relatively uniform sampling of this hydrophobic surface. Comparatively, virtually no $^1$H from the part of the surface expected to be water-exposed appear to be involved in any NOE interaction with the detergent. It appears that the limit between the micelle-exposed and water-exposed surfaces of gramicidin A occurs at the level of the tryptophan indols that do interact with the detergent in our experiments and consistently are supposed to be H-bonded to lipid carbonyl in membranes (Hu and Cross, 1995). It is interesting to compare the data of Figure 5 with those of Figure 6 in which the gramicidin A surface groups have been color-coded according to their hydrophobicity. Qualitatively, there seems to be a fair correlation between the distribution of $^1$H yielding NOEs with detergent on the molecular surface and the local hydrophobicity of this surface.

*Information gained on the gramicidin A membrane topography from detergent–peptide NOESY contacts*

In the NMR spectrum of sodium dodecylsulfate, the $\alpha$ and $\beta$ methylene $^1$H as well as the chain terminal methyl group yield resolved single $^1$H resonances (while all other methylene $^1$H correspond to an unresolved line). These methylene and methyl groups are expected to have average positions that correspond respectively to the micelle surface and interior. It was therefore investigated whether through space correlations between these specific detergent $^1$H and gramicidin A $^1$H may provide information concerning the topography of particular residues of the peptide inside the detergent micelle. The information that can be gathered is quite limited. Obviously, as judged from Table 1, no spatial selectivity is found in the hydrophobic surface $^1$H sampled by the detergent methyl. Indeed, such $^1$H are scattered almost uniformly on this surface (i.e. from Val 1 to Trp 15). However, as already, mentioned, it appears that the tryptophan indol $^1$H are sampled only at 35 °C and not at 25 °C which may indicate that selectivity is better at lower temperature. In the case of the detergent $\alpha$ methylene, the spatial selectivity appears still limited but more satisfactory since only peptide surface $^1$H significantly closer to the extremity of the pore are involved (i.e. from Ala 5 to Trp 15).

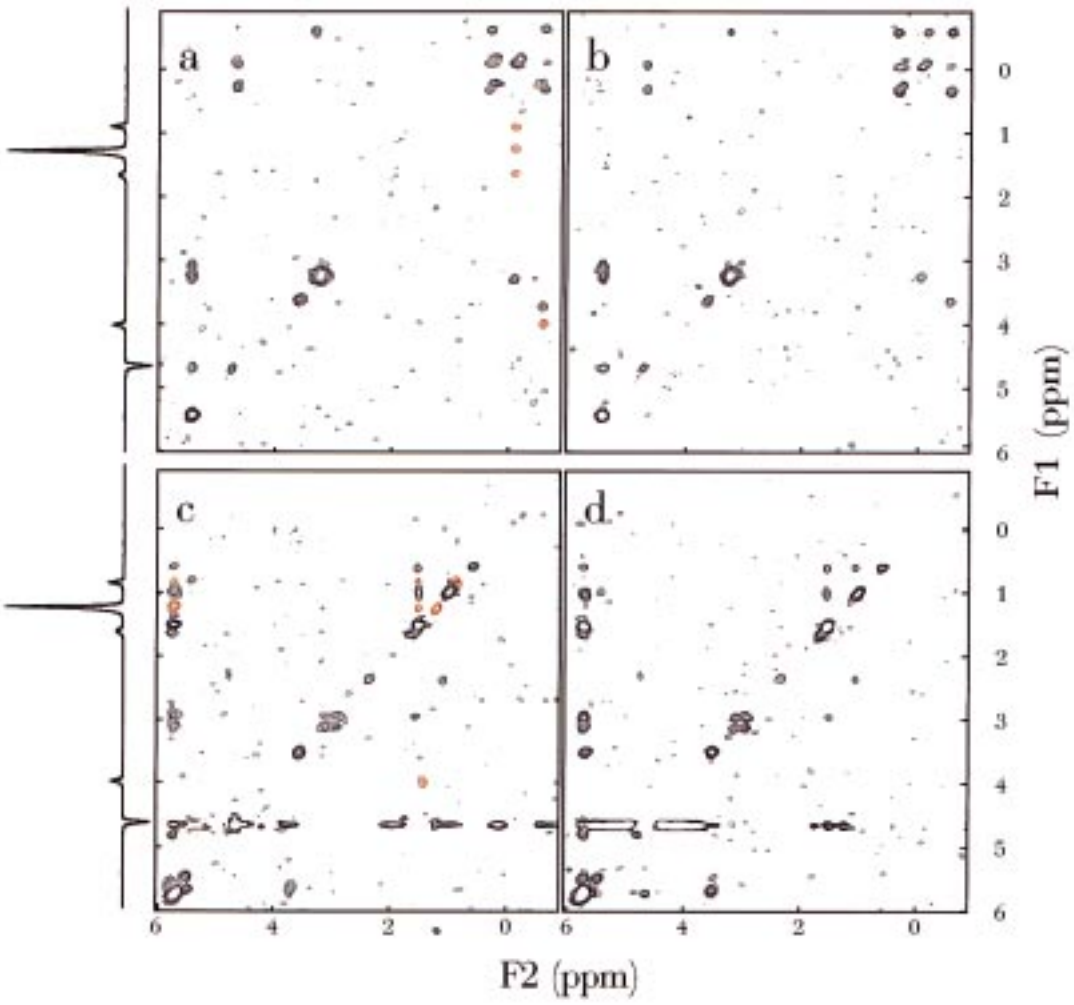


*Figure 4.* F1-F2 planes from the F3-band-selective NOESY-NOESY spectrum (both mixing times 100 ms) of gramicidin A in non-deuterated (left side: a, c) and deuterated (right side: b, d) sodium dodecylsulfate at 35 °C corresponding to the F3 frequency of the Leu 10 amide $^{1}$H (a, b) and of the Ala 5 α $^{1}$H (c, d). Cross peaks corresponding to detergent–peptide intermolecular correlations are drawn in red. The 1D spectrum of the corresponding sample in the detergent aliphatic region is drawn on the side.

## Discussion and conclusions

The purpose of the present study is the measurement of the detergent-exposed surface and of the topography of solubilized membrane peptides and small proteins by NMR. In order for this study to have biochemical interest, it is therefore required that the embedded portion of the polypeptide be similar in a detergent micelle and a lipid bilayer. Numerous studies indicate that this is the case at least qualitatively (for a review see Moller et al., 1986). However, quantitative differences are likely to occur. For example, detergents usually have shorter paraffinic chains than phospholipids so that the width of the hydrophobic part of detergent micelles is smaller than that of lipid bilayers. Moreover, the hydrophilic surface of natural lipid bilayers may contain large sugar headgroups that may be in contact with the hydrophilic portions of membrane proteins. Therefore, delineation of the bilayer-exposed areas of a membrane protein or peptide from data obtained in detergent should be consirered as qualitative.

Measurements of NOESY correlations between a solubilized membrane peptide or protein and a protonated detergent have been performed in two previous studies for qualitative estimations of the micellar topography. Papavoine et al. (1994) used classical homonuclear 2D NOESY to monitor M13 coat

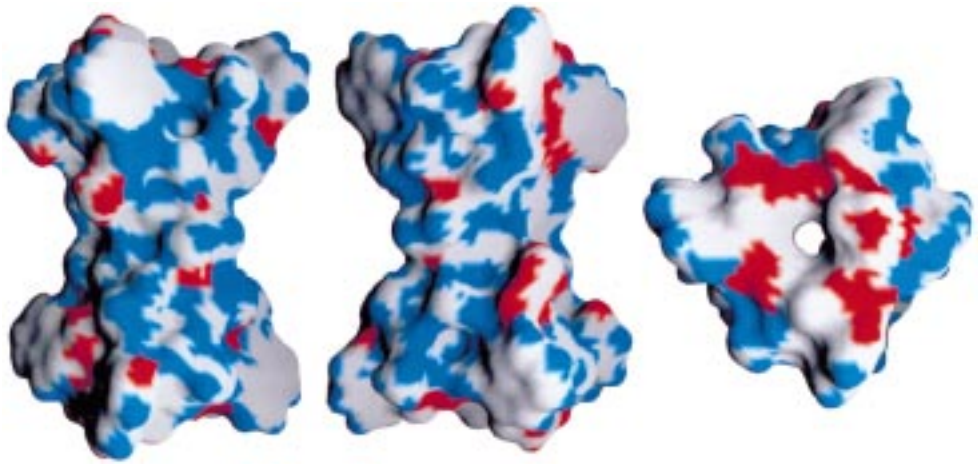

*Figure 5.* Molecular surface representation of gramicidin A in its transmembrane channel structure according to Arseniev et al. (1985). The atomic contribution to the molecular surface is drawn in blue for $^{1}$H yielding at least one NOE with a detergent $^{1}$H at 35 or 25 °C, in red for $^{1}$H yielding no such NOE and in grey for all other atoms.

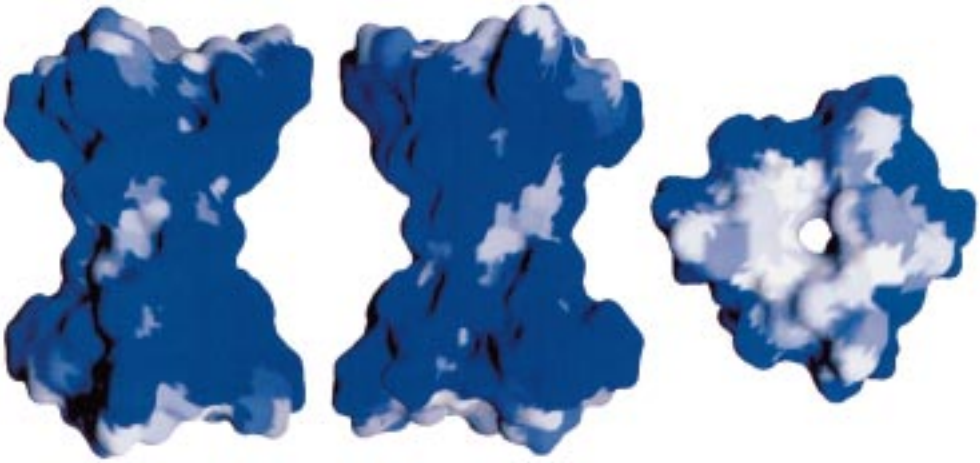

*Figure 6.* Molecular surface representation of gramicidin A in its transmembrane channel structure according to Arseniev et al. (1985). Each group of the molecular surface is colored according to its hydrophobicity which was calculated as described in Materials and methods. The hydrophobicity was color-scaled linearly from white (RGB value 111), corresponding to the less hydrophobic groups (i.e. peptide amino groups), to blue (RGB value 001), corresponding to the more hydrophobic groups (i.e. leucine or valine methyls).

protein-sodium dodecylsulfate interactions. Due to artefacts introduced by the hydrogenated detergent, a fivefold dilution in deuterated analog was necessary with an inherent loss of sensitivity which led to the use of very long mixing times (500 ms) to observe very weak intermolecular NOEs between the detergent main methylene peak and resonances from a few protein residues. Comparatively, the second approach used by these authors, namely paramagnetic broadening with spin labels, appeared more efficient in reporting on protein bilayer topography. Williams et al. (1996) used NOESY-HSQC and NOESY-HMQC experiments on doubly $^{13}$C-$^{15}$N labeled Ike coat protein solubilized in lysophosphatidylglycerol, the signal of which was filtered out by the heteronuclear editing. Several NOEs between the detergent and the protein were detected which could be used to delineate the protein arrangement in the micelle. A complication of this approach is of course that it requires isotopic labeling of the membrane protein or peptide (although

of course heteronuclear NMR is applicable to a wider range of protein molecular weights). The approach used in the present study to observe detergent–protein through-space correlations combines the simplicity of homonuclear methods with the sensitivity of using a fully protonated detergent.

For gramicidin A in sodium dodecylsulfate, the intensities of cross peaks corresponding to detergent–peptide through-space correlations appear to be very sensitive to temperature. A possible explanation for such sensitivity is, as suggested by band-selective ROESY experiments, the fact that at least one of the correlation times modulating the intermolecular dipolar interaction is close to the inverse of the resonance angular frequency, i.e. ~0.5 ns in our case. Using previous data from NMR relaxation measurements on sodium dodecylsulfate (Soderman et al., 1988; Monduzzi et al., 1990), a dwell time of 1.0 ns for a 2D lateral diffusion process (Davoust et al., 1983) can be calculated for micellar detergent molecules. This value is reasonably close to the inverse of the resonance angular frequency. This may indicate that detergent lateral diffusion, i.e. on–off exchange at the peptide boundary, may indeed contribute to the temperature dependance of detergent–peptide NOEs. However, further studies are necessary to clarify this point.

Provided that the proper temperature is selected (i.e. 35 or 25 °C in our case), our results on the gramicidin A/sodium dodecylsulfate system indicate that relevant data can be obtained on the bilayer-exposed surface of a membrane peptide or small protein by band-selective NOESY and NOESY-NOESY. The detergent-accessible surface reported by these experiments appears to be compatible with the bilayer-exposed surface expected from current assumptions on the membrane arrangement of the gramicidin cation channel (Killian, 1992). There is also a good correlation between such detergent-exposed areas and the distribution of local hydrophobicity on the surface of the gramicidin A pore.

The information gathered concerning the topography of gramicidin A surface residues by comparing NOEs involving distinct detergent $^1$H appears much more limited. This can be explained by the motion of detergent molecules inside micelles, documented from NMR relaxation data (Soderman et al., 1988; Monduzzi et al., 1990) and molecular dynamics calculations (Wendoloski et al., 1989). Each methylene segment typically has a low order parameter so that, for example, the detergent terminal methyl undergoes a quasi-isotropic motion within the micelle interior. Also, each detergent molecule undergoes significant transverse motion so that the headgroup region can partially get buried into the micelle interior. This may explain the non-existent or limited selectivity of intermolecular NOEs involving specific detergent $^1$H. Improvement of the spatial selectivity of such topography studies might be obtained at lower temperatures were the mobility of detergent molecules would be decreased.

The approach described here would greatly benefit from the use of higher field strengths. This would in particular lead to an increase in resolution both directly and through the possibility, afforded by the higher sensitivity to use more dilute and less viscous samples. Such a resolution improvement would allow the use of lower temperatures, thereby increasing the overall sensitivity of the method as well as its accuracy for the study of peptide topography. It might also eliminate the need to perform experiments with the deuterated detergent as control, so that the method might be applicable with detergents not available in deuterated form.

## References


Arseniev, A.S., Barsukov, I.L., Bystrov, V.F., Lomize, A.L. and Ovchinnikov, Y.A. (1985) *FEBS Lett.*, **186**, 168–174.

Boelens, R., Vuister, G.H., Koning, T.M.G. and Kaptein, R. (1989) *J. Am. Chem. Soc.*, **111**, 8525–8526.

Connoly, M.L. (1983) *Science*, **221**, 709–713.

Davoust, J., Seigneuret, M. and Hervé, P. (1983) *Biochemistry*, **22**, 3137–3145.

Delaglio, F., Grzesiek, S., Vuister, G., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Ghose, A.K., Pritchett, A. and Crippen, G.M. (1988) *J. Comput. Chem.*, **9**, 80–90.

Henry, G.D. and Sykes, B.D. (1994) *Methods Enzymol.*, **239**, 515–535.

Hu, W. and Cross, T.A. (1995) *Biochemistry*, **34**, 14147–14155.

Hubbard, S. and Thornton, J.M. (1993) Naccess, Computer Program, Department Biochemistry and Molecular Biology, University College, London, U.K.

Ketchem, R.R., Hu, W. and Cross, T.A. (1993) *Science*, **261**, 1457–1460.

Killian, J.A. (1992) *Biochim. Biophys. Acta*, **1113**, 391–425.

Killian, J.A., Trouard, T.P., Greathouse, D.V., Chupin, V. and Lindblom, G. (1994) *FEBS Lett.*, **348**, 161–165.

Le Guernevé, C. and Seigneuret, M. (1996) *J. Biomol. NMR*, **8**, 219–222.

Le Guernevé, C. and Seigneuret, M. (1996) *Biophys. J.*, **71**, 2633–2644.

Lomize, A.L., Orekhov, V.I. and Arseniev, A.S. (1992) *Bioorg. Khim.*, **18**, 182–200.

Marion, D., Ikura, M., Tschudin, R. and Bax, A. (1989) *J. Magn. Reson.*, **85**, 393–401.

Moller, J.V., Le Maire, M. and Andersen, J.P. (1986) In *Progress in Protein–Lipid Interactions*, Vol. 2 (Eds, Watts, A. and De Pont, J.M.M.), Elsevier, Amsterdam, pp. 147–196.

Monduzzi, M., Ceglie, A., Lindman, B. and Soderman, O. (1990) *J. Colloid Interface Sci.*, **136**, 113–123.

Neuhaus, D. and Williamson, M. (1989) *The Nuclear Overhauser Effect in Structural and Conformational Analysis*, VCH Publishers, New York, NY.

Nicholls, A., Sharp, K. and Honig, B. (1991) *Proteins Struct. Funct. Genet.*, **11**, 281–292.

Opella, S.J., Kim, Y. and McDonnell, P. (1994) *Methods Enzymol.*, **239**, 536–560.

Papavoine, C.H.M., Konings, R.N.H., Hilbers, C.W. and van de Ven, F.J.M. (1994) *Biochemistry*, **33**, 12990–12997.

Piotto, M., Saudek, V. and Sklenar, V. (1992) *J. Biomol. NMR*, **2**, 661–665.

Rance, M. (1987) *J. Magn. Reson.*, **63**, 557–567.

Roumestand, C., Mispelter, J., Austruy, C. and Canet, D. (1995) *J. Magn. Reson.*, **B106**, 68–71.

Soderman, O., Carlstrom, G., Olsson, U. and Wong, T.C. (1988) *J. Chem. Soc. Faraday Trans. I*, **84**, 4475–4481.

Seigneuret, M. and Levy, D. (1995) *J. Biomol. NMR*, **5**, 345–352.

Wendoloski, J.J., Kimatian, S.J., Schutt, E. and Salemme, F.R. (1989) *Science*, **243**, 636–638.

Williams, K.A., Farrow, N.A., Deber, C.M. and Kay, L.E. (1996) *Biochemistry*, **35**, 5145–5157.