ELSEVIER

Biochimica et Biophysica Acta 1416 (1999) 48–56

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

NMR structure of C-terminally tagged gramicidin channels

Frances Separovic [a,*], Stephen Barker [b], Matthew Delahunty [a], Ross Smith [b]

[a] *School of Chemistry, University of Melbourne, Parkville, VIC 3052, Australia*
[b] *Department of Biochemistry, University of Queensland, QLD 4072, Australia*

Received 1 June 1998; received in revised form 16 October 1998; accepted 16 October 1998

Abstract

A biotin group was covalently attached to the C terminus of gramicidin A (gA) through a linker arm comprising a glycine residue with either one (gAXB) or two caproyl groups (gAXXB). High-resolution two-dimensional NMR spectroscopy was used to determine the structure of these modified gA analogues and [Lys^{16}]gramicidin A (gA-Lys) in sodium dodecyl-d_{25} sulphate micelles. Gated gA ion channels based on linking a receptor group to these gA analogues have been used recently as a component in a sensing device. The conformations of the gA backbones and amino acid side chains of lysinated gA and biotinylated gA in detergent micelles were found to be almost identical to that of native gA, i.e. that of an N-terminal to N-terminal (head to head) dimer formed by two right-handed, single-stranded $\beta^{6.3}$ helices. The biotin tail of the gAXB and gAXXB and the lysine extremity of gA-Lys appeared to lie outside the micelle. Thus it appears that the covalent attachment of functional groups to the C terminus of gA does not disrupt the peptide's helical configuration. Further, single channel measurements of all three gA analogues showed that functioning ion channels were preserved within a membrane environment.

Keywords: Gramicidin A; Membrane; Ion channel; Nuclear magnetic resonance; Structure

1. Introduction

Gramicidin A (gA) is a linear polypeptide of 15 alternating L- and D-amino acid residues isolated from *Bacillus brevis*, where it functions as an antibiotic and takes part in the regulation of gene activity [1]. Gramicidin acts as a defence mechanism against Gram-positive bacteria forming, in the cellular membranes of these competitors, ion channels that create an influx of sodium and potassium ions that destroys the cell. The amino acid sequence of gA is: HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-$NHCH_2CH_2OH$.

The structure of gA is strongly solvent dependent. For example, the crystal structure of gA complexed with Cs^+ is a left-handed, antiparallel, double-stranded (APDS) β helix with 6.4 amino acid residues per turn [2]. Langs et al. [3] found that gA with no complexed ions also formed left-handed APDS helices but with 5.6 amino acids per turn. In organic solvents gA exists as a mixture of double stranded helices and monomers [4–6]. In lipid membranes, gA forms ion channels selective to monovalent cations ($Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$) [7]. The gA ion channel structure has been characterised in vesicles and micelles as a head-to-head right-handed β helical

* Corresponding author. Fax: +61 (3) 93475180; E-mail: f.separovic@chemistry.unimelb.edu.au

0005-2736/99/$ – see front matter
PII: S0005-2736(98)00207-7

dimer 2.6 nm in length with 6.3 amino acids per turn [8]. This $\beta^{6.3}$ helix (also known as a $\pi_{L,D}$ helix) is so named because the hydrogen bonding pattern is consistent with that present in a β-sheet structure. The alternating L,D sequence results in the hydrophobic side chains pointing out into the hydrophobic core of the membrane, with the amide and carbonyl groups directed along the channel axis, creating a hydrophilic pore of 0.40 nm diameter. Modification of the N terminus of gA inhibits channel function [9] by disrupting the helical dimer, but modification or substitution to groups at the C terminus has a lesser effect.

The crystal structure of the ion channel has not been solved, despite the existence of gA-lipid co-crystals [10]. However, high-resolution solution-state NMR structures have been determined for gA in SDS (sodium dodecyl sulphate) micelles [8]. The circular dichroism (CD) spectrum of gA in phosphatidylcholine bilayers is specific to the β channel conformation [11]. The CD spectrum from gA incorporated in SDS micelles and lysolecithin micelles is almost identical [8] and is evidence that gA in SDS micelles forms the channel conformation and that in many ways the micellar system mimics the lipid bilayer membrane. Studies of gA in SDS micelles by ^{1}H NMR have shown the dimer to be a right-handed $\beta^{6.3}$ helix [8]. Solid state NMR studies of gA have confirmed that the same structure is also present in lipid bilayers [12,13].

Current investigations are being carried out using gA as a part of a biosensor [14]. The biosensor consists of a receptor attached to a gA channel embedded in a bilayer lipid membrane (BLM). Binding of an analyte inhibits or disrupts dimer formation (and hence channel function) and restricts ion flow across the lipid membrane, resulting in a detectable increase in impedance. The gated-ion channel sensor was originally demonstrated by the addition of streptavidin to biotinylated gA incorporated within a BLM supported on a gold electrode [14]. Specific binding between biotin and the protein streptavidin is well characterised [15]. In the biosensor, the biotinylated gA acts as a receptor for streptavidin. The present work describes the conformation of gA modified at the C terminus by the attachment of biotin molecules via a linker to the ethanolamine group. The linker arm for this study comprised either one (gAXB) or two caproyl groups (gAXXB) attached to a glycine residue.

A further modification of the C terminus of gA, gA-Lys, included a lysine group attached directly to tryptophan 15. Lysine was chosen because it is a polar, hydrophilic amino acid with a mobile side chain and thus should be accessible at the membrane surface, and also because the chemistry involved in attaching lysine to a receptor and to gA is relatively simple. In practice, as part of a sensor, gA is modified by attachment of an antibody fragment and linker chain to a lysine at the C terminus and incorporated into a BLM. L-Lysine was selected over the D-configuration as it was intended that lysine act as a linker and not merely an extension of the gA chain. The L-form of the amino acid breaks the important L,D sequence of gA and may disrupt the channel conformation of gA, and hence it was important to ascertain the structure of gA-Lys [Val^{1}Lys^{16}] gramicidin A in SDS micelles.

The aim of this study was to determine the three-dimensional (3D) structure of biotinylated and lysinated gA. High-resolution 2D NMR spectroscopy was used to examine modified gA and native gA with a view to determining how modification of gA affects its 3D conformation. Interproton distance constraints determined from the NMR data were used with distance geometry and energy minimisation to generate models of gA and modified gA.

2. Materials and methods

Lysinated gA was obtained from Dr Charles Tansey, University of Sydney, Australia. The peptide was synthesised using described procedures [16], and purified using isocratic reverse-phase HPLC with 25% water in methanol. Peptide purity was assessed by analytical reverse-phase HPLC, electrospray mass spectrometry (MW 1967.4) and high-resolution NMR and judged to be ≥99% pure. Preliminary NMR experiments were run with 6 mg of gA-Lys in deuterated methanol-d_4 (99.8% D, Aldrich, Milwaukee, WI) (approx. 3 mM). Following these experiments, the sample was dried under nitrogen followed by vacuum pumping and used for further experiments. Biotinylated gA, gAXB and gAXXB [14] were synthesised by Dr Lionel King, CSIRO

Food Science and Technology, Sydney, Australia. Both samples were made by covalently attaching a biotin molecule to the C terminus of gA through a linker arm comprised of either one or two caproyl groups attached to a glycine residue (Fig. 1).

These gA analogues were incorporated into sodium dodecyl sulphate (SDS-d_{25}, Cambridge Isotope Laboratories, Andover, MA) micelles by dropwise addition of a 50 mM of peptide solution in deuterated 2,2,2-trifluoroethyl alcohol-d_3 (99% D, dispersion of SDS-d_{25} (98% D) in H_20 containing 10% ^2H_2O. The samples were then diluted with water to a final concentration of 5 mM gramicidin and 0.25 M SDS in 2,2,2-trifluoroethanol-d_3/water (molar ratio 1:16 TFE:water). Due to limited availability of gAXB, the final molar concentration of peptide in this sample was 4 mM. These conditions produce micelles that contain no more than two gramicidin molecules per SDS micelle and are the same as those used by Arseniev et al. [8]. The pH of the sample was then adjusted to 6.5 in order that proton-deuterium exchange was sufficiently slow to allow amide protons to be observed. These conditions also facilitated comparison with previous data for gA.

One- and two-dimensional NMR experiments were carried out using Bruker (Karlsruhe, Germany) AMX and DRX 500 MHz spectrometers, and a Varian (Palo Alto, CA) Unity INOVA 400 MHz spectrometer using 5 mm probes. Proton chemical shifts were measured with reference to sodium 3-trimethylsilyl propionate (2,2,3,3-d_4) as an internal standard. All experiments were conducted without spinning the sample. All spectra were recorded at 55°C in order to give narrower linewidths and to compare results with previous data for gA and its analogues [8]. Phase-sensitive double quantum filtered (DQF) COSY spectra [17] were obtained on the Bruker AMX 500 and DRX 500 with t1 incrementally changed over 512 experiments (384 experiments/768 FIDs (F1) for gA-Lys). The water resonance was suppressed by presaturation during the relaxation delay (1 s). The spectrum was recorded over a sweep width of 6024 Hz (5700 Hz), collected in 4K (2K) data points (F2) over a period of 23 (65) h, using TPPI (time proportional phase incrementation) phase cycling [18]. The digital resolution was ≈12 Hz/point in F1 and 1.5 Hz/point in F2. The 90° pulse was determined to be 12 μs for the Bruker experiments.

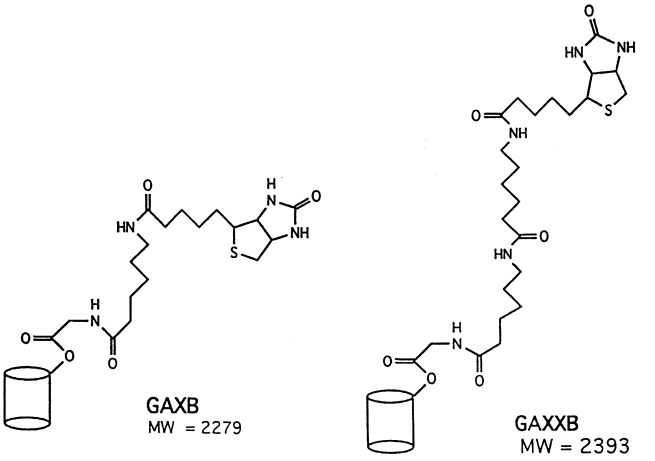

Fig. 1. Biotinylated gA, gAXB and gAXXB, where the gramicidin (gA) is represented by a cylinder.

^{1}H NOESY experiments [17] were recorded at both 500 MHz and 400 MHz. Bruker NOE data was obtained using the standard NOESY water presaturation experiment, over 256 FIDs (128 increments). The water resonance was suppressed by presaturation during the relaxation delay (1 s) and mixing time (40 ms). The TPPI method of phase cycling was used for quadrature detection. Varian experiments were obtained using a NOESY pulse sequence that utilised pulsed field gradients to suppress the water resonance. One data set was recorded over 236 increments in t1 over 42 h and another with 512 increments in 94 h. Both NOESY spectra were recorded over a sweep width of 5000 Hz collected in 2K data points and used the States-Haberkorn method of phase cycling [19]. The typical 90° pulse for all Varian experiments was determined to be approx. 7 μs. Typically, the 2D experiments involved the acquisition of 256 transients/scans per FID.

CALIBA (Calibration of NOE intensity vs. distance bonds) software was used to convert NOESY data, in the form of peak volumes, to upper distance constraints [20]. The volume of cross-peaks between geminal pairs of β and γ protons was used to calibrate the relationship between peak volume and distance (0.18 nm). Interproton distance calculations were made assuming that the strength of NOESY cross-peaks is inversely proportional to the sixth power of the interproton distance. DIANA (distance geometry algorithm for NMR applications) software was used to produce energy minimised structures [20]. A large number of structures (100) were gener-

ated and compared in order to determine a root mean square deviation (RMSD) for each sample. The five most similar structures were converted to PDB files using COFIMA (coordinate file manipulation) software [20] and visualised on a Silicon Graphics Indigo workstation.

3. Results

DQF-COSY spectra and NOESY spectra were used to assign the resonances of all protons in the molecule by sequential resonance assignment [21], with the aid of EASY software [22]. There was a high degree of identity between the chemical shifts of most of the protons of gA and the modified gAs (Fig. 2). The exceptions are the ethanolamine protons where the linker to biotin is attached in gAXB and gAXXB; and Leu-10 in gA-Lys where the presence of the additional amino acid, Lys-16, shifts the resonance. The good agreement between proton chemical shifts in both the backbone and the side chain for all the peptides is strong evidence that the three-dimensional structure of gA [8] has been retained in biotinylated and lysinated gramicidin A.

Resonance assignments could not be made for $N\varepsilon H_2$ protons on the lysine side chain of gA-Lys as these protons exchange rapidly with the solvent. In addition, the resonances from the caproyl linker of gAXB and gAXXB were not assigned due to the high degree of equivalence between the aliphatic protons. The proton chemical shifts of biotin attached to gA were identical to within 0.03 ppm to those of unmodified biotin in water at pH 6.5 and 55°C. This indicates that the biotin on biotinylated gA is situated in the water phase outside the SDS micelles.

Table 1
Significant NOE connectivities between sequentially distant amino acid residues in gA, gAXB, gAXXB and gA-Lys

	gA	gAXB	gAXXB	≠gA-Lys
Main chain				
Formyl HCO...D-Val-6 CαH	+	+	+	+
L-Val-1 NH...L-Ala-5 NH[a]	+	+	+	+
Gly-2 NH...D-Val-8 CαH	+	+	+	+
Gly-2 $C\alpha H_2$...D-Leu-4 CαH[a]	+	Not resolved	+	+
L-Ala-3 CαH...L-Trp-9 NH	+	+	+	+
D-Leu-4 NH...D-Leu-10 CαH	+	Not resolved	Not resolved	+
L-Ala-5 CαH...L-Trp-11 NH	+	+	+	+
L-Val-7 CαH...L-Trp-13 NH	+	Not resolved	+	+
D-Val-8 NH...D-Leu-14 CαH	+	+	+	+
Others				
L-Val-1 NH...L-Ala-5 $C\beta H_3^a$	+	+	+	+
L-Val-1 $C\gamma H_3$...L-Val-7 CβH	+	+	+	+
L-Ala-3 $C\alpha H_3$...L-Trp-9 CβH	+	Not resolved	+	+
L-Ala-3 $C\alpha H_3$...L-Trp-9 C4H	+	+	+	+
L-Ala-5 $C\alpha H_3$...L-Trp-11 CβH	+	+	+	+
L-Ala-5 $C\alpha H_3$...L-Trp-11 C4H	+	+	+	+
D-Val-6 NH...D-Leu-12 $C\delta H_3$	+	+	+	+
D-Val-6 CβH...D-Leu-12 $C\delta H_3$	+	+	+	+
D-Val-6 $C\gamma H_3$...L-Trp-13 C4H	+	+	+	+
D-Val-6 $C\gamma H_3$...L-Trp-13 C5H	+	Not resolved	+	+
D-Val-8 NH...D-Leu-14 $C\delta H_3$	+	+	+	+
D-Val-8 CβH...D-Leu-14 $C\delta H_3$	+	+	+	+
D-Val-8 $C\gamma H_3$...L-Trp-15 C4H	+	+	+	+
D-Val-8 $C\gamma H_3$...L-Trp-15 C5H	+	+	+	+
L-Trp-9 C2H...L-Trp-15 CβH	+	NO	NO	+
L-Trp-9 N1H...Ethanolamine CβH	+	NO	NO	NO[b]

[a]NOE between different monomers.
[b]Not applicable since gA-Lys does not have an ethanolamine group.

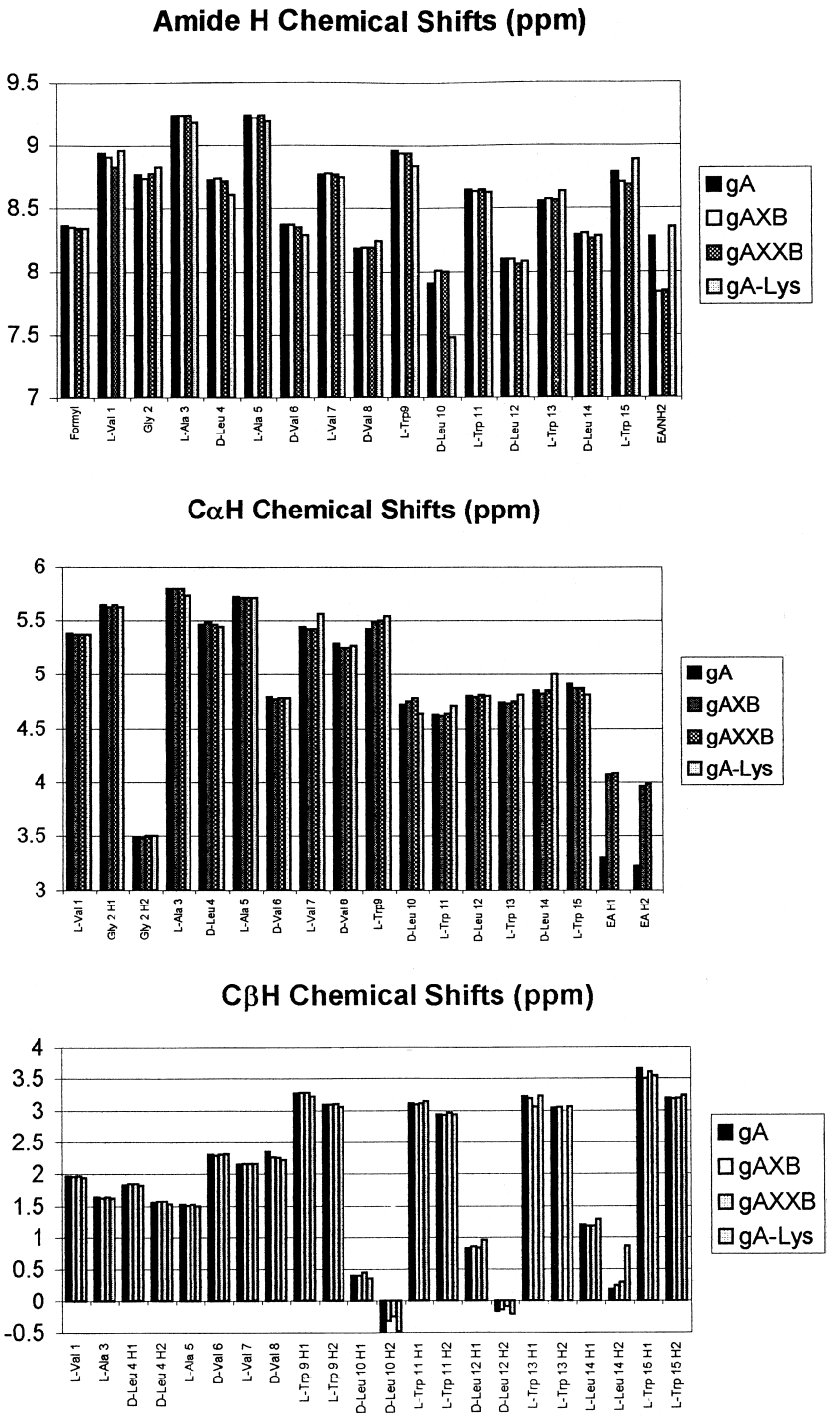

Fig. 2. Chemical shifts ($\delta \pm 0.03$ ppm) of (a) the backbone amide protons, (b) the CαH, and (c) the CβH of gA, gAXB, gAXXB and gA-Lys in SDS micelles, pH 6.5 at 55°C. The lysine protons (NH at 8.38 ppm, CαH at 4.51 ppm, and CβH at 1.75 and 1.96 ppm) are not included in the graphs. Since gA-Lys does not have an ethanolamine group (EA), the comparison to the terminal NH_2 has been included.

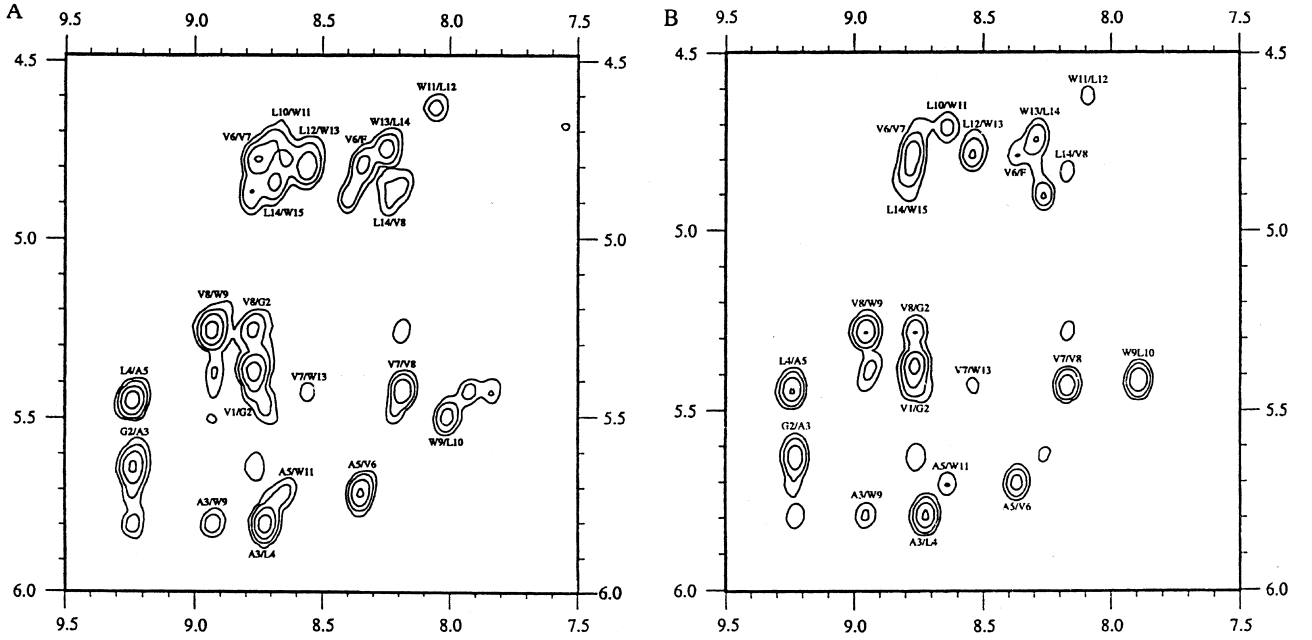

Fig. 3. Expansion of contour plot showing fingerprint region (F2 = 7.5–9.5 ppm, F1 = 4.5–6.0 ppm) of a NOESY spectrum of: (A) gAXXB and (B) gA in SDS micelles. $N_iH...C\alpha_{i-1}H$ connectivities are labelled, F2/F1.

Analysis of NOESY spectra revealed a similar pattern of cross-peaks for all samples. Complete backbone proton assignments were made using sequential NOEs. NOE cross-peaks were observed between every NH backbone proton and the Cα proton of the preceding residue. NOEs were also observed between the $N_{i+1}H$ and the $C_i\beta H$ for Ala-3/Gly-2, Leu-4/Ala-3, Val-6/Ala-5, Leu-12/Trp-11, Trp-13/Leu-12 and Leu-14/Trp-13, and for the formyl H to Val-1 NH. NOE cross-peaks were observed between protons of sequentially distant residues (Table 1). The existence of NOEs between N_iH and C_jH, where $j = i+6$ for even i, and $j = i-6$ for odd i, e.g., Trp-9 NH....Ala-3 CαH and Val-8 NH....Leu-14 CαH (Fig. 3), indicated that the helix was right-handed. The observed short- and middle-range NOEs (Table 1) were consistent with the existence of a right-handed helix with about six residues/turn.

Other significant NOE cross-peaks arose between various Cβ, Cγ and Cδ protons of sequentially distant residues, and also between hydrogens on the tryptophan rings to Cα and Cγ protons of sequentially distant residues (Table 1). Intermolecular NOEs were detected for Gly-2 CαH...Leu-4 CαH, Val-1 NH...Ala-5 NH, and for Val-1 NH...Ala-5 CβH. The existence of these intermolecular connectivities signifies that the channel is a head-to-head dimer. Some difficulty did arise due to the similarity in chemical shift of several protons, causing an overlap of NOESY cross-peaks. Between 130 and 150 upper distance constraints were determined for each sample.

In gA-Lys, no NOE connectivity was observed between Leu-10 NH...Lys-16 CαH. This indicates that Lys-16 CαH does not point back towards Leu-10 NH as might have been expected for D-lysine, or that the residue is rapidly interconverting through a number of conformations. No NOE connectivities were recorded between any proton of the lysine residue, apart from Lys-16 NH...Trp-15 CαH, to protons of other residues. Only intraresidue connectivities were observed for lysine side chain protons. An NOE connectivity was observed for the terminal amide NH_2 back to Trp-11 CαH. No NOE cross-peaks were observed for the biotin in gAXB or gAXXB, suggesting that this part of the molecule is not constrained by the micelle.

NOE-derived upper distance constraints were used to produce energy-minimised models. The average global backbone RMSDs ranged between 0.266 nm for gA and 0.289 nm for gAXXB with global heavy atom RMSDs between 0.423 nm and 0.429 nm. The

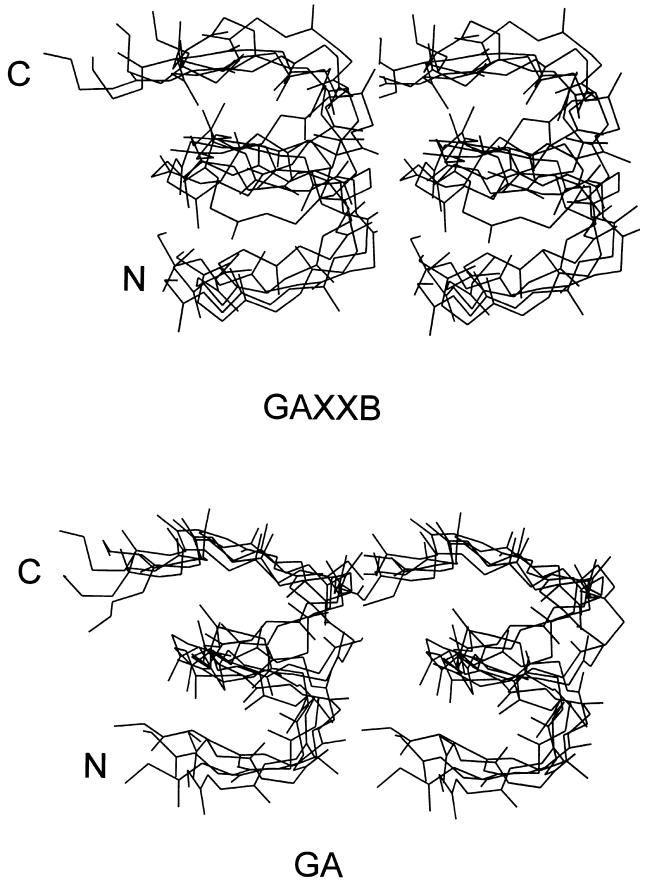

Fig. 4. Superposition of five energy minimised models of the backbone structure of gAXXB and gA showing a stereo view of the hydrophobic face. Global backbone RMSDs were ≈0.28 nm for each sample.

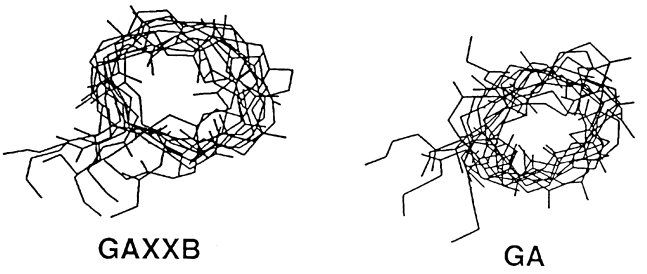

Fig. 5. An axial view down the hydrophilic core of gAXXB and the gA channel.

higher figures for the latter reflect the greater degree of disorder in the side chains compared to the backbone. A superposition of five energy-minimised backbone structures of gA and gAXXB is shown in Figs. 4 and 5, demonstrating that the modified gA analogues maintain the same helical fold as native gA.

4. Discussion

The good correlation between chemical shifts for almost all of the protons within gA and the biotinylated and lysinated gAs is strong evidence that the three-dimensional channel structure of gA [8] is retained in the modified gAs. For both gAXB and gAXXB there is a shift in the ethanolamine protons due to the covalently attached linker groups to the biotin. The similarity of the proton chemical shifts observed for free biotin and the biotin moiety attached to gA, suggests that the biotin tail of the modified gA is not confined within the SDS micelle. Likewise, in the case of lysinated gA, it appears that the lysine group is in the water phase. The only resonance showing a dramatically different chemical shift in gA-Lys was that of the amide proton of Leu-10. This is not surprising as its environment has changed with the replacement of the ethanolamine with the lysine residue at the 16 position. The different configuration (L instead of D) for this even numbered residue has resulted in a chemical shift upfield from that of every other amide proton.

The pattern of NOE connectivities in biotinylated and lysinated gA was almost identical to that in native gA [8], further indication of adoption of the same three-dimensional conformation. NOEs were observed between protons that were six residues apart and consistent with a right-handed helix. NOEs between the two monomers confirmed that in all cases, the structure was an N terminus head-to-head dimer.

No NOEs were observed between biotin protons, suggesting that the biotin has a shorter correlation time than the peptide portion of the biotinylated gA samples, and is moving freely in the aqueous phase external to the micelle. It has been shown that acylation of gA at the C terminus [23] produces a mobile portion of the acyl chain beyond the point of covalent attachment. The acyl chain buries itself within the lipid bilayer but in the case of biotinylated gA, this is precluded by the more polar nature of the linker chain, and the biotin, therefore, remains in the aqueous phase.

In gA-Lys, an NOE connectivity was observed from the terminal amide protons to Trp-11 CαH, suggesting that the addition of the lysine residue

has not disrupted the helix, and the terminal group continues the spiralling of the helix. Consequently, this leads to the expectation that the lysinated gA dimer possesses an additional NH...C=O hydrogen bond: Leu-10 C=O to NH_2 (terminal), in continuation of the hydrogen bonding pattern. However, no such NOE was observed, nor were NOEs observed between Leu-10 NH and Lys-16 CαH. Since the lysine is the L-isomer, the Cα proton does not point back towards Leu-10 NH. However, if the molecule adopted a fairly rigid conformation an NOE would have been expected between the Cβ protons and Leu-10 NH. As there is no such connectivity, this suggests that either the Lys-16 side chain is too mobile to show an NOE, or that the Lys-16 residue adopts another conformation (or range of conformations).

The absence of NOE connectivities from Lys-16 to any other residue, and the rapid exchange of the C-terminal amide protons is preliminary evidence that the lysine side chain *could be* directed away from the dimer and into the water phase. This conformation would see the terminal NH_2 group located upwards and back toward Leu-10 NH, and hence accessible for linker chemistry. Accompanied by, or independent of this, may be a change in the orientation of Trp-15 C=O. Both of these changes could result in the Leu-10 amide proton becoming more shielded, and moving upfield as observed.

Orientation of the lysine side chain back towards Leu-10 (and the molecule in general) is likely to be sterically and energetically unfavourable for a polar amino acid side chain. NOEs between Lys-16 and other residues would be expected if the lysine were so positioned. Alternatively, the $N\varepsilon H_2$ may be hydrogen bonding back to other functional groups within the molecule. Again, if this were the case, NOEs would have been observed. The most likely orientation, and that which is considered ideal for operation of the linker, would be that the Cα proton points toward the pore and the lysine side chain is directed away from the dimer and directly into the water phase, roughly parallel to the channel axis. A comparison with chemical shifts of the side chain protons for free lysine in water was made and indicated that the lysine side chain in gA-Lys is in the water phase.

Only the peptide portions of the modified gA could be modelled due to the limited number of NOEs observed from the covalently attached linker groups. Both the lysine and biotin portions of the modified gA appeared to be in the water phase, the ideal location for attachment of receptor groups for sensing applications. A global RMSD of ≈ 0.28 nm was obtained, and despite the relatively large value, the models clearly show that the modified gA maintains the same helix structure as native gA.

Having determined that modified gA retains the three-dimensional ion channel conformation of gA, and thus has the potential to act as an efficient ion channel, a further step is to measure ion conductance. Early studies have shown that lysinated gA functions as an ion channel in BLMs, but 'noise' (flickering) was encountered in the single channel measurements (P. Osman, D. Milojevic, unpublished data). This could possibly be attributed to movement of the lysine side chain and possible obscuring of the pore (either by the side chain or by the Cα protons as postulated). Biotinylated gA analogues in BLMs behaved similarly to native gA with current amplitudes of 0.6 pA and lifetime of ≈ 0.5 s using 0.1 M saline (P. Osman, unpublished data). Impedance measurements using biotinylated gA incorporated in BLMs attached to gold electrodes show that these ion channels are functioning and gated off using streptavidin as the receptor for biotin [14].

From this study it can be concluded that biotinylated and lysinated gAs incorporated into sodium dodecyl sulphate micelles adopt the conformation of a right-handed head-to-head $\beta^{6.3}$ helical dimer. The NMR data are consistent with the three-dimensional structure for gAXB, gAXXB and gA-Lys being similar to that of native gA. As a result, the modified gA analogues function effectively as ion channels in lipid bilayer membranes and are being used as gated ion channels in biosensor applications [14].

Acknowledgements

The authors extend special thanks to Dr Charles Tansey and Dr Lionel King, Cooperative Research Centre for Molecular Engineering and Technology, Sydney, Australia for donating the modified gramicidin analogues. Special thanks also go to Dr T.K.

Lim, University of Melbourne, for his assistance in operating the NMR spectrometers.

References

[1] H. Ristow, W. Pschorn, J. Hansen, U. Winkel, Nature 280 (1979) 165–166.
[2] B.A. Wallace, K. Ravikumar, Science 241 (1988) 182–187.
[3] D.A. Langs, Science 241 (1988) 188–191.
[4] V.F. Brystov, A.S. Arseniev, Tetrahedron 44 (1988) 925–940.
[5] B.A. Wallace, Biopolymers 22 (1983) 397–402.
[6] B.A. Wallace, Biophys J. 45 (1984) 114–116.
[7] V.B. Myers, D.A. Haydon, Biochim. Biophys. Acta 274 (1972) 313–322.
[8] A.S. Arseniev, I.L. Barsukov, V.F. Brystov, A.L. Lomize, Y.A. Ovchinnikov, FEBS 186 (1986) 168–174.
[9] J.S. Morrow, W.R. Veatch, L. Stryer, J. Mol. Biol. 132 (1979) 733–738.
[10] B.A. Wallace, R.W. Janes, J. Mol. Biol. 217 (1991) 625–627.
[11] B.A. Wallace, W.R. Veatch, E.R. Blout, Biochemistry 20 (1981) 5754–5760.
[12] L.K. Nicholson, T.A. Cross, Biochemistry 28 (1989) 9379–9385.
[13] B.A. Cornell, F. Separovic, A.J. Baldassi, R. Smith, Biophys. J. 53 (1988) 67–76.
[14] B.A. Cornell, V.L.B. Braach-Maksvytis, L.G. King, P.D.J. Osman, B. Raguse, L. Wieczorek, R.J. Pace, Nature 387 (1997) 580–583.
[15] N.M. Green, in: M.C. Anson, J.T. Edsell (Eds.), Advances in Protein Chemistry, Academic Press, New York, 1975, pp. 85–133.
[16] F. Separovic, J. Gehrmann, T. Milne, B.A. Cornell, S.Y. Lin, R. Smith, Biophys. J. 67 (1994) 1495–1500.
[17] A.E. Derome, Modern NMR Techniques for Chemistry Research, Pergamon Press, New York, 1987.
[18] A. Derome, M. Wilkinson, J. Mag. Reson. 88 (1990) 177–185.
[19] D.J. States, R.A. Haberkorn, D.J. Ruben, J. Magn. Reson. 48 (1982) 286–292.
[20] P. Guntert, J. Mol. Biol. 217 (1991) 517–530.
[21] K. Wuthrich, NMR of Proteins and Nucleic Acids, John Wiley and Sons, Zurich, 1986.
[22] C. Eccles, P. Guntert, M. Billeter, K. Wuthrich, J. Biomol. NMR 1 (1991) 106–119.
[23] R.E. Koeppe II, T.C. Bas Vogt, D.V. Greathouse, J.A. Killian, B. de Kruijff, Biochemistry 35 (1996) 3641–3648.