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Single channel and monolayer studies of acylated gramicidin A: influence of the length of the alkyl group

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Abstract. Three different gramicidin A analogues bearing acyl chains of various length on the ethanolamine moiety have been studied by investigating their single channel behaviour and their monolayer properties. It is shown that the single channel conductance does not depend on the substitution of the ethanolamine OH group and that the channel lifetime is roughly proportional to the length of the alkyl chain. The monolayer study indicates that acylation of gramicidin A produces compounds which have medium-dependent conformations. These acylated compounds are miscible with lipids, while GA is not, and the surface potential is not modified by the esterification of the alcohol group.

Key words: Gramicidin A analogues – Monolayers – Lipid-peptide interactions

Introduction

In addition to the unusual alternation of D and L residues found in linear gramicidins (HCO-Val[1]-Gly[2]-Ala[3]-D-Leu[4]-Ala[5]-D-Val[6]-Val[7]-D-Val[8]-Trp[9]-D-Leu[10]-Trp[11]-D-Leu[12]-Trp[13]-D-Leu[14]-Trp[15]-NHC_2H_4OH for gramicidin A or GA) (Sarges and Witkop 1965), the antibiotic is characterized by the presence of an ethanolamine moiety at the C-terminus of the peptide. Although surprizing, it appears that the alcohol function has no effect on the conductance properties (Trudelle et al. 1987) of the gramicidin molecule, and thus the functional role of the OH group is not clear. The recent isolation of gramicidin K (Koeppe et al. 1985) from commercial gramicidin D suggests that this acylated gramicidin could be the biological precursor of gramicidin A. The fact that the presence of acyl groups on the C-terminus of other channel forming peptides, namely trichorzianines belonging to the peptaibol family, was also detected (Bodo, personal communication) supports this idea. In addition, gramicidin K shows interesting properties when incorporated into lipid bilayers: while the conductance is very close to that of gramicidin A, the channel lifetime is strongly increased, indicating that the lipid-peptide interactions are modified upon acylation. It appeared therefore that acylated gramicidin A could be a good tool for investigations related to the mechanism of formation of the pores and to the determination of the nature of the peptide-lipid interactions involved in the case of transmembrane peptides. As gramicidin K is built of a mixture of various acyl groups, we decided to study gramicidin A which is esterified by fatty acids of various length: acetic acid, undecanoic acid and hexadecanoic acid leading to compounds referred to hereafter as GA(1), GA(10) and GA(15), respectively. We describe here their single channel characteristics together with investigations dealing with lipid-peptide interactions through monolayer studies and compare their behaviour with that of gramicidin A.

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Materials and methods

– The various gramicidins were kindly provided by Dr. R. Lazaro and obtained by esterification of gramicidin D (Sigma, St. Louis, MO) by the required fatty acid.
– Single channel experiments were performed on black lipid membranes made from a 2% solution of glyceryl monooleate (Sigma) in decane using Teflon cells filled with the aqueous electrolyte solutions. All measurements were made on symmetrical systems using Ag/AgCl electrodes. The membrane areas were about $2-3 \cdot 10^{-4}$ cm^2 and a current to voltage convertor (Keithley model 427) was used as current amplifier. Gramicidins were added from solutions in ethanol to the aqueous phases.
– Force-area measurements were obtained by spreading a solution of gramicidin/GMO (1 to 2 mg/ml in $CHCl_3$ or CH_3OH or mixtures of both) on a Teflon Langmuir trough with a starting molecular density of $\approx 2 \cdot 10^{-3}$ mol $\cdot$ Å^{-2}, and the film was compressed continuously with a Teflon barrier at a compression rate of $5-10$ Å$^2 \cdot$ mol$^{-1} \cdot$ min^{-1}. The surface pressure was measured using a tensiometer (Prolabo, Paris) based on the

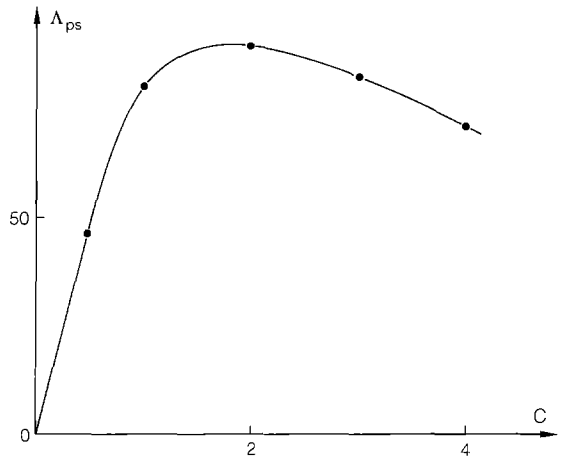

Fig. 1. Variation of the conductance of GA(10), extrapolated at zero applied voltage, with the CsCl concentration

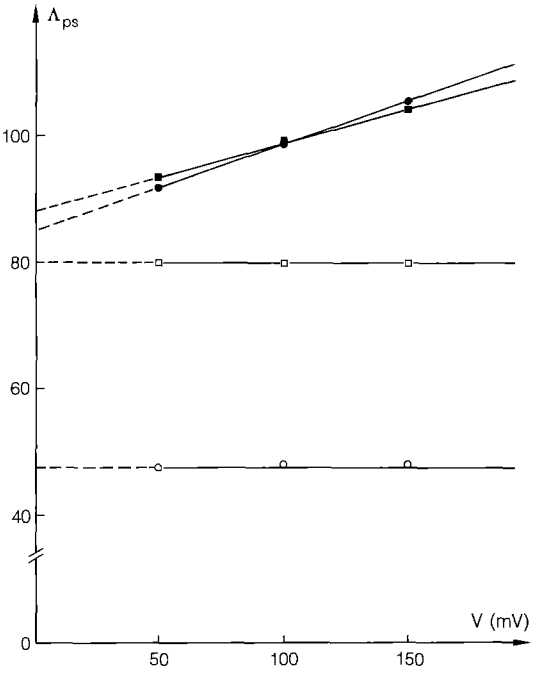

Fig. 2. Variations at various CsCl concentrations of the conductance of GA(10) with the applied voltage. ○, 0.5 *M*; □, 1 *M*; ■, 2 *M*; ●, 3 *M*

Wilhelmy method. The surface tension of the pure water was 72 mN/m.

– Surface potential measurements were made simultaneously with the force-area measurements using two identical Americium 241 air-ionizing electrodes which were placed on both sides of the barrier and connected to a Keithley model 619 voltmeter with an impedance $>2 \cdot 10^{13}\ \Omega$. Both, tension and surface potential variations were recorded on an X-Y-Y′ Kipp & Zonen (Bohemia, NY) BD/91 recorder. Reproducibility was checked by varying the composition of the solvent mixture and the spreading conditions. The given results are the average of 3 to 5 measurements.

– Infrared spectra were recorded on a Bomem model DA8 or Bruker spectrometer working in the FT mode and under vacuum. Samples were prepared by transfer at constant pressure (10 mN/m) of 1 to 7 monolayers onto CaF_2 plates using the procedure described by Briggs et al. (1986).

Results and discussion

Single channel experiments

As expected on the basis of the results reported for gramicidin P, which differs from gramicidin A by the substitution of the ethanolamine group by methylamine (Trudelle et al. 1987) and K (Koeppe et al. 1985), all three acylated gramicidins give rise to single channel events. Although some differences, when compared to GA (Hladky and Haydon 1972) are noticed at the high salt concentrations ($>1\ M$) the general trend concerning the Λ_0-c relationship is the same (Λ_0 is the conductance at zero applied potential and c the salt concentration) (Fig. 1). The identity between the conductances of GA and the acylated compounds for all the cations examined (Na^+, K^+ and Cs^+) confirms the conclusion reached by the study of GP, which indicated that the ethanolamine moiety, particularly its OH function, has no influence on the conductance properties of the gramicidin channel. As mentioned above, small differences with GA occur at high salt concentrations where the conductance shows a small and linear voltage dependence (Fig. 2). The significance of this dependence is still unclear but could reflect an increase of the flexibility of the peptide backbone due to the alkyl chains, the presence of which will modify the interactions between the ionophore and the lipid as will be discussed below.

Table 1. Average lifetimes of the various gramicidin analogues in GMO/decane (1.25%) membranes; CsCl 1 *M*. Average based on 75 events for each sample

Sample	GA	GA(1)	GA(10)	GA(15)
Average lifetime in ms	40	1 200	2 800	3 600

From the point of view of the channel lifetime, the introduction of an acyl group at the C-terminus of gramicidin leads to an increase of the duration of the open state and the lengthening of the alkyl chain induces an increase of the channel lifetime (Table 1). In order to determine the causes of this lifetime increase we undertook a monomolecular film study at the air water interface, the monomolecular films being composed of both the gramicidin and the lipid.

Surface properties investigations

The force-area plots at various GA/GMO ratios (χ) are shown in Fig. 3 and the mean molecular area of GA/GMO films at 10 mN · m^{-1} as a function of the monolayer composition is shown in Fig. 4. The general trend is the same as that already reported for GA/DMPC mix-

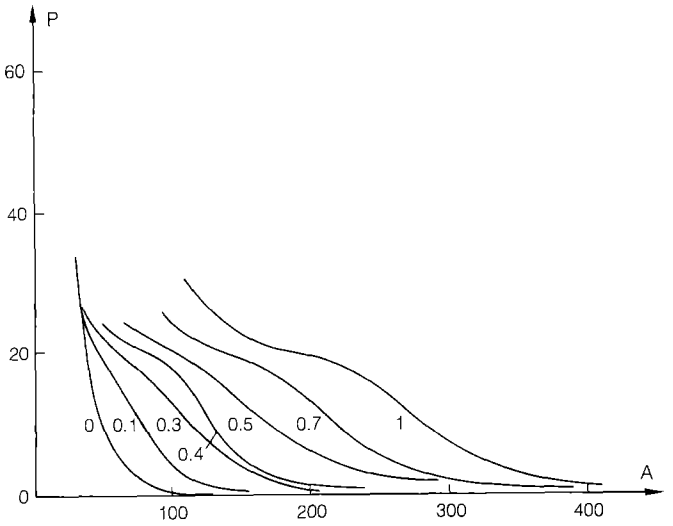

Fig. 3. Variations of the surface pressure P (mN/m) with the molecular area A (Å) at various GA/GMO ratios as indicated on the figure

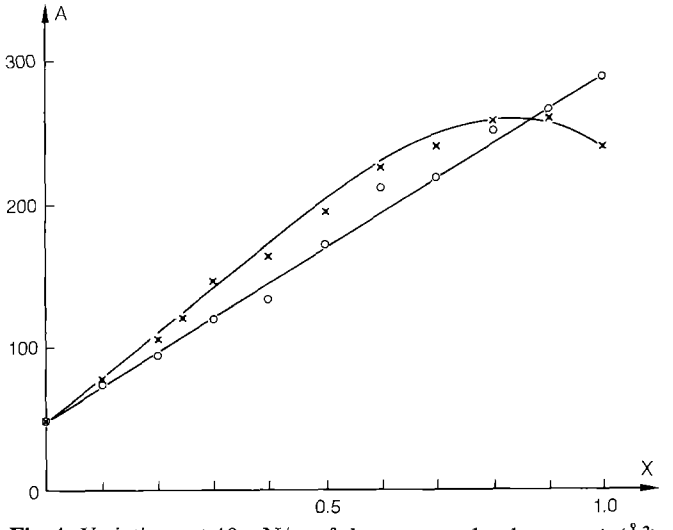

Fig. 4. Variations at 10 mN/m of the mean molecular area A ($Å^2$) with the peptide/lipid ratio (χ in molar fraction) for (o) GA and (×) GA(10)

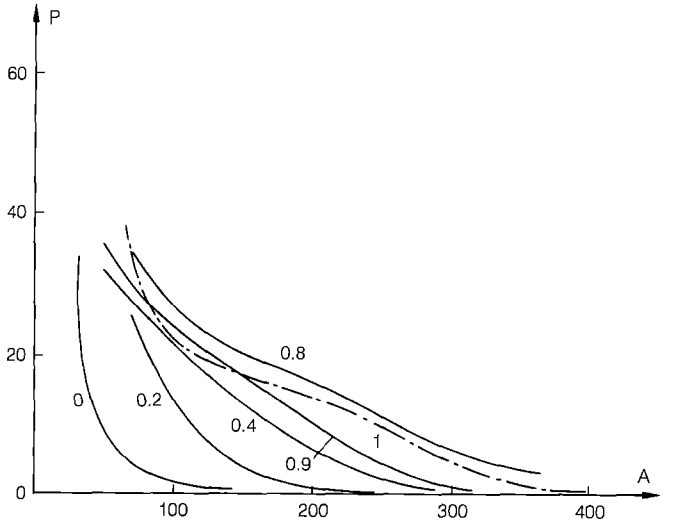

Fig. 5. Variations of the surface pressure P (mN/m) with the molecular area A ($Å^2$) at various GA(10)/GMO ratios as indicated on the figure

Table 2. Variation of the collapse pressure with the composition (χ) of the monolayer for GA (10)

χ	0	0.25	0.5	0.8	1
Collapse pressure (mN/m)	45	22.8	20.7	16.8	14.6

tures (Van Mau et al. 1988) although some differences in the collapse pressure can be noticed (18–19 $mN \cdot m^{-1}$ in the present case which has to be compared with 14–15 $mN \cdot m^{-1}$ obtained previously). The origin of this difference may arise from some uncheckable conditions used for the recovery of the material during or after recrystallisation. Nevertheless, from examination of Figs. 3 and 4 it appears clear that, within the experimental precision, the mean molecular area varies linearly with the composition (i.e. with the GA/GMO ratio) of the monolayer and that no detectable variation of the collapse pressure occurs. As already mentioned (Van Mau et al. 1988), on the basis of Crisp's rule (Gaines 1965) it can be concluded that GA and GMO are not miscible.

When examining the acylated compounds, two different situations occur. The first one, which is found for GA(1) reveals, that within the experimental uncertainties, this analogue is indistinguishable from GA, a slight increase of the collapse pressure can also be detected (15–16 $mN \cdot m^{-1}$ for a monolayer of pure GA(1) and 17–18 $mN \cdot m^{-1}$ for a mixed monolayer at a GA(1)/GMO ratio of 0.6). When the length of the alkyl chain is increased a clear modification of the behaviour is observed. For simplification reasons only the results obtained for GA(10) will be discussed in detail, those obtained for GA(15) are very similar. The force-area plots and the variation of the mean molecular area with the peptide/lipid ratio are shown in Figs. 4 and 5, respectively. Beside a slight but significant increase of the collapse pressure when χ is lowered (see Fig. 5 and Table 2), the mean molecular area at 10 mN/m does not show a linear variation with χ. Such a situation is very close to that reported for mixtures of oleic acid and its oxidation product (Gaines 1965) or of lecithin and a spin probe derivative of palmitic acid (Chatelain et al. 1979). In all these situations both components of the monolayer are miscible and give rise to more expanded structures. Therefore, the finding of a positive deviation from additivity would, a priori, indicate that GA(10) and GMO interact and are miscible and also that repulsive interaction energies between the two components prevail in the monolayer.

However, closer examination of Fig. 4 reveals two puzzling points. The first one is related to the striking similarity between the variations of the mean molecular area with χ, when χ is varied between 0 and 0.7, for both GA and GA(10), although a very small deviation from linearity occurs for GA(10). The second point concerns the lowering, on going from GA to GA(10), of the molecular area when the monolayers are made of pure peptide (230 and 200 $Å^2$ respectively). These observations question the above conclusion which is deduced only from the feature of the variation of the mean molecular area with χ.

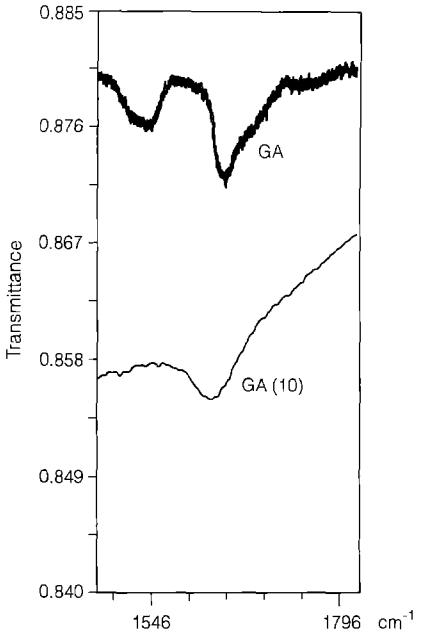

Fig. 6. Infrared spectra of transferred pure monolayers of GA and GA(10)

The fact that the addition of an acyl chain at the C-terminus of GA leads to a lowering of the molecular area therefore requires an analysis of the conformational states of the peptides at the air–water interface with and without lipid.

For these investigations we used the transfer technique onto CaF_2 plates in order to make infrared spectroscopy studies. In the case of GA, as expected, the infrared spectrum is always characterized by the presence of an amide I band centered at $1648 \pm 2\ cm^{-1}$. When examined under identical conditions, the situation for GA(10) appears more complicated. Indeed, when $\chi = 1$, the infrared spectrum shows an amide I band at $1634 \pm 2\ cm^{-1}$ while it is shifted toward higher wavenumbers ($1646 \pm 2\ cm^{-1}$) for $\chi = 0.6$ (Fig. 6). These findings clearly indicate that a transconformation of GA(10) occurs when χ is varied from 0.6 to 1. By analogy with observations made on the model polypeptide poly-γ-benzyl-D-L-glutamate (Heitz et al. 1975; Lotz et al. 1976) for which the various conformations, single and double stranded helices, have been unambiguously identified in the solid state by fiber X-ray diffraction, it can be stated that the structures characterized by the 1634 and $1648\ cm^{-1}$ amide I bands correspond to the double $\Pi\Pi_{DL}$ (Veatch et al. 1974) and single Π_{DL} stranded helices respectively, the latter very probably corresponding to the Π^6_{DL} helix proposed earlier by Urry (1971). It must be added that the same situation occurs for solutions of this model polypeptide (Heitz and Spach 1975, 1977). This conclusion appears to conflict with that proposed by Dhathathreyan et al. (1988). These authors claim that "The presence or absence of the shoulder near $1680\ cm^{-1}$ is more important than the shift of the amide I band in distinguishing between the two conformations." Therefore, they conclude that the presence of an amide I band at $1648\ cm^{-1}$ could also reflect the existence of a double helical structure as a shoulder is still present at $1680\ cm^{-1}$. In our opinion, this conclusion is erroneous for two main reasons: the first one is given above while the second one is related to the fact that in the case of GA we are dealing with a peptide of finite length where the end effects can play an important role. For a Π^6_{DL} helix of gramicidin in the monomeric state, 6 of the 16 carbonyl groups are not engaged in hydrogen bonds and may therefore be responsible for the $1680\ cm^{-1}$ contribution.

In addition, the conclusion made on the basis of the infrared study is also consistent with the finding of a slight but significant lowering of the molecular area on going from GA to GA(10) which is in agreement with single and double stranded helices, respectively. Therefore, it can be stated that:

– when in monolayers with and without lipid, GA is always in the single stranded form (Urry's type),
– for GA(10) under identical conditions a transition from double to single helix occurs upon addition of lipid,
– an examination restricted only to the variation of the mean molecular area with the composition of the monolayer can lead to erroneous conclusions.

As to the positions of the gramicidin molecules at the air–water interface, the clearest situation occurs for pure GA(10) monolayers. In the double helical model, an amide I band at $1634\ cm^{-1}$ is indicative of an antiparallel β structure, the two strands are therefore in an antiparallel arrangement. Thus, for symmetry reasons, the GA(10) molecules are aligned at the interface with their helical axis parallel to the interface while the acyl chains point toward the air. From an examination restricted only to the molecular areas it is not possible to decide whether or not the same orientation occurs for the single stranded forms. Further investigations are required to clarify this latter point.

Concerning the surface potential, for all samples with or without GMO, the same surface potential (240–250 mV) has been detected. This result indicates that the conformational state of the ionophore has no influence on the surface potential which is probably mainly governed by the polar aromatic side-chains. This result is also consistent with the fact that all examined molecules give rise to single channel events of the same amplitude.

Conclusion

From the present investigations it can be concluded that the acylation of the ethanolamine moiety has no influence on the conductance of the gramicidin channel, thus confirming earlier observations made with the natural compound gramicidin K. However, the introduction of a long hydrocarbon chain at the C-terminus of gramicidin strongly modifies the monomer-dimer equilibrium and the interactions of the ionophore with the lipid, GMO in our case, produces compounds which are miscible with lipids. The former point is illustrated by the fact that monolayers of pure GA and GA(10) are built of peptide in the single and double stranded helices respectively while the latter phenomenon can be related to the increase of the channel lifetime on going from, GA which is

not miscible with lipids, to acylated GA. This increase of the channel lifetime may be related to the anchoring effect due to the presence of the acyl groups which are able to interact with the hydrophobic part of the lipids. The longer the acyl chain, the stronger the interactions, hence reducing the possibility of "squeezing" the peptide out of the lipid medium (Van Mau et al. 1988). Finally, the fact that all compounds lead to the same surface potential is in agreement with the finding of identical conductances for all analogues.

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