SINGLE CHANNELS OF 9, 11, 13, 15-DESTRYPTOPHYL-PHENYLALANYL-GRAMICIDIN A

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ABSTRACT Analysis of the single-channel behavior of an analogue of gramicidin A in which all four tryptophyl residues are substituted by phenylalanyl suggests that the nature of the side chains may play an important role in the ion translocation process. Indeed, while infrared spectroscopy indicates that both peptides have very similar backbone conformations, they have different single-channel characteristics. The unit conductance of the analogue is much smaller than that of the natural product. Moreover, contrary to gramicidin A, it is voltage dependent.

INTRODUCTION

The single channel behavior of gramidicin A (Sarges and Witkop, 1965 *a*)

HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp
1 2 3 4 5 6 7 8 9

-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NHC$_2$H$_4$OH
10 11 12 13 14 15

is now widely described (Hladky and Haydon, 1972; Haydon and Hladky, 1972; Bamberg et al., 1976) and is characterized by a defined unit conductance which, however, seems to fluctuate to lower conductance states, possibly due to different orientations of the tryptophyl side chains (Busath and Szabo, 1981). This conductance seems to depend on the chemical composition of the antibiotic as suggested by the results obtained on gramicidin B and C in which residue 11 is phenylalanyl or tyrosyl, respectively. For gramicidin B the conductance is smaller than for gramicidin A, whereas the difference is insignificant for gramicidin C (Bamberg et al., 1976). However, more recently, it was claimed by Tredgold et al. (1977) that the nature of the aromatic side groups attached to residues 9, 11, 13, and 15 does not influence the single-channel conductivity with the following analogues: gramicidin L (residue 13 is phenylalanyl) and gramicidin M (residues 9, 11, 13, and 15 are all phenylalanyl). The latter analogue was of a great interest to us as it does not contain any tryptophyl residue and should be more suitable for conformational investigations especially with regard to spectroscopic techniques. Therefore, we synthesized the same analogue, actually its mirror image, called hereafter gramicidin M$^-$. We report now preliminary data concerning the single-channel behavior of this analogue, which strongly differs from that described previously (Tredgold et al., 1977).

MATERIAL AND METHODS

Samples

Gramicidin A is commercial gramicidin from Sigma Chemical Co., St. Louis, MO. Gramicidin M$^-$ was synthesized by the solid phase technique. Each coupling step was repeated twice, and possible unreacted amino groups were blocked by acetylation. Removal of the pentadecapeptide from the resin was carried out by treatment with 1:1 (vol/vol) ethanolamine-ethanol mixtures (Tredgold et al., 1977) for 10 h, followed by washings with chloroform. When repeated six times, the recovery of the peptide was nearly quantitative. Formylation of the terminal amino group was performed as reported previously by Sarges and Witkop (1965 *b*).

The ethanolic solution of the crude peptide was filtered through Dowex (Dow Chemical Co., Midland, MI) 50W X 2 and 2 X 2 columns (1 × 25 cm). Further purification was achieved on a Sephadex LH 60 (2.5 × 100 cm) column using a 1:1 chloroform-methanol mixture as eluting solvent. Amino acid analysis after hydrolysis for 4 d at 110°C in 5.6 N HCl yielded these results. Gly, calculated 1.00, found 0.94; Ala, calculated 2.00, found 2.13; Val, calculated 4.00, found 4.00; Leu, calculated 4.00, found 4.26; Phe, calculated 4.00, found 3.94.

Single-Channel Experiments

Black lipid membranes were formed from a 2% solution of glycerylmonooleate (Sigma) in decane in Teflon cells filled with 1 M aqueous electrolyte solutions. The membrane areas were ~0.3–0.5 × 10^{-3} cm^2. A Keithley model 427 (Keithley Instruments, Inc., Cleveland, OH) current amplifier was used and the information was stored on a microcomputer Apple II (Apple Computer Inc., Capertino, CA). Frequency sampling was 10–50 Hz. The polypeptides were added from ethanol (gramicidin A) or dioxane (gramicidin M$^-$) solutions to the aqueous phases.

RESULTS AND DISCUSSION

In our experiments, for applied voltages between 0 and 325 mV, we found the single-channel characteristics for grami-

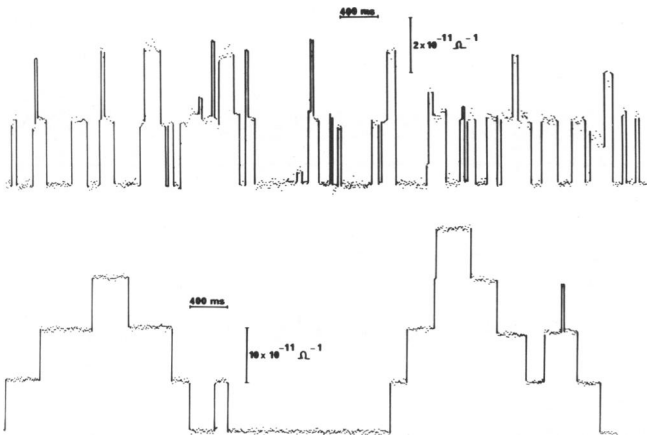

FIGURE 1 Fluctuation of the transmembrane current in the presence of small amounts of gramicidin A (lower trace) and gramicidin M^- (upper trace) in 1 M CsCl. Applied voltage 300 mV.

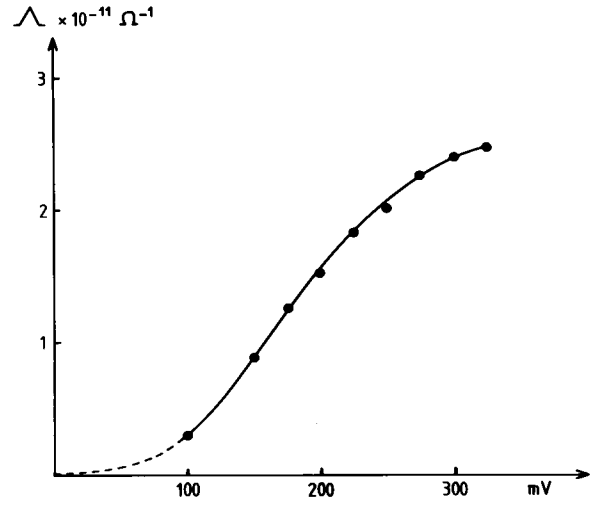

FIGURE 2 Variations of the single-channel unit conductance with the applied potential for gramicidin M^- in 1 M CsCl. Below 100 mV the curve has been estimated from measurements of the conductivity with larger amounts of gramicidin M^-.

cidin A ($4.9 \times 10^{-11}\ \Omega^{-1}$ and $9.1 \times 10^{-11}\ \Omega^{-1}$ in 1 M KCl and CsCl, respectively) (Bamberg et al., 1976) although for gramicidin M^-, discrete fluctuations of the transmembrane current could be detected with certainty only over 100 mV. On Fig. 1 we have reported a trace obtained for gramicidin M^- (upper trace) and, for comparison purposes, that obtained for gramicidin A in the same conditions (lower trace). This figure clearly shows that both peptides behave differently in the lifetimes as well as in the conductance of the channels. The latter point is in complete discrepancy with the behavior reported by Tredgold et al. (1977) for gramicidin M, but agrees with the trend expected from gramicidin B (Bamberg et al., 1976). It must be mentioned here that results obtained in Dr. Läuger's laboratory with another sample of gramidicin M^- also showed a conductance that was considerably smaller than that of gramicidin A (Bamberg and Läuger, personal communication).[1] Further, as shown in Fig. 2, the unit single-channel conductance of gramicidin M^- is voltage dependent although it is constant for gramicidin A. This is true for the two alkali ions that have been studied (K^+ and Cs^+), the ratios $\Lambda\ K^+/\Lambda\ Cs^+$ being nearly identical for both peptides.

Such a behavior is yet unexplained and was unexpected in view of our preliminary conformational investigations with the aid of infrared spectroscopy. Actually gramicidin A and M^- possess very similar spectra (see Fig. 3). Following the attributions of the amide I bands, the band centered ~1633 cm^{-1} corresponds to the dimer form and the one ~1648 cm^{-1} to the monomer form (Sychev et al., 1980). Thus, both compounds undergo analogous dimerization processes, the rates of which depend on the solvent. The equilibrium is reached in a few hours in dichloromethane, but in a few weeks in chloroform or dioxane. Further the monomer-dimer ratio is also solvent dependent, the dimer being more favored in dichloromethane than in chloroform or dioxane. This suggests that gramidicins A and M^- have very close backbone conformations. However, owing to their ionophoric behavior, which is quite different, as described above, we are led to conclude that the side chains of the residues in positions 9, 11, 13,

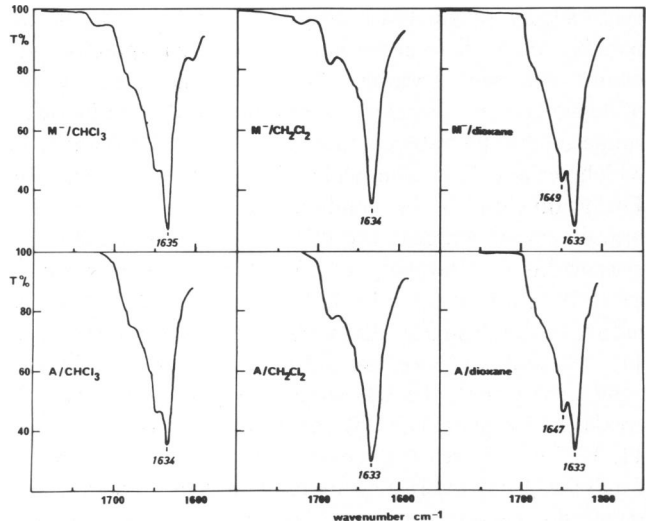

FIGURE 3 Infrared spectra in various solvents of gramicidin M^- (upper part) and A (lower part) previously dissolved and recast from hexafluoroisopropanol in a glass flask. The spectra were recorded 2 wk after dissolution. c = 1 mg/mL in 1 mm cells.

[1]Our experience with gel permeation chromatography on Sephadex LH 60 of gramicidin M^- has shown that columns on which natural gramicidin A had been previously chromatographed must be discarded for further chromatographic experiments because the eluted products are then polluted with gramicidin A. This may offer an explanation for the results of Tredgold.

and 15, i.e., tryptophyl for gramicidin A and phenylalanyl for gramicidin M^-, may play a significant role in the ion translocation process.

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