LINEAR GRAMICIDINS AT THE AIR–WATER INTERFACE

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ABSTRACT The behavior of four linear gramicidins, which differ by the nature of their 9, 11, 13, and 15 aromatic residues, together with a covalent "head to tail" retro GA-DAla-GA dimer, has been examined at the air–water interface. It is shown that all four "monomers" have almost the same molecular area, which is compatible with either a single-stranded or a double-stranded helical model, whereas it is suggested that retro GA-DAla-GA could adopt another conformation. The surface potential measurements agree with those of different groups of molecules characterized by their single-channel behaviors.

INTRODUCTION

It was recently shown that the single-channel characteristics of linear gramicidins HCO-LVal-Gly-LAla-DLeu-LAla-DVal-LVal-DVal-LX-DLeu-LX-DLeu-LX-DLeu-LX-NH-CH_4OH (Sarges and Witkop, 1965) depend on the chemical nature of the aromatic residue X. When X = Trp (gramicidin A or GA) (Hladky and Haydon, 1972) or Tyr (GT) (Trudelle and Heitz, 1987), although the conductances differ by their amplitudes, they are almost independent of the applied voltage, at least in the 0–150-mV range, whereas they are strongly voltage dependent when X = Phe (GM or GM^-) (Heitz et al., 1982) or Tyr-(OBzl)(GT') (Trudelle and Heitz, 1987). These different behaviors can be analyzed in terms of the relative height of the barriers along the ionic channel (Heitz et al., 1986) and thus on differences in the rate-determining steps. For example, in GA, cation binding is the rate-determining step (Urban and Hladky, 1979), whereas in GM^- it is the translocation process (Heitz et al., 1986). The precise origin of these differences is still unknown, although it was suggested that the dipoles of the side chains could play a role (Mazet et al., 1984; Barrett Russell et al., 1986). To obtain information on this point and the influence on the side chains, we investigated the four above-mentioned linear gramicidins together with a covalent dimer retro GA-DAla-GA (HO-C_2H_4-CO-LTrp-DLeu-LTrp-DLeu-LTrp-DLeu-LTrp-DVal-LVal-DVal-LAla-DLeu-LAla-Gly-LVal-DAla-LVal-Gly-LAla-DLeu-LAla-DVal-LVal-DVal-LTrp-DLeu-LTrp-DLeu-LTrp-DLeu-LTrp-NH-C_2H_4OH) (Lelièvre et al., 1986) at the air–water interface with particular attention to the surface potential.

MATERIALS AND METHODS

GA was commercial (Sigma Chemical and Co., St. Louis, MO) and recrystallized before use. GM^-, GT, GT', and the retro GA-DAla-GA dimer have the same origin as described in Heitz et al. (1982) (GM^-), (Trudelle and Heitz, 1987) (GT and GT'), and Lelièvre et al. (1986) (dimer).

Solutions of the various gramicidins were either obtained by dissolution of 0.5–1 mg in 150 μl of methanol (GA, GT, and retro GA-DAla-GA), which was diluted up to 500 μl with chloroform, or by dissolution in 350 μl of chloroform (GM^- and GT'), which was diluted with 150 μl of methanol.

Force–Area Measurements

Aliquots of the gramicidin solutions with a starting molecular density of $\sim 2 \cdot 10^{-3}$ mol $Å^{-2}$, were spread on a Teflon Langmuir trough, and the film was compressed continuously with a Teflon barrier at a compression rate of 5–10 $Å^2$ mol^{-1} min^{-1}. The surface pressure was measured using a tensiometer (Prolabo, Paris) based on the Wilhelmy method.

Surface Potential Measurements

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 curves were recorded on an X-Y-Y' Kipp & Zonen (Bohemia, NY) BD/91 recorder.

Reproducibility was checked by varying the solvent and the spreading conditions. The given results are the average of five measurements.

RESULTS AND DISCUSSION

Surface Pressure–Area Relationship

Fig. 1 shows the force–area plots of the four gramicidin molecules at the air–water interface. Curves *b* and *c*, which correspond to GM^- and GT, respectively, are characterized by a shoulder for a pressure of ~ 20 dyn·cm^{-1}. This discontinuity is attributed to the expansion–condensation transition and corresponds to a close-packed situation (Gaines, 1965) with a molecular area of 225 ± 25 $Å^2$ (see Table I for the detailed values). Curve *a* (GA), which is identical to that reported earlier by Kemp et al. (1972), is similar to curve *d* (GT'). In the latter case the expansion–condensation transition is characterized by an inflection point at 12.5 dyn·cm^{-1} with a molecular area nearly

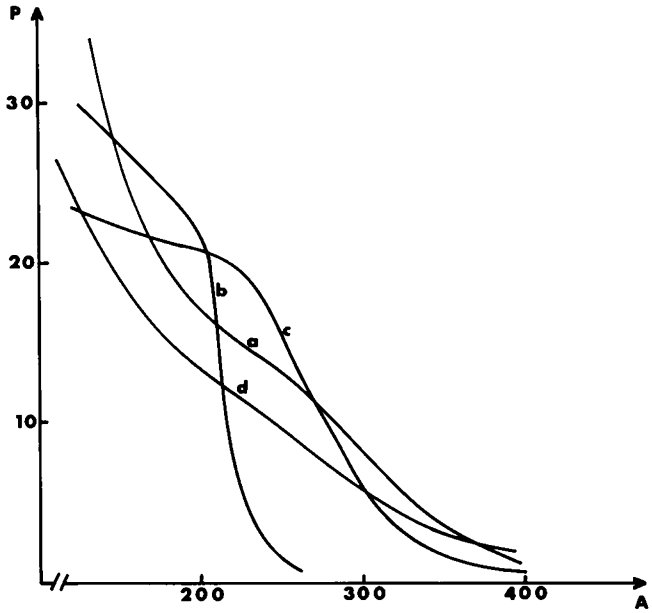

FIGURE 1 Variations of the surface pressure as a function of the molecular area for various linear gramicidins. (*a*) GA; (*b*) GM^-; (*c*) GT; (*d*) GT'. *P* in dyn·cm^{-1}, *A* in Å^2.

identical to that determined above (Table I). The molecular area determined here for gramicidin A conflicts with that reported by Kemp et al. (1972) (~150 Å^2). The discrepancy between the two molecular areas of GA lies in the determination of the expansion–condensation transition. In our opinion the results of Kemp et al. are erroneous because these authors determined the area at a pressure higher than that of the collapse (Kemp and Wenner, 1976).

For the pressure corresponding to the close-packed monolayers, Table I and Fig. 1 reveal the existence of two groups of gramicidins: GA and GT', and GM^- and GT. For the former group, the monolayers are highly compressible probably due to the long side chains (GT') or the side chain–water interactions involving the indole NHs (GA). GM^- and GT are substantially less compressible because they have smaller side-chains, and the high values of the collapse pressures (21–22 dyn·cm^{-1}) indicate a hydrophobicity higher than that for GA or GT'. However, it must be noted that curve *c*, obtained for GT, suggests that this analogue is less rigid than GM^- because of interactions of the tyrosine side chain with the surrounding water. About the retro GA-DAla-GA dimer, the general trend is the same as for GA but at higher molecular areas (Fig. 2 and Table I).

TABLE I
CHARACTERISTIC DATA OF THE VARIOUS LINEAR GRAMICIDINS AT THE AIR–WATER INTERFACE

	GA	GM^-	GT	GT'	Retro GA-DAla-GA
Molecular area (Å^2)*	250	200	210	220	390
Surface pressure (*dyn/cm*)*	12.5	22	21	11.5	11.5
Surface potential (*mV*)*	192	285	167	322	195

*In the close-packed monolayer state.

As already mentioned above, the molecular area of the close-packed monolayers of the various gramicidins differs from that given by Kemp et al. (1972), thus our conclusions too. The present findings indicate that the molecules are aligned parallel to the water surface either in Urry's model (Urry, 1971; Urry et al., 1971) or in the double-helical conformation (Veatch et al., 1974), both being undistinguishable because of many similar physical characteristics (Wallace, 1986). A molecular area of ~250 Å^2 cannot account for gramicidin molecules with helices perpendicular to the interface (Kemp et al., 1972; Brasseur et al., 1986), which require a lower (~150 Å^2) molecular area.

The molecular area of retro GA-DAla-GA (~390 Å^2), which is less than twice that of GA, may indicate that the molecule adopts a conformational state different from that of the shorter molecules, as suggested by infrared spectroscopy (Trudelle and Heitz, 1985).

Indeed, whereas GA or GM^- shows, when dissolved in chloroform, an amide I band at 1,634 or 1,648 cm^{-1} depending on the concentration, the dimer shows a spectrum that remains identical at any concentration (5–0.05 mg/ml) namely an amide I band at 1,631 cm^{-1}.

Surface Potential Measurements

The surface potential–area plots, which were recorded simultaneously with the force–area plots, are shown in Fig. 3. This figure reveals again the existence of two groups of gramicidins, which are characterized by different values of the surface potential in the close-packed state. These two groups, the compositions of which differ from those deduced from the force–area measurements, consist of GA–GT and GM^-–GT' the surface potential of the latter

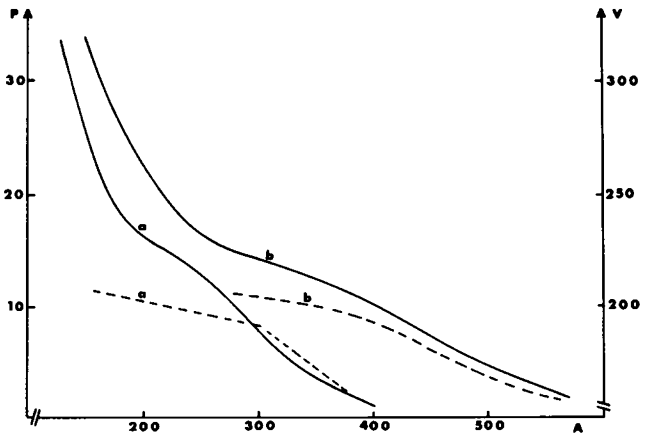

FIGURE 2 Variations of the surface pressure (—) and surface potential (···) as a function of the molecular area for (*a*) gramicidin A, and (*b*) retro GA-DAla-GA. *P* in dyn·cm^{-1}, *V* in mV, *A* in Å^2.

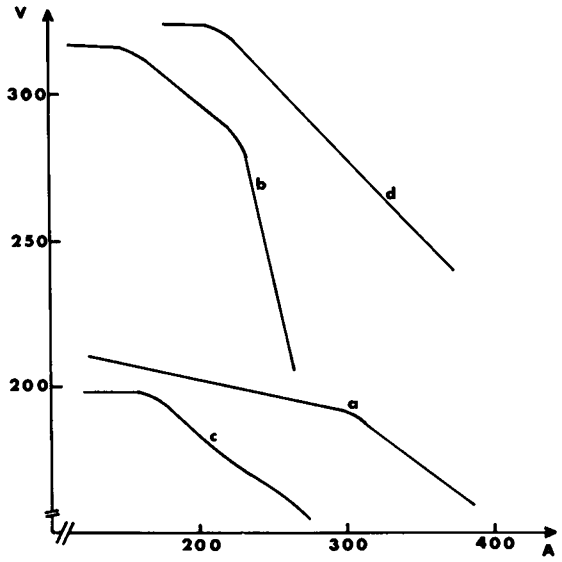

FIGURE 3 Variation of the surface potential as a function of the molecular area for various linear gramicidins. (*a*) GA; (*b*) GM^-; (*c*) GT; (*d*) GT'. *V* in mV, *A* in $Å^2$.

group is higher than that of the former. This observation is reminiscent of the single-channel behavior of these four linear gramicidins: the conductances of GA and GT are high and almost independent of the applied voltage, whereas those of GM^- and GT' are lower and strongly voltage dependent. It is therefore tempting to relate these single-channel characteristics to the variations of the surface potential induced by varying the nature of the aromatic side chains. For the surface potential, GA and retro GA-DAla-GA behave identically (Table I and Fig. 2), suggesting that the latter molecule could give rise to high single-channel conductances, i.e., with amplitudes the same order of magnitude as those of GA and GT. This point will be discussed in detail in future papers. Presently, one must be cautious in proposing a relationship between surface potential and single-channel characteristics because no lipids are involved in the experiments described here; the surface potential is the result of the vertical components of the dipole moments that arise from the side chains and therefore could only reflect various water–side chains interactions.

CONCLUSION

By investigations made on a series of linear gramicidins at the air–water interface, it is shown that the molecular areas of the various analogues are compatible with the proposed transmembrane channel models (Urry, 1971; Veatch et al., 1974), with the molecules aligned parallel to the water surface. Surface potential measurements reveal the existence of different groups of gramicidins, the characteristics of which can be related to the single-channel behavior. Further investigations involving lipids are now in progress to confirm this latter point. The same experiments performed using the retro GA-DAla-GA dimer suggest that this molecule could adopt a conformation that differs from that of the GA analogues.

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