Biochimica et Biophysica Acta 855 (1986) 317–324
Elsevier

BBA 72987

Orientation of gramicidin A at the lysophosphatidylcholine/water interface: a semi-empirical conformational analysis

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(Received September 30th, 1985)

Key words: Gramicidin A; Lysophosphatidylcholine-gramicidin A monolayer; Conformational analysis; Lipid/water interface

The mode of orientation of gramicidin A in the $\beta^{6.3}$ conformation in a monolayer of lysophosphatidylcholine was studied on the basis of conformational data. The approach involves localization of the centers of hydrophobicity and hydrophilicity of the gramicidin molecule, and takes into account the dielectric constant discontinuity of the interface. The most probable orientation is that with the C-terminal, tryptophan-rich, end directed towards the hydrophobic medium. In this orientation, four lysophosphatidylcholines surround a gramicidin monomer leading to a complex with an overall cylindrical shape compatible with the bilayer structure found in aqueous dispersions of mixtures of these molecules (Killian, J.A., De Kruijff, B., Van Echteld, C.J.A., Verkleij, A.J., Leunissen-Bijvelt, J. and De Gier, J. (1983) Biochim. Biophys. Acta 728, 141–144).

Introduction

Gramicidin A is a linear pentadecapeptide which in a dimer conformation forms ion-selective channels in membranes [1–3]. The mode of insertion of gramicidin in a lipid bilayer has been extensively studied and four classes of models have been proposed: the amino-terminal to amino-terminal $\beta^{6.3}$ helical dimer [4,5], the carboxy terminal to carboxy terminal helical dimer [6] and parallel [7,8] and anti-parallel [8] double helices. The N-N terminal $\beta^{6.3}$ helical dimer was originally proposed by Urry et al. [4] as the channel conformation and is presently largely accepted on the basis of ^{13}C-NMR [4,5,9], infrared [10] and circular dichroism [11] studies. However, there are two aspects of the molecule which are difficult to understand with such an orientation. Firstly, intuitively a location of the carboxy terminal part at the membrane/water interface seems unlikely, in view of the concentration of the four hydrophobic tryptophan residues at that end of the molecule. Secondly, the profound effect of gramicidin on lipid polymorphism, e.g., a bilayer stabilization in lysophosphatidylcholine (lysoPC) systems [12,13] and hexagonal H_{II} phase induction in phosphatidylcholine and phosphatidylethanolamine systems can be understood [14,15] assuming a conical shape of the molecule in which the bulky tryptophans are located towards the center of the bilayer. With these apparent discrepancies in mind, we calculated the most probable orientation of gramicidin at the lysoPC/water interface. For that, we used the conformational analysis approach as applied earlier to pure lipid systems but now extended to include one gramicidin molecule in the $\beta^{6.3}$ conformation.

Methods

We started from the gramicidin A modeled according the coordinates corresponding to a left-handed β helix structure [16]. Its orientation at the air/water interface was defined as described earlier [17,18].

From the transfer energy of atoms or groups of atoms from an hydrophobic into an hydrophilic medium, we defined the hydrophilic center ($\dot{C}_w$) by the following equation:

$$\dot{C}_w = \frac{\sum_i E^+_{tr_i} \dot{r}_i}{\sum_i E^+_{tr_i}}$$

in which $\dot{r}_i$ are the coordinates of the atom i which has a positive transfer energy of $E^+_{tr_i}$. The hydrophobic center located in the hydrocarbon domain ($\dot{C}_{HC}$) is defined by the same equation, except that now only atoms (j) with a negative transfer energy were taken into account. The molecule was oriented with the line joining the hydrophilic and the hydrophobic centers perpendicular to the interface [18].

The interface position $\dot{I}$ is defined by the equation:

$$\frac{\sum_i E^+_{tr_i}}{|\dot{C}_w - \dot{I}|} = \frac{\sum_j E^-_{tr_j}}{|\dot{C}_{HC} - \dot{I}|}$$

The structure of the isolated lipid molecule and its orientation at the air/water interface have been established as described previously [18,20,21]. The total conformational energy is calculated as the sum of the London-Van der Waals energy of interaction between non-mutually-bonded atoms, the electrostatic interaction between atomic point charges, the potential energy of rotation of torsional angles and the transfer energy.

The procedure used to surround one gramicidin A with lipid molecules has been described elsewhere [17] and can be summarized as follows (Fig. 1: (a) The gramicidin A (G A) position was fixed and the lipid (L) position was modified along the X-axis. Each distance change was equal to 0.05 nm. (b) For each separating distance, the lipid molecule was rotated in steps of 30° around its long axis and around gramicidin. Among all possible orientations, only the structure of minimum energy was considered. (c) The lipid was now allowed to move along the Z-axis perpendicular to the interface. Again, only the structure of energy minimum was considered. (d) Finally, the orientation of the long axis of the lipid molecule was varied with respect to the Z-axis.

The intermolecular energy of interaction is calculated as the sum of the following terms: (a) The London-Van der Waals energy of interaction between atoms associated to different molecules. Buckingham's pairwise atom-atom interaction functions have been used:

$$E_{vdW} = \sum_{ij} \left[A_{ij} \exp(-B_{ij} r_{ij}) - C_{ij} r_{ij}^{-6} \right]$$

where i, $j = 1, 2, \ldots$ are atoms, r_{ij} their distances from each other, and A_{ij}, B_{ij} and C_{ij} are coefficients assigned to atom pairs. The values of these coefficients have been reported by Liquori and co-workers [22,23]. Like other quantum-mechanical results [24], these values emerge in part as the solution of the Sehrödinger equation and in part as heuristic variables. They have been applied with success to conformational analysis of molecular crystals, proteins, polypeptides and lipids [17, 25–27]. In order to compensate for the decrease of the function E_{vdW} at small r_{ij}, we have imposed an arbitrary cut-off value of $E_{vdW} = 100$ kcal/mol at $r_{ij} < 1$ Å.

(b) The generalized Keesom-Van der Waals interaction or electrostatic interaction between atomic point charges:

$$E_{eb} = 332 \left(\sum_{ij} \frac{e_i e_j}{r_{ij} \epsilon} \right)$$

where e_i and e_j are expressed in electron charge units and r_{ij} in Å. The values of the atomic point charges are similar to the values used for polypeptides [28]. To simulate the membrane interface, we have assumed a dielectric constant (ϵ) equal to 3 above the interface (I), while at the atom most deeply immersed in the aqueous phase, a plane was lined where the dielectric constant is assumed to be 30. Between these two planes, the dielectric constant was assumed to increase lin-

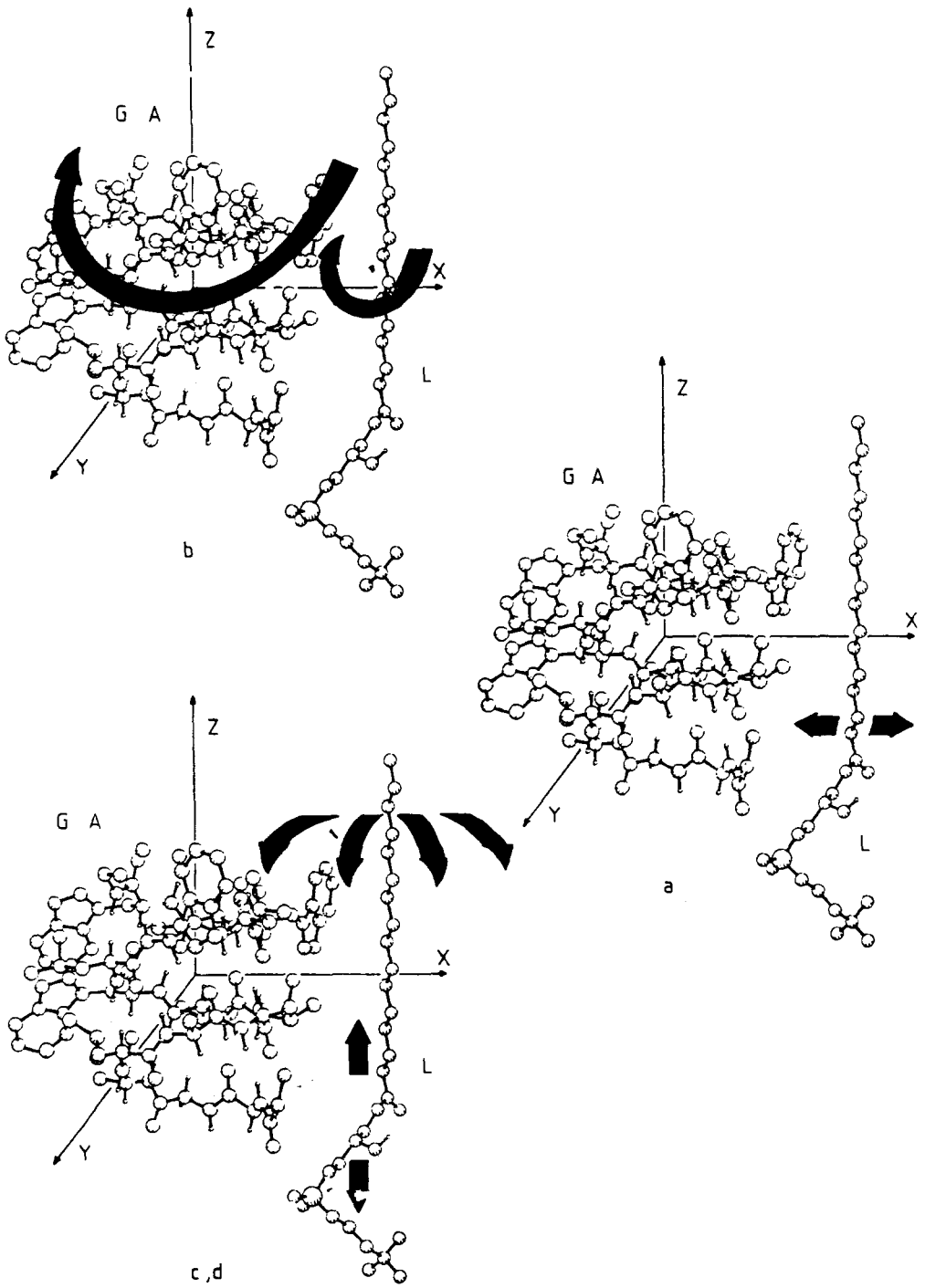


Fig. 1. Assemblage procedure of gramicidin A (G A) and palmitoyllysophosphatidylcholine (L).

early along the Z-axis perpendicular to the interface.

(c) The transfer energy of atoms or groups of atoms from an hydrophobic phase to an hydrophilic phase. The values of the transfer energies are similar to those determined experimentally by numerous authors and are summarized elsewhere [19]. Then, the packing of the lipid and gramicidin molecule was maintained and the orientation of a second lipid molecule around them was considered. We limited for computing-time considerations this approach to the number of lipids sufficient to surround one gramicidin A molecule. The mean molecular area occupied by the hydrophobic and hydrophilic moieties was estimated by projection on the X-Y plane using a grid of squares, each with 1 Å side. Calculations were made on a CDC Cyber 170 Computer coupled to a Calcomp 1051 drawing table (Computing Center of Brussels Free University) with the PLUTO drawing pro-

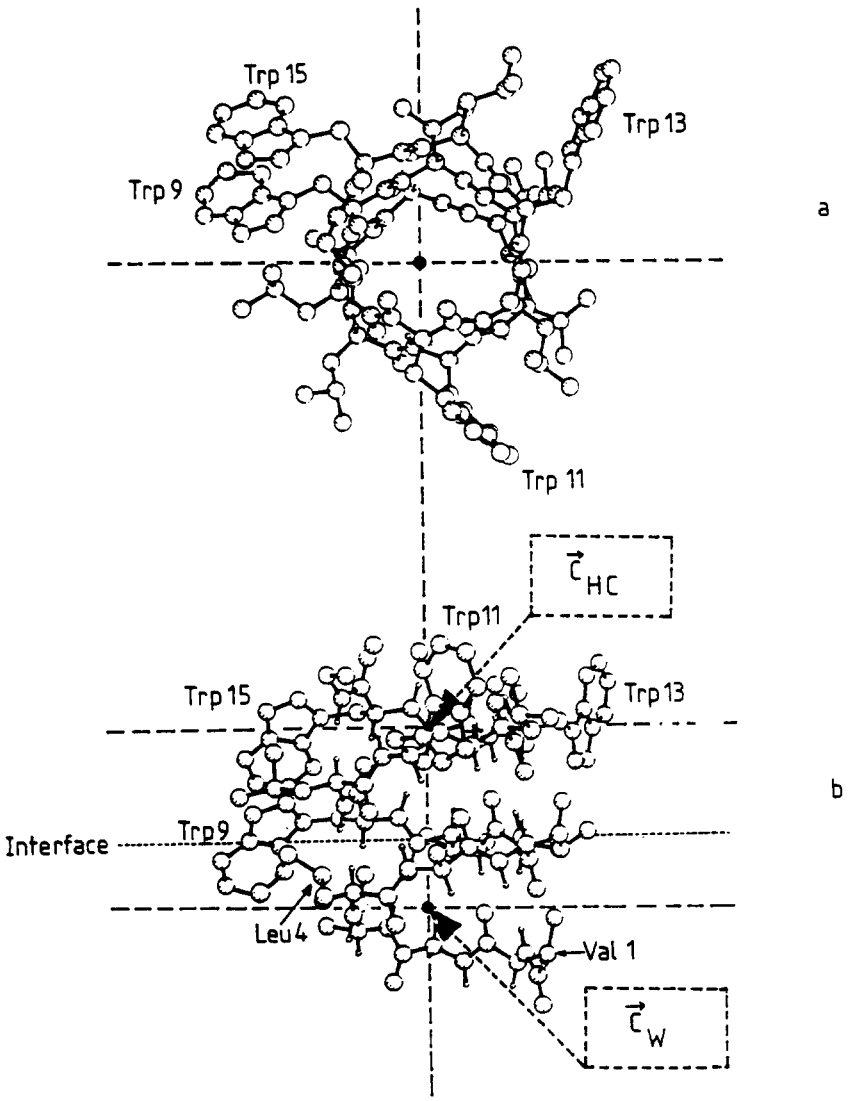


Fig. 2. Molecular structure of gramicidin A in a top view (a) and in a side view (b) indicating the position of the hydrophobic center ($\vec{C}_{HC}$) and the hydrophilic center ($\vec{C}_w$). Gramicidin A is a pentadecapeptide with the chemical structure: HCO-LVal$_1$-Gly$_2$-LAla$_3$-DLeu$_4$-LAla$_5$-DVal$_6$-LVal$_7$-DVal$_8$-LTrp$_9$-DLeu$_{10}$-LTrp$_{11}$-DLeu$_{12}$-LTrp$_{13}$-DLeu$_{14}$-LTrp$_{15}$-NHCH$_2$CH$_2$OH.

gram (Motherwell, B.C. and Clegg, W., (1978) Pluto, Cambridge, U.K.).

Results and Discussion

Isolated gramicidin A molecule at the air/water interface

Gramidicin A was oriented in the left-handed β-helical structure at the air/water interface as described in Methods using the data published by Venkatachalam and Urry [16]. From the hydrophobic transfer energy ($\Sigma_j E^-_{tr_j} = 229.6$ kcal/mol) and the hydrophilic transfer energy ($\Sigma_i E_{tr_i} = 142.2$ kcal/mol) associated to the entire molecule, we

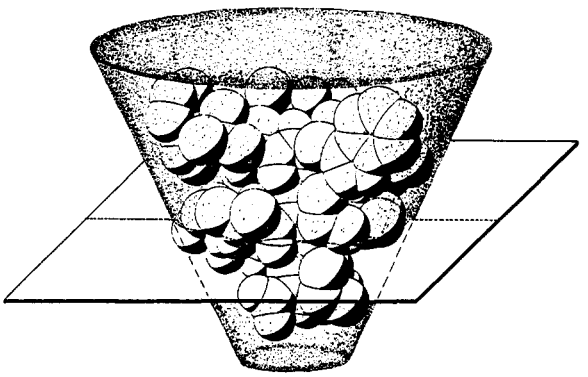


Fig. 3. Space-filling drawing of gramicidin A showing the cone-shaped dynamical structure of the molecule. The plane delineates the hydrophobic (above) and the hydrophilic (below) medium.

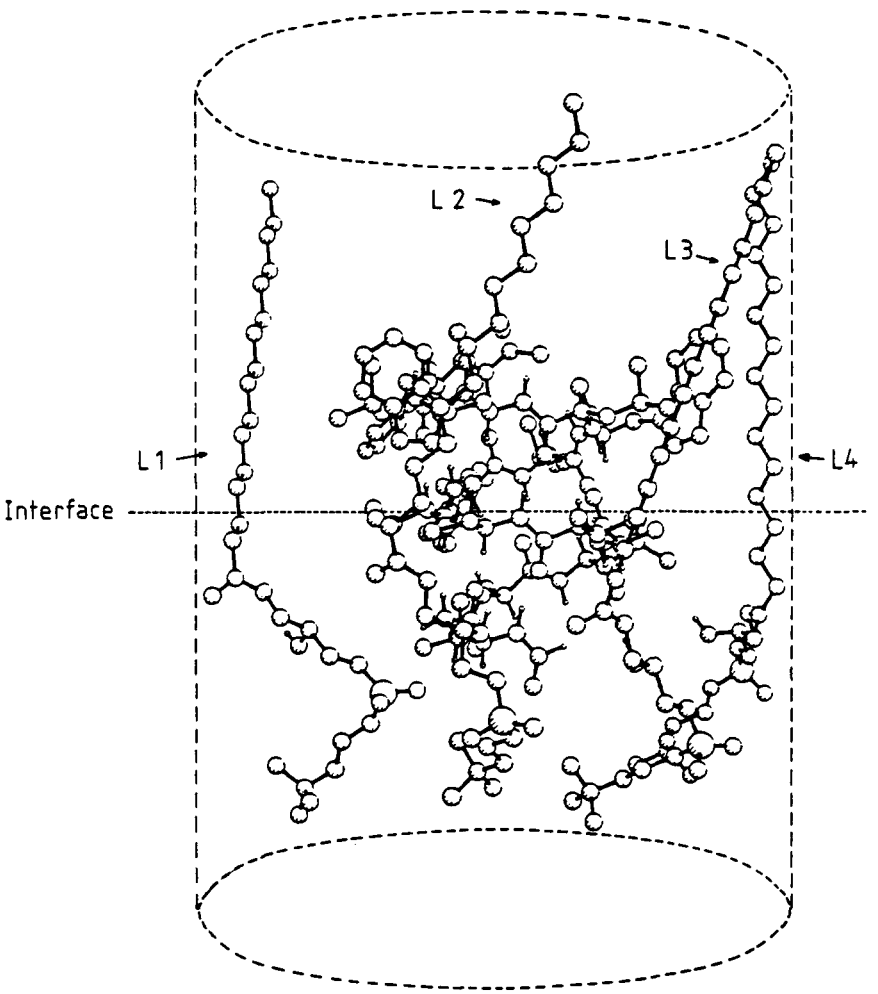


Fig. 4. Computer visualization (side view) of the mode of insertion of gramicidin A (structure I) in palmitoyllysophosphatidylcholine (1:4 molar ratio). L1, L2, L3 and L4 are the four palmitoyllysophosphatidylcholine molecules.

calculated the position of the hydrophobic and hydrophilic centers. The most probable orientation of the isolated gramicidin A at the air/water interface, which was calculated to run through tryptophan 9, is that with the hydrophobic center located in the hydrophobic medium, i.e., with the C-terminal, tryptophans 11, 13 and 15 bearing part pointing towards the hydrophobic medium (Fig. 2). Fig. 3 gives a representation of the gramicidin A structure in real volume, showing the cone-shaped structure of the molecule. The calculated area of the hydrophobic and hydrophilic moiety responsible for this cone-shaped structure were equal to 137 and 47 $Å^2$. This compares favorably with the values of 130 [29] and 145 $Å^2$/molecule [30] reported as limiting values at the air/water interface. The calculated energy required to orient gramicidin A with the tryptophans directed towards the hydrophilic medium was calculated to be 32 kcal/mol which is of the order of magnitude of the energy required to reorient lipids with the acyl chains pointing into the aqueous phase (unpublished results).

Isolated palmitoyllysophosphatidylcholine at the air/water interface

The calculation of this structure has been performed previously [21]. The isolated palmitoyllysophosphatidylcholine adopts a cone-shaped structure as a consequence of the large difference between the calculated area of the hydrophobic (20 $Å^2$) and hydrophilic (59 $Å^2$) moieties [21].

Gramicidin A-palmitoyllysophosphatidylcholine at the air/water interface

The assembly procedure has been applied to gramicidin A and palmitoyllysophosphatidylcholine. We assumed that gramicidin A could adopt

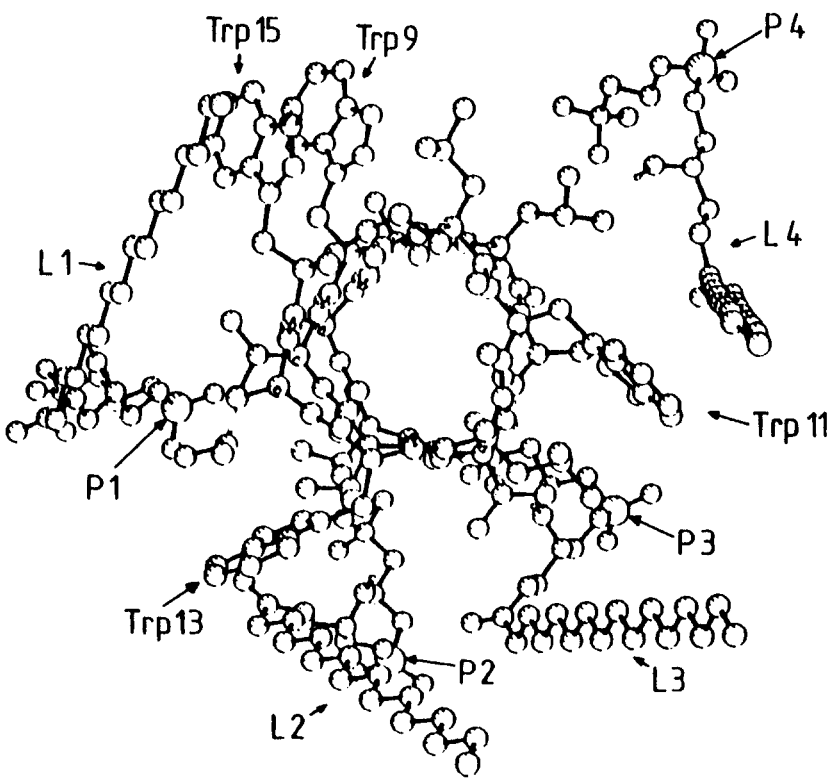
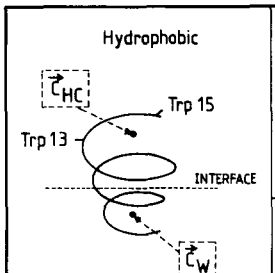
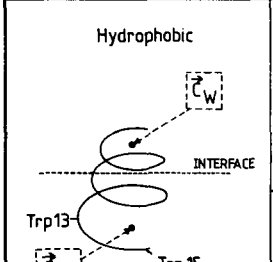


Fig. 5. Computer visualization (top view) of the mode of insertion of gramicidin A (structure I) in palmitoyllysophosphatidylcholine (1:4 molar ratio). L1, L2, L3 and L4 represent the four palmitoyllysophosphatidylcholine molecules, and P1, P2, P3 and P4 the phosphates associated to each lipid.

two orientations, one with the tryptophans oriented towards the hydrophobic phase (structure I) and one with the tryptophans oriented towards the hydrophilic (aqueous) phase (structure II). Four lysoPC molecules were calculated to surround one gramicidin A in structure I. In this 4 : 1 molar ratio complex, the total calculated area occupied by the hydrophobic moieties (lipid acyl chain and tryptophan residues located above the interface) and the hydrophilic moieties (lipid polar headgroups and amino acids located below the interface) are, respectively, equal to 250 and 240 $Å^2$. The dynamical shape associated to such structures is cylindrical (Figs. 4 and 5), which is compatible with a bilayer organization in agreement with the experimental data [12]. It is interesting that the same stoichiometry of four lysophosphatidylcholines per gramicidin monomer was experimentally found in the lamellar complex formed between these molecules [12]. It should be mentioned that three lysophosphatidylcholine molecules interact directly with the tryptophan moieties (L2-Trp-13, L1-Trp-15, L4-Trp-11). The fourth lysophosphatidylcholine is located in the proximity of Leu-12. For structure II of gramicidin A, the conformational analysis showed that six lysoPC are required to surround one gramicidin A. Since the area occupied per hydrophilic moiety is larger (137 $Å^2$) than the hydrophobic moiety (47 $Å^2$), a dynamical shape similar to that of lysoPC is obtained, making a lamellar organization highly unlikely. Furthermore, in this orientation, the gramicidin A-lysoPC energy of interaction is calculated to be 5.7 kcal/mol lysoPC lower than that obtained for the gramicidin A (structure I)-lysoPC complex (Table I). Also, if the stoichiometry of the two lysoPC-gramicidin A complexes is taken into account, the energy of interaction is higher (4.4 kcal/mol) for the gramicidin A (structure I)-lysoPC complex. However, it might be argued that dimer formation, which was not taken into account in the present calculation, could cause an inversion of this structure energetically to be more favorable. We calculated the energy of dimerization of structures I and II of gramicidin A as a function of rotation and translation of one monomer with respect to the other one, as described by Venkatachalam and Urry [16]. Clearly, the calculation demonstrated a preference for the left-handed β-helix NH_2-terminal to NH_2-terminal dimer, as compared to the COOH-terminal to COOH-terminal. The difference of interacting energy between the two forms is equal to 2 kcal/mol. This is considerably less than the difference of 5.7 kcal/mol for the two orientations of the lysoPC-gramicidin complex, making it unlikely that dimer formation would lead to a N-N-terminal association of gramicidin in the complex with lysoPC. In summary, the present calculations clearly suggest a preferred orientation of the gramicidin molecule in the β[6.3]

TABLE I

E_{int} is the mean energy of interaction between one gramicidin A and one palmitoyllysophosphatidylcholine. It is the sum of the Van der Waals interaction between atoms associated to different molecules, The electrostatic interaction between atomic point charges and the transfer energy of atoms or groups of atoms from an hydrophobic phase to an hydrophilic phase. C_{HC} and C_w define the hydrophobic and the hydrophilic centers.

		Lyso
	<E_{int}> (Kcal/mol)	-14.9
	Number of lipids surrounding one Gramicidin A	4
	<E_{int}> (Kcal/mol)	-9.2
	Number of lipids surrounding one Gramicidin A	6

helical structure with its C-terminal end directed towards the hydrophobic medium, in good agreement with the data on the effect of gramicidin on lipid polymorphism [12–15], but in contradiction with other studies [4,5,9–11]. The reason for this discrepancy is unknown, but might be related to the ability of the gramicidin molecule to adopt a variety of different structures depending on the environmental conditions. However, it should be noted that the conformational analysis methods we have used, are still crude and, for instance, do not take into account a possible change in structure of the entire gramicidin-lysophosphatidylcholine aggregate as a result of changes in gramicidin conformation induced by the interaction with the lipid. Furthermore, the present calculations predict an all-*trans* acyl chain as the most probably conformation of the lipid in interaction with gramicidin (Figs. 4 and 5). This might be in conflict with recent ^{2}H-NMR experiments which showed that in the lamellar gramicidin-lysophosphatidylcholine (1 : 4) complex, the acyl chains display an average order parameter profile characteristic of the liquid-crystalline phase [31]. Future refinements of this conformational approach could possibly resolve this discrepancy. However, it is also clear that definitive statements concerning the interrelationship between the orientation of gramicidin and the structure of the gramicidin-lysophosphatidylcholine complex will require, in adition, experimental data using covalently bound N-N and C-C gramicidin dimers.

Acknowledgements

It is our pleasure to acknowledge Professor D.W. Urry for having provided us with an update list of the atomic coordinates of gramicidin A.

References

1 Urry, D.W. (1971) Proc. Natl. Acad. Sci. USA 68, 672–676
2 Hladky, S.B. and Haydon, D.A. (1972) Biochim. Biophys. Acta 274, 294–312
3 Apell, H.J., Bamberg, E. and Alpes, H. (1979) J. Membrane Biol. 50, 271–285
4 Urry, D.W., Trapane, T.L. and Prasad, K.U. (1983) Science 221, 1064–1067
5 Weinstein, S., Wallace, B.A., Blout, E.R.N., Morrow, J. and Veatch, W.R. (1979) Proc. Natl. Acad. Sci. USA 76, 4230–4234
6 Bradley, R.J., Urry, D.W., Okamoto, K. and Rapaka, R. (1978) Science 200, 435–436
7 Veatch, W.R., Fossel, E.T. and Blout, E.R. (1974) Biochemistry 13, 5249–5256
8 Arseniev, S.A., Barsukov, I.L. and Bystrov, V.F. (1985) FEBS Lett. 180, 33–39
9 Urry, D.W., Trapane, T.L., Romanowski, S., Bradley, R.J. and Prasad, K.U. (1983) Int. J. Peptide Protein Res. 21, 16–23
10 Naik, V.M. and Krimm, S. (1984) Biochem. Biophys. Res. Commun. 125, 919–925
11 Wallace, B.A. (1983) Biopolymers 22, 397–402
12 Killian, J.A., De Kruijff, B., Van Echteld, C.J.A., Verkleij, A.J., Leunissen-Bijvelt, J. and De Gier, J. (1983) Biochim. Biophys. Acta 728, 141–144
13 Pasquali-Ronchetti, E., Spisni, A., Casali, E., Masotti, L. and Urry, D.M. (1983) Biosci. Rep. 3, 127–133
14 Van Echteld, C.J.A., Van Stigt, R., De Kruijff, B., Leunissen-Bijvelt, J., Verkleij, A.J. and De Gier, J. (1981) Biochim. Biophys. Acta 648, 287–291
15 Van Echteld, C.J.A., De Kruijff, B., Verkleij, A.J., Leunissen-Bijvelt, J. and De Gier, J. (1982) Biochim. Biophys. Acta 692, 126–138
16 Venkatachalam, C.M. and Urry, D.W. (1983) J. Comp. Chem. 4, 461–469
17 Brasseur, R., Goormaghtigh, E. and Ruysschaert, J.M. (1981) Biochem. Biophys. Res. Commun. 103, 301–310
18 Brasseur, R., Vandenbosch, C., Van den Bossche, H. and Ruysschaert, J.M. (1983) Biochem. Pharmac. 32, 2175–2180
19 Tanford, C. (1973) The Hydrophobic Effect. Formation of Micelles and Biological Membranes, Wiley, New York
20 Brasseur, R. and Deleers, M. (1984) Proc. Natl. Acad. Sci. USA 81, 3370–3375
21 Brasseur, R., De Kruijff, B. and Ruysschaert, J.M. (1984) Biosci. Rep. 4, 256–267
22 Liquori, A.M., Giglio, E. and Mazzarella, L. (1968) Nuovo Cimento 55B, 475–480
23 Giglio, E., Liquori, A.M. and Mazzarella, L. (1968) Nuovo Cimento 56B, 57–62
24 Scordamaglia, R., Gavollone, F. and Clemente, E. (1977) J. Chem. Soc. 99, 5545
25 Giglio, E. (1969) Nature 222, 339–341
26 Liquori, A.M. (1969) Rev. Biophys. 2, 65–92
27 De Coen, J.L. and Ralston, E. (1971) Biopolymers 16, 1929–1935
28 Hopfinger, A.J. (1973) Conformational Properties of Macromolecules, Academic Press, New York
29 Cornell, B.A., Sacré, M.M., Peel, W.E. and Chapman, D. (1978) FEBS Lett. 90, 29–35
30 Kemp, G., Jacobson, K.A. and Wenner, C.E. (1972) Biochim. Biophys. Acta 255, 493–501
31 Killian, J.A., Borle, F., De Kruijff, B. and Seelig, J. (1986) Biochim. Biophys. Acta 854, 133–142