Biochimica et Biophysica Acta, 728 (1983) 141–144
Elsevier Biomedical Press

BBA Report

BBA 70080

MIXTURES OF GRAMICIDIN AND LYSOPHOSPHATIDYLCHOLINE FORM LAMELLAR STRUCTURES

J.A. KILLIAN [a], B. DE KRUIJFF [b], C.J.A. VAN ECHTELD [a], A.J. VERKLEIJ [b], J. LEUNISSEN-BIJVELT [c] and J. DE GIER [a]

[a] *Department of Biochemistry,* [b] *Department of Molecular Biology, and* [c] *Department of Molecular Cell Biology, State University of Utrecht, Tansitorium 3, Padualaan 8, NL-3584 CH Utrecht (The Netherlands)*

(Received September 2nd, 1982)

Key words: Gramicidin; Lysophosphatidylcholine; Bilayer structure; ^{31}P-NMR; Freeze-fracture

It is shown by ^{31}P-NMR and freeze-fracture electron microscopy that in aqueous dispersions of mixtures of gramicidin and palmitoyllysophosphatidylcholine lamellar structures are formed which contain four lysophosphatidylcholine molecules per gramicidin monomer.

Lipid-protein interactions are generally assumed to play decisive roles in the structure and function of biological membranes. Therefore the interaction between, in particular, intrinsic membrane proteins and lipids is a subject of considerable interest. Gramicidin, a hydrophobic linear pentadecapeptide, has been widely used as a model for the hydrophobic segment of membrane proteins [1,2]. It is known that gramicidin in phosphatidylcholine bilayers is generally organized in NH_2-terminal to NH_2-terminal π_6 (L, D) helical dimers [3,13]. Recently it has been shown that gramicidin lowers the bilayer to hexagonal H_{II} phase transition temperature of phosphatidylethanolamines [4]. This hexagonal H_{II} phase promoting ability of gramicidin is also manifested in phosphatidylcholine dispersions. In the absence of the peptide these lipids organize in a lamellar phase, but upon incorporation of gramicidin the hexagonal H_{II} phase is induced when the acyl chain length exceeds 16 carbon atoms [5].

The phase preference of lipids has been related to the average shape of the molecules [6,10]. Bilayer preferring lipids are thought to have an overall cylindrical shape. Hexagonal H_{II} phase type of lipids are thought to be conical with the polar headgroup at the smaller end of the cone. Lipids such as lysophosphatidylcholine, with a relatively large hydrophilic moiety, prefer micellar organizations in excess water. It should be realized that the concept of molecular shape is inclusive and takes into account dynamic properties of the lipids as well as headgroup hydration and intermolecular interactions such as hydrogen bonding.

The shape-structure relationship applies also to mixed lipid systems. For instance, mixtures of lysophosphatidylcholines and the cone shaped cholesterol form bilayers [7].

In the frame of the shape concept two explanations have been offered for the hexagonal H_{II} phase promoting ability of gramicidin [4]. Firstly, it has been proposed that when the length of the hydrophobic part of the bilayer exceeds the length of the dimer, due to meniscus formation the phospholipid/gramicidin entity adopts more of a cone shape. A second possibility is that the gramicidin molecule itself has a conical shape, thereby favouring hexagonal H_{II} phase formation. To discriminate between these possibilities and to get further insight in the effect of gramicidin on the structure of membrane lipids, we investigate in this study the interaction of gramicidin with palmitoyllysophosphatidylcholine dispersions.

It will be shown that a mixture of gramicidin

and lysophosphatidylcholine forms bilayers when dispersed in aqueous solution. Gramicidin from *Bacillus brevis*, which is a mixture of gramicidins A, B and C was obtained from Sigma (St. Louis, MO, U.S.A.). 1-Palmitoyl-*sn*-glycero-3-phosphocholine (lysophosphatidylcholine) was obtained by hydrolysis of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (which was synthesized as described elsewhere [8]), using phospholipase A_2 from *Naja naja* (a kind gift of Professor Dr. G.H. de Haas). Both lipids were purified by HPLC [9].

A variable amount of gramicidin and 35 μmol of lysophosphatidylcholine were dissolved in chloroform and evaporated to dryness in a 10-mm NMR tube under a stream of nitrogen, followed by overnight storage under high vacuum. Dispersions were prepared by adding 1.3 ml of a 100 mM NaCl, 25 mM Tris/acetic acid, 0.2 mM EDTA, pH 7.0 buffer containing 25% (v/v) ^2H_2O to the dry lipids. This was followed by incubation for several hours at room temperature to allow swelling of the lipids after which the dispersion was gently vortexed.

^{31}P-NMR spectra of aqueous dispersions of mixtures of gramicidin and lysophosphatidylcholine at varous molar ratios are shown in Fig. 1. Pure lysophosphatidylcholine forms micelles in solution, which, due to fast tumbling and lateral diffusion of the lipids, give rise to a narrow isotropic NMR signal. When the phospholipid is mixed with gramicidin a second spectral component becomes visible which is characterized by a high-field peak and a low-field shoulder. Such asymmetrical spectra are typical for phospholipids organized in extended lamellar structures in which the chemical shift anisotropy is only partially averaged by rapid axial rotation of the phosphate moiety. Upon increasing the gramicidin concentration the bilayer component increases such that when the molar ratio of gramicidin to lysophosphatidylcholine exceeds 1 : 4, the isotropic signal is absent. That the sharp signal at intermediate gramicidin concentrations is due to small structures, possibly pure lysophosphatidylcholine micelles, could be conluded from centrifugation experiments. When the samples were spun for 15 min at 27000 × g at 4°C a quantitative separation could be obtained between structures remaining in the supernatant which gave rise to an isotropic

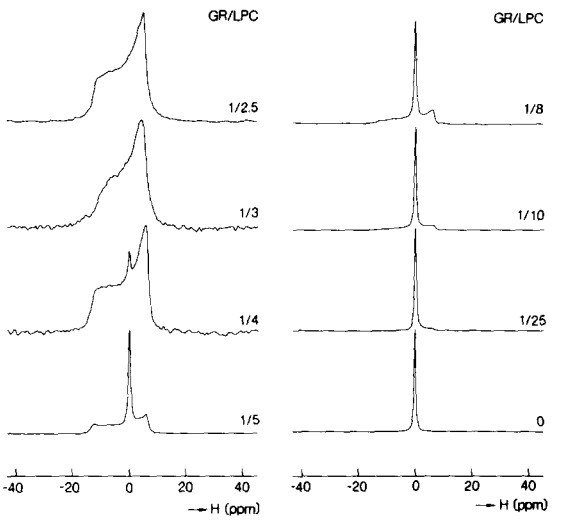

Fig. 1. Proton noise decoupled 81.0 MHz ^{31}P-NMR spectra of aqueous dispersions of gramicidin and lysophosphatidylcholine at various molar ratios, recorded on a Bruker WP 200 spectrometer using a gated decoupling method with an input power of 5 W during 10% of the interpulse time. 3000–20000 transients were recorded with a 1-s interpulse time, a 90° pulse angle and a 25 kHz spectral width at a temperature of 25°C, using 4 K data points. Upon increasing the relaxation delay to 5 s no significant changes of relative peak intensities were observed. To all free induction decays an exponential multiplication was applied, resulting in a 50 Hz line broadening. All samples contained 35 μmol phospholipid with a variable amount of gramicidin. The molar ratio of both components is indicated in the figure. 0 ppm corresponds to the chemical shift of the ^{31}P-NMR resonance position of pure lysophosphatidylcholine micelles.

signal and pelleted structures which produced the 'bilayer' type of spectrum. The relative amount of 'bilayer' and isotropic signal in the spectra shown in Fig. 1 were independent of temperature in the range of 0–65°C.

Another interesting feature is that the residual chemical shift anisotropy ($\Delta\sigma$) of the bilayer was 16–20 ppm, which is only half of that reported for diacylphospholipid dispersions [6,10]. Such small values of $\Delta\sigma$ have been reported previously for gel state lysophosphatidylcholine bilayers and for bilayers of lysophosphatidylcholine/cholesterol mixtures and demonstrate increased motional freedom of the phosphate part of the lipid molecule [7].

Fig. 2 shows the relative amount of 'bilayer' signal as a function of the gramicidin content. There is a linear increase of the 'bilayer' compo-

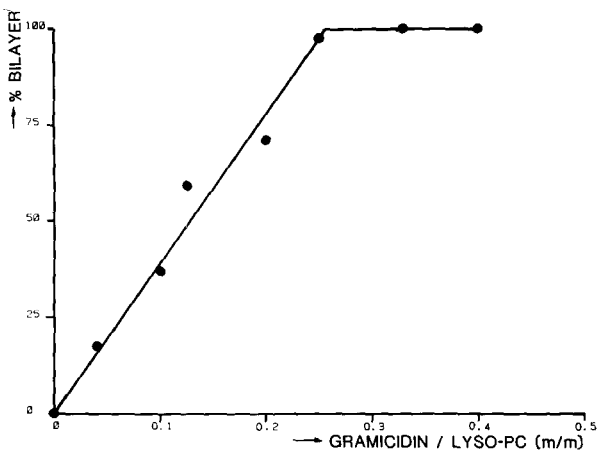

Fig. 2. Relative amount of 'bilayer' type of ^{31}P-NMR signal in aqueous dispersions of gramicidin and lysophosphatidylcholine at various molar ratios as derived from ^{31}P-NMR spectra presented in Fig. 1. The relative amount of 'bilayer' component was calculated from the amount of isotropic signal which was determined either by computer subtraction or by measuring directly the part of the total integral corresponding to the area of isotropic signal. From the data points of the samples with molar ratios of gramicidin to lysophosphatidylcholine from 0 to 0.25 a linear relationship was derived. The slope was calculated by a linear regression method to be 389.3 ± 11.3 corresponding to a 100% 'bilayer' component at a molar ratio of 0.256 ± 0.007.

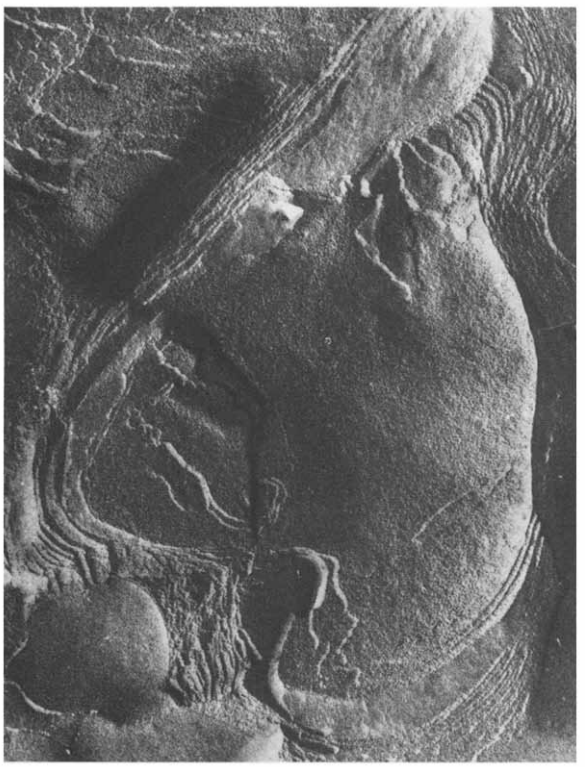

Fig. 3. Freeze-fracture electron micrograph of an aqueous dispersion of a mixture of gramicidin and lysophosphatidylcholine in a molar ratio of 1 : 4. The sample was quenched from 25°C using the one-sided propane-jet procedure described by Pscheid et al. [14]. Magnification: 43000×.

nent with the amount of gramicidin present till at a gramicidin to lysophosphatidylcholine ratio of approximately 0.25 the isotropic component is completely absent. Using a linear regression method it could be calculated that each gramicidin molecule causes 3.9 ± 0.1 lysophosphatidylcholine molecules to adopt a structure giving rise to a 'bilayer' type of ^{31}P-NMR signal.

The various samples also showed macroscopic differences. Whereas pure lysophosphatidylcholine formed a clear solution in water, gramicidin/lysophosphatidylcholine mixtures in the range of 1 : 25 to 1 : 5 formed white turbid dispersions. At molar ratios of 1 : 4 to 1 : 2.5 the samples consisted of an excess water and a translucent material which sedimented at the bottom of the NMR tube.

Further evidence for the bilayer structure of the gramicidin-lysophosphatidylcholine complex was obtained from freeze fracturing electron microscopy. Fig. 3 clearly shows the presence of large multilamellar vesicles in the sample with a molar ratio of 1 : 4. A relatively large amount of cross-fractures is observed, which might indicate rather close stacking of the bilayers.

Preliminary small angle X-ray diffraction measurements, carried out as described elsewhere [5] showed a broad diffraction band, centered at 35 Å for the pelleted 1 : 10 sample. In the sample with a molar ratio of gramicidin to lysophosphatidylcholine of 1 : 2.5 a sharper band at 110 Å and a weak band at 35 Å were visible. The measurements demonstrate the presence of an ordered structure in the samples and are consistent with the presence of multilayered structures.

The present study clearly demonstrates the ability of gramicidin to form extended bilayer structures with lysophosphatidylcholine which on its own prefers to adopt a micellar organization. This is a remarkable property of gramicidin as

lysophospholipids by their detergent action normally solubilize intrinsic membrane proteins to form micellar aggregates.

Sonicated lysophosphatidylcholine/gramicidin mixtures have been extensively used in spectroscopic studies on the conformation and properties of gramicidin. In these studies the dimeric state of gramicidin and the channel activity appeared to be retained in the presence of the lysophosphatidylcholine [11,12].

In view of the shape concept [6] the present finding that gramicidin forms stable bilayers with lysophosphatidylcholine, suggests that gramicidin is cone shaped. However, as the COOH-terminal part is bulkier than the NH_2-terminal part, due to the presence of the tryptophan residues, this would imply an orientation of the NH_2-terminal at the membrane/water interface, which is opposite to the reported [3,13] configuration. Studies with desformylgramicidin and the covalently bonded dimer might further elucidate the conformation of the gramicidin in these systems as well as the origin of the 1 : 4 stoichiometry of the lamellar gramicidin-lysophosphatidylcholine complex.

We would like to thank Dr. H. Rijnbout for performing the X-ray experiments and Mr. W.S.M. Geurts van Kessel for synthesizing and purifying the lysophosphatidylcholine.

References

1 Chapman, D., Cornell, B.A., Eliasz, A.W. and Perry, A. (1977) J. Mol. Biol. 113, 517–538
2 Rice, D. and Oldfield, E. (1979) Biochemistry 18, 3272–3278
3 Urry, D.W., Goodall, M.C., Glickson, J.D. and Mayers, D.F. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 1907–1911
4 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
5 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
6 Cullis, P.R. and De Kruijff, B. (1979) Biochim. Biophys. Acta 559, 399–420
7 Van Echteld, C.J.A., De Kruijff, B., Mandersloot, J.G. and De Gier, J. (1981) Biochim. Biophys. Acta 649, 211–220
8 Van Deenen, L.L.M. and De Haas, G.H. (1964) Adv. Lipid Res. 2, 168–229
9 Geurts van Kessel, W.S.M., Tieman, M. and Demel, R.A. (1981) Lipids 16, 58–63
10 Seelig, J. (1978) Biochim. Biophys. Acta 515, 105–140
11 Cavatorta, P., Spisni, A., Casali, E., Lindner, L., Masotti, L. and Urry, D.W. (1982) Biochim. Biophys. Acta 689, 113–120
12 Urry, D.W., Spisni, A., Khaled, M.A. and Long, M.M. (1979) Int. J. Quantum Chem.: Quantum Biol. Symp. 6, 289–303
13 Weinstein, S., Wallace, B.A., Morrow, J.S. and Veatch, W.R. (1980) J. Mol. Biol. 143, 1–19
14 Pscheid, P., Schudt, C. and Plattner, H. (1981) J. Microsc. 121, 149