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Solvent determined conformation of gramicidin affects the ability of the peptide to induce hexagonal H_{II} phase formation in dioleoylphosphatidylcholine model membranes

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It is shown by ^{31}P-NMR and small angle X-ray scattering that induction of an hexagonal H_{II} phase in dioleoylphosphatidylcholine model membranes by external addition of gramicidin A′ depends on the solvent which is used to solubilize the peptide. Addition of gramicidin from dimethylsulfoxide or trifluoroethanol solution leads to H_{II} phase formation whereas addition of the peptide from ethanol does not. This solvent dependence is shown by circular dichroism to be correlated with the peptide conformation. The channel conformation appears to be responsible for H_{II} phase formation by gramicidin.

Gramicidin A is an effective promoter of hexagonal H_{II} phases in model and biological membranes [1]. This effect is a consequence of the specific chemical structure of this pentadecapeptide, in particular with respect to the presence of the four tryptophans at the C-terminal part of the molecule [1–3]. It was proposed that these bulky residues would provide a pronounced cone shape to the molecule which, within the shape-structure concept of polymorphism [4], together with the peptide's tendency to self associate into cylindrical structures [5–7] would explain the H_{II} phase-inducing ability of the molecule.

The molecular properties of gramicidin in a membrane environment will depend on the exact conformation of the peptide. Since gramicidin can adopt different conformations in different solvents, we thought it of interest to compare the effect of gramicidin addition from different solvents on the peptide's ability to induce the H_{II} phase in model membranes. As test lipid dioleoylphosphatidylcholine (DOPC) was chosen because of the well characterized H_{II} phase formation in hydrated mixed films with gramicidin [3,6,8]. Solvents studied were dimethylsulfoxide (DMSO) in which the peptide adopts a monomeric conformation, the exact nature of which is still not known [9–12], trifluoroethanol in which gramicidin is considered to have a similar conformation [9] and ethanol in which a mixture of conformers exists the dominant one most likely being an antiparallel β-double helix [12,13].

Abbreviations: CD, circular dichroism; DMSO, dimethylsulfoxide; DOPC, dioleoylphosphatidylcholine.

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Gramicidin A′ (natural mixture) was obtained from Sigma (St. Louis, MO) and used as such. Solvents and all other reagents were of analytical grade. 6-Carboxyfluorescein (carboxyfluorescein) (Eastman Kodak Co., Rochester, NY, U.S.A.) was purified by active carbon treatment, recrystallization from water/methanol (2 : 1, v/v) and Sephadex LH20 column chromatography [14]. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine was synthesized and purified as described elsewhere [15] and purified with HPLC [16]. The structure of the gramicidin-DOPC systems was investigated by ^{31}P-NMR and small angle X-ray scattering. An aliquot of a gramicidin solution (9 mM) was dropwise added to 40 μmol DOPC dispersed by vortexing in 60 ml vigorously stirred buffer (150 mM NaCl, 10 mM Tris-HCl, 0.2 mM EDTA (pH 7.4)). After incubation for 30 min at roomtemperature the dispersions were centrifuged (17 500 × *g*; 20 min at 4 °C) and the pellet was either transferred to a 10 mm NMR tube and measured on a Bruker MSL-300 NMR spectrometer [6] or mounted in the slit of a steel cuvet enclosed between cellophane sheets and measured in a Kratky X-ray camera [5]. Unless otherwise indicated all experiments were carried out at 20 °C.

Carboxyfluorescein leakage was used to detect gramicidin-induced changes in barrier properties of DOPC vesicles. Carboxyfluorescein was entrapped by preparing large unilamellar vesicles (average size approx. 200 nm) using the extrusion technique [17] with a dispersion of 20 μmol of DOPC in 1 ml 40 mM NaCl, 40 mM Tris acetate (pH 7.0) containing 75 mM carboxyfluorescein. The external carboxyfluorescein-buffer was replaced by incubation buffer (77.5 mM NaCl, 40 mM Tris acetate (pH 7.0)) by means of a Sephadex G-50 column (1.5 × 20 cm). To 3 ml of the incubation buffer, 4–8 μl of a vesicle suspension (2–4 mM phospholipid) was added and subsequently small aliquots of gramicidin (2 mM) were added with immediate vigorous mixing. Carboxyfluorescein release was measured as an increased emission due to a dequenched fluorescence [18]. The total amount of carboxyfluorescein entrapped was determined by lysing the vesicles by the addition of 50 μl 10% Triton X-100.

Circular dichroism (CD) was used to monitor the conformation of gramicidin and was carried out as described before [19] using small unilamellar DOPC vesicles to reduce scattering artefacts [20,21]. A 12.5 mM DOPC dispersion in distilled water was sonicated on ice in aliquots of 1–2 ml with a Heat Systems Ultrasonics, Inc. sonicator cell disrupter (W-225R), using a macrotip at power level 7 for 3–5 min. After centrifugation (15 000 × *g*, 30 min at 4 °C) 0.125 μmole gramicidin was added slowly to the supernatant from a stock solution in trifluoroethanol or ethanol (9 mM) or DMSO (40 mM) with a microsyringe and under vortexing. The gramicidin/DOPC ratio was varied by dilution of the lipid vesicle solution with distilled water prior to gramicidin addition. After 15 min incubation at roomtemperature the samples were briefly resonicated (power setting 4) and centrifuged (15 000 × *g*, 15 min at 4 °C), yielding a clear supernatant of which 100–200 μl was used for CD measurements and the remainder for gramicidin [3] and phosphorus determinations [22]. In all cases the lipid recovery in the supernatant was greater than 95% whereas the recovery of gramicidin varied from almost quantitative when added from DMSO or trifluoroethanol to only about 40% when gramicidin was added as an ethanolic solution. That in these experiments the peptide in the supernatant is associated with the lipids is demonstrated by control measurements in which no gramicidin was recovered in the supernatant when the peptide was added from the different solvents in the absence of lipid.

Fig. 1 shows that immediately after the addition of gramicidin to DOPC from either DMSO or trifluoroethanol in a 1/10 molar ratio with respect to the phospholipid there is a change from a typical 'bilayer' to a composite 'bilayer/H_{II}' ^{31}P-NMR line shape (see Ref. 23 for description of line shapes) indicating a bilayer to hexagonal H_{II} phase transition for a part (approx. 30%) of the lipids. Similar results have been reported upon hydration of mixed DOPC-gramicidin 10/1 films dried from chloroform/methanol mixtures [2,3, 6,8]. Further incubation does not result in changes in the ^{31}P-NMR spectrum. When gramicidin is added to the DOPC dispersion as an ethanolic solution a completely different picture emerges. In this case no H_{II} ^{31}P-NMR line shape can be detected up to several hours after addition of the peptide. Only a small broad spectral component is

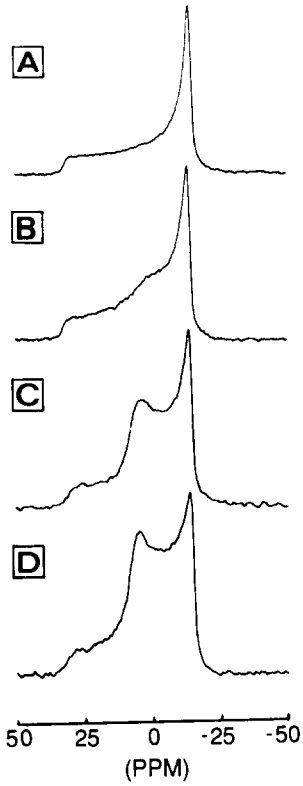

Fig. 1. 121.5 MHz ^{31}P-NMR spectra obtained from aqueous dispersions of DOPC (A) and DOPC to which gramicidin 1/10 (mol/mol) phospholipid was added externally from ethanol (B), trifluoroethanol (C) or DMSO (D). Spectra were recorded within 1 h after addition of gramicidin. The 0 ppm position corresponds to isotropically moving DOPC molecules as present in sonicated vesicles.

observed centered around 0 ppm and superimposed on a 'bilayer' type of ^{31}P-NMR signal (Fig. 1B). Incubation of this sample at a slightly elevated temperature (35°C) results in the gradual loss of the central component and the appearance of a 'H_{II}' type of ^{31}P-NMR line shape which after 24 h has an intensity comparable to that obtained for DOPC immediately after external addition of gramicidin from DMSO or trifluoroethanol (data not shown).

Small angle X-ray scattering profiles obtained from DOPC vesicles to which gramicidin was added from trifluoroethanol or DMSO (gramicidin/lipid 1:10, molar) show three reflections with d values of 63.9, 36.7 and 32.0 Å which relate as $1:1/\sqrt{3}:1/2$ and indicate the coexistence of hexagonal H_{II} phase and lamellar structures [24].

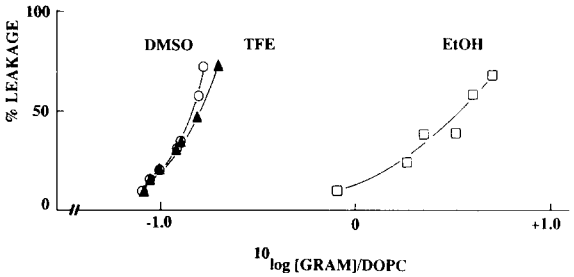

Fig. 2. The percentage of carboxyfluorescein release from DOPC vesicles induced by gramicidin (2 mM) added from ethanol (□), trifluoroethanol (TFE) (▲) and DMSO (○) after 10 min after addition of varying amounts of gramicidin (molar ratios are given).

The scattering profiles were similar to that reported earlier [25]. In conjunction with the ^{31}P-NMR data discussed above, the H_{II} phase specific $(1/\sqrt{3})$ reflection was absent in the diffraction patterns obtained from peptide-lipid dispersions to which gramicidin was added from an ethanolic solution (data not shown). In these latter samples the $(1/\sqrt{3})$ reflection gradually appeared after several hours of incubation at 35°C. These observations differ qualitatively from earlier data [25] which showed some H_{II} phase formation by gramicidin already within 2 h when added externally from ethanolic solution. Comparable spectra are in the present approach obtained only after 5 h of incubation at 35°C. This may be the consequence of slight differences in the experimental protocols.

It is interesting to note that the same differences in lipid structure modulating ability of gramicidin can be observed when the peptide is added externally from trifluoroethanol or ethanol as when it is incorporated via hydration of a dry mixed peptide-lipid film prepared from these same solvents (data not shown). Addition of the organic solvents themselves did not lead to any change in ^{31}P-NMR line shape or small-angle X-ray scattering patterns.

Gramicidin induced H_{II} phase formation is parallelled by a release of entrapped carboxyfluorescein from DOPC vesicles. After a short lag time of 1–2 min a steep sigmoidal increase in fluorescence emission is observed which reaches a plateau after 4–5 min. Fluorescence measurements show that gramicidin, dissolved in trifluoro-

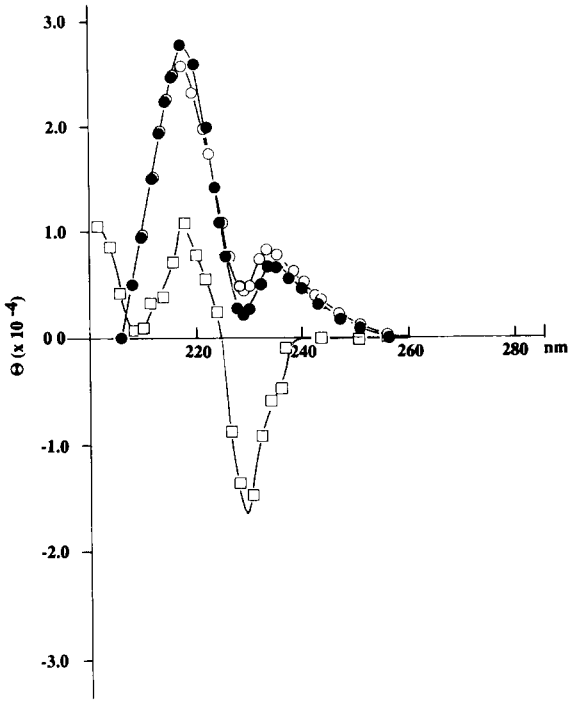

Fig. 3. Circular dichroism spectra of gramicidin added to DOPC vesicles in a molar ratio of 1/25 of peptide to lipid from a solution of trifluoroethanol (●), DMSO (○) and ethanol (□). The CD measurements were carried out at room temperature using a 0.2 mm pathlength cell. Due to the high solvent absorption, the samples in which DMSO was present could not be measured at wavelength shorter than 215 nm.

ethanol or DMSO, induces leakage of carboxyfluorescein at molar ratios exceeding 1/15 mol of peptide to phospholipid whereas external addition of gramicidin dissolved in ethanol causes carboxyfluorescein release increase only at molar ratios as high as approximately 1 : 1 (Fig. 2). A full account of the effect of gramicidin on carboxyfluorescein release in relation to H_{II} phase formation and channel function will be given elsewhere.

Because it was impossible to produce, even by prolonged (re)sonication, DOPC-gramicidin dispersions of sufficient clarity at peptide concentrations where H_{II} phase formation occurs, lower peptide to lipid ratios had to be used in the CD measurements.

Fig. 3 shows the CD patterns of gramicidin, added from trifluoroethanol, DMSO and ethanol to DOPC sonicated vesicles in a 1/25 molar ratio of peptide to lipid. Upon addition from trifluoroethanol and DMSO very similar CD spectra are observed for gramicidin characterized by positive peaks at about 218 and 235 nm and a negative extremum at 229 nm. This spectrum is considered to be representative of the channel conformation (β6.3 conformation) of gramicidin [26]. When the peptide is added from an ethanolic solution a different CD pattern emerges, characterized by a large negative peak at 229 nm, a weaker positive peak at 218 nm and positive ellipticity below 208 nm, demonstrating that peptide is now present in another yet unknown but possibly antiparallel double-helical conformation. Incubation at elevated temperature resulted in a time-dependent change in CD spectrum towards that of the channel conformation. Similar results were reported by Masotti et al. [19] upon external addition of gramicidin in trifluoroethanol and ethanol to egg-phosphatidylcholine vesicles and egg-lysophosphatidylcholine micelles. In trifluoroethanol and DMSO the spectra did not change when the initial gramicidin/lipid molar ratio was altered to 1/50. In ethanol, however, such an alteration resulted in a slight decrease in absolute intensity of the negative peak at 229 nm and a slight increase in ellipticity of the positive maximum at 218 nm. Both the direction and the small magnitude of this change in the CD pattern upon decreasing the gramicidin/lipid ratio, indicate that the observed differences between the spectra obtained after addition from trifluoroethanol and DMSO on one hand and from ethanol on the other, cannot be a result of differences in the gramicidin/DOPC ratios in these samples.

That the observed differences are also not due to the presence of solvent is demonstrated by the fact that when gramicidin and lipid are co-dissolved in different organic solvents, subsequent drying and hydration of these samples results in similar conformational differences of the peptide as found upon external addition of gramicidin from these same solvents (Killian, J.A. and Urry, D.W., work in preparation).

In conclusion, the combined results demonstrate that the conformation of gramicidin is very important for the peptide-lipid interaction. Extrapolation of the CD data towards higher peptide concentrations, suggests that for H_{II} phase induc-

tion the peptide has to be in the channel conformation.

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References

1 Killian, J.A. and De Kruijff, B. (1986) Chem. Phys. Lipids 40, 259–284
2 Killian, J.A., Timmermans, J.W., Keur, S. and De Kruijff, B. (1985) Biochim. Biophys. Acta 820, 154–156
3 Killian, J.A., Burger, K.N.J. and De Kruijff, B. (1987) Biochim. Biophys. Acta 897, 269–284
4 Cullis, P.R. and De Kruijff, B. (1979) Biochim. Biophys. Acta 559, 399–420
5 Killian, J.A. and De Kruijff, B. (1985) Biochemistry 24, 7880–7890
6 Chupin, V., Killian, J.A. and De Kruijff, B. (1987) Biophys. J. 51, 395–405
7 Brasseur, R., Killian, J.A., De Kruijff, B. and Ruysschaert, J.M. (1987) Biochim. Biophys. Acta 903, 11–17
8 Killian, J.A. and De Kruijff, B. (1985) Biochemistry 24, 7890–7898
9 Urry, D.W., Glickson, J.D., Mayers, D.F. and Haider, J. (1972) Biochemistry 11, 487–493
10 Fossel, E.T., Veatch, W.R., Ovchinnikov, Y.A. and Blout, E.R. (1974) Biochemistry 13, 5264–5275
11 Heitz, F., Heitz, A. and Trudelle, Y. (1986) Biophys. Chem. 24, 149–160
12 Veatch, W.R. and Blout, E.R. (1974) Biochemistry 13, 5257–5263
13 Veatch, W.R., Fossel, E.T. and Blout, E.R. (1974) Biochemistry 13, 5249–5256
14 Ralston, E., Hjelmeland, L.M., Klausner, R.D., Weinstein, J.W. and Blumenthal, R. (1981) Biochim. Biophys. Acta 649, 133–137
15 Van Deenen, L.L.M. and De Haas, G.H. (1964) Adv. Lipid Res. 2, 168–363
16 Dekker, C.J., Geurts van Kessel, W.S.M., Klomp, J.P.G., Pieters, J. and De Kruijff, B. (1983) Chem. Phys. Lipids 33, 93–106
17 Mayer, L.D., Hope, M.J. and Cullis, P.R. (1986) Biochim. Biophys. Acta 858, 161–168
18 Weinstein, J.N., Yoshikami, S., Henkart, P., Blumenthal, R. and Hagins, W.A. (1977) Science 195, 489–491
19 Masotti, L., Spisni, A. and Urry, D.W. (1980) Cell Biophys. 2, 241–251
20 Urry, D.W. (1985) in The Enzymes of Biological Membranes (Martonosi, A.N., ed.), pp. 229–257, Plenum Publishing Corporation, New York
21 Wallace, B.A. and Mao, D. (1984) Anal. Biochem. 142, 317–328
22 Fiske, C.H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375–389
23 Seelig, J. (1978) Biochim. Biophys. Acta 515, 105–140
24 Luzzati, V. (1980) in Biological Membranes (Chapman, D., ed.) pp. 71–123, Academic Press, New York
25 Killian, J.A., Verkleij, A.J., Leunissen-Bijvelt, J. and De Kruijff, B. (1985) Biochim. Biophys. Acta 812, 21–26
26 Urry, D.W., Trapane, T.L. and Prasad, K.U. (1983) Science 221, 1064–1067