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Interrelationships between tyrocidine and gramicidin A′ in their interaction with phospholipids in model membranes

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Key words: Tyrocidine–phospholipid interaction; Gramicidin A′–tyrocidine interaction; Gramicidin A′–phospholipid interaction; NMR, ^{31}P-; Carboxyfluorescein release; Model membrane; Differential scanning calorimetry; (*B. brevis*)

(1) The interaction of tyrocidine with different lipids is studied in model membranes and the results are compared to the gramicinid–lipid interaction. (2) The tyrocidine–dielaidoylphosphatidylethanolamine interaction gives rise to a population of phospholipids with a lower gel to liquid-crystalline transition temperature and to an abolition of the bilayer to H_{II} phase transition, resulting in a macroscopic organization with dynamic and structural properties different from those of the pure lipid. (3) Tyrocidine has a strong fluidizing effect on the acyl chains of phosphatidylcholines, manifested by a decrease in enthalpy of the main thermotropic transition. (4) No evidence of a gramicidin A′-like lipid-structure modulating activity was found. However, tyrocidine inhibits the formation by gramicidin of an H_{II} phase in dioleoylphosphatidylcholine model membranes. Instead, a cubic type of lipid organization is observed. (5) Tyrocidine greatly perturbs the barrier properties of dioleoylphosphatidylcholine model membrane. (6) Gramicidin A′ reverses the effect of tyrocidine on membrane permeability by forming a complex in the model membrane with an apparent 1:1 stoichiometry. (7) The results suggest that both peptide antibiotics, which are produced by *Bacillus brevis* ATC 8185 prior to sporulation, show antagonism in their effect on membrane structure similar to their effect on superhelical DNA (Bogh, A. and Ristow, H. (1986) Eur. J. Biochem. 160, 587–591. The possible underlying basic mechanism is indicated.

Introduction

Bacillus brevis (strain ATC 8185) produces two types of peptide antibiotics at the onset of the process of sporulation, namely the gramicidins and the tyrocidines. The linear gramicidins are pentadecapeptides consisting of an alternating L- and D-amino acid sequence with the N- and C-termini, respectively, blocked by formyl and ethanolamine groups. The structure of gramicidin A is

HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-

D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NH-CH_2-CH_2-OH

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Abbreviations: DEPE, dielaidoylphosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; DEPC, dielaidoylphosphatidylcholine; lysoPC, lysophosphatidylcholine; DOPC, dioleoylphosphatidylcholine; Pipes, piperazine-1,4-bis(2-ethanesulfonic acid); DSC, differential scanning calorimetry; T_m, temperature at the maximum of the thermotropic peak; ^{31}P-NMR, ^{31}P-nuclear magnetic resonance; LUVs, large unilamellar vesicles; DMSO, dimethyl sulfoxide.

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Gramicidins B and C have their Trp-11 replaced by phenylalanine and tyrosine, respectively. In the natural mixture of gramicidin (gramicidin A′) the relative amount of gramicidin A, B and C is 80%, 5% and 15%, respectively. Tyrocidines are cyclic decapeptides with the following structure

D-Phe-L-Pro-X-Y-L-Asn-L-Gln-L-Trp-L-Val-L-Orn-L-Leu

In the tyrocidines A, B and C the amino acids in position X and Y correspond to L-Phe-D-Phe, L-Trp-L-Phe and L-Trp-L-Trp, respectively.

It has been proposed that these polypeptides are involved in gene regulation during the transition from vegetative growth to sporulation [1]. Tyrocidine has been shown to inhibit RNA synthesis [2], which effect is reversed by addition of gramicidin A′ [3]. It is known that gramicidin A′ is able to inhibit transcription by binding to the δ-subunit of the RNA polymerase [4,5]. These together with recent findings concerning the effect of these polypeptides on DNA supercoiling [6] have lead to the proposal that gramicidin A′ could interact with the tyrocidine molecule causing a weakening of the DNA-tyrocidine complex [6].

From the very hydrophobic amino acid sequence an interaction of these peptides with the membranes can be expected. In case of gramicidin A′ there is abundant evidence that the peptide forms ion-permeable channels in natural and artificial membranes [7,8]. Furthermore, gramicidin A′ has been shown to have a dramatic influence on lipid structure [9]. The polypeptide induces bilayer formation in lysophosphatidylcholine, strongly promotes H_{II} phase formation in phosphatidylethanolamines and induces an H_{II} phase in phosphatidylcholines with fatty acyl chains longer than 16 carbon atoms (see Ref. 10 for a review). Virtually nothing is known about the effect of tyrocidine on membranes.

The aim of this paper is to study the interaction of the tyrocidine molecule with phospholipid model systems of different composition and to compare the tyrocidine–lipid with the gramicidin–lipid interaction. Furthermore, we studied the possible molecular interaction between both polypeptides in model membranes to test for possible complex formation and antagonism.

Materials and Methods

Tyrocidine (tyrocidine -HCl, mixture of components A, B and C) was obtained from Serva (Heidelberg, F.R.G.) and gramicidin A′ and carboxyfluorescein from Sigma (St. Louis, MO, U.S.A.). 1-Palmitoyl-*sn*-glycero-3-phosphocholine (lysophosphatidylcholine, lysoPC), 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine (dielaidoylphosphatidylethanolamine, DEPE), 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (dielaidoylphosphatidylcholine, DEPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (dipalmitoylphosphatidylcholine, DPPC), and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (dioleoylphosphatidylcholine, DOPC), were synthesized according to the procedures described in Refs. 11 and 12. The lipids were purified by HPLC as described in Ref. 28 and judged chromatographically pure by the use of HPTLC.

All other chemicals were of analytical grade.

Differential scanning calorimetry (DSC)

Samples containing 5 μmol of phospholipid and the appropriate amount of tyrocidine in chloroform/methanol (1 : 1, v/v) were dried under a stream of N_2 and stored overnight under vacuum to remove the last traces of solvent. The samples were then dispersed in 1 ml of 100 mM NaCl, 25 mM Pipes, 0.1 mM EDTA buffer (pH 7.4) at 45 °C. Subsequently, the samples were spun for 30 min at 4 °C at 17000 rpm. The pellet was then carefully transferred to stainless steel capsules, and measured in a Perkin-Elmer DSC-4 calorimeter equipped with a Perkin-Elmer Thermal Analysis Data Station, using an empty capsule as reference. The instrument was calibrated using indium as standard. The samples were scanned with a heating rate of 5 K/min. The range of temperatures studied was from 20 °C to 75 °C (DEPE samples), from 15 °C to 55 °C (DPPC samples) and from 0 °C to 25 °C (DEPC samples). Successive scans yielded identical thermograms, the second scan was usually used for transition enthalpy calculations. After the measurements, the capsules were opened and the samples were dissolved in chloroform/methanol (1 : 1, v/v). After subsequent perchloric acid hydrolysis, the amount of phospholipid originally present in the sample was determined by the method of Fiske and SubbaRow [13].

^{31}P-Nuclear magnetic resonance (^{31}P-NMR)

20 μmol of lyso-PC or 40 μmol of DEPE and the appropriate amount of tyrocidine were mixed in a final volume of 300 μl of chloroform/methanol (1:1, v/v) or chloroform, respectively, in a small tube (5 cm length, 8 mm outer diameter) and evaporated under vacuum to dryness in a rotavapor, followed by overnight storage under vacuum to remove the last traces of solvent. 100 μl of distilled water or buffer (100 mM NaCl, 25 mM Pipes, 0.1 mM EDTA (pH 7.4)) in the case of lysoPC or DEPE, respectively, was added to the obtained lipid film. The samples were kept for 60 min at 37°C for lysoPC or 45°C for DEPE to hydrate the phospholipid. The samples were then centrifuged at 25°C during 60 min at 3000 rpm to settle the hydrated phospholipid at the bottom of the tube. The tube was then placed inside a conventional 10 mm NMR tube.

DOPC samples were prepared as follows. 40 μmol of phospholipid (and the appropriate amount of tyrocidine when necessary) were dried under a stream of N_2 in a round bottom flask and stored overnight under vacuum to remove the last traces of solvent. To the obtained lipid film 40 ml of buffer (100 mM NaCl, 25 mM Pipes, 0.1 mM EDTA (pH 7.4)) was added and liposome formation was achieved by gentle shaking the solution during 5 min at room temperature. The sample was then placed on a magnetic stirrer and tyrocidine or gramicidin A′ in a DMSO solution (0.3% w/v) were externally added to the liposome solution at room temperature. After 10 min of stirring the samples were kept during 10 min at room temperature to further equilibration. Then the samples were spun for 30 min at 4°C at 17000 rpm, and the obtained pellet was suspended in 1 ml of the original buffer and transferred to a conventional 10-mm, NMR tube. Control experiments performed by addition of pure DMSO showed no effect on the ^{31}P-NMR spectra when compared with that of the pure phospholipid.

Proton noise decoupled 121.5 MHz ^{31}P-NMR spectra were recorder on a Bruker MSL-300 spectrometer as described before [14]. A spectral width of 40 KHz, a 14 μs 90° pulse, a memory of 4K data points and an interpulse delay of 1 s were used. 2000 free induction decays were accumulated in case of lysoPC samples and 4000 in case of DEPE and DOPC ones. Prior to Fourier transformation an exponential multiplication was applied resulting in a 100 Hz line broadening. 0 ppm corresponds to the chemical shift of the ^{31}P-NMR resonance position of pure lysoPC micelles.

Small-angle X-ray diffraction

X-ray diffraction experiments were performed on samples used for NMR and on freshly prepared ones on a Kratky camera with a 10×0.2 mm Cu/K_α beam (40 kV, 20 mA) equipped with a position sensitive detector (Leti) interfaced to a microcomputer as described before [15]. X-ray diffraction profiles at different temperatures were obtained from 5–10 min exposure times after 15 min of temperature equilibration.

Freeze-fracture electron microscopy

Freeze-fracturing was performed on similar samples as used for ^{31}P-NMR after plunge freezing with a Reichert Jung KF80 without the use of cryoprotectants. Replicas were analyzed with a Philips 420 microscope.

Carboxyfluorescein release

10 μmol of DOPC were dispersed in 1 ml of 75 mM carboxyfluorescein, 10 mM Tris-acetate (pH 7.4) buffer and large unilamellar vesicles (LUVs) were prepared by 10-times extrusion through a polycarbonate filter (Nucleopore, 0.4 μm pore size) using an Extruder (Lipex Biomembranes. Inc., Canada). The untrapped carboxyfluorescein was separated from the carboxyfluorescein-containing LUVs and the external solution replaced by 75 mM NaCl, 10 mM Tris-acetate (pH 7.4) using gel filtration over a 1.5×15 cm Sephadex G-50 column. Tyrocidine and/or gramicidin in a DMSO solution (0.3% w/v) were externally added to the vesicles (DOPC concentration 5–7 μM) and the release of carboxyfluorescein from the vesicles was determined fluorimetrically at room temperature in a Perkin-Elmer LS-5 Luminescence Spectrometer using an emission and excitation wavelength of 513 nm and 430 nm, respectively. Carboxyfluorescein release from the vesicles causes dequenching of the fluorescence of the probe and results in an increase in emission signal. This signal is linear with the concentration of the

carboxyfluorescein released [16]. Complete carboxyfluorescein release was obtained by the addition of Triton X-100. Addition of pure DMSO in control experiments did not show any effect on carboxyfluorescein release.

Results

Interaction between tyrocidine and phosphatidylethanolamine systems

The presence of low amounts of tyrocidine (molar ratio DEPE/tyrocidine > 25 : 1) does not perturb the calorimetric profile of the thermotropic gel to liquid-crystalline transition of DEPE ($T_m = 37 \pm 0.2$° C) but it has a pronounced effect on the bilayer to H_{II} phase transition (Fig. 1). The polypeptide causes a decrease of the size of the peak such that at a molar DEPE/polypeptide of 25 : 1 the transition can no longer be observed. The transition temperature ($T_m = 59 \pm 1$° C) is not affected. At higher peptide concentration (DEPE/tyrocidine ratios < 25 : 1) a new transition occurs at the low-temperature side of the gel to liquid-crystalline phase transition. The size of this new peak increases at the expense of the main peak when the amount of tyrocidine is increased. At the highest amount of polypeptide tested (molar ratio of 5 : 1) only one peak located at temperatures intermediate between those of the previous two components is present.

For pure DEPE the enthalpies for both transitions were estimated to be 7.5 ± 0.5 kcal/mol and

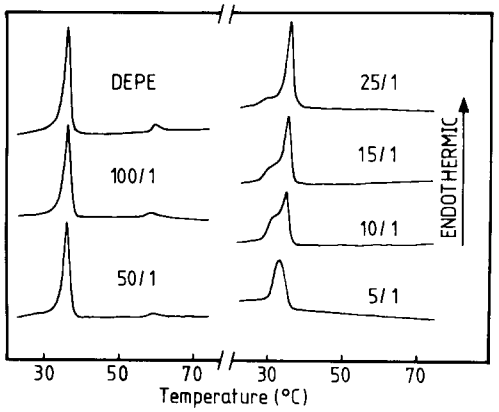

Fig. 1. The DSC thermograms of DEPE and DEPE-tyrocidine mixtures. Molar DEPE/tyrocidine ratios are indicated on the curves. The curves were normalized for the same amount of phospholipid in each case.

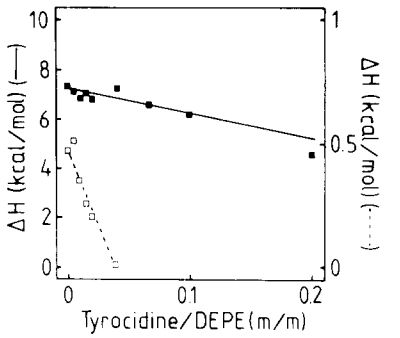

Fig. 2. Effect of incorporation of tyrocidine on the enthalpy of the gel to liquid-crystalline (■) and bilayer to H_{II} phase (□) transition of DEPE.

0.5 ± 0.07 kcal/mol, respectively. Incorporation of tyrocidine (Fig. 2) produces only a small effect on the enthalpy of the total gel to liquid-crystalline transition (sum of both peaks). The presence of the largest amount of polypeptide tested decreases the enthalpy to a value of 4.5 kcal/mol. The enthalpy of the bilayer to H_{II} phase transition decreases proportionally with the tyrocidine content such that it is abolished at a 25 : 1 molar ratio.

As reported previously [17], pure DEPE when organized in hydrated bilayer structures gives rise to an asymmetrical ^{31}P-NMR line shape with a high-field peak and a low-field shoulder and a $\Delta\sigma$ of approx. 40 ppm characteristic of an axially symmetrical chemical shift tensor (Fig. 3). In the

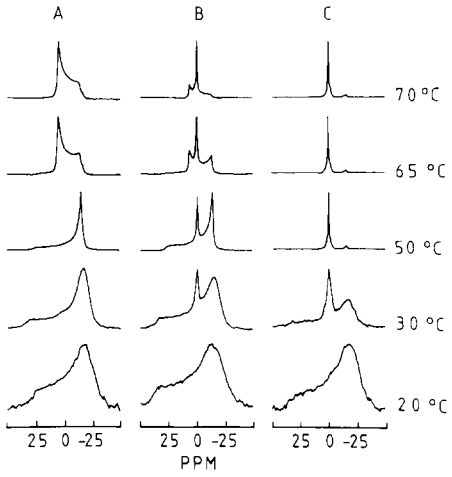

Fig. 3. ^{31}P-NMR spectra of pure DEPE (A) and DEPE/tyrocidine systems at a molar ratio of 50 : 1 (B) and 10 : 1 (C) at different temperatures. The spectra were normalized to the same signal height.

gel state the lineshape is broadened, possibly due to increased ($^1H-^{31}P$) dipolar interactions. In the H_{II} phase, due to rapid lateral diffusion around the tubes of which this phase is composed, the chemical shift anisotropy is further averaged resulting in a lineshape with a reversed asymmetry, i.e. a high-field shoulder and a low-field peak, with a 2-fold reduction in absolute value of $\Delta\sigma$. The presence of tyrocidine gives rise to an isotropic spectral component above 25°C (Fig. 3). The lack of ^{31}P-NMR signal in the sample supernatant after centrifugation indicated that this isotropic signal does not arise from small free tumbling lipid-peptide structures. The intensity of the isotropic component is proportional to the tyrocidine concentration. The data demonstrate that the spectrum originating from the DEPE molecules in the L_α or H_{II} phase is replaced, by tyrocidine, for a spectrum characteristic of isotropically moving phospholipid molecules present in macroscopically large peptide-lipid aggregates.

To further characterize the DEPE–tyrocidine interaction, small angle X-ray diffraction was used. Pure DEPE showed the typical diffraction pattern profiles corresponding to the different structural organization which have been previously reported [15,18]. The presence of tyrocidine at a 50 : 1 molar ratio, resulted in a similar diffraction profile when compared to pure DEPE. At a 10 : 1 molar ratio, in the gel state, similar reflections were observed for the pure phospholipid. However, at temperatures above the gel to liquid-crystalline transition no sharp reflections were observed anymore, instead broad scattering bands centered at 76 Å and 56 Å were found (data not shown). These data thus demonstrate that tyrocidine upon interaction with DEPE eliminates the $L_\alpha \rightarrow H_{II}$ transition and induces in the liquid-crystalline state a new lipid organization in which the PE molecules undergo isotropic motion.

Interaction between tyrocidine and phosphatidylcholine systems

In order to get insight into the mode of interaction of tyrocidine with the acyl chains of phosphatidylcholines, DSC experiments were carried out. The thermotropic pretransition of DPPC is abolished at low concentrations of polypeptide and can no longer be detected at molar ratios < 100–50 : 1 (Fig. 4). Increasing amounts of tyrocidine results in a broadening of the main gel to liquid-crystalline transition and in a decrease of the size of the peak, but do not significantly change the position of the peak maximum (T_m = 41.7 ± 0.4°C) (Fig. 4).

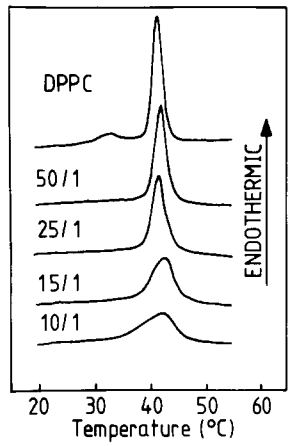

Fig. 4. The DSC thermograms of DPPC and DPPC-tyrocidine mixtures. Molar ratios are indicated on the curves. The curves were normalized for the same amount of phospholipid in each case.

In order to facilitate a comparison with the DEPE data, similar experiments were carried out using DEPC. The effect of tyrocidine on this phospholipid was similar to the one described above for DPPC, a broadening and decrease in size of the main transition with un unchanged peak maximum (T_m = 15 ± 1°C). Different from DPPC, in the range of 50–25 : 1 (DEPC/polypeptide) a shoulder in the higher part of the transition was observed (data not shown).

The enthalpies for the gel to liquid crystalline transition of pure DPPC and DEPC were estimated to be 8 ± 0.5 kcal/mol and 7.1 ± 0.5 kcal/mol, respectively. The presence of tyrocidine (Fig. 5) produces similar effects for both phospholipids. Incorporation of low amounts of polypeptide produces an initial linear decrease of the transition enthalpy. When the amount of tyrocidine reaches a molar ratio of approx. 10 : 1, incorporating of higher amounts of polypeptide only results in a slightly further decrease of enthalpy producing a deflection in the curve.

The possible effect of tyrocidine on lipid polymorphism was further investigated by means of

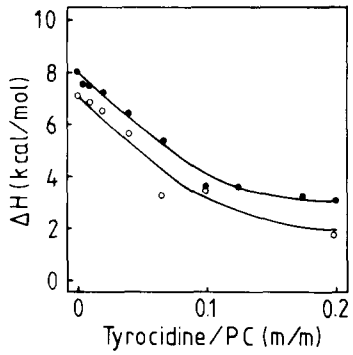

Fig. 5. Effect of tyrocidine incorporation on the enthalpy of the gel to liquid-crystalline transition of DPPC (●) and DEPC (○).

^{31}P-NMR studies on DOPC and lysoPC tyrocidine systems. The ^{31}P-NMR spectrum of pure DOPC liposomes shown in Fig. 6A has a lineshape in which the intensity of the chemical shift position of phospholipid molecules oriented perpendicular to the magnetic field ($\sigma \approx -12$ ppm) is greatly enhanced. This results in a loss of the characteristics lineshape typical for a random collection of bilayers and an axially symmetrical chemical shift tensor. This effect is due to magnetic ordering of the bilayers parallel to the field [19]. Incorporation of tyrocidine in the DOPC liposomes at a molar ratio of 1:10 either by external addition (Fig. 6D) or by mixed film hydration (not shown) does not change the phospholipid phase but only causes a loss of the magnetic orientation. This behaviour strongly contrasts the well-documented [20] gramicidin-induced H_{II} phase formation in this system (Fig. 6C). Pure lysoPC forms micelles in aqueous solution, which due to fast tumbling and lateral diffusion of the lipids gives rise to a narrow isotropic ^{31}P-NMR signal [21]. The presence of tyrocidine does not change the overall lineshape of the peak. The polypeptide only causes a concentration-dependent increase in linewidth of the spectra from 37.5 Hz for pure lysoPC to 300.5 Hz for the highest amount of tyrocidine tested (4:1 molar ratio) indicating a more restricted motion of the phospholipid possibly because of an increase in micelle size (data not shown). No evidence for a bilayer induction evidenced by a ^{31}P-NMR spectrum characteristic of an axially symmetrical chemical shift tensor which is only partially averaged by fast axial rotation, as has been reported for gramicidin A′ [21], was found.

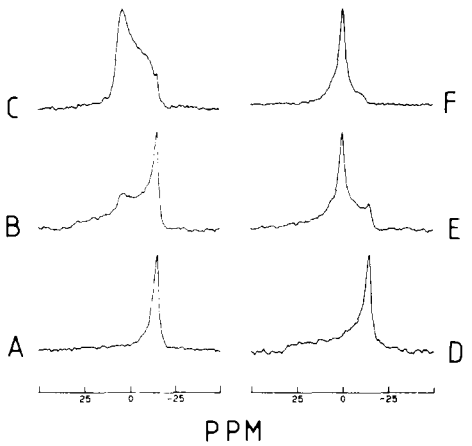

Fig. 6. ^{31}P-NMR spectra of different DOPC/polypeptide systems. Pure DOPC (A); DOPC/external addition of gramicidin A′ at a molar ratio of 50:1 (B) and 10:1 (C): external addition of tyrocidine at a molar ratio of 10:1 D); external addition of gramicidin A′ to a performed DOPC/tyrocidine (10:1) system at a DOPC/gramicidin A′, molar ratio of 50:1 (E) and 10:1 (F). The spectra were obtained at 25 °C and normalized to the same signal height.

Interaction between tyrocidine and gramicidin A′ in model DOPC membranes

Polymorphism. Since tyrocidine and gramicidin have completely different effects on DOPC structure (Fig. 6), we investigated by ^{31}P-NMR whether one peptide could influence the lipid structure modulating activity of the other peptide. Interestingly, the presence of tyrocidine in the DOPC bilayer prevents the formation of a H_{II} phase by external addition of gramicidin. Instead, a new lipid organization is formed, which is sedimentable by low-speed centrifugation and in which the phospholipids undergo fast isotropic reorientation (compare Figs. 6B, E and C, F). Freeze-fracture analysis of the samples revealed that this material give rise to particulate fracture faces where at some points the particles appear to be organized with a cubic symmetry (Fig. 7). Such morphology is typical for inverted non-bilayer phases which occur under conditions intermediate between those resulting in bilayer or H_{II} organization [22]. At some parts in the replica of the tyrocidine-gramicidin-DOPC sample (1:1:10, molar ratio) some residual H_{II} phase could be observed (not

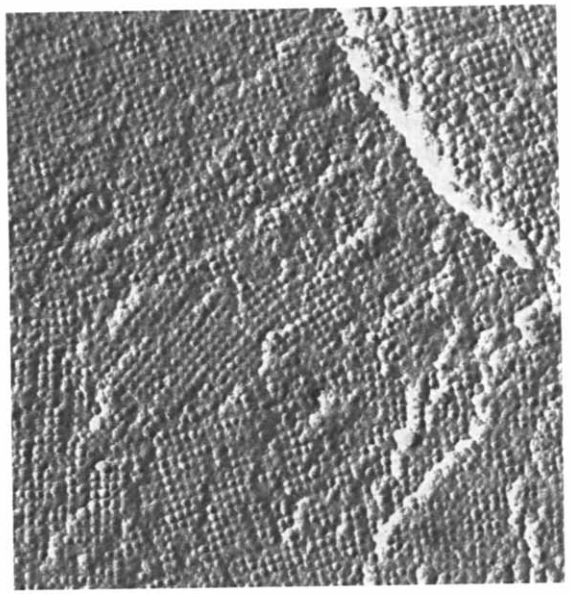

Fig. 7. Freeze-fracture morphology of a tyrocidine-gramicidin-DOPC sample (1 : 1 : 10, molar ratio) prepared as described in the legend of Fig. 6. Final magnification 72000×.

shown). Consistent herewith is the occurrence of a residual 'H_{II}-type' X-ray diffraction pattern. The new 'isotropic' structure apparently lacks sufficient long-range order as no new diffraction lines were found (not shown).

Barrier properties. We next studied the effect of tyrocidine on the barrier properties of DOPC model membranes. Despite the fact that tyrocidine does not affect the macroscopic organization of DOPC it is a lytic agent as it causes a marked concentration dependent release of carboxyfluorescein from unilamellar vesicles prepared from this lipid (Fig. 8). Since our ^{31}P-NMR results indicated that both peptides interacted with each other in a membrane environment we studied the effect of gramicidin addition on the tyrocidine-induced carboxyfluorescein release from DOPC vesicles. Fig. 9 demonstrates that gramicidin, which on its own under these conditions only slightly enhances the carboxyfluorescein release, blocks the tyrocidine-induced carboxyfluorescein release when added any time after tyrocidine.

To get insight into the stoichiometry of this inhibitory interaction, a series of experiments was carried out in which the amount of vesicles and tyrocidine was kept constant while varying the gramicidin concentration. The amount of tyrocidine molecules was fixed in the 2–3 mol% range which is the linear part of the tyrocidine-induced carboxyfluorescein release curve (Fig. 8). A scheme of such an experiment is shown in Fig. 10. With the symbols defined in this figure it is possible to estimate for conditions of an excess of tyrocidine the stoichiometry of the complex from:

$$\frac{F_{T+G} - F_G}{F_T} \cdot T = A \qquad (1)$$

and

$$\frac{T - A}{G} = N \qquad (2)$$

where F_T, F_G and F_{T+G} are the increments of

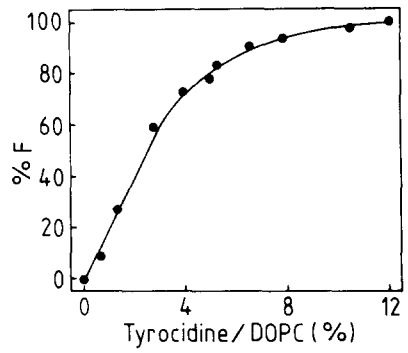

Fig. 8. Increase in fluorescence due to release of carboxyfluorescein from DOPC-LUVs versus molar percentage of externally added tyrocidine at 25 °C. 0% and 100% correspond to the fluorescence of the vesicles without tyrocidine and after solubilization with Triton X-100, respectively. Each point represents a different experiment and the data were taken 15 min after the addition of tyrocidine.

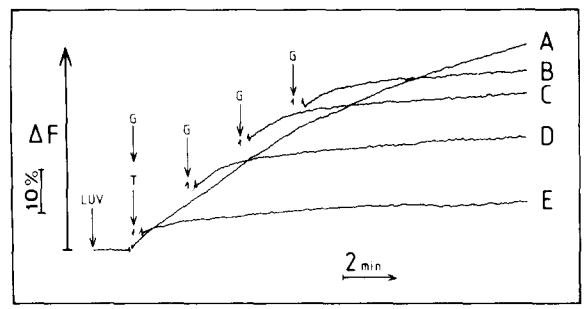

Fig. 9. Curves for the release of carboxyfluorescein from DOPC-LUVs upon addition of 2.3 mol% of tyrocidine (A) and subsequent addition of 2.3 mol% of gramicidin A′ at 6 min (B), 4 min (C), 2 min (D) and 0 min (E) after the addition of tyrocidine. LUV, DOPC large unilamellar vesicles; T, tyrocidine and G, gramicidin A′.

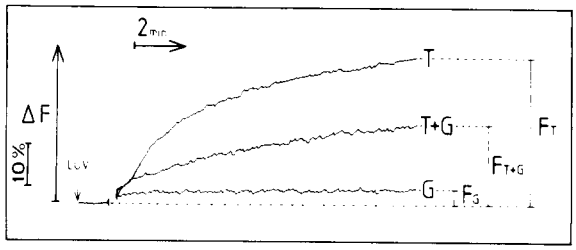

Fig. 10. Schematic representation of a typical experiment for the calculation of the stoichiometry of the tyrocidine-gramicidin A′ complex, showing the utilized parameters. T, tyrocidine; G, gramicidin A′ (at $t = 15$ min). For further details see text.

fluorescence (after 15 min) corresponding to the addition of T nmol of tyrocidine, to G nmol of gramicidin A′ and to T and G nmol of tyrocidine and gramicidin A′ added simultaneously: A is the number of nmol of tyrocidine which remain active (uncomplexed to gramicidin A′) and N is the number of molecules of tyrocidine inhibited per molecule of gramicidin A′. Assuming that carboxyfluorescein release by the T − G complex is similar to that of the free gramicidin A′ (thus very low) for molar tyrocidine to gramicidin A′ ratios $(T/G) > 1$ the value for N was found to be 1.1 ± 0.24 ($n = 15$) which indicates that the complex of tyrocidine-gramicidin A′ has an apparent 1 : 1 stoichiometry. For molar T/G ratios $\leqslant 1$ it was found that an apparent $12 \pm 4\%$ ($n = 9$) of the tyrocidine molecules remained active independent of the concentration of gramicidin A′ used. This indicates that in the presence of an excess of gramicidin A′ tyrocidine in the complex with gramicidin has about 12% of the lytic activity of free tyrocidine.

Discussion

In this study it was shown that tyrocidine is able to interact strongly with different lipids in model membrane systems. It perturbs gel state packing of both phosphatidylcholines and phosphatidylethanolamines. The tyrocidine induced decrease in ΔH of the gel to liquid-crystalline phase transition is maximal for phosphatidylcholines similar to the effect of gramicidin [15] suggesting that the stronger intermolecular head group interactions between PE molecules interfere with the peptide–PE interaction. In contrast to gramicidin, tyrocidine causes the appearance of a new low-temperature gel to liquid-crystalline phase transition in DEPE indicative of a more stronger fluidizing effect of tyrocidine.

Tyrocidine furthermore appears to be highly lytic towards unilamellar DOPC vesicles as it caused an efficient carboxyfluorescein release.

Further pronounced differences between tyrocidine and gramicidin in their interaction with membrane lipids were apparent in studies on lipid polymorphism. Whereas gramicidin promotes H_{II} phase formation in DEPE and DOPC model membranes and induces bilayers in aqueous mixtures with lysoPC [10], tyrocidine did not show any of these effects, once more emphasising the high degree of specificity of the gramicidin induced changes in lipid structure [23].

Only in case of DEPE did tyrocidine change lipid structure. Above 25 °C the L_α and H_{II} phase is replaced by another lipid organization which is macroscopically large and in which the phospholipid molecules undergo fast isotropic reorientation.

The chemical structure of tyrocidine is related to that of the cyclic decapeptide gramicidin S produced by another strain of *B. brevis* (ATC 9999). Therefore, it is of interest to compare the tyrocidine–lipid interaction described in this paper to the gramicidin S–lipid interaction published previously. Early [24] and recent [25] DSC experiments carried out with DPPC/gramicidin S have shown that the peptide does not perturb to a large extent the thermotropic transition of the lipid. Together with NMR data this suggests that gramicidin S interacts with the phosphate moiety of the lipid head group leading to a location of the molecule at the membrane surface. However, our DSC data on tyrocidine support a strong interaction with the acyl chains of PC. This difference in interaction between these two structurally similar cyclic peptide antibiotics might be related to the finding that gramicidin S in contrast to tyrocidine does not induce sporulation in the bacteria [26].

It has been recently shown [6] that gramicidin A′ reverses the tyrocidine-induced relaxation as well as the packing of the DNA of the bacteria. In this study we showed that gramicidin A′ blocks the tyrocidine-induced release of carboxy-

fluorescein from DOPC vesicles by forming a complex with an apparent 1:1 stoichiometry in the membrane. Furthermore H_{II} phase formation induced by high concentrations of gramicidin in DOPC model membranes is inhibited by the presence of tyrocidine. The new 'isotropic' structure present in DOPC-tyrocidine-gramicidin mixtures resembles the inverted cubic phases which are intermediates between the bilayer and H_{II} phase. In that sense tyrocidine causes the DOPC-gramicidin complex to be organised in a more bilayer like organization.

The tryptophans of gramicidin are essential for the lipid-structure modulating activity of gramicidin [23,27], because they determine the shape of the molecule and most likely cause, by ring stacking interactions, a self assembly of the peptide into specific structures. In view of the abundant presence of aromatic amino acids in both gramicidin and tyrocidine we propose that ring stacking interactions are responsible for the observed complex formation between these polypeptides. The obvious speculation then is that such interactions could in *B. brevis* play an essential role in gene regulation and protection of its membrane against the high concentrations of these potentially harmful hydrophobic polypeptides. Furthermore, gramicidine–tyrocidine interactions could be of importance for modulation of the antibiotic activity of these polypeptides.

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