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Effects of the antibiotic peptide microcin J25 on liposomes: role of acyl chain length and negatively charged phospholipid

María R. Rintoul, Beatriz F. de Arcuri, Roberto D. Morero *

Departamento de Bioquímica de la Nutrición, Instituto Superior de Investigaciones Biológicas, Instituto de Química Biológica 'Dr. B. Bloj', Facultad de Bioquímica, Química y Farmacia, (CONICET/UNT), Chacabuco 461, 4000 S.M. de Tucumán, Argentina

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Abstract

This paper reports the effects of microcin J25 (MccJ25) on the microviscosity and permeability of phospholipid vesicles of different compositions. The results obtained indicate that MccJ25 interacts with egg L-α-phosphatidylcholine (PC) vesicles as demonstrated by peptide intrinsic fluorescence determinations. The interaction depends on the lipid composition of the vesicles. MccJ25 interaction induces a significant fluidity increase of egg PC vesicles. This effect is time and concentration dependent. Both trimethyl ammonium 1,6-diphenyl-1,3,5-hexatriene and 1,6-diphenyl-1,3,5-hexatriene gave the same results. The microviscosity of L-α-phosphatidylcholine dipalmitoyl small unilamellar vesicles (SUVs) was affected while that of L-α-phosphatidylcholine dimyristoyl vesicles was not, indicating that the effect was strongly dependent on the chain length of fatty acids. On the other hand, negatively charged L-α-phosphatidyl-DL-glycerol (PG) vesicles remarkably inhibited the peptide effect. Nevertheless vesicles composed of L-α-phosphatidylethanolamine:PG:cardiolipin (7:2:1), a composition resembling bacterial membrane, were sensitive to the MccJ25 effect. MccJ25 effectively dissipated the valinomycin-induced membrane potential, but induced only a modest leakage (5%) of the trapped Tb^{+3}–dipicolinic acid complex. These results indicate that the peptides interact and perturb the bilayer of SUVs. The relationships between this effect and bactericidal action remain to be elucidated.

Keywords: Microcin; Antibiotic; Liposome; Peptide; Lipid–protein interaction

1. Introduction

Microcins form a miscellaneous group of low molecular mass peptide antibiotics produced by diverse strains of Enterobacteriaceae, mostly *Escherichia coli* [1]. They have been classified into immunity groups (A, B, C, D, E and H) [2]. Underlying this classification is the assumption that each immunity group represents a unique structure with a unique mode of action. Microcin J25 (MccJ25) is the single representative of the immunity group J [3]. This microcin, produced by an *E. coli* strain isolated from human feces is highly active on some pathogenic *Salmonella*

Abbreviations: DMPC, L-α-phosphatidylcholine dimyristoyl; PC, L-α-phosphatidylcholine; PG, L-α-phosphatidyl-DL-glycerol; PE, L-α-phosphatidylethanolamine; DPPC, L-α-phosphatidylcholine dipalmitoyl; DLPC, L-α-phosphatidylcholine dilauroyl; CL, cardiolipin; DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, trimethyl ammonium 1,6-diphenyl-1,3,5-hexatriene; DPA, dipicolinic acid; MccJ25, microcin J25

* Corresponding author. Fax: +54-81-248-025;
E-mail: rdmore@unt.edu.ar

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and *Shigella* species [3]. MccJ25 production occurs during the stationary phase of growth [3]. Four plasmid genes involved in MccJ25 production and immunity have been defined [4]. The nucleotide sequencing of the MccJ25 production genes has been determined [5].

The structure of MccJ25 was elucidated based on mass spectrometry and nuclear magnetic resonance spectroscopy [6]. This study shows MccJ25 to be a highly hydrophobic cyclic peptide of 21 unmodified amino acid residues. The MccJ25 sequence is cyclo-(GYFSIPTGIGVFYEPVHGAGG) [5,6]. Since only tyrosine residues determine the peptide fluorescence, it is a class A peptide. The quantum yield of a tyrosyl residue is extremely sensitive to the environment and structural group around it [7].

The detailed mechanism of cell death by the peptide is not clearly known yet. At minimal inhibitory concentration, MccJ25 induces susceptible cell filamentation, suggesting that the molecular target is a factor directly involved in cell division. However, a possible effect at the membrane level cannot be discarded due to the high peptide hydrophobicity. In addition, there have been various reports of natural peptides with small molecular weights that induce the permeabilization of membranes, e.g. colicin A [8], magainins [9], nisin [10], bacteriocins [11], sapecins [12], defensins [13] and even some microcins [1,2].

We are interested in the membrane-perturbing ability of MccJ25. Here, we studied microcin's fluorescent tyrosine by measuring its interaction with membrane models. In addition, the effect on the acyl chain microviscosity and the membrane potential was investigated. These experiments enabled an evaluation of the membrane interaction of MccJ25.

2. Materials and methods

2.1. Materials

L-α-Phosphatidylcholine (PC), L-α-phosphatidylethanolamine (PE), L-α-phosphatidyl-DL-glycerol (PG) and cardiolipin (CL) were purchased from Avanti Polar Lipids. All lipids were pure, as checked by thin layer chromatography, and used without further purification. Valinomycin was purchased from Sigma USA, and the fluorescent dyes 3,3′-dipropylthiadicarbocyanine iodide ($DiSC_3(5)$), merocyanine 540, 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene, *p*-toluenesulfonate (TMA-DPH) from Molecular Probes.

2.2. Purification of microcin

MccJ25 was typically purified from 1 l cultures of *E. coli* K-12 strain MC4100 [*F⁻araD 139 Δ(argF-lac)205 λ⁻ flbB5301 ptsF25 relA1 rpsL150 deoC1*] harboring pTUC 202, a high-copy-number recombinant plasmid [4]. The cells were grown in M9 minimal medium to the stationary phase and, after removing the bacteria by centrifugation at $12\,000\times g$ for 20 min, the supernatant was applied to a preparative C8 cartridge (BontElut, Varian, Les Ulis, France). The column was consecutively washed with methanol:water, 20:80 (v/v) and then 45:55 (v/v), before the microcin was eluted with methanol:water, 80:20 (v/v). This crude MccJ25 was further purified by reverse phase-high performance liquid chromatography (RP-HPLC) on a semi-preparative μBundapack C18, 10 μm, 300×19 mm column (Waters) with acetonitrile:water (TFA 0.1%) 20:100 (v/v) gradient as eluent and a flow rate of 2 ml/min, using a Gilson liquid chromatograph. The purity of the microcin preparation was 97%, which appeared homogeneous in two different systems of analytical RP-HPLC. The MccJ25 purified with this procedure was used to elucidate its cyclic structure and the amino acid sequence [6].

2.3. Preparation of liposomes

Lipids dissolved in $CHCl_3$:methanol (9:1, v/v) dried under nitrogen stream, were resuspended in 20 mM Tris–HCl, pH 7.4. To obtain small unilamellar vesicles (SUVs), the formed multilamellar liposomes were subjected to sonication for 20 min, with intervals of 15 s, at 5°C, using a Branson sonicator. To eliminate large particles, the suspension was centrifuged at $12\,000\times g$ in a fixed angle rotor centrifuge.

The liposomes with DPH or TMA-DPH were prepared by adding a fluorescent probe to the lipid suspension before drying the mixture. After resuspension the lipid/probe molar ratio was 0.1%.

2.4. Fluorescence spectroscopy

MccJ25 was excited at 275 nm and the intrinsic fluorescence spectra or emission at single wavelength (310 nm) were recorded. Corrections were made for buffer fluorescence and dilutions. These measurements were performed using an SLM Instruments 4800 spectrophotofluorometer. The bandwidth was generally 4.0 nm for the fluorescence spectra. MccJ25 spectra were measured in the absence and presence of different liposome concentrations. The spectra were corrected subtracting liposome contribution and instrument response factors.

The steady-state DPH or TMA-DPH fluorescence polarization (P) measurements were obtained using an SLM Instruments 4800 fluorimeter T-format (SLM Aminco, Urbana, IL, USA) equipped with a thermostatic cuvette holder. The excitation wavelength was 360 nm and all the fluorescence was collected through a cutoff filter (Corning 3-73). Polarization values were calculated according to standard procedure [14]. The MccJ25 effect was recorded immediately after the addition to the liposome suspension.

2.5. Leakage of liposome contents

The release of liposomal content was measured using a Tb^{+3}–dipicolinic acid (DPA) assay [15]. Phospholipid vesicles containing the fluorescent complex Tb^{+3}–DPA, suspended in 20 mM Tris–HCl, 1 mM EDTA buffer, pH 7.4, were placed into a quartz cuvette. The fluorescence decrease (excitation wavelength: 270 nm, emission wavelength: 530 nm) due to leakage of the complex was measured immediately and after 30 min of MccJ25 addition.

2.6. Membrane potential generation in liposomes

Liposomes prepared in 50 mM potassium phosphate buffer (pH 7.4), were diluted 100-fold in 50 mM sodium phosphate buffer (pH 7.4). The final concentration of phospholipid was 50 μM. Upon addition of the K^+ ionophore valinomycin (37 or 5 nM) a membrane potential ($\Delta\Psi$, interior negative) can be generated. The reaction was performed at 25°C in an SLM Instruments 4800 spectrophotofluorometer, and the $\Delta\Psi$ was followed with the incorporation of the fluorescent dyes $DiSC_3(5)$ (500 nM) (excitation wavelength: 622 nm; emission wavelength: 675 nm) or merocyanine 540 (37 nM) (excitation wavelength: 550 nm; emission wavelength: 580 nm).

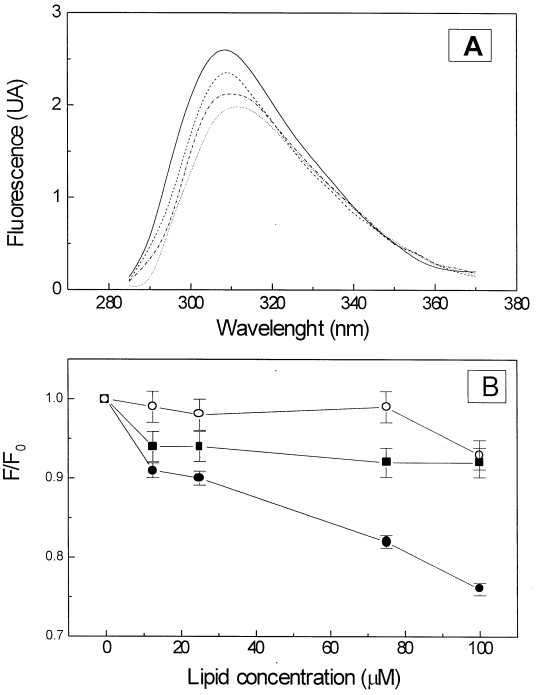

Fig. 1. Interaction of MccJ25 with liposomes. (A) Intrinsic fluorescence emission spectra of MccJ25 in 20 mM Tris–HCl buffer, pH 7.4, in the absence (solid line) and in the presence of 12.5 μM (dashed line), 50 μM (dot–dash line) and 100 μM (dotted line) of egg PC vesicles. Excitation wavelength: 275 nm. Peptide concentration: 15 μM. (B) Ratio of the fluorescence emission intensity at 310 nm of MccJ25 versus concentration of egg PC (-●-), DMPC (-+-) and DLPC (-○-) vesicles. F_0 and F are the fluorescence intensity in the absence and in the presence of liposomes, respectively. Values are expressed as mean ± S.D. ($n = 5$).

3. Results

3.1. Steady-state fluorescence associated with interaction of MccJ25 to liposomes

Fig. 1A shows typical intrinsic fluorescence spectra of MccJ25 at neutral pH in the absence and presence of an increasing concentration of SUV egg PC. The emission maximum of the MccJ25 fluorescence spectra was 310 nm. The tyrosine fluorescence shown by MccJ25 is relatively weak, $R_{tyr} = 0.25$ (see [7]). Upon

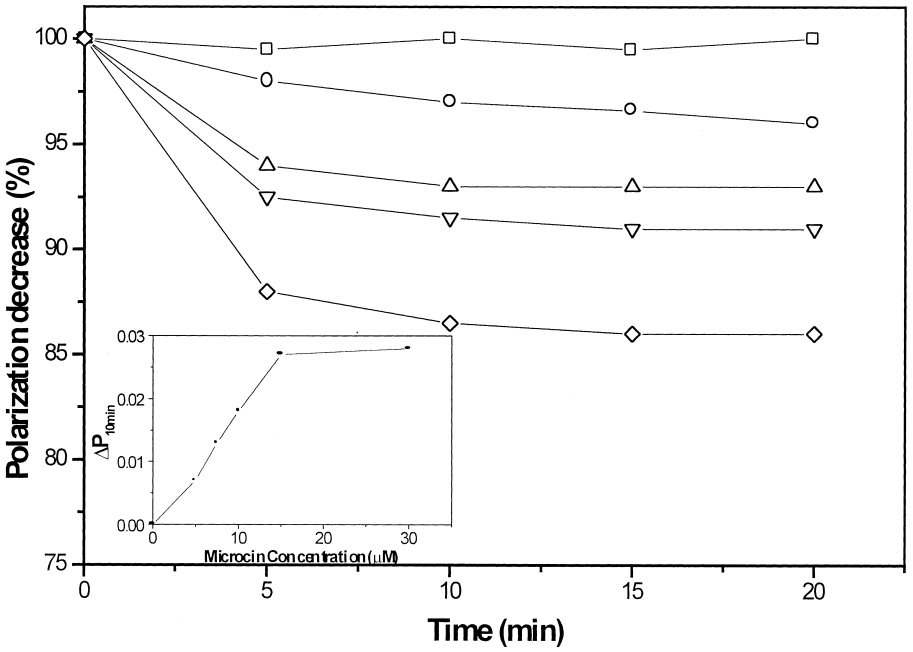

Fig. 2. Effect of MccJ25 on the fluorescence polarization values of DPH incorporated into egg PC liposomes as a function of time. DPH-labeled egg PC liposomes resuspended in 20 mM Tris–HCl buffer, pH 7.4, were incubated at 30°C in the absence of MccJ25 (-□-) and in the presence of 5 μM (-○-), 7 μM (-△-), 10 μM (-▽-) and 15 μM (-◇-) MccJ25. At defined times the DPH polarization values were determined. Insert: $\Delta P_{10\,min}$ is plotted against the MccJ25 concentration ($\Delta P_{10\,min} = P_0 - P_{10\,min}$; P_0 and $P_{10\,min}$ are the fluorescence polarization values in the absence or presence of MccJ25).

addition of egg PC liposomes into a solution containing MccJ25 peptide, the steady-state intensity of tyrosine fluorescence decreases. The drastic decrease of the fluorescence intensity observed seems to be clearly dependent on the lipid vesicle composition. In fact, when tyrosine fluorescence represented by the F/F_0 ratio is plotted against the lipid concentration of different composition vesicles, a 25% decrease of the F/F_0 ratio was obtained with 100 μM egg PC vesicles. Similar results were obtained with L-α-phosphatidylcholine dipalmitoyl (DPPC) vesicles and PE:PG:CL (7:2:1) vesicles (not shown). However, the same concentration of L-α-phosphatidylcholine dimyristoyl (DMPC) vesicles or L-α-phosphatidylcholine dilauroyl (DLPC) vesicles did not change the ratio significantly (Fig. 1B).

3.2. Effect of MccJ25 on the microviscosity of phospholipid vesicles

The fluorescence depolarization of DPH and TMA-DPH can be used as an indicator for the relative microviscosity of the phospholipid bilayer [16,17]. Fig. 2 shows the time course profile of decreasing fluorescence polarization values of DPH incorporated into small unilamellar PC vesicles after the addition of different concentrations of MccJ25. In the absence of peptide, the polarization values remained constant for several hours. In contrast, after peptide addition an abrupt decrease is observed during 5 min, followed by a further decrease until a plateau is reached after 10 min. As can be seen in Fig. 2, insert, the extension changes of polarization values at 10 min (ΔP) were dependent on the MccJ25 concentration (maximum effect around 15 μM).

DPH is known to be located within the hydrophobic core of the lipid bilayer. On the other hand, cationic TMA-DPH is anchored in close proximity to the lipid bilayer surface [18]. We have used, separately, both fluorescent probes to determine the effects of MccJ25 on the core and on the surface of the egg PC bilayer. Experiments were carried out at 30°C. Similar results were obtained with both fluorescent probes, indicating that the perturbation induced by the polypeptide involved either the interior or the surface of the membrane (results not shown).

3.3. Dependence of the lipid composition vesicles on the MccJ25 effect

SUVs of different phospholipid composition were used to study the dependence of the vesicle charge and physical state of the lipid on the action of MccJ25. Table 1 shows the change of DPH polarization values (ΔP) after 10 min of incubation of liposomes composed of different phospholipid with MccJ25. The experiments were carried out at 30°C.

As described previously, addition of MccJ25 to pure egg PC vesicles, decreases significantly DPH polarization values. In contrast, egg PG vesicles which confer a high-density negative charge on the vesicle surface were resistant to the peptide-induced polarization changes. However, with egg PC:egg PG (7:3), a binary mixture where the negative density charge on the liposome surface was diminished, the MccJ25 effect was observed again. In addition, vesicles composed of a complex phospholipid mixture of PE:PG:CL (7:2:1), which resemble the bacteria membrane, were sensitive to the MccJ25 effect.

Results with SUVs composed of pure species of DLPC, DMPC and DPPC are also included in Table 1. MccJ25 induced a DPH polarization value decrease when acting on DPPC liposomes, showing the importance of the acyl chain length. Additional experiments showing the importance of the acyl chain length can be seen in Fig. 3. The effect of MccJ25 on the polarization value (ΔP) after a 10 min incubation observed with egg PC liposomes was inhibited by the incorporation of DMPC. The inhibition was dependent of the PC/DMPC relationship used.

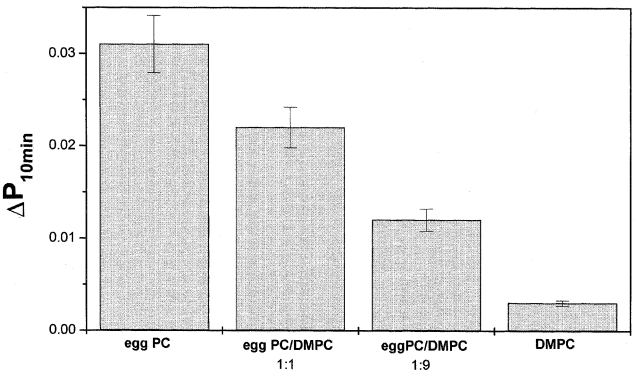

Fig. 3. Influence of fatty acid length chain on the $\Delta P_{10\,min}$ values induced by MccJ25. Pure egg PC, egg PC:DMPC (1:1), egg PC:DMPC (1:9) or DMPC-liposomes labeled with DPH, as described in Section 2, were incubated at 30°C with MccJ25 at a final concentration of 15 μM. The $\Delta P_{10\,min}$ was calculated as mentioned above. The results are expressed as the mean ± S.D. ($n = 4$).

The physical state of lipids does not seem to correlate with the MccJ25 effect. The maximum decrease obtained is about 0.026 steady-state polarization units with lipids in either the gel or liquid/crystal phase (see Table 1).

3.4. Effect of MccJ25 on the permeability of phospholipid vesicles

Permeability studies were carried out by two different methods: (A) dissipation of the membrane potential generated in liposomes by valinomycin and

Table 1
Different polarization values of DPH incorporated into liposomes of different phospholipid composition

Liposome composition	Net charge	Physical state	$\Delta P_{10\,min}$
Egg PC	zwitterion	liquid/crystal	0.026 ± 0.001
Egg PG	negative	liquid/crystal	0.002 ± 0.001
Egg PC/egg PG (7:3)	zwitterion	liquid/crystal	0.027 ± 0.002
Egg PC/egg PE (7:3)	zwitterion	liquid/crystal	0.027 ± 0.003
Egg PE/egg PG (7:3)	negative	liquid/crystal	0.018 ± 0.003
Egg PE/egg PG/CL (7:2:1)	negative	–	0.020 ± 0.003
DLPC	zwitterion	liquid/crystal	0.003 ± 0.001
DMPC	zwitterion	liquid/crystal	0.006 ± 0.002
		gel	0.003 ± 0.001
DPPC	zwitterion	gel	0.031 ± 0.004

The DPH-labeled liposomes were incubated with 10 μM MccJ25 at 30°C, and $\Delta P_{10\,min}$ were calculated by subtracting P values obtained at 0 and 10 min of incubation. Polarization values of DMPC vesicles in the gel state were determined at 10°C. Values are expressed as mean ± S.D. ($n = 4$).

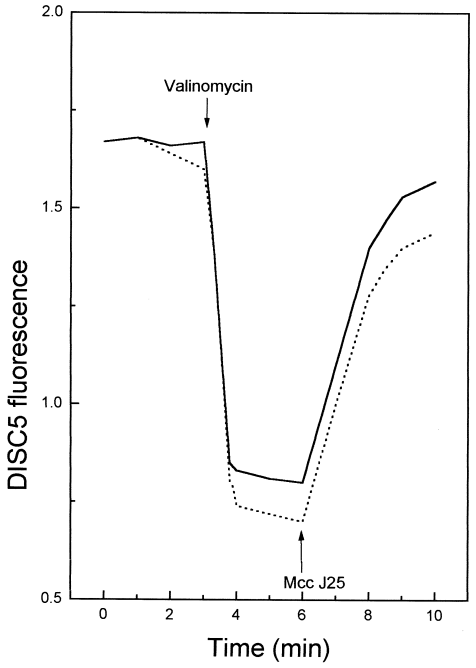

Fig. 4. Effect of MccJ25 on the membrane potential ($\Delta\Psi$, inside negative) in liposomes. K^+-loaded egg PC (solid line) and PC/PG/CL (7:2:1) liposomes (dotted line) in 50 mM potassium phosphate (pH 7.4) were diluted 100-fold in 50 mM sodium phosphate (pH 7.4). At the indicated times valinomycin (37 nM) or MccJ25 (15 μM) was added. Changes in membrane potential were measured as fluorescence changes of the indicator probe, $DiSC_3(5)$ (excitation wavelength: 622 nm, emission wavelength: 675 nm), as described in Section 2.

(B) the leakage of low molecular weight markers from SUVs using the fluorescent complex Tb^{+3}–DPA (MW 530), as described in Section 2.

The influence of MccJ25 on egg PC or PC:PG:CL (7:2:1) liposomes with artificially imposed K^+ diffusion potential was investigated. K^+-loaded liposomes were diluted 200-fold with isotonic buffer, potassium-free, according to Section 2. Upon addition of the K^+ ionophore valinomycin, a $\Delta\Psi$ (inside negative) was generated as indicated by the potential-sensitive fluorescent probe $DiSC_3(5)$ (Fig. 4). The membrane potential imposed by valinomycin was stable for up to 2 h (data not shown). After addition of MccJ25 (15 μM), a significant dissipation of the $\Delta\Psi$ can be seen. Identical results were obtained with the fluorescent probe, merocyanine 540 (not shown). This clearly indicates that MccJ25 makes the liposomal membrane permeable to ions. No fluorescence changes were observed with K^+-loaded liposomes diluted in K^+ buffer upon the addition of valinomycin or MccJ25 (data not shown).

MccJ25 was unable to induce a significant leakage of the Tb^{+3}–DPA complex. The aqueous content release was never higher than 5% for all the vesicle compositions tested (result not shown).

4. Discussion

Intrinsic fluorescence, a very sensitive technique for studying lipid–protein interaction [19], has shown that MccJ25 positively interacts with phospholipid vesicles. The observed decreased tyrosine fluorescence, induced by the presence of egg PC vesicles, indicates the environment modification of the phenolic residue of tyrosine, resulting from structural changes or self-quenching aggregates of the peptide on the membrane surface. Furthermore, the change of the intrinsic fluorescence of MccJ25 was dependent on the phospholipid composition. As seen in Fig. 1B, decreased tyrosine fluorescence has not been observed with DMPC and DLPC vesicles.

MccJ25 interacts with egg PC vesicles inducing microviscosity changes assayed by polarization fluorescence of DPH and TMA-DPH incorporated into the vesicles. This effect was time and concentration dependent. The microviscosity change was also found to be strongly sensitive to the lipid composition of the bilayer. The experiments clearly showed that the peptide interacts with egg PC and DPPC vesicles modifying their microviscosity, but not with DMPC, DLPC and PG vesicles. In addition, incorporation of DMPC into egg PC vesicles inhibited the MccJ25 effect. In this respect, two general conclusions can be deducted: (i) microviscosity changes were dependent on the presence of a negative charge on the vesicle surface, since PG vesicles did not show any MccJ25 effect. At pH 7.4, MccJ25 should be negatively charged, due to the carboxylic group of the glutamate residue. Therefore, the inhibitory effect of PG can be explained in terms of electrostatic repulsion between the same charges of the phospholipid and the peptide. It is important to emphasize that the incorporation of up to 30% of PG into PC vesicles or a mixture of PE:PG:CL (7:2:1), a composition similar to the bacteria membrane, did not modify the peptide effect. (ii) Microviscosity changes

were dependent on the fatty acid length of the phospholipid vesicles, since the effect was observed with DPPC vesicles but not with DMPC and DLPC vesicles. The fact that DLPC, DMPC and DPPC, although having the same head group, behave differently suggests that some physicochemical properties of the bilayer (depending on the acyl chain length), rather than specific molecular recognition by the peptide, regulate the MccJ25 effect.

We show that MccJ25 conducts transmembrane ion movements in egg PC liposomes and this activity is specific for small ion solutes, since leakage of the Tb^{+3}–DPA complex is practically null.

Several mechanisms have been proposed for peptide-induced membrane permeabilization and considerable controversy still exists [20]. Alamethicin forms the barrel-stave channel [21], cecropins are suggested to act by the 'carpet-like mechanism' [22] and 18L model peptides deteriorate the bilayer barrier property imposing positive and negative curvature on the membrane [23]. The results presented here do not allow us to classify the MccJ25 action within one of the described mechanisms, but show that there is no need for an integral membrane component that serves as a receptor for the peptide to permeabilize the membrane.

It is well known that a number of proteins mediate septum formation during cell division [24]. Microcin of the type J group such as MccJ25 is thought to mediate its bactericidal effect by interfering with cell division [3], however, additional effects on cell membrane permeabilization cannot be discarded. In this paper we show that MccJ25 interacts with artificial phospholipid vesicles inducing microviscosity changes and further disordering of the bilayer structure up to membrane permeabilization. If this action is pertinent to the mode of action of MccJ25 in vivo, it remains to be elucidated and is under study in our laboratory.

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References

[1] C. Asencio, J.C. Perez-Díaz, M.C. Martinez, F. Baquero, A new family of low molecular weight antibiotics from Enterobacteria, Biochem. Biophys. Res. Commun. 69 (1976) 7–14.
[2] F. Baquero, F. Moreno, The microcins, FEMS Microbiol. Lett. 23 (1984) 117–124.
[3] R.A. Salomón, R.N. Farías, Microcin 25, a novel antimicrobial peptide produced by *Escherichia coli*, J. Bacteriol. 174 (1992) 7428–7435.
[4] J.O. Solbiati, M. Ciaccio, R.N. Farias, R.A. Salomón, Genetic analysis of plasmid determinants for microcin J25 production and immunity, J. Bacteriol. 178 (1996) 3661–3663.
[5] J.O. Solbiati, M. Ciaccio, R.N. Farias, J.E. Gonzalez-Pastor, F. Moreno, R.A. Salomon, Sequence analysis of the four plasmid genes required to produce the circular peptide antibiotic microcin J25, J. Bacteriol. 181 (1999) 2659–2662.
[6] A. Blond, J. Péduzzi, C. Goulard, M.J. Chiuchiolo, M. Barthélémy, Y. Prigent, R.A. Salomón, R. Farías, F. Moreno, S. Rebuffat, The cyclic structure of microcin J25, a 21-residue peptide antibiotic from *Escherichia coli*, Eur. J. Biochem. 259 (1999) 747–755.
[7] R.W. Cowgill, Tyrosyl fluorescence in protein and model peptide, in: R.F. Chen, H. Edelhoch (Eds.), in: Biochemical Fluorescence, Vol. 2, ch. 9, Marcel Dekker, New York, 1976.
[8] C.J. Lazdunski, D. Baty, V. Geli, J. Morlon, R. Lloubes, S.P. Howard, M. Knibiehler, M. Chartier, S. Varenne, M. Frenette, J.L. Dasseux, F. Pattus, The membrane channel-forming colicin A: synthesis, secretion, structure, action and immunity, Biochim. Biophys. Acta 947 (1988) 445–464.
[9] R.A. Cruciani, J.L. Baker, S.R. Durell, G. Raghunathan, H.R. Guy, M. Zasloff, E.F. Stanley, Magainin 2, a natural antibiotic from frog skin, forms ion channels in lipid bilayer membranes, Eur. J. Pharmacol. 226 (1992) 287–296.
[10] F.H. Gao, T. Abee, W.N. Konings, Mechanism of action of the peptide antibiotic nisin in liposomes and cytochrome *c* oxidase-containing proteoliposomes, Appl. Environ. Microbiol. 57 (1991) 2164–2170.
[11] A.J.M. Driessen, H.W. van den Hooven, W. Kuiper, M. van de Kamp, H.-G. Sahl, R.N.H. Konings, W.N. Konings, Mechanistic studies of antibiotic-induced permeabilization of phospholipid vesicles, Biochemistry 34 (1995) 1606–1614.
[12] Y. Hirakura, J. Alvarez-Bravo, S. Kurata, S. Natori, Y. Kirino, Selective interaction of synthetic antimicrobial peptides derived from sapecin B with lipid bilayers, J. Biochem. 120 (1996) 1130–1140.
[13] K. Hristova, M.E. Selsted, S.H. White, Critical role of lipid composition in membrane permeabilization by rabbit neutrophil defensins, J. Biol. Chem. 272 (1997) 24224–24233.

[14] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Plenum Press, New York, 1983, pp. 112–128.
[15] J. Bentz, N. Duzgunes, S. Nir, Kinetics of divalent cation induced fusion of phosphatidylserine vesicles correlation between fusogenic capacities and binding affinities, Biochemistry 22 (1983) 3320–3330.
[16] M. Shinitzki, Y. Brenholz, Fluidity parameters of lipids region determined by fluorescence polarization, Biochim. Biophys. Acta 515 (1987) 367–394.
[17] K. Florine-Casteel, W. Feigenson, On the use of partition coefficients to characterize the distribution of fluorescent membrane probes between coexisting gel and fluid lipid phase: an analysis of the partition behavior of 1,6-diphenyl-1,3,5-hexatriene, Biochim. Biophys. Acta 914 (1988) 102–109.
[18] R.R.C. New, in: R.R.C. New (Ed.), Liposomes: A Practical Approach. Introduction, IRL, Oxford University, Oxford, 1990, pp. 1–32.
[19] W.C. Galley, Heterogeneity in protein emission spectra, in: R.F. Chen, H. Edelhoch (Eds.), Biochemical Fluorescence, vol. 2, ch. 8, Marcel Dekker, New York, 1976.
[20] K. Lohner, R.M. Epand, Membrane interactions of hemolytic and antibacterial peptides, Adv. Biophys. Chem. 6 (1997) 53–66.
[21] M.S. Sansom, The biophysics of peptide models of ion channels, Prog. Biophys. Mol. Biol. 55 (1991) 139–235.
[22] Y. Shai, Molecular recognition between membrane-spanning polypeptides, Trends Biol. Sci. 20 (1995) 460–465.
[23] I.V. Polozov, A.I. Polozova, E.M. Tytler, G.M. Anantharamaiah, J.P. Segrest, G.A. Wooley, R.M. Epand, Role of lipids in the permeabilization of membranes by class L amphipathic helical peptides, Biochemistry 36 (1997) 9237–9245.
[24] M. Matsuhashi, M. Wachi, F. Ishino, Machinery for cell growth and division: penicillin-binding proteins and other proteins, Res. Microbiol. 141 (1990) 89–102.