Microcin J25 membrane interaction: Selectivity toward gel phase

Fernando Dupuy, Roberto Morero *

Departamento Bioquímica de la Nutrición, INSIBIO-CONICET/UNT e Instituto de Química Biológica Dr. Bernabé Bloj, Universidad Nacional de Tucumán,
Chacabuco 461 San Miguel de Tucumán, Tucumán T4000ILI, Argentina

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ABSTRACT

The interaction of the tryptophan-containing variant of microcin J25, MccJ25 I13W, with phosphatidylcholine membranes was studied by fluorescence spectroscopy techniques. The peptide was able to interact with dimiristoylphophatidylcholine and dipalmitoylphosphatidylcholine liposomes only when the membranes were in gel phase, as was demonstrated by the blue shift of the intrinsic fluorescence of MccJ25 I13W. The binding isotherm showed a cooperative partition of the peptide toward the membrane and the binding constant increased as the temperature decreased and the order parameter increased. No interaction with liquid crystalline membranes was observed. Studies of dynamic quenching of the fluorescence indicated that the peptide penetrated the lipid bilayer and was located primarily in the interfacial region. Our results suggest that MccJ25 I13W interacts with gel phase phospholipids and increases both its own affinity for the bilayer and the membrane permeability of small ions.

1. Introduction

Antimicrobial peptides are synthesized and secreted by organisms among plant, animal and prokaryotic kingdoms and have the ability to kill microorganisms. The mechanism of action of these peptides is highly variable, probably because of the few features in common they share with each other. The ability to permeabilize the plasma membrane of target cells precisely is a very general feature. In order to explain how peptides interact with and disrupt membranes, two types of models have been proposed [1–3]. The first model is termed the 'carpet' model, where at low concentrations, peptides lay across the surface of the membrane and, once a critical concentration is reached, disrupt bilayers in a detergent-like manner causing the formation of micelles and ultimately the disintegration of the membrane [4]. The second type is known as the barrel-stave model and involves the formation of discrete oligomeric pores that allow ions and other molecules to cross the membrane [3]. Variations of this are the 'toroidal pore' model, whereby the pore is composed of both peptide monomers and lipid head groups, and the aggregate model in which the peptides and lipids form informal aggregates within the membrane that permit ion leakage or peptide translocation across the membrane. In the beginning, membrane permeabilization was believed to be the sole event responsible for cell killing, although it was actually probed for only certain peptides. Moreover, it was shown that in some cases, depolarization of the plasma membrane alone did not ensure viability loss of sensible cells [5], suggesting that the killing effect mediated by antimicrobial peptides would involve multitarget strategies.

Microcins are a miscellaneous group of low-molecular weight peptides with antibiotic activity. They are produced by a number of *Enterobacteriaceae* species, mostly *Escherichia coli* strains [6,7]. Although most of the microcins inhibit enzymatic pathways, membrane interaction was also postulated to be part of their mechanism of action. Microcin J25 (MccJ25) is a 21-amino acid antimicrobial peptide that target certain human pathogens such as *Salmonella* and *Shigella* [8]. The sequence is rich in hydrophobic residues and the backbone is folded in an unusual lasso distinctive structure [9–11]: the γ-carboxyl of the side chain of Glu^8 and the N-terminal Gly^1 are covalently bonded and form an eight residue ring that is threaded by C-terminal tail (Fig. 1). This folding is maintained by steric hindrance between the aromatic side chains of Phe^{19} and Tyr^{20}, located on each side of the ring, and the residues of the ring [9–11]. MccJ25 exerts antimicrobial activity by means of a dual mechanism of action [12]: the peptide inhibits transcriptional activity by obstructing the RNA polymerase secondary channel [13–15] and affects, independently, the cytoplasmic membrane of *Escherichia coli* and *Salmonella enterica* serovars [12,16,17]. In this regard, it was shown that MccJ25 causes dissipation of the membrane electrical potential of *S. enterica* and inhibition of respiratory chain enzymes such as NADH, succinate and lactate dehydrogenase, decreasing oxygen consumption rates. The fact that MccJ25 is a membrane-active peptide was also supported by studies carried out on model systems, such as liposomes and Langmuir monolayers [18,19].

In this work, we performed a study of the interaction of a tryptophan-containing derivative of microcin J25, MccJ25 I13W, with model membranes by means of fluorescence spectroscopic methods. The results

* Corresponding author. Tel./fax: +54 381 4248921.
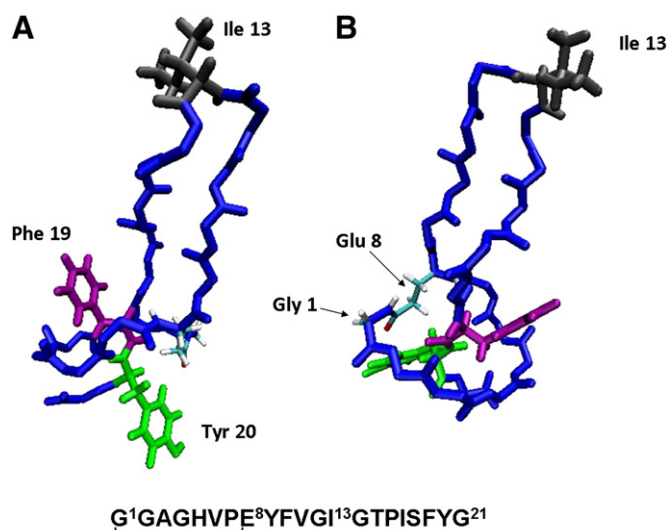 E-mail address: rdmorero@fbqf.unt.edu.ar (R. Morero).

Fig. 1. Microcin J25 structure obtained from PDB file 1Q71. Lateral view (A) depicts the bulky side chains of residues Phe[19] and Tyr[20] and the residue Ile[13] which was replaced by tryptophan. Upper view (B) depicts the residue Ile[13] and the N-terminal ring, formed by amide bond between the sidechain of residue Glu[8] and the N-terminal Gly[1].

show unusually strong selectivity of peptide toward phospholipids bilayers in gel phase.

2. Materials and methods

2.1. Materials

1, 2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-sn-glycero-3 phosphocholine (DMPC) were obtained from Avanti Polar Lipids (Alabaster, AL). All chemicals used were of analytical grade. Potassium phosphate monobasic (KH$_2$PO$_4$), sodium phosphate dibasic (Na$_2$HPO$_4$), sodium chloride, Triton X-100, the spin labels 5-doxyl stearic acid (5DS) and 16-doxyl stearic acid (16DS) and N-acetyl-tryptophanamide (NATA) were purchased from Sigma-Aldrich (St. Louis, MO). 3,3′-Dipropylthiadicarbocyanine iodide (DiSC$_3$ (5)) was purchased from Molecular Probes–Invitrogen.

2.2. Mutagenesis and peptides purification

MccJ25 and MccJ25 I13W were purified from 2-l cultures of strain SBG231, harboring the corresponding plasmids as previously described [19]. The purity of the peptides was evaluated by analytical high performance chromatography in two different configurations of a Gilson HPLC system as follows: 20 μl of a 0.1 mg ml^{-1} solution of either MccJ25 or its variant, MccJ25 I13W, was loaded either on a μBondapak C18 column (10 μm, 3.9×300 mm Waters) and eluted in a linear gradient from 20 to 80% (v/v) methanol in trifluoroacetic acid (TFA) 0.1% (v/v), or on a X-Terra MS C8 column (5 μm, 4.6×250 mm, Waters) and eluted in a linear gradient from 0 to 60% (v/v) acetonitrile in 0.1% (v/v) TFA. Both MccJ25 and MccJ25 I13W peptides purified in our laboratory eluted as a single peak in these analytical chromatography systems.

2.3. Liposome preparation

DPPC (2 mM) and DMPC (2 mM) in chloroform were used as stock solutions for the preparation of dried films. The stock solutions were aliquoted to the bottom of clean glass and the solvent was evaporated under a gentle stream of N$_2$ gas. Trace of the solvent was removed under vacuum during 3 h. The dried lipid films were hydrated by adding the appropriate volume of buffer for a 1 mM phospholipid dispersion and by heating and vortexing the sample about 10 °C above

the transition temperature of each lipid. The hydrated multillamelar suspension was then sonicated with a Branson tip sonicator for 30 min at temperatures higher than the transition temperature of the lipids. The small unilamellar vesicles were centrifuged at 8000×g for 10 min in order to eliminate titanium particles and multilamellar vesicles. Large unilamellar vesicles were prepared by extruding 10 times multilamellar vesicles through 100 nm polycarbonate filters (Whatman), in a stainless steel extruder operated under high pressure N$_2$ flow and heated above the main transition temperature of the phospholipids.

2.4. Fluorescence measurements

Fluorescence was recorded on an ISS-PC1 photon-counting spectrofluorimeter. Emission and excitation slits were 1 mm width (8 nm FWHH) and emission intensity background corrections were performed by subtracting the corresponding control without peptide using the Vinci software provided by the manufacturer. A computer-controlled stirrer device ensured constant mixing of the medium. The temperature inside the cuvette was measured with a thermocouple and adjusted to the desired value with a circulating water bath (Cole Parmer).

Peptide and phospholipid concentrations were always lower than 10 μM and 1 mM, respectively, in order to avoid inner filter effects ($\varepsilon_{278 \text{ nm, MccJ25 I13W}} = 8714 \text{ M}^{-1}\text{cm}^{-1}$) and undesired light scattering in the different assays. Peptide solutions and vesicles suspensions were prepared in 10 mM KH$_2$PO$_4$, 100 mM Na$_2$HPO$_4$, 0.2 mM EDTA, pH 7.4 (phosphate buffer).

Fluorescence quenching assays were carried out at 27 °C by adding increasing amounts of cesium chloride from a 5 M stock solution to a mixture containing peptide and phospholipids vesicles in a 1:200 molar ratio. Peptide and quencher final concentration were 2.5 μM and 400 mM, respectively. The decrease of the fluorophore emission intensity produced by the increasing volume of the mixture during titration was negligible with respective to the cesium quenching effect.

From the fluorescence values obtained ($\lambda_{\text{emission}} = 353$ nm; $\lambda_{\text{excitation}} = 288$ nm) at different concentrations of cesium, Stern–Volmer graphs were constructed according to the following equation:

$$\frac{F_0}{F} = 1 + K_{\text{SV}}[Q]$$

where F_o and F are the peptide fluorescence intensities in the absence and in the presence of the quencher at concentration $[Q]$, respectively. The Stern–Volmer constant (K_{SV}) was calculated from the slope of the curve and is proportional to the quencher accessibility to the peptide.

The depth of insertion of MccJ25 I13W into the lipid bilayer was studied by quenching of the peptide intrinsic fluorescence by the spin label probes 5-DS and 16-DS. The fluorescence emission at 335 nm, excited at 288 nm, was measured after addition of successive aliquots of methanolic stock solutions of the spin labels probes. The actual concentration of the probes inside the hydrophobic core of the phospholipids membranes (Q)$_l$ was calculated according to their corresponding partition coefficient ($K'_{\text{pQ}} = 12570$ and $K'_{\text{pQ}} = 3340$, for 5-DS and 16-DS, respectively) [20].

$$[Q]_l = \left(\frac{K'_{\text{pQ}}}{1 + K'_{\text{pQ}} \gamma[L]} \right) \cdot [Q]$$

2.5. Calculation of the partition coefficient of MccJ25 I13W

The peptide binding to the lipid bilayer was quantified by measuring the fluorescence increase at 335 nm as liposome aliquots were added. The increase in fluorescence intensity is proportional to the amount of peptide incorporated into the vesicles [21,22], so a binding