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Penetration and interactions of the antimicrobial peptide, microcin J25, into uncharged phospholipid monolayers

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Abstract

Microcin J25 forms stable monolayers at the air–water interface showing a collapse at a surface pressure of 5 mN/m, 220 mV of surface potential, and 6 fV per squared centimeter of surface potential per unit of molecular surface density. The adsorption of microcin J25 from the subphase at clean interfaces leads to a rise of 10 mN/m in surface pressure and a surface potential of 220 mV. From these data microcin appears to be a poor surfactant per se. Nevertheless, the interaction with the lipid monolayer further increase the stability of the peptide at the interface depending on the mode in which the monolayer is formed. Spreading with egg PC leads to nonideal mixing up to 7 mN/m, with hyperpolarization and expansion of components at the interface, with a small excess free energy of mixing caused by favorable contributions to entropy due to molecular area expansion compensating for the unfavorable enthalpy changes arising from repulsive dipolar interactions. Above 7 mN/m microcin is squeezed out, leaving a film of pure phospholipid. Nevertheless, the presence of lipid at 10 and 20 mN/m stabilize further microcin at the interface and adsorption from the subphase proceeds up to 30 mN/m, equivalent to surface pressure in bilayers.

Keywords: Langmuir monolayers; Gibbs monolayers; Microcin; Phosphatidyl choline; MccJ25; Antimicrobial peptide

1. Introduction

The microcins are a family of low-molecular-weight peptides produced by diverse strains of Enterobacteriaceae, mostly *Escherichia coli*. Their biological characterization has shown that they are molecules with antibiotic activity, mainly active against bacterial genera or species phylogenetically related to the producing strains [1,2]. In more general terms the microcins share common biological properties with other antibiotic peptides such as magainin, melittin, and defensin. All of these peptides have different amino acid sequences, structures, and sizes. The biological activities of most of these peptides seem to be directly correlated to their capacity to form an amphiphilic ordered structure that allows them to associate and penetrate cellular membranes [3,4].

Microcin J25, a peptide of 21 amino acids, was previously reported as a head-to-tail cyclic structure [5]. On the basis of biochemical studies, extensive analysis by mass spectrometry, and NMR, the MccJ25 structure was recently revised [6–8]. MccJ25 contains a backbone cyclization between Glu8 and the N-terminus. This creates an embedded ring that is threaded by the C-terminal tail of the molecule, forming a noose-like feature. Slippage of the noose is prevented by steric constraints imposed by the sidechains of Phe19 and Tyr20, irreversibly trapping the threaded segment within the cycle.

The detailed mechanism of cell death caused by this peptide is not clearly known and we are working to get information on this subject. Recently, our laboratory provided

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convincing evidence that RNA polymerase is the target for MccJ25 action in *E. coli*. MccJ25 inhibits transcription by binding in and blocking the secondary channel and preventing the traffic of nucleoside triphosphate substrates to the catalytic center of the enzyme [9,10]. The C-terminal carboxyl group and histidyl residue appear to be important for antimicrobial activity [11]. Both charges are close in the three-dimensional structure and the interaction between them might provide further stability to the fold and the highly hydrophobic nature of this molecule overall [7]. Furthermore, and according to our previous work, the peptide interacts with phosphatidyl choline bilayer changing the lipid microviscosity and this effect is dependent on the hydrocarbon chain length [12]. Additionally, we have shown that the peptide exerts a strong effect on membrane permeability of *Salmonella newport* but not on *E. coli*, suggesting a differential mechanism between both strains [13]. We decided to extend our previous biophysical studies investigating the interaction of MccJ25 with phosphatidyl choline monolayers. At present there are no microcins-monolayer studies described. In particular, we used a Wilhelmy type of film balance to determine possible interactions with lipid interfaces, a validated technique to explore lipid interactions with antibiotics [14], toxic peptides [15], and protein molecules in general [16]. Our results show for the first time that MccJ25 is able to spontaneously penetrate from the subphase an egg PC monolayer, and when spread in mixed monolayers leads to marked cross-sectional molecular area expansion and hyperpolarization. This allows gaining insights on the interaction between the peptide and the components of the membrane and it might help in clarifying whether antibiotic activity might be related to changes of the physical state of the membrane.

2. Materials and methods

2.1. Chemicals

The peptide MccJ25 was obtained from the supernatant of *E. coli* K-12 MC4100 (F^- araD 139 Δ(argF-lac)205 λ^- flbB5301 $ptsF_{25}$ relA1 prsL150 deoC1) harboring pTUC203, a low-copy-number recombinant plasmid with the MccJ25 cloned [12,17]. The cells were grown in M9 minimal media [18], supplemented with glucose (0.2%) and thiamine (1 μg/ml). MccJ25 was purified according to the procedure previously reported [12]. This procedure yielded a preparation which appeared homogeneous in two different systems of analytical RP-HPLC [5]. Concentrations of peptide solutions in methanol were determined by absorbance determination at 278 nm (ε_{278} 3340 $M\,L^{-1}$) [19–22]. The egg PC was obtained according to [23]. A thin-layer chromatogram of PC with a variety of solvent systems did not reveal any impurities [24].

2.2. Compression isotherms

Egg phosphatidylcholine monolayers were spread from a chloroform–methanol 1:1 solution directly over the surface with a Hamilton microsyringe. Pure MccJ25 was spread from a methanol solution. The mixture (egg PC:MccJ25) in different proportions was spread from a solution of chloroform-methanol 1:1. The subphase was Tris–HCl 20 mM, pH 7.0, and the temperature was maintained at 25 °C. The surface pressure (Wilhelmy plate method via platinized-Pt plate), the area enclosing the monolayer, and the surface potential (via millivoltmeter with air-ionizing ^{241}Am plate and calomel electrode pair) were automatically measured with a Monofilmmeter control unit, Mayer Feintechnique, Gottingen, Germany. The output was recorded continuously and simultaneously with a double-channel X-Y-Y recorder. The excess free energy of mixing (ΔG_m^{ex}) was calculated as the difference between the area under the experimental and the ideal surface pressure–molecular area isotherms, integrated between 1 and 7 mN/m (Eq. (1)) [14]. These conditions reduce errors arising from the rather variable gaseous region of the isotherms below 1 mN/m, which can be markedly dependent on technical artifacts during spreading. Thus, the values discussed herein for this parameter include only the liquid-expanded and -condensed states of coherent films and leave out the contribution from the gaseous states of the isotherms,

$$\Delta G_m^{ex} = \int_1^7 A_{Mcc-PC}\,d\pi - X_{Mcc}\int_1^7 A_{Mcc}\,d\pi - X_{PC}\int_1^7 A_{PC}\,d\pi, \quad (1)$$

where π is the surface pressure and A_{PC}, A_{Mcc}, and A_{Mcc-PC} are the molecular areas at surface pressure π for PC, MccJ25, and the mixed monolayer, respectively. X_{Mcc} and X_{PC} are the MccJ25 and PC molar fractions, respectively.

The free energy of mixing for an ideal system (ΔG_m^i) and the real free energy of mixing (ΔG_m) were calculated using Eqs. (2) and (3), respectively [25],

$$\Delta G_m^i = RT X_{Mcc}\ln X_{Mcc} + RT X_{PC}\ln X_{PC}, \quad (2)$$

$$\Delta G_m = \Delta G_m^i + \Delta G_m^{ex}, \quad (3)$$

where R is the gas constant and T is the absolute temperature.

The excess of configurational entropy (ΔS_c^{ex}), entropy of mixing for an ideal system (ΔS_m^i), and entropy of mixing (ΔS_m) were calculated respectively with the equation

$$\Delta S_c^{ex} = R\ln A/A_i, \quad (4)$$

$$\Delta S_m^i = -R(X_{Mcc}\ln X_{Mcc} + X_{PC}\ln X_{PC}), \quad (5)$$

$$\Delta S_m = \Delta S_m^i + \Delta S_c^{ex}, \quad (6)$$