Interaction of polymyxin B and A deacilated derivative with monolayers of bacterial lipids

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ABSTRACT. Polymyxin B (PxB) is a polycationic peptide antibiotic produced by gram-positive bacteria Bacillus polymyxa, and is highly selective against gram-negative organisms. In the present work we have studied the interaction of PxB and PxB-nonapeptide (NP), with lipid monolayers of a lipidic extract from Escherichia coli, a bacteria susceptible to PxB. Kinetics of insertion of both peptides at different surface pressures are compared. Also, the effect of peptides on the phase behaviour of the monolayers is determined from the compression isotherms.

1. INTRODUCTION

Polymyxin B (PxB) (figure 1-a) is a cyclic amphipathic decapeptide with five positively charged side chains and an acyl chain at the N-terminus [1]. It is produced by Gram-positive Paenibacillus polymyxa and it is highly selective against Gram-negative organisms. Polymyxins have been in prevalent use for several decades, however there is no indication of the antibiotic resistance in clinical isolates. They act on membranes, yet the antibacterial mechanism is not stablished. Binding of PxB to surface lipopolysaccharides, and consequent disruption of the outer membrane of Gram-negative organisms [2] to permit their entry into periplasmatic space of gram-negative organisms, is a necessary but not a sufficient condition for the antimicrobial effect; PxB nonapeptide (NP) (figure 1-b) disrupts the outer membrane but it is not an antibiotic [2,3].

![Figure 1-a. Polymyxin B](image1)

![Figure 1-b. Polymyxin B nonapeptide](image2)

Studies with model membranes have provided compelling evidence that these cationic peptides interact with anionic phospholipid of bacterial membranes, yet they are not
ionophores. They induce leakage of the cytoplasmic contents at significantly higher concentrations than the antimicrobial concentrations [4].

In the present work we have studied the interaction of PxB and PxB-nonapeptide (NP), with lipid monolayers of a lipidic extract from *Escherichia coli*, a bacteria susceptible to PxB. Kinetics of insertion of both peptides at different surface pressures are compared. Also, the effect of peptides on the phase behaviour of the monolayers is determined from the compression isotherms.

2. MATERIALS AND METHODS

2.1 Materials
Polymyxyn B (PxB) and PxB nonapeptide were purchased from Sigma Aldrich (St. Louis, Mo, USA). *E. coli* total lipid extract (57.5% phosphatidylethanolamine (PE), 15.1% of phophatidylglycerol (PG), 9.8% cardiolipin (CL) and 17.0% others) was obtained from Avanti Polar Lipids (Alabaster, Al. USA).

Spreading solvent was chloroform. Lipid films were prepared from chloroform solutions of approximate concentration of 1 mg.ml⁻¹. Monolayers were spread on 10 mM TRIS (tris[hydroxymethyl]amino-methane) buffer subphase, pH = 8.0.

2.2 Methods
Monolayer studies were performed on a NIMA technology Langmuir film balance equipped with a Wilhemy plate, a pression sensor PS4, a ST 9000 tensiometer and a software trough. The temperature of the experiments was always 21±0.5 °C.

2.2.1. Surface activity measurements
These experiments were performed in a cylindrical trough (70 ml volume) with mechanical stirring. The trough was filled with 10 mM TRIS buffer solution, pH 8.0, and increasing volumes of concentrate antibiotic solutions were injected directly underneath through a lateral hole. Pressure increase was recorded continuously for 120 min. Each run was carried out in triplicate and reproducibility was usually within 0.1-0.2 mN.m⁻¹
2.2.2. Penetration kinetics

The same conditions mentioned above were used but for this case a lipid monolayer was present at the air-water interface. Monolayers of E. coli extract were formed spreading lipidic solutions in chloroform to reach a required initial pressure of 5, 10, 20 or 32 mN.m⁻¹.

2.2.3. Compression isotherms

The compression isotherms were carried on a PTFE trough of surface area 525 cm² and volume 240 cm³. Monolayers of E. coli extract were formed by applying small drops of the spreading solutions on the 10 mM TRIS buffer subphase (pH=8.0) with a microsyringe (Hamilton Co, Reno, USA). After five minutes, the monolayers were continuously compressed (symmetrical compression) with an area reduction rate of 80 cm².min⁻¹. The films were compressed up to their collapse pressure. Each run was repeated three times and standard deviation was typically < 0.5%.

3. RESULTS AND DISCUSSION

3.1. Surface activity

PxB when injected in an aqueous subphase modified the surface pressure of the system in a concentration dependent way while NP showed a very low surface activity that was independent of the peptide concentration in the subphase (fig. 1). From these values the surface excess was calculated applying the Gibbs equation:

\[ \Gamma = \frac{\Delta \pi}{RT \Delta \ln c} \ (\text{mol/m}^2) \]

where \( R = 8.31 \times 10^3 \) mN . cm . K⁻¹ . mol⁻¹, \( T \) is the temperature (K), \( \Delta \Pi \) is the surface pressure increase and \( c \) is the molar concentration and are given in table 1.

![Fig. 1. Surface activity of PxB (*) and NP (o). Results in the figure correspond to 80 minutes after injection.](image-url)
Table 1: Surface activity of PxB in TRIS 10 mM pH 8.0 buffer. a PxB concentrations in the subphase; b Surface pressure increase at 80 minutes from PxB injection; c Surface excess.

<table>
<thead>
<tr>
<th>PxB (μM)</th>
<th>Δπ (mN/m)</th>
<th>Γ (mol/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>2.6</td>
<td>1.75x10⁻⁷</td>
</tr>
<tr>
<td>1.0</td>
<td>2.8</td>
<td>1.63x10⁻⁷</td>
</tr>
<tr>
<td>2.0</td>
<td>6.0</td>
<td>3.18x10⁻⁷</td>
</tr>
<tr>
<td>3.0</td>
<td>6.2</td>
<td>3.12x10⁻⁷</td>
</tr>
<tr>
<td>4.0</td>
<td>7.5</td>
<td>3.64x10⁻⁷</td>
</tr>
<tr>
<td>5.0</td>
<td>7.7</td>
<td>3.64x10⁻⁷</td>
</tr>
<tr>
<td>5.9</td>
<td>8.5</td>
<td>3.94x10⁻⁷</td>
</tr>
<tr>
<td>7.8</td>
<td>8.7</td>
<td>3.91x10⁻⁷</td>
</tr>
</tbody>
</table>

3.2. Penetration kinetics

After injection of PxB beneath monolayers a clear insertion process was detected (fig. 2). As expected, there was a strong dependence of the maximum surface increase achieved
on the initial monolayer surface pressure, but pressure increase were lower than those obtained in absence of monolayer. The monolayer in fact acts as a barrier hindering the incorporation of antibiotic molecules into the surface. The insertion process can be analysed by iterative least-square fit to $\Delta \pi = A \cdot (1 - e^{-k_1 t}) + B \cdot (1 - e^{-k_2 t})$ where $A$ and $B$ are the $\Delta \pi$ corresponding to two processes, with half times of $0.69/k_1$ and $0.69/k_2$, respectively, being $k_1$ and $k_2$ the specific rate constants for each process. Results are summarised in table 2. When the NP was injected no changes in the surface pressure was observed. That indicates the inability of NP to insert in the $E. coli$ extract monolayer.

<table>
<thead>
<tr>
<th>$\pi_1$ (mN/m)</th>
<th>$t_{1/2}$ (min) = $0.69/k_1$</th>
<th>$t_{1/2}$ (min) = $0.69/k_2$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.92 (82%)</td>
<td>15.30 (18%)</td>
<td>0.994</td>
</tr>
<tr>
<td>10</td>
<td>0.90 (100%)</td>
<td>----</td>
<td>0.953</td>
</tr>
<tr>
<td>20</td>
<td>0.85 (100%)</td>
<td>----</td>
<td>0.969</td>
</tr>
<tr>
<td>32</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

Table 2. Kinetic parameters for the insertion of PxB in $E. coli$ total extract monolayers at different surface pressures.

### 3.3. Compression isotherms

The studies performed using compression isotherms let us to check the ability of the $E. coli$ lipid extract to produce a stable monolayer with pressure collapse at 49 mN.m$^{-1}$. The monolayer compressibility indicates that for area values until 360 Å$^2$.molec$^{-1}$ the monolayer present a gas phase behaviour and for minor values are the liquid-expanded state until to collapse pressure. The presence of PxB in the subphase at 2µM concentration produced an expansion of the monolayer indicative that an insertion process is produced. The collapse pressure remains constant at 49 mN.m$^{-1}$. Similar behaviour was described before for derivative lipopeptidic antigens of hepatitis A viruses [5]. When NP at the same concentration was injected in the subphase no changes in the $E. coli$ lipid extract monolayer were observed. These results are
indicative of the importance of hydrophobic interaction over electrostatic attractions and can be seen in figure 3 A and B.

Fig. 3. A: Compression isotherms of *E. coli* lipid extract on 10 mM TRIS buffer, pH = 8.0, subphase (-) and when the PxB at 2µM concentration are present in the subphase (Q). When NP was in the subphase the compression isotherm is the same as *E. coli* lipid extract (-). B: Compressibility, C$_s^{-1}$ (mN./m), of a monolayer of *E. coli* lipid extract on TRIS buffer pH = 8.0 subphase and when the PxB at 2 µM concentration was present in the subphase (---). When NP at 2 µM was present in the subphase the compressibility of the monolayer are the same as *E. coli* lipid extract (-).

4. REFERENCES