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Nanoscale investigation of the interaction of colistin with model phospholipid membranes by Langmuir technique, and combined infrared and force spectroscopies

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ABSTRACT

Colistin (Polymyxin E), an antimicrobial peptide, is increasingly put forward as salvage for severe multidrug-resistant infections. Unfortunately, colistin is potentially toxic to mammalian cells. A better understanding of the interaction with specific components of the cell membranes may be helpful in controlling the factors that may enhance toxicity. Here, we report a physico-chemical study of model phospholipid (PL) mono- and bilayers exposed to colistin at different concentrations by Langmuir technique, atomic force microscopy (AFM) and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). The effect of colistin on chosen PL monolayers was examined. Insights into the topographical and elastic changes in the PL bilayers within time after peptide injection are presented via AFM imaging and force spectra. Finally, changes in the PL bilayers' ATR-FTIR spectra as a function of time within three bilayer compositions, and the influence of colistin on their spectral fingerprint are examined together with the time-evolution of the Amide II and νC═O band integrated intensity ratios. Our study reveals a great importance in the role of the PL composition as well as the peptide concentration on the action of colistin on PL model membranes.

1. Introduction

Colistin (polymyxin E, $C_{52}H_{98}N_{16}O_{13}$, structure shown in Fig. S1, Supplementary Data) is a cationic cyclic peptide belonging to the polymyxin family. It is known not only for its action against gram negative bacteria, but also for its toxic effects on mammalian eukaryotic cells [1,2]. The action of colistin is mainly leaded by interactions with the cell membrane that eventually cause an increased permeability of the cell membrane [3]. The composition and organization of cell membranes are complex, consisting of mixtures of phospholipids, glycolipids, and proteins. Such complexity makes the task of deciphering the specific role of individual components difficult. PLs are found in prokaryotic and eukaryotic cells as one of the major components. Here, we have selected a model of the cell membrane to gain insights into the importance of lipid composition on the colistin interaction with membrane. Because the use of colistin is hampered due to claimed reports of toxicity, it is of basic and applied interest to get more insight in the interaction between the peptide and PLs such as phosphatidylcholines. Different drugs have been studied together with PL model membranes of different compositions [4–6]. Because of their multiple advantages, such as the precise control of the monolayer packing density, Langmuir film method is commonly used for studying specific aspects of biological phenomenon at lipid membrane interface [7–9]. Langmuir films of PLs can be formed at the air/liquid interface and their physico-chemical properties can be investigated through isotherms measured by barrier compression. The monolayers can be further analysed with Brewster angle microscopy (BAM) and electric surface potential [9,10]. BAM is a microscopic technique relying on the reflectivity differences of water and monolayer surfaces. P-polarized light at a Brewster's angle of incidence results in a minimum reflectivity from water surface and thus when monolayer is present, the optical properties of the interface are altered, resulting in increased reflectivity of the regions covered by the monolayer. This allows a visual investigation of the monolayer structure, such as domain growth [11,12]. Electric surface potential is used to study the difference in electrical potential above and below a monolayer at the air/liquid interface and is measured by

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observing the potential difference between a vibrating plate placed above the monolayer and a counter electrode immersed in the subphase [13]. Although monolayers are useful in the examination of drug–membrane interfacial interactions, they do not represent the complexity of a two leaflet cell membrane [14]. Supported lipid bilayers are also widely used as model systems to mimic biological membranes. This kind of model lipid membranes yields complementary information compared to lipid monolayers. These systems can be investigated with several analytical techniques and their physicochemical properties can be compared with those of cell membranes [15]. Among these analytical techniques, atomic force microscopy (AFM) is a method of choice for characterizing the physico-chemical behaviour at the nanoscale, including drug–membrane interactions [16–20]. Not only is AFM able to produce high-resolved imaging of lipid bilayers at the nanometer scale, but it also provides quantitative information on nanomechanical properties of supported lipid systems in an aqueous environment [21–23]. Furthermore, it allows monitoring and quantifying biomembrane mechanical and morphological properties of the membrane in the presence of other compounds [18,22,24]. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) is a useful complementary tool, because it allows gaining information on the structure of liposomes and lipid bilayers deposited in aqueous media on the surface of an internal reflexion element [25–28]. However, a glance at the literature reveals that there is very little information on the infrared spectroscopy studies of PL bilayer formation. Infrared spectroscopy is especially powerful in studying lipid–protein and lipid–peptide interactions and conformational changes [29,30]. Furthermore, polarization can be used to gain more knowledge on the orientation and organization of lipid layers in various conditions via the calculation of dichroic ratios of selected vibrational modes [31–34].

The scope of the present work was to address the interactions of colistin with different PLs such as 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) [35], and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC). The action of colistin was investigated by combining three different types of techniques in a complementary manner. PL monolayers were examined by compression isotherms, BAM and electric surface potential. The bilayer studies were carried out in liquid condition [36–38] in real time observations through AFM (by both morphology and mechanic monitoring) and ATR-FTIR.

2. Materials and methods

2.1. Chemicals

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, ≥99%), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE, ≥97%), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC, ≥99%), colistin sulfate salt, tris(hydroxymethyl)aminomethane (Tris, 99.9%), NaCl, anhydrous $CaCl_2$, spectrometric grade chloroform and methanol were purchased from Sigma-Aldrich. At room temperature (20 ± 1 °C), DPPC (transition temperature 41 °C) and DPPE (transition temperature 63 °C) are in a gel-like state (L_{β}), whereas DOPC (transition temperature −17 °C) containing unsaturated hydrocarbon chains, is in liquid disordered state (L_{α}) [16,39,40].

2.2. Sample preparation

The colistin sulfate salt was dissolved into TRIS buffer (10 mM Tris, 150 mM NaCl, pH 7.4) with a concentration of 1.25 g/L to obtain a mother solution. For Langmuir film studies, DPPC, DPPE, DOPC and equimolar mixtures of DPPC/DOPC and DPPE/DOPC phospholipids were dissolved in chloroform at a concentration of 1 mM. The solutions were further stored at 4 °C before use. For bilayer studies, unilamellar liposomes of DPPC, DPPE and DOPC were prepared: the lipids were dissolved in chloroform and deposited on the wall of a rotating round bottom flask under a stream of nitrogen gas. The flasks were then kept under vacuum for 3 h to avoid traces of chloroform, and finally filled with 10 mL of TRIS–Ca buffer (10 mM Tris, 150 mM NaCl, 1 mM $CaCl_2$, pH 7.4) to obtain a lipid suspension with a concentration of 1 mM. Suspension of small unilamellar vesicles (SUVs) was obtained by sonication of the lipid solution until clarity (3 cycles of 2 min) using a 500 W probe sonicator at 35% of the maximal power (VibraCell 505, Fisher Bioblock Scientific, France). During this procedure the suspension was kept in an ice bath to limit heating. Finally, the SUVs suspension was filtered on 0.2 μm nylon filters to eliminate titanium particles and then stored at 4 °C until use (storage did not exceed 15 days). Supported DPPC, DOPC, DPPC/DOPC (1:1, v/v), and DPPE/DOPC (1:1, v/v) bilayers were obtained by the vesicle fusion method [23]. The prepared suspensions of liposomes were let to spontaneously deposit onto the surface (65 °C pre-heated well-cleaved mica in an AFM closed fluid cell, or Ge crystal enclosed in a batch infrared cell) by incubating the liposome solutions at 65 °C for 45 min. Preheated (65°C) TRIS buffer was then added into the cell, and the cell was gradually let to cool down to room temperature after which it was either rinsed or not with TRIS buffer.

2.3. Compression isotherms and Brewster angle microscopy

The surface pressure (Π) and electric surface potential (ΔV) measurements were carried out with a KSV 2000 Langmuir balance (KSV Instruments, Ltd., Helsinki, Finland). Compression isotherms were determined for pure lipid monolayers as well as their equimolar (1:1) mixtures in pure water and in 50 μM colistin subphase with a Teflon trough [6.5 cm (l) × 58 cm (w) × 1.0 cm (d)] holding two hydrophilic Delrin barriers for symmetric compression. The system was equipped with an electrobalance, from which a platinum Wilhelmy plate (perimeter 3.94 cm) was hanging as a surface pressure sensor, and a surface potential measuring head with a vibrating electrode (KSV SPOT1). A stainless steel plate immersed 4 mm below the surface was used as a counter electrode. The system was kept in a Plexiglas cabinet and trough temperature was kept constant at 20 ± 0.1 °C. Prior to each experiment, the trough and the barriers were washed by soaked cotton in chloroform and ethanol and then rinsed with excess amount of Milli-Q water. The platinum plate was carefully cleaned between each run by rinsing with Milli-Q water and ethanol, and finally heating to a red-hot glow in a propane flame to eliminate any organic contaminants. All solvents used for cleaning the trough and the barriers were of analytical grade. Any residual surface-active impurities were removed from subphase surface by sweeping and suction. The stability of the surface potential signal was checked before each experiment after cleaning the subphase surface. After the ΔV signal had stabilized and the surface pressure fluctuation was lower than 0.2 $mN \cdot m^{-1}$ during compression stage, monolayers were spread from calibrated solutions using microsyringe (Hamilton Co., USA). After 10 min of equilibration time, the films were compressed at the rate of 10 $mm \cdot min^{-1}$ by the two symmetrically advancing barriers (5 $mm \cdot min^{-1}$ per barrier). A computer and KSV Instruments software were used to control the experiments. Each compression isotherm was performed at least three times. The standard deviation was 2.6 $mN \cdot m^{-1}$ for surface pressure, 2.5 $Å^2$/molecule for mean molecular area and 0.01 V for surface potential measurements. Changes in the mechanical properties of the monolayers were studied through the values of compressibility modulus ($c_s^{-1} = -A(\partial \Pi / \partial A)_T$) [41] and the collapse parameters, Π_{coll}, A_{coll} and ΔV_{coll} were determined directly from the compression isotherms. During compression, the morphology of the films was imaged with a Brewster angle microscope (KSV Optrel BAM 300, Helsinki, Finland) using *p*-polarized light from a class IIIb 10-mW laser at 632.8 nm, the lateral resolution of the instrument being 1 μm.

2.4. Atomic force microscopy

In order to study the topography and elasticity of lipid bilayers, two lipid systems were used: DPPC/DOPC and DPPE/DOPC. The bilayers