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Interaction of non-aqueous dispersions of silver nanoparticles with cellular membrane models

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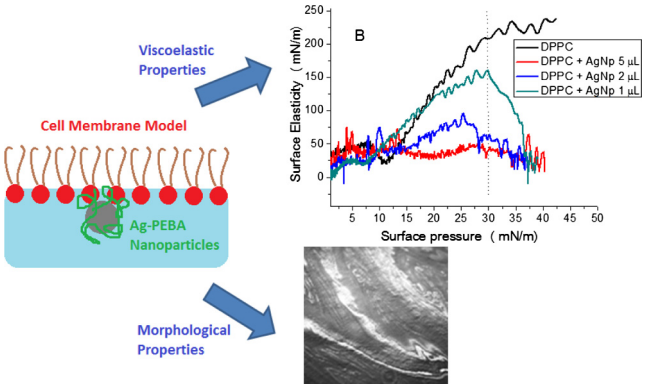
GRAPHICAL ABSTRACT



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ABSTRACT

In this work, silver nanoparticles (AgNPs) dispersed in non-aqueous media and stabilized with polyether block polymers amide (PEBA) were incorporated in Langmuir monolayers of dipalmitoylphosphatidylcholine (DPPC), which served as a cell membrane model. The AgNPs presented surface activity, disturbing the viscoelastic properties of the floating film. They expanded the monolayers decreasing their surface elasticity as observed with surface pressure-area isotherms. Polarization modulation reflection-absorption spectroscopy showed that the permanence of AgNPs at the air-water interface is favored by PEBA, affecting both the hydrophilic and the hydrophobic groups of the phospholipid. Brewster angle microscopy showed that the AgNPs lead to the formation of aggregates at the air-water interface, establishing domains that shear with each other due to the low lateral viscosity of irregular and non-monomolecular domains. These data can be correlated to the possible toxicity and microbicide effect of AgNPs in lipidic surfaces such as in mammalian and microbial membranes.

1. Introduction

Nanomaterials are systems with size ranging from 1 to 100 nm that present particular physical and chemical properties and can be used in numerous applications [1]. Particularly, silver nanoparticles (AgNPs) are employed in surgical and hygienic supplies due to their antimicrobial properties [2,3]. Although their bactericidal effect is well known, the molecular mechanism of action on bacterial membranes has not yet been fully understood. Scanning and transmission electron microscopies have been employed to study the biocidal effect of silver nanoparticles prepared in aqueous media against *E. Coli* [4] and others types of bacterium [5]. These

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reports demonstrated that AgNPs are able to destroy the bacterial membrane and to penetrate the cell wall as well as the plasmatic membrane, releasing silver ions. Particularly, the bactericidal effect of silver ions has been the issue of several investigations. It has been proposed that it may be associated to some factors, such as inactivation of vital enzymes [6,7], loss of ability of DNA to replicate [8], as well as structural changes in the cell membranes [8,9]. Silver nanoparticles have also been used for electronic [10], and catalytic [11] devices, as well for biosensing [12] and medical imaging [13]. Consequently, data on specific molecular interactions between nanoparticles and lipids are crucial not only to understand, at the molecular level, mechanisms of action, but also to investigate the formation of metallic particles confined in bioinspired structures for optoelectronic devices. Furthermore, it is also important to understand how silver nanoparticles interact with mammalian cells. It is reported that they can cause inflammation, denaturation of proteins, oxidative stress and disruption of the cell membranes [14]. As these mechanisms are not comprehended in detail, and the cell membrane is naturally complex, the use simplified models should be convenient to describe cellular responses.

Examples of such models include black lipid membranes, liposomes and supported lipid bilayers on flat surfaces [15–20]. A complementary model to these ones is the formation of lipid Langmuir monolayers at the air-water interface. The use of these systems to mimic biomembranes has become popular in view of the complexity of *in vivo* systems, in which distinguishing the effects from the many components in a membrane is not straightforward. Langmuir films permit easy manipulation of the chemical composition and surface density [21], but papers using lipid monolayers to investigate the action of silver nanoparticles are still scarce [22]. As a result, Langmuir films composed of lipids are an interesting tool to mimic the first barrier encountered by nanoparticles in contact with the cell as a microbicide. In this context, recently, Girón et al. [22] proposed that AgNPs act on fluid saturated lipid monolayers of dimyristoylphosphatidylcholine (DMPC), expanding the films, whose effects are dependent on the capping molecules.

In this present work, silver nanoparticles were produced in non-aqueous media and stabilized with a polyether block polymers amide (abbreviated as PEBA). They were then incorporated in lipid monolayers at the air-water interface. Dipalmitoylphosphatidylcholine (DPPC) was chosen since this lipid has been employed for several kinds of cell models at the air-water interface, including mammalian [23] and microbial [24] ones. Surface-area pressure-area isotherms, polarization modulated infrared reflection-absorption spectroscopy (PM-IRRAS) and Brewster angle microscopy BAM) were employed for investigation on the thermodynamic, structural and morphological properties of the supramolecular system. It is worth mentioning that the non-aqueous AgNP dispersion used in this work is reported to present antimicrobial activity against *E. coli* and *S. aureus* microorganisms, even in low concentrations [25], but no study on the molecular interaction of these nanoparticles with membrane models in found in the literature.

2. Materials and methods

The water employed in this work was purified using a MilliQ-Plus system (resistivity 18.2 MΩ cm, pH 5.5).The phospholipid DPPC, dipalmitoylphosphatidylcholine, (Sigma-Aldrich) was dissolved in chloroform (Synth, PA) to result in a concentration of 0.5 mg/mL. The silver nanoparticle dispersion was prepared following a procedure already described in the literature [25]. Succinctly, PEBAX-2533 (Arkema) was dissolved in 1-butanol (Aldrich, 99%) in 4% (w/w) with vigorous stirring and under reflux (24 h). To a 10 mL sample of this PEBAX-2533 solution, it was added 14.7 mM of $AgNO_3$ (Aldrich, 99%) and the mixture was refluxed during 30 min. To remove the unreacted silver salt from the dispersion, it was washed 5 times with 10 mL of deionized water. The polymer PEBAX-2533 is based on polyamide-12 (PA-12) (Mw = 530, 20 wt%) and polytetramethyleneoxide (PTMO) (Mw = 2000, 80 wt%). Transmission Electron Microscopy of the nanoparticles was reported in Ref. [25] and showed spherical and non-aggregated nanoparticles with diameters ranging from 5 to 10 nm. Zeta potential determined for these dispersions indicated that the nanoparticles are not charged.

Pre-determined aliquots of the lipid solutions were spread drop-by-drop on the surface of the aqueous buffer solution in order to form Langmuir monolayers supported on water subphase with a mini-KSV Langmuir trough equipped with a surface pressure sensor (the Wilhelmy method). For preliminary tests, the nanoparticle dispersion was spread on the air-water interface to test its surface activity. For the preparation of mixed monolayers, lipids and nanoparticles were co-spread separately at the air-water interface. After at least 10 min allowed for solvent evaporation, mobile barriers were actioned to compress the air-water interface at a rate of 5 $Å^2$ $molecule^{-1}$ min^{-1}. The surface pressure (π) were measured as long as the film area (A) decreased, obtaining then π-A isotherms. For polarization modulation infrared reflection-absorption spectroscopy (PM-IRRAS) studies, the monolayer was compressed until 30 mN/m. This surface pressure was maintained during the obtainment of the spectra. For that, a KSV PMI 550 instrument (KSV Instruments, Ltd., Helsinki, Finland), that operates with a modulation frequency of 84 kHz, and an incidence angle to the normal was 75° was employed. A minimum of 600 scans were obtained for each spectrum, with a resolution of 8 cm^{-1}, with the incoming light continuously modulated between the p and s polarization, allowing simultaneous measurements of the spectra for both polarizations. Control experiments using only the capping polymer (without Ag) were performed in order to check the role of PEBA on the surface activity of the dispersions containing the nanoparticles. For that, PEBA solutions, in the same concentration existing in the dispersions, were co-spread with DPPC in a similar procedure employed for AgNPs, and tensiometry and vibrational experiments were performed in order to verify their specific surface activity on DPPC monolayers. Brewster Angle Microscopy (BAM) images were obtained with a micro-BAM from KSV-Instruments at a surface pressure of 30 mN/m. This value of surface pressure was chosen because it corresponds to the lateral pressure of cell membranes [26].

All experiments were carried out at a controlled room temperature (25 °C). Each surface pressure-area isotherm, spectrum, and BAM image was obtained at least three times to ensure the reproducibility of the experiments. Only isotherms, spectra, and images that were highly reproducible are shown in this manuscript.

3. Results and discussion

3.1. Tensiometry and thermodynamic parameters

Fig. 1 shows the surface pressure-area isotherms for AgNPs spread on the air-water interface in several selected volumes of spreading. It can be noted irregular curves, with the surface pressure increasing with compression. Increasing the volumes spread on the interface leads to higher values of surface pressure. Particularly for the isotherms obtained for spread volumes of 5 and 50 μL, it can be observed that during the compression there are some stages on the curve where the surface pressure decreases upon compression, followed by an increase of the surface pressure with further compression. This indicates a dynamic process of desorption and re-adsorption of the AgNPs with probable formation of aggregates. Cycles of compression and decompression (not shown)