Interaction between antimicrobial polyelectrolytes and membrane models studied by SFG vibrational spectroscopy

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New antimicrobial strategies are crucial due to the increasing microorganism resistance to antibiotics. Antimicrobial polymers have many advantages when compared to other small biocides [1], and therefore they have great potential for technological applications. In particular, water-soluble derivatives of chitosan, such as chitosan oligomers (CO), are cationic biopolymers obtained from renewable sources that are promising candidates to a wide spectrum antimicrobial agent [2]. Unlike chitosan, which is mainly bioactive at acidic pH, CO remain cationic and bioactive at physiological pH. Nevertheless, the exact mechanism by which this polymer acts on the cell membranes remains unknown at the molecular level.

In this work we investigate the molecular interaction between CO and a biomimetic cell membrane model (Langmuir Film). For comparison, another synthetic cationic polylelectrolyte with antibacterial properties, PAH – poly(alylamine hydrochloride), has been investigated. We have carried out Sum-Frequency Generation (SFG) Spectroscopy on Langmuir Films of phospholipids on pure water and on antimicrobial containing subphases. SFG Spectroscopy allows obtaining the vibrational spectrum of interfacial molecules (lipid Langmuir Film and molecules interacting with it – water and antimicrobials), without any contribution from the bulk molecules, and is quite sensitive to the conformation of membrane lipids [3]. A zwitterionic phospholipid (DPPC) was used to model human-like membranes, while a negatively charged phospholipid (DPPG) modeled bacterial-like membranes.

Surface pressure-area isotherms showed that both PAH and CO led to a small expansion of DPPC monolayers and a significant expansion of the DPPG film, with CO causing a more dramatic effect on DPPG. SFG spectra in the CH stretch range showed that the lipid chain conformation remained always well ordered in all cases, despite membrane expansion. This indicates that CO were inserted in the monolayer, forming islands of CO within the lipid film. Changes in the SFG spectral lineshape of OH stretches for the interfacial water molecules indicated that PAH adsorption on DPPG films was able to overcompensate the lipid negative charge and led to an overall surface charge reversal. The SFG spectra of the phosphate groups also indicated that in pure water the DPPC headgroups had a more ordered orientation than in the case of DPPG. Nevertheless, upon interaction with the cationic polyelectrolytes, the DPPG headgroups also become ordered, with a preferential orientation towards the subphase. Experiments with the antimicrobials injected in the subphase under a condensed Langmuir film indicated that CO were also capable of monolayer penetration, albeit causing a reduced film expansion. This comparison indicates that the choice of experimental methodology affects the outcome, but both may be complementary, as they may represent different phases of a biomembrane lifecycle. The detailed view provided here for the molecular interaction of these polyelectrolytes with lipid films may shed light on the mechanism of their biocidal activity and aid on a rational design of new antimicrobial polymers.

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