Using infrared spectroscopy to investigate the interaction between chitosan and mixed DMPA-cholesterol films

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Chitosan (Chi) has been used in various applications associated with biology and medicine, e.g. as a fat reducer and wound healer. In most of such applications, the action at the molecular level is unknown, especially owing the complexity in the interactions between chitosans and the cells. One possible way to learn about the action is to investigate the interaction with cell membrane models, which for chitosan has been done with Langmuir and Langmuir-Blodgett (LB) films of phospholipids[1,2]. In this study we employed infrared spectroscopy *"in situ"* for characterizing mixed Langmuir films of dimyristoyl phosphatidic acid (DMPA) and cholesterol (Chol) formed on acetate buffer subphases that contained 0.2 mg/mL of Chi. Polarization-modulated infrared spectroscopy measurements were performed with a PMI 550 instrument from KSV (Finland) with the incident laser at 80°. Chitosan was found to induce ordering of neat DMPA monolayers, thus making the hydrophobic chains to be further aligned. This was inferred by the increase in the intensity ratio of the CH_3:CH_2 peaks. Significantly, the infrared peaks for PO_3 shifted to lower wavenumbers upon compression, indicating that PO_3-H_2O interactions were replaced by PO_3-Chi electrostatic interactions. Also, an extinction of NH bands above 20 mN/m indicates apparent Chi depletion from the interface. For pure cholesterol films, Chi did not affect significantly the OH, CH_2 and CH_3 peaks, but a NH signal was detected. For DMPA:Chol films on pure buffer, the CH_3 shoulder was absent, indicating a less ordered film, with cholesterol triggering DMPA dissociation leading to electrostatic repulsion, and hence monolayer expansion. With Chi in the subphase, the mixed film was more ordered, with the CH_3 peak at 2883 cm^{-1} being restored (Figure 1). This effect suppresses the expanding action from cholesterol with Chi-DMPA electrostatic interactions being stronger than for DMPA-Chol. Also, the decrease in the intensity of PO_3 peaks indicates that Chi penetrated into the lipid tails. Hence, Chi affected the polar region and was not expelled from the interface even for high surface pressures. It is therefore demonstrated Chi interacts with lipids from membranes via mechanisms involving hydrophobic, dipole and mainly electrostatic interactions.

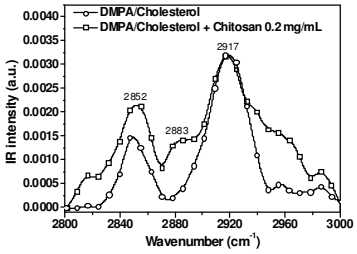

Figure 1. PM-IRRAS spectra for mixed 1:1 DMPA:Cholesterol Langmuir film on a pure acetate buffer (○) and onto 0.2 mg/mL Chi solution in the same buffer (□)

Keywords: Chitosan, Langmuir, Phospholipids, Cholesterol and Membrane models.
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