Using Infrared Spectroscopy to Investigate the Removal of beta-Lactoglobulin from Membrane Models by Chitosan

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Langmuir and Langmuir-Blodgett (LB) films made from lipids have been currently used to mimic biological interfaces, specially cell membranes and lipid droplets. The Langmuir technique allows easy control of several parameters such as composition, surface elasticity, and lateral pressure, and has been used to investigate interaction among biomolecules at the molecular level, which include lipids, proteins, drugs, nucleic acids and polysaccharides. Among the polysaccharides, we highlight chitosan, a natural cationic polymer that may be applied in several areas from nanobiotecnhology to medicine, among which as a fat-reducer agent for food industry. Many of chitosan biological activities depend on the interaction with biomembranes, but up to now it has not been possible to obtain molecular-level evidence of chitosan action. In this present study, we employ Langmuir lipid monolayers as biointerface models and show that chitosan is able to remove β-lactoglobulin (BLG) from the negatively charged dimyristoyl phosphatidic acid (DMPA) and dipalmitoyl phosphatidyl glycerol (DPPG). In previous works, we proved that chitosan can interact with phospholipids Langmuir monolayers [1]. Here, we proved the ability of chitosan to remove BLG from membrane through surface pressure isotherms, surface elasticity, and polarization-modulated infrared reflection-absorption spectroscopy (PM-IRRAS) measurements in the Langmuir monolayers, in addition to quartz crystal microbalance and fluorescence spectroscopy measurements for Langmuir-Blodgett (LB) films transferred onto solid substrates. Some specificity was noted in the removal action since chitosan was unable to remove BLG incorporated into neutral dipalmitoyl phosphatidyl choline (DPPC) and cholesterol monolayers, and had no effect on horseradish peroxidase and urease interacting with DMPA. Use of PM-IRRAS could detect the amide groups from BLG at the first step, when the protein was incorporated to the lipid monolayer. In the second step, chitosan was adsorbed at the mixed BLG-lipid monolayer and removed the protein from the air-water interface. This could be proved by PM-IRRAS observing the disappearing of amide groups. These findings may imply relevant biological implications to explain why chitosan can remove BLG from lipophilic environments, as reported in the recent literature [2].

Keywords: Biological Membranes, Langmuir-Blodgett films, Proteins, Langmuir Films

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