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Studying the interaction of chitosans with different degrees of acetylation with lipid monolayers at physiological pH

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Various events take place in a biological membrane, and different techniques can be used to study them. For instance, Langmuir monolayers is a membrane mimetic system that resembles half of a membrane – taken as a lipid bilayer. They are especially interesting for obtaining information at the molecular level, and are very useful to investigate interactions between membrane lipids and other biomolecules. (1) One of these molecules is chitosan, a natural polysaccharide obtained by the partial deacetylation of chitin. It displays many interesting properties, such as antimicrobial, anti-tumor, wound healing and fat binder. Because of its poor solubility in non-acidic pH, there is an effort to synthesize chitosans that keep their biological properties and are soluble at a broader pH range. In this work, we use two chitosans with different degrees of acetylation (35% and 15%, named Ch35% and Ch15%, respectively) that have a quasi-ideal random pattern of acetylation, full water solubility and unusually high weight average molecular weight, synthesized by Fiamingo *et al.* (2) Lipid monolayers with different compositions are used as bacteria and mammal membrane models, in order to investigate their interaction with these chitosans, at pHs 7.4 –more biologically relevant– and 4.5 –in the range of most of the scientific work. The lipids chosen were the zwitterionic dipalmitoyl phosphatidyl choline (DPPC) and dipalmitoyl phosphatidyl ethalonamine (DPPE); the anionic dipalmitoyl phosphatidyl glycerol (DPPG); the Escherichia coli total lipid extract and the lipopolysaccharide (LPS) from *E. coli* J5 (Rc strains). Ch35% or Ch15% were added to the subphases at concentrations of 0, 10^{-5}, 10^{-3} or 10^{-1} mg mL^{-1}. The later was only used in monolayers containing *E. coli* extract or LPS. In a published work (3), we already described the interaction of Ch35% with DPPC, DPP, DPPG and the *E. coli* extract. The presence of chitosan in the subphase, caused an expansion of the films, and, generally, increased their rigidity. Unexpectedly, the interaction was weaker for the negatively charged DPPG and *E. coli* extract, when compared to the neutral DPPC and DPPE. That was related to the low capacity of protonation of this chitosan, considering its relatively high degree of acetylation. Now, as expected, we verified that Ch15% affected the monolayers more strongly, which we attribute to its lower degree of acetylation. Also, monolayers containing LPS were highly affected, which may indicate that the antibacterial action of chitosan is more related to its interaction with the outer membrane, rather than with the inner. Unlike neat phospholipids, these monolayers became more rigid in the presence of chitosan. This also happened with the *E. coli* extract, which, as LPS, is highly heterogeneous, so their molecules are harder to pack. Thus, chitosans appear to facilitate their packing, probably by decreasing charge repulsion. Finally, chitosan properties proved to be determinant to their interaction with lipid monolayers, and will be important to each of its applications.

Palavras-chave: Langmuir monolayers. Chitosan. Bacterial and mammal membranes.

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