INFLUENCE FROM THE MOLECULAR WEIGHT OF CHITOSAN ON ITS INTERACTIONS WITH CELL MEMBRANE MODELS

A. PAVINATTO[1], J. A. DE M. DELEZUK[2], F. J. PAVINATTO[1], S. P. CAMPANA-FILHO[2], O. N. OLIVEIRA JR[1].

[1] Universidade de São Paulo, Instituto de Física de São Carlos, São Carlos – SP. e-mail: adriana@ursa.ifsc.usp.br
[2] Universidade de São Paulo, Instituto de Química de São Carlos, São Carlos - SP

Chitosans own most of their applications in the biomedical and pharmaceutical areas due to their biocompatibility and biodegradability [1]. Of particular importance are the interactions with cell membranes, probably via electrostatic forces between the positively charged amine groups from chitosan and the negatively charged phospholipids of the membrane [2]. The bactericide action of chitosan has also been found to apply to low-molecular weight compounds with the repeating units of chitosan [3].

In this study we evaluate the influence from the molecular weight of chitosan upon the interaction with cell membrane models made with phospholipid Langmuir monolayers. This was performed by using the glucopyranose units present (or found) in the chitosan chains, namely 2-acetamido-2-deoxy-D-glucopiranose (AcG ou GlcNAc) and 2-amino-2-deoxy-D-glucopiranose (G ou GlcN), which were acquired from Sigma and used without further purifications. The low molecular weight chitosan sample (CLMW) used in this study was obtained by ultrasound-assisted depolymerization.

The Langmuir films were prepared from the phospholipid dimyristoyl phosphatidic acid (DMPA). The AcG and G units were dissolved in the subphase in two molar ratios: i) 0.95G + 0.5AcG and ii) 0.73G + 0.27AcG. Films of DMPA were also studied over a subphase containing CLMW in different concentrations, ranging from 0.05 mg/ml to 0.30 mg/mL. The Langmuir films were produced in a Langmuir trough KSV 5000 placed in clean room class 10,000 at a temperature of 20º C ± 1. The spreading DMPA solution in chloroform had a concentration of 0.5 mg/mL, of which 150 µL were spread on a buffered subphase (Theorell-Stenhagen, pH 3.0) containing the AcG and G units and CLMW. The trough is equipped with a surface pressure sensor (Wilhelmy) and a Kelvin probe to measure surface potential. The data could be compared to results for high molecular weight chitosans with degree of acetylation of 27% and 5%, in a way that the direct influence from the molecular weight could be assessed.

The surface pressure isotherms for the Langmuir films with DMPA + AcG/G units (0.95G/ 0.5AcG) show practically no effect from the AcG/G units (Fig.1), as if no AcG and G units adsorbed on the DMPA film, in sharp contrast to the results for high molecular weight chitosan.

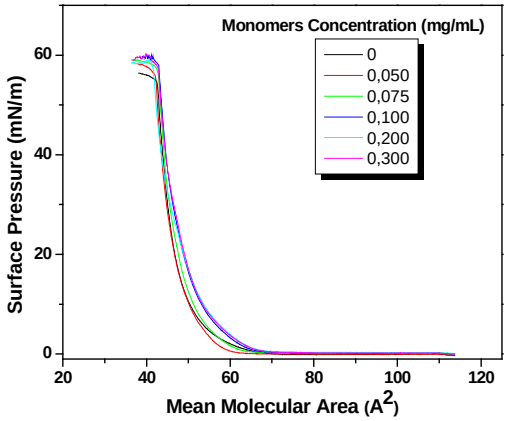

Fig. 1. Surface pressure isotherms for DMPA films spread on subphases containing 0.95G/0.5 AcG.

In spite of this behavior, the AcG/G units have a strong contribution to the surface potential measurements, probably due to the higher charge density in the subsurface. The same result is observed when the subphase contains a solution of 0.73G/0.27AcG. From the comparison of these results with those obtained with chitosan it is concluded that, in addition to the proportion of G and AcG units, the molecular weight and other characteristics of long chain chitosans (conformation) are also important for the interaction.

ACKNOWLEDGMENTS

This work was supported by FAPESP, CNPq, CAPES and INEO

REFERENCES

1. RINAUDO, M., Chitin and chitosan: Properties and applications**,** *Progress in Polymer Science*, 31, 603 (2006).
2. PAVINATTO, F.J., Interaction of chitosan with cell membrane models ate the air-water interface*, Biomacromolecules,* 8, 1633-1640, (2007).
3. JEON, Y-J, Antimicrobial effect of chitooligosaccharides produced by bioreactor*, Carbohydrate Polymers*, 44, 71-76, (2001).