Irradiation effect on Methylene Blue-containing DOPC:CL different biomembrane models

Schmidt, T. F.[1,3*], Wilke, N.[2], Maggio, B.[2], Marques, C.[3], Itri, R.[4]
[1] *Universidade Federal do ABC, Santo André, SP, Brasil.*
[2] *Universidad Nacional de Cordoba, Cordoba, Argentina.*
[3] *Institut Charles Sadron, Université de Strasbourg, CNRS, Strasbourg, France.*
[4] *Instituto de Física, Universidade de São Paulo, São Paulo, SP, Brasil.*
*e-mail address: thais0010@gmail.com

It is well known that Reactive Oxygen Species (ROS) [1-2] lead to lipid peroxidation in cell membranes [3-4], and in particular to hydroperoxidized lipids (LOOH) [5-7], but the mechanisms leading to expansion or condensation of the lipid bilayers have not yet been determined. ROS are also important components of Photodynamic Therapy (PDT), a clinical method that relies on the administration of a sensitizer molecule to generate the oxidative species singlet oxygen. In this work the photosensitizer Methylene Blue (MB) was incorporated into the subphase of pure and mixed monolayers of dioleyl-phosphatydil-choline (DOPC) and Cardiolipin (1,1',2,2'-tetramyristoyl cardiolipin-sodium salt, CL), to analyze the conformations of the photosensitizer-lipid systems before and after irradiation in a Langmuir trough. In addition, the irradiation effect was also analyzed in lipid bilayer systems by studying mixed DOPC:CL giant unilamellar vesicles(GUVs) prepared by the gel-growth method [8]. For the case of monolayers, molecular expansion following irradiation showed lipid peroxidation by formation of hydroperoxide groups with the peroxide group located at air-water interface. Expansion was observed in pure CL monolayers and in DOPC: CL 1:1 mixtures. Monolayer compaction after irradiation was observed for pure DOPC monolayers and for 1:2 and 2:1 DOPC:CL mixtures, showing the formation of more hydrophilic smaller molecules, the nonanoic acids, which can leave the monolayer to the bulk subphase. In the case of giant vesicles, the beginning of the process shows increased area of the membrane accompanied by fluctuations. At the end of the irradiation process, oxidation leads to the opening of large, micrometer size pores, followed by loss of contrast. The kinetics of oxidation is faster for vesicles with higher Cardiolipin content, promoted by the attraction electrostatic interactions between the anionic CL lipid and the cationic MB photosensitizer.

Acknowledgements: FAPESP process nº2008/07240-9 and Capes process nº7119127.

References

[1] Sabatini, K., Mattila, J-P, Megli, F.M., Kinnunen, P.K.J., *Biophysical Journal* **90**, 4488-4499 (2006).
[2] Caetano, W., Haddad, P.S., Itri, R., Severino, D., Vieira, V.C., Baptista, M.S., Schröder, A.P., Marques, C.M., *Langmuir* **23**, 1307-1314 (2007).
[3] Riske, K. A., Sudbrack, T. P., Archilha, N. L., Uchoa, A. F., Schroder, A. P., Marques, C. M., Baptista, M. S., Itri, R., *Biophysical Journal* **97**, 1362-1370 (2009).
[4] Haluska, C. K., Baptista, M. S., Fernandes, A. U., Schroder, A. P., Marques, C. M., Itri, R., *Biochimica et Biophysica Acta. Biomembranes* **1818**, 666-672 (2011).
[5] Beranova, L., Cwiklik, L., Jurkiewicz, P., Hof, M., Jungwirth, P., *Langmuir Lett*. **26**(9), 6140-6144 (2010).
[6] Khandhelia, H., Mouritsen, O., G., *Biophysical Journal* **96**, 2734-2743 (2009).
[7] Markovic, D., *J. Serb. Chem. Soc.* **71**(4), 349-356 (2006).
[8] Horger, K.S., Estes, D.J., Capone, R., Mayer, M., *Journal of American Chemical Society* **131**, 1810-1819 (2009).