Na,K-ATPase-Liposomes: lipid and lipid-protein microdomains imaged by Atomic Force Microscopy.

Sebinelli, H.G., Bolean. M., Borin, I.A., Ramos, A.P. and Ciancaglini, P.
Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (FFCLRP), Universidade de São Paulo (USP), Ribeirão Preto, Brasil.

Introduction: Na,K-ATPase or sodium-potassium pump is an integral plasma membrane protein found in almost all animal cells. Proteoliposomes samples were prepared in different lipid compositions to observe the NKA microdomains in the mimetic membranes interface. AFM tapping mode technique provided complementary phase and topographical images.

Objective: To use AFM tapping mode technique to image NKA in proteoliposomes surface.

Material and Methods: Na,K-ATPase was solubilized with C12E8 detergent. After purification, the enzyme was reconstituted in lipid bilayers by co-solubization with 10mg.mL^{-1} in the compositions of DPPC and DPPC-DPPE. To fix and to avoid shape changes in the liposomes and proteoliposomes, 12% glutaraldehyde was used.

Results and Discussion: The analysis of phase images from both liposomes compositions DPPC and DPPC:DPPE (molar ratio1:1) showed no phase contrast and a smooth surface. In the topographical images, DPPC liposomes presented predominant spherical shapes. In liposomes composed of DPPC:DPPE, small points were formed in most of the vesicles. Changes in the surface were imaged in DPPC-NKA, where darker areas suggested different chemical compositions that can be associated with the presence of the protein. Phase and topographical images showed that these darker areas matched the protrusions areas in the lipid surface.

DPPC:DPPE-NKA imaged different protrusions clusters, they were aggregated and well-defined structures in both image modes, phase and topographical. In the phase mode, the areas with distinct mechanical properties appeared only as thin boarders between the protrusions, showed as darker interstices.

Conclusions: AFM tapping mode provided high resolution images where areas of different chemical compositions in phase mode images were correlated with protrusions in topographical images. The different chemical compositions have specific mechanical properties that indicate the NKA presence in the proteoliposome membrane in all lipid compositions. It´s known that membrane proteins interact with specific lipids that lead to the formation of the lipid-protein microdomains.

Keywords: AFM tapping mode, Liposomes, Na,K-ATPase

Financial supports: CNPq, CAPES and FAPESP