Effects of Annexin V in Mimetic Systems of Matrix Vesicles: Study by Advanced Fluorescence Techniques

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Introduction: Biomineralization process is initiated in matrix vesicles (MVs). Annexin V (AnxA5), present in MVs membranes, mediates Ca^{2+} influx in them by calcium channels. The phospholipids dipalmitoylphosphatidylcholine (DPPC) and –serine (DPPS) are present in MVs membranes and AnxA5 function is dependent of its lipid environment and concentration of Ca^{2+}. **Objective:** Study the AnxA5 interaction with model membranes of MVs influenced by the presence of Ca^{2+}. **Material and Methods:** Proteoliposomes formed by large unilamellar vesicles (extruded with 200 nm) of pure DPPC or DPPC/DPPS 9:1 mol%, and 10-20 µg/mL of AnxA5 were used. The interaction of AnxA5 with the bilayer was studied using fluorescence methods as fluorescence anisotropy, Förster resonance energy transfer (FRET) and fluorescence lifetime imaging microscopy (FLIM). The probes NBD-lipids were used, with the dye in the polar head (NBD-PE), in the sixth and twelfth carbon of one acyl chain (C6 and C12-NBD-PC). To determine conformational changes in the protein interaction with membranes, the intrinsic tryptophan residue (Trp) of AnxA5 was used. For FRET experiments the probe Rhodamine-lipid (Rh-PE) was used as an energy acceptor for the NBD-lipids donors and DPH for the Trp donor. **Results and Discussion**: The probe NBD in lipid environment has two fluorescence lifetimes, one in the order of 3 and other 10 to 12 ns, and the longer lifetime is more affected by presence of AnxA5. FLIM images show the size of proteoliposomes in comparison to liposomes, as obtained by dynamic light scattering, and the lower fluorescence lifetime values in the presence of the acceptor. Changes in fluorescence in the presence of AnxA5 indicate an interaction of the protein with the membrane depending on where the dyes are located. **Conclusion:** The changes in lifetimes and in FRET efficiency in presence of AnxA5 suggest that there is change in lipid-lipid interaction promoted by the protein.

Keywords: Annexin V, Fluorescence, Proteoliposomes

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