Effect of different sterols on the ATPase activity of TNAP responsible for the propagation of the biomineralization

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Introduction: Tissue-nonspecific alkaline phosphatase (TNAP) is a GPI enzyme present in matriz vesicles that hydrolyse ATP or PP_i to generate phosphate during biomineralization. **Objective:** to evaluate the incorporation of TNAP into liposome compounds DPPC, DOPC and different sterols (cholesterol (Chol); cholestenone (AChol) and Ergosterol (Ergo)), and compare the kinetic properties of TNAP for the ATP and PP_i substrates, while embedded in these different lipid environments. **Materials and methods:** TNAP was liposome-incorporated, as previously described by Bolean et al. (2010) and kinetic parameters for the hydrolysis of ATP were determined. **Results and Discussion:** By comparing catalytic efficiencies TNAP by hydrolysis of ATP incorporated into liposomes composed of DPPC and DPPC:Chol36%, revealed that the presence of cholesterol increases by approximately 4x the catalytic efficiency values (570-1800$M^{-1}s^{-1}$). The same behavior can be observed in the presence of other sterols such as DPPC:Achol36% and DPPC:Ergo36% (2700-1000$M^{-1}s^{-1}$, respectively). We emphasize that the presence of cholesterol led to an increase in the spread of the mineral of around 3.4x. There was an increase of approximately 10x in catalytic efficiency values of ATP hydrolysis by TNAP when compared with DOPC vesicles and DOPC:CHOL36% (120-1000$M^{-1}s^{-1}$). The same behavior can be observed in the presence of other sterols such as DOPC:Achol36% and DOPC:Ergo36% (8x and 10x, respectively).
For DOPC-Proteoliposomes, the catalytic efficiency found for TNAP was about 4 times lower compared to DPPC. But if one correlates mineralization, it can be noted that these proteoliposomes composed by DOPC result in 2.8x higher ability. In vesicles with unsaturated lipids containing sterols, mineralization spreading was more effective compared with proteoliposomes containing only DPPC. **Conclusion:** Considering the kinetics and mineralization results shown it is possible to suggest that a crystalline liquid phase (lipid+cholesterol or cholestenone) could be more effective in spreading the mineral.

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