Cholesterol Effect in the Reconstitution of Alkaline Phosphatase in DPPC-Liposomes: Correlation of Protein Incorporation and Thermodynamic Parameters.

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Alkaline phosphatase (TNAP), which is located in matrix vesicles originated in osteogenic cells, plays a key role in calcifying bone and cartilage. Studies revealed that lipid composition of matrix vesicles might be controlled by the presence of cholesterol. In this work, we studied the influence of cholesterol in the reconstitution of p-NPPase activity of TNAP in DPPC-liposomes, using differential scanning calorimetry (DSC). DPPC:cholesterol liposomes (9:0-9:5 molar ratio) were prepared by extrusion (~100 nm diameter), to a final concentration of 10 mg/mL. DPPC-liposomes revealed a critical transition temperature (T_c) of 41.5°C, enthalpy (?H) and entropy (?S) variation of 6.8019 Kcal.mol^{-1} and 0.0216 Kcal.K^{-1}.mol^{-1}, respectively. The gradual increase in cholesterol decreased ?H and ?S values (5.9354 Kcal.mol^{-1} and 0.0189 Kcal.K^{-1}.mol^{-1}, respectively), destabilizing the liposomes. A slight increase in the T_c was observed, since it reaches 47^{o}C for 9:5 DPPC:cholesterol, resulted by the increase of the area per molecule in gel state. TNAP (179.5 µg/mL) reconstitution was done with 1:10000 protein:lipid molar ratio (1 h incubation time, 25ºC), resulting in 75% of the p-NPPase activity of the enzyme being incorporated. The presence of cholesterol makes the insertion of the TNAP into liposomes difficult, reducing its incorporation in about 30% at DPPC:cholesterol 9:5. DSC analyses of the proteoliposomes show that the presence of the enzyme also destabilizes the systems, as seen by the reduction in ?H and ?S values, but did not significantly alter the T_c. Thus, these systems can be efficiently used to understand the role of lipids and TNAP during the biomineralization process.
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Key words: Alkaline phosphatase, liposome, DSC.