Cholesterol regulates phosphatase alkaline incorporation on DPPC Langmuir monolayers.

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Introduction: Matrix vesicles (MVs) are extracellular vesicles site-responsible for the initial mineral deposition in the biomineralization process. MVs are significantly enriched with tissue-nonspecific alkaline phosphatase (TNAP). The enzyme adsorption and catalytic activity of TNAP may be regulated by cholesterol (CHOL).
Objectives: Study the CHOL effect on the TNAP incorporation into cell membrane models using the Langmuir monolayer technique.
Materials and methods: Surface pressure-surface area (π-A) isotherms of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) or binary monolayers containing DPPC:CHOL were recorded at 25.0ºC in a 216cm^2 Langmuir trough (Insight-Brazil) using pure MilliQ® water or 5 mmol.L^{-1} Tris-HCl buffer with 150 mmol L^{-1} NaCl and 15 mmol L^{-1} $MgCl_2$ (pH8.5) as subphase in the presence and absence of TNAP.
Results and discussion: The compressional modulus (Cs^{-1}) values of DPPC monolayers at 30 mN m^{-1} were decreased (the fluidity of the monolayers was increased) when 20mol% of CHOL was added to the monolayer, whereas the Cs^{-1} values increased (reduced fluidity) when 50mol% of CHOL was added into the monolayer. The excess Gibbs free energy of the mixture at 30 mN m^{-1} showed a positive value at 20mol% of CHOL, meaning non-favorable DPPC-CHOL interactions, and a negative value at 50mol% of CHOL, evidencing favorable interactions. These results follow the trends in the Cs^{-1} changes. In the presence of TNAP, an area expansion was observed when CHOL was added to the DPPC monolayer. At DPPC:CHOL(4:1) molar ratio the TNAP incorporation was higher than for the 1:1 mixture, which is also in agreement with the Cs^{-1} data.
Conclusions: At small amounts of CHOL the interactions between DPPC and CHOL are non favorable and the lipid monolayer is fluidified, favoring TNAP incorporation. On the other hand, at high CHOL amounts the monolayer is more rigid and the TNAP incorporation is diminished. These results evidence the possibility of regulating the enzyme incorporation into cell membrane models by controlling the CHOL concentration.

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Keywords
Cholesterol, alkaline phosphatase, Langmuir monolayer