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BBM N°4

Effect of natural terpenes on Bovine erythrocyte acetylcholinesterase (BEA) activity from bovine erythrocyte ghost membranes (BEM). Possible unspecific mechanism that tunes the BEA catalytic activity.

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BEA is a GPI-anchored enzyme that hydrolyzessericacetylcholine. The 'anionic' subsite in the active site determines the specificity with respect to the choline moiety through electrostatic interactions. Since a) changes on the molecular environment of GPI-anchored enzymes affect their kinetic parameters and b) monoterpenes (MT) affects biomembrane order and electrostatics according to their dipole moment modulus and orientation, here we tested the effects of MTs (1-8 cineol, CIN and camphor, CAM) on the hydrolysis of acethylthiocholine (ATC, Ellman's method)catalyzed by BEA present in BEM. The affinity of the BEA-ATC complex in the absence of MTs (K_M=0.1) was significantly affected by CIN which resulted a stronger inhibitor (K_M= 0.81) than CAM (K_M=0.11) (both at 0.3mM). Moreover, CIN exhibited an IC_{50}=0.3mM whereas the IC_{50} of CAM was >> 0.6 mM.Measurements of the fluorescence anisotropy (A) of DPH and TMA-DPH in BEM, demonstrated that both MTs affected the organization of the inner regions of the bilayer (both MTs reduced about a 10% the A_{DPH}) but not the polar head group region ($A_{TMA-DPH}$ was almost unaffected). The effect of MTs on the lateral pressure (π) and surface potential (DV) vs Area compression isotherms in Langmuir films were also studied. In the presence of CIN, the transition found in the control π-Aisotherm become less cooperative and the $\pi_{collapse}$ decreased. At low π, the slopes of both isotherms (π-A and DV-A) changed; e.g.we found a DDV~20mV with respect to the control without CIN. At high π,CIN and control isotherms convergedsuggesting the CINmolecules expulsion from the film upon compression. CAM did not produce significant effects on DV, but expanded slightly the whole π-A isotherm up to the collapse point. Concluding, the inhibitory activity of CIN on BEA may be related with its effect on the membrane order and electrostatics which may be interfering unspecificallywith the BEA-ATC electrostatic interaction at the active site.

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