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A NEW LANGMUIR-SCHAEFER-BASED METHOD DEVELOPED FOR CATALYTIC STUDIES OF ACETYLCHOLINESTERASE IN PLANAR FILMS OF ERYTHROCYTE MEMBRANES

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Previously we reported that the catalytic activity of bovine erythrocyte acetylcholinesterase (BEA) located in Langmuir-Blodgett films (LB) of bovine erythrocyte membranes (BEM), LBBEA, depended on the curvature and packing of the molecular environment. Moreover, the specific activity of LBBEA was much lower than that of BEA in suspensions of BEM vesicles (SBEA). So, the present work was aimed at maximizing the specific activity of BEA recovered from the transfer of a Langmuir film (LF) from the air-aqueous interface to alkylated solid surfaces and improving the precision of the enzymatic assays. Three main changes were introduced to the previously assayed method. a) Phosphate saline buffer (PBS), pH 7.4 was used instead of H2O as the subphase over which was spread the BEM to form the LF, assuming that this composition, closer to physiological conditions, would be more effective than water in preserving the BEA protein structure/activity and the LF organization. b) BEA in LF films (LFBEA) was transferred from air-PBS interface to hydrophobic flat surfaces by the Langmuir-Schaefer technique (LS) to obtain LSBEA samples. c) A new device was designed to allow performing the whole enzymatic activity assay using a unique LS film as well as the reading of the absorbance values in the same container. The LF of BEM at the air-PBS interface, compared with LF formed over H2O, showed surface pressure vs area (π-A) isotherms more expanded at low π, more compressible, with a bi-dimensional transition at lower π and lower minimal A. The surface potential reached 250 mV at the collapse point in both conditions (H2O and PBS). The specific activity resulted SBEA>>LSBEA>LBBEA. The use of PBS in the subphase and the transfer of LF at π=35mN/m instead of 10 mN/m improved the recovery of specific activity in LSBEA and LBBEA. The homogeneity of BEA distribution in LSBEA samples highly improved the precision of the kinetic parameters determined in different molecular packing conditions.

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