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ENZ3_Surfactant type influence on the catalytic activity of soybean lipoxygenase

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The catalytic activity of enzymes is influenced by many physical and chemical factors. Model systems are used to study this activity based on the properties of amphiphilic molecules to self-organize in solution, such as micellar systems [1]. Lipoxygenases (LOX) are dioxygenases which catalyze the initial reaction of oxidation of polyunsaturated fatty acids, containing a 1,4-cis,cis-pentadiene system yielding primary products with conjugated dienehydroperoxides [2]. In food, these enzymes play an important role in oxidative processes, therefore several technological processes have been developed for the purpose of eliminating or reducing its activity [3-4]. The objectives of this work were to study the influence of different type of surfactants on the soybean LOX catalytic activity and to determine the kinetic parameters associated with the oxidation reaction. The results of this work indicated that the LOX activity depends on the nature of the surfactant and its concentration. Activity values obtained for Brij 35 and Tween 20 micelles were superior to those of SDS and AOT. Maximum activity values were obtained at 2.5; 0.8; 3; and 1 mM of SDS, Brij 35, Tween 20 and AOT, respectively. The non-ionic surfactants promote the activity, whereas ionic surfactants decrease or totally inhibit this activity.

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ENZ4_β-galactosidase activity againts different substrates and in the presence of lipid interfaces

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Previously we demonstrated that the activity of a soluble wild-type *E. coli* β-galactosidase (β-Gal_{wt}) against both lactose (the natural substrate) as ortho-nitrofenilgalactopiranósido (ONPG, artificial substrate) increases in the presence of multilamellar vesicles (MLVs) composed of neutral and charged phospholipids. The aim of this study was to compare the activity of a recombinant β-Gal (β-Gal_{His6}) against two different substrates in the presence of MLVs of different lipid composition. β-Gal_{-His6} was overexpressed in *E.coli,* and the six histidine residues (His-tag) fused to the carboxyl terminus facilitated purification by ion metal affinity chromatography (IMAC). The enzyme activity was measured by visible spectrophotometry, in the absence or presence of MLVs of pure egg phosphatidylcholine (EPC interface) or at 80:20 molar ratio with dioleoylphosphatidyl glycerol ($EPC_{80}/DOPG_{20}$) negative zwitterionic interface). Kinetic parameters were determined by fitting the michaelian model to the experimental data using nonlinear regression. Our results showed that the enzyme activity was more efficient against ONPG compared to lactose ($kcat/K_{MONPG} > kcat/K_{MLactosa}$) as previously described for other beta galactosidase. An activation of the enzymatic activity but a decrease in the substrate affinity were observed in the presence of lipid interfaces against both substrates. Those effects were enhanced by the presence of charged interfaces favored by electrostatic interactions mediated by the presence His residues of the enzyme. The nature of the substrate not qualitatively affected the kinetics of the reaction catalyzed by β-Gal_{-His6} in the presence of lipid interfaces.

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