Structural and Biochemical Studies of Hypothetical Lipase CT-43 Based on Candida antarctica Lipase B (CalB) Sequence

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Introduction: Lipases are enzymes that hydrolyze triglycerides at a lipid-water interface. Study of these biocatalysts is important due to their high stability and specificity in diverse conditions, being relevant to industries. In biodiesel ones, lipase B from Candida antarctica (CalB) stands out, although its use in large scale is still expensive. Looking for new lipases with important properties to bioprocesses, we used the primary sequence of CalB as a model and selected the CT-43 hypothetical lipase from Bacillus thuringiensis to study its structural and biochemical features.

Material and Methods: CT-43 was cloned into pET28a and pETM30-GST vectors. Expression tests were performed using different strains of E. coli, IPTG concentration and temperatures. Due to the low solubility of CT-43 (pET28a), the addition of urea was required to cell lysis and purification by affinity chromatography. Thereafter, refolding was done using different protocols. CT-43 (pETM30-GST) was expressed in a soluble fraction and its purification was performed with the same protocol in absence of urea. Biochemical and structural analysis was monitored by enzymatic activity and fluorescence. Results and Discussion: Best expression results were achieved using BL21(DE3) strains at 37°C and 18°C for CT-43 (pET28a) and (pETM30-GST), respectively. Both constructions presented enzymatic activity, indicating successful refolding for CT-43 (pET28a), although more tests will be necessary to surely insert this protein in lipase family. Structural studies of CT-43 showed a fluorescence spectrum characteristic of denatured proteins to both constructions. Conclusions: Preliminary results indicate that CT-43 is possibly a lipase and its structural behavior by fluorescence indicates that this enzyme has tryptophans exposed to the solvent. Circular dichroism experiments will be performed with different denatured agents and temperatures to obtain informations about the stability of this protein. The improvement of purification will be necessary to increase final concentration of this enzyme to lead off studies by NMR.

Keywords: Biocatalysts, Lipases, Structural studies

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