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CHEMICAL MODIFICATION OF LIPASE B OF *CANDIDA ANTARCTICA* IN SOLID PHASE FOR IMPROVING BIOCHEMICAL PROPERTIES

Oveimar Barbosa[1], Nazzoly Rueda[1], Rodrigo Torres[1], Claudia Ortiz[2], and R. Fernandez-Lafuente[3*]

1 Universidad Industrial de Santander, Escuela de Química; grupo de investigación en Bioquímica y Microbiología, Bucaramanga, Colombia; 2 Universidad Industrial de Santander, Escuela de Bacteriología, grupo de investigación en Bioquímica y Microbiología, Bucaramanga, Colombia; 3 Instituto de Catálisis-CSIC, Campus UAM-CSIC, Cantoblanco, Madrid, Spain.

* rfl@icp.csic.es

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INTRODUCTION

Chemical modification of enzymes is a strategy widely used in biocatalysis for design of biocatalysts with improved biochemical properties. when the chemical modification is performed on immobilized enzymes, we can protect the enzyme from side-effects that are normally produced with modification of the soluble form of the enzymes[1]. In this work, we modified CALB through two different methodologies, and the effect of these modification on the activity, stability and specificity of the enzyme was studied[2].

RESULTS AND DISCUSSION

The effect of chemical modification on the enzyme activity of CALB immobilized on octyl-agarose (OC) and Cyanogen Bromide-agarose (BrCN) were evaluated. In **Table 1** are shown changes in enzyme activity of CALB by chemical modification produced by modification (100%) with EDA and TNBS.

Table 1. Changes in relative activity of CALB (using as substrate p-NPB) produced by chemical modification of the different immobilized biocatalysts of CALB. 100% of enzyme activity corresponds to activity of non-modified OC-CALB.

Type of Agarose Support	CALB	CALB-EDA	CALB-TNBS	CALB-TNBS-EDA	CALB-TNBS-EDA-TNBS
OC	100	140±3	83±4	115±5	70±2
BrCN	100	49±2	63±3	59±2	58±1

In Table 2 are shown enzyme activity and enantio-selectivity of the different derivatives on (R/S)-Methyl mandelate. Modification of lipase caused different activity and enatioselectivity results, demonstrating that both activity and selectivity of lipase are affected depending on the type of enzyme modification

Table 2. Activity and enantioselectivity of modified OC-CALB derivatives.

	Reaction Condition					
	pH 5		pH 7		pH 9	
Type of Biocatalyst	Activity[a]	E[b]	Activity[a]	E[b]	Activity[a]	E[b]
CALB	44.8±1.0	11.2±0.2	48.4±1	25.5±0.2	11.6±0.5	8.9±0.1
CALB-EDA	22.6±0.5	7.3±0.1	30.6±1.5	35.6±0.2	9.1±0.5	11.4±0.1
CALB-TNBS-	71.3±1.0	16.2±0.2	33.3±1	22.2±0.2	10.9±0.5	15.2±0.1
CALB-TNBS-EDA	25.2±1.0	7.6±0.1	29.8±1	27±0.3	11.2±0.5	12.4±0.1
CALB-TNBS-EDA-TNBS	25.8±0.5	6.1±0.1	27.5±0.5	27.5±0.3	11±0.5	12.2±0.1

[a] **µmoles/g biocatalyst/min using 10 mM (R/S)-Methyl mandelate as substrate**

[b] **Enantiomeric (E) =kcat R/kcat S.**

CONCLUSION

This work exemplifies as chemical modification on solid phase allows modulating biochemical properties of lipases, affecting activity, stability and enantioselectivity of these enzymes.

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