The evaluation of the stability of the lipase of *Fusarium verticillioides* immobilized on inert supports

Janaina P. Borges[1](PG), Daniela A. B. Martins[1](PQ), Eleni Gomes[2](PQ), Mauricio Boscolo[2](PQ), Roberto da Silva[2](PQ)*, José M. Guisan[3](PQ).

[1]IQ/UNESP, Department of Biochemistry and Chemical Technology – Rua Prof. Francisco Degni, 55 – CEP: 14800-060, Araraquara/SP, Brazil.

[2]IBILCE/UNESP, Department of Chemistry and Environmental Sciences – Rua Cristóvão Colombo, 2265 – CEP: 15054-000, São José do Rio Preto/SP, Brazil.

[3]Departament of Catalysis, CSIC (Consejo Superior de Investigaciones Científicas), Campus UAM, 28049, Madrid, Spain.

Key Words:*Lipase, hydrolytic activity, immobilization.*

Introduction

Lipases are enzymes of a class of hydrolases that act on the ester bonds of glycerides of long chains. Normally they are activated by a water-oil interface. The immobilization and the use of organic solvents in the reaction medium have a major effect on the lipase structure. This may influence the catalytic capacity and modulate its selectivity. Furthermore, the effect of solvent on the activity and selectivity of this enzyme can vary between lipases from different sources. The objective of this work was to immobilize the lipase of *Fusarium verticillioides* in hydrophilic and hydrophobic inert carriers through adsorption and evaluate both the thermal stability and also its stability in 50% ethanol of the derivatives (Table 1) in relation to the free enzyme.

Results and Discussion

The enzyme was immobilized on hydrophobic and hydrophilic carriers. 10 g of each adsorbent was added to 100 mL enzyme solution (enzyme dissolved in 10 mM phosphate buffer, pH 7.0). The decreased amount of enzyme present in the supernatant was evaluated, and its incorporation to the carriers according to Rodrigues[1] et al (2009). Hydrophobic supports showed a better immobilization percentage, with the exception of C18 (Table 1). As most lipases have a "lid" at the active site with hydrophobic characteristics, it was expected that these carriers would present a higher percentage of lipase absorption. Support PEI had the highest recovered activity, 31 % of initial activity, but also other proteins were immobilized. The low value of the recovered activity may be due to the conformation of the immobilized enzyme to the support, leaving the active site less exposed to the reaction. After immobilization, the derivatives that showed recovered activity were submitted to evaluation for stability at the optimal temperature of the lipase (35°C) for 7 hours and compared to the free enzyme.

Table 1: Immobilization and desorption of the lipase on inert supports.

Carrier	Immobil. yield (%)	Immobilized protein (%)	Recov. activity (%)	Desorption
Hydrophilic				[NaCl] mM
Q-seph.	71,9	79,0	12,4	200
PEI	78,1	90,0	31,4	300
DEAE	60,1	92,6	16,8	100
Duolite	76,4	96,0	2,01	-----
CM-seph.	81,0	84,0	8,30	700
Hydrophobic				% triton X 100
Octyl	89,8	50,0	4,70	0,8
Fenyl	98,0	63,0	-----	0,8
C18	74,3	86,0	-----	0,4
Lew. 1600	93,2	88,0	-----	-----
Lew. 105	97,6	100	21,3	-----

The derivative of Lewatit 105 showed 100% activation after 3 hours of experiment, falling after 5 hours. However, when kept in ethanol the PEI derivative has a very distinct profile, with a hyper-activation of 150 % in its activity after 30 minutes of reaction, but its activity drops to 50 % at the end of 6 hours, showing the derivative to be the most unstable. The Octyl and Lewatit 105 derivatives remained stable throughout the experiment, with activity always above the initial showing a positive effect of ethanol on these hydrophobic derivatives.

Conclusions

The hydrophobic supports showed better yields of immobilization, but most of the carriers tested did not show recovered hydrolytic activity. This may be due to the reaction medium, since lipases are activated in organic media. The Lewatit 105 support was more stable in both stability tests, being ideal for use in hydrolytic reactions.

Thanks

The authors would like to thank CAPES for their financial support.

[1]Rodrigues R. C.; Godoy C. A.; Volpato G.; Ayub M. A. Z.; Lafuente R. F.; Guisan J. M. Process Biochem. **2009**, 44, 963.