Available online at www.sciencedirect.com
ScienceDirect
Journal of Biotechnology 128 (2007) 908–918
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

An approach to address *Candida rugosa* lipase regioselectivity in the acylation reactions of trytilated glucosides

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Received 11 April 2006; received in revised form 17 July 2006; accepted 17 August 2006

Abstract

Candida rugosa lipase crude preparations (CRL) catalyse the regioselective acylation of methyl 6-*O*-trytil β-D-glucopyranoside in organic solvents, using vinyl acetate as acyl donor. The ratio of the two products formed, namely methyl 2-*O* acetyl 6-*O*-trytil β-D-glucopyranoside and methyl 3-*O* acetyl 6-*O*-trytil β-D-glucopyranoside was found to be markedly affected by the nature of the reaction medium. In hydrophobic solvents values up to 80% of the monoacetylated product in position C-3 were obtained compared to less than 30% in solvents with low hydrophobicity. Computational studies were carried out to simulate the interactions between methyl 6-*O*-trytil β-D-glucopyranoside and both CRL and the solvents, in order to rationalize the experimental results.
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Keywords: Lipase; Organic solvents; Regioselectivity; Glucosides; Molecular modelling

1. Introduction

In the last years, the use of enzymes as biocatalysts in non conventional media has become one of the most important research fields within biotechnological applications (Wescott and Klibanov, 1994; Singh et al., 1994; Dordick, 1991; Zacks and Klibanov, 1984). It is well known that through a specific choice of the reaction medium (solvent engineering), it is possible to modulate, and in some cases reverse, substrate specificity, enantioselectivity, prochiral selectivity, regioselectivity and chemo selectivity of enzyme-catalysed reactions (Margolin and Klibanov, 1986; Margolin et al., 1991; Kirchner et al., 1985; Karandijand et al., 1986; Ooshima et al., 1985; Russel and Klibanov, 1988). While the use of solvent engineering to control enzyme enantioselectivity has been described in great detail, there are only few studies concerning the control of enzyme regioselectivity by changing the reaction

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doi:10.1016/j.jbiotec.2006.08.019

medium (Ryu et al., 1990; Tor et al., 1990; Burke et al., 1989; Dordick, 1989; Cernia et al., 1996; Mc Hugh and Krukonis, 1985; Williams, 1981; Mc Hugh, 1985).

Among the enzymes being more largely applied in industrial transformations, lipases find interesting applications in regioselective acylation of polyfunctional compounds. Among these, monosaccharides represent a very interesting class of molecules to be employed as new drugs. The chemical selective modification of them usually requires several protecting and deprotecting steps, since they have multiple hydroxyl groups of comparable chemical reactivity.

In general, lipases catalyse acylation reactions on glycosides with a preference for primary hydroxyl functions and regioselective acylation at O-6 may be achieved easily (Gotor and Pulido, 1991; Sharma and Chattopadhyay, 1993; Wong and Whitesides, 1994). However, the specificity of lipases in regard to secondary hydroxyl groups has also been examined and high degrees of regioselectivity were achieved using 6-deoxy sugars and glycosides protected at their primary positions (Ciuffreda et al., 1990; Nicotra et al., 1989; Iacazio and Roberts, 1993; MacManus and Vulfson, 1995).

In this framework, we focused our attention on the study of transesterification reactions catalysed by wild type microbial lipases by using monoprotected glycosides, as substrates, in reaction media with different hydrophobicity and the findings of one of these studies are reported in this paper.

A molecular modelling approach was applied to rationalize the experimental results. It took into account the possibilities that the observed effect could be rationalized either on the basis of different conformational restraints imposed on the substrate by the different solvent media or of a direct influence of solvent hydrophobicity on the regioselectivity of enzyme-substrate interactions. Even the possibility of a combination of both effects was taken into account.

2. Materials and methods

2.1. Chemicals

Lipase (EC 3.1.1.3) from *Candida rugosa* (CRL), was purchased from Sigma type VII (890 U/mg). Silica gel TLC plates were obtained from Merck. All other chemicals, silica gel (60 mesh), organic solvents, analytical or HPLC grade, used in this work were supplied by Aldrich Chemical.

2.2. Enzymatic activity assay

The lipolytic activity was assayed by alkalimetric final titration. The assay mixture, containing buffer (2.5 ml) (PBS, pH 7.4, 20 mM), tributyrin (0.5 ml) and enzyme solution (0.1 ml, 20 mg/ml) was incubated at 37 °C under magnetic stirring (300 rpm) for 30 min. The reaction mixture was stopped with acetone/ethanol mixture 1:1 (2.5 ml) and titrated with NaOH (0.05 M) in the presence of phenolphthalein as indicator using an automatic burette (Methrom).

2.3. Enzymatic acetylation in organic solvents

The reactions were performed by dissolving the trityl glycoside (1) in organic solvent in the presence of the acetylating agent, vinyl acetate (alcohol–ester 1:10 molar ratio) and incubating with CRL (15 mg) in a thermostatic bath with magnetic stirring (600 rpm) at 40 °C. The conversion was monitored qualitatively by TLC and quantitatively by HPLC by periodically analysis of 50 μl aliquots of the reaction mixtures dissolved in of acetonitrile (1 ml). Blank experiments (without enzyme) were also run.

2.4. Analytical methods

TLC was performed on Silica Gel 60 precoated on aluminium sheets (Merck) using $CHCl_3$–MeOH mixtures of appropriate polarity as eluent. Products were visualised using the aqueous solution of H_2SO_4 (4%, v/v) followed by heating at 150 °C for 5 min.

Quantitative analysis was performed by HPLC on a C-18 reversed phase column using acetonitrile/water 70/30 as eluent, flow 1 ml/min. An ELSD detector (evaporative, light, scattering detector) at 50°, *P* (atm) air = 2–2.5 atm was used. Products (two monoacetates) and reagents (tritylated glicosided) were eluted over a period of 5 min. The retention times (min) of the methyl 6-*O*-trytil β-D-glucopyranoside and of the mixture of two monoesters were respectively of 3.54 and 4.77 min.

Preparative column chromatography was performed using silica gel.

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