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ORIGINAL PAPER

Effective immobilisation of lipase to enhance esterification potential and reusability

Ashok Kumar, Vikrant Sharma, Prachi Sharma, Shamsher S. Kanwar*

Department of Biotechnology, Himachal Pradesh University, Shimla 171 005, India

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A commercial lipase, "Lipolase T100", was immobilised onto silica by means of physical adsorption. The silica-bound lipase was subsequently exposed to 1 vol. % glutaraldehyde (pentane-1,5-dial). The silica was loaded repeatedly with the Lipolase T100 in 0.05 M Tris buffer (pH 8.5) until saturation was achieved. During the 1st, 2nd, 3rd, 4th, and 5th cycles of loading of silica with the enzyme, the protein-binding on the silica achieved 51.73 %, 48.27 %, 26.92 %, 10.73 %, and 4.29 %, respectively. The synthesis of methyl salicylate (methyl 2-hydroxybenzoate) and linalyl ferulate (3,7-dimethylocta-1,6-dien-3-yl 4-hydroxy-3-methoxycinnamate) carried out at 45 °C under shaking with mole ratios of 200 mM of acid and 500 mM alcohol in DMSO using 15 mg mL^{-1} of hyper-activated biocatalyst resulted in yield(s) of 77.2 % of methyl salicylate and 65.3 % of linalyl ferulate in the presence of molecular sieves. The hyper-activated biocatalyst was more efficient than the previously reported silica-bound lipase with minimum leaching of the enzyme from the reaction mixture. The K_m and V_{max} of the free (0.142 mM and 38.31 μmol min^{-1} mL^{-1}, respectively) and silica-bound lipase (0.043 mM and 26.32 μmol min^{-1} mg^{-1}, respectively) were determined for the hydrolysis of *p*-NPP. During repeated esterification studies using silica-bound lipase, yields of 50.1 % of methyl salicylate after the 5th cycle, and 53.9 % of linalyl ferulate after the 7th cycle of esterification were recorded. In the presence of molecular sieves (30 mg mL^{-1}) in the reaction mixture, the maximum syntheses of methyl salicylate (77.2 %) and linalyl ferulate (65.3 %) were also observed. In a volumetric batch scale-up, when the reaction volume was increased to 50 mL, 44.9 % and 31.4 % yields of methyl salicylate and linalyl ferulate, respectively, were achieved.

Keywords: Lipolase T100, silica, glutaraldehyde treatment, immobilisation, esterification, DMSO, methyl salicylate, linalyl ferulate

Introduction

Lipases find potential applications in bioprocesses largely due to their availability and stability in organic as well as in aqueous media (Aulakh & Prakash, 2010; Verma & Kanwar, 2010; Kumar & Kanwar, 2012a, 2012b). The immobilisation of lipases currently attracts much commercial interest as it facilitates reuse and makes for easy separation of the biocatalyst, in addition to improving the thermal and chemical stability of the bound lipase (Kanwar et al., 2005a, 2005b). However, selection of the immobilisation approach is based on the effectiveness of the biocatalyst, cost of the immobilisation procedure, toxicity of the immobilisation reagents and the desired final properties of the immobilised biocatalyst (Panzavolta et al., 2005; Bučko et al., 2012). The immobilisation could resolve some of the problems encountered when using enzymes as industrial biocatalysts such as efficient biocatalyst recovery, enzyme stability, enzyme selectivity, and inhibition by the medium and/or product(s). In a recent study, it was observed that the immobilisation of *R. oryzae* lipase onto silica aerogels markedly increased its stability at high temperature and over a wide pH range. The immobilised enzyme also exhibited a high tolerance to a polar solvent and retained its

*Corresponding author, e-mail: kanwarss2000@yahoo.com