

COVALENT IMMOBILISATION OF LIPASE ON DIFFERENT SUPPORTS

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Abstract - Lipase (EC 3.1.1.3) from *Candida rugosa* was covalently immobilised on polymeric membranes (cellulose filter, crude cellulose, regenerated cellulose, cellulose acetate, starch and collagen) using 1,1' - Carbonyldiimidazole (CDI) as activating agent. Immobilised lipase activities obtained suggest that this activator is excellent for the carboxyl and hydroxyl groups present in these membranes. Other activating agents (Carbodiimide metho-p-toluene sulfonate (CMC) and Glutaraldehyde) were used to activate collagen membranes for lipase immobilisation, with similar catalytic performances.

Keywords - Lipase, immobilisation, membrane, 1,1' - carbonyldiimidazole

I. INTRODUCTION

Lipases (EC 3.1.1.3) catalyse both the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids. Important commercial applications of lipases are found in detergent formulations, in the manufacture of food ingredients, in the modification of fats and oils, in pitch control in the pulp and paper industry and as biocatalysts in biotransformations (Gilbert, 1993). In common to other enzymes, the lipase preparations for commercial applications should be highly active and show a high turnover. Therefore, efforts have long been made to immobilise lipases for an easy recovery and reutilization. Most of the techniques developed for immobilisation of proteins have emphasised the stability of the immobilised species rather than the activity. An immobilised enzyme may have a lower specific activity than the soluble counterpart, which may be due to several reasons, including diffusion limitations, conformational changes, reduced conformational mobility, modification of optimum pH or an immobilised orientation which results in steric hindrance of the binding site. Covalent immobilisation can be an alternative to other immobilisation techniques when a strong binding of the

lipase is required. In such cases, the support physical characteristics must be extensively analysed for the effects on the activity and stability of the lipase. Polymeric membranes have been used as carriers for lipase immobilisation (Pronk *et al.* 1988; Rucka and Turkiewicz, 1990; Guit *et al.* 1991; Bouwer *et al.* 1997; Carneiro-da-Cunha, *et al.* 1999). The effect of several membrane supports on covalent immobilisation of the lipase from *Candida rugosa* is reported here.

II. MATERIALS AND METHODS

Materials

Lipase (EC 3.1.1.3) from *Candida rugosa* (type VII) in a powder form, Triton X-100, Tris-HCl buffer, cellulose acetate and 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-p-toluene sulfonate (CMC), 1,1' - Carbonyldiimidazole (CDI) and Dimethyl Sulfoxide (DMSO) were obtained from Sigma (USA). Glutaraldehyde solution at 25%, glycerol and potato starch were purchased from Merck (D). Triolein was from Fluka (D).

Cellulose filter (Type Nr. 589³) was from Schleicher & Schüll (D). Regenerated cellulose and pig tripe (collagen membrane), were purchased from a local shop. Crude cellulose (~80% cellulose and ~20% hemicelluloses) was kindly donated by Stora Celbi (P).

Cellulose acetate and starch membranes were prepared *in situ* before use while the others were purchased and used directly.

Methods

Preparation of the cellulose acetate membranes

Membranes of cellulose acetate were prepared by evaporation of a 10% (w/v) solution in tetrahydrofuran, spread over glass plates at room temperature (Gil *et al.*, 1992).

Preparation of the starch membranes

Starch membranes were prepared by a modification of Sommerfeld and Blume's (1992) method as follows: