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IMMOBILISATION OF *Candida cylindracea* LIPASE ON A NEW RANGE OF CERAMIC SUPPORTS.

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SUMMARY

Lipase from *C. cylindracea* was covalently immobilised to a number of surface-treated ceramic supports (3-10 mg.(g dry wt support)$^{-1}$). At room temperature, the immobilised lipase could convert R,S-citronellol and butyric acid to citronellyl butyrate at rates in the range 7-51 μmol.(mg lipase.min)$^{-1}$. The lipase maintained 90-100% of its initial activity over a period of 150 days.

INTRODUCTION

A new range of surface-treated ceramic supports for enzymes (the Biofix E series) has been produced (Adams *et al.*, 1988). Two examples of these are Biofix E1 and E2, which are synthesised from kaolinite. E1 particles are spherical (20-50 μm diameter) whilst E2 particles are granular (mm-sized); both particles have pore sizes in the range 20-50 nm. Biofix E1 and E2 were supplied in the native form or surface-treated with either polyethylene amine (PEA), polyethylene imine (PEI) or 3-aminopropyltriethoxysilane (GAPTES). These surface treatments allow the enzymes to be bound via glutaraldehyde coupling. The suitability of Biofix E1 and E2 as enzyme supports was demonstrated by assessing the catalytic activity of a lipase from *C. cylindracea* (Borckerhoff & Jensen, 1974). The activity was monitored by following the formation of citronellyl butyrate from citronellol and butyric acid (Nishio *et al.*, 1987). The reaction cannot take place in an aqueous medium because water is one of the products and citronellol is poorly water-soluble (Cambou & Klibanov 1984). Approaches to overcome this general problem have included using biphasic aqueous-organic solvent mixtures (Klibanov & Cambou 1987), modifying a lipase such that it can remain soluble in an organic solvent (Nishio et al. 1987) and immobilising the lipase (Kawamoto *et al.*, 1987).

MATERIALS AND METHODS

Candida cylindracea lipase was a gift from Biocatalysts Ltd (Treforest Industrial Estate, Pontypridd, CF37 5UT). All chemicals were obtained from Aldrich, Sigma or BDH. Three different methods were used to treat E1 and E2 with GAPTES: (1) 5 ml GAPTES + 100 ml acetone + 20 g support were mixed. The acetone was removed by rotary evaporation and the support dried at 115 deg C. (2) 40 ml GAPTES + 360 ml water + 20 g support. This mixture was heated at 75 deg C for 2 h, then the support was washed and dried at 115 deg C. (3) 100 ml GAPTES + 900 ml toluene + 20 g support. The mixture was refluxed for 4 h at 110 deg C. The support was washed in acetone and dried at 115 deg C.

The lipase was immobilised on the surface-treated supports as follows: 0.5g support was agitated gently with 10 ml of 2.5% (w/v) glutaraldehyde in 0.05M Na_2HPO_4 (pH 7) for 1 h at room temperature. The glutaraldehyde solution was removed and the support washed thoroughly with 5 x 20 ml single-distilled water (SDW). 5 ml of a 10 $mg.ml^{-1}$ lipase solution, in the above buffer (Sample L), was added to the support which was then agitated gently for 3 h. The lipase solution was removed (Sample P) and 20 ml SDW added to the support (Sample W). The samples were analysed for protein by the Lowry method and bound protein estimated as L-(P+W). The values in the protocol were scaled-up in proportion when larger quantities were required. The activity of the bound lipase was assessed by following the formation of citronellyl butyrate from R,S-citronellol and butyric acid (in the ratio 5:1 v/v), and the immobilised lipase was either agitated gently in bottles or packed into columns through which the reaction mixture was passed. The formation of citronellyl butyrate was analysed by GLC using a column of OV1 on Gas ChromQ (PhaseSep, Queensferry, Clwyd).

RESULTS AND DISCUSSION

It was important to ascertain which of the surface treaments could bind the maximum amount of lipase and whether this corresponded with the maximum formation of citronellyl butyrate in 24 hours. 2 g of Biofix E1 or E2 were used in this experiment and the results are shown in Table 1. Sil 1, 2 or 3 denote the different methods of GAPTES treatment described in the Methods section. For E1, treatments Sil 1 and Sil 2 gave optimum results, whilst for E2, treatments Sil 2 and Sil 3 proved to be most effective. The use of PEI and PEA for the surface-treatment of Biofix E1 and E2 gave poor results in comparison to those with GAPTES. For further studies it was decided to use supports treated with GAPTES by the Sil 2 method and with PEI. The PEI treatment was chosen in case the GAPTES samples proved not to be stable over a longer period of use.

To test the activity and stability of the immobilised lipase, five columns were prepared using E1 or E2 treated with GAPTES or PEI. Data concerning these columns are given in Table 2. The activity of the lipase in the small columns was assessed by circulating through the columns a mixture of 1 ml butyric acid and 5 ml R,S-citronellol for 24

hours at room temperature (16-28 deg C). 50 µl samples were taken and analysed for the production of citronellyl butyrate. 10 ml butyric acid and 50 ml R,S-citronellol were circulated through the larger column. When the columns were not in use they were stored either at 4 deg C or at room temperature. Figure 1 shows that four of the columns essentially maintained their initial activity over a period of 150 days; only the E1-PEI column failed to retain any activity. The fluctuations in activity (especially in the E2-Sil columns) were probably due to the variations in room temperature; 80-100 days corresponded to mid-summer. Fig 1 also demonstrates that the GAPTES surface-treatment allowed higher activities to be expressed by the lipase than did surface-treatment with PEI. Continuous use of the E2-Sil column showed that the lipase activity decreased by 15% over a period of 650 hours. Further experiments with the large E2-Sil column demonstrated that 95% depletion of the butyric acid could be achieved within 4 days.

Thus, the new ceramic supports Biofix E1 and E2, which have been surface-treated with GAPTES or PEI, are suitable for the immobilisation of lipase. Once immobilised, the lipase can maintain its initial activity for at least 150 days when converting R,S-citronellol and butyric acid to citronellyl butyrate. This process has the advantage that the lipase does not have to be derivatised and the reaction does not have to occur in organic (co)solvents.

Column Type	E2-Sil	E1-Sil	E2-PEI	E1-PEI	E2-Sil(L)
Amount of Biofix (g)	2.0	2.8	2.0	3.0	10.0
Amount of lipase bound (mg)	10.7	18.1	10.2	5.9	62.0
Storage temperature (deg C)	4	4	4	4	16-28

Table 2: The types of columns used in this investigation. Key: E2-Sil(L) denotes the larger column.

Sample	Bound Lipase (mg.(g dry wt support)$^{-1}$)	% Conversion
Biofix E1		
Sil 1	7.8 ± 0.5	73.1 ± 4.8
Sil 2	8.1 ± 0.0	69.2 ± 3.7
Sil 3	8.3 ± 0.3	51.0 ± 2.4
PEA	5.0 ± 0.0	10.9 ± 1.2
PEI	3.4 ± 0.2	13.5 ± 1.4
Biofix E2		
Sil 1	5.1 ± 0.5	19.4 ± 0.0
Sil 2	10.6 ± 0.2	60.8 ± 7.3
Sil 3	10.5 ± 0.3	51.1 ± 0.0
PEA	6.5 ± 0.5	30.4 ± 3.6
PEI	6.8 ± 0.2	28.0 ± 1.2

Table 1: The amount of lipase which can be bound to different surface-treated samples of Biofix E1 and E2, and the percent conversion of citronellol to citronellyl butyrate by these samples after 24 hours. Reactions were carried out in duplicate in 20 ml Universal bottles as described in the Methods section.

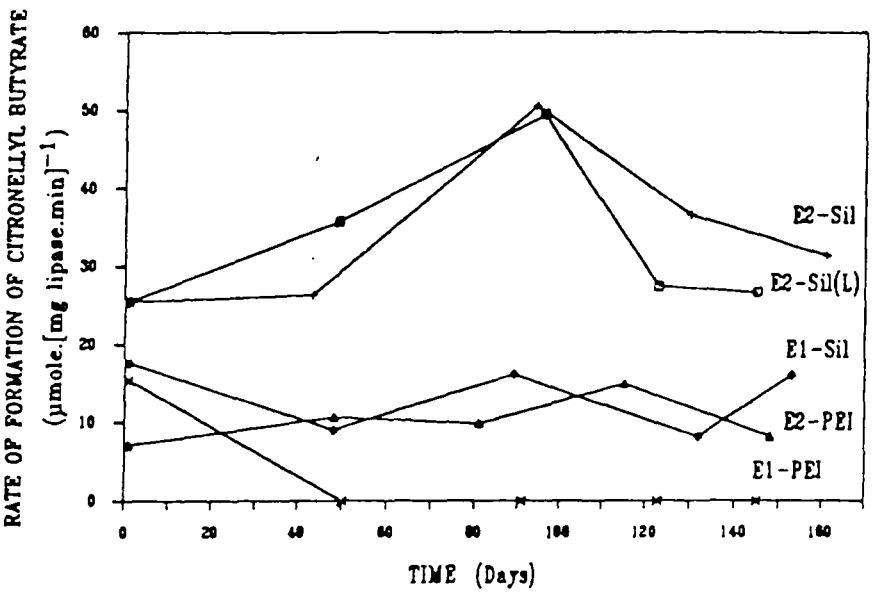

Figure 1: Longevity of lipase immobilised on surface-treated Biofix E1 and E2 and assessed in columns. The types of columns (E2-Sil etc) and their storage conditions are described in Table 2.

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