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Article

The Use of Immobilized Lipases on Chrysotile for Esterification Reactions

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Lipases (CCL e PPL) foram imobilizadas em crisotila, um silicato de magnésio, e utilizadas na esterificação de vários alcoóis alifáticos, obtendo-se os produtos com bons rendimentos a 25 °C. A reutilização do sistema também foi avaliada.

Lipases (CCL and PPL) were supported on chrysotile, a magnesium silicate, and used in the esterification of a series of aliphatic alcohols, and the products were obtained in a good yields at 25 °C. The re-use of the system was also evaluated.

Keywords: *lipases, immobilization, chrysotile*

Introduction

The use of enzymes in organic conversions is a subject of growing interest in synthetic chemistry. Recent studies have particularly focused on the utilization of these biocatalysts in organic media[1-4].

The immobilization of enzymes on a solid matrix has been widely investigated, and a variety of supporting media has been tested[5-7]. Functional ceramics, such as glass beads[8], acrylamide and acryloxysuccinimide (PAN)[9] cross-linked copolymers, epoxide-containing acrylamide beads (Eupergit C)[10], and carbohydrate-based supports[11] are commonly used for covalent enzyme immobilization. More recently, enzymes immobilized in polythiophene in microemulsion[12] and in alginate gels[13] were used in glucose biosensors. Glucose oxidase was also immobilized in electrodes with the pre-polymer polyurethane (PU-6)[14] and poly(N-isopropylacrylamide-co-vinyl)ferrocene (PAF)[15]. L-glutamate dihydrogenase (GLDH) was immobilized in an isothyocyanate-modified controlled pore glass for the determination of glutamic acid[16]. A hollow fiber reactor was also used for the immobilization of soluble enzyme glucose-isomerase in the study of fructose-glucose isomerization[17].

We have been studying new forms of immobilizing enzymes in non-aqueous media, and have successfully utilized microemulsion-based-gels (MBG)[18-19] to carry out enantioselective esterifications in hexane with catalytic amounts of lipases[20].

We have recently investigated a new and inexpensive supporting material for the immobilization of enzymes. Chrysotile is an inexpensive magnesium silicate, previously employed by Moran *et al.* in the enantioselective resolution of phenylketones and in the reduction of α-azidopropiophenone by immobilized Baker's yeast[21-23]. In the present communication we report the use of this material as an enzyme-supporting medium in the esterification of a variety of alcohols by different acids. For this purpose, two enzymes were used, *Candida cylindracea* (CCL, now called *Candida rugosa*[24]) and *Porcine pancreatic* (PPL) lipases.

Experimental

Candida cylindracea lipase (CCL), (Type VII 900 units/mg solid, 4,865 units/mg of protein) and *Porcine pancreatic* lipase (PPL), (Type II with 15 units/mg of solid, 70 units/mg of protein) were obtained from Sigma Chemical Co. (St. Louis, Missouri, United States). All solvents and reagents were of analytical grade.

The ^{1}H-NMR spectra were recorded on a Bruker AC 200 spectrometer with tetramethylsilane (TMS) as the internal

standard, and the UV spectra on a Beckman DU 65 spectrometer.

Enzyme immobilization

Crude chrysotile (purchased from Sama's mine, Goias, Minas Gerais, Brazil) was washed under a strong water flow on a sieve (0.0062 mm) for 10 min, and sonicated at 25 kHz for 30 min[25]. The two lipases were immobilized on chrysotile as follows: 0.5 g of enzyme in 25 mL of water was added to 1.0 g of chrysotile, and the resulting suspension was shaken for 12 h at 25 °C. The solid material was then filtered and dried in air. The estimate of the amount of lipase adsorbed by the chrysotile was by UV spectra (260 nm and 258 nm for CCL and PPL, respectively). 30-40% of the enzyme was adsorbed by this procedure. These values are the average of five experiments.

Esterification reactions: general procedure

The enzyme-catalyzed esterifications were carried out by using equal amounts (5 mmol) of the acid and alcohol in n-hexane (25 mL), in the presence of the dry support chrysotile containing enzyme (1 g), as prepared above. The suspension was shaken at 25 °C for 24 h in a Dubnoff bath. After filtration and standard work-up (washing with $NaHCO_3$ solution, then water, drying with Na_2SO_4, and evaporating the solvent), the crude product was analysed by ^{1}H-NMR, and the yield was estimated from the relative proportion of the ester and the alcohol in the spectra.

Results and Discussion

A variety of esters and a thioester were obtained in good yields (Table 1). Ideal substrates were lipophilic acids and primary alcohols. Esterifications with acetic and benzoic acid with n-pentanol and n-octanol formed no products, and lower yields were obtained with secondary alcohols (not shown). The low yields obtained in the esterification of propanoic acid with different alcohols indicate that the chain length of the acid is a determining factor in this system. The esterification of decanoic acid with 2-propanol, 2-pentanol and 3-pentanol with PPL and CCL as the catalyst, supported on chrysotile was also investigated. No product was observed in the esterification of 2- and 3-pentanol. 2-propylhexanoate was formed in a 20% yield after 24 h of reaction at 25 °C under the same conditions. Using *Chromobacterium viscosum* lipase (CV), immobilized in chrysotile 2-propyl hexanoate was obtained in a quantitative yield. These results show that CCL and PPL work more properly in esterification reactions with primary alcohols, while lipase CV is better for secondary alcohols. Similar results were obtained by using lipases immobilized in microemulsion-based gels[19]. The esterification of oleic acid with several alcohols in various organic solvents was studied by enzyme-catalyzed reactions involving the immobilization of the enzyme in microemulsion-based gels (MBG). The esters were obtained in high yields when CV

Table 1. Esterifications reactions using lipases CCL and PPL supported on chrysotile at 25 °C.

Acid	Alcohol	Yield (%)
Acetic	n-Pentanol	0
	n-Pentanol	56
Propanoic	n-Octanol	60
	n-Undecanol	40
	n-Pentanol	≅100
	n-Pentanol*	82
Hexanoic	n-Octanol	98
	n-Undecanol	98
	n-Pentanol	≅100
	n-Pentanol*	75
Decanoic	n-Octanol	86
	n-Undecanol	90
	n-Octanethiol	92
	n-Pentanol	85
	n-Pentanol*	90
Tetradecanoic	n-Octanol	93
	n-Octanol*	98
	n-Undecanol	85
	n-Pentanol	85
Hexadecanoic	n-Octanol	75
	n-Undecanol	85
Benzoic	n-Pentanol	0
	n-Octanol	0

Reaction time: 24 h. Yield calculated by ^{1}H-RMN*. PPL.

lipase was employed, *e.g. Pseudomonas fluorescens* and *Microbial* lipases, whereas CCL and PPL formed no esters under the same conditions. Comparing the two systems of immobilization, *e.g.* MBG and chrysotile, the results seem to indicate that chrysotile is a better support for CCL and PPL immobilization. This support was also used in the esterification of decanoic acid with octanethiol, and the corresponding thioester was obtained in a 92% yield at 36 °C after 24 h (Table 1). In this case the use of higher temperatures is necessary, probably due to differences in the electronegativity of oxygen and sulfur. It is also important to mention that even if lipases were used with lower activity than those used in microemulsion-based gels (MBG)[19], the esters were formed in very good yields.

We have also tested for the re-use of these catalysts. A sample of *Candida cylindracea* lipase absorbed on chrysotile can be re-utilized ten times in the reaction of hexanoic acid and n-pentanol at 25 °C. The observed yields

were practically constant in all esterifications and the products were obtained in the range of 80-100%.

Conclusions

The above results indicate that CCL and PPL may be successfully immobilized in chrysotile and used in the esterification reactions of a variety of aliphatic acids and alcohols. In previous work, CCL has been used for the resolution of open-chain halogenated acids via esterification and for the resolution of racemic open-chain secondary alcohols via transesterification[26] in organic solvents, as well as for the resolution of menthol in aqueous and organic solvents[27]. PPL is selective for primary alcohol esters, but it is not appropriate for secondary alcohols, as indicated in one case where the enzyme activity for secondary alcohol ester substrates is totally abolished when pure PPL is used[28].

Chrysotile is an inexpensive and abundant mineral in Brazil. The ease of the experimental procedure and the stability of the polymer catalyst are attractive features for the use of chrysotile as an enzyme-supporting medium in biocatalytic conversions. This support, as noted, may be used with more hydrophilic solvents and substrates than those with MBG, where it was observed, for example, that acetic and benzoic acid destroy the organo-gel[19]. Furthermore, chrysotile can be reutilized several times. We are now investigating other properties of this system in our laboratory and expanding the use of this support in the resolution of racemic alcohols and acids.

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