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Mesoporous materials as host for an entrapped enzyme

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Abstract

Three mesoporous materials, silica, alumina and titania, were used as host for a lipase and the enzyme-loaded particles were employed as catalyst for esterification of caprylic acid using a mixture of glycerol and water as reaction medium. The reaction proceeded well with all three oxides but alumina gave considerably higher conversion than the other two. Hydrophobized silica gave an even higher degree of esterification. The degree of esterification obtained is believed to depend on the microenvironment of the enzyme. When alumina, which is positively charged under the conditions used, and hydrophobized silica are used as host material, the negatively charged lipase can be assumed to be adsorbed at the walls of the pores. The water activity is believed to be lower at the solid surface than in the middle of the pores, where the enzyme is situated when silica and alumina are used as host material. It is shown that the lipase is not irreversibly entrapped in the pores of the mesoporous materials. When the particles are removed by filtration after completed reaction and subsequently washed with an aqueous buffer, the enzyme is leached out. The lipase can be immobilized in the pores, however, by cross-linking *in situ* inside the pores using glutaraldehyde as cross-linking agent. Mesoporous materials loaded with cross-linked lipase can be reused several times with only marginal loss of activity.

Key words: Lipase; Heterogenized; Entrapment; Biocatalysis; Monoglyceride; Monocaprylin; Caprylic acid; Esterification; Mesoporous material; Silica; Alumina; Titania

1. Introduction

In bioorganic synthesis, the catalyst, i.e. the enzyme, can be used in either free or immobilized state. This parallels chemical reactions in general for which the catalyst can be either specific atoms at the surface (heterogeneous catalysis) or catalytically active molecules in solution (homogeneous catalysis), the latter usually being metal-organic substances. Homogeneous catalysts have some attractive properties, such as high selectivity and good accessibility to catalytically active sites [1]. However, heterogeneous catalysts have many advantages over the homogeneous ones, such as easy catalyst regeneration and good stability to harsh conditions.

A specific problem with enzymes when used free in solution, i.e., as homogeneous catalysts, is their susceptibility to protease catalyzed breakdown [2]. Most of the problems associated with free enzyme can be circumvented by immobilization of the enzyme. The degradation rate will usually be much decreased and the work-up becomes equivalent to that when heterogeneous catalysts are used. The carrier-bound enzyme can simply be removed from the reaction mixture by filtration or centrifugation and then reused.

Immobilization of enzymes to a support material is usually carried out by either of three routes: covalent binding, adsorption, or encapsulation/entrapment [3]. Immobilization by covalent bonds and also adsorption by electrostatic interactions may severely affect the active site, which can lead to loss of activity [4]. These procedures may also create diffusional restrictions that may impair the activity [5]. Immobilization by adsorption to a hydrophobic surface will usually not directly affect the active site but may have other consequences. Such an interaction is based on van der Waals forces between the enzyme and the support

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and the nature of the surface is crucial for successful immobilization, as well as for long term retention of enzymatic activity [6]. Too weak interaction between the enzyme and the surface may lead to leakage of the enzyme; too strong interaction may cause the enzyme to gradually change its conformation such that the activity decreases, a phenomenon known as denaturation.

Encapsulation or entrapment relates to techniques of trapping the catalyst in the pore space of a carrier material [7]. The technique can be used with both organic and inorganic support materials and a variety of encapsulation procedures have been developed. An encapsulated enzyme is normally resistant to peptidase catalyzed biodegradation and can usually be recovered and reused many times.

There are a few previous reports in the literature on the use of mesoporous materials for entrapment of enzymes [1]. These all relate to silica. Diaz and Balkus were probably the first to load a mesoporous silica material with an enzyme and they studied entrapment of three different enzymes, cytochrome *C*, papain, and trypsin [8]. For trypsin they found a much enhanced retention of activity: no activity decrease was seen after one week in entrapped state while free trypsin in solution was totally deactivated after 24 h. Wang and Caruso used mesoporous silicas for entrapment of catalase, peroxidase, cytochrome *C*, and lysozyme and obtained good activity and long durability of the enzymes [9]. They demonstrated resistance towards protease-catalyzed breakdown, which, as discussed in the Introduction, is seen as one of the main advantages with the use of immobilized enzymes.

In addition to entrapment of an enzyme into ready-made mesoporous silica, attempts have been made to immobilize the enzyme by having it present during the formation of the porous inorganic material. Blin et al. immobilized glucose oxidase by such a procedure [10] and Mureseanu and coworkers have reported incorporation of lactose [11]. Both groups have shown that the ordering of the material is not markedly affected by the presence of the protein. They also demonstrated that the enzyme retains its activity well.

In this communication, we report a procedure for ''heterogenizing'' a lipase by entrapment into the pores of a mesoporous oxide material. The pores are hydrophilic and can be filled with an aqueous solution of the enzyme. Fine particles of the lipase-loaded mesoporous material are then suspended in a solution of a hydrophobic substrate and the reaction occurs at the oil-water interface, i.e., at the pore openings. Three different oxides and different geometries are tested and the results are compared with results from the use of non-porous particles at which surface the enzyme is merely adsorbed.

The lipase is likely not to interact strongly with the oxide material; thus, it can be assumed to be in a more native state than when covalently immobilized to a solid support. However, since the enzyme is not firmly attached to the surface, it may leach out of the pores on washing with water. In order to prevent this, the enzyme is cross-linked *in situ* inside the pores. It is shown that particles containing such cross-linked enzyme can be recirculated with good retention of the catalytic activity.

2. Materials and methods

2.1. Chemicals

Lipase from *Rhizomucor miehei* (4210 units/mg solid), glycerol (99%), 4-nitrophenylpalmitate, and glutaraldehyde were from Sigma, St. Louis, MO. Caprylic acid, monocaprylin, dicaprylin and tricaprylin were obtained from Larodan, Malmö, Sweden.

2.2. Synthesis of mesoporous materials

Silica with hexagonal geometry was synthesized using tetraethylorthosilicate (TEOS) as silica source and the triblock copolymer Pluronic 105 from BASF, Germany as structure directing agent. Pluronic 105 is poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide) with the composition $(EO)_{37}(PO)_{58}(EO)_{37}$. Hexagonal alumina and titania were made from $AlCl_3$ and $TiCl_4$, respectively, using Pluronic 123 as structure directing agent. Pluronic 123 is poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide) with the composition $(EO)_{20}(PO)_{70}(EO)_{20}$, and was also obtained from BASF. Cubic silica was made from TEOS using Pluronic 123 as structure directing agent. Detailed description of the syntheses of the hydrophilic mesoporous oxide materials has recently been published [12].

Hydrophobic hexagonal mesoporous silica was prepared by addition of ethanol to the solid silca particles (75 ml 99.5% ethanol to 1.5 g silica). Chlorotrimethylsilane (4.3 g) was added together with five drops of triethylamine that serves as catalyst and the mixture was stirred at 70 °C for 3 h. The product was filtered, washed three times with small amounts of ethanol, and then dried at room temperature. A proper degree of silylation was confirmed by FTIR, as described previously [12].

The non-porous beads that were used as reference materials were synthesized according to literature methods [13].

The mesoporous materials were characterized with respect to BET surface area, pore size and pore size distribution according to previously published procedures [12]. The results are collected in Table 1. Small angle X-ray scattering and transmission electron microscopy were used for identification of the materials, as also given in Ref.[8].

2.3. Entrapment of lipase

A 2 ml solution of 1 mg/ml lipase in 0.1 M phosphate buffer at pH 7.0 was added to 0.15 g mesoporous or nonporous material. The sample was stored overnight at 4 °C in screw-capped vials and then filtered using a paper filter with 0.10 mm mesh size.