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Oxidation of indole using chloroperoxidase and glucose oxidase immobilized on SBA-15 as tandem biocatalyst

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Abstract

In recent years, enzymatic oxidations are being evaluated for production of fine chemicals and pharmaceuticals. For industrial applications, heterogeneous biocatalysts are favored for several reasons, e.g. easy separation and recycling of the catalyst. In the present work, chloroperoxidase (CPO) from *Caldariomyces fumago* was immobilized on the mesoporous molecular sieve SBA-15 and tested in the oxidation of indole to 2-oxindole using hydrogen peroxide as oxidant. The deactivation of peroxidases by external addition of peroxides was circumvented by in situ hydrogen peroxide generation. H_2O_2 was produced by glucose oxidation with glucose oxidase (GOx) immobilized on SBA-15. By the use of this tandem reaction, CPO deactivation was largely suppressed due to the "sensitive" hydrogen peroxide generation. The conversion of indole was maximal ($X_{indole} = 92\%$) at pH = 5.5 with a selectivity $S > 99\%$ to 2-oxindole. Furthermore, it is found that the tandem catalyst consisting of a physical mixture of CPO-SBA-15 and GOx-SBA-15 can be recycled several times without significant loss of activity.

Keywords: Tandem enzyme reaction; Immobilization; Oxidation; SBA-15; Chloroperoxidase; Glucose oxidase

1. Introduction

The enzyme chloroperoxidase (CPO) is a heme-containing protein which belongs to the group of heme haloperoxidases. Haloperoxidases exist in nature; they are produced by many different organisms including bacteria [1]. With respect to their prosthetic group, the haloperoxidases can be subdivided into several categories: one major group, the heme haloperoxidases feature a ferriprotoporphyrin IX system as their active site [2,3]. The enzyme is glycosylated and the dimensions determined by single crystal X-ray diffraction are approximately $5.3 \times 5.5 \times 3.1$ nm [4]. It has been shown that CPO is capable of performing oxidative dehydrogenation and oxidative halogenation reactions [5–7]. The most promising feature is the broad variety of oxygen transfer reactions catalyzed by CPO [8]. Although CPO exhibits a versatile catalytic activity, its utilization in industrial processes and preparative organic chemistry is still lacking. Major reasons therefore are the expenses for the production of CPO, the difficulties in recovering the native enzyme from the crude reaction mixture and – in the case of oxidation reactions – its deactivation due to the presence of peroxides. Therefore, the development of a heterogeneous biocatalyst is necessary and in this context two main problems have to be addressed: (1) the development of an immobilization technique for CPO which suppresses leaching and (2) the implementation of a reaction procedure which reduces enzyme deactivation.

In recent years, considerable attention was given to the immobilization of CPO and other proteins onto porous solids including mesoporous silicas and carbons [10]. Chloroperoxidase was bond covalently to aminopropyl glasses [11], adsorbed on different types of talc or in reverse micelles [12] and immobilized on the mesoporous molecular sieves SBA-15, SBA-16, MCM-48 and MCF [13]. In our previous publication, we reported the immobilization

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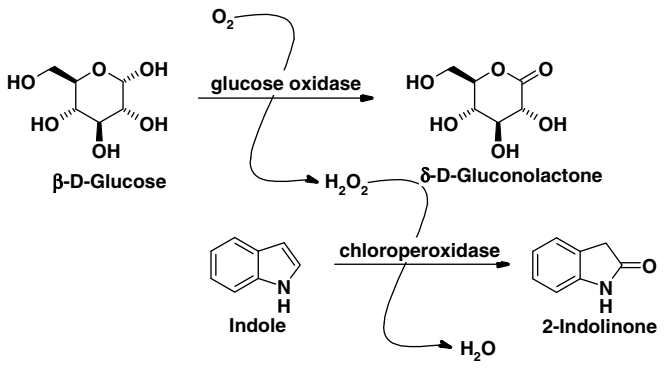
of CPO on the mesoporous molecular sieve SBA-15. The resulting catalyst was applied for the oxidation of indole to 2-oxoindole using hydrogen peroxide or *tert*-butyl hydroperoxide as oxidants. The performance of the immobilized enzyme was found to be superior to native chloroperoxidase with respect to maximum conversion and pH range applicable [14]. Sheldon et al. reported the immobilization of glycosylated enzymes via reaction with isocyanate groups in a polyurethane prepolymer [15]. CPO was covalently bound to silica gel derivatized with 3-glycidoxypropyltrimethoxysilane via reaction with the epoxide groups under mild conditions [16]. New strategies for synthesizing solid biocatalysts, e.g. involving the build-up of a microporous silica gel cage around CPO [17] and the immobilization of CPO on mesoporous sol–gel glasses possessing a highly ordered porous structure were published [18].

In order to avoid the deactivation of CPO by hydrogen peroxide a new tandem reaction was invented by the group of Sheldon, who studied the stability of peroxidases by coimmobilization with glucose oxidase into polyurethane foams [25]. It is found that CPO deactivation can be suppressed by application of this tandem reaction most probably by maintaining a lower local peroxide concentration. Glucose oxidase catalyzes the oxidation of β-D-glucose to δ-D-glucono-1,5-lactone and hydrogen peroxide, using molecular oxygen as electron acceptor (cf. Scheme 1) [23]. As a test reaction, a prominent example for the ability of CPO to oxidize activated C–H bonds was chosen: the oxidation of indole to 2-oxindole (2-indolinone) [9,21,22]. The non-enzymatic synthesis of 2-oxindole requires harsh conditions or complex inorganic catalysts to be successful since oxidation of the electron-rich 3-position is typically favored. Fujita et al. synthesized 2-oxindole from 3-(2-aminophenyl)-1-propanol by using $[Cp^*RhCl_2]_2/K_2CO_3$ (Cp^* = pentamethylcyclo-pentadienyl) as catalyst with a yield of 80% [19]. A mild synthesis of 2-oxoindole by Wolff–Kishner reduction of isatin derivates was published by Crestini and Saladino [20]. Here, a 2,3-dioxoindole derivative is required as reactant.

In this work, the oxidation of indole to 2-oxindole was employed as a test reaction in order to study the activity of CPO immobilized on SBA-15 in a tandem reaction with glucose oxidation by immobilized glucose oxidase (GOx) in order to generate H_2O_2 in situ.



Scheme 1. Tandem reaction with immobilized chloroperoxidase and glucose oxidase.

Glucose oxidase has been highly purified from the extracts of *Aspergillus niger* [24]. GOx is a dimeric protein with a molecular weight of 160 kDa containing one tightly bound flavin adenine dinucleotide (FAD) per monomer as cofactor. The enzyme is glycosylated with a carbohydrate content of 16 wt.%. The monomeric molecule is a compact spheroid with approximate dimensions of $6.0 \times 5.2 \times 3.7$ nm; the corresponding dimensions of the dimer are $7.0 \times 5.5 \times 8.0$ nm.

Initially, the physical adsorption of CPO and GOx on the mesoporous silica molecular sieve SBA-15 is studied. The bi-enzymatic catalysts obtained by physically mixing CPO-SBA-15 and GOx-SBA-15 were tested in the selective oxidation of indole to 2-oxoindole using the CPO–GOx-tandem reaction. It is found that fast deactivation of the immobilized CPO is avoided and that the catalyst can be recycled four times without significant loss of activity.

2. Experimental section

2.1. Synthesis of the support

SBA-15 was synthesized according to procedures published in previous publications [26–28]. A typical synthesis was performed as follows: In a polypropylene bottle ($V = 500$ ml), 4 g of the amphiphilic triblock copolymer P123 were dispersed in 30 g of water and stirred for 4 h until the copolymer was completely dissolved. Thereafter, 120 g of a 2 M aqueous HCl solution were added and the mixture was stirred for another 2 h. Then 9.5 g (45.6 mmol) of tetraethyl orthosilicate (TEOS) were slowly added under stirring. The resulting gel was aged in a water bath at 40 °C for 24 h and finally heated in an oven for 48 h at 100, 130 or 150 °C. The obtained material is referred to as SBA-15-*X* ($X = 100$, 130, 150 °C). The resulting product was washed with 100 ml of ethanol and the as-synthesized SBA-15 was recovered by vacuum suction filtration over black ribbon filter paper. The material was then washed two times with 400 ml of a water–ethanol mixture (3:1 v:v) and dried at 100 °C for 24 h. Finally, the template was removed by calcination at 540 °C in air. The catalysts were characterized by X-ray powder diffraction (Bruker AXS D5005; 2θ was varied between 0.8 and 6 °) and nitrogen sorption at −196 °C using a Quantachrome Autosorb 1 instrument.

2.2. Adsorption of GOx and CPO

Ten milligrams of SBA-15 and 10 units (U) of GOx were suspended in 5 ml of a 0.1 mM aqueous citrate buffer or phosphate buffer solution. After shaking the samples for 24 h in a water bath at 25 °C, the solid was separated from the supernatant liquid by centrifugation. The obtained supernatant liquids were tested for GOx activity. The solid