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Asymmetric bioreduction of acetophenones by Baker's yeast and its cell-free extract encapsulated in sol–gel silica materials

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ABSTRACT

Baker's yeast (BY) encapsulated in silica materials was synthesized using a yeast cell suspension and its cell-free extract during a sol–gel reaction of tetramethoxysilane with nitric acid as a catalyst. The synthesized samples were fully characterized using various methods, such as scanning electron microscopy, nitrogen adsorption–desorption, Fourier transform infrared spectroscopy, thermogravimetry, and differential thermal analysis. The BY cells were easily encapsulated inside silica-gel networks, and the ratio of the cells in the silica gel was approximately 75 wt%, which indicated that a large volume of BY was trapped with a small amount of silica. The enzyme activity (asymmetric reduction of prochiral ketones) of BY and its cell-free extract encapsulated in silica gel was investigated in detail. The activities and enantioselectivities of free and encapsulated BY were similar to those of acetophenone and its fluorine derivatives, which indicated that the conformation structure of BY enzymes inside silica-gel networks did not change. In addition, the encapsulated BY exhibited considerably better solvent (methanol) stability and recyclability compared to free BY solution. We expect that the development of BY encapsulated in sol–gel silica materials will significantly impact the industrial-scale advancement of high-efficiency and low-cost biocatalysts for the synthesis of valuable chiral alcohols.

1. Introduction

Considerable effort has been devoted to the fabrication of nanomaterials with well-defined morphologies for specific applications, including nanocapsules that simultaneously provide the advantages of hollow and porous systems [1–3]. Such nanocapsules can consequently be used as storage spaces or reaction chambers while supplying the necessary paths for the design of controlled uptake/release systems. Particular interest in the performance of chemical reactions in these confined environments has led to the discovery of catalyst-containing nanomaterials such that a diffusional product/substrate exchange between the inner space and the bulk solution occurs in an efficient way [4–9].

Sol–gel glass offers a better way to immobilize biomolecules present within its porous, optically transparent matrix, and has also demonstrated functional activity of encapsulated biomolecules [10–14]. This advantage of sol–gel glass stems from the fact that it is prepared under simple sol–gel processing conditions, which introduce the possibility of tailoring the glass on the basis of specific requirements. Because of this inherent versatility, sol–gel-derived glass can serve as a potential host matrix for biosensors and biocatalysts [15,16]. This approach is unique compared to the conventional methods, which involve adsorption onto glass surfaces, entrapment in polymer matrices, and impregnation in porous glass powders, such as mesoporous silica materials, because encapsulation is based on the growth of siloxane polymer chains around the biomolecule within an inorganic oxide network. Furthermore, because of the porous nature of the sol–gel network, the encapsulated species remain accessible and can interact with external chemical species.

Chiral alcohols are useful intermediates and auxiliaries in the production of fine chemicals. Moreover, the synthesis of chiral fluoro-organic compounds, which are important in research on biological chemistry and in the development of medicines, is one of the most important aspects of modern organofluorine chemistry because of fluorine's unique effect on biological activity [17–20]. The trifluoromethyl group is a very important substituent in organic chemistry. The chemoenzymatic approach to asymmetric synthesis of fluorinated compounds is increasingly used in synthetic strategies, and the use of an enzyme as a routine chiral catalyst for asymmetric synthesis is well documented [21–25].

One of the most attractive methods to the synthesis of pure alcohol enantiomers is via the catalytic enantioselective reduction of the corresponding prochiral ketones [26,27]. Although chiral transition-metal complexes have successfully been employed for this purpose, the use of biocatalytic reduction has some

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advantages because it proceeds at room temperature and does not require high pressures of hydrogen. Several oxidoreductases are capable of efficiently reducing ketones with a combination of cofactors such as nicotinamide adenine dinucleotide phosphate reduced form (NADPH) or nicotinamide adenine dinucleotide reduced form (NADH). However, the use of whole cells, such as baker's yeast (BY) (*Saccharomyces cerevisiae*), for chiral reduction is more economically attractive on account of its availability, low cost, and ease of handling and disposal [28–31]. Howarth et al. reported about BY immobilized in sodium alginate capsules for the asymmetric reduction of prochiral ketones in moderate yields [32]. Recently, Yang et al. described the immobilization of BY in sol–gel SiO_2, and evaluated the viability of BY inside SiO_2. However, the biocatalytic activities of immobilized BY for organic compounds such as ketones and alcohols have not been investigated [33].

Here we report on the asymmetric bioreductions of acetophenone and its fluorinated derivatives with both BY and NADPH regeneration system encapsulated in sol–gel silica materials. The obtained silica materials were fully characterized by field-emission scanning electron microscopy (FE-SEM), Fourier-transform infrared spectra (FT-IR), thermogravimetry (TG), and differential thermal analysis (DTA). The reactivity and enantioselectivity for several ketones of BY encapsulated in sol–gel silica materials were investigated; moreover, their solvent stability and recyclability were explored in detail. To the best of our knowledge, the reactivity and enantioselectivity for acetophenone and its fluorinated derivatives by BY, its cell-free extract (BYCF), and BYCF/NADPH regeneration systems encapsulated in sol–gel silica materials have not yet been reported.

2. Material and methods

2.1. Chemicals

Baker's yeast (dry yeast), D-glucose-6-phosphate (disodium salt), and glucose-6-phopsphate dehydrogenase (from yeast) were purchased from Oriental Yeast Co., Tokyo, Japan. Tetramethoxysilane (TMOS) was obtained from Shin-Etsu Chemical Co., Tokyo, Japan. Nicotinamide adenine dinucleotide phosphate reduced form (β-NADPH), nicotinamide adenine dinucleotide phosphate oxidized form (β-$NADP^+$), and acetophenone (1) were acquired from Wako Pure Chemical Industries, Tokyo, Japan. All materials were of analytical grade and used as received without further purification.

2.2. Preparation of Baker's yeast encapsulated sol–gel silica materials

The silica sol was prepared as follows: 0.5 mL of TMOS was added to a solution containing 65 μL of 0.1 M HNO_3 and 0.25 mL of distilled water, and the mixture was then sonicated for 5 min and cooled at 4 °C for 24 h. Then, 0.5 mL of the prepared silica sol was mixed with 3 mL of BY solution (0.5 g of dry yeast dissolved in 3 mL of 0.1 M phosphate buffer pH 7), and the suspension was gently mixed by hand for 15 s. The gelation occurred within 30 min at room temperature, and the gel was aged at 4 °C for 48 h. The silica gel obtained was refrigerated before use in activity measurements and material characterization.

2.3. Characterization of the materials

The morphology of the products was characterized using a field-emission scanning electron microscopy (FESEM, Hitachi S-4700, Hitachi, Tokyo, Japan). The Brunauer–Emmett–Teller (BET) specific surface area of the samples was determined using nitrogen (N_2) adsorption–desorption isotherms acquired on a Micromeritics TriStar 3000 analyzer (SHIMADZU, Tokyo, Japan). FT-IR spectra were obtained using an MFT-2000, JASCO, Tokyo, Japan. For FT-IR analyses, the samples were pelletized with KBr (sample/KBr = 1:100) by using a hydraulic press. TG and DTA were performed on an SSC-5200, SEIKO Instrument, Tokyo, Japan. Peaks were collected from room temperature to 1050 °C (heating rate 10 °C/min).

2.4. Asymmetric reduction of acetophenone (1) and its fluorinated derivatives (2–4) via BY encapsulated in sol–gel silica materials

The phenyl α-fluorinated ketones 2, 3, and 4 were prepared from fluorinated esters and the Grignard reagent in good yields according to the procedure described in our previous study [22]. The previously described BY encapsulated in sol–gel silica was added to a mixture of 5 mg acetophenone (1–4, in 0.5 mL of methanol) and 50 mg D-(+)-glucose in 3 mL of 0.1 M phosphate buffer pH 7. After being stirred on a reciprocal shaker at 120 rpm and 30 °C for 2 days, the mixture was extracted two times with ethyl acetate, and the extract was dried with anhydrous sodium sulfate. The mixture was filtered, and the filtrate was evaporated in vacuo. The residue was subjected to gas chromatography to determine the conversion rate and enantiomeric excess. Gas chromatograph was equipped with a flame ionization detector and a CP-cyclodextrin-2,3,6-M-19 column (0.25 mm × 50 m, 0.25 μm film, GL Sciences, Tokyo, Japan). A high-purity helium carrier gas was used at a constant flow rate (1 mL/min), and the temperatures of the column, injector, and detector were set at 145, 200, and 200 °C, respectively. The retention times were as follows: (*S*)-1a, 13.9 min; (*R*)-1a, 14.2 min; (*S*)-2a, 14.5 min; (*R*)-2a, 14.9 min; (*S*)-3a, 16.3 min; (*R*)-3a, 16.5 min; (*S*)-4a, 18.6 min; (*R*)-4a, 18.9 min. The reductive reaction of acetophenone by free BY solution prepared from 0.5 g of dry yeast dissolved in 3 mL of same buffer pH 7 was performed as a control. Each reported value is the mean of the results of at least three experiments.

2.5. Preparation of BYCF extract and its encapsulation in silica gel

Two grams of BY was dissolved in 7 mL of 0.1 M phosphate buffer pH 7, and 5 mL of glass beads ($\phi \sim 0.5$ mm) was added to the suspension. The suspension was mixed strongly with a vortex mixer for 30 s and was cooled for 60 s on ice; this protocol was repeated four times. After the crude mixture was centrifuged at 12,000 rpm for 15 min at 4 °C, the supernatant (approximately 4.5 mL) was obtained as the cell-free extract of BY.

2.6. Asymmetric reduction via BYCF systems

The cell-free extract (3.0 mL), obtained as described in Section 2.5, was added to a silica sol (0.5 mL), and the following procedure was similar to that described in Section 2.2. The reductive reaction was performed using two types of BYCF systems.

2.6.1. BYCF system 1

The reductive reaction via BYCF inside silica gel was performed as follows: a mixture of 5 mg trifluoromethyl phenyl ketone (4, dissolved in 0.5 mL of methanol) and 8.5 mg β-NADPH was added to 3.0 mL of 0.1 M phosphate buffer pH 7. After being stirred on a reciprocal shaker at 120 rpm and 30 °C for 24 h, the conversion rate (%) and enantiomeric excess (%ee) were determined by a method similar to that described in Section 2.4.

2.6.2. BYCF system 2

The cell-free extract (3.0 mL), obtained as described in Section 2.5, and glucose-6-phopsphate dehydrogenase (10 μL, 10 units)