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Covalent immobilization of chloroperoxidase onto magnetic beads: Catalytic properties and stability

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Abstract

Amino groups containing magnetic beads were used in covalent immobilization of the enzyme "chloroperoxidase (CPO)" which is one of a few enzymes that can catalyse the peroxide dependent oxidation of a wide spectrum of organic and inorganic compounds. The magnetic poly(glycidylmethacrylate-methylmethacrylate-ethyleneglycol dimethacrylate), magnetic p(GMA-MMA-EGDMA) beads were prepared via suspension polymerization in the presence of ferric ions. The magnetic beads were characterized with scanning electron microscope (SEM), Fourier transform infrared (FTIR), Mössbauer spectroscopy and vibrating sample magnetometer (VSM). The magnetic beads were derivatized sequentially with ammonia and glutaraldehyde, and CPO was covalently immobilized on the support via reaction of the amino groups of the enzyme under mild conditions. The effect of various parameters including pH, temperature and enzyme concentration on the immobilization efficiency of CPO onto glutaric dialdhyde activated magnetic beads was evaluated. Magnetic measurement revealed that the resultant CPO-immobilized magnetic beads were superparamagnetic with a saturation magnetization of 18.2 emu/g. The analysis of FTIR spectra confirmed the binding of CPO on the magnetic beads. The maximum amount of immobilized CPO on the magnetic beads was 2.94 mg/g support. The values of Michaelis constants K_m for immobilized CPO was significantly larger, indicating decreased affinity by the enzyme for its substrate, whereas V_{max} values were smaller for the immobilized CPO. However, the CPO immobilized on the magnetic beads resulted in an increase in enzyme stability with time.

Keywords: Magnetic beads; Enzyme immobilization; Chloroperoxidase; Kinetic parameters; Enzyme stability

1. Introduction

The use of magnetic beads for the immobilization of enzymes and cells has received attention for industrial manufacturing of biocatalysts-processed products [1–4]. In addition, functional magnetic beaded support materials have become increasingly attractive in enzyme immobilization technology. These applications are mainly based on the magnetic properties of the solid-phase that enables to achieve a rapid separation in a magnetic field. Moreover, the magnetic supports can be easily stabilized in a fluidized bed reactor for continuous operation of enzyme by applying an external magnetic field, and the use of magnetic supports can also reduce the capital and operation costs [5–9]. The enhancement of the enzyme operational stability and easy recovery from reaction mixture are among the requirements for the commercial use of an enzyme. The covalent immobilization of an enzyme to magnetic support can be an effective way to improve the enzyme stability.

Chloroperoxidase (E.C. 1.11.1.10) is a heme-containing enzyme that exhibits catalase, peroxidase and cytochrome P450 activities besides the halogenation reaction [10–12]. CPO has 321 amino acids with predominantly acidic residues and a p*I* in the range of 3.2–4.0. It is a 42.000 molecular weight glycoprotein produced by *Caldariomyces fumago*. CPO is able to catalyze reactions such as heteroatom oxidation (N- and S-oxidation), epoxidations, hydroxylation, and oxidation of alcohols and indole. Like most peroxidases, CPO can be used in fields such as analytical diagnosis, pharmaceuticals and removal of toxic wastes [13,14].

Immobilization of a variety of peroxidases has been reported on to synthetic and natural supports materials, but little

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work has been carried out on CPO immobilization [12–16]. In this study, acrylate based magnetic beads were prepared by copolymerization of monomers (i.e., glycidylmethacrylate, methylmethacrylate and ethyleneglycol dimethacrylate) via suspension polymerization. The epoxy groups of the magnetic beads were converted into amino groups in the presence of ammonia during thermal precipitation iron oxide crystal in the beads' structures. The aminated magnetic beads were used for the covalent immobilization of CPO via glutaric dialdehyde coupling and the immobilization of CPO via covalent attachment onto the magnetic beads from solutions was investigated in a batch system. Here, we describe the effects of immobilization conditions on the activity of CPO, and the resultant immobilized CPO was characterized under different reaction conditions.

2. Experimental

2.1. Materials

Chloroperoxidase (CPO; EC 1.11.1.10; from *Caldariomyces fumago* (∼3000 units/ml)), monochlorodimedone (MCDM), bovine serum albumin (BSA) and polyvinyl alcohol (PVA; MW: 50.000) were purchased from Sigma–Aldrich Chem. Co. (St. Louis, USA) and were used without further purification. Glycidyl methacrylate (GMA) methylmethacrylate (MMA), ethyleneglycoldimethacrylate (EGDMA) and α-α′-azo-isobisbutyronitrile (AIBN) were obtained from Fluka AG (Switzerland). The monomers GMA and MMA distilled under reduced pressure in the presence of hydroquinone and stored at 4 °C until use. All other chemicals were of analytical grade and were purchased from Merck AG (Darmstadt, Germany).

2.2. Preparation, activation and characterizations of magnetic p(GMA-MMA-EGDMA) beads

The magnetic p(GMA-MMA-EGDMA) beads were prepared in two sequential steps as described previously [9]. In the first step, ferric-p(GMA-MMA-EGDMA) beads were prepared via suspension polymerization. The discontinuous phase contained GMA (7.5 ml), MMA (7.5 ml), EGDMA (7.5 ml; as cross-linker) and 5.0% polyvinyl alcohol (20 ml, as stabilizer) was mixed together with 0.2 g of AIBN as initiator in 20 ml of toluene. The aqueous dispersion medium comprised of $FeCl_3$ solution (0.3 M, 400 ml) which was used as a precursor for the thermal iron-oxide precipitation in the beads. The polymerization reactor was placed in a water bath and heated to 65 °C. The reactor was then equipped with a mechanical stirrer, nitrogen inlet and reflux condenser. The polymerization reaction was maintained at 70 °C for 2.0 h and then at 80 °C for 1.0 h. After the reaction, the resultant beads were filtered under suction and washed with distilled water and ethanol.

In the second step, magnetic p(GMA-MMA-EGDMA) beads were prepared by conventional co-precipitation reaction of iron oxide in the beads (Fig. 1). For the co-precipitation reaction, 5.0 g $FeCl_2$ was dissolved in purified water (100 ml) and then was transferred into a reactor containing ferric-p(GMA-MMA-EGDMA) beads (15 g) in $NH_3{\cdot}H_2O$ (50 ml, 25% w/v). The reactor was equipped with reflux condenser and it was refluxed under nitrogen atmosphere at three different sequential temperatures (i.e., at 40 °C, 50 °C and 90 °C for 2 h) while continuous stirring. Finally, the synthesized magnetic p(GMA-MMA-EGDMA) beads were separated from the reaction medium, washed in ethanol solution (50%; 250 ml) for 3 h, and then washed with purified water.

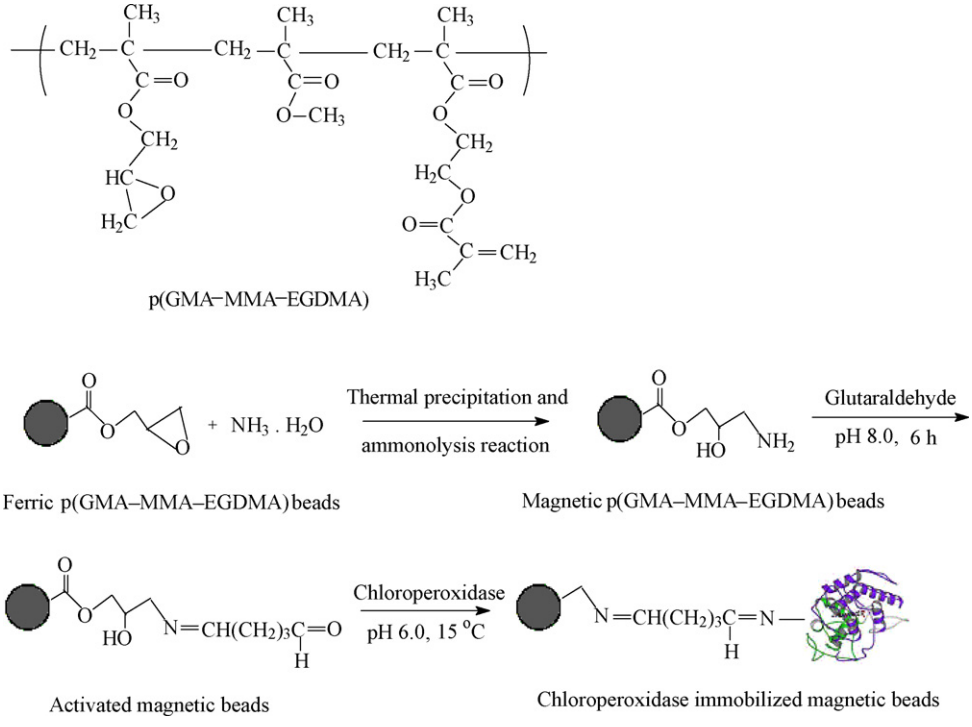

Fig. 1. The reaction schemes for immobilization of CPO onto magnetic beads.