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Oxime-functionalized cryogel disks for catalase immobilization

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ABSTRACT

Catalase is a protective enzyme against oxidative stress and converts hydrogen peroxide into water and molecular oxygen. In the current study, catalase immobilization was applied onto the oxime-functionalized cryogel disks. Cryogel disks were produced by free radical polymerization. After cutting as circular disks, oxime ligand (4-biphenylchloroglyoxime, BPCGO) was attached and oxime-functionalized cryogel disks were obtained. After optimization of several immobilization parameters such as pH, initial catalase concentration, temperature and ionic strength, maximum catalase load was detected as 261.7 ± 11.2 mg/g for cryogel disk at pH 5.0. Activity studies indicated that immobilization enhanced the enzyme activity in basic pH region, the temperature range of 15–35 °C and at ionic strengths between 0.2 and 1.0 M NaCl. *K*m was detected as 9.9 and 11.0 mM and V_{max} was 357.1 and 769.2 μmol min^{-1} for free and immobilized catalase, respectively. k_{cat} and Km/k_{cat} values showed that immobilization enhanced the catalytic efficiency. Storage stability experiments demonstrated that immobilization increased the usability period. Furthermore, catalase desorption was achieved by 1.0 M NaSCN at pH 8.0 successfully and catalase adsorption capacity of oxime-functionalized cryogel disk was decreased by 9.9% at the end of 5 adsorption-desorption cycle.

1. Introduction

Enzymes are biological and green catalysts with high efficiency and selectivity under mild conditions [1] although they have adverse properties such as instability, non-reusability, and inability to isolate from enzyme solution in free form [2]. Different strategies such as immobilization, chemical modification, and engineering of the protein and/or its medium can be applied to increase enzyme stability. Immobilized enzymes are produced by attaching to inert insoluble material [3]. Immobilized enzymes in industrial applications have the advantages such as lower quantity of required enzyme, easier recovery from reaction medium, and reusability with regard to free form [4,5]. Adsorption, entrapment, encapsulation, cross-linking, and covalent binding methods are preferably applied for enzyme immobilization [6]. Performance of immobilized enzymes is mostly based on the properties and microenvironment of the solid support material. Thus, immobilization conditions could be regulated for biomolecule-support material interaction to obtain optimum adsorption capacity and high stability [7].

Catalase (hydrogen peroxide oxidoreductase) is a unique enzyme found in plants and animal tissues. It is a protective enzyme against oxidative stress and converts hydrogen peroxide into water and molecular oxygen. Catalase contains four subunits, each having heme-group as a prosthetic group, and has a molecular weight of 240 kDa and an isoelectric point (pI) of 5.4 [8–10]. Catalase find several applications in medical, bioremediation, textile, food, and pharmaceutical industries [11]. In food industry, immobilized catalase is generally used for the removal of hydrogen peroxide after cold pasteurization and in milk pasteurization [12,13]. Immobilized catalase can be used to reduce environmental pollution with hydrogen peroxide removal from bleaching effluents [14]. Additionally, immobilized catalase is used for the production of gluconic acid with glucose oxidase [15], synthesis of dihydroxyacetone phosphate with l-α- glycerophosphate oxidase [16], and production of phenylpyruvic acid with d-aminoacid oxidase [17]. In biosensor field, catalase is used for H_2O_2 detection [10,18], glucose biosensor with glucose oxidase [18,19], γ-aminobutyric acid biosensor with d-glutamate oxidase [20], and glycolic acid biosensor with glycolate oxidase [21]. Catalase immobilization has been studied with several materials such as titania submicrospheres [2], nanofibers [7], magnetic composite beads [8], carbon nanotube films [9], chitosan membrane [10], electrospun nanofibers [11], modified florisil [12], hydrophilic mesoporous monoliths [22], polysulfone membrane [23], cryogel [24], and magnetic polymeric nanospheres [25].

Inorganic supports, synthetic polymers, and natural macromolecules are used as support materials in enzyme immobilization processes. Cryogels are polymeric gels synthesized in the frozen solutions of monomeric or polymeric precursors by cryotropic gelation. Cryotropic gel

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formation can occur during freezing, storage in the frozen state, or thawing stages, and it provides polymeric gels with varied morphology in comparison with traditional gels prepared in non-frozen solutions. The fundamental characteristic of cryogelation is solvent crystallization. These solvent crystals act as porogens, and melting of crystals supplies cavities causing macroporous structure. The interconnected macroporous and sponge-like nature of cryogels provides an advantage for cryogel applications such as chromatographic matrices and/or biocatalysts in both aqueous and organic solvents [26,27]. It is apparent that enzyme immobilization into the sponge-like morphology of cryogels enables unhindered diffusion in the macropores [27,28]. Furthermore, osmotic, chemical, and mechanical stability of macroporous cryogels offers many challenging options for biotechnologists [26].

Oximes has been growingly critical reagents in analytical, biomedical, and antimicrobial applications and drawn great interest with their usage as liquid crystals and dyes. Several colored chelates are commonly formed from different types of oximes such as as α-dioximes, α-keto oximes, amino oximes, and some transition metal salts that have usage in analytical applications [29]. Nowadays, oxime formations are easily used for interaction of biomolecules in various probes. Oximes can find application in F-labeling peptides and proteins [30], syntheses of sensors [31], organophosphate poisoning [32], modification of nanoparticles [33,34], derivation of carbohydrates for mass spectrometric analysis [35], and preparation of protein conjugates [36].

In this study, 4-biphenylchloroglyoxime (BPCGO) ligand attached p(HEMA) cryogel disks were utilized for catalase immobilization. Cryogel disks were selected due to their interconnected macroporous structure for the immobilization of the catalase which is a large protein with four subunits. Immobilization conditions were optimized by changing pH, initial catalase concentration, temperature, and ionic strength. Furthermore, the effects of pH, temperature, and ionic strength on enzyme activity were determined for both the free and the immobilized catalase. Kinetic parameters were also identified.

2. Materials and methods

2.1. Materials

2-hydroxyethyl methacrylate (HEMA, ≥99%) and *N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diamine (TEMED, ≥99%) was purchased from Fluka A.G. (Buchs, Switzerland). N,N′-Methylenebis (acrylamide) (MBAAm), ammonium persulfate (APS), and catalase (hydrogen peroxide oxidoreductase-E.C.1.11.1.6) were purchased from Sigma (St. Louis, USA). All other chemicals were of analytical purity. Deionized water which was used in this study was prepared using a Milli-Q ultra pure water purification system from Millipore (Milford, MA, USA).

2.2. Production of cryogel disks

Cryogel disks were produced by free radical polymerization started with APS and TEMED as initiator/activator pair [37,38]. HEMA and MBAAm were used as monomer and cross-linker, respectively. Briefly, HEMA (2.6 mL) and MBAAm (0.566 g) were dissolved in deionized water (30 mL). APS (0.04 g, 1% (*w*/w) of the monomer) was added, and polymerization solution was cooled in an ice bath for 5 min. TEMED (50 μL, 1% (*w*/*v*) of the monomer) was added to polymerization solution by stirring magnetically and was kept stirred for 2–3 more min. The final solution was placed between two glass plates separated with 1.5 mm thick spacers and left to polymerization at −16 °C. At the end of 24 h, p(HEMA) cryogel membrane was thawed at room temperature and washed with water and methanol. Finally, cryogel disks were obtained by shearing in circular shape.

2.3. Attachment of 4-biphenylchloroglyoxime (BPCGO) ligand to cryogel disks

BPCGO ligand was synthesized and characterized as described in the study of Karipcin and Arabalı [29]. The attachment of BPCGO was performed in the following manner. BPCGO ligand (100 mg) was dissolved in acetonitrile (ACN, 4 mL) and diluted with water (26 mL). Cryogel disks which were placed into BPCGO solution were incubated at 60 °C with shaking at 200 rpm for 1 h. After NaCl (150 mg) addition, cryogel disks were incubated for another 30 min. Na_2CO_3 (15 mg) was added, and the final solution was shaken at 70 °C for 2 h. At the end of ligand attachment process, cryogel disks were washed several times with water to remove excess ligand and dried in an oven at 50 °C. Dry p(HEMA)-BPCGO cryogel disks (20 ± 2 mg) were stored at 4 °C until use. The chemical structure of BPCGO ligand and photographs of BPCGO ligand, p(HEMA), and p(HEMA)-BPCGO cryogel disks are given in Fig. 1.

2.4. Characterization of p(HEMA)-BPCGO cryogel disks

FTIR spectra of BPCGO ligand, p(HEMA), and p(HEMA)-BPCGO cryogel disks were obtained by using a FTIR spectrophotometer (Perkin Elmer spectrum 100 FT-IR spectrometer) with universal ATR sampling accessory.

The surface morphology of BPCGO ligand and p(HEMA)-BPCGO cryogel disks were examined using SEM. The samples were initially dried in air at 25 °C. A part of the dried samples were mounted on a SEM sample mount and was sputter-coated for 2 min. The surfaces of the samples were scanned with SEM (Phillips, XL-30S FEG, Germany) at the desired magnification.

Cryogel disks are sponge-like and elastic materials. They can easily swell with water and can be squeezed by hand. Thus, swelling ratio, porosity, and macropore porosity are characteristic properties of cryogel disks. The swelling degree and water content of p(HEMA)-BPCGO were determined according to the study by Plieva et al. [39]. The dry p(HEMA) and p(HEMA)-BPCGO cryogel disks were filled with ionized

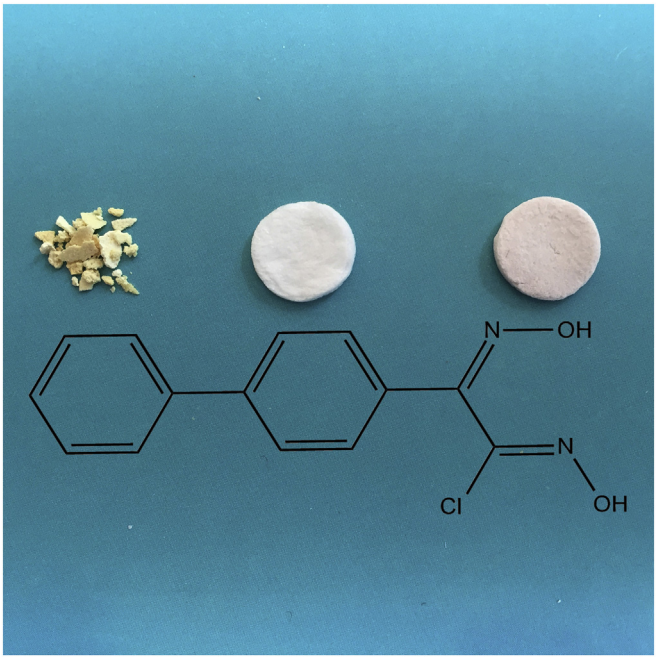

Fig. 1. Chemical structure of BPCGO ligand and photographs of BPCGO ligand, p(HEMA), and p(HEMA)-BPCGO cryogel disks.