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Immobilization of mushroom polyphenol oxidase on poly(allyl glycidyl ether-co-ethylene glycol dimethacrylate) macroporous beaded copolymers

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Abstract

Functional, macroporous, beaded copolymers containing epoxy groups were synthesized for immobilization of polyphenol oxidase (PPO) from edible mushroom (*Agaricus bisporus*). The effect of incorporation of two different sets of monomers such as glycidyl methacrylate (GMA) and allyl glycidyl ether (AGE) and the effect of cross-linking agent ethylene glycol dimethacrylate (EGDM) with varying cross-link densities on binding and expression of mushroom PPO activity were studied. The effect of porogen viz. cyclohexanol and hexanol on PPO immobilization was studied. AGE copolymers with hexanol as a porogen were found to give higher binding and expression of PPO activity than GE polymers. Cross-linking of amino groups of enzyme with 5% glutaraldehyde for 6 h gave a stable binding of PPO on AGE-75(Hex) polymer with storage half-life of approximately 25 days. Under optimum conditions, AGE-75(Hex) polymer gave 70.3% of activity yield while percent retention of PPO activity was found to be 83.5%. Immobilized PPO showed a broader pH, higher temperature and excellent storage stability.

Keywords: Enzyme immobilization; Mushroom PPO; Epoxy-activated support; Cross-linking agent

1. Introduction

Polyphenol oxidase (PPO) or tyrosinase (EC 1.14.18.1 and EC 1.10.3.1) is a copper enzyme which catalyses the hydroxylation of monophenols to *o*-diphenols and the oxidation of the latter to *o*-quinones using oxygen [1,2]. PPO activity has attracted interest for use in the synthesis of value-added products such as the substituted catechol, L-DOPA for the treatment of Parkinson's disease [3]. Furthermore, a number of other catechols have found applications as fine chemicals or as starting materials for pharmaceutical drug synthesis [4]. PPO also plays an important role in the formulation of

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cleaning agents for wastewater-containing polyphenols [5]. Besides, PPO-based amperometric biosensors have proved to be very useful for the determination of phenols and substituted phenols at low levels [6,7].

PPO is a moderately unstable enzyme, which is susceptible to degradation at elevated temperature and extreme conditions of pH. It also tends to lose its catalytic activity upon storage [8,9], which ultimately restricts its commercial applications. Immobilization confers stability to the enzyme against denaturation by preventing conformational changes and protecting it in a confined microenvironment. Furthermore, immobilization of enzymes is known to offer several advantages such as ease of separation from a reaction mixture, improved stability and repeated reuse in continuous processes, enabling greater control over catalytic processes and process economics [10].

Enzymes have been immobilized on various supports either by physical adsorption, covalent binding or entrapment. The immobilization of enzymes inside porous supports via covalent binding may improve the enzyme stability by limiting external access to proteolytic enzymes and by preventing enzyme stacking and aggregation. The enzyme is also protected from gross interactions with external interfaces (air, oxygen, immiscible organic solvents, etc.) [11]. The extent of stabilization depends on the enzyme's structure, the immobilization method and the type of support. During the last decade numerous supports for immobilization have been investigated [12,13].

Porous polymers have been studied extensively as supports for immobilization of enzymes [14,15]. The porosity of these polymer supports has a direct bearing on diffusion of substrate and product through them, which affects rate of enzymatic conversion. It is reported that presence of meso and/or macropores is a key requirement to minimize diffusional limitations [15]. Reactive functional groups assist the formation of a permanent covalent bond between the polymer and the enzyme without affecting its tertiary structure. Polymer containing epoxide side groups are of technical interest as they provide almost ideal conditions for stable immobilization of enzymes both at laboratory and industrial scale. There are several advantages of epoxy-activated supports in terms of storage stability, reactivity with different protein groups (amino, thiol, phenolic ones) that lead to the formation of stable covalent linkages. Moreover, the epoxy (oxyranyl) group is perhaps the preferred functional group since covalent binding of enzyme proceeds under very mild experimental conditions (e.g. pH 7) without significant loss in catalytic activity [11].

Glycidyl methacrylate (GMA) and allyl glycidyl ether (AGE) are commonly preferred monomers, which can be easily modified into various functional groups. GMA and AGE are bifunctional molecules containing terminal epoxy groups. Besides the terminal epoxy group, glycidyl methacrylate monomer has acrylic functionality whereas allyl glycidyl ether contains allyl functionality. The acrylic/allyl functionality allows copolymerization with a variety of other vinyl monomers in aqueous and non-aqueous systems and the resulting polymers give a unique combination of epoxy functionality with an acrylic backbone. As polymerization of GMA or AGE proceed, the epoxy groups of the GMA become useful for the introduction of various functional groups, such as amines, alcohols [16–18] and proteins [19,20]. Moreover, theses copolymers can also be employed for grafting of natural/synthetic polymers and reported in the literature for immobilization of variety of enzymes like lipase [21], urease [22], trypsin [23], etc.

In the present study, PPO was immobilized on epoxy-activated macroporous polymers in order to stabilize the catalytic activity of enzyme upon storage. Different compositions of epoxy-activated poly(GMA-co-EGDM) and poly(AGE-co-EGDM) macroporous beaded copolymers were evaluated for binding and expression of mushroom PPO. Two different types of hydrophobic epoxy copolymers were synthesized by varying cross-link densities while cyclohexanol and hexanol were used as the pore generating solvent (porogen). This resulted in a series of porous beads with different pore volume, pore size, surface area and varying distribution and concentration of reactive epoxy pendent groups. The effects of these varying conditions on the immobilization of mushroom PPO are reported.

2. Materials and methods

2.1. Materials

Glcidyl methacrylate (GMA), allyl glycidyl ether (AGE) and ethylene dimethacrylate were obtained from Sigma–Aldrich (USA), 2,2′-azobis(isobutyronitrile) (AIBN) (SISCO, India) was used as initiator, cyclohexanol (S.D. Fine Chem., India), poly vinyl pyrrolidone (PVP, K-90) Aldrich Chemical Co., USA. All other chemicals were of analytical grade from local suppliers.