



Wood mimetic hydrogel beads for enzyme immobilization



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ABSTRACT

Wood component-based composite hydrogels have potential applications in biomedical fields owing to their low cost, biodegradability, and biocompatibility. The controllable properties of wood mimetic composites containing three major wood components are useful for enzyme immobilization. Here, lipase from *Candida rugosa* was entrapped in wood mimetic beads containing cellulose, xylan, and lignin by dissolving wood components with lipase in [Emim][Ac], followed by reconstitution. Lipase entrapped in cellulose/xylan/lignin beads in a 5:3:2 ratio showed the highest activity; this ratio is very similar to that in natural wood. The lipase entrapped in various wood mimetic beads showed increased thermal and pH stability. The half-life times of lipase entrapped in cellulose/alkali lignin hydrogel were 31- and 82-times higher than those of free lipase during incubation under denaturing conditions of high temperature and low pH, respectively. Owing to their biocompatibility, biodegradability, and controllable properties, wood mimetic hydrogel beads can be used to immobilize various enzymes for applications in the biomedical, bioelectronic, and biocatalytic fields.

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1. Introduction

Composites are engineered materials that have two or more constituents with different physical or chemical properties (Šimkovic, 2008). Recently, biopolymer-based composites have received considerable attention for applications in biomedical fields, as they are biocompatible, biodegradable, and possess a high degree of functionality (Kim, An, Won, Kim, & Lee, 2012; Lee, Miyauchi, Dordick, & Linhardt, 2010). Biopolymers are naturally obtainable macromolecules including polysaccharides, polyphenols, polyesters, polyamides, and proteins. The inherent biodegradability of these materials makes biopolymers particularly promising for developing environmentally friendly materials (Simmons et al., 2011). However, the preparation of such biopolymer-based composite materials remains a challenge because of the low solubility of many biopolymers in conventional hydrophobic or hydrophilic solvents such as hexane, acetone, dimethylsulfoxide, and *N,N*-dimethylacetamide.

Lignocellulosic biomass, such as agricultural residues, forestry wastes, waste paper, and energy crops, has long been recognized for

its potential value as a sustainable source of biopolymer composites (Himmel et al., 2007; Li, Wang, & Zhao, 2008). Lignocellulose consists of three major biopolymers, that is, cellulose, hemicellulose, and lignin, which have distinct chemical, physical, and structural properties. Cellulose, a linear polysaccharide of D -glucose residues linked by β -(1 \rightarrow 4)-glycosidic bonds, is the most abundant renewable biopolymer on earth and its wood content ranges from 41% to 50% (Brown, 2004). It has excellent thermal and mechanical properties and biocompatibility and is a promising material for biochemical engineering because of economic and scientific reasons (Gemeiner, Stefuca, & Bales, 1993). Hemicelluloses are heterogeneous, branched polymers of pentoses, hexoses, and acetylated sugars, with xylans being the most plentiful of the hemicelluloses. They are as commonly found as cellulose, but their molecular weight is lower than that of cellulose and their wood content ranges from 25% to 35% (Saha, 2003). Lignin is an aromatic network polymer composed of phenylpropanoid units and helps to bind cellulose and hemicelluloses together. Its wood content ranges from 18% to 35% (Chandra et al., 2007; Pettersen, 1984). Lignin is more hydrophobic than cellulose and hemicelluloses, and its properties are dependent on the extraction methods used. Although lignin is usually recognized as waste material, in recent years, many studies on the application of lignin have been reported (Chung & Washburn, 2012; Lora & Glasser, 2002; Stewart, 2008). To prepare

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lignocellulose-based materials, the development of solvents with high dissolving power for lignocellulose is very important, because lignocelluloses are highly difficult to handle due to their crystalline structure.

Recently, we reported the preparation of lignocellulose-based materials and biomimetic synthetic wood composites containing cellulose, hemicellulose, and lignin by using ionic liquids (ILs) (Ahn et al., 2012; Kang et al., 2013; Lee, Kim, Lee, Lee, & Park, 2011; Park et al., 2011; Simmons et al., 2011). Cellulose/starch/lignin film, lignocellulose aerogel, and all-wood composites were also prepared by using ILs (Wu, Wang, Li, Li, & Wang, 2009; Li et al., 2011; Lu et al., 2012; Shibata, Yamazoe, Kuribayashi, & Okuyama, 2013). ILs are organic salts that usually melt below 100 °C. The interest in ILs stems from their potential application as “green solvents” due to their high thermal stability and non-volatility (Sheldon, Lau, Sorgedragger, & van Rantwijk, 2002). In chemical processes, ILs exhibit excellent physical characteristics, including the ability to dissolve polar and non-polar organic, inorganic, and polymeric compounds (Lee, Doherty, Linhardt, & Dordick, 2009). ILs have also been shown to be capable of dissolving lignocellulosic biomasses. In our previous work, three major components of wood were fully dissolved in 1-ethyl-3-methylimidazolium acetate ([Emim][Ac]) and successfully reconstituted to various forms such as hydrogel, thin film, and microfiber by molding, film casting, and electrospinning (Lee et al., 2011; Park et al., 2011; Simmons et al., 2011). Fabrication of homogeneous composites from cellulose/hemicellulose/lignin was achieved via an uncomplicated process by using [Emim][Ac]. Biomimetic synthetic wood films containing cellulose, xylan, and lignin showed smoother surface textures, higher water resistance, and higher tensile strengths than cellulose films. In particular, tailor-made synthetic wood composites with controllable properties, including hydrophilicity/hydrophobicity, dielectric constant, cohesiveness, and light protection, could be prepared by changing the ratio of cellulose, xylan, and lignin (Simmons et al., 2011). A variety of additives, such as polyethylene glycol, multi-walled carbon nanotube, chitosan, porphyrin, and poly(3-octylthiophene), could also be entrapped in the synthetic wood composites by simple dissolution or by dispersion of the additives to the wood solution containing the three wood components (Lee et al., 2011; Park et al., 2011; Simmons et al., 2011). Biomimetic synthetic wood composites can provide great opportunities to develop environmentally friendly and biocompatible materials.

Enzymes have been recognized as efficient and environmentally friendly catalysts because of their high specificity and catalytic activity under mild conditions. However, the industrial applications of enzymes have been limited due to their low stability and difficult recovery for subsequent use. Enzyme immobilization is the most commonly used strategy for overcoming these drawbacks (Sheldon, 2007). Entrapment, an immobilization technique, can be defined as the physical restriction of an enzyme within a confined polymer network such as a hydrogel. Hydrogels are three-dimensional polymer networks that are swollen because of the presence of large amounts of water. On the basis of the source, hydrogels can be divided into natural polymer-based gels and synthetic polymer-based gels. On the basis of the cross-linking method, hydrogels can be divided into chemical gels and physical gels. Recently, biopolymer-based hydrogels have received considerable attention in biomedical fields, including tissue engineering, drug delivery systems, contact lenses, and biosensors because of their safety, hydrophilicity, biocompatibility, and biodegradability (Chang & Zhang, 2011; Vlierberghe, Dubruel, & Schacht, 2011). Various polysaccharide hydrogels, such as alginate, chitosan, agarose, carrageenan, and gelatin have been employed for the entrapment of a number of enzymes such as lipases, lactases, invertases, and peroxidases (Betigeri & Neau, 2002; Jegannathan, Chan, & Ravindra, 2009; Cheirsilp, Jeamjounkhaw, & H-Kittikun, 2009;

Matto & Husain, 2009; Won, Kim, Kim, Park, & Moon, 2005; Pulat & Akalin, 2013). However, only a few studies have been performed on the entrapment of enzymes in non-derivatized cellulose hydrogels. Recently, Turner, Spear, Holbrey, and Rogers (2004) attempted to use [Bmim][Cl] to entrap laccase in a cellulose membrane. The enzyme was entrapped in cellulose but showed low residual activity because of IL-induced denaturation. More recently, we reported the successful entrapment of lipase in cellulose/chitosan, cellulose/agarose, and cellulose/carrageenan composites by using biocompatible [Emim][Ac] (Kim et al., 2012). Cellulose/biopolymer composite hydrogels proved to be good supports for the entrapment of lipase and have many potential applications.

In this work, lipase from *Candida rugosa*, which has many industrial applications in the fields of pharmaceuticals, cosmetics, food, perfumery, and bioremediation, was entrapped in wood mimetic composite hydrogels for the first time. Knowledge regarding which properties of synthetic wood composites can be modified may be beneficial for increasing the activity and stability of the immobilized lipase. The effects of the source and composition of each component in the wood mimetic composite hydrogel on the activity and stability of the entrapped lipase were also investigated in the present study.

2. Experimental

2.1. Materials

Cellulose (microcrystalline cellulose; cotton linter with medium fiber form; cotton linter with long fiber form), xylan from birchwood, alkali lignin, organosolv lignin, [Emim][Ac], *p*-nitrophenyl butyrate, and lipase from *C. rugosa* were purchased from Sigma-Aldrich (St. Louis, MO, USA). Isopropanol, acetic acid, sodium acetate trihydrate, and acetonitrile were purchased from Samchun Pure Chemical (Gyeonggi-do, South Korea). *p*-Nitrophenol was obtained from Kanto Chemical (Tokyo, Japan). Milled wood lignins (MWLs) from *Pinus rigida* and *Populus albaglandulosa* were prepared according to the Bjorkman method (Bjorkman, 1956). All other chemicals used in this study were of analytical grade and were used without further purification.

2.2. Preparation of wood component-based composite hydrogel beads containing lipase

To prepare wood component-based composite hydrogel beads containing lipase, we used our previously reported method with slight modifications (Kim et al., 2012). Various contents of cellulose, xylan, and lignins were dissolved in 5 mL of [Emim][Ac] while stirring at 80 °C for 1 h. Transparent solutions containing wood components were dried under vacuum at 60 °C to remove air bubbles. One milliliter of each wood component solution was mixed with 50 mg of lipase powder at room temperature. The resultant mixture was then added drop-wise into 1 L of distilled water at a rate of 50 μ L/min with vigorous stirring by using a 1-mL plastic syringe with a 26-gauge needle and a syringe pump (LSP01-2A; Longer Pump; China). The hydrogel beads were cured in distilled water for 30 min. Washing was conducted three times, and the absence of [Emim][Ac] was confirmed by measuring the optical density of the washing solution at 211 nm.

2.3. Determination of lipase activity

The hydrolytic activity of the entrapped lipase was determined by conducting a spectrophotometric assay. A hydrogel bead containing lipase was placed in a 50-mL Falcon tube containing 9.5 mL of 0.1 M phosphate buffer (pH 7.0). The reaction was

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