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Preparation and characterization of cross-linked enzyme aggregates (CLEAs) of recombinant poly-3-hydroxybutyrate depolymerase from *Streptomyces exfoliatus*

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ABSTRACT

Cross-linked enzyme aggregates of poly-3-hydroxybutyrate (PHB) depolymerase from *Streptomyces exfoliatus* (PhaZ$_{Sex}$–CLEAs) have been prepared. Acetone was used as the precipitating agent, while addition of bovine serum albumin (BSA) facilitated CLEAs formation. Conditions for enzyme precipitation and cross-linking have been optimized, and confocal scanning microscopy showed a homogeneous enzyme distribution in the biocatalyst. Obtained PhaZ$_{Sex}$–CLEAs presented an average size of 50–300 μm, showing a high PHB depolymerase activity of 255 U/g wet biocatalyst at 40 °C and pH 7.0. Temperature-activity profile of PhaZ$_{Sex}$–CLEAs at pH 8.0 showed that the highest activity for *p*NPB hydrolysis was achieved at 60 °C, whereas pH-activity profile at 40 °C indicated that highest activity for PHB hydrolysis was achieved at pH 7.0. Additionally, immobilized biocatalyst could be recycled at least for 20 consecutive batch reactions without loss of catalytic activity, and showed higher pH and temperature stability, and better tolerance to several organic solvents than its soluble counterpart.

1. Introduction

Preparation of cross-linked enzyme aggregates (CLEAs) has emerged as a promising carrier-free immobilization strategy (Sheldon et al., 2005; Sheldon, 2007a). As a matter of fact, CLEA technology has been proposed as an alternative to conventional immobilization methods on solid carriers (Sheldon, 2007b). In this sense, enzyme attachment to solid supports greatly reduces the specific and volumetric activity of the biocatalysts, whereas self-immobilized enzyme particles show many clear advantages, such as highly concentrated enzyme activity in the biocatalyst, high stability and low production cost due to the exclusion of an additional support (Wang et al., 2010). Firstly developed in 2000 (Cao et al., 2000), CLEA technology is a very simple method which consists of enzyme precipitation with addition of a salt or an organic solvent, followed by cross-linking of the resulting aggregates with bifunctional reactive agents such as glutaraldehyde. As regards the latter, is capable of reacting with the ε-amine groups of lysine residues to yield an insoluble biocatalyst with high activity. In some cases, such cross-linking reaction may modify essential residues which results in significant loss of enzymatic activity. Then, an alternative cross-linking reagent must be selected. In other cases, cross-linking may be not expectedly effective with enzymes with low ε-amine content, thus resulting in CLEAs with low mechanical stability, or releasing enzyme molecules into the reaction media. These problems can be sorted out aggregating the enzyme in the presence of certain additives with large number of amine groups such as polyionic polymers (Wilson et al., 2009) or bovine serum albumin (Shah et al., 2006; Cabana et al., 2007; Dong et al., 2010; Majumder and Gupta, 2010).

Polyhydroxyalkanoate depolymerases are microbial enzymes which degrade polyhydroxyalkanoates (PHAs), biopolyesters produced by a wide range of bacteria when the environmental conditions are not optimal for growth (Anderson and Dawes, 1990; Madison and Huisman, 1999; Jendrossek and Handrick, 2002). The most common and well-studied PHA is poly-(*R*)-3-hydroxybutyrate (PHB), which is an amorphous polymer *in vivo* that becomes partially crystalline after release from accumulating cells, e.g., after cell lysis. Crystalline PHB, referred as "denatured" PHB, can only be degraded by specialized PHB-degrading microorganisms that secrete extracellular depolymerases (EC 3.1.1.75) into the environment (Jendrossek and Handrick, 2002; Tokiwa and Calabia, 2004). Application of PHB depolymerases to produce chiral (*R*)-3-hydroxybutyric acid from PHB as starting material has been attracting much attention in biotechnology. In this sense, (*R*)-3-hydroxybutyric acid has proven to exhibit antimicrobial, insecticidal, and antiviral activities (Chen and Wu, 2005), and can be used as a chiral building block for the synthesis of fine chemicals such as antibiotics, vitamins, flavors, and pheromones (Steinbüchel and Valentin, 1995; Lee et al., 1999; Ren et al., 2005, 2010).

A large number of streptomycetes strains have been distinguished by its ability to hydrolyze PHB (Klingbeil et al., 1996;

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