Applied Surface Science 444 (2018) 42–47

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Full Length Article

Sodium alginate-grafted β-cyclodextrins as a matrix for immobilized *Arthrobacter simplex* for cortisone acetate biotransfromation

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ARTICLE INFO

Article history:
Received 18 January 2018
Revised 18 February 2018
Accepted 4 March 2018
Available online 7 March 2018

Keywords:
Sodium alginate
β-Cyclodcxtrin
Graft
Biotransformation
Recycle

ABSTRACT

Cyclodextrins (CDs) are used to resolve the low aqueous solubility of steroids, but the high cost of CDs is still a limiting factor in biotransformation process. This study, which is based on grafting and immobilization techniques, focused on synthesizing for the first time sodium alginate (SA)-grafted β-CD (SA-β-CD) and alginate-grafted β-CD for the immobilization of *Arthrobacter simplex* (*ASP*) cells (SA-β-CD-cells) and subsequent recycling of CDs and cells. FTIR spectium and X-ray diffraction proved that β-CD was successfully grafted with SA, whereas the grafting yield of β-CD was 10.3 μmol g^{-1}. SA-β-CD could increase the solubility of CA by 3.5-fold, whereas the transformation rate was enhanced by 10%. The conversion ratio of CA was over 92% after the SA-β-CD recycling for nine cycles. In addition, after SA-β-CD-cells were applied in biocatalytic reactions for eight cycles, the conversion ratio of CA was over 90%. These advantages suggest great potential for using both grafting and immobilized techniques in steroid transformation.

1. Introduction

As the second largest drug category after antibiotics, steroids are vital in treating and preventing various diseases, like coronary heart disease, breast and prostate cancer. Although the chemical method has been widely used in the pharmaceutical industry, recent attention is focused on preparation steroid intermediates by employing "green" and efficient enzymatic processes [1]. Using microbial transformation of steroid compounds has many advantages, including yielding products of high purity at low cost and production of steroid-active pharmaceutical ingredients and key intermediates, when compared with using the traditional chemical synthesis process [2]. Cyclodextrins (CDs), which are wildly used as solubilizers and stabilizers of several steroid drugs during biotransformation, can selectively include a wide range of guest molecules into their hydrophobic cavity to increase the degree of conversion [3–6]. Although CDs have advantages over aqueous media, their high cost is still a limiting factor in their utilization. Therefore, developing appropriate methods is imperative for the widespread application of CDs in microbial steroid transformation.

Grafting technology focuses on design and synthesis and has been widely applied in medicine for drug delivery [7–9]. CDs, as environment-friendly auxiliaries, can be grafted onto macromolecules with crosslinkers [10–12], which then combine the advantageous properties of sodium alginate (SA) to form synthetic polymers [13]. Previous studies have suggested that grafting technology may contribute to the stability of the material [14], whereas increase the adsorption capacity[15]. Grafting CDs onto natural polymer materials could be accomplished by placing a water-soluble powder material into a water-insoluble polymer to achieve recycling. SA, which is a linear and anionic polysaccharide consisting of two kinds of 1,4-linked hexuronic acid residues capable of forming hydrogels with divalent cations, has been used as an immobilizing agent for microorganisms and cells [16–18]. In addition, immobilized microorganisms have been widely used for biosynthesis and biotransformation [19,20]. The main advantages of using immobilized microorganisms are their high cell density, enhanced the stability of the biocatalyst, and facilitation of recovery and reuse [21,22]. Introducing functional agents into CDs using SA as a carrier and then immobilizing cells to form gel beads could result in the recycling of CDs and cells. Therefore, the exploitation of the combined grafting and immobilization techniques could fully utilize CDs and cells in bioconversion reactions.

In this study, the 1-dehydrogenation of cortisone acetate (CA) by *Arthrobacter simplex* (*ASP*) CPCC 140,451 was selected as the experimental model. We synthesized SA-grafted β-CD (SA-β-CD) and immobilized *ASP* cells (SA-β-CD-cells) in SA-β-CD, and

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https://doi.org/10.1016/j.apsusc.2018.03.028
0169-4332/

epichlorohydrin, as cross-linking agents, to form gel beads. The optimum grafting process was determined by biotransformation tests, and the efficiencies of the two gel beads were evaluated. The process of recycling SA-β-CD and SA-β-CD-cells during CA biotransformation was also studied.

2. Methods

2.1. Materials

CA standard with ≥98% purity was provided by Xianju Pharmaceutical Company Ltd. (Zhejiang, China). All other reagents and solvents were purely chromatographic or of analytical grade.

2.2. Bacterial strain and cultivation

ASP CPCC 140,451 was stored in our laboratory and was prepared using a method with two cultivation steps, as previously described [3]. The *ASP* resting cells were harvested after being centrifuged thrice at 4 °C, rinsed, and resuspended in 100 mM Tris–HCl buffer (pH 7.5).

2.3. Preparation of SA-β-CD and immobilized cell gel beads

A total of 0.9 g of SA and 2.8 g of β-CD were added to 50 mL deionized water. Then, epichlorohydrin and 10 mL NaOH solution (0.5 mol L^{-1}) were slowly added. The gelation solution was shaken in a water bath for 3.5 h at a constant temperature of 70 °C. The obtained solution was cooled to room temperature. Then, SA-β-CD gel beads were formed by extruding the mixture into a $CaCl_2$ solution (0.1 mol L^{-1}) with a syringe and then letting the concoction to solidify for 2 h. The gel beads were filtered and washed with Tris-HCl buffer. To prepare the SA-β-CD-cell gel beads, *ASP* cell suspensions were added to the prepared mixture of cooling gel per a previously described method [23]. The same procedure was used to prepare the SA-β-CD-cell gel beads. The Schematic representation of preparing SA-β-CD and SA-β-CD-cells gel beads are illustrated in Fig. 1. All gel beads were refrigerated at 4 °C.

2.4. Effect of SA-β-CD on CA solubility

To determine the effect of SA-β-CD on CA solubility, excess CA was added to the aqueous phase (20 mL) with SA-β-CD in 250 mL shake flasks at 32 °C and 180 rpm for 24 h, as previously described [24]. The shake flasks were tightly sealed. The samples were filtered through a 0.2 μm filter, dried in vacuum, and then redissolved in mobile phase methanol/water (80:20, v/v). The total concentration of CA in the filtrate was analyzed using high-performance liquid chromatography (HPLC) (Agilent 1100, USA). The area of each peak was measured by the integrator and transformed to concentration using a standard CA curve.

2.5. The activity of dehydrogenase

ASP cells were immobilized in SA-β-CD gel beads. The activity of dehydrogenase compared with control (free cells) was determined by triphenyl tetrazolium chloride (TTC) analysis measured at 485 nm as previously described [3]. The samples were added 2 mL 0.5 % (w/v) TTC and 1 mL Tris-HCl, and after incubating at 37 °C for 40 min. The reaction was terminated by 0.5 mL formaldehyde, and then centrifuged for 5 min. The precipitate was washed once with Tris-HCl and then extracted with 80% acetone. The absorbance of the combined supernatants was measured at 485 nm. The level of dehydrogenase activity is indicated by OD_{485}. The greater the absorbance, the higher the dehydrogenase activity.

2.6. Biotransformation of CA and reuse of β-CD and cells

CA (0.06 g) and 3 g SA-β-CD were added into a 20 mL of cell suspension at 34 °C and 180 rpm for 8 h. Free cells incubated without β-CD were used as the control. For the biotransformation of immobilized cells, CA (0.06 g) and 10 mL SA-β-CD-cell gel beads were added into 20 mL deionized water at 34 °C and 180 rpm for 10 h. Immobilized cells incubated without β-CD were used as control. After the activity of immobilized cells had decreased, the plastic beads were placed into the fermentation medium, activated for 20 h, and then solidified again for 2 h.

The samples, which were taken at every 2 h, were extracted with ethyl acetate, redissolved in the mobile phase (dichlorome thane/ether/methanol, 86:12:3.6, v/v/v) and analyzed using HPLC, as described by Zhang et al. [25]. All the experiments were conducted in triplicate to ensure data quality. The collected SA-β-CD and SA-β-CD-cell gel beads were washed with Tris-HCl buffer (pH 7.5) and used in the next cycle.

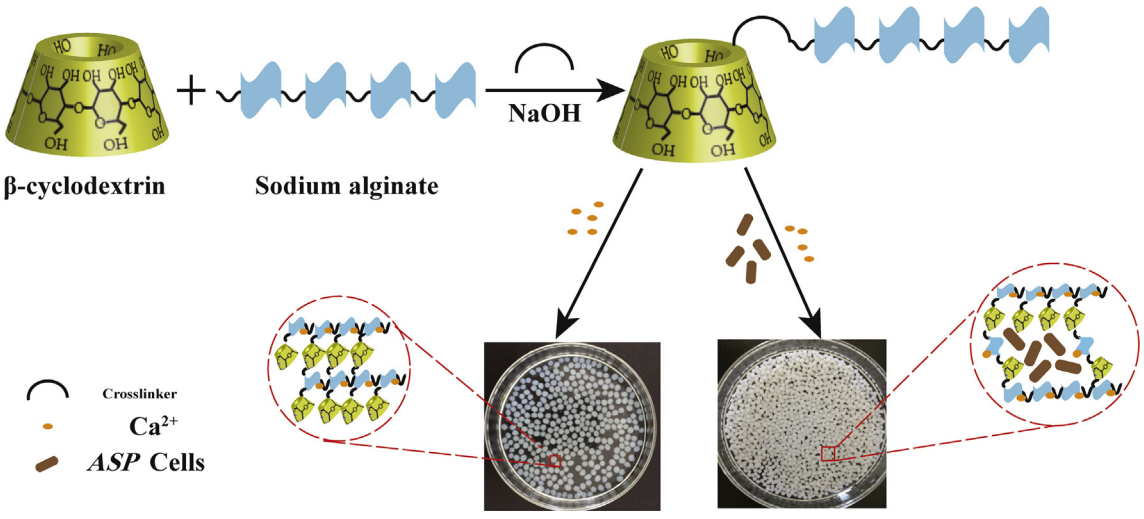

Fig. 1. The Schematic representation of preparing SA-β-CD and SA-β-CD-cells gel beads.