Journal of Molecular Catalysis B: Enzymatic xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Dextransucrase immobilized on activated-chitosan particles as a novel biocatalyst

Natália G. Graebin, Diandra de Andrades, Marina C. Bonin, Rafael C. Rodrigues, Marco A.Z. Ayub*

Biotechnology, Bioprocess, and Biocatalysis Group, Food Science and Technology Institute, Federal University of Rio Grande do Sul, Av. Bento Gonçalves 9500, PO Box 15090, ZC 91501-970 Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 15 August 2016
Received in revised form
12 December 2016
Accepted 13 December 2016
Available online xxx

Keywords:
Dextransucrase
Enzymatic covalent immobilization
Chitosan
Dextran
Oligosaccharides

ABSTRACT

Dextransucrase from *Leuconostoc mesenteroides* B-512F was covalently immobilized on glutaraldehyde-actived chitosan particles. The best initial protein loading (400 mg/g of dried support) showed 197 U/g of catalytic activity. The optimal reaction pH and temperature of this new biocatalyst were determined to be 4.5 and 20 °C, respectively. Regarding the thermal stability, the immobilization enhanced enzyme protection against high temperatures, whereas glucose and maltose acted as stabilizers. The biocatalyst was stable under storage at 5 °C for a month. The biocatalyst presented good operational stability, retaining up to 40% of its initial activity after ten batch cycles of reaction to obtain oligosaccharides. These results suggest the use of the immobilized dextransucrase on chitosan particles as a promising novel biocatalyst to produce dextran and oligosaccharides.

1. Introduction

Glucansucrases, belonging to the glycoside-hydrolase GH70 family of enzymes, are extracellular enzymes, recognized as GRAS for food applications, and are produced by lactic acid bacteria, especially of the genera *Leuconostoc*, *Streptococcus*, *Lactobacillus,* and *Weisella* [1]. Among the glucansucrases, dextransucrase (sucrose: 1,6-α-D-glucan 6-α-D-glucosyltransferases, EC 2.4.1.5) has become the most intensively studied enzyme, especially the one from *Leuconostoc mesenteroides* strain B-512F, because of the characteristics of dextrans and oligosaccharides obtained by this enzyme, [2–4]. This enzyme catalyzes mainly polymerization and acceptor reactions. Polymerization comprises the transfer of glucosyl moieties, in presence of sucrose, onto α-glucans [5,6], whereas in the acceptor reaction, the glucosyl moieties are transferred to the non-reducing end of acceptor molecules producing oligosaccharides [7].

There is a growing interest in the enzymatic synthesis of dextrans and oligosaccharides because these products are so far difficult to be obtained via chemical synthesis. These molecules have an extensive use in the food, feed, cosmetic, and pharmaceutical industries [8–10]. Dextrans can act as functional ingredients as stabilizing, emulsifying, and gelling agents [11]. Concerning oligosaccharides, many of them hold prebiotic potentials, being selectively digested by *Bifidobacteria* and *Lactobacillus* in the intestine of humans and animals, stimulating the growth of these beneficial bacteria and, as a consequence, improving the host health [12–15].

In order to assure continuous processing and reuse of the biocatalyst in industrial applications of dextransucrase, an effective enzyme immobilization technique would be required. Reports on the literature show that the most studied immobilization technique for dextransucrase is the process of enzyme encapsulation. Comparing this technique with other immobilization systems, such as covalent binding and adsorption, the encapsulation presents higher immobilization yields, which varies from 57% to 98% [16–20]. However, this method presents some limitations, especially concerning substrates and products diffusion problems, swelling of particles, protein leakage to the medium, as well as low operational stability under continuous operation [16,21–23].

Another important technique for enzyme immobilization is the covalent attachment using glutaraldehyde, which is the most used approach to immobilize many enzymes [24]. The linkage of enzyme and activated-support takes place between the most reactive amino group exposed on the enzyme structure, which usually is the terminal amino group [24,25]. The covalent attachment methodology is simple to be carried out and efficient, the glutaraldehyde-protein

* Corresponding author.
E-mail address: mazayub@ufrgs.br (M.A.Z. Ayub).

http://dx.doi.org/10.1016/j.molcatb.2016.12.007
1381-1177/

bonds formed are stable, promoting good stabilization factors. This approach can be used in very different reaction conditions [24,26,27].

Several supports have already been evaluated to covalently bind dextransucrase, among them the Bio-Gel P-2 [28], polyacrylamide gel, cellulose acetate membranes, polysulfone hollow fibers [29,30], akylamine porous silica [31], and porous silica activated with α-aminopropyl and glutaraldehyde [32,33]. Most of these studies reported low immobilization yields, low specific activities and poor operational stabilities. The main problems associated with these types of immobilizations might be associated with the dextran layer linked to the enzyme that covers the reactive groups on its surface blocking the interaction with the carrier [4,34,35]. Some authors studied the rupture of the complex dextransucrase-dextran in order to make available the amino acids on the enzyme surface, thus allowing the immobilization via covalent binding [19,23].

Chitosan, a cationic polymer of β-1,4 glycosidic linkages, is obtained from chitin, a major natural structural component of the invertebrates exoskeleton and of fungi cell wall [36]. This polymer has been successfully applied for enzyme immobilization to be used in food and food process, because of its non-toxicity, high protein affinity, and biocompatibility and biodegradability properties [37–39]. Moreover, the enzyme can be strongly linked to the chitosan surface [40], and the major part of the immobilized enzyme is located on the external surface, which can effectively reduce mass-transfer limitations [41]. Several studies from our group reported the increase in thermal and operational stabilities of different enzymes when immobilized on glutaraldehyde-activated chitosan particles [27,42,43]. Additionally, lysozyme, stem bromelain, and lipases are other examples of enzymes that are used in food applications, which were immobilized on chitosan-based supports [44,45]. To the best of our knowledge, there are no reports regarding the use of activated chitosan as support for the immobilization of dextransucrase.

Considering all these aspects, the objective of this work was to study the covalent immobilization of dextransucrase on chitosan particles. The immobilization procedure followed two steps: first, the hydrolysis of the dextran-layer around dextransucrase molecules was carried out using dextranase; then, the immobilization of the enzyme proceeded on glutaraldehyde-activated chitosan particles. Optimal pH and temperature, the thermal and storage stabilities, and the operational stability of the immobilized biocatalyst in repeated reaction batches of oligosaccharides formation were also tested.

2. Experimental

2.1. Materials

Chitosan from shrimp shells (>75% deacetylated) was purchased from Sigma–Aldrich (USA). Dextranase Plus L (Novozymes A/S) were kindly provided by LNF Latino Americana (Bento Gonçalves, Brazil). Glutaraldehyde (50% mass fraction solution) was bought from Dinâmica Ltda. (Brazil). Sucrose, glucose, and maltose (HPLC grade) were obtained from Sigma–Aldrich (St. Louis, USA). All other chemicals were of analytical grade, purchased from readily available commercial sources.

2.2. Microorganism, culture medium and enzyme production

A strain of *L. mesenteroides* B-512F obtained from Fundação Tropical de Pesquisas e Tecnologia André Tosello (Campinas, SP, Brazil) was used for dextransucrase production. The strain was preserved as frozen samples in MRS (de Man, Rogosa, and Sharpe) medium and 20% (volume fraction) of glycerol.

The culture medium used for microbial growth and enzyme production was composed of (in g/L): sucrose, 50; yeast extract, 20; $MgSO_4 \cdot 7H_2O$, 0.20; $MnSO_4 \cdot 2H_2O$, 0.01; $FeSO_4 \cdot 7H_2O$, 0.01; $CaCl_2 \cdot 2H_2O$, 0.02; NaCl, 0.01; K_2HPO_4, 20, pH adjusted to 6.7 with phosphoric acid 10% (volume fraction) as proposed by Rabelo et al. [46].

Batch cultivations of *L. mesenteroides* B-512F were carried out in stirred tank bioreactors (Biostat B model, B. Braun Biotech International, Germany) to produce the enzyme. Pre-inoculum was prepared by transferring one isolated colony of *L. mesenteroides* B-512F to 500 mL conical flasks containing 100 mL of the culture medium and incubated in an orbital shaker at 180 rpm for 16 h at 30 °C. From this culture, a volume of 200 mL with a cell concentration corresponding to OD_{600} = 1.0 was added to the bioreactor, which was filled with 1.8 L of culture medium. Culture conditions were based on the work of Rabelo et al. [46], who defined the best temperature for bacterial growth and enzyme production as 30 °C. Under these conditions, the dextransucrase production was 7.5-fold higher than that reported by Goyal et al. [47]. Other cultivation parameters were controlled pH at 6.7, agitation speed of 500 rpm, and air flow of 0.5 L air/min for 6 h. This pH value was kept by the addition of a solution containing sucrose (300 g/L) and NaOH (120 g/L), to optimize the enzyme production, following procedures described by Rabelo et al. [46]. At the end of feeding, culture proceeded for another 2 h to allow the pH to reach 5.2, the optimal pH to preserve enzyme activity. At this point, cells were harvested by centrifugation at 12,000 × *g* for 15 min at 4 °C to recover the supernatant. The enzyme was partially purified from the culture broth by the precipitation using polyethylene glycol (PEG 1500–50% mass fraction), according to Paul et al. [48] with some modifications. The samples were centrifuged at 4500 × *g* for 15 min at 4 °C. The salts and media components were not removed. After the centrifugation, the denser (bottom) phase contained dextran along with approximately 90% of dextransucrase activity, whereas the upper phase contains glucose, fructose, and polyethylene glycol (PEG), according to reports by Paul et al. [48]. The enzymatic extract prepared in this way was described as being free of levansucrase activity. The supernatant containing the concentrated enzyme (enzymatic extract) was stored at −20 °C until further analyses. The obtained enzymatic preparation, containing 49 U/mL (specific activity of 7.9 U/mg protein), was used for the immobilization protocol.

2.3. Synthesis of chitosan spheres for immobilization

The chitosan particles were prepared by dripping the chitosan solution into the alkaline coagulation solution, as described by Klein et al. [27]. The prepared particles had spherical shape with a diameter of approximately 2 mm and pore sizes varying from 20 to 200 Å, and a dry weight of 0.343 ± 0.008 mg per sphere. The activation was carried out incubating the chitosan particles with 5% of glutaraldehyde solution at 25 °C for 3 h, as reported by Lorenzoni et al. [42].

2.4. Dextransucrase immobilization

Because dextran is a polymer, and its removal by dialysis is a complex process, it was chosen the treatment using dextranase prior to the immobilization. Therefore, before the immobilization, the dextran layer surrounding the enzyme molecule was removed using Dextranase Plus L [23]. The dextranase was added to the solution containing dextransucrase and the reaction proceeded for 8 h at 5 °C. After this treatment, the specific activity of dextran-free dextransucrase was 7.9 U/mg. The immobilization was carried out incubating the activated-chitosan particles with the enzyme solution, properly diluted in activity buffer (50 mM sodium acetate, pH

Please cite this article in press as: N.G. Graebin, et al., Dextransucrase immobilized on activated-chitosan particles as a novel biocatalyst, J. Mol. Catal. B: Enzym. (2016), http://dx.doi.org/10.1016/j.molcatb.2016.12.007