Journal of Molecular Catalysis B: Enzymatic 57 (2009) 242–249

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Catalytic properties of the immobilized *Talaromyces thermophilus* β-xylosidase and its use for xylose and xylooligosaccharides production

Mohamed Guerfali, Ines Maalej, Ali Gargouri, Hafedh Belghith*

Laboratoire de Génétique Moléculaire des Eucaryotes, Centre de Biotechnologie de Sfax, PB "K" 3038 Sfax, Tunisia

ARTICLE INFO

Article history:
Received 12 May 2008
Received in revised form
25 September 2008
Accepted 25 September 2008
Available online 4 October 2008

Keywords:
β-Xylosidase
Xylooligosaccharides
Immobilization
Talaromyces thermophilus

ABSTRACT

The present study explores the efficiency of *Talaromyces thermophilus* β-xylosidase, in the production of xylose and xylooligosaccharides. The β-xylosidase was immobilized by different methods namely ionic binding, entrapment and covalent coupling and using various carriers. Chitosan, pre-treated with glutaraldehyde, was selected as the best support material for β-xylosidase immobilization; it gave the highest immobilization and activity yields (94%, 87%, respectively) of initial activity, and also provided the highest stability, retaining 94% of its initial activity even after being recycled 25 times. Shifts in the optimal temperature and pH were observed for the immobilized β-xylosidase when compared to the free enzyme. The maximal activity obtained for the immobilized enzyme was achieved at pH 8.0 and 53 °C, whereas that for the free enzyme was obtained at pH 7.0 and 50 °C. The immobilized enzyme was more thermostable than the free β-xylosidase. We observed an increase of the K_m values of the free enzyme from 2.37 to 3.42 mM at the immobilized state. Native and immobilized β-xylosidase were found to be stimulated by Ca^{2+}, Mn^{2+} and Co^{2+} and to be inhibited by Zn^{2+}, Cu^{2+}, Hg^{2+}, Fe^{2+}, EDTA and SDS. Immobilized enzyme was found to catalyze the reverse hydrolysis reaction, forming xylooligosaccharides in the presence of a high concentration of xylose. In order to examine the synergistic action of xylanase and β-xylosidase of *T. thermophilus*, these two enzymes were co-immobilized on chitosan. A continuous hydrolysis of 3% Oat spelt xylan at 50 °C was performed and better hydrolysis yields and higher amount of xylose was obtained.

1. Introduction

Enzymes are biocatalysts with high specificity, catalytic efficiency and bio-degradability and are becoming popular in diverse industrial and medical applications particularly for their abilities to speed up the rate of chemical reactions by lowering the activation energy [1]. However, the effective use of enzymes has often been restricted because of certain shortenings, such as their non-reusability, instability and sensitivity to denaturation. Their use has also been deterred by the high cost associated with their isolation, purification and characterization, as well as with the difficult recovery of active enzymes from reaction mixtures.

These restrictions, which remain a challenge for the application of free enzymes in biotransformation and chemical processes, have actually been eased by the use of immobilized enzymes. More succinctly, the immobilization of enzymes on various water-insoluble supports have been reported to improve biocatalysts long-term stability, to allow enzyme reusability and application in continuous operations, and to minimize the time and cost burdens associated with those activities [2].

This immobilization can be accomplished by physical or chemical methods [3]. Based on their characteristics, the currently used protein immobilization techniques can be classified into five broad categories; physical adsorption [4], entrapment in a matrix [5], ionic binding [6], covalent binding [7] and cross-linking [8]. The later involves the formation of covalent bonds between the support material and enzyme molecules through the employment of a bi- or multifunctional reagent.

Enzyme immobilization is often accompanied by a number of changes that affect enzymatic activity, optimum pH, affinity to the substrate, etc. The extent of these changes is known to depend upon the type of the enzyme and carrier support as well as on the conditions in which immobilization is performed [9].

β-Xylosidase (EC 3.2.1.37) is one of the component enzymes of the hemicellulase complex. It catalyzes the hydrolysis of xylooligosaccharides, such as xylobiose and xylotriose, to xylose by recognizing the xylosyl residue at the non-reducing end and by cleaving the β-1,4 glycosidic bonds [10]. β-Xylosidase is, therefore, a key enzyme in the xylanolytic system with a great potential in many biotechnological applications, particularly in paper pulp,

* Corresponding author. Tel.: +216 74 875 818x1090; fax: +216 871 816.
E-mail address: hafeth.belghith@cbs.rnrt.tn (H. Belghith).

1381-1177/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2008.09.011

food, beverage, and animal foodstuff bioconversion industries [11]. β-Xylosidases are often classified into five families: 3, 39, 43, 52, and 54, based on amino acid sequence similarities [12,13]. These enzymes, as well as other glycosidases, have recently been proven to be powerful glycosynthases that can be used efficiently in the synthesis of oligosaccharides [14].

β-Xylosidases are particularly important as they exhibit the dual function of catalyzing both the hydrolysis and synthesis reactions. The immobilized enzyme used for the hydrolysis of xylo-oligosaccharides prepared from xylan [15,16] can also be used in the synthesis of xylo-oligosaccharides and alkyl-xylosides by transglucosylation reaction [17].

Only few studies have reported that, using a high xylose concentration, this enzyme can catalyze a condensation reaction and produce xylobiose, including β-xylosidase of *Aspergillus niger* and *Sporotrichum thermophile* [18,19].

The present study was undertaken to explore the immobilization of the β-xylosidase of *Talaromyces thermophilus* and to evaluate its hydrolytic efficiency for xylose production when compared to the free enzyme. The first step involved the immobilization of this β-xylosidase of *T. thermophilus* on different supports and the subsequent identification of its properties (kinetic parameters, temperature and pH optimum, thermal stability and reusability) compared to those of the free enzyme. The second step involved the application and subsequent evaluation of the immobilized enzyme for xylose production and xylooligosaccharide synthesis by oat spelt xylan hydrolysis and condensation reaction, respectively.

2. Materials and methods

2.1. Chemicals

Chitosan, chitin, xylan (beechwood, oat spelt), *p*-nitrophenyl β-D-xylopyranoside (*p*NPX), *p*-nitrophenol, glutaraldehyde, acrylamide, and series of oligosaccharides used as standard for (HPLC) chromatography were obtained from Sigma. Gelatin was from Amersham. DEAE-cellulose and DEAE-sephadex were from Pharmacia.

2.2. Microorganism and culture conditions

The present study reports on a newly isolated thermotolerant fungal strain from a soil sample collected in the thermal station of El Hamma in the south of Tunisia. The fungal isolate was identified as *T. thermophilus Stolk* by CBS (Centraalbureau voor schimmelculturen, Holland). The *T. thermophilus* was cultivated in a modified liquid Mandels medium [20]: KH_2PO_4, 1 g; K_2HPO_4, 2.5 g; $(NH_4)_2SO_4$, 1.4 g; $MgSO_4{\cdot}7H_2O$, 0.3 g; $CaCl_2$, 0.3 g; yeast extract, 1 g; urea, 0.7 g; Tween 80, 1 ml; water, 1 l and 2% wheat bran. The pH of the medium was 7.0 and was supplemented with 1 ml of an oligoelements solution with $MnSO_4$, 1.6 g/l; $ZnSO_4$, 1.4 g/l; $FeSO_4$, 5 g/l and $CoCl_2$, 2 g/l. The enzyme production was carried out in 500-ml flasks containing 100 ml of culture medium that was incubated at 50 °C and at an agitation rate of 160 rpm for 5 days.

2.3. Enzyme preparation protocol

The production of β-xylosidase by *T. thermophilus* strain in submerged fermentation was examined. The strain was grown on optimized nutrient medium containing wheat bran as a carbon source. Cultivation was performed at 50 °C for 5 days. The extracellular proteins were recovered by centrifugation and the supernatant was treated with ammonium sulfate (80% saturation). The precipitate was collected by centrifugation at 9000 rpm for 15 min, dissolved in 20 mM phosphate buffer, pH 6.0, and then dialyzed overnight against the same buffer. The dialyzed enzyme solution was loaded on a DEAE-Cellulose column (1.25 × 28 cm) pre-equilibrated with 20 mM phosphate buffer, pH 8. The column was extensively washed with the same buffer. β-xylosidase activity was eluted with a gradient of 0–1 M NaCl in the same buffer, at a flow rate of 24 ml/h. The active fractions were pooled, concentrated using PEG 6000 and dialyzed overnight against 20 mM phosphate buffer, pH 6.0. The β-xylosidase obtained from the ion exchanger was further purified by gel filtration HPLC (Shodex, Protein kw P 802.5, 8 mm × 300 mm) equilibrated and eluted by 50 mM phosphate buffer, pH 6.0 at a flow rate of 0.8 ml/min. The highly active β-xylosidase fractions were pooled, concentrated and used as purified enzyme for subsequent studies. The purified enzyme has a high specific activity of 147.5 U/mg proteins.

2.4. Enzyme immobilization

T. thermophilus β-xylosidase was immobilized by different methods on different supports. The immobilization steps and enzyme storage were carried out at 4 °C. The supernatants and washing volumes were pooled after each step and non-immobilized activity was determined.

2.4.1. Ionic binding (DEAE-cellulose and DEAE-sephadex)

1 g resin DEAE-cellulose or DEAE-sephadex was washed twice with 50 mM phosphate pH 7.0 and centrifuged for 2 min at 4600 × *g*. The resin was mixed with 1 ml of the enzyme preparation (1.5 U/ml, 46 μg/ml) and 2 ml phosphate buffer for 20 min under agitation. The mixture was then washed twice with 25 mM phosphate buffer and centrifuged for 2 min at 4600 rpm.

2.4.2. Entrapment in polyacrylamide gel

Immobilization in polyacrylamide gel was achieved by mixing 3 ml of a solution of acrylamide and bis-acrylamide (30:1), 4 ml water, 2 ml 100 mM Tris–HCl buffer pH 7 and 1 ml enzyme (1.5 U/ml). Polymerization was achieved by the addition of 100 μl ammonium persulfate at 1% and 6 μl TEMED. The gel film was polymerized at 4 °C on a surface of 5 cm × 5 cm and cut into small blocks. The gel pieces were washed twice with 25 mM phosphate buffer, pH 7.0, before use.

2.4.3. Covalent coupling (chitosan, chitin and gelatin)

Chitosan (0.5 g) was dissolved in 50 ml of 0.1 M HCl containing 2.5% (v/v) glutaraldehyde (GA) at 30 °C for 2 h. The solublized chitosan was precipitated by the addition of 1 ml NaOH (1.0 mol/l). The precipitate was separated by centrifugation (10 min at 6000 rpm) and washed with distilled water to remove excess of GA. The wet chitosan was mixed with 2.0 ml (1.5 U/ml) of the enzyme solution and stirred at 4 °C for 24 h. The unbound enzyme was removed by washing with phosphate buffer 20 mM until no protein or activity was detected [21]. This protocol was repeated in the presence of different GA concentrations (0.5–4%) to investigate the latter's effect on the immobilization and activity yields of the enzyme.

Chitin (0.5 g) was shaken with 5 ml 2.5% (v/v) GA. It was then collected by centrifugation (10 min at 6000 rpm) and washed with distilled water to remove excess of GA. The wet chitin was mixed with 2.0 ml of the enzyme solution at 4 °C for 24 h. The unbound enzyme was removed by washing with distilled water as described earlier [21].

The gelatin powder (5%, w/v) was swelled in 5 ml (50 mM) phosphate buffer (pH 7.0) and heated at 50 °C for 10 min to ensure its complete solubilization. The mixture was then cooled and the enzyme was added (1.5 U/ml). After the thorough mixing of the enzyme, the required amount of organic cross-linker (0.6% w/v) glutaraldehyde was added. The mixture was constantly stirred and