Bioresource Technology 102 (2011) 4277–4280

Contents lists available at ScienceDirect

Bioresource Technology

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

Short Communication

Synergistic production of L-arabinose from arabinan by the combined use of thermostable endo- and exo-arabinanases from *Caldicellulosiruptor saccharolyticus*

Yu-Ri Lim, Soo-Jin Yeom, Young-Su Kim, Deok-Kun Oh *

Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, South Korea

ARTICLE INFO

Article history:
Received 24 October 2010
Received in revised form 7 December 2010
Accepted 8 December 2010
Available online 22 December 2010

Keywords:
L-Arabinose production
Arabinan
α-L-Arabinofuranosidase
Endo-1,5-α-L-arabinanase
Caldicellulosiruptor saccharolyticus

ABSTRACT

The optimum conditions for the production of L-arabinose from debranched arabinan were determined to be pH 6.5, 75 °C, 20 g l^{-1} debranched arabinan, 42 U ml^{-1} endo-1,5-α-L-arabinanase, and 14 U ml^{-1} α-L-arabinofuranosidase from *Caldicellulosiruptor saccharolyticus* and the conditions for sugar beet arabinan were pH 6.0, 75 °C, 20 g l^{-1} sugar beet arabinan, 3 U ml^{-1} endo-1,5-α-L-arabinanase, and 24 U ml^{-1} α-L-arabinofuranosidase. Under the optimum conditions, 16 g l^{-1} L-arabinose was obtained from 20 g l^{-1} debranched arabinan or sugar beet arabinan after 120 min, with a hydrolysis yield of 80% and a productivity of 8 g l^{-1} h^{-1}. This is the first reported trial for the production of L-arabinose from the hemicellulose arabinan by the combined use of endo- and exo-arabinanases.

1. Introduction

L-Arabinose can potentially inhibit obesity and prevent or treat diseases associated with hyperglycemia (Brudnak, 2002) because it inhibits sucrose digestion and prevents the elevation of blood glucose levels induced by sucrose intake (Osaki et al., 2001). The sugar can be used as a low-calorie sweetener. Thus, L-arabinose has potential as a useful sugar in the food industry.

Endo-1,5-α-L-arabinanase (EC 3.2.1.99) hydrolyzes the hemicellulose arabinan backbone to arabino-oligosaccharide or L-arabinose (Hong et al., 2009). α-L-Arabinofuranosidase (EC 3.2.1.55), as exo-arabinanase, generates L-arabinose from arabino-oligosaccharide via the hydrolysis of the non-reducing termini of the α-L-arabinofuranosyl residues as side chains of arabinan (Lim et al., 2010; Numan and Bhosle, 2006). However, the production of L-arabinose from arabinan via the combined use of endo- and exo-arabinanase enzymes has not been attempted.

In this study, the production of L-arabinose from debranched arabinan or sugar beet arabinan was optimized by the combined use of endo-1,5-α-L-arabinanase and α-L-arabinofuranosidase.

2. Methods

2.1. Bacterial strains, plasmid, and culture conditions

Caldicellulosiruptor saccharolyticus DSM 8903, *Escherichia coli* ER2566, and plasmid pET-28a(+) were used as the gene sources of endo-1,5-α-L-arabinanase and α-L-arabinofuranosidase, host cells, and expression vector, respectively. The recombinant *E. coli* for α-L-arabinofuranosidase or endo-1,5-α-L-arabinanase expression was cultivated in a 2-l flask containing 500 ml of Luria–Bertani (LB) medium and 25 μg ml^{-1} of kanamycin at 37 °C with agitation at 200 rpm. When the optical density of the bacteria reached 0.5 at a wavelength of 600 nm, isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 0.1 mM to induce enzyme expression, after which the culture was incubated with shaking at 150 rpm at 16 °C for 16 h.

2.2. Purification of enzymes

Two clones harboring individual enzymes had been processed individually to purify individual enzymes. The grown cells possessing endo-1,5-α-L-arabinanase or α-L-arabinofuranosidase were harvested and disrupted by sonication in a lysis buffer (pH 8.0) containing 50 mM NaH_2PO_4 and 300 mM NaCl with the addition of 1 mg ml^{-1} lysozyme. After centrifugation at 13,000*g* for 20 min at 4 °C, the supernatant was used as a crude extract. The

* Corresponding author. Tel.: +82 2 454 3118; fax: +82 2 444 6176.
E-mail address: deokkun@konkuk.ac.kr (D.-K. Oh).

0960-8524/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2010.12.039

crude extract was subsequently heated at 75 °C for 5 min, and the suspension was centrifuged at 13,000*g* for 20 min. The supernatant was applied to a His-trap column (Amersham Biosciences, Uppsala, Sweden) equilibrated with the lysis buffer (pH 8.0). The bound protein was eluted at 4 °C with linear gradient between 10 and 250 mM imidazole at 1 ml min^{-1}. The active fractions were collected and dialyzed at 4 °C for 16 h against 50 mM citrate/phosphate buffer (pH 6.0). The resultant solution was used as the purified enzyme of endo-1,5-α-L-arabinanase or α-L-arabinofuranosidase. The purification step using the column was conducted using a fast protein liquid chromatography system (Bio-Rad, Hercules, CA, USA) at 4 °C.

2.3. Enzyme assay

The reactions with endo-1,5-α-L-arabinanase were performed at 75 °C for 10 min in 50 mM citrate/phosphate buffer (pH 6.5) containing 0.1 U ml^{-1} enzyme and 1 g l^{-1} debranched arabinan. Total released reducing sugar levels were determined via the 3,5-dinitrosalicylic reagent method. One unit (U) of endo-1,5-α-L-arabinanase activity was defined as the amount of enzyme required to liberate 1 μM L-arabinose per min at 75 °C and pH 6.5. The reactions with α-L-arabinofuranosidase were performed at 80 °C for 10 min in 50 mM citrate/phosphate buffer (pH 5.5) containing 1 mM *p*-nitrophenyl-α-L-arabinofuranoside (*p*NP-Araf) and 0.1 U ml^{-1} enzyme and the activity was determined by release of *p*-nitrophenol (*p*NP). One unit of α-L-arabinofuranosidase activity was defined as the amount of enzyme required to liberate 1 μM of *p*NP per min at 80 °C and pH 5.5.

2.4. Optimization of reaction conditions for L-arabinose production

Unless otherwise indicated, all reactions were performed at 75 °C for 10 min in 50 mM citrate/phosphate buffer containing 1 g l^{-1} arabinan at pH 6.5 for debranched arabinan (Lot number 80601, Megazyme, Wicklow, Ireland) and at pH 6.0 for sugar beet arabinan (Lot number 80901, Megazyme) with the optimal unit ratio of endo-1,5-α-L-arabinanase to α-L-arabinofuranosidase. The effect of the ratio of endo-1,5-α-L-arabinanase to α-L-arabinofuranosidase on L-arabinose production was investigated using the solutions of enzymes which were obtained by mixing 20 U ml^{-1} endo-1,5-α-L-arabinanase and 10 U ml^{-1} α-L-arabinofuranosidase for debranched arabinan and 18 U ml^{-1} endo-1,5-α-L-arabinanase and 36 U ml^{-1} α-L-arabinofuranosidase for sugar beet arabinan ranging from 0:100–100:0 (v/v).

The effects of pH and temperature on L-arabinose production were investigated by varying the pH from 4.5 to 7.0, and the temperature from 60 to 90 °C. The effect of temperature on enzyme stability was monitored as a function of incubation time by maintaining the enzyme solution at five different temperatures (65, 70, 75, 80, and 85 °C). The effects of the concentrations of enzymes on L-arabinose production with 20 g l^{-1} arabinan were evaluated by varying the concentrations of endo-1,5-α-L-arabinanase and α-L-arabinofuranosidase from 0.15 and 0.45 U ml^{-1} to 35 and 105 U ml^{-1}, respectively, for debranched arabinan and varied from 0.3 and 2.4 U ml^{-1} to 8 and 64 U ml^{-1}, respectively, for sugar beet arabinan. The reactions were performed for 1 h.

2.5. Analytical methods

The concentrations of L-arabinose, arabinobiose, arabinotriose, arabinotetraose, and arabinopentaose were determined by a Bio-LC system (Dionex ICS-3000, Sunnylvale, CA, USA) with an electrochemical detector equipped with a CarboPac PA1 column (4 × 250 mm, Dionex) and eluted with 0.1 M NaOH (0–5 min), followed by a linear gradient (5–35 min) of sodium acetate (0–0.2 M) at 1 ml min^{-1} at 30 °C.

3. Results and discussion

3.1. Effect of the ratio of endo-1,5-α-L-arabinanase to α-L-arabinofuranosidase on L-arabinose production

Endo-1,5-α-L-arabinanase or α-L-arabinofuranosidase was purified from crude extract obtained from harvested cells as a soluble protein via heat treatment and His-trap affinity chromatography column. Endo-1,5-α-L-arabinanase and α-L-arabinofuranosidase were purified with final purification of 39- and 23-fold, yields of 14% and 11%, and specific activities of 11.0 and 28.3 U mg^{-1}, respectively. The effect of the ratio of endo-1,5-α-L-arabinanase to α-L-arabinofuranosidase on L-arabinose production were investigated using the purified enzymes of 11.0 U mg^{-1} endo-1,5-α-L-arabinanase and 28.3 U mg^{-1} α-L-arabinofuranosidase. The maximum L-arabinose production was observed at 12 U ml^{-1} endo-1,5-α-L-arabinanase and 4 U ml^{-1} α-L-arabinofuranosidase with the optimal unit ratio of 3:1 for debranched arabinan and observed at 4 U ml^{-1} endo-1,5-α-L-arabinanase and 32 U ml^{-1} α-L-arabinofuranosidase with the optimal unit ratio of 1:8 for sugar beet arabinan. Thus, the hydrolysis reactions for arabinan were performed at the optimal unit ratio in all subsequent experiments.

3.2. Effects of pH and temperature on L-arabinose production

The maximum activity of debranched arabinan was observed at pH 6.5 and 75 °C, and that of sugar beet arabinan was observed at pH 6.0 and 75 °C. The half-lives of the two-enzymes system for debranched arabinan at 65, 70, 75, 80, and 85 °C were 851, 234, 88, 8, and 0.08 h, and those for sugar beet arabinan at 65, 70, 75, 80, and 85 °C were 1037, 277, 64, 0.61, and 0.11 h, respectively. The pH and temperature properties of endo-1,5-α-L-arabinanase, α-L-arabinofuranosidase, and two-enzyme system with endo-1,5-α-L-arabinanase and α-L-arabinofuranosidase from *C. saccharolyticus* are summarized in Table 1.

The maximum endo-1,5-α-L-arabinanase activities from the genus *Bacillus* (Leal and de Sa-Nogueira, 2004; Seo et al., 2010; Takao et al., 2002) and *Aspergillus niger* (Dunkel and Amadó, 1994) were observed at pH 6.0 and 4.8, respectively. The optimum temperature for endo-1,5-α-L-arabinanase from *Bacillus thermodenitrificans* (Takao et al., 2002) was 70 °C as the previously highest optimum temperature, thus demonstrating that endo-1,5-α-L-arabinanase from *C. saccharolyticus* exhibited the highest optimum temperature among the reported endo-1,5-α-L-arabinanases. The maximum activities of thermostable α-L-arabinofuranosidases were observed at a pH range of 5.0–7.0. The maximum activity of α-L-arabinofuranosidase from *Thermotoga maritima* was observed at 90 °C, which was the highest optimum temperature among the reported α-L-arabinofuranosidases (Miyazaki, 2005).

3.3. Effects of the concentrations of enzymes and substrate on L-arabinose production

With increasing the concentrations of the enzymes, the levels for the production of L-arabinose from arabinan increased. However, at above 42 U ml^{-1} endo-1,5-α-L-arabinanase and 14 U ml^{-1} α-L-arabinofuranosidase for debranched arabinan and at above 3 U ml^{-1} endo-1,5-α-L-arabinanase and 24 U ml^{-1} α-L-arabinofuranosidase for sugar beet arabinan, L-arabinose production reached plateaus. The hydrolysis yield decreased with increasing the substrate concentration from 0 to 50 g l^{-1}, while L-arabinose production was maximal at 20 g l^{-1} arabinan (Fig. 1). When the