ELSEVIER

Process Biochemistry 40 (2005) 359–364

PROCESS
BIOCHEMISTRY

www.elsevier.com/locate/procbio

Production, purification and characterization of a minor form of xylanase from *Aspergillus versicolor*

Eleonora Cano Carmona*, Mauricio Batista Fialho, Érika Bicalho Buchgnani, Glauciane Danusa Coelho, Márcia Regina Brocheto-Braga, João Atílio Jorge

Departamento de Bioquímica e Microbiologia, Instituto de Biociências, UNESP, Caixa Postal 199, 13.506-900, Rio Claro, SP, Brazil

Received 17 June 2003; received in revised form 12 November 2003; accepted 3 January 2004

Abstract

A strain of *Aspergillus versicolor* produces a xylanolytic complex containing two components, the minor component being designated xylanase II. The highest production of xylanase II was observed in cultures grown for 5 days in 1% wheat bran as carbon source, at pH 6.5. Xylanase II was purified 28-fold by DEAE-Sephadex and HPLC GF-510 gel filtration. Xylanase II was a monomeric glycoprotein, exhibiting a molecular mass of 32 kDa with 14.1% of carbohydrate content. Optimal pH and temperature values for the enzyme activity were about 6.0–7.0 and 55 °C, respectively. Xylanase II thermoinactivation at 50 °C showed a biphasic curve. The ions Hg^{2+}, Cu^{2+} and the detergent SDS were strong inhibitors, while Mn^{2+} ions and dithiothreitol were stimulators of the enzyme activity. The enzyme was specific for xylans, showing higher specific activity on birchwood xylan. The Michaelis–Menten constant (K_m) for birchwood xylan was estimated to be 2.3 mg ml^{-1} while maximal velocity (V_{max}) was 233.1 μmol mg^{-1} min^{-1} of protein. The hydrolysis of oat spelt xylan released only xylooligosaccharides.
Published by Elsevier Ltd.

Keywords: Aspergillus versicolor; Xylanase; Endoxylanase; Enzyme purification

1. Introduction

The primary component of hemicellulose is xylan, a heteropolysaccharide formed by a main chain of linked β-1,4 xylose residues, and a variety of substituents, differing from plant to plant. It may constitute up to 30% cellular walls in hard wood types and annual plants. Therefore, considerable amounts of xylan are found in solid agricultural and agroindustrial residues, as well as in effluents released during wood processing, which, due to frequent inappropriate discard, cause great damage to the ecosystem [1,2].

Xylan biodegradation is performed by a xylanolytic complex, which is primarily produced by fungi and bacteria. Hydrolysis of the main chain is accomplished by the action of endo-1,4-β-xylanases (1,4-β-D-xylan xylanohydrolases, EC 3.2.1.8), which release xylooligosaccharides of different sizes [1,3,4].

Basic and applied research on microbial hemicellulases has not only produced significant scientific knowledge, but also revealed their enormous biotechnological potential. Xylanases, associated or not with other enzymes, can be used in the food industry in order to enhance the digestibility of animal feeding, as well as in the textile industry, among other applications. Special attention has been given to their use in the pulp and paper industry for bleaching purposes, resulting in a decrease of chlorine utilization and consequently lowering environmental impact [4–6].

A strain of *Aspergillus versicolor* produces high levels of xylanolytic activity associated with low cellulolytic activity, an essential characteristic for certain industrial applications [7]. The xylanolytic complex of this fungus consists of two enzymes, the more abundant of which, xylanase I, has been purified and characterized [8]. The aim of this work was the establishment of optimal conditions for the production, purification and biochemical characterization of xylanase II, the minor component of *A. versicolor* xylanolytic complex.

2. Materials and methods

2.1. Organism and growth

Conidia of *A. versicolor* were obtained from cultures in Vogel solid medium [9] containing 1.5% (w/v) sucrose.

* Corresponding author. Tel.: +55-19-3526-4175; fax: +55-19-3526-4176.
E-mail address: ecarmona@rc.unesp.br (E.C. Carmona).

0032-9592/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.procbio.2004.01.010

Liquid cultures were prepared in the same medium with 1% (w/v) wheat bran as carbon source and pH was adjusted to 6.5 or 5.8 as indicated in the text and legends. Erlenmeyer flasks (250 ml) containing 50 ml of medium were inoculated with 2 ml of the spore suspension (10^7 spores/ml) and incubated at 30 °C for 5 or 9 days, or as indicated in the text and legends. The mycelium was removed by vacuum filtration and the filtrate was used as crude enzyme extract.

2.2. Enzymic assay

Xylanase activity was assayed at 55 °C using 2% (w/v) birchwood xylan or oat spelt xylan in 100 mM sodium phosphate buffer pH 6.0. The reducing sugars released were quantified by the dinitrosalicylic acid method [10], using xylose as standard. One enzymic activity unit was defined as the enzyme amount that releases 1 μmol of reducing sugar per minute. Specific activity was expressed as unit per milligram of protein.

2.3. Determination of neutral carbohydrates and protein

Total carbohydrate content was determined by phenol sulphuric method [11] with glucose as standard. Protein concentration was determined by the method of Lowry et al. [12] using bovine serum albumin as standard.

2.4. Separation of the xylanolytic complex components

The culture filtrate obtained after different cultivation conditions was dialyzed against 50 mM Tris–HCl buffer pH 7.0, and submitted to a batch assay with DEAE-Sephadex A-50 equilibrated in the same buffer. Xylanase I, which does not bind to the resin under these conditions, was removed by centrifugation at $6000 \times g$ for 15 min at 4 °C and the resin was exhaustively washed with the same buffer. The xylanase I-rich supernatants were pooled to estimate xylanolytic activity. The resin-bound components (including xylanase II) were eluted by three washes with 50 mM Tris–HCl buffer pH 7.0, containing 0.7 M NaCl, followed by centrifugation at the conditions described above. The pooled supernatants were also assayed for xylanolytic activity. Total xylanolytic activity corresponded to the sum of the activities of both supernatant pools.

2.5. Purification of xylanase II

The culture filtrate (140 ml) was dialyzed against 50 mM Tris–HCl buffer pH 8.8 and chromatographed in a DEAE-Sephadex A-50 column (1.0 cm × 13.8 cm) pre-equilibrated with the buffer at a flow rate of 20 ml/h. The bound proteins were eluted with a NaCl gradient (0.0–0.6 M) in the same buffer. The fractions with higher activity were pooled and concentrated approximately 10 times through reverse osmosis using polyethylene glycol (MW 15,000–20,000). The concentrated sample was dialyzed against 50 mM ammonium acetate buffer pH 6.8, and submitted to a gel filtration in HPLC Shimadzu CLASS-LC10, using a Shodex Asahipak GF-510 HQ column equilibrated in the same buffer.

2.6. Electrophoresis

SDS-PAGE was carried out in an acrylamide gradient (5–20%) as described by Hames and Rickwood [13]. Molecular weight standards were: phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and α-lactalbumin. Proteins were stained with Coomassie brilliant blue R-250.

2.7. Optimal temperature and pH, and thermal stability

Optimal assay temperature was determined by performing a standard activity assay in a temperature range from 25 to 70 °C. In order to determine optimal pH, the enzymic assay was carried out at different pH values, at 55 °C. McIlvaine buffer was used for pH 4.0–8.0, Tris–HCl buffer for pH 8.0–9.0 and glycine–NaOH buffer for pH 9.0 and 9.5.

2.8. Effect of potential inhibitors and activators on purified xylanase II activity

The effect of various compounds on xylanase activity was determined by performing the enzyme assay in 50 mM sodium acetate buffer pH 5.5, with birchwood xylan as substrate at final concentrations of 2 and 10 mM. Residual activity was expressed as the percentage of the activity observed in the absence of any compound.

2.9. Kinetic parameters

The effect of oat spelt xylan concentration, ranging from 2.0 to 40 mg ml^{-1}, on xylanase activity was evaluated under optimal assay conditions. The kinetic parameters (Michaelis–Menten constant, K_m, and maximal reaction velocity, V_{max}) were estimated by linear regression from double-reciprocal plots according to Lineweaver and Burk [14]. The initial velocities (V_0) were expressed in μmol ml^{-1} min^{-1} and the maximum velocity was converted into μmol mg^{-1} min^{-1} of protein.

2.10. Analysis of xylan hydrolysis products

The products of oat spelt xylan hydrolysis were analyzed by thin-layer chromatography on silica gel G-60 with ethyl acetate/acetic acid/formic acid/distilled water (9:3:1:4, v/v/v/v) as the mobile phase. Xylose and xylobiose were used as standards, and thymol blue as a front indicator. Development was performed with 0.2% (w/v) orcinol in sulphuric acid/methanol (1:9, v/v) [15].