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Immobilization of a recombinant endo-1,5-arabinanase secreted by *Aspergillus nidulans* strain A773

André Ricardo de Lima Damásio[a], Benevides Costa Pessela[b], César Mateo[c], Fernando Segato[d], Rolf Alexander Prade[e], Jose Manuel Guisan[c], Maria de Lourdes Teixeira de Moraes Polizeli[f,*]

[a] *Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto Universidade de São Paulo, Ribeirão Preto SP, Brazil*
[b] *Departamento de Biotecnología y Microbiología de los Alimentos, Instituto de Ciencias de la Alimentación, CIAL-CSIC, Calle Nicolás Cabrera 9, Campus UAM, Cantoblanco, 28049 Madrid, Spain*
[c] *Departamento de Biocatálisis, Instituto de Catálisis y Petroleoquímica-CSIC, Campus UAM, Cantoblanco, 28049 Madrid, Spain*
[d] *Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE), Centro Nacional de Pesquisa em Energia e Materiais (CNPEM), Brazil*
[e] *Department of Microbiology and Molecular Biology, Oklahoma State University, Stillwater, OK, USA*
[f] *Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Avenida Bandeirantes 3900, Monte Alegre, 14.040-901 Ribeirão Preto, SP, Brazil*

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ABSTRACT

An endo-1,5-arabinanase (*abnA*) encoding gene from *Aspergillus niveus* was identified, cloned and successfully expressed in *Aspergillus nidulans* strain A773. Based on amino acid sequence comparison, the 34-kDa enzyme could be assigned to CAZy GH family 43. Characterization of purified recombinant endo-1,5-arabinanase (AbnA) revealed that it is active at a wide pH range (pH 4.0–7.0) and an optimum temperature at 70 °C. The immobilization of the AbnA was performed via covalent binding onto agarose-modified supports: glyoxyl iminodiacetic acid–Ni^{2+}, glyoxyl amine, glyoxyl (4% and 10%) and cyanogen bromide activated sepharose. The yield of immobilization was similar on glyoxyl amine and glyoxyl (96%), and higher than glyoxyl iminodiacetic acid–Ni^{2+} (43%) support. The thermal inactivation of these immobilized preparations showed that the stability of the AbnA immobilized on glyoxyl 4 and 10% was improved by 4.0 and 10.3-fold factor at 70 °C. The half-life of glyoxyl 4% derivative at 60 °C was >48 h (pH 5), 9 h (pH 7) and 88 min (pH 9). The major hydrolysis product of debranched arabinan or arabinopentaose by glyoxyl agarose-immobilized AbnA was arabinobiose.

1. Introduction

Plant structural polysaccharides are the most abundant source of renewable carbon in the biosphere and represent a valuable industrial substrate in several applications, such as bio-energy production, pulp and paper, food technology, detergent, textile, nutritional or medical research and organic synthesis. Arabinan is a pectic polysaccharide consisting of a backbone of α-1,5-linked L-arabinofuranosyl units, which are further decorated with α-1,2- and α-1,3-linked arabinofuranosides. In the plant cell wall, arabinan is generally linked to rhamnogalacturonan, and often substituted with terminal phenolic esters, particularly feruloyl or coumaroyl, which can dimerize oxidatively to form links between the rhamnogalacturonan polymers [1].

Enzymes from plant, fungal and bacterial sources that are capable of degrading arabinans and/or arabinose-containing polysaccharides have been described and reviewed [2]. Two major enzymes hydrolyze arabinan: endo-α-1,5-L-arabinanase (EC 3.2.1.99) is specific for α-1,5-L-arabinofuranosidic linkages, and hydrolysis α-1,5-L-arabinan by an endo mechanism. α-L-arabinofuranosidase (3.2.1.55) releases terminal non-reducing α-L-arabinofuranosyl residues present in arabinans, arabinogalactans and arabinoxylans. Arabinanases and arabinofuranosidases are classified by CAZy [5,6] as being members of glycoside hydrolase families GH3, GH43, GH51, GH54 and GH62, which display a wide range of enzymatic activities [3,4].

Multipoint covalent attachment is one of the most interesting approaches to link immobilization and stabilization of enzymes, therefore, stabilization factors ranging between 100 and 1,000,000 have been reported [5–8]. Theoretically, an enzyme molecule attached to a rigid support through many covalent linkages, and via very short spacer arms, should be highly stabilized, that is, the enzyme residues involved in the covalent immobilization should keep their relative positions unaltered during any conformational changes of the protein promoted by any distorting agent (heat, organic solvents, etc.) [8].

* Corresponding author. Tel.: +55 16 36024680; fax: +55 16 36024886.
E-mail address: polizeli@ffclrp.usp.br (M. de Lourdes Teixeira de Moraes Polizeli).

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This report describes the secretion of an endo-1,5-arabinanase (AbnA) in *Aspergillus nidulans* strain A773 (*pyrG*^{89}). pEXPYR plasmids carrying *abnA* gene was transformed into the host strain and directed protein towards the extracellular medium. It was examined the effect of maltose overexpression followed by studies of temperature and pH, immobilization on modified-agarose supports and analysis of released products from debranched arabinan.

2. Experimental

A. nidulans strain A773 (*pyrG89;wA3;pyroA4*) was purchased from the Fungal Genetics Stock Center (FGSC, St. Louis, MO). The zeocin (pheomycin) was purchased from Invivogen (ant-zn-1) and all other chemicals from the best source possible (Sigma Aldrich, Megazyme and Fisher Scientific). Sepharose 4 or 10BCL and Cyanogen bromide (CNBr)-activated sepharose 4B was purchased from GE Healthcare (Uppsala, Sweden).

2.1. Microbial strains, plasmids and culture conditions

Aspergillus niveus was cultured in defined minimum medium (MM) [9] supplemented with 1% glucose at 37 °C. *A. nidulans* strain A773 (*pyrG*^{89}) was cultured in complete medium (CM) as previously described [10]. One Shot® TOP10 Chemically Competent *Escherichia coli* (Invitrogen, The Netherland) was used to propagate plasmid and to clone purified polymerase chain reaction (PCR) products. The PyrG-containing vector pExPYR was used for expression of AbnA in *A. nidulans*.

2.2. Cloning of endo-1,5-arabinanase-encoding gene

In order to isolate the genomic DNA, *A. niveus* grown in CM-glucose medium, the mycelia was freezed with liquid nitrogen and harvested followed by treatment with 600 μl of genomic extraction solution (10% of 0.5 M EDTA and 1% SDS). The suspension was heated at 68 °C for 10 min, centrifuged at 13,000 × *g* for 5 min. and the supernatant transferred into a fresh tube. A volume of 40 μl of 5 M potassium acetate was added, mixed by inversion and placed on ice for 10 min. The suspension was centrifuged again at 13,000 × *g* for 5 min and the supernatant transferred into a fresh tube. After that, it was added 2.5× vol 95% EtOH and 70% EtOH twice to wash. The pellet was well dried and DNAse-free water added.

The coding sequence for the gene abnA was amplified with the polymerase chain reaction (PCR) using Platinum® Pfx DNA Polymerase (Invitrogen). The primer set used for *abnA* amplification was 5′-NNN **GCG GCC GC**T ATG CCA ACC CGG GGT CGT G-3′ and 5′-NNN **TCT AGA** GGC TAA TTA TCA TAC AAC TGG CCA TCC GCT AGA GAA ATC-3′, bearing restriction sites for directional cloning using *NotI* and *XbaI* sites (indicated in bold). The following touchdown PCR cycle parameters were used: denaturing at 95 °C for 1 min, annealing at 60 °C for 30 s and extension at 68 °C for 3 min, with repetition of previous cycles excepting by lowering the annealing temperature by 2 °C for every cycle until an annealing temperature of 52 °C was reached, then 28 cycles of 95 °C for 30 s, 52 °C for 30 s and 68 °C for 3 min, followed by a final extension at 68 °C for 10 min.

2.3. Secretion and purification of AbnA from A. nidulans

After confirmation of cloning into pExPYR plasmid by sequencing (accession number JN222917), the plasmid was transformed into *A. nidulans* [11]. Positive transformants were isolated by their ability to grow in the presence of 100 μg/ml of zeocin. Protein expression was carried out in an Erlenmeyer flask using MM with 100 μg/ml of zeocin with 2% maltose as inducer. The AbnA production was monitored by analyzing the culture supernatant by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and debranched arabinan hydrolysis.

To follow with the AbnA purification, *A. nidulans* was grown in 1 L of MM for 72 h at 37 °C. The mycelium was filtered with Wathman paper n° 1 and the crude extract was dialyzed against water and lyophilized. It was ressuspended in 4 mL of 10 mM ammonium acetate buffer pH 5.0 and applied on a BioGel P100 (Bio-Rad) column (44.9 × 1.2 cm) equilibrated with the same buffer. The protein elution was carried with the same buffer at a flow rate of 0.8 mL h^{-1}. Fractions with activity were concentrated by ultra-centrifugation (10 kDa cut off; Amicon – Millipore, Massachusetts) and analyzed to SDS–PAGE homogeneity.

2.4. Enzyme characterization

Activity against debranched arabinan and others complex carbohydrates were determined for reducing sugar production using dinitrosalicylic acid reagent (DNS) [12]. The reaction mixture, consisting of 50 μl substrate (1%, w/v) in 50 mM ammonium acetate buffer, pH 5.0 and 50 μl enzyme solution, was incubated at 60 °C in a water bath for 5 min. The reaction was stopped by adding 0.1 ml dinitrosalicylic acid and immediately boiled for 5 min. The reducing sugars released as enzyme activity were measured at 540 nm after 1 ml water addition and cooling. One unit of enzymatic activity was defined as the amount of enzyme which produced 1 μmol/min of reducing sugars. Against p-nitrophenyl arabinofuranoside or arabinopyranoside the reaction was assayed using 50 μl of 8-mM substrate and 50 μl of enzyme for 5 min. Reactions were stopped by adding 100 μl saturated sodium tetraborate, and products were measured by absorbance at 405 nm. To determine the optimum pH and temperature profiles, the enzymatic reaction was carried out at different pH in McIlvaine's [13] buffer system (pH 4.0–10.0) and various temperatures (30–80 °C). The protein content was measured by Bradford method [14].

2.5. Activation of agarose with epoxy groups

All the experiments were performed using the same agarose batch and the error was always lower than 10%. For this work, 10 g of agarose 4 or 10 BCL was suspended in 44 mL water, 16 mL acetone, 3.28 g NaOH, 0.2 g $NaBH_4$ and 11 mL epichlorohydrin. The suspension was stirred mildly for 16 h and washed with an excess of water. For quantification of the activated epoxy groups, 1 g of the support was treated with 10 mL 0.5 M H_2SO_4 for 2 h to hydrolyze the epoxy groups. Then, this hydrolyzed support was oxidized with $NaIO_4$, as previously described [15]. The number of epoxy groups was calculated by the difference in periodate consumption between the hydrolyzed support and the initial epoxy support. Periodate consumption was quantified using potassium iodide as previously described [16].

2.6. Modification of agarose supports with different reactive groups

Agarose epoxy-supports were modified with different moieties. In all cases, the ratio of the modifying reactive solution to the support was 1/10 (v/w).

2.6.1. Cationic supports

The epoxy-agarose support was modified with 1 M triethylamine in 50% water/acetone at pH 12 for 24 h at 25 °C.

2.6.2. Anionic supports

The epoxy-agarose support was treated with 0.5 M iminodiacetic acid (IDA) at pH 11 for 24 h at 25 °C.

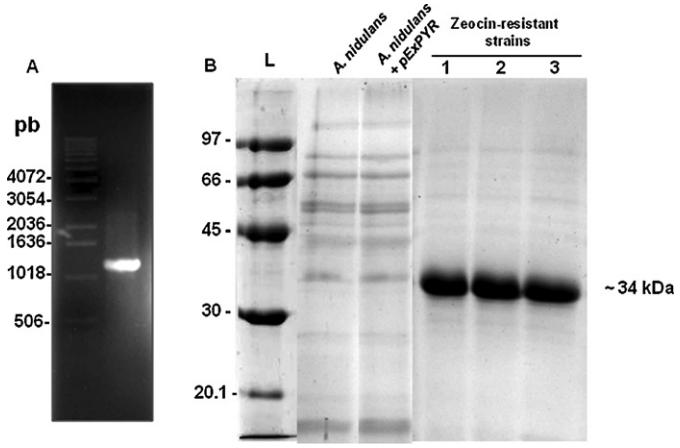

Fig. 1. Cloning and secretion of AbnA. (A) Endo-1,5-arabinanase-encoding gene amplified by PCR from *A. niveus* genomic DNA. (B) Recombinant AbnA secretion by *A. nidulans* strain A773. L, ladder; (1–3) pEXPYR plasmids carrying *abnA* was transformed into *A. nidulans* A773 (*pyrG*^{89}). Recombinants were grown on media containing 2% maltose for 2 days and secreted proteins analyzed by SDS–PAGE. The protein pattern of wild-type and a pExPYR-transformed strain of *A. nidulans* were used as control.

2.6.3. Metal chelate supports

The anionic supports were modified with a 30 mg/mL solution of 4 different metallic salts ($CuSO_4$, $NiCl_2$, $ZnCl_2$ and $CoCl_2$) at pH 7.0, 25 °C for 1 h.

2.7. Monofunctional supports

The epoxy-agarose supports were blocked with 5% mercaptoethanol at pH 8.7 and 25 °C for 16 h. Finally the supports were oxidized with the appropriate amount of sodium periodate, and washed with water.

2.8. Immobilization of the enzyme on CNBr activated sepharose

A solution of 10 mL of enzyme was diluted with 5 mL of 0.25 M sodium phosphate buffer at pH 7. This final solution was added to one gram of CNBr-activated sepharose 4B for 15 min at 4 °C under gentle stirring. After that, the immobilized preparation was filtered and treated several times with 20 mL of washing solution (0.1 M sodium bicarbonate, 0.5 M NaCl at pH 8.4). Afterwards, the derivative was filtrated and this preparation was incubated on 5 mL of blocking solution (1 M ethanolamine, pH 8) for 2 h. Finally, the derivative was washed exhaustively with distilled water. More than 95% of enzyme was immobilized.

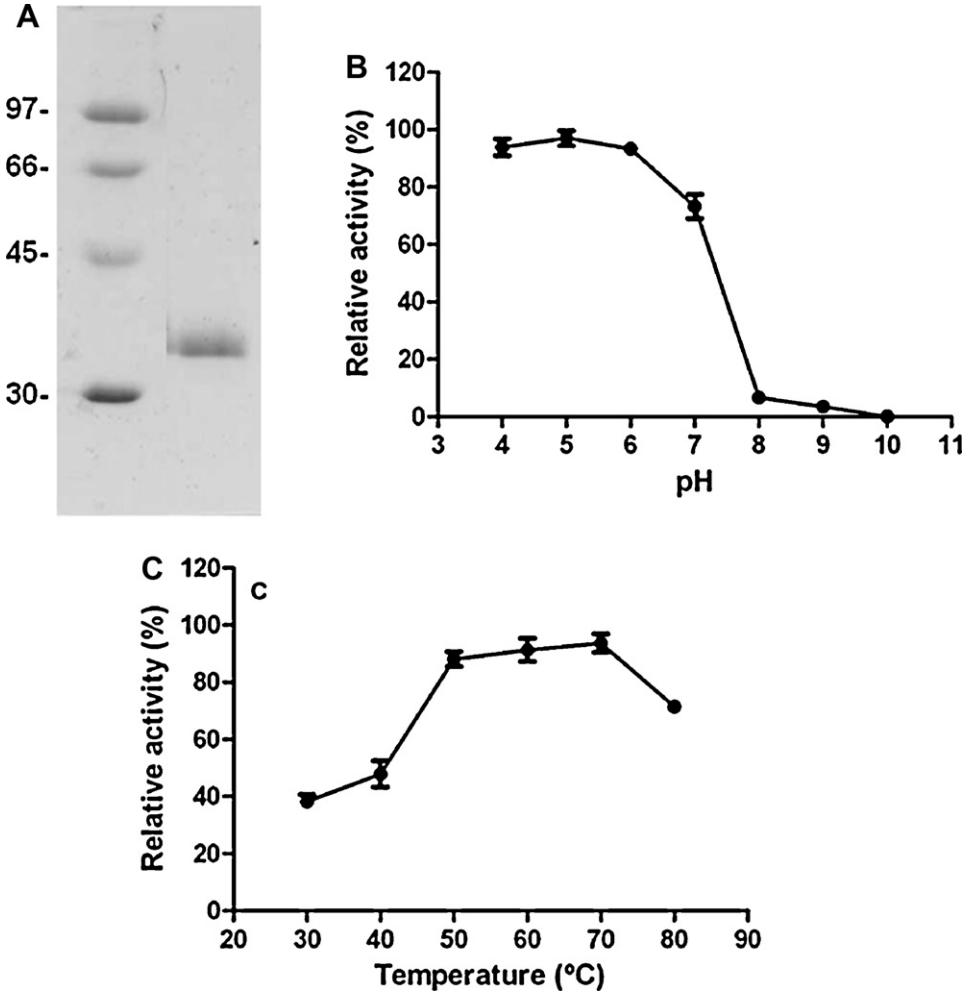

Fig. 2. Purification and characterization of AbnA. (A) 12% SDS–PAGE after AbnA BioGel P-100 filtration chromatography. Effect of pH (B) and temperature (C) on debranched arabinan hydrolysis by purified recombinant AbnA.

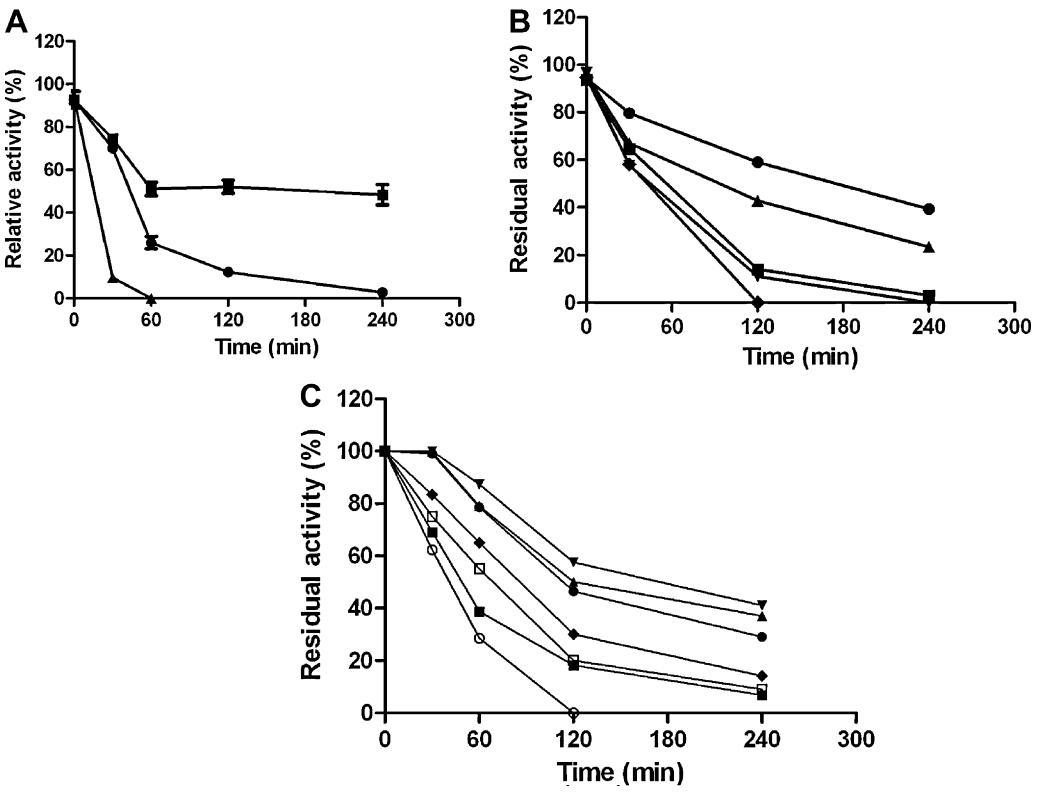

Fig. 3. Immobilization and stabilization of AbnA. (A) Immobilization courses of AbnA on modified agarose: (■) glyoxyl IDA–Ni^{2+}, (●) glyoxyl agarose and (▲) glyoxyl amine. Experiments were performed in 10 mM sodium phosphate at pH 7 for glyoxyl IDA–Ni^{2+} and glyoxyl amine, and pH 10 for glyoxyl agarose, both at 25 °C. (B) Thermal inactivation courses of different immobilized preparations of AbnA. Experiments were performed in 10 mM sodium phosphate at 70 °C and pH 5. (●) glyoxyl agarose, (▲) glyoxyl amine, (■) glyoxyl IDA–Ni^{2+}, (▼) CNBr activated sepharose and (♦) free AbnA. (C) Thermal inactivation courses of AbnA-glyoxyl agarose derivative after different times of immobilization at pH 10. Times of incubation: (●) 1 h, (▲) 2 h, (▼) 4 h, (♦) 6 h, (□) 12 h. Free AbnA (○) and the derivative CNBr activated sepharose (■) were used for comparison.

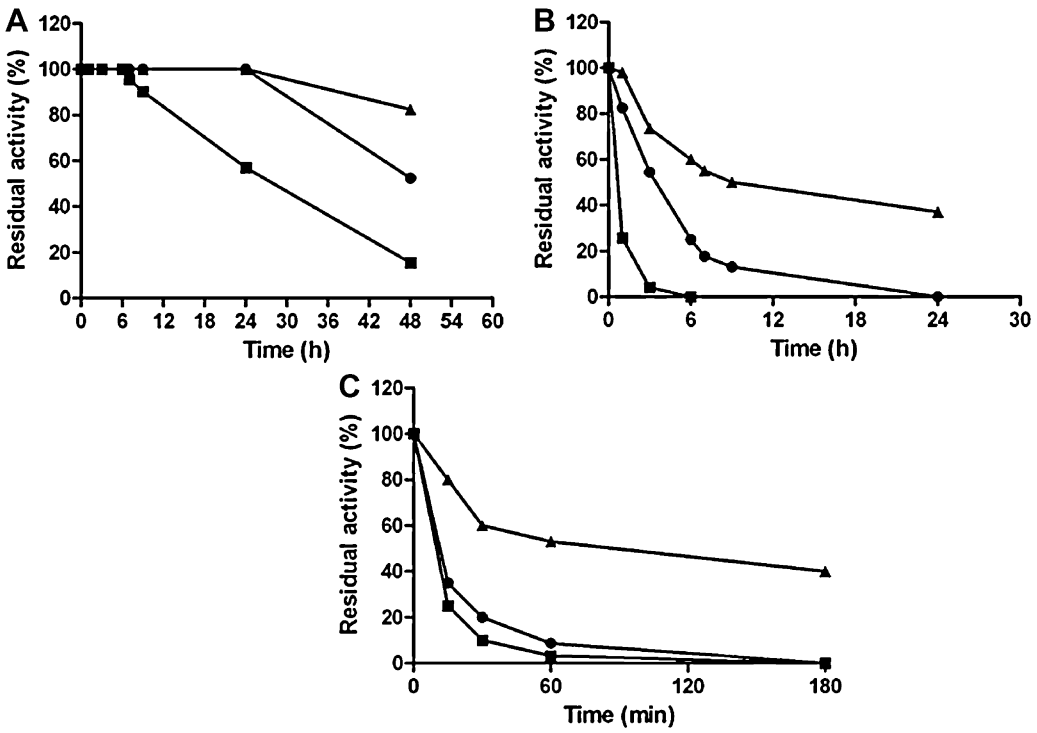

Fig. 4. Influence of pH on thermal inactivation of AbnA-glyoxyl agarose derivative. The AbnA-glyoxyl agarose derivative immobilization course was carried out for 4 h at pH 10 (▲). Free AbnA (■) and the CNBr activated sepharose derivative (●) were used for comparison. The thermal inactivation was carried at 60 °C and pH 5.0 (A), pH 7.0 (B) and pH 9.0 (C).

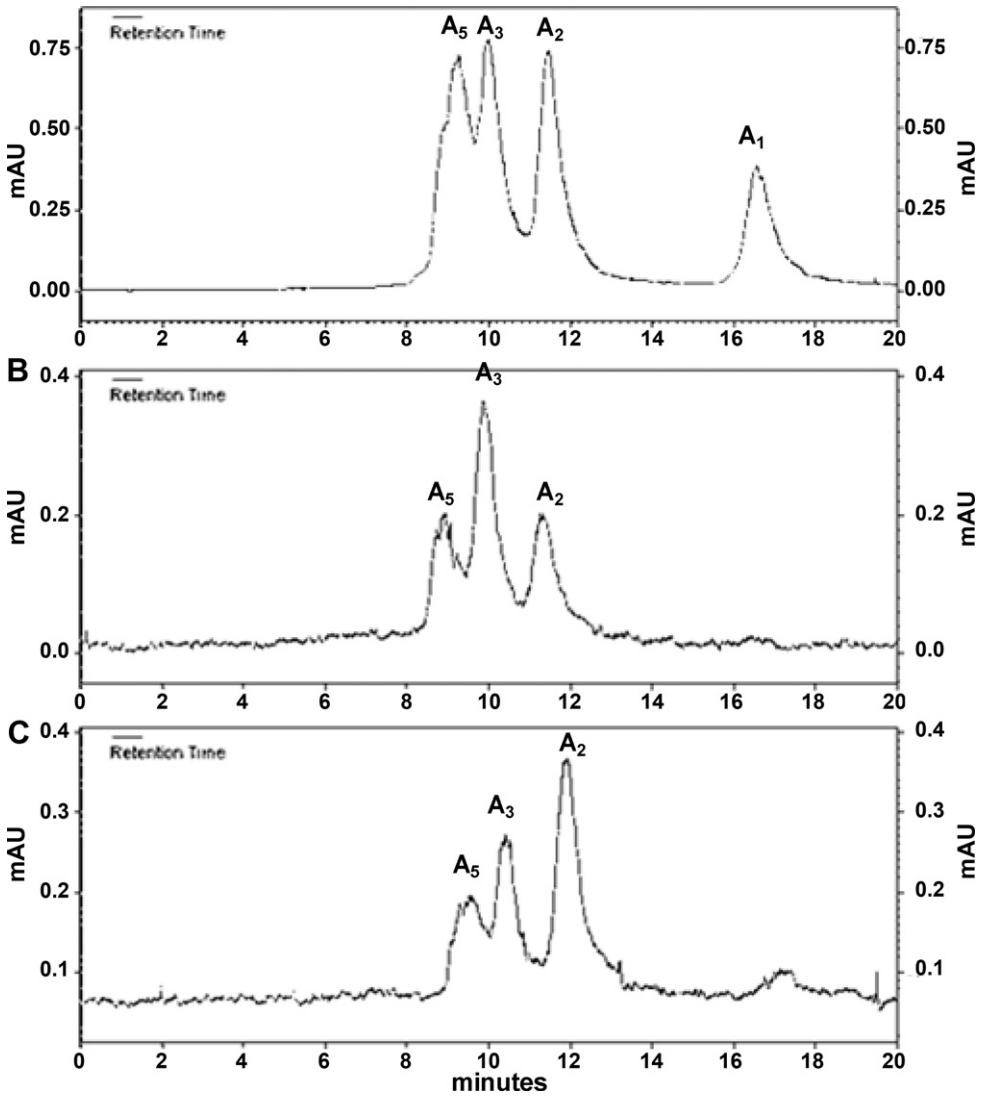

Fig. 5. HPLC analyses of arabinopentaose hydrolysis products by AbnA. Arabinopentaose (0.5%) in 5 mM sodium acetate buffer, pH 5, was incubated with AbnA, at 5 min (B) and 10 h (C); the elution times of the standards arabinose (A_1), arabinobiose (A_2), arabinotriose (A_3) and arabinopentaose (A_5) are indicated (A).

2.9. Enzymatic hydrolysis of arabinopentaose and debranched arabinan

High-pressure liquid chromatography (HPLC) was used to identify the products generated by AbnA from arabinopentaose and debranched arabinan. The substrates (1 mg/mL) were incubated with AbnA at 70 °C. At regular times, samples were withdrawn and heated in a boiling water bath for 5 min to stop the reaction. The reaction mixtures were then loaded on a SUPELCOGEL-Ca column (30 cm × 7.8 mm; Sigma–Aldrich, St. Louis, MO, USA), and the oligosaccharides were eluted with Milli-Q water as mobile phase at a flow rate of 0.5 ml/min at 80 °C. The sugars were detected with "Light Scattering" detector and were identified and quantified by comparing their elution times with those of standard amounts of arabinose and arabino-oligosaccharides, up to arabinopentaose.

2.10. Thermal stability of enzyme preparations

The inactivations were carried out in 0.25 mM of sodium phosphate buffer at different temperatures and pH. At different times, samples were withdrawn and their activity was tested with debranched arabinan as substrate as described in methods. The remaining activity was calculated as the ratio between activity at a given time and activity before incubation.

3. Results and discussion

3.1. AbnA secretion in A. nidulans

The *A. niveus* genomic DNA was recently sequenced by our group. These data are currently under assembly and annotation. After an initial analysis, it was observed a high identity with *Aspergillus fumigatus* genomic DNA (around 96%). This analysis allowed us design the abnA primers based on *A. fumigatus* Af293 data base from NCBI. The gene encoding AbnA was amplified (Fig. 1A) from *A. niveus* genomic DNA by PCR and ligated into the pExPYR plasmid at the *NotI* and *XbaI* restriction sites which allows protein expression driven by the glucoamylase promoter and secretion based on the recombinant native glucoamylase signal peptide sequence. The plasmid was introduced through integrative transformation into the *A. nidulans* (strain A773) genome and recombinants selected on MM supplemented with 100 μg/ml of zeocin. Selected zeocin resistant integrants were submitted to induction (maltose), AbnA production and secretion analyzed by

SDS–PAGE (Fig. 1B). The *abnA* gene was 1233 bp in length showing three introns (269-418/899-950/1049-1093). The CDS was 966 bp in length coding to 321 amino acids. The *A. nidulans* post-translational machinery was enough to process the introns. It was identified a signal peptide (N-terminal 19 amino acids) by SignalP 4.0 Server [17] with the cleavage site predicted between Gly19 and Tyr20. The primary structure did not reveal a Ser/Gly-rich linker or a CBD region typically found in modular hydrolases. The mature protein had a calculated molecular weight of 34 kDa and a p*I* of 5.18 by "Compute pI/Mw" [18]. Multiple sequence alignment (ClustalW2 [19]) showed 95% amino acid identity to *Neosartorya fischeri* NRRL 181 arabinanase, followed by *Aspergillus clavatus* 86%, *Penicillium chrysogenum* Wisconsin 83%, *A. nidulans* FGSC A4 84%, *Aspergillus niger* 76%, *Aspergillus oryzae* RIB40 70%, *Chaetomium globosum* CBS 148.51 58% and *Aspergillus terreus* NIH2624 81%. Pfam analysis [20] found that AbnA belongs to GH43 family.

3.2. Purification and properties of the recombinant AbnA secreted by *A. nidulans*

The estimated molecular weight of the purified recombinant AbnA was 34 kDa, the same predicted from the deduced amino acid sequence (Fig. 2A) [21–23].

The optimal pH was measured at 60 °C in the presence of 1% debranched arabinan as substrate. Fig. 2B shows that AbnA displayed a working pH ranging from 4.0 to 7.0, with an optimum at pH 5.0. The AbnA operational temperature was measured at their optimal pH, with an optimum at 70 °C (Fig. 2C). Using debranched arabinan as the substrate, the apparent K_m and V_{max} values were determined to be 4.9 ± 0.3 mg/ml and 263.8 ± 0.8 U mg^{-1}, respectively. These high V_{max} and low K_m values, make this enzyme more interesting than arabinanases previously described [22–24].

3.3. Immobilization and stabilization of AbnA on modified glyoxyl-agarose supports

The immobilization yields of AbnA on glyoxyl IDA-Ni^{2+}, glyoxyl and glyoxyl amine supports were 43%, 96% and 100%, respectively (Fig. 3A). The short clusters of potential metal-binding residues such as histidines, cysteines, aspartates and glutamates can explain the low immobilization yield on glyoxyl IDA-Ni^{2+}. The immobilization on glyoxyl agarose was quite fast; in fact after 120 min, 96% of the enzyme had been immobilized [25].

Considering the strict dependence of the immobilization rate on glyoxyl agarose on the density of Lys residues [26], the content of Lys residues on the enzyme surface was analyzed in PyMOL Molecular Graphics System 1.1, based on high resolution crystal structure of 1,5-alpha-L-arabinanase from *Geobacillus stearothermophilus* available at the PDB (Protein Data Bank) [PDB structure code: 3cu9A]. The enzyme presented a high content in Lys groups exposed to the medium. In all cases, most of the enzyme immobilized on the supports retained very high activity (from 85% to 100%). The thermal inactivation of these immobilized preparations showed that the stability of the AbnA immobilized on glyoxyl agarose was improved by 1.6 and 4.0-fold factor (based on half-life) compared to the glyoxyl amine and free enzyme, respectively (Fig. 3B). The R groups from Lys residues present a p*Ka* around 10.28, multipoint covalent binding of proteins on glyoxyl agarose is dependent of deprotonated Lys residues. AbnA was incubated with glyoxyl agarose in sodium bicarbonate buffer at pH 10.2. It was analyzed crescents incubation times at pH 10.2 and what was its influence on the thermal stability of the glyoxyl derivatives at 70 °C (Fig. 3C). The optimal incubation time was 4 h, and this derivative stability was quite similar (≤1.5-fold) with incubations for 1 h or 2 h. However, increasing the time, the derivatives lose stability. The influence of pH under glyoxyl derivative thermal stability

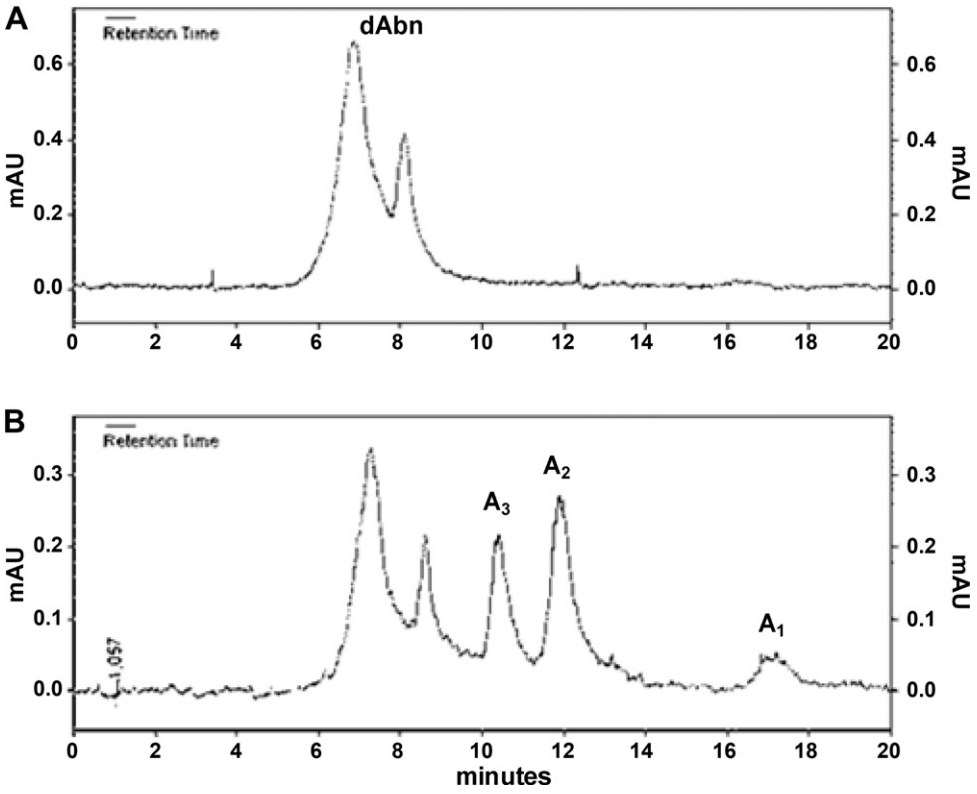

Fig. 6. HPLC analyses of the hydrolysis products of debranched arabinan (sugar beet) by AbnA. Debranched arabinan (0.5%) in 5 mM sodium acetate buffer, pH 5, was incubated with AbnA at 12 h (B). The elution times of the debranched arabinan (dAbn) are indicated in (A) and the elution times of the arabinose (A_1), arabinobiose (A_2), arabinotriose (A_3) and arabinopentaose (A_5) are presented in Fig. 5.

Table 1
Stabilization of different AbnA derivatives.

Activation[a]	Support[b]	μEqs glyoxyl/wet gr.[c]	Activity[d]	Stability[e]
CNBr	4% agarose	–	100	1
Glyoxyl IDA–Ni^{2+}	4% agarose	24	89	1.4
Amino-glyoxyl	4% agarose	24	93	2.5
Glyoxyl	4% agarose	24	93	4
Glyoxyl	10% agarose	180	82	10.3

[a] (1) Mild immobilization enzymes on CNBr-activated sepharose (15 min, at pH 7.0 and 4 °C), very likely, the enzyme is immobilized by a one-point attachment involving the amino terminal residue. (2) Amino-glyoxyl. The enzyme is firstly immobilized at pH 7.0 by anionic exchange through the region having the highest negative charge. Then a multipoint attachment between Lys present in the adsorbed region and the support is promoted by incubation under alkaline conditions. (3) Glyoxyl agarose. The enzyme is multipoint covalently immobilized, at pH 10, through the region having the highest amount of Lys groups.

[b] Different agarose supports: 4% agarose are the ones having thin fibers as morphology. 10% agarose have thicker fibers and hence they are able to promote a higher geometrical congruence between the enzyme and the support.

[c] Concentration of glyoxyl groups in the support: 24 Eqs is the result of activation with epichlorohydrin where 16 μEqs are epoxy groups converted in ionized amino groups. 40 μEqs are 4% agarose gels fully activated with glyoxyl groups. 180 μEqs are 10% agarose gels fully activated with glyoxyl groups.

[d] Activity is the percentage of activity of immobilized derivatives regarding to the soluble enzyme that has been immobilized.

[e] Stabilization is the ratio between half-life times of different derivatives and the half-life time of pure soluble enzyme.

at 60 °C was investigated. The half-life was >48 h, 9 h and 88 min, at pH 5.0 (Fig. 4A), 7.0 (Fig. 4B) and 9.0 (Fig. 4C), respectively. To date there is no reported data for immobilization of arabinanases to compare our results.

The low agarose-activation degree was firstly used for a correct comparison between different immobilization protocols by using glyoxyl-agarose and glyoxyl amine or chelate-glyoxyl. However after the observation that glyoxyl support was the best (immobilization of the enzyme through the region with the highest amount of Lys residues) we tried improving the stabilization by increasing the degree of activation of the support by using 10% agarose. The stability of the AbnA immobilized on glyoxyl agarose (10% 180 μEqs glyoxyl/wet g) was improved by 10.3-fold compared to the free enzyme (Table 1).

To determine the mode of action of the AbnA-glyoxyl agarose derivative, hydrolysis products released from arabinopentaose and debranched arabinan were analyzed by HPLC. During the course of arabinopentaose hydrolysis, arabinotriose and arabinobiose were detected as unique products (Fig. 5). The released products from debranched arabinan after incubation with AbnA-glyoxyl agarose derivative were also performed. Likewise, arabinobiose and arabinotriose were the major products, with trace amount of arabinose (Fig. 6) [27–29]. The capacity of hydrolysis of natural substrates, as arabinan, is an important characteristic of AbnA-glyoxyl agarose derivative to application on industrial process.

AbnA did not hydrolyze arabinofuranosyl or arabinopyranosyl aryl substrates (data not shown). To determine the AbnA substrate specificity, reducing sugar amount was measure after hydrolysis of corresponding native glycosidic bonds (wheat arabinoxylan, debranched arabinan, xylan form birchwood, xyloglucan from tamarind and starch). The enzyme could not hydrolyze wheat arabinoxylan, xylan from birchwood, xyloglucan or starch and showed substrate specificity towards debranched arabinan (data not shown) [30].

4. Conclusions

Here, we reported cloning, secretion, characterization and immobilization of an endo-1,5-arabinanase from *A. niveus*. The properties of this enzyme were compared to some previously studied arabinanases. The host *A. nidulans* strain A773 showed an AbnA over-secretion to culture medium, making easy the purification process. The enzyme is a true endo-1,5-arabinanase with a catalytic domain characteristic of family GH43. Our study demonstrated that the glyoxyl agarose support is very suitable for a rapid multipoint covalent immobilization between AbnA and the support, since the enzyme presented Lys groups exposed in its surface. 10% glyoxyl agarose derivative was 10.3-fold more stable than the free enzyme at 70 °C, based on half-life time. AbnA endo-action was confirmed after hydrolysis of arabinopentaose and debranched arabinan, where was observed arabinotriose and arabinobiose as major products. This is the first report of immobilization of an endo-1,5-arabinanase on glyoxyl agarose.

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