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Characterization of α-L-fucosidase and other digestive hydrolases from *Biomphalaria glabrata*

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ABSTRACT

Schistosoma mansoni is one of the major agents of the disease Schistosomiasis, which is one of the major global public health concerns. *Biomphalaria glabrata* is an obligate intermediate mollusc host of *S. mansoni*. Although the development of *S. mansoni* occurs in the snail hepatopancreas, studies that focus on this organ remain limited. In this study, we biochemically identified five distinct carbohydrases (amylase, maltase, α-glucosidase, trehalase, and α-L-fucosidase), lipases, and peptidases in the *B. glabrata* hepatopancreas and focused on the isolation and characterization of the activity of α-L-fucosidase. The isolated α-L-fucosidase has a molecular mass of 141 kDa, an optimum pH of 5.8, and is inhibited by Tris, fucose, and 1-deoxyfuconojirimycin. *B. glabrata* α-L-fucosidase is an exoglycosidase that can hydrolyze the natural substrate fucoidan to fucose residues. It presented K_m values of 48.4 μM to 4-Methylumbelliferyl α-L-fucopyranoside and 0.55 mM to p-nitrophenyl-α-L-fucopyranoside. Thus, α-L-fucosidase has a high activity in the hepatopancreas of *B. glabrata*, and the differential expression of this enzyme between susceptible and resistant strains indicates that besides its digestive role, α-L-fucosidase may also be important in host/parasite interactions.

1. Introduction

Schistosomiasis, a serious public health problem, is second only to malaria (WHO, 2013; http://www.who.int). Globally, 200 million people are infected with the snail-transmitted, water-borne parasitic helminth *Schistosoma mansoni*, and severe infections claim 20,000 lives annually. Multiple studies show that during infection in mammalian or mollusc hosts, the different life-cycle stages of *S. mansoni* present a series of glycoconjugates that play important roles in the host-parasite interplay (Van Die et al., 2010). For example, glycosphingolipids extracted from cercariae and eggs are known to contain a series of fucose residues (Weiss et al., 1986; Wuhrer et al., 2002).

Studies on *S. mansoni* and the snail *Biomphalaria glabrata* have focused mainly in *Biomphalaria's* hemocytes and defense mechanisms. In snails, hepatopancreas is an organ that provides the most favorable conditions for the development and multiplication of parasites by providing abundant food supply to sporocysts (Becker, 1980). Studies that focus on the hepatopancreatic environment as an interface for parasite-host interactions remain limited. Recently, Myers et al. (2008) investigated the role of proteolytic enzymes from *B. glabrata* in the development of the *S. mansoni* and its distribution in the hepatopancreas, ovotestis, albumen gland, and cell-free plasma (hemolymph). The authors also identified clones corresponding to *B. glabrata* cellulase, elastase, disintegrin and metallopepetidase, lysozyme, α-L-fucosidase, and serine peptidase in the EST data. The dependence of the fucose residues present on glycoconjugates on host-parasite interactions and the evidence of a possible hepatopancreatic α-L-fucosidase that can catalyze the removal of these fucose residues, led us to hypothesize that this α-L-fucosidase may facilitate the removal of the fucose residues from the glycosphingolipids present in *S. mansoni* in order to diminish host/parasite interaction.

α-L-fucosidase (EC 3.2.1.51) is a ubiquitous lysosomal glycoside hydrolase (GH) from family 29, (Shaikh et al., 2013) and catalyzes

Abbreviations: MUB, 4-methylumbelliferyl butyrate; DMPTB, dimercapto-1-propanol tributyrate; MUFUC, 4-methylumbelliferyl α-L-fucopyranoside; MUGLU, 4-methylumbelliferyl α-D-glucopyranoside; NPFUC, *p*-nitrophenyl α-L-fucopyranoside; BgFUC, α-L-fucosidase from *Biomphalaria glabrata*.

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the removal of L-fucose from the non-reducing α-1,2; α-1,3; α-1,4; or α-1,6 ends of oligosaccharides or glycoconjugates. Some species also present secreted forms of α-L-fucosidase, including human FUCA2 (Liu et al., 2009). In addition to, other soluble fucosidases have been identified as digestive enzymes in some invertebrates such as molluscs (Reglero and Cabezas, 1976; Endo et al., 1993; Berteau et al., 2002), mites (Bowman, 1987), and ticks (Moreti et al., 2013).

In this study, we identified and isolated the digestive α-L-fucosidase from *B. glabrata*, in the hepatopancreas of this mollusc. We also characterized the digestive activity of the hepatopancreatic α-fucosidase to better understand its physiological role. In addition, we quantified other distinct digestive hydrolases from the hepatopancreas of *B. glabrata.*

2. Materials and methods

2.1. Chemicals

Buffer salts, detergents, protein inhibitors, fucose, deoxyfuconojirimycin, and the substrates were purchased from Sigma-Aldrich (USA).

2.2. Samples

Feeding snails (*B. glabrata;* shells of 14 mm) (Cantinha et al., 2010) were immobilized on ice and dissected to isolate their digestive system. The tissues were homogenized in a Potter–Elvehjem homogenizer in ultra-filtered water (Millipore). The samples were then centrifuged at 16,100 × *g* for 20 min at 4 °C. The soluble fraction was used as the enzyme source.

2.3. Hydrolase assays and protein estimation

The protein levels were estimated according to methods described by Smith et al. (1985), using egg albumin as the standard. All the enzymatic assays were performed at 30 °C. For each measurement, incubations were performed for at least four different time points, and the initial rates were calculated. One unit of enzyme (U) is defined as the amount of enzyme that hydrolyzes the substrate to generate 1 μmol of the product/min. The hydrolases tested and their substrate and assay conditions are listed in Table 1. The release of fluorescent products was followed in a Gemini XPS spectrofluorimeter (Molecular Devices, USA). Colorimetric measurements were performed using a SpectraMax 190 spectrophotometer (Molecular Devices, USA).

2.4. Effect of pH on hydrolase activities

The effect of pH variation on all the hydrolase activities was determined using at least 16 different pH solutions. The following buffers (0.05 M) were used: glycine–HCl (pH 2.0–2.5); citrate–phosphate (pH 2.5–7.0), Tris–HCl (pH 7.0–9.0), and glycine–NaOH (pH 9.0–10).

2.5. Effect of Tris on glycosidase activities

Gel filtration fractions were assayed for α-fucosidase and α-glucosidase in the presence and absence of 20 mM Tris. MUFUC and MUGLU were used as substrates. The activity was measured as described in Table 1.

2.6. Isolation of α-L-fucosidase activity

Enrichment of α-L-fucosidase activity was achieved by fractionation using 40% ammonium sulfate and hydrophobic chromatography. Samples were first mixed with ammonium sulfate solution and kept for 18 h at 4 °C. The samples were centrifuged at 16,100 × *g* for 20 min at 4 °C. The soluble fraction was then subjected to hydrophobic chromatography (HiTrap Butyl column) on an FPLC System (GE Life Sciences) equilibrated with 0.05 M citrate-phosphate buffer containing 1.4 M ammonium sulfate. The proteins were eluted in a 25 mL gradient from 1.4–0 M $(NH_4)_2SO_4$ in the same buffer at a flow rate of 1.0 mL/min and 1.0 mL fractions were collected.

2.7. SDS-PAGE, native gel electrophoresis (PAGE), and gel filtration

Samples containing α-L-fucosidase activity were combined with a sample buffer containing 60 mM Tris–HCl buffer at pH 6.8, 2.5% SDS, 10% v/v glycerol, and 0.005% (w/v) bromophenol blue; and boiled for 5 mins. The samples were loaded onto a 12% (w/v) polyacrylamide gel slab containing 0.1% SDS (Laemmli, 1970) and electrophoresed at 200 V. Native gels (without β-mercaptoethanol and SDS) were run at a constant voltage of 100 V at 10 °C and silver-stained for proteins (Blum et al., 1987) or subjected to gel activity assay, as described below. After native electrophoresis, the gels

Table 1
Assay conditions and methods used in the determination of hydrolases from *Biomphalaria glabrata*[*].

Enzyme	Substrate	Concentration	pH	Group determined	Reference
Maltase	Maltose	10 mM	5.0	Glucose	Dalquist (1968)
Amylase	Starch	1%	6.0	Reducing groups	Noelting and Bernfeld (1948)
	Starch	1%	7.5		
	Glycogen	1%	6.0		
Trehalase	Trehalose	7 mM	5.0	glucose	Dalquist (1968)
α-Glucosidase	4-MU-α-Glucopyranoside	0.05 mM	5.5	4-Methylumbelliferone	Baker and Woo (1992)
α-L-Fucosidase	4-MU-α-L-fucopyranoside	0.025 mM	5.5	4-Methylumbelliferone	Baker and Woo (1992)
Lipase	4-MU-butirate	1 mM	8.5	4-Methylumbelliferone	Vaneechoutte et al. (1988)
	DMPTB	0.22 mM	7.5	Thiol groups	Choi et al. (2003)
Aminopeptidase	Leu-pNA	0.96 mM	8.5	*p*-Nitroanilide	Erlanger et al. (1961)
Trypsin	Z-FR-MCA	10 μM	8.5	7-Amino 4-methyl coumarin	Alves et al. (1996)
Chymotrypsin	Suc-AlaAlaProPhe-MCA	10 μM	8.0	7-Amino 4-methyl coumarin	Alves et al. (1996)
Peptidase	Casein-FITC	0.2%	8.0	FITC	Twining (1984)
Carboxypeptidase	Z-Gly-Phe	10 mM	8.0	phenylalanine	Nicholson and Kim (1975)
Cathepsin L[**]	Z-FR-MCA	10 μM	5.0	7-Amino 4-methyl coumarin	Alves et al. (1996)
Cathepsin B[**]	Z-RR-MCA	10 μM	5.0	7-Amino 4-methyl coumarin	Alves et al. (1996)

[*] Assays were performed at 30 °C at the indicated pH values. The buffers (0.05 M) were used: citrate-phosphate (pH 2.5–7.0). Tris–HCl (7.0–9.0), and glycine-NaOH (pH 9.0–10). Incubations were carried out for at least four different periods of time and the initial rates calculated. One U of enzyme is defined as the amount that catalyses the cleavage of 1 μmol of product/min.

[**] Assay buffers containing 3.0 mM EDTA and 3.0 mM cysteine.

were washed three times in 0.05 M citrate–phosphate buffer (pH 5.5) at 4 °C. A filter paper was moistened in MUFUC solution as described in the enzyme assay in Section 2.3. The filter paper was then superimposed onto the gel and incubated for 5 min at 25 °C and then fluorescence of free methylumbelliferone was photographed using the Gel Documentation system (MiniBis Pro-DNR Bio-Imaging Systems). The Mr values were calculated according to Shapiro et al. (1967) using a low range marker (BioRad). Gel filtration chromatography using a Superdex G75 column (GE) was performed in an FPLC system (GE) to determine the molecular mass of the hydrolases. Soybean trypsin inhibitor (21.5 kDa), ovalbumin (45 kDa), bovine serum albumin (66 kDa) were used as the molecular mass standards.

2.8. Specificity and kinetic characterization of *B. glabrata* α-L fucosidase

Besides the MUFUC assay, the specificity of *B. glabrata* α-fucosidase was tested using its natural substrate, fucoidan, and the competitive inhibitors, fucose and deoxyfuconojirimicyn. Fucoidan from *Fucus vesiculosus* (Sigma) was solubilized in 0.05 M citrate-phosphate buffer (pH 5.5) containing 0.02% sodium azide and mixed with the homogenized *B. glabrata* samples. Hydrolysis was performed at 37 °C for 120 h (Berteau et al., 2002; Moreti et al., 2013). The samples: control (fucoidan in citrate phosphate buffer without enzymes under the same hydrolysis condition), experimental (fucoidan in citrate phosphate buffer with the enzymes under the same hydrolysis condition), and standard (5.0 mM fucose), were boiled for 5 min and lyophilized by vacuum centrifugation. The experimental, control, and standard samples were then solubilized in ultrapure water and spotted on to an Alugram Sil G TLC plate (Macherey—Nagel, Germany) using a mixture of butanol–ethanol–water (50:30:20) as solvent (Genta et al., 2003). After the chromatographic separation, the results were visualized with a phenol–sulfuric acid reagent (Stahl, 1969).

The effect of substrate concentration on the enriched α-L-fucosidase activity was determined using at least 15 different substrate concentrations. K_m and V_{max} values (mean and S.E.M.) were determined by linear regression using the Enzfitter (Elsevier, Biosoft) software.

For determining the K_i value using deoxyfuconojirimycin as the inhibitor, enriched α-L-fucosidase was incubated with 5 different concentrations of deoxyfuconojirimycin: 50, 25, 5, 2.5, 1.25, and 0.5 nM in five different concentrations of the substrate: 150, 75, 40, 30, 20, and 10 μM. The K_m and V_{max} values were determined by fitting the data in the Michaelis–Menten equation, fitting the data in the Michaelis–Menten equation using the Enzfitter software. K_i values were determined from replots of slopes and/or intercepts of Lineweaver–Burk plots against the inhibitor concentration (Segel, 1975).

3. Results

3.1. Enzymes involved in the digestive process

Thirteen distinct substrates were used to identify the enzymes involved in the digestive process of *B. glabrata*. In order to understand carbohydrate digestion, we focused on the α-glycosidases. Amylase was found to be the most active enzyme (Table 2). There was no significant difference in the efficiency of hydrolysis between the two amylase substrates, starch and glycogen. Amylase products are usually hydrolyzed by maltase and α-glucosidases. Both the activities were identified in the digestive tract of *B. glabrata*. We also measured the activity of α-L-fucosidase, which was the second major glycosidase. Although we identified the presence of a trehalase, which hydrolyzes α-1,1-glycosidic bonds in disaccharides, its activity was very low. The lipase activity was assayed using DMPTB and MUB. The hydrolysis of DMPTB indicates the presence of triacylglycerol lipases. The highest activity observed on MUB could be due to the presence of other esterases.

In the case of proteolytic enzymes, casein-FITC was used as a natural and generic substrate for endopeptidases. Specific substrates and assay conditions were used for the identification of serine and cysteine peptidases. The highest activity was observed using Z-FR-MCA as a substrate in acidic conditions in the presence of cysteine and EDTA that favors the activity of cysteine peptidase. The final digestion of the proteolysis products was analyzed by measuring aminopeptidase and carboxypeptidase. The activity of aminopeptidase was higher than that of carboxypeptidase.

The optimum pH of amylase, aminopeptidase, and lipase activities is slightly alkaline, and is 7.9; 7.8; and 7.7, respectively (Fig. 1). Cysteine peptidase, α-L-fucosidase, and α-glucosidase have an acidic optimum pH of 5.0, 6.0, and 6.0, respectively. Trypsin activity using BApNa as the substrate showed that it is an alkaline proteolytic enzyme. Separation of the homogenized samples of the digestive tract of *B. glabrata* on gel filtration chromatography

Table 2
Activity of different digestive enzymes from the digestive system of *Biomphalaria glabrata*[*].

Enzyme	Substrate	Activity (mU/hepatopancreas)	Specific activity (mU/mg protein)	Specific activity (mU/mg h[**])
α-Amylase	Starch[1]	1630 ± 90	690 ± 32	43.0
	Starch[2]	3165 ± 260	1333 ± 60	83.0
	Glycogen	1919 ± 300	1259 ± 16	50.0
α-Glucosidase	MU-α-glucoside	160 ± 19	86 ± 5	4.2
Trehalase	Trehalose	Lh	Lh	Lh
Maltase	Maltose	71 ± 2	25 ± 2	1.9
α-L-Fucosidase	MU-α-L-fucoside	371 ± 20	151 ± 5	9.7
Lipase	MU-Butyrate	855 ± 41	556 ± 22	22.3
	DMPTB	7.5 ± 0.7	4 ± 0.4	0.2
Peptidase	casein-FITC	Lh	Lh	Lh
Chymotrypsin	Suc-AlaAlaProPhe-MCA	nh	nh	nh
Trypsin	BApNa	4.5 ± 0.5	1 ± 0.14	0.1
Cysteine peptidase	Z-FR-MCA	30,000 ± 3300	15,000 ± 1500	783
Cysteine peptidase	Z-RR-MCA	nh	nh	nh
Aminopeptidase	Leu-pNa	9.8 ± 0.3	4 ± 0.3	0.3
Carboxypeptidase	Z-GlyPhe	6.7 ± 0.2	3 ± 0.4	0.2

[*] Results are means and range corresponding to determinations in at least three different biological samples. Assay conditions are listed in Table 1. Lh: means low hydrolysis at assay conditions and nh indicates absence of hydrolysis products in any assay conditions tested.
[**] Results are means of activity divided by an average mass of *Biomphalaria glabrata* hepatopancreas.
[1] pH 6.0.
[2] pH 7.5.

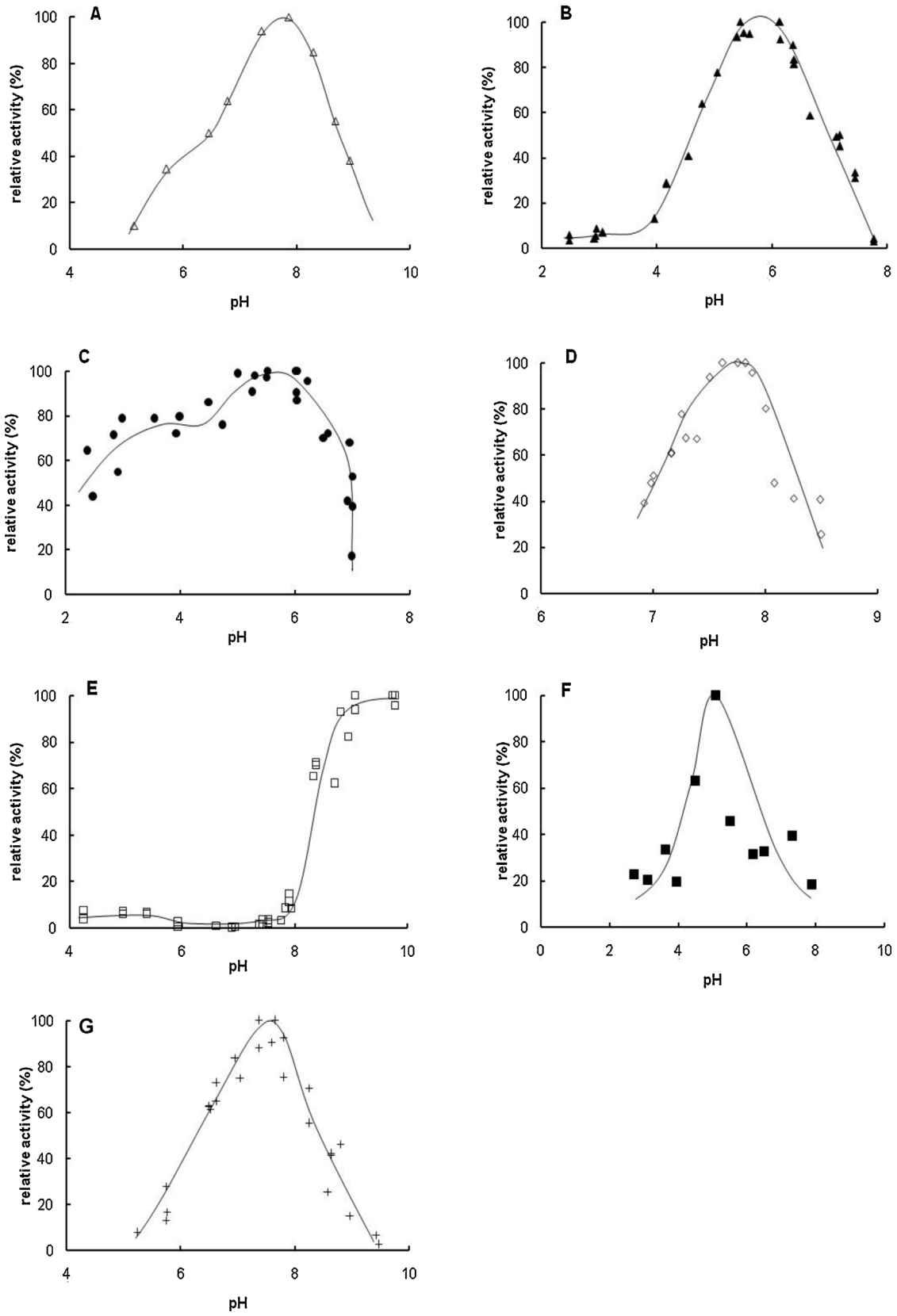

Fig. 1. Effect of pH on the activity of different enzymes from the digestive system of *B. glabrata:* (A) α-amylase, (B) α-glucosidase, (C) α-L-fucosidase, (D) lipase, (E) trypsin, (F) cathepsin L, and (G) aminopeptidase.

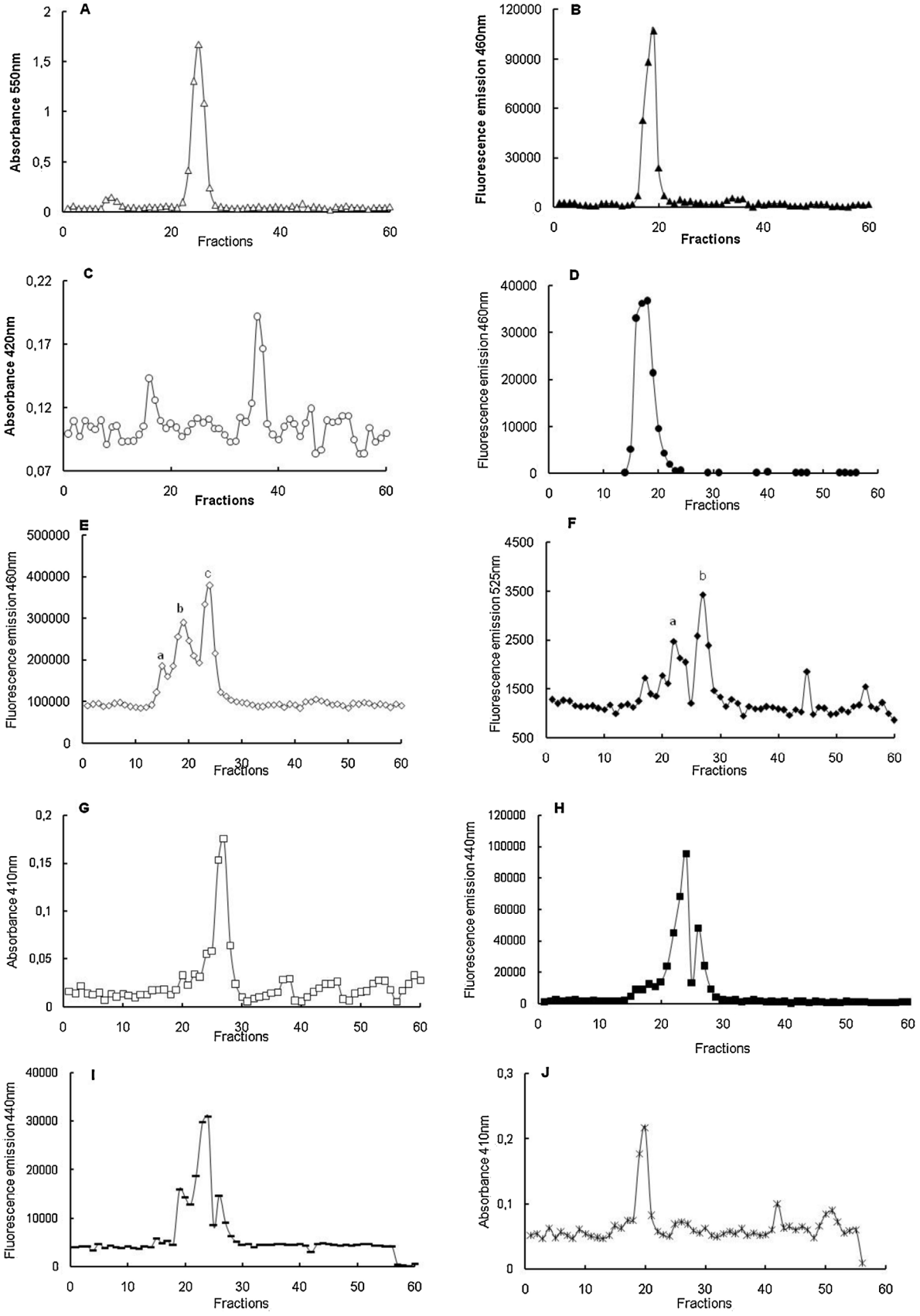

Fig. 2. Enzymatic activity from digestive system of *B. glabrata* after separation on gel filtration (Superdex G75 column) chromatography: (A) α-amylase, (B) α-glucosidase, (C) trehalase, (D) α-L-fucosidase, (E) lipase, (F) peptidase, (G) trypsin, (H) cathepsin L, (I) cathepsin B and (J) aminopeptidase.

Table 3
Molecular mass determined by gel filtration chromatography of distinct digestive enzymes from *Biomphalaria glabrata*.

Enzyme	Molecular mass (kDa)
α-Amylase	26
α-Glucosidase	110
trehalase	290
α-L-fucosidase	141
Lipase	290[a]
	111[b]
	33[c]
Peptidase	54[d]
(casein)	16[e]
Trypsin	16
Cysteine peptidase	33
Aminopeptidase	87

a, b and c are isoforms identified of lipase on gel filtration separation. d and e are distinct peptidases active on casein-FITC as substrates.

using a Superdex G75 column allowed mass determination of these enzymes (Fig. 2 and Table 3).

3.2. Isolation and characterization of α-L-fucosidase

The incubation of the soluble fraction of the homogenate samples with fucoidan, a natural substrate for fucosidases, resulted in the production of fucose residues (Fig. 3A), indicating that the effect observed on MUFUC is because of the activity of an exoglycosidase and not a fucoidanase. Native electrophoresis and gel activity of this sample (Fig. 3B and C) resulted in one activity band on MUFUC, indicating one major fucosidase. In order to isolate this fucosidase activity, the soluble fraction of a homogenate sample was subjected to ammonium sulfate fractionation (Fig. 4A). Both the resuspended pellet and the soluble fractions were tested for their fucosidase activity. Only the soluble fraction presented fucosidase activity with a recovery of 97% and a 7-fold yield. The soluble ammonium sulfate fraction was also subjected to SDS-PAGE (Fig. 4B). The samples were then applied on to a HiTrap Butyl column. The HiTrap Butyl fractions were assayed with MUFUC and MUGLU (Fig. 4C and D) and presented a recovery of 90%. The effect of the activity on MUFUC and MUGLU was also measured in the fractions from gel filtration chromatography in the presence of 40 mM Tris as a glycosidase inhibitor (Fig. 5). Data obtained from gel filtration, hydrophobic separation, and Tris inhibition validates the result that the hydrolysis of MUFUC is actually catalyzed by an α-fucosidase and not by an α-glucosidase with a broad specificity (Fig. 5A and B).

The active fractions obtained after the HiTrap Butyl separation were pooled and used to characterize the isolated activity. The optimum pH of the isolated α-L-fucosidase was 5.8 (Fig. 6A), as previously measured in the crude sample. The specificity of fucosidase was tested using fucose and deoxyfuconojirimycin as inhibitors in the HiTrap Butyl fractions and an inhibition of 46% and 95%, respectively, was observed (Fig. 6B). K_m and V_{max} were determined in the presence of different concentrations of MUFUC and NPFUC, resulting in K_m and V_{max} values of 48.4 ± 5.8 μM and 1100 ± 66 mU (Fig. 6C) and 0.55 ± 0.08 and 1.6 ± 0.12 (Fig. 6D) to MUFUC and NPFUC, respectively. Inhibition of the *B. glabrata* α-L-fucosidase by deoxyfuconojirimycin is a competitive inhibition with a K_i value of 20 nM (Fig. 6E).

4. Discussion

4.1. Digestion in Biomphalaria glabrata

Evidence from transcriptome analyses demonstrates that digestive enzymes can differ between lineages of *B. glabrata* that are resistant and susceptible to *S. mansoni* (Lockyer et al., 2007; Myers et al., 2008; Ittiprasert et al., 2010). In order to verify their activities and properties, we identified and characterized digestive enzymes involved in lipid, carbohydrate, and protein digestion. We were able to measure the activity of endopeptidases, specifically cysteine peptidases such as cathepsin B and cathepsin L, and serine peptidases including trypsin. The activities of the exopeptidases, carboxypeptidase and aminopeptidase, were also identified. These

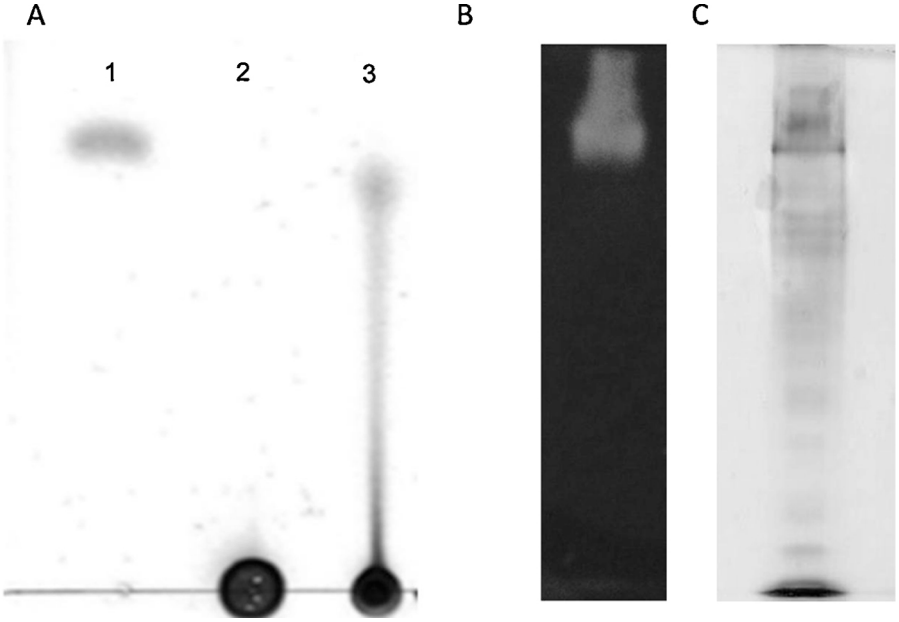

Fig. 3. Fucosidase activity from *B. glabrata*. (A) Thin-layer chromatography of the products of fucoidan hydrolysis by *B. glabrata* α-L-fucosidase ((1) standard: L-fucose; (2) negative control, fucoidan without enzyme; (3) product of fucoidan hydrolysis by *B. glabrata* α-L-fucosidase). (B) On gel α-L-fucosidase activity after native electrophoresis separation of soluble fraction from the homogenate sample of the digestive system from *B. glabrata* (C) Silver staining of the native electrophoresis separation of soluble fraction from the homogenate sample of the digestive system from *B. glabrata*.

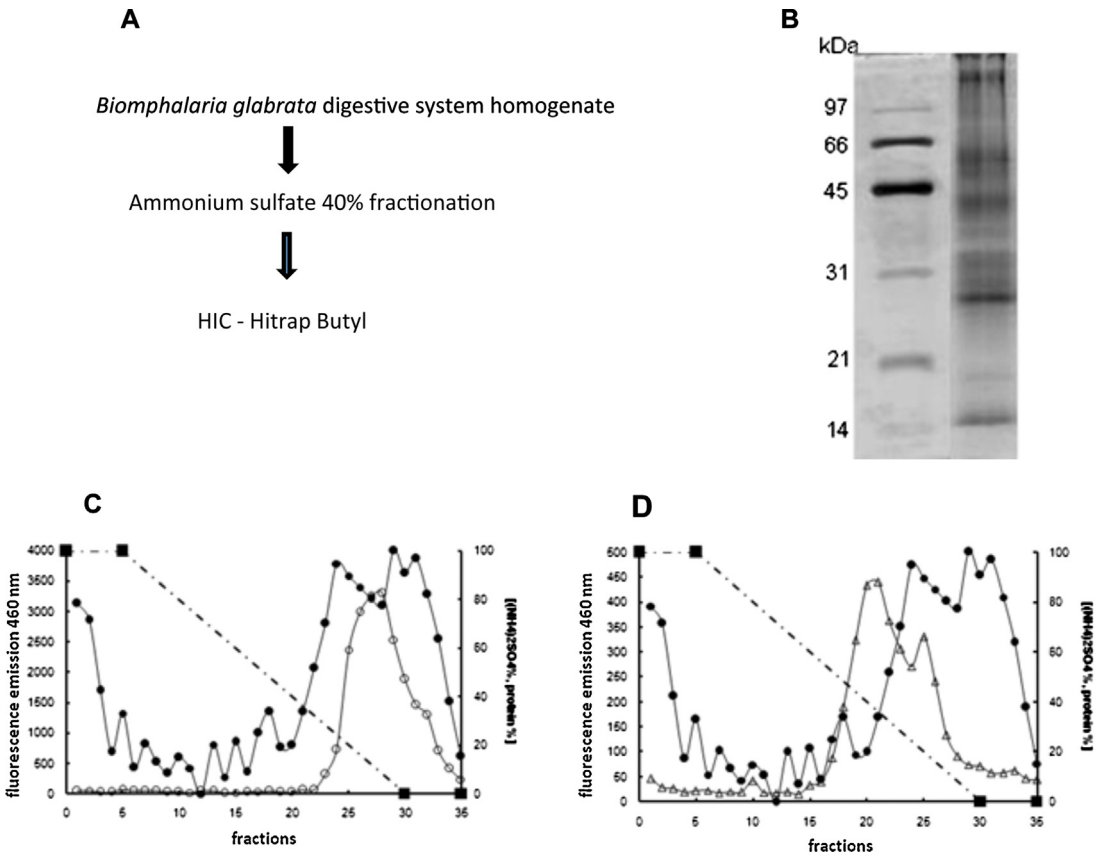

Fig. 4. Isolation of α-L-fucosidase. (A) Schematic presentation of the isolation steps. (B) Silver stained SDS-PAGE of the supernatant from the ammonium sulfate fractionation. Chromatographic separation on a Hitrap Butyl column of the supernatant from the ammonium sulfate fractionation of the samples exhibiting activity on MUFUC after ammonium sulfate fractionation. (C) Activity of α-L-fucosidase (○); Substrate: MU-α-L-fucoside (●) relative protein profile. (D) Activity of α-glucosidase (Δ); Substrate: MU-glucoside. (■) Decrescent ammonium sulfate gradient.

two enzymes are possibly involved in the degradation of products from cathepsins and trypsin activities. The carboxypeptidase activities were lower than our expectations based on data from cDNA sequence analysis. However, this may be related to differences in the diet provided to the animals, the different substrates used to measure carboxypeptidase activity, and susceptibility to *S. mansoni* infections.

For the first time, the lipase activity in the *B. glabrata* hepatopancreas was measured and characterized. Tunholi-Alves et al. (2013) show that there is a significant change in the lipid content of both the hemolymph and the digestive glands of *B. glabrata* during infection. This may be related to lipid processing from diet by distinct digestive lipases. Using gel filtration chromatography separation, we observed three distinct activities on MUB with molecular masses of 290 kDa, 111 kDa, and 30 kDa. The measured activities on both the substrates are probably due to the activity of a mixture of distinct lipases with an optimum alkaline pH ranging from 7.5 to 8.0. This value suggests that the activities of these lipases are similar to those of the pancreatic lipases. The presence of a group of enzymes catalyzing lipid digestion and related to multigenic families is common in many organisms, including mammals (Bakala N'Goma et al., 2012) and invertebrates such as insects (Horne et al., 2009; Christeller et al., 2011).

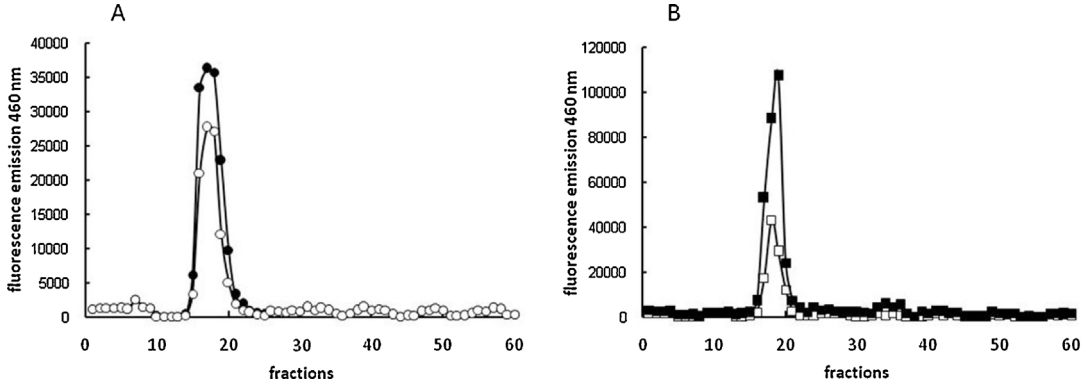

Fig. 5. Tris inhibition on fractions obtained from a separation on a Superdex G75 gel filtration column. (A) α-L-Fucosidase activity: (●) control; (○) Assays containing 20 mM Tris. Substrate: MU-α-L-fucoside. (B) α-Glucosidase activity: (■) control; (□) Assays containing 20 mM Tris. Substrate: MU-α-L-glucoside.

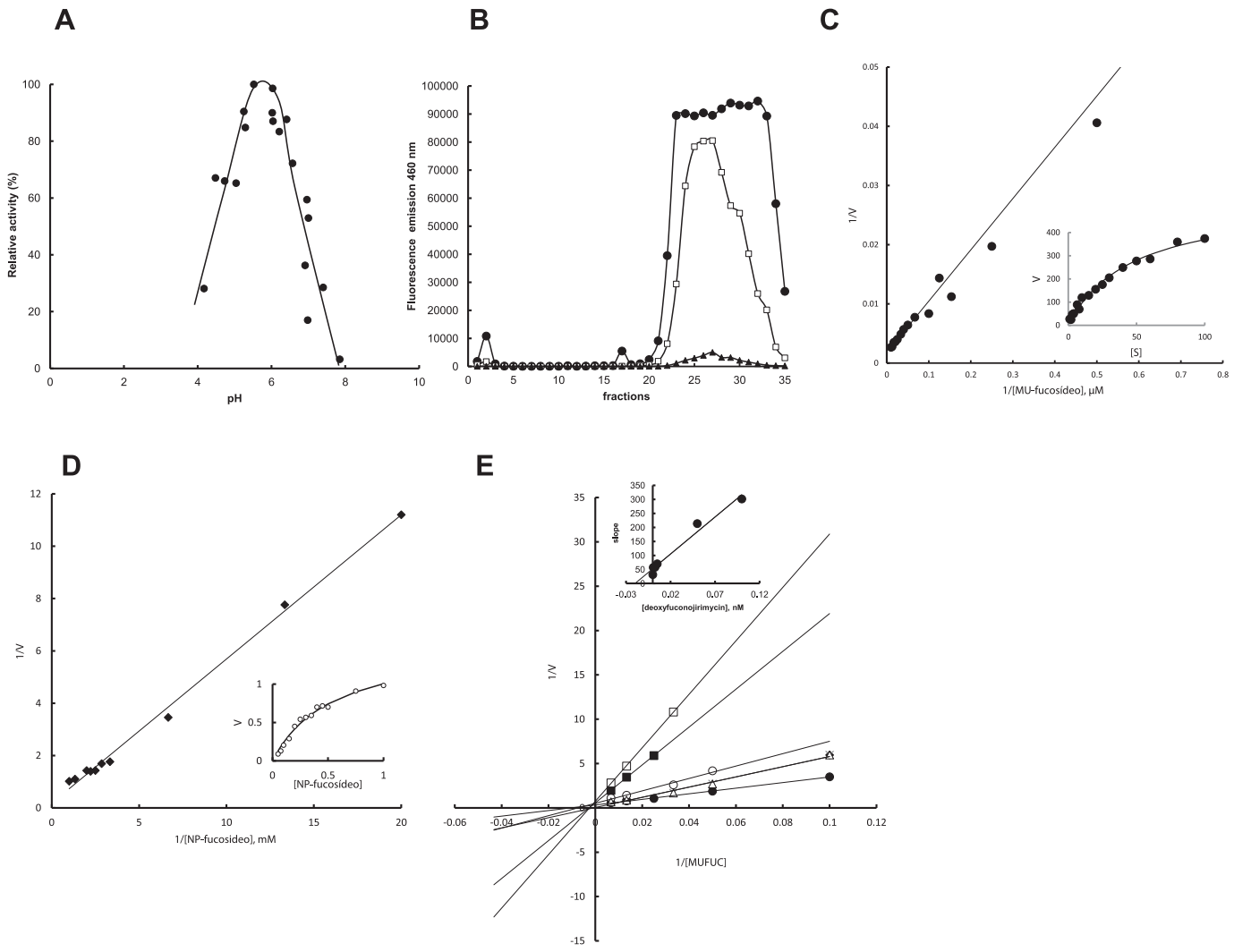

Fig. 6. Properties of the isolated α-L-fucosidase activity from *B. glabrata*. (A) Effect of pH on the activity of α-L-fucosidase isolated activity. (B) Inhibition profile by 1 mM fucose (□) and 65 μM deoxyfuconojirimycin (▲) of active fraction after hydrophobic chromatography on MUFUC (●). (C) Effect of MUFUC concentration on the activity of α-L-fucosidase. Lineweaver–Burk plot of the MUFUC activity. Inset: Michaelis–Menten plot. (D) Effect of NPFUC concentration on the activity of α-L-fucosidase. Lineweaver–Burk plot of the NPFUC activity. Inset: Michaelis–Menten plot. (E) Effect of 1-deoxyfuconojirimycin on α-L-fucosidase activity from *Biomphalaria glabrata*. K_i value = 20 nM.

A high activity of distinct carbohydrases, mainly amylase, α-glucosidase, and α-fucosidase, was identified. Amylase presented a low molecular mass in gel filtration chromatography. It is possible that this is an artifact of the interaction between the enzyme and the column matrix. Amylase hydrolyzed both starch and glycogen and presented an optimal pH of 7.8. This value is slightly higher than the optimum pH value of amylases (Schomburg et al., 2013) and indicates a more alkaline phase of digestion.

We were also able to identify the activity of α-L-fucosidase. The presence of a differentially expressed transcript of α-L-fucosidase in the hepatopancreas of *B. glabrata* strains that are resistant and susceptible to *S. mansoni* has already been reported by Myers et al. (2008). In addition, several α-L-fucosidases from the digestive tract of different species of molluscs, including the gastropods *Turbo cornutus* (Iijima et al., 1961); *Helix pomatia* (Marnay et al., 1964); and *Pomacea canaliculata* (Endo et al., 1993; Hirata et al., 1996) and the bivalves *Chamelea gallina* (Reglero and Cabezas, 1976) and *Pecten maximus* (Berteau et al., 2002), have been characterized. Due to the high activity of α-L-fucosidase and its differential expression in the resistant strains of *B. glabrata*, we isolated and characterized this enzyme in order to understand its potential role in the interaction between *S. mansoni* and *B. glabrata*.

4.2. α-L-Fucosidase and digestion in molluscs

The characterization of the activity of the isolated *B. glabrata* α-L-fucosidase (BgFUC) demonstrates that this digestive fucosidase has an optimum pH of 5.8. This acidic pH is typically found in different fucosidases. The isolated fucosidase presented a molecular mass of 141 kDa, as determined by gel filtration chromatography. This high molecular mass of α-L-fucosidases usually indicates an oligomerization process (Sulzenbacher et al., 2004; Moreti et al., 2013). The evidence of oligomerization was also corroborated by the analysis of native and SDS-PAGE.

BgFUC was able to hydrolyze fucoidan from *F. vesiculosus* and release fucose as the sole reaction product, confirming its activity as an exoglycosidase. In the case of its substrate affinity, BgFUC presented a K_m of 48.4 μM when MUFUC was used as the substrate. This value is similar to that of α-L-fucosidase of human and mouse liver and human spleen (Johnson and Alhadeff, 1991). Results of experiments using NPFUC indicate that BgFUC presents a similar K_m value to other mollusc α-L-fucosidases. BgFUC is inhibited by 1.0 mM fucose. However, only a 30% inhibition was observed. This low inhibition is distinct of K_i values reported to fucosidase inhibition by fucose (K_i of *Pecten maximus* is 240 μM) (Berteau et al., 2002).

Attempts to measure the K_i values of fucose showed that BgFUC is not competitively inhibited by fucose (data not shown) and a detailed characterization is necessary. These results may also be related to the transfucosylation process. Deoxyfuconojirimycin completely inhibited BgFUC at 65 μM and presented a K_i of 20 nM to 1-deoxyfuconojirimycin, which is similar to *Pecten maximus* fucosidase with a K_i of 26 nM and also to bovine and human fucosidases (Berteau et al., 2002).

4.3. Function of fucosidases in vector species

Pan (1965), and Becker (1980) demonstrated that the hepatopancreas of *B. glabrata* provides the most favorable conditions for the development and multiplication of *S. mansoni* by providing the sporocysts with abundant food supply, allowing for a fast parasitic growth.

Glycoconjugates located on the cell surface are involved in important cellular and molecular processes such as cell adhesion, cell growth, proliferation, and cell–cell interactions, including parasite–host interactions (Weiss et al., 1986). *S. mansoni* produces a variety of complex carbohydrates such as glycoproteins and glycolipids, many of which are highly fucosylated, in the different stages of its life-cycle (Wuhrer et al., 2002; Lehr et al., 2007; Peterson et al., 2009; Van Die et al., 2010).

Lehr et al. (2008) found a significant increase in fucosylated glycoconjugates in the hepatopancreas of infected snails in comparison to the non-infected ones. The authors proposed that this increase is related to the synthesis of these molecules by the parasite, as part of its strategy of molecular mimicry survival. The high degree of fucosylation of carbohydrates in both the parasite and host and the differential expression of fucosidase in the hepatopancreas of *B. glabrata* resistant strains (Lockyer et al., 2007; Myers et al., 2008) may indicate an involvement of fucosidase and fucosyl transferases in the parasite/host interactions.

An example of the role of fucose and α-L-fucosidase in parasite-host interactions is the infection of human gastric mucosa by *Helicobacter pylori*. These bacteria induce the secretion of FUCA2 from the infected gastric cells (Liu et al., 2009). Depletion experiments of FUCA2 show that this enzyme is essential for the adhesion of *H. pylori*.

The dependence of fucose/fucosidase to parasite/host interaction may also be important in invertebrate vectors such as ticks. Pedra et al. (2010) investigated the dependence of fucose on the interaction of *Anaplasma phagocytophilum* to the colonization of ticks midgut. The authors demonstrated that *A. phagocytophilum* modulates the expression of three α-1,3-fucosyltransferases and uses α-1,3-fucosylation to colonize the ticks. Fungi and other bacteria also contain fucose that is involved in parasite-host interactions (Järvinen et al., 2001; Ruiz-Palacios et al., 2003; Chessa et al., 2009).

Fucosidase is normally a lysosomal enzyme. However, in some vectors, infections, and some diseases like cancer, this enzyme appears in its soluble form. Our group previously identified the activity of a soluble α-L-fucosidase in the digestive tract of the tick *Amblyomma cajennense* (Moreti et al., 2013), which possibly indicates that this enzyme has a digestive function and is also involved in parasite/host interactions. We also analyzed the genome of the tick species, *Ixodes scapularis*, the vector of anaplasmosis, babesiosis, Lyme disease, and Powassan virus (Center for Disease Control and Prevention—http://www.cdc.gov/ticks/geographic_distribution.html). A search for α-fucosidases in the genome and transcriptome data from *Ixodes scapularis* revealed at least 10 distinct sequences of α-L-fucosidases. Similarly, sequences of digestive fucosidases were also found in *Rhipicephalus pulchellus* and the mite *Metaseiulus occidentalis*, indicating an important role of these enzymes in ticks (Genbank, 2014; http://www.ncbi.nlm.nih.gov/genbank).

The role of fucose/fucosidase in parasite/host interactions and its increased activity in the *B. glabrata* hepatopancreas underscores the importance of this enzyme. Additional studies on the ability of fucosidases to hydrolyze *Schistosoma* glycoconjugates and the use of techniques such as RNAi and qPCR may facilitate a comprehensive understanding of the role of this enzyme in *Biomphalaria/Schistosoma* interactions.

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