

## Biochemical properties of extracellular $\alpha$ -mannosidases from the digestive fluid of *Rhynchophorus palmarum* larvae

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### Abstract

Three extracellular  $\alpha$ -mannosidases (EC 3.2.1.24) were purified from the digestive fluid of oil palm weevil larvae, *Rhynchophorus palmarum* (L.) and characterized in order to explore their potential for biotechnological application. Three steps procedure including size exclusion, anion exchange and hydrophobic interaction chromatography were used. The enzymes named *RpIM1*, *RpIM2* and *RpIM3* had native molecular weights of approximately 125, 72 and 111 kDa, respectively and functioned as dimeric (*RpIM1*) and monomeric (*RpIM2* and *RpIM3*) structures. The three  $\alpha$ -mannosidases displayed acidic and mesophilic activities by using *para*-nitrophenyl- $\alpha$ -D-Mannopyranoside as substrate. Apparent  $K_M$  values were found to be 0.13, 0.22 and 0.16 mM for *RpIM1*, *RpIM2* and *RpIM3*, respectively.  $Ba^{2+}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Zn^{2+}$  and ethylene diamine tetra acetic acid (EDTA) had no effect on the three enzyme activities whereas detergents and reducing agents were inhibitors. *RpIM1*, *RpIM2* and *RpIM3* were more sensitive to swainsonine and cleaved  $\alpha$ -1,2;  $\alpha$ -1,3 and  $\alpha$ -1,6 linked mannobioses. These characteristics prompted us to categorize them as catabolic  $\alpha$ -mannosidases. Thus, they would help in the larval oil palm weevil nutrition allowing digestion by the removal of mannose residues in the beetle's diet. Also, these enzymes could find potential application in biological, biochemical and pharmaceutical area.

**Key words:** extracellular  $\alpha$ -mannosidases, characterization, oil palm weevil, *Rhynchophorus palmarum*, Coleoptera, Curculionidae.

### Introduction

The  $\alpha$ -mannosidases play an essential role in the processing of N-glycans to complex and hybrid oligosaccharides. The  $\alpha$ -mannosidases involved in N-glycans processing are categorized as class I and class II, with different substrate specificities, intracellular locations, sizes, cations requirements, amino acid sequences and sensitivities to plant alkaloid inhibitors (Moremen *et al.*, 1994; Herscovics, 1999; Moremen, 2000). According to the sensitivity of  $\alpha$ -mannosidases to plant alkaloid inhibitors, the enzymes are categorized into two distinctive classes. The azapyranose analogues, such as kifunensine (Kif) and 1-deoxymannojirimycin (DMNJ), are potent inhibitors of class I  $\alpha$ -mannosidases (Bischoff and Kornfeld, 1984; Elbein *et al.*, 1990). In contrast, those sensitive to swainsonine (Sw) and 1,4-dideoxy-1,4-imino-D-mannitol (DIM) which are azafuranose analogues belong to class II  $\alpha$ -mannosidases (Tulsiani *et al.*, 1982a; Cenci di Bello *et al.*, 1989). Based on the amino acid sequence alignments, class I and class II  $\alpha$ -mannosidases belong to family 47 and family 38, respectively in Henrissat's glycosidases classification (Moremen *et al.*, 1994; Henrissat and Bairoch, 1996). On the other hand, microbial  $\alpha$ -mannosidases have been widely studied (Keskar *et al.*, 1996) and fallen into two groups. In group A, the enzymes hydrolyse non-reducing terminal  $\alpha$ -mannosidic linkages regardless of the aglycon moiety. It includes those enzymes which act

on *para*-nitrophenyl- $\alpha$ -D-Mannopyranoside (*pNP*- $\alpha$ -Man) and do not have strict linkage specificity. In group B, the enzymes hydrolyse specifically linked  $\alpha$ -D-mannosidic bonds.

Apart from the vital role in glycoproteins synthesis and degradation,  $\alpha$ -mannosidases are currently an important therapeutic target for the development of anti-cancer agents (Goss *et al.*, 1995, 1997). The major potential application of  $\alpha$ -mannosidases involves the strategies for treating the lysosomal storage disorder  $\alpha$ -mannosidosis by enzyme replacement therapy. Indeed,  $\alpha$ -mannosidosis results from deficient activity of  $\alpha$ -mannosidase. The disease is characterized by massive intracellular accumulation of mannose-rich oligosaccharide, that is oligosaccharides carrying  $\alpha$ -1,2;  $\alpha$ -1,3 and  $\alpha$ -1,6 mannosyl residues at the non-reducing termini (Saint-Pol *et al.*, 1999; Sun *et al.*, 1999; Hirsch *et al.*, 2003). In this respect, the broad specific  $\alpha$ -mannosidases become useful for application requiring removal of all  $\alpha$ -mannosyl linkages. It is suggested that  $\alpha$ -mannosidase and N-acetyl- $\beta$ -hexosaminidase (isolated from latex) be used synergistically in the medical treatment of fungal diseases in the most severe cases to boost the known  $\beta$ -hexosaminidase antifungal action (Giordani *et al.*, 1991).

$\alpha$ -mannosidases have been reported from various sources, *e.g.* plants (Woo *et al.*, 2004; Ahi *et al.*, 2007), fungi (Reyna *et al.*, 2000; Tatara *et al.*, 2003), yeast (Kelly and Herscovics, 1998), animals (Weng and

Spiro, 1993; Tremblay *et al.*, 2007) and human cells (Winchester *et al.*, 1993; Liao *et al.*, 1996). Concerning insects, high  $\alpha$ -mannosidase activities have been well characterized in *Spodoptera frugiperda* (J.E. Smith) (Jarvis *et al.*, 1997; Kawar *et al.*, 2001) and in *Drosophila melanogaster* Meigen cells (Foster *et al.*, 1995; Rabouille *et al.*, 1999). Elsewhere, this enzyme has also been detected in the leaf cutting ant [*Acromyrmex subterraneus* (Forel)] larvae (Erthal Jr. *et al.*, 2007). Although insect  $\alpha$ -mannosidases have been subjected to previous studies, any work relative to extracellular  $\alpha$ -mannosidases from *Rhynchophorus palmarum* (L.) (Coleoptera Curculionidae) larvae has received the same attention to date. The weevil larvae are widely found in southern Asia, America, Melanesia and Africa, where they feed on broad range of palms including coconut, sago, date and oil palm. In some areas within these regions, it has been recorded as a serious pest of introduced palms (Oehlschlager *et al.*, 2002).

In this study, different chromatographic and biochemical methods were employed to purify and characterize three swainsonine sensitive  $\alpha$ -mannosidases from the digestive fluid of oil palm weevil larvae. The present paper will help on these enzymes categorization, their real importance in food digestion in order to explore their potential for biotechnological application.

## Materials and methods

### Insects

Oil palm weevil larvae were collected locally from their host trees (oil palm *Elaeis guineensis* Jacquin) in Côte d'Ivoire.

### Chemicals

*para*-nitrophenyl- $\alpha$ -D-Mannopyranoside (*p*NP- $\alpha$ -Man) and the other synthetic aryl-glycoside substrates, Mannobioses containing disaccharides linked (2-O- $\alpha$ -D-mannopyranosyl-D-mannopyranoside, 3-O- $\alpha$ -D-mannopyranosyl-D-mannopyranoside and 6-O- $\alpha$ -D-mannopyranosyl-D-mannopyranoside), swainsonine (Sw), 1,4-dideoxy-1,4-iminomannitol (DIM) and 1-deoxymannojirimycin (DMNJ) were purchased from Sigma-Aldrich. D (+) Mannose was from Panreac Quimica. Kifunensine (*Kitasatosporia kifunense*) (Kif) was obtained from Calbiochem. Sephacryl S-100 HR, Sephacryl S-200 HR, DEAE-Sepharose Fast Flow and Phenyl-Sepharose 6 Fast Flow gels were provided from Pharmacia Biotech. Bovine Serum Albumin (BSA) was obtained from Fluka Biochemika. Standard molecular weights proteins were provided from Bio Rad. Silicate gel 60 for thin-layer chromatography (TLC) was purchased from Merck. All the other reagents used were of analytical grade.

### Enzymes extraction

Oil palm weevil larvae digestive fluid was collected with a syringe, filtered through cotton wool and then centrifuged at 10,000 g for 30 min. The supernatant was used as the crude extract and stored at 4 °C.

### Enzymes purification

All the purification procedure was carried out in cold room. To the crude extract was added solid ammonium sulphate to 80% saturation. The mixture was stirred for at least eight hours and centrifuged at 10,000 g for 15 min. The pellet was suspended in 20 mM sodium acetate buffer pH 5.0 and loaded onto a Sephacryl S-100 HR column (1.5 cm  $\times$  67 cm) equilibrated with the same buffer. Fractions of 1 ml were collected at a flow rate of 0.2 ml/min and, those containing the  $\alpha$ -mannosidase activity were pooled.

The pooled fractions were applied to the following chromatography, a DEAE Sepharose Fast Flow column (2.5 cm  $\times$  4.5 cm) equilibrated with 20 mM sodium acetate buffer pH 5.0. The column was washed at a flow rate of 3 ml/min with two bed volumes of equilibration buffer to remove unbound proteins. Bound proteins were then eluted with a stepwise salt gradient (0.1, 0.2, 0.25, 0.3 and 1 M) of NaCl in 20 mM sodium acetate buffer pH 5.0 and fractions of 1 ml were collected. Three peaks of  $\alpha$ -mannosidase activity were obtained. To each pooled active fractions, solid ammonium sulphate was slowly added to give a final concentration of 1 M and the resulted enzyme solution was applied on a Phenyl Sepharose 6 Fast Flow column (1.5 cm  $\times$  3.2 cm) previously equilibrated with 20 mM sodium acetate buffer pH 5.0 containing 1 M ammonium sulphate. The column was washed with a reverse stepwise gradient of ammonium sulphate concentration (from 1 to 0 M) in the same sodium acetate buffer at a flow rate of 1 ml/min and fractions of 1 ml were collected.

The active fractions of each isoform were pooled, dialyzed overnight at 4 °C against 20 mM sodium acetate buffer pH 5.0 and constituted the purified enzymes.

### Enzymes assay

All enzyme reactions were performed at 37 °C for 20 min in appropriate 100 mM sodium acetate buffer pHs containing 0.8 mM of *p*NP- $\alpha$ -Man. After prewarming the mixture at 37 °C for 5 min, the reactions were initiated by adding enzyme solution. The final volume was 150  $\mu$ l and the reactions were stopped with 2 ml of sodium carbonate 2% (w/v). Enzymes activities towards 0.8 mM of *p*NP- $\alpha$ -Man were determined by measuring the released *para*-nitrophenol (*p*NP) at 410 nm using a spectrophotometer GENESIS 5. *p*NP was used as standard.

One unit of activity was defined as the amount of enzyme that hydrolyzes 1  $\mu$ mol of substrate per min under the assay conditions. The specific activity was expressed as unit of activity per mg of protein.

### Proteins estimation

Protein concentrations and elution profiles from chromatographic columns were determined by the Folin method (Lowry *et al.*, 1951). Bovine serum albumin (BSA) was used as the standard protein.

### Polyacrylamide gel electrophoresis (PAGE)

Electrophoresis was carried out by the method of Laemmli (1970) on 7.5% and 10% (w/v) acrylamide gels under denaturing and non-denaturing conditions,

respectively. In denaturing conditions, samples were incubated for 5 min at 100 °C with SDS-PAGE sample buffer containing 2-mercaptoethanol. Concerning non-denaturing conditions, samples were mixed just before running in sample buffer without 2-mercaptoethanol and SDS. Silver staining was used to localize protein bands (Blum *et al.*, 1987). The standard molecular weights (Bio-Rad) comprising myosin (200 kDa),  $\beta$ -galactosidase (116.25 kDa), phosphorylase b (97.4 kDa), BSA (66.2 kDa) and ovalbumin (45.0 kDa) were used.

#### Native molecular weights determination

The purified enzymes were applied to gel filtration on Sephacryl S-200 HR column (0.8 cm  $\times$  35 cm) equilibrated with 20 mM sodium acetate buffer (pH 5.0) to estimate the native molecular weights. Elution was done at a flow rate of 0.2 ml/min and fractions of 0.5 ml were collected. Standard molecular weights (SIGMA) used for calibration were  $\beta$ -amylase from sweet potato (206 kDa), BSA (66 kDa), ovalbumin from egg white (45 kDa) and cellulase from *Aspergillus niger* (26 kDa).

#### pH and temperature optima

The effect of pH on the enzyme activities was determined by performing the hydrolysis of *para*-nitrophenyl- $\alpha$ -D-Mannopyranoside in a series of buffers (100 mM) at various pH values ranging from 3.0 to 8.0. The buffers used were sodium acetate buffer from pH 3.6 to 5.5; sodium phosphate buffer from pH 5.6 to 8.0 and sodium citrate buffer from pH 3.0 to 6.0. The pH values of each buffer were determined at 25 °C.

The effect of temperature on  $\alpha$ -mannosidase activities was performed in 100 mM acetate buffer (appropriate pHs) over a temperature range of 30 to 80 °C using *pNP*- $\alpha$ -Man (2.5 mM) under the enzyme assay conditions.

#### pH and temperature stabilities

The pH stability of each  $\alpha$ -mannosidase was studied in pH range of 3.0 to 8.0 with 100 mM buffers. The buffers were the same as in pH and temperature optima study (above). After 2 h preincubation at room temperature, residual  $\alpha$ -mannosidase activities were measured at 37 °C for 20 min by adding substrate *para*-nitrophenyl- $\alpha$ -D-Mannopyranoside.

The thermal inactivation was determined at 37 °C and at each enzyme optimum temperature. Enzymes in appropriate buffers (pHs) were exposed to each temperature for 0 to 180 min. Aliquots were withdrawn at intervals (30 min) and immediately cooled.

Concerning thermal denaturation tests, the aliquots of each enzyme were preheated at different temperatures ranging from 30 to 80 °C for 25 min.

Residual activities, determined in the three cases at 37 °C under the enzyme assay conditions, were expressed as percentage activity of zero-time control of untreated enzymes.

#### Effect of some chemical agents

To determine the effect of various compounds (metal ions, detergents, reducing agents and  $\alpha$ -mannosidase

specific inhibitors) as possible activators or inhibitors of the purified  $\alpha$ -mannosidases, each enzyme solution was preincubated at 37 °C for 30 min with the compounds and then the activity was assayed under the enzyme assay conditions. The residual activities were expressed as percentage refers to a control without chemical agents.

#### Substrate specificity and kinetic parameters determinations

The substrate specificity was determined by incubating each purified enzyme with substrates *para*-nitrophenyl- $\alpha$ -D-Mannopyranoside, *para*-nitrophenyl- $\alpha$ -D-Glucopyranoside, *para*-nitrophenyl- $\alpha$ -L-Fucopyranoside, *para*-nitrophenyl- $\alpha$ -D-Galactopyranoside, *para*-nitrophenyl- $\alpha$ -L-Arabinopyranoside, *para*-nitrophenyl- $\beta$ -D-Fucopyranoside, *para*-nitrophenyl- $\beta$ -D-Glucopyranoside, *para*-nitrophenyl- $\beta$ -D-Galactopyranoside, *para*-nitrophenyl- $\beta$ -D-Xylopyranoside (2.5 mM) at 37 °C for 20 min in 100 mM sodium acetate buffer with appropriate pHs.

The kinetic parameters ( $K_M$ ,  $V_{max}$  and  $V_{max}/K_M$ ) were determined at 37 °C in 100 mM sodium acetate buffer with appropriate pHs. The hydrolysis of synthetic aryl substrates was quantified on the basis of released *pNP*, as the standard enzymes assay.  $K_M$  and  $V_{max}$  were determined from a Lineweaver-Burk plot using seven concentrations varying between 0 and 6 mM of the substrate *pNP*- $\alpha$ -Man.

#### Characterization of hydrolytic specificity

The hydrolytic specificity was determined at 37 °C by incubating 0.45  $\mu$ g of each purified enzyme preparation with 1 mM of 2-O- $\alpha$ -D-mannopyranosyl-D-mannopyranoside, 3-O- $\alpha$ -D-mannopyranosyl-D-mannopyranoside or 6-O- $\alpha$ -D-mannopyranosyl-D-mannopyranoside, in 100 mM sodium acetate buffer (at appropriate pHs) for up to 24 h. Samples (5  $\mu$ l) were removed at regular time intervals and applied to TLC plates to monitor the hydrolysis of different linked disaccharides. The TLC plates were run with butanol-acetic acid-water (9:3.75:2.25, v/v/v) and developed with naphto-resorcinol in ethanol and H<sub>2</sub>SO<sub>4</sub> 20% (v/v). The sugar spots were visualized at 110 °C for 5 min.

## Results

### Enzymes purification

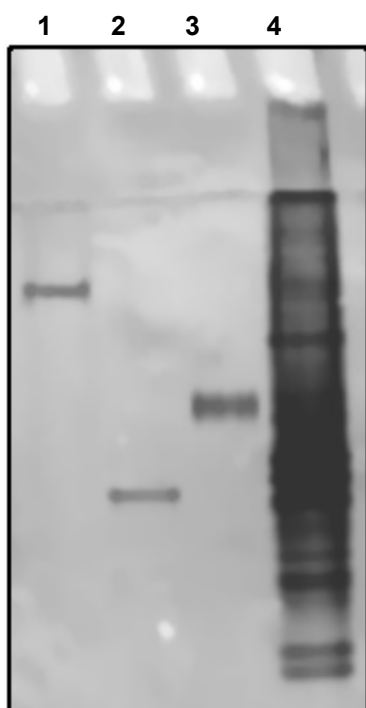
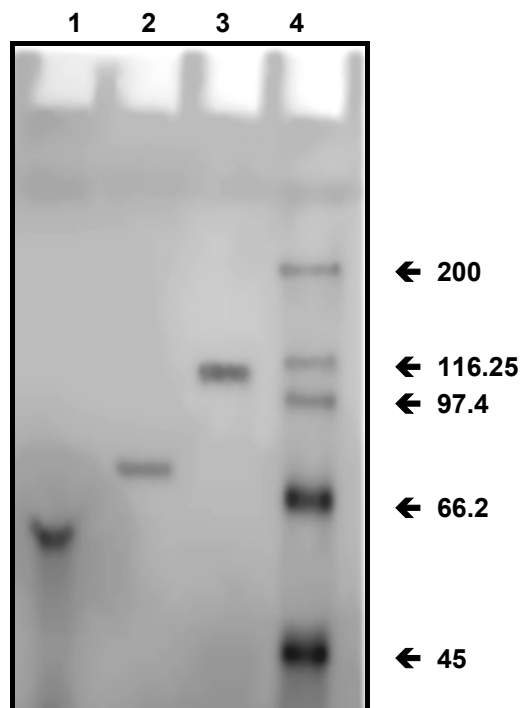
Purification results of digestive  $\alpha$ -mannosidases from the oil palm weevil larvae are summarized in table 1. Three isoenzymes were purified from the crude extract and the purification procedure involved three chromatographic steps for each enzyme.

The peak of  $\alpha$ -mannosidase activity resolved on gel filtration (Sephacryl S-100 HR) as first step was designated *Rp*/M. Pooled fractions were collected and then applied onto DEAE-Sepharose Fast Flow, an anion exchange chromatography. Three peaks showing bounded  $\alpha$ -mannosidase activities were eluted with 0.20, 0.25 and 0.30 M of NaCl, respectively. The three isoforms were termed *Rp*/M1, *Rp*/M2 and *Rp*/M3 based on their elution order (data not shown).

**Table 1.** Purification procedure of  $\alpha$ -mannosidases from the digestive fluid of *R. palmarum* larvae.

Purification steps	Total protein (mg)	Total activity (UI)	Specific activity (UI/mg)	Purification fold	Yield (%)
Crude extract	86.03	42.24	0.48	1	100
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (80%)	72.35	36.64	0.51	1.06	88.80
Sephacryl S-100 HR					
<i>RpIM</i>	17.89	26.43	1.48	3.08	64.00
DEAE-Sepharose Fast Flow					
<i>RpIM1</i>	0.93	1.25	1.34	2.79	3.00
<i>RpIM2</i>	0.33	0.64	1.94	4.04	1.60
<i>RpIM3</i>	1.02	0.69	0.68	1.42	1.70
Phenyl-Sepharose 6 Fast Flow					
<i>RpIM1</i>	0.03	0.33	12.22	25.46	0.80
<i>RpIM2</i>	0.04	0.41	11.39	23.73	1.00
<i>RpIM3</i>	0.04	0.13	3.17	6.61	0.30

UI = 1  $\mu$ mol of *p*NP per min.

**Figure 1.** Native-PAGE analysis of purified  $\alpha$ -mannosidases from the digestive fluid of *R. palmarum* larvae. The samples were loaded onto a 10% gel. Lane 1, *RpIM1*; Lane 2, *RpIM2*; Lane 3, *RpIM3*; Lane 4, crude extract.**Figure 2.** SDS-PAGE analysis of purified  $\alpha$ -mannosidases from the digestive fluid of *R. palmarum* larvae. The samples were loaded onto a 7.5% gel. Lane 1, *RpIM1*; Lane 2, *RpIM2*; Lane 3, *RpIM3*; Lane 4, Markers. Numbers on the right indicate the molecular weight (kDa) of the markers.

An ultimate hydrophobic interaction chromatography on a Phenyl-Sepharose 6 Fast Flow column as third step was necessary to each isoform for purification. The purified enzymes named *RpIM1*, *RpIM2* and *RpIM3* were eluted with 0.61, 0.02 and 0.41 M of ammonium sulphate, and were enriched about 26, 24 and 7-fold, respectively (table 1).

Each isoenzyme showed a single protein band by silver staining on native polyacrylamide gel electrophoresis (figure 1).

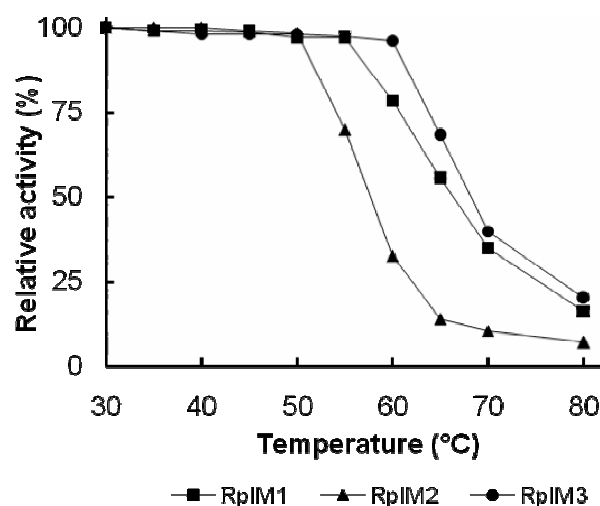
#### Molecular weights estimation

After SDS-PAGE analysis under reducing conditions, each  $\alpha$ -mannosidase (*RpIM1*, *RpIM2* and *RpIM3*) showed a single protein band. Their relative molecular weights were estimated to be 62, 76 and 105 kDa, respectively (figure 2).

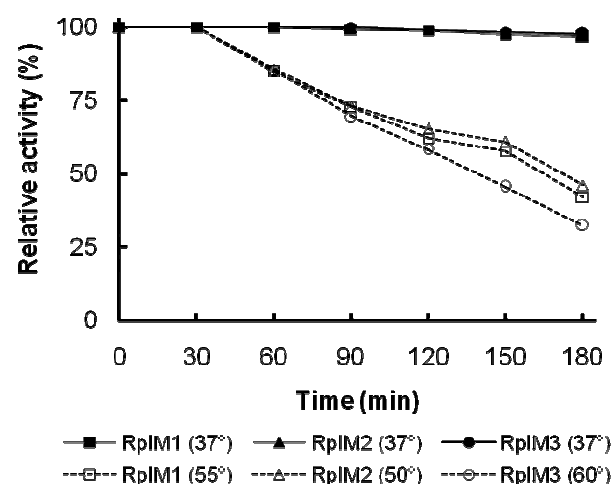
Molecular weights of the native enzymes, as determined by gel filtration, were approximately 125 kDa (*RpIM1*), 72 kDa (*RpIM2*) and 111 kDa (*RpIM3*) (table 2).

**Table 2.** Physicochemical properties of  $\alpha$ -mannosidases from the digestive fluid of oil palm weevil larvae.

Physicochemical properties	<i>RpIM1</i>	<i>RpIM2</i>	<i>RpIM3</i>
Optimum temperature ( $^{\circ}\text{C}$ )	55	50	60
Optimum pH	5.0	5.0	4.5
pH stability	4.4 - 5.2	4.6 - 5.2	4.3 - 4.8
$Q_{10}$	1.8	1.7	1.8
Activation energy (kJ/mol)	47.0	42.9	50.4
Molecular weights (kDa)			
SDS-PAGE	62	76	105
Gel filtration	125	72	111



**Figure 3.** Thermal denaturation of  $\alpha$ -mannosidases from the digestive fluid of *R. palmarum* larvae. The experiments were carried out at temperatures ranging from 30 to 80  $^{\circ}\text{C}$ . The enzymes were preincubated at each temperature for 25 min and the remaining activity measured at 37  $^{\circ}\text{C}$  under the enzyme assay conditions.



**Figure 4.** Thermal inactivation of  $\alpha$ -mannosidases from the digestive fluid of *R. palmarum* larvae. Each enzyme was separately preincubated at 37  $^{\circ}\text{C}$  and its optimum temperature, in 100 mM sodium acetate buffers (pH 4.5 or 5.0). At the indicated times, aliquots were withdrawn and the residual activity measured at 37  $^{\circ}\text{C}$  under the enzyme assay conditions.

### Effect of pH and temperature

The effect of pH and temperature on the three  $\alpha$ -mannosidase activities is shown in table 2. The three isoenzymes were found to be acidic and mesophilic. At 37  $^{\circ}\text{C}$ , the purified enzymes showed best stability over pH values ranging from 4.3 to 5.2, conserving at least more than 90% of total activities. Their maxima activities were observed at 55, 50 and 60  $^{\circ}\text{C}$  for *RpIM1*, *RpIM2* and *RpIM3*, respectively. Values of temperature coefficients ( $Q_{10}$ ) calculated were found to be around 1.8 for the three  $\alpha$ -mannosidases. From Arrhenius plot, values of 47, 42.9 and 50.4 kJ/mol were calculated for the activation energy of *RpIM1*, *RpIM2* and *RpIM3*, respectively (table 2).

The thermal stability was investigated by incubating each enzyme at various temperatures for 25 min. The three  $\alpha$ -mannosidases retained 100% of their activities to temperatures up to their optima. Above, their activities declined rapidly as the temperature increased. Nevertheless, the enzymes were not completely inactivated even at 80  $^{\circ}\text{C}$  (figure 3).

A long-term stability study indicated that at 37  $^{\circ}\text{C}$  and in appropriate optimum pHs, the three  $\alpha$ -mannosidases remained stable for 180 min. However, at their optimum temperatures, each enzyme is stable for 30 min and retained about 75% of its activity after 80 min preincubation. Above this preheating time,  $\alpha$ -mannosidases were less stable showing half-life (50% of activity) nearby 150 min for *RpIM3* and 180 min for *RpIM1* and *RpIM2* (figure 4).

### Effect of some metal ions, chelating and reducing agents and detergents

The effect of some chemical agents on  $\alpha$ -mannosidases from oil palm weevil larvae digestive fluid using *para*-nitrophenyl- $\alpha$ -D-Mannopyranoside as substrate was examined.

Most of chemicals tested ( $\text{Ba}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ , EDTA, dithiothreitol, polyoxyethylen-9-lauryl ether and polyoxyethylen-10-oleyl ether) were without effect on the enzymes activity. Nevertheless,  $\text{Cu}^{2+}$ , 5,5'-dithio-2,2'-dinitro-dibenzoic acid (DTNB), L-cysteine and SDS were found to be the common inhibitors (60-100% inhibition observed on the three  $\alpha$ -mannosidase activities), while *para*-chloromercuribenzoic acid (*p*CMB), tetradecyl trimethyl ammonium bromide and hexadecyl trimethyl ammonium bromide displayed 70 to 80% of inhibition on *RpIM2* and *RpIM3*. Nonidet-P40, a non-ionic detergent, showed 34% of inhibition on *RpIM3* activity at the concentration of 1% (w/v). Only 2-mercaptoethanol displayed a particular stimulatory effect on *RpIM1* (137%) at the concentration of 0.1% (v/v).

### Effect of $\alpha$ -mannosidase specific inhibitors

The influence of  $\alpha$ -mannosidase specific inhibitors such as 1-deoxymannojirimycin (DMNJ), kifunensine (Kif), swainsonine (Sw) and 1,4-dideoxy-1,4-imino-mannitol (DIM) on the enzymes activities is presented in table 3.

Hydrolysis of *p*NP- $\alpha$ -Man by *RpIM1*, *RpIM2* and *RpIM3* was strongly inhibited by the azafuranose ana-

**Table 3.** Effect of specific inhibitors on the activity of the  $\alpha$ -mannosidases from the digestive fluid of *R. palmarum* larvae.

Specific inhibitors	Concentration ( $\mu$ M)	Relative activity (% of blank)		
		<i>Rp/M1</i>	<i>Rp/M2</i>	<i>Rp/M3</i>
Blank	0	100	100	100
Swainsonine	0.05	91	77	97
"	0.5	29	15	57
"	10	0	2	5
"	20	0	0	2
1,4-dideoxy-1,4-iminomannitol	0.05	100	99	100
"	0.5	91	71	93
"	10	39	15	43
"	20	23	10	10
Kifunensine	0.05	100	99	100
"	0.5	97	99	100
"	10	92	99	100
"	20	88	86	96
1-deoxymannojirimycin	0.05	100	98	100
"	0.5	100	98	100
"	10	100	95	100
"	20	80	93	100

**Table 4.** Kinetic parameters of  $\alpha$ -mannosidases from the digestive fluid of *R. palmarum* larvae towards *p*NP- $\alpha$ -Man. The Michaelis-Menten constant ( $K_M$ ) and the maximum velocity ( $V_{max}$ ) are expressed as mM and units/mg of protein, respectively.

$\alpha$ -mannosidases	$K_M$ (mM)	$V_{max}$ ( $\mu$ mol/min/mg)	$V_{max}/K_M$ (ml/min/mg)
<i>Rp/M1</i>	0.13	0.73	5.62
<i>Rp/M2</i>	0.22	2.46	11.18
<i>Rp/M3</i>	0.16	1.32	8.25

logues of mannose such as Sw and DIM when used in the reaction mixture at 20  $\mu$ M. These inhibitions vary from 77 to 100%. In contrast, the azapyranose analogues as Kif and DMNJ had not such effect at the same concentration (0-20% of inhibition observed).

#### Substrate specificity and kinetic parameters

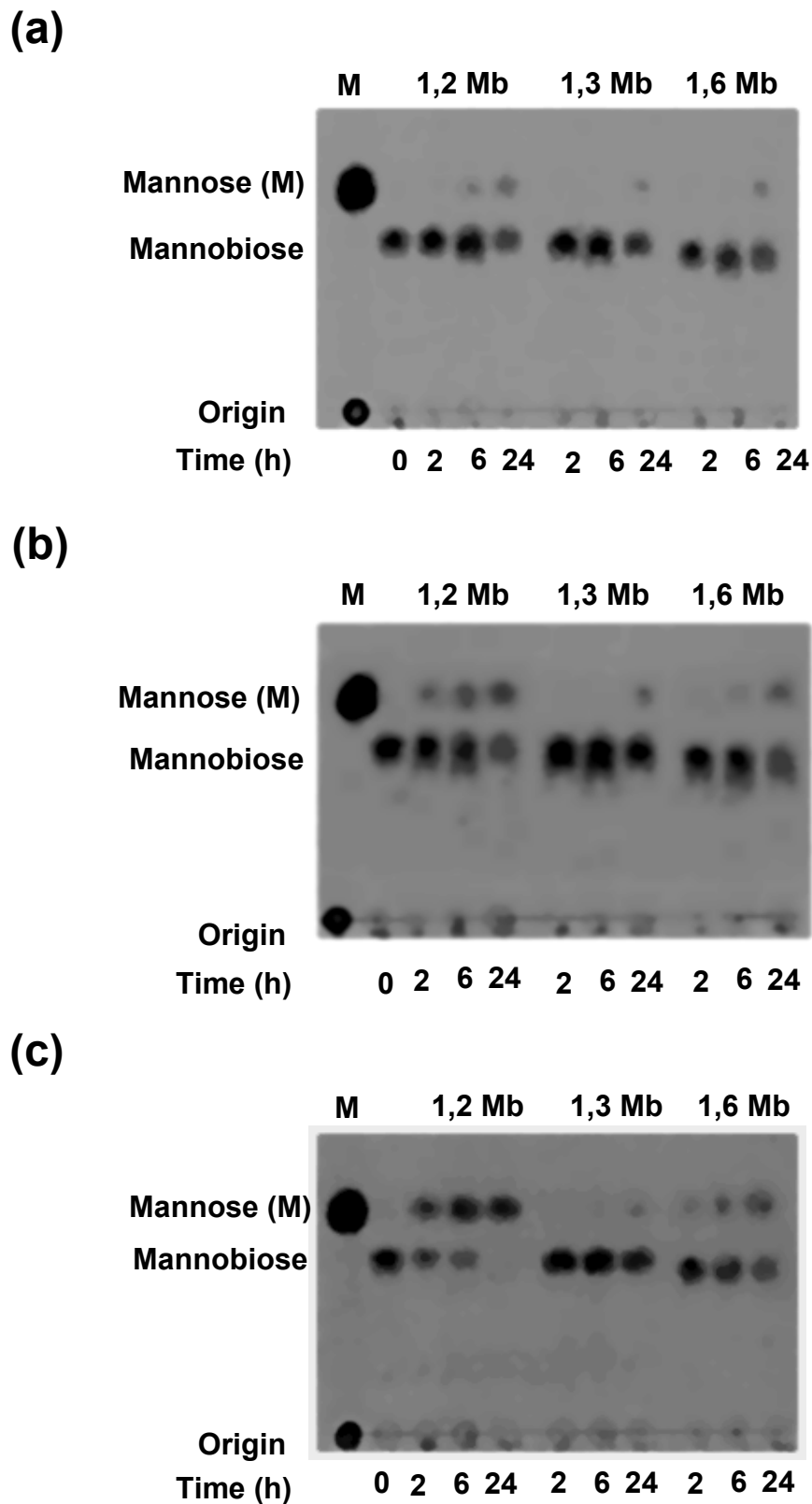
Purified  $\alpha$ -mannosidases were assayed for hydrolytic activities towards a variety of synthetic and natural substrates. No detectable activities towards *para*-nitrophenyl glucopyranosides (*para*-nitrophenyl- $\alpha$ -D-Glucopyranoside, *para*-nitrophenyl- $\alpha$ -L-Fucopyranoside, *para*-nitrophenyl- $\alpha$ -D-Galactopyranoside, *para*-nitrophenyl- $\alpha$ -L-Arabinopyranoside, *para*-nitrophenyl- $\beta$ -D-Fucopyranoside, *para*-nitrophenyl- $\beta$ -D-Glucopyranoside, *para*-nitrophenyl- $\beta$ -D-Galactopyranoside, *para*-nitrophenyl- $\beta$ -D-Xylopyranoside) were observed except *para*-nitrophenyl- $\alpha$ -D-Mannopyranoside (data not shown) on which Lineweaver-Burk-plot in range of concentrations 0 to 6 mM showed  $K_M$  and  $V_{max}$  values of the  $\alpha$ -mannosidases (table 4). The  $K_M$  values were 0.13, 0.22 and 0.16 mM for isoforms *Rp/M1*, *Rp/M2* and *Rp/M3*, respectively.

On the other hand, the linkage specificity was investigated using natural substrates such as disaccharides

2-O- $\alpha$ -D-mannopyranosyl-D-mannopyranoside (Man- $\alpha$ -1,2-Man), 3-O- $\alpha$ -D-mannopyranosyl-D-mannopyranoside (Man- $\alpha$ -1,3-Man) and 6-O- $\alpha$ -D-mannopyranosyl-D-mannopyranoside (Man- $\alpha$ -1,6-Man). Following incubation with *Rp/M1*, *Rp/M2* and *Rp/M3*, released mannose was separated by TLC (figure 5). All the  $\alpha$ -mannosidases (*Rp/M1*, *Rp/M2* and *Rp/M3*) showed broad specificity under acidic conditions by cleaving  $\alpha$ -1,2;  $\alpha$ -1,3 and  $\alpha$ -1,6 Mannobioses at different rates. However, their hydrolytic on  $\alpha$ -1,2 Mannobiose was greater than on  $\alpha$ -1,3 or  $\alpha$ -1,6 Mannobioses after 24 h (figure 5).

#### Discussion

$\alpha$ -mannosidases from insects have been largely studied physiologically with regard to their hydrolytic activities in crude extracts and many other properties (Ferreira *et al.*, 1988; Silva and Terra, 1994; Francis *et al.*, 2002; Erthal Jr. *et al.*, 2007) to understand their importance in protein glycosylation, both in N-glycans elongation and in catabolism of mannosylated oligosaccharides (Moremen *et al.*, 1994). However, few enzymatic studies have been realized and no work relative to oil palm weevil  $\alpha$ -mannosidase has been so far reported. In this respect, three extracellular  $\alpha$ -mannosidases were purified from the larvae of oil palm weevil and their properties were examined. The purification procedure of these enzymes involved three steps including size exclusion, anion exchange and hydrophobic interaction chromatography. The two latter chromatographies were crucial to purify the digestive  $\alpha$ -mannosidases. Indeed, the anion-exchange chromatography on a DEAE-Sepharose Fast Flow column enabled the enzyme activity to be separated into three different isoforms (*Rp/M1*, *Rp/M2* and *Rp/M3*), while hydrophobic interaction chromatography on Phenyl-Sepharose 6 Fast Flow led to separate each



**Figure 5.** TLC plates showing the hydrolytic activity of the purified  $\alpha$ -mannosidases from the digestive fluid of *R. palmarum* larvae towards different linked disaccharides. Experiments were monitored with 45  $\mu$ g of each enzyme, over 24 h incubation time at 37 °C in appropriate pHs. Separation of hydrolysed products was realized with butanol-acetic acid-water 9:3.75:2.25 (v/v/v). The plates were developed with naphtho-resorcinol in ethanol and H<sub>2</sub>SO<sub>4</sub> 20% (v/v). The sugar spots were visualized at 110 °C for 5 min. (a), *RpIM1* activity; (b), *RpIM2* activity; (c), *RpIM3* activity. 1,2 Mb,  $\alpha$ -D-Manp(1,2)-D-Manp; 1,3 Mb,  $\alpha$ -D-Manp(1,3)-D-Manp; 1,6 Mb,  $\alpha$ -D-Manp(1,6)-D-Manp; M, mannose.

digestive  $\alpha$ -mannosidase from the other proteins and impurities. Similar results concerning Phenyl-Sepharose gel has been reported for purification of the endopeptidase Thr-N from *Archachatina ventricosa* (Gould) digestive fluid (Niamké *et al.*, 1999), endoxylanase and other glycosidases of *Macrotermes subhyalinus* (Rambur) workers (Kouamé *et al.*, 2005; Faulet *et al.*, 2006) and digestive  $\beta$ -galactosidase from *R. palmarum* larvae (Yapi *et al.*, 2007).

Regarding molecular properties of the purified enzymes,  $\alpha$ -mannosidases *Rp/M2* and *Rp/M3* have monomeric structures. Except rat liver  $\alpha$ -mannosidase I and jack bean  $\alpha$ -mannosidase which are tetrameric polypeptides with different subunits (Tulsiani *et al.*, 1982b; Kimura *et al.*, 1999), most of  $\alpha$ -mannosidases are monomeric (Bischoff and Kornfeld, 1986). As for  $\alpha$ -mannosidase *Rp/M1*, it functions as a dimeric structure.

The purified  $\alpha$ -mannosidase activities on *pNP- $\alpha$ -Man* were maximal in acidic region. This behaviour is in accordance with the majority of insect larvae  $\alpha$ -mannosidases e.g. *Rhagium inquisitor* (L.) (Coleoptera) and *Rhynchosciara americana* (Wiedemann) (Diptera) (Chipoulet and Chararas, 1985; Terra and Ferreira, 1994). In contrast, only few insect  $\alpha$ -mannosidases such as those from *D. melanogaster* and *S. frugiperda* which are both localized in Golgi apparatus have been reported to perform maximal activity near neutral pH region.

Since the enzymes behaved like lysosomal  $\alpha$ -mannosidases and, also retained more than half of their hydrolytic activities near neutral pH region (data not shown), they would be advantageous for treating the lysosomal storage disorder  $\alpha$ -mannosidosis by enzyme replacement. Indeed, the enzyme replacement therapy consists of a lysosomal  $\alpha$ -mannosidase, which is formulated in an isotonic solution (Neufeld, 2004).

The optimum temperatures of these enzymes are in good agreement with those of the majority of  $\alpha$ -mannosidases and other glycosidases purified from plants (Jagadeesh *et al.*, 2004; Ahi *et al.*, 2007), microbials (Yoshida *et al.*, 1993; Gaikwad *et al.*, 1995) and insects (Faulet *et al.*, 2006; Yapi *et al.*, 2007) which are most of the time mesophilic enzymes. The digestive  $\alpha$ -mannosidase activities from the oil palm weevil larvae were tested after a long time of pre-warming (150-180 min). Most published reports determine thermal stability over a very short incubation time, usually 10 min (Ichishima *et al.*, 1981; Yoshida *et al.*, 1993; Maruyama *et al.*, 1994), but a long-term stability study gives more meaningful results, especially useful when lengthy concentration or purification steps (polyethylene glycol concentration or gel filtration) have to be employed.

The behaviour of the enzymes in presence of divalent cations tested and cation chelator (EDTA) is tempting to speculate that the concerning divalent cations are not necessary for enzyme catalytic activities. However, the sensitivity of the enzymes to *pCMB* and *DTNB* leads to assume that -SH groups participate probably in the enzymic reaction. This assumption is supported by the fact that L-cysteine, an amino acid containing -SH group, inhibited the concerning enzymes activity. In contrast, the particular enhanced effect of 2-mercaptoethanol on *Rp/M1* activity suggests that, hydroxyl group of this molecule participate in this enzyme reaction compare to

L-cystein which is also a small molecule as opposed to *pCMB* and *DTNB*.

Inhibitory effect is also noticed when detergents were tested on the three enzymes activities. These results contrast with those reported for rat liver  $\alpha$ -mannosidases (Bonay *et al.*, 1992). So, the concerning detergents should be avoid in these enzymes preparations. Nevertheless, the particular inhibitory effect (34%) that displays a non-ionic detergent (nonidet-P40) on *Rp/M3* activity attracted our attention. This could be probably due to the enzyme's structure. Indeed, it is possible that the active site of the enzyme contains sites for non-ionic ligands as in bovine serum albumin structure (Wasylewski and Kozik, 1979). Thus, nonidet-P40 may affect the activity of *Rp/M3* by modifying its catalytic environment. Inhibition by this neutral detergent at extremely lower concentration (0.005%) had previously been reported on a rat calcium-activated ATPase (Batchelor and Stanworth, 1981).

The sensitivity of digestive  $\alpha$ -mannosidases from *R. palmarum* larvae to Sw and DIM (specific inhibitors of  $\alpha$ -mannosidases) largely matches the inhibition profile of lysosomal  $\alpha$ -mannosidases previously characterized.

It is well known that pKa of Sw is 7.4 and thus, it would be fully ionized at pH 5.0 (Dorling *et al.*, 1980). This may account for the apparent specificity of Sw for *R. palmarum* larvae  $\alpha$ -mannosidases which show maximum activity at pH 4.5 and 5.0. Indeed, the ionization of Sw could improve its fixation in the catalytic centre of the enzymes. Using molecular models, it can be seen that the configuration of secondary hydroxyl groups of Sw is identical to that in DIM (Cenci di Bello *et al.*, 1989). This observation supports the similar effect of DIM on the three digestive enzymes. However, the moderate effect of DIM may be explained by the accessibility of these compounds to the catalytic centre. DMNJ and Kif, both pyranose analogues, are normally not known to inhibit class II  $\alpha$ -mannosidases (Daniel *et al.*, 1994; Moremen *et al.*, 1994). Taken together, these results suggest that the three  $\alpha$ -mannosidases from the digestive fluid of oil palm weevil larvae belong to the catabolic group of  $\alpha$ -mannosidases with close resemblance to lysosomale  $\alpha$ -mannosidases.

$\alpha$ -mannosidases *Rp/M1*, *Rp/M2* and *Rp/M3* are restricted towards the synthetic aryl-substrate (*pNP- $\alpha$ -Man*) normally acted upon by  $\alpha$ -mannosidase, since they did not possess any other tested glycosidase activities. Based on their substrate specificity and their high activities in acidic regions (pH 4.5; 5.0), these enzymes behaved like lysosomal  $\alpha$ -mannosidases. This hypothesis is supported by the fact that other extracellular enzymes are hydrolases and one finds glycosidase, phosphatase and protease activity. As for the lysosome hydrolases to which they bear a close resemblance, they are not very specific (Forestier, 1992). Indeed, these  $\alpha$ -mannosidases display a broad natural substrate specificity by hydrolysing  $\alpha$ -1,2;  $\alpha$ -1,3 and  $\alpha$ -1,6 mannobioses linkages. Lysosomal  $\alpha$ -mannosidases with these characteristics have previously been identified and purified from several sources as *Dictyostelium discoideum* (Schatzle *et al.*, 1992) and a variety of mammalian tissues (Opheim and Touster, 1978; De Gasperi *et al.*, 1991).

The three broad specific  $\alpha$ -mannosidases are thought to



be very important and are presumably involved in cleaving the carbohydrate moieties of glycoproteins and in finishing the digestion of  $\alpha$ -mannosylated compounds contained in *R. palmarum* larvae's feeding habits and diets.

This study showed that the three  $\alpha$ -mannosidases (*Rp/M1*, *Rp/M2* and *Rp/M3*) purified from the digestive fluid of oil palm weevil larvae behaved like lysosomal  $\alpha$ -mannosidases by combination of a low pH optimum, broad natural substrate specificity, activity towards the synthetic aryl-substrate pNP- $\alpha$ -Man and a sensitivity to Sw. These enzymes due to their characteristics above could be useful for bioindustrial mannose removal by recycling glycoproteins and mannosylated compounds. They could also find application in pharmaceutical industries since they expressed lysosomal  $\alpha$ -mannosidase activity. In addition, the inhibition profile of these enzymes should much contribute to the *R. palmarum* larvae's control.

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## References

- AHI A. P., GONNETY T. J., FAULET B. M., KOUAMÉ L. P., NIAMKÉ S. L., 2007.- Biochemical characterization of two  $\alpha$ -mannosidases from breadfruit (*Artocarpus communis*) seeds.- *African Journal of Biochemistry Research*, 1: 106-116.
- BATCHELOR K. W., STANWORTH D. R., 1981.- The effect of nonidet P40 on the function of rat peritoneal mast cells in vitro.- *British Journal of Pharmacology*, 72: 95-100.
- BISCHOFF J., KORNFELD R., 1984.- The effect of 1-deoxymannojirimycin on rat liver  $\alpha$ -mannosidases.- *Biochemical and Biophysical Research Communications*, 25: 324-331.
- BISCHOFF J., KORNFELD R., 1986.- The soluble form of rat liver  $\alpha$ -mannosidase is immunologically related to endoplasmic reticulum membrane  $\alpha$ -mannosidase.- *Journal of Biological Chemistry*, 261: 4758-4765.
- BLUM H., BEIER H., GROSS B., 1987.- Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels.- *Electrophoresis*, 8: 93-99.
- BONAY P., ROTH J., HUGGUES C. R., 1992.- Subcellular distribution in rat liver of a novel broad-specificity ( $\alpha$ -1,2;  $\alpha$ -1,3 and  $\alpha$ -1,6) mannosidase active on oligomannose glycans.- *European Journal of Biochemistry*, 205: 399-407.
- CENCI DI BELLO I., FLEET G., NAMGOONG S. K., TANDANO K. I., WINCHESTER B., 1989.- Structure-activity relationship of swainsonine. Inhibition of human  $\alpha$ -mannosidases by swainsonine analogs.- *Biochemical Journal*, 159: 855-861.
- CHIPOULET J. M., CHARARAS C., 1985.- Survey and electrophoretic separation of the glycosidases of *Rhagium inquisitor* (Coleoptera: Cerambycidae) larvae.- *Comparative Biochemistry and Physiology*, 80B: 241-246.
- DANIEL P. F., WINCHESTER B., WARREN C. D., 1994.- Mammalian  $\alpha$ -mannosidase: multiple forms but a common purpose.- *Glycobiology*, 4: 551-566.
- DE GASPERI R., AL-DAHER S., DANIEL P. F., WINCHESTER B. G., JEANLOZ R. W., WARREN C. D., 1991.- The substrate specificity of the bovine and feline lysosomal  $\alpha$ -D-mannosidases in relation to mannosidosis.- *Journal of Biological Chemistry*, 266: 16556-16563.
- DORLING P. R., HUXTABLE C. R., COLEGATE S. M., 1980.- Inhibition of lysosomal  $\alpha$ -mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens*.- *Biochemical Journal*, 191: 649-665.
- ELBEIN A. D., TROPEA J. E., MITCHELL M., KAUSHAL G. P., 1990.- Kifunensine, a potent inhibitor of the glycoprotein processing mannosidase I.- *Journal of Biological Chemistry*, 265: 15599-15605.
- ERTHAL JR. M., SILVA C. P., SAMUELS R. I., 2007.- Digestive enzymes in larvae of the leaf cutting ant, *Acromyrmex subterraneus* (Hymenoptera: Formicidae: Attini).- *Journal of Insect Physiology*, 53: 1101-1111.
- FAULET M. B., NIAMKÉ S., GONNETY T. J., KOUAMÉ L. P., 2006.- Purification and biochemical characteristics of a new strictly specific endoxylanase from termite *Macrotermes subhyalinus* workers.- *Bulletin of Insectology*, 59: 17-26.
- FERREIRA C., RIBEIRO A. F., GARCIA E. S., TERRA W. R., 1988.- Digestive enzymes trapped between and associated with the double plasma membranes of *Rhodnius prolixus* posterior midgut cells.- *Insect Biochemistry*, 18: 521-530.
- FORESTIER J. P., 1992.- Les enzymes de l'espace extracellulaire du stratum corneum.- *International Journal of Cosmetic Science*, 14: 47-63.
- FOSTER J. M., YUDKIN B., LOCKYER A. E., ROBERTS D. B., 1995.- Cloning and sequence analysis of GMII, a *Drosophila melanogaster* homologue of cDNA encoding murine Golgi alpha mannosidase II.- *Gene*, 154: 183-186.
- FRANCIS B. R., PAQUIN L., WEINKAUF C., JARVIS D. L., 2002.- Biosynthesis and processing of *Spodoptera frugiperda*  $\alpha$ -mannosidase III.- *Glycobiology*, 12: 369-377.
- GAIKWAD S. M., KESKAR S. S., KHAN M. I., 1995.- Purification and characterization of  $\alpha$ -mannosidase from *Aspergillus sp.*- *Biochimica Biophysica Acta*, 1250: 144-148.
- GIORDANI R., SEIPAI M., MOULIN T. J., REGLI P., 1991.- Antifungal action of *Carica papaya* latex: isolation of fungal cell wall hydrolysing enzymes.- *Mycoses*, 34: 469-477.
- GOSS P. E., BAKER M. A., CARVER J. P., DENNIS J. W., 1995.- Inhibitors of carbohydrate processing: a new class of anticancer agents.- *Clinical Cancer Research*, 1: 935-944.
- GOSS P. E., REID C. L., BAILEY D., DENNIS J. W., 1997.- Phase IB clinical trial of the oligosaccharide processing inhibitor swainsonine in patients with advanced malignancies.- *Clinical Cancer Research*, 3: 1077-1086.
- HENRISSAT B., BAIROCH A., 1996.- Updating the sequence-based classification of glycosyl hydrolases.- *Biochemical Journal*, 316: 695-696.
- HERSCOVICS A., 1999.- Importance of glycosidases in mammals glycoprotein biosynthesis.- *Biochimica Biophysica Acta*, 1473: 96-107.
- HIRSCH C., BLOM D., PLOEGH H. L., 2003.- A role for N-glycanase in the cytosolic turnover of glycoproteins.- *The EMBO Journal*, 22: 1036-1046.
- ICHISHIMA E., ARAI M., SHIGEMATSU Y., KUMAGAI H., SUMIDA-TANAKA R., 1981.- Purification of an acidic alpha-D-mannosidase from *Aspergillus saitoi* and specific cleavage of 1,2-alpha-D-mannosidic linkage in yeast mannan.- *Biochimica Biophysica Acta*, 658: 45-53.
- JACOB Q. S., SCUDDER P., 1994.- Glycosidases in structural analysis.- *Methods in Enzymology*, 230: 280-299.
- JAGADEESH B. H., PRABHA T. N., SRINIVASAN K., 2004.- Activities of glycosidases during fruit development and ripening of tomato (*Lycopersicon esculentum* L.): implication in fruit ripening.- *Plant Science*, 166: 1451-1459.
- JARVIS D. L., BOHLMAYER D. A., LIAO Y. F., LOMAX K. K., MERKEL R. K., WEINKAUF C., MOREMEN K. W., 1997.- Isolation and characterization of a class II alpha-mannosidase cDNA from lepidopteran insect cells.- *Glycobiology*, 7: 113-127.
- KAWAR Z. S., MOREMEN K. W., JARVIS D. L., 2001.- Insect cells encode a class II alpha-mannosidase with unique properties.- *Journal of Biological Chemistry*, 276: 16335-16340.

- KELLY S. J., HERSCOVICS A., 1998.- Glycoprotein biosynthesis in *Saccharomyces cerevisiae*. Purification of the alpha-mannosidase which removes one specific mannose residue from Man<sub>9</sub>GlcNAc.- *Journal of Biological Chemistry*, 263: 14757-14763.
- KESKAR S. S., GAIKWAD S. M., KHAN M. I., 1996.- Microbial  $\alpha$ -mannosidases.- *Enzyme and Microbial Technology*, 18: 602-604.
- KIMURA Y., HESS D., STURM A., 1999.- The N-glycans of jack bean  $\alpha$ -mannosidase structure, topology and function.- *European Journal of Biochemistry*, 264: 168-175.
- KOUAMÉ L. P., KOUAMÉ F. A., NIAMKÉ S. L., FAULET B. M., KAMENAN A., 2005.- Biochemical and catalytic properties of two  $\beta$ -glycosidases purified from workers of the termite *Macrotermes subhyalinus* (Isoptera: Termitidae).- *International Journal of Tropical Insect Science*, 25: 103-113.
- LAEMMLI U. K., 1970.- Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 658-685.
- LIAO Y. F., LAL A., MOREMEN K. W., 1996.- Cloning, expression, purification and characterization of the human broad specificity lysosomal acid  $\alpha$ -mannosidase.- *Journal of Biological Chemistry*, 271: 28348-28358.
- LOWRY O. H., ROSEBROUGH N. J., FARRA L., RANDALL R. J., 1951.- Protein measurement with Folin-phenol reagent.- *Journal of Biological Chemistry*, 193: 265-275.
- MARUYAMA Y., NAKAJIMA T., ICHISHIMA E., 1994.- A 1,2-alpha-D-mannosidase from a *Bacillus* sp.: purification, characterization and mode of action.- *Carbohydrate Research*, 251: 89-98.
- MOREMEN K. W., 2000.-  $\alpha$ -mannosidases in asparagine-linked oligosaccharide processing and catabolism, pp. 81-117. In: *Oligosaccharides in chemistry and biology: a comprehensive handbook* (Ernst B., Hart G., Sinay P., Ed.).- Academic Press, New York, USA.
- MOREMEN K. W., TRIMBLE R. B., HERSCOVICS A., 1994.- Glycosidases of the asparagine-linked oligosaccharide processing pathway.- *Glycobiology*, 4: 113-125.
- NEUFELD E. F., 2004.- Enzyme replacement therapy, pp. 327-338. In: *Lysosomal disorders of the brain* (Platt F. M., Walkley S. V., Ed.).- Oxford University Press, Oxford, UK.
- NIAMKÉ S., SINE J-P., GUIONIE O., COLAS B., 1999.- A novel endopeptidase with a strict specificity for threonine residues at the P1' position.- *Biochemical and Biophysical Research Communications*, 256: 307-312.
- OEHLISCHLAGER A., CHINCHILLA C., CASTILLO G., GONZALEZ L., 2002.- Control of red ring disease by mass trapping of *Rhynchophorus palmarum* (Coleoptera: Curculionidae).- *Florida Entomologist*, 85: 507-513.
- OPHEIM D. J., TOUSTER O., 1978.- Lysosomal alpha-D-mannosidase of rat liver. Purification and comparison with the golgi and cytosolic alpha-D-mannosidases.- *Journal of Biological Chemistry*, 253: 1017-1023.
- RABOUILLE G., KUNTZ D. A., LOCKYER A. E., WATSON R., SIGNORELLI T., ROSE D. R., VAN DER HEUVEL M., ROBERTS D. B., 1999.- The *Drosophila* GMII gene encodes a Golgi  $\alpha$ -mannosidase II.- *Journal of Cell Science*, 112: 3319-3330.
- REYNA A. B. V., OROZCO R. B., MÉNDEZ C. C., ROMERO E. L., CARREON A. F., 2000.- Processing of the Man<sub>10</sub>GlcNAc (M<sub>10</sub>) oligosaccharide by  $\alpha$ -mannosidases from *Candida albicans*.- *FEMS Microbiology Letters*, 185: 37-41.
- SAINT-POL A., CODOGNO P., MOORE S. E. H., 1999.- Cytosol to lysosome transfer of free polymannose-type oligosaccharides, kinetic and specificity studies using rat liver lysosomes.- *Journal of Biological Chemistry*, 274: 13547-13555.
- SCHATZLE J., BUSH J., CARDELLI J., 1992.- Molecular cloning and characterization of the structural gene coding for the developmentally regulated lysosomal enzyme,  $\alpha$ -mannosidase, in *Dictyostelium discoideum*.- *Journal of Biological Chemistry*, 267: 4000-4007.
- SILVA C. P., TERRA W. R., 1994.- Digestive and absorptive sites along the midgut of the cotton seed sucker bug *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae).- *Insect Biochemistry and Molecular Biology*, 24: 493-505.
- SUN H., YANG H., ASKINS M. E., 1999.- Retrovirus vector mediated correction and cross correlation of lysosomal  $\alpha$ -mannosidase deficiency in human and feline fibroblasts.- *Human Gene Therapy*, 10 (1999), 1311-1319.
- TATARA Y., LEE B. R., YOSHIDA T., TAKAHASHI K., ICHISHIMA E., 2003.- Identification of catalytic residues of Ca<sup>2+</sup> independent 1,2- $\alpha$ -D-mannosidase from *Aspergillus saitoi* by site-directed mutagenesis.- *Journal of Biological Chemistry*, 278: 25289-25294.
- TERRA W. R., FERREIRA C., 1994.- Insect digestive enzymes: properties, compartmentalization and function.- *Comparative Biochemistry and Physiology*, 109B: 1-62.
- TREMBLAY L. O., KOVACS E. N., DANIELS E., WONG N. K., SUTTON-SMITH M., MORRIS H. R., DELL A., MARCINKIEWICZ E., SEIDAH N. G., C. MCKERLIE C., HERSCOVICS A., 2007.- Respiratory distress and neonatal lethality in mice lacking Golgi  $\alpha$ -1,2-mannosidase IB involved in N-glycan maturation.- *Journal of Biological Chemistry*, 282: 2558-2566.
- TULSIANI D. R. P., HARRIS T. M., TOUSTER O., 1982a.- Novel mannosidase inhibitor blocking conversion of high mannose to complex oligosaccharides.- *Journal of Biological Chemistry*, 257: 7936-7939.
- TULSIANI D. R. P., HUBBARD S. C., ROBBINS P. W., TOUSTER O., 1982b.-  $\alpha$ -mannosidases of rat liver Golgi membranes.- *Journal of Biological Chemistry*, 257: 3660-3668.
- WASYLEWSKI Z., KOZIK A., 1979.- Interaction of bovine serum albumin with alkyl glucosides studied by equilibrium dialysis and infrared spectroscopy.- *European Journal of Biochemistry*, 95: 121-126.
- WENG S., SPIRO R. G., 1993.- Demonstration that a kifunensine-resistant  $\alpha$ -mannosidase with a unique processing action on N-linked oligosaccharides occurs in rat liver endoplasmic reticulum and various cultured cells.- *Journal of Biological Chemistry*, 268: 25656-25663.
- WINCHESTER B., AL-DAHER S., CARPENTER C., CENCI DI BELLO I., CHOI S. S., FAIRBANKS A. J., FLEET G. W. J., 1993.- The structural basis of the inhibition of human  $\alpha$ -mannosidase by azafuranose analogues of mannose.- *Biochemical Journal*, 290: 743-749.
- WOO K. K., KIMURA Y., SHINTARO H., YOSHINOBU K., 2004.- Purification of a Co (II)-sensitive  $\alpha$ -mannosidase from *Ginkgo biloba* Seeds.- *Bioscience Biotechnology and Biochemistry*, 68: 2547-2556.
- YAPI D. Y. A., NIAMKÉ S. L., KOUAMÉ L. P., 2007.- Biochemical characterization of a strictly specific beta-galactosidase from the digestive juice of the palm weevil *Rhynchophorus palmarum* larvae.- *Entomological Science*, 10: 343-352.
- YOSHIDA T., INOUE T., ICHISHIMA E., 1993.- 1,2- $\alpha$ -D-mannosidase from *Penicillium citrinum*: molecular and enzymic properties of two isoenzymes.- *Biochemical Journal*, 290: 349-354.

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