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Purification and biochemical characterization of a specific alpha-glucosidase from the digestive fluid of larvae of the palm weevil, *Rhynchophorus palmarum* L.

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Abstract

A alpha-glucosidase was purified from the digestive fluid of the palm weevil *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae) by chromatography on anion-exchange, gel filtration, and hydrophobic interaction columns. The preparation was shown to be homogeneous on polyacrylamide gels. alpha-glucosidase is a monomeric protein with a molecular weight of 60.60 kDa based on its mobility in SDS-PAGE and 61.05 kDa based on gel filtration. Maximal alpha-glucosidase activity occurred at 45°C and pH 5.6. The purified alpha-glucosidase was stable at 37°C and its pH stability was in the range of 4.0–5.6. The enzyme readily hydrolysed *p*-nitrophenyl-α-D-glucoside, maltose, maltodextrins and required strictly alpha-gluco configuration for activity. It cleaved glucose-glucose alpha-(1–2) linkages better than alpha-(1–4), alpha-(1–1), alpha-(1–3) and β-(1–6) linkages. The catalytic efficiency (V_{max}/K_M) values for *p*-nitrophenyl-α-D-glucoside, maltose, maltotriose, maltotetraose, maltopentose, maltohexose, Saccharose and Kojibiose were respectively 474.00, 36.81, 45.79, 45.85, 31.15, 6.63, 184.61 and 109.17. Alpha-glucosidase was capable of catalysing transglucosylation reactions. The yields in transglucosylation reactions at 37 °C were very high and could attain 62% with2-phenylethanol as glucosyl acceptors. This alpha-glucosidasehydrolyzed the products formed. It seems that the products formed were the phenylethyl-α-D-glucoside. These results suggest that alpha-glucosidase from the digestive fluid of the palm weevil *Rhynchophorus palmarum* is an exoglucosidase which catalyse the splitting of the α-glucosyl residue from the non reducing terminal of the substrate to liberate α-glucose.

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Introduction

The occurrence of different digestive enzymes in the alimentary canal of insects is frequently said to depend mainly on the chemical composition of the diet ingested by the animals (Wigglesworth, 1972). A consequence of this view is the belief that adaptation to a habitat is more important than phylogenetic traits in determining the kinds of digestive enzymes found in insect guts (Terra & Ferreira, 1994). In the alimentary canal, alpha-glucosidases seems to be an essential tool for the partitioning of carbohydrates from the diet into carbon nutrition and osmoregulation (Ashford *et al.*, 2000). In this instance, this enzyme is the final enzyme involved in the metabolism of starch, or perhaps other carbohydrates, to glucose. Intestinal alpha-glucosidases are involved in the final step of the carbohydrate digestion to convert these into monosaccharides which are absorbed from the intestine (Akinloye *et al*., 2012).

Some alpha-glucosidases preferentially hydrolyse alpha-linked di-, oligo-, and/or polyglucans as substrate, while others preferentially cleavage heterogeneous substrates such as aryl glucosides and sucrose (Chiba, 1988; Frandsen & Svensson, 1998). This enzyme was also capable of catalysing transglycosylation to produce alpha-1,4 linked maltotriose and alpha-1,6 linked isomaltooligosaccharides (Johnson *et al.*, 2010) and some alpha-glucosidases show clear transglycosylation activity (Kato *et al.*, 2002).

This enzyme can be found in the midgut and salivary glands of insects (Lagadic & Chararas, 1988; Ghadamyari *et al.*, 2010; Ramzi & Hosseininave, 2010; Saberi *et al.*, 2012; Asadi *et al.*, 2012) as well as hypopharynxgeal glands of *Apis melifera* L. (Baker & Lehner 1972; Terra *et al.* 1996). So far, alpha-glucosidases have been isolated and characterised from many insects including *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridase), *Pyrhocoridae zeamais* (Coleoptera: Curculionidae), *Apis mellifera* (Hymenoptera: Apidae), *Drosophila melanogaster* (Diptera: Drosophilidae), and *Glyphodes pyloalis* (Lep.:Pyralidae) (Huber & Mathison 1976; Tanimura *et al.* 1979; Baker 1991; Silva & Terra 1995; Ghadamyari *et al.,* 2010).

However, there are no researches on the purification and characterization of alpha-glucosidases in the digestive juice of the palm weevil *Rhynchophorus palmarum (Coleoptera: Curculionidae)* larvae. In this study, we attempted to purify and characterize the alpha-glucosidase from the digestive fluid of larvae of the palm weevil *R. palmarum*. This was done in order to find new alpha-glucosidase for use in glycobiotechnology and propose a biological role for the characterized enzyme in the degradation of starch.

Materials and methods

Chemicals

Substrates

saccharose, maltose, maltotriose, maltotetraose, maltohexaose, maltoheptaose, trehalose, kojibiose, nigerose, isomaltose, cellobiose, Laminaribiose, Arabino-galactane, carboxymethylcellulose, inulin, laminarin, xylan, lichenan, starch, glucose containing, substrates and *p*-nitrophenyl-glycopyranosides were purchased from Sigma Aldrich (St. Louis, MO, USA). DEAE-Sepharose CL-6B, Sephacryl-S100 HR, phenyl Sepharose CL-6B gels were obtained from Pharmacia-LKB Biotech (Uppsala, Sweden). The chemicals used for polyacrylamide gel electrophoresis (PAGE) were from Bio-Rad (Milan, Italy). All other chemicals and reagents were of analytical grade.

Biological material

R. palmarum larvae were obtained from the commercial oil palm, *Elaeis guineensis* Jacq. (Arecales: Arecaceae), plantation near the Université Nangui Abrogoua (Abidjan, Côte d'Ivoire). They were collected directly from the palm weevil and dissected in the laboratory.

Enzyme samples

R. palmarum larvae were rinsed in cold water and blotted with filter paper. Guts were dissected in cold 0.9% NaCl (w/v) solution and digestive content was

removed and stirred in the presence of 0.9% NaCl (w/v) solution before centrifugation at 6,000 x g for 30 min. The supernatant was then stirred with 100 mM acetate buffer pH 5.6 for 30 min. The homogenate was centrifuged at 10,000 x g for 30 min. The collected supernatant constituted the crude extract. After freezing at –180°C in liquid nitrogen, the crude extract was stored at –20°C.

Enzyme assays

Under the standard test conditions, alpha-glucosidase activity was measured by the release of *p*-nitrophenol from the substrate *p*-nitrophenyl-α-D-glucopyranoside. An assay mixture (275 **µ**l) consisting of a 100 mM acetate buffer (pH 5.6), 1.25 mM *p*-nitrophenyl-α-D-glucopyranoside and enzyme solution, was incubated at 37°C for 10 min. The control contained all reactants except the enzyme. Determination of other *p*-nitrophenyl glycosidase activities was carried out under the same experimental conditions. The reaction was stopped by the addition of 1M sodium carbonate (2 ml), and absorbance of the reaction mixture was measured at 410 nm.

Oligo-saccharidase activity was determined by measuring the amount of glucose liberated from oligosaccharide by incubation at 37°C for 10 min in a 100 mM acetate (pH 5.6), containing 10 mM oligosaccharide. The amount of glucose was determined by the glucose oxidase-peroxidase method (Kunst *et al.* 1984) after heating the reaction mixture at 100°C for 5 min.

Polysaccharidase activity was assayed by the dinitro salicylic acid procedure (Bernfeld, 1955), using 1% (w/v) polysaccharide (arabino-galactan, carboxymethylcellulose, inulin, lichenan, laminarin, xylan and starch) as substrate. The enzyme (100 **µ**l) was incubated for 30 min at37°C with 200 **µ**l buffer (100 mM acetate, pH 5.6) and100 **µ**l polysaccharide. The reaction was stopped by addition of 300 **µ**l dinitro salicylic acid and heating in boiling water for 5 min. The absorbance was read at 540 nm after cooling on ice for 5 min.

One unit of enzyme activity was defined as the amount of enzyme capable of releasing one **µ**mol of *p*-nitrophenol or glucose per min under the defined reaction conditions. Specific activity was expressed as units per mg of protein (U/mg of protein).

Protein assays

Protein concentrations and elution profiles from chromatographic columns were determined by the Lowry method (Lowry *et al.*,1951) using bovine serum albumin as a standard.

Purification of enzyme

All the purification procedure was carried out in the cold room. The crude extract of *R. palmarum* larvae was loaded onto an anion-exchange chromatography using a DEAE-Sepharose CL-6B column (2.5 cm x 4.5 cm), equilibrated with 20 mM sodium acetate buffer (pH 5.6). 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, 0.3, 0.5, 0.7 and 1 M) of NaCl in 20 mM sodium acetate buffer (pH 5.6), and fractions of 3 mL were collected. One peak of alpha-glucosidase activity was obtained.

On the one hand, the unbound alpha-glucosidase activity was submitted to ammonium sulphate precipitation at 80% saturation overnight in a cold room. The mixture was stirred for at least 8 h and centrifuged at 10,000 g for 15 min. The pellet was suspended in 1 mL of 20 mM sodium acetate buffer (pH 5.6) and loaded onto a Sephacryl S-100 HR column (1.6 cm × 64 cm), a gel filtration chromatography, equilibrated with the same buffer. Fractions of 1 mL were collected at a flow rate of 0.25 mL/min, and those containing the alpha-glucosidase activity were pooled. To the pooled active fractions, solid ammonium sulphate was slowly added to give a final concentration of 1.7 M and the resulting enzyme solution was subsequently applied on a Phenyl SepharoseCL-6B column (1.4 cm × 5.0 cm) previously equilibrated with 20 mM sodium acetate buffer (pH 5.6) containing 1.7 M of ammonium sulphate salt. The column was washed with a reverse stepwise gradient

of ammonium sulphate concentrations (from 0–1.7 M) dissolved in the same sodium acetate buffer at a flow rate of 1 mL/min and fractions of 1 mL were collected. The pooled active fractions were dialyzed overnight against 20 mM sodium acetate buffer (pH 5.6) and constituted the purified enzyme solution.

Homogeneity and molecular weight determination

To check purity and determine molecular weight, the purified enzyme was analysed using SDS-PAGE electrophoresis on a 10% separating gel and a 4% stacking gel (Hoefer mini-gel system; Hoefer Pharmacia Biotech, www.hoeferinc.com), according to the procedure of Laemmli (1970) at 10°C and constant current 20 mM. Proteins were stained with silver nitrate according to Blum *et al.* (1987). The sample was denatured by a 5 min treatment at 100°C. Electrophoretic buffers contained sodium dodecyl sulfate (SDS) and beta-mercaptoethanol.

The native molecular weight of the enzyme was determined using HPLC gel filtration chromatography. The TSK (Sigma-Adrich) column (2.5 cm × 52 cm; QC-PAKGFC 200) was equilibrated with 20 mM acetate buffer(pH 5.6) containing sodium azide 0.5 % (w/v) and calibrated with beta-amylase (200 kDa), alcohol dehydrogenase(150 kDa), bovine serum albumin (66 kDa), ovalbumin (48.8 kDa) and cytochrome C (12.4 kDa). Fractions of 0.5 ml were collected at a flow rate of 0.5 ml/min.

Temperature and pH optima

The effect of pH on alpha-glucosidase activity was determined by measuring the hydrolysis of p-nitrophenyl-α-D-glucopyranoside in a series of buffers at various pH values ranging from pH 3.6 to 8.0. The buffers used were acetate buffer (100 mM) from pH 3.6 to 5.6 and phosphate buffer (100 mM) from pH 5.6 to 8.0. The pH values of each buffer were determined at 37°C. Alpha-glucosidase activity was measured at 37°C under the standard test conditions. The effect of temperature on alpha-glucosidase activity was followed in 100 mM acetate buffer pH 5.6 over a temperature range of 30 to 80°C using 1.25 mM p-nitrophenyl-α-D-glucopyranoside under the standard test conditions.

pH and temperature stabilities

The stability of alpha-glucosidase was followed over the pH range of 3.6 to 8.0 in 100 mM buffers. The buffers were the same as those used in the study of the pH and temperature optima. After 2 h incubation at 37°C, aliquots were taken and immediately assayed for residual alpha-glucosidase activity. The thermal stability of the enzyme was determined at 37 and 45°C after exposure to each temperature for a period from 10 to 140 min. The enzyme was incubated in 100 mM acetate buffer pH 5.6.Aliquots were drawn at intervals and immediately cooled in ice-cold water. Residual activities, determined in both cases at 37°C under the standard test conditions, are expressed as percentage activity of zero-time control of untreated enzyme.

Determination of kinetic parameters

The kinetic parameters (K_M, V_{max} and V_{max}/ K_M) were determined in 100 mM acetate buffer (pH 5.6) at 37°C. Hydrolysis of *p*-nitrophenyl-α-D-glucopyranoside was quantified on the basis of released *p*-nitrophenol as in the standard enzyme assay. Maltose, Saccharose and Kojibiose hydrolysis was quantified by determination of released glucose, determined with oxidase-peroxidase method (Kunst *et al.* 1984) after heating the reaction mixture at 100°C for 5 min. K_M and V_{max} were determined from Lineweaver-Burk plot using different concentrations of *p*-nitrophenyl-alpha-D-glucopyranoside (1–10 mM) and oligosacharides (1–20 mM).

Effect of chemical agents

The enzyme was incubated with 1 mM or 1% (w/v) of different chemical agents for 20 min at 37°C (various cations in the form of chlorides). After incubation, the residual activity was determined by the standard enzyme assay using *p*-nitrophenyl-α-D-glucopyranoside as a substrate. The activity of enzyme assayed in the absence of the chemical agents was taken as 100%.

Transglucosylation reaction

The ability of alpha-glucosidase from digestive juice of the palm weevil *R. palmarum* larvae to catalyse transglucosylation reactions was tested with maltose and saccharose as glucosyl donor and 2-phenylethanol as the glucosyl acceptor. In a typical experiment, the transglucosylation reaction was carried out at 37°C in 2 ml 100 mM acetate buffer or 100 mM phosphate buffer containing an appropriate amount of α-glucosidase corresponding to 15 units of the considered enzymatic activity and various concentrations of glucosyl donor (maltose and saccharose) and of glucosyl acceptor (2-phenylethanol) (Kouamé *et al.* 2001, 2005). The progress of the reaction was monitored at different times (between 1 and 36 h) by withdrawing aliquots (100 µl) which were heated at 100°C for 5 min. After filtration through a 0.45 µm hydrophilic Durapore membrane (Millipore, www.waters.com), the reaction mixture (20 µl) was analysed quantitatively by HPLC at room temperature. Chromatographic separation of sugars (maltose and saccharose) were performed on a Supelcosyl LC-NH2 (5 µm) column (0.46 x 25 cm) from Supelco (www.sigmaaldrich.com) using acetonitrile/water (75:25; v/v) as the eluent, and monitored by refractometric detection. The flow rate was maintained at 0.75 ml min^{-1}. 2-phenylethanol (PE) and phenylethylglucoside (PEGlc) were analysed on a Thermo Hypersil (www.thermo.com) (5 µm) column (0.46 x 25 cm) using methanol/water (35: 65, v/v) as the eluent. Chromatographic separations were monitored at257 nm using a constant flow rate of 0.45 $mlmin^{-1}$ (Kouamé *et al.* 2001, 2005).

The transglucosylation percentage (T) may be expressed as:

$T = 2\,[PEGlc] \times 100 / [PEGlc] + [Glc]$

where [PEGlc] and [Glc] are concentrations of phenylethylglucoside and glucose respectively (Kouamé *et al.* 2001, 2005).

Results and discussion

Purification procedures

The purification procedure of alpha-glucosidase from digestive juice of the palm weevil *R. palmarum* larvae involved three steps including anion-exchange, size exclusion and hydrophobic interaction chromatographies; the results are summarized in Table 1. One peak of alpha-glucosidase activity was resolved at 0.3 M of NaCl concentration in 20 mM sodium acetate buffer (pH 5.6) on DEAE-Sepharose CL-6B (data not shown).

Table 1. Purification procedure of the purified alpha-glucosidase from digestive juice of the palm weevil *Rhynchophorus palmarum* larvae

Purification steps	Total activity [a](Units)	Total protein (mg)	Specific activity (Units/mg)	Purification fold	Yield (%)
Crude extract	28.08	57.36	0.49	1	100
DEAE Sepharose CL-6B	22.92	18.18	1.26	2.57	81.62
Ammonium sulfate precipitation	21.24	14.24	1.49	3.04	75.64
Sephacryl-S100 HR	9.67	0.3	32.23	65.77	34.43
Phenyl-Sepharose CL-6B	5.65	0.06	94.16	20.12	19.12

a. One unit equals 1 µmol of *p*NP release per min.

The pooled fractions, after this step, were precipitated in 80% ammonium sulphate and applied to a Sephacryl S-100 HR gel, one peak showing alpha-glucosidase activity was eluted (data not shown). The *R. palmarum* larvae alpha-glucosidase activity was finally purified by Phenyl-SepharoseCL-6B hydrophobic interaction chromatography (data not shown). After this step, enzyme specific activity was enriched 20.12-fold with a recovery of 19.12 % over the starting material (Table 1). The specific activity of purified alpha-glucosidase from *R. palmarum* larvae was higher than those obtained for two alpha-glucosidases purified from *Apis cerana indica* larval midgut (Chanchao *et al.*, 2008) and Midgut alpha-

Glucosidase from *Naranga aenescens* (Memarizadeh *et al.,* 2014).

The enzyme showed a single protein band on SDS-PAGE gel electrophoresis staining with silver nitrate (Fig.1). This result confirmed that this enzyme was purified to homogeneity.

Molecular weight determination

SDS-PAGE profile of purified enzyme is depicted in fig. 1. After SDS-PAGE analysis under reducing conditions, alpha-glucosidase from *R. palmarum* larvae showed a single protein band.

Table 2. Some physicochemical characteristics of the purified alpha-glucosidase from digestive juice of the palm weevil *Rhynchophorus palmarum* larvae.

Physicochemical properties	Values
Optimum temperature (°C)	45 °C
Optimum pH	5.6
pH stability	4.0-5.6
Molecular weight (kDa)	
SDS-PAGE	60.60
Gel filtration	61.05
Activation energy (kJ/mol)	36.67
Q_{10}	1.31
Inhibitor agents	Hg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Ba^{2+}, DTNB[a], *p*CMB[b], SDS[c], β-mercaptoethanol, EDTA[d]
Best yield of transglucosylation	62

SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis .a: 5,5'-dithio-2,2'dinitro-dibenzoïc acid ; b: Sodium *para*chloro mercuri benzoate; c: Sodium dodecyl sulphate, d: ethylene diamine tetra acetic acid.

Table 3. Activities of the purified alpha-glucosidase from digestive juice of the palm weevil *Rhynchophorus palmarum* larvae on synthetic chromogenic substrates.

Substrat	Relative activity
p-nitrophenyl-α-D-glucoside	100
p-nitrophenyl-β-D-lucoside	0
p-nitrophenyl-β-D-mannoside	0
p-nitrophenyl-α-D-mannoside	0
p-nitrophenyl-β-D-galactoside	0
p-nitrophenyl-α-D-galactoside	0
p-nitrophenyl-α-D-fucoside	0
p-nitrophenyl-β-D-fucoside	0
p-nitrophenyl-α-L-arabinoside	0
p-nitrophenyl-β-L-arabinoside	0
p-nitrophenyl-β-D-xyloside	0
p-nitrophenyl-α-D-xyloside	0

Its relative molecular weight was estimated to be 60.6kDa (Table 2). On the other hand, the molecular weight determined by HPLC was 61.05 kDa for native enzyme (Table 2). This result was different from that reported for purified alpha-glucosidases from *A. cerana indica* (68 kDa) (Chanchao *et al.*, 2008), *Apis mellifera* L (98 kDa) (Nishimoto *et al.*, 2001) and *Diatraea saccharalis* (54kDa) (Carneiro *et al.*, 2004). Since the values obtained from the two methods were approximately the same, the enzyme was thought to be monomeric.

Table 4. Activities of the purified alpha-glucosidase from digestive juice of the palm weevil *Rhynchophorus palmarum* larvae on oligosaccharide and polysaccharide substrates.

Substrat	Relative activity (%)
Maltose [Glcα(1-4)Glc]	100
Maltotriose [Glcα(1-4)Glcα(1-4)Glc]	112
Maltotetraose [Glcα(1-4)Glcα(1-4)Glcα(1-4)Glc]	126
Maltopentaose[Glcα(1-4)Glcα(1-4)Glcα(1-4)Glcα(1-4)Glc]	119
Maltohexaose[Glcα(1-4)Glcα(1-4)Glcα(1-4)Glcα(1-4) Glcα(1-4)Glc]	105
Maltoheptaose[Glcα(1-4)Glcα(1-4)Glcα(1-4)Glcα(1-4) Glcα(1-4) Glcα(1-4)Glc]	96
Trehalose[Glcα(1-1)Glc]	12
Kojibiose[Glcα(1-2)Glc]	120
Nigerose[Glcα(1-3)Glc]	8
Isomaltose[Glcα(1-6)Glc]	6
Saccharose[Glcα(1-2)Fru]	146
CellobioseGlcβ(1-4)Glc	0
laminarin	0
Arabino-galactane	0
Carboxymethylcellulose	0
Inuline	0
Lichenane	0
Xylane	0
starch	0

Glc. Glucose.

Effect of pH and temperature

The optimum values of pH for studying alpha-glucosidase activity are presented in table 2. The enzyme activity was maximal at pH 5.6. This optimum pH value was similar on the whole to this reported alpha-glucosidase from *A. mellifera* (Nishimoto *et al.*, 2001). Most of alpha-glucosidase, extracted from insects, exhibits pH optima ranging from 4.5 to 7.0 (Frandsen & Svensson, 1998; Ghadamyari *et al.*, 2010; Ramzi & Hoseininaveh, 2010; Saberi *et al.*, 2012).

Table 5. Kinetic parameters of the purified alpha-glucosidase from digestive juice of the palm weevil *Rhynchophorus palmarum* larvae towards *p*-nitrophenyl-α-D-glucopyranoside, Maltose, Saccharose and Kojibiose.

Substrat	K_M (mM)	V_{max} (U/mg)	V_{max}/ K_M (U/mM x mg)
p-nitrophenyl-α-D-glucopyranoside	0.30	142.20	474.00
Maltose	1.31	48.23	36.81
Maltotriose	1.14	52.21	45.79
Maltotetraose	1.08	49.52	45.85
Maltopentose	1.42	44.23	31.15
Maltohexose	3.21	21.28	6.63
Saccharose	0.42	77.54	184.61
Kojibiose	0.57	62.23	109.17

The zone of stability of the pH of the alpha-glucosidase is between pH 4 and 5.6 (Table 2). The pH stability margin is wide; it might be beneficial to synthesis reactions or hydrolysis which uses purified α-glucosidase in biotechnology processes.

The optimum temperature of the purified alpha-glucosidase of *R. palmarum* larvae was at 45°C (Table2). This optimum temperature value was similar on the whole to those reported for other insect alpha-glucosidases from *N. aenescens* (Memarizadeh

et al 2014) and *Limicolaria flammea* (Saki *et al*., 2014).

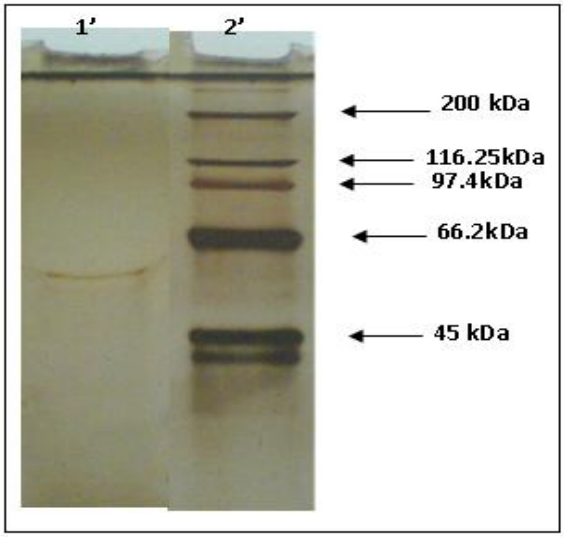

Fig. 1. SDS-PAGE analysis of the purified alpha-glucosidase from digestive juice of the palm weevil *Rhynchophorus palmarum* larvae. The sample was loaded onto a 10 % gel. Lane 1, purified alpha-glucosidase, Lane 2, numbers on the right indicate the molecular weights (kDa) of protein markers.

The thermal inactivation study at pH 5.6 indicated that, alpha-glucosidase remained fully stable for 140 min at 37 °C (Fig. 2). However, at 45 °C (its optimum temperature) the enzyme was less stable and lost about 80% of its hydrolytic activity after 100 min of pre-incubation.

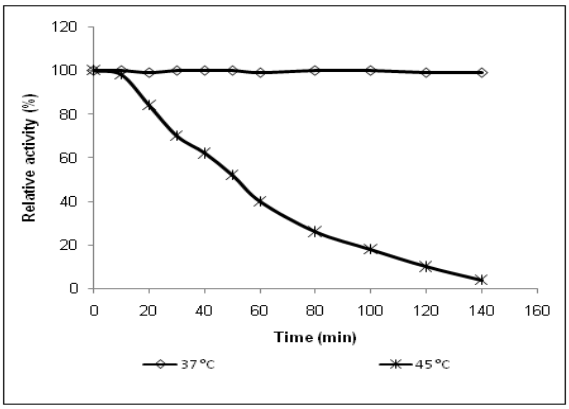

Fig. 2. Thermal inactivation of the purified alpha-glucosidasefrom digestive juice of the palm weevil *Rhynchophorus palmarum* larvae.

Kinetic parameters values of alpha-glucosidase

R. palmarum larvae alpha-glucosidase did not attack the following *p*nitrophenyl glycosides: beta-glucoside, alpha-and beta-galactoside, alpha-and beta-mannoside, alpha-and beta-xyloside, alpha-and beta-L-arabinoside, alpha-and beta-fucoside (Table3); nor the following oligosaccharide: cellobiose; nor the following polysaccharides: arabinogalactan, carboxymethylcellulose, inulin, lichenan, laminarin, xylan and starch (Table 4). However, it was very active on *p*-nitrophenyl-alpha-D-glucopyranoside, maltose, maltodextrins, saccharose and kojibiose (Table 3; 4). Low activity was observed towards Trehalose, Nigerose and Isomaltose(Table 4). This result suggests that the alpha-glucosidase is an exoglycosidase with a high specificity for the alpha-anomeric configuration of the glycosidic linkage. This pattern seems to reflect the activity of the alpha-glucosidases from *Thermus caldophilus* GK24 (Oyekanmi *et al*., 2001) and from cockroach, *Periplaneta americana (Kouamé et al.,* 2005*)*.

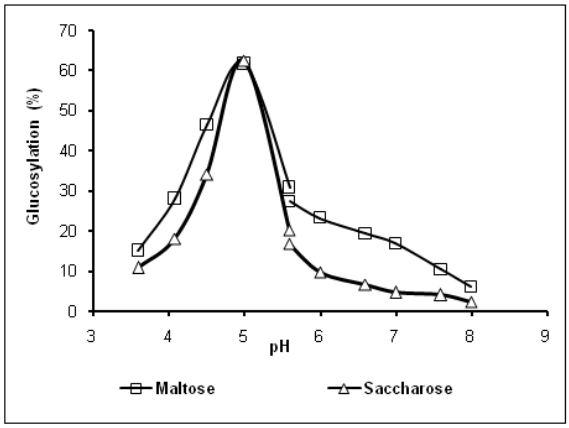

Fig. 3. Effect of pH on transglucosylation reaction catalyzed by the purified alpha-glucosidase from digestive juice of the palm weevil *Rhynchophorus palmarum* larvae with glucosyl donor (maltose and saccharose).

Kinetic parameters values of alpha-glucosidase

The kinetic constants (V_{max} and k_m) for alpha-glucosidase from *R. palmarum* larvae were determined by incubating fixed amount of enzyme with varied concentrations of *p*-nitrophenyl-alpha-D-glucopyranoside (1–10 mM) and oligosacharides (1–20 mM). The enzyme followed the Michealis Menten kinetics of catalysis. The K_m value of purified alpha-glucosidase from *R. palmarum* larvae for *p*NαG substrate was calculated at 0.30 mM and it showed much higher affinity of purified enzyme to substrate

over crud enzyme. Furthermore, the K_m value is within the range of the majority of other alpha-glucosidases. For instance, the K_m values of purified alpha-glucosidases from *Apis cerana japonica* and *N. aenescens*were 1 and 0.54 mM, when *p*NαG was used as a substrate (Wongchawalit *et al.*, 2006; Memarizadeh *et al.*, 2014). The values of K_M for saccharose (0.42 mM) and for maltose (1.31 mM) were lower than the values measured for the same substrates and the alpha-glucosidase purified from *Quesada gigas* midgut (10.8 mM and 5.46 mM, respectively) (Fonseca *et al.*, 2010). The values for the ratios of V_{Max}/K_M for the different oligo maltodextrins suggest that the *R. palmarum* larvae alpha-glucosidase efficiently hydrolyses glucose chains up to five residues; lower efficiencies were detected as the size of the chain increased. Although the order of greatness of the ratios is larger in the cicada, the purified *Dysdercus peruvianus* alpha-glucosidase exhibits a very similar kinetic profile (Silva & Terra, 1995).

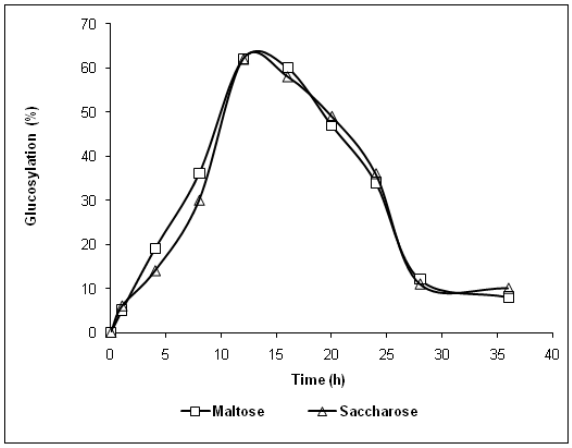

Fig. 4. Time course of 2-phenylethylglycoside synthesis by the alpha-glucosidase from digestive juice of the palm weevil *Rhynchophorus palmarum* larvae with glucosyl donor (maltose and saccharose).

Effect of chemical agents on enzyme activity

Chemical agents Fe, Cu, Zn, Mn, Ba, DTNB, *p*CMB, SDS, β-mercaptoethanol and EDTA showed an inhibitory effect on alpha-glucosidase activity (Table 2). Others had no effect on enzyme activity (data not show). The action of EDTA could be explained by its interaction with a metal ion housed in the structure of alpha-glucosidase from *R. palmarum* larvae. This result indicates that the alpha-glucosidase is a metalloenzyme. This enzyme also contains thiol groups in its structure because it is inhibited by agents such as *P*CMB and DTNB. The presence of thiol groups in the essential conformation of the enzyme is shown also by the inhibitory action of the Hg^{2+} ion. Indeed, the reduction of enzyme activity by Hg2+ ion indicates that thiol groups are not only located in the active center of the enzyme but these thiol groups participate in the catalytic act (Yang & Liu, 2004).

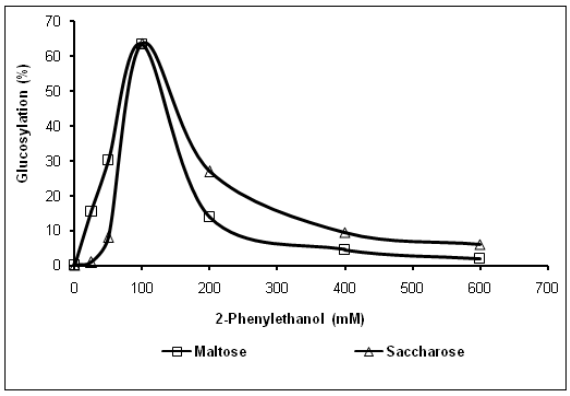

Fig. 5. Effect of the glucosyl acceptor (2-phenylethanol) concentration on transglucosylation reaction catalyzed by the alpha-glucosidase from the digestive juice of the palm weevil *Rhynchophorus palmarum* larvae with glucosyl donor (maltose and saccharose).

Transglucosylation reaction

The ability of alpha-glucosidase from the digestive juice of the palm weevil *R. palmarum* larvae to catalyse transglucosylation reactions was tested with maltose and saccharose as glucosyl donor and with 2-phenylethanol as glucosyl acceptor. The effect of pH on the yield of the transglucosylation was studied (Fig. 3). Maximum transglucosylation was obtained in the pH 5.0 (Fig. 3), which is different from that of the hydrolysis reaction (pH 5.6). This result was similar on the whole to this reported alpha-glucosidases from *Macrotermes subhyalinus* and *P. americana* (Kouamé *et al.*, 2001; 2005). The rate of hydrolysis is more highly favoured than the rate of transglucosylation product formation. Glucosylation kinetics were also studied as a function of the incubation time. In the initial stage of the reaction, much phenylethylglucoside was synthesized by the

transglucosylation activity of the enzyme (Fig. 4). However, as the reaction proceeded, the transglucosylation product was gradually hydrolysed by the same enzyme. The alpha-glucosidase hydrolysed the products formed (Fig. 4). It seems that the product formed is the phenylethyl-alpha-D-glucoside. This result suggests that the enzyme catalyses the splitting of alpha-glucosyl residue from-non-reducing terminal of the substrate to liberate alpha-glucose. This comportment indicates that this enzyme operated by a mechanism involving the retention of the anomeric configuration (Kouamé *et al.*, 2005). The efficiency of the alpha-glucosidase from the digestive juices of the palm weevil *R. palmarum* larvae in catalysing of transglucosylation reactions was also largely dependent on the respective concentrations of glucosyl donor (maltose and saccharose) and glucosyl acceptor (2-phenylethanol; Fig. 5; 6). The best yields (62%; Table 2) were obtained with a concentration of around 400 mM of glucosyl donor (maltose and saccharose) and 100 mM of glucosyl acceptor (2-phenylethanol; Fig. 5; 6).

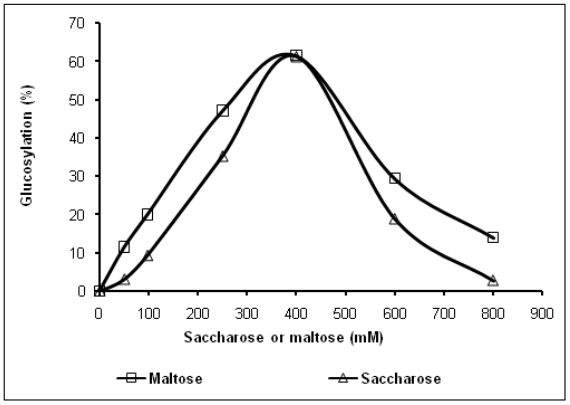

Fig. 6. Effect of the glucosyl donor (maltose and saccharose) concentration on transglucosylation reaction catalyzed by the alpha-glucosidase from the digestive juice of the palm weevil *Rhynchophorus palmarum* larvae.

Conclusion

The alpha-glucosidase from *R. palmarum* larvae appears to be distinct from other alpha-glucosidases so far reported in terms of substrate specificity and transglucosidase activity. This enzyme could be used as a tool in the structural analysis of D-glucose containing oligosaccharide chains of glycoproteins, glycolipids and starch.

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