African Journal of Biotechnology Vol. 7 (15), pp. 2707-2716, 4 August, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Purification and physicochemical properties of α-amylase from cockroach, *Periplaneta americana* (LINNAEUS), for starches saccharification

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Accepted 17 April, 2008

An α-amylase was purified from the American cockroach *Periplaneta americana* (Linnaeus) to homogeneity by four steps purification via ammonium sulphate crude extract precipitation, Sephacryl S-100 HR gel permeation chromatography, anion exchange chromatography on DEAE-Sepharose CL-6B and hydrophobic interaction chromatography on phenyl Sepharose CL-4B. The purification was approximatively 38.42 fold with a 24.31% yield. Optimums pH and temperature of the purified α-amylase were found to be 5.6 and 55°C, respectively. The enzyme was stable up to 55°C and its pH stability was in range of 5.6 - 6.6. The K_M and Vmax of the enzyme with soluble starch as substrate were 5 mg/ml and 100 µmol/min/mg, respectively, and the energy of activation (Ea), was 50.32 Kj/mol. The α-amylase was inhibited by Tris, Fe^{3+}, Ba^{2+}, Mo^{+} and EDTA. While Ca^{2+}, K^{+}, Cu^{2+}, Mg^{2+} and *para*-hydroxymercuribenzoate (*p*HMB) activated the enzyme. Analysis of the amylolytic reaction products by HPLC showed the presence of maltose and maltodextrin but not glucose in the starch hydrolysate (2 h of reaction). This result indicated that the amylolytic enzyme of *P. americana* is an α-amylase (an endoamylase). The purified α-amylase hydrolysed maltopentose, maltohexose and maltoheptose. Maltose, maltotriose and maltotetrose were not hydrolysed by this enzyme. Therefore, the purified α-amylase is active only on substrates with more than four residues of glucose.

Key words: α-Amylase, *Periplaneta americana,* cockroach, enzyme purification, saccharification, maltopentaose.

INTRODUCTION

The American cockroach, *Periplaneta americana* (Linnaeus), is the largest of the common peridomestic cockroaches measuring on average 4 cm in length. 47 species are included in the genus *Periplaneta* (Bell and Kilogramme, 1981). The cockroaches dwell outside but will wander indoors for food and water or during extremes in weather condition. Trees, woodpiles, garbage facilities, and accumulation of organic debris around homes provide adequate food water and harbourages for peridomestic cockroaches such as the American cockroach (Hagenbuch et al., 1988). *P. Americana* can become a public health problem due to its association with human waste and disease, and its ability to move from sewers into homes and commercial establishments. It can also spoil food and cause allergies (Bell and Kilogramme, 2001). Their presence in the habitats is of epidemiological significance. At least, 22 species of path-

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ogenic human bacteria, virus, fungi and protozoans, as well as five species of helminthic worms, have been isolated from field collected American cockroaches (Rust et al., 1991).

This insect has been extensively studied due to its economical and medical importance (Bernard and Thomas, 1988; Jahargirdar et al., 1990; Bidat et al., 1993; Changlin et al., 1998; Iraneta et al., 1999). Nevertheless, its digestive system, which is a valuable source of importance about the species, is yet poorly understood.

The American cockroach is an omnivorous and a scavenger, which eat almost anything. It prefers sweets and has been observed eating paper, boots, hair, bread, fruit, old rice, putrid sake, cloth and dead insects (Bell and Kilogramme, 1981). Wang et al. (2000) inhibited the amylase activity of the digestive tube of *P. Americana* using synthetic polypeptidases which inhibited the α-amylase of the Mexican plant seeds *Hypochondriacus amarantus*. Lima et al. (2003) localised the amylase activity of *P. Americana* in two regions of the midgut: the caecal epithelium and the anterior ventricular epithelium. In these regions, the enzyme was observed in the columnars cells, especially in areas above the nucleus and in the gut lumen near the brush border. Recently, Kouamé et al., (2004) studied the synergism of *P. americana* α-amylase and α-glucosidase hydrolysis of starches. To study the capacity of *P. americana* α-glucosidase to transfert glucosyls residues, the same authors, (Kouamé et al., 2005) purified to homogeneity and partially characterized this enzyme. This enzyme exhibited higher transfer activity with maltose and saccharose in high yield (67%).

In this work, we described the purification to homogeneity and some biochemicals properties of the α-amylase from *P. americana* for starches saccharification.

MATERIALS AND METHODS

Soluble starch, amylose, amylopectin, pullulan, glucose, maltose and DEAE Sepharose CL-6B were obtained from Sigma Chemicals Co. Sephacryl S-100 HR and Phenyl-sepharose CL-4B was obtained from Pharmacia biotech. Protein molecular weight standards were obtained through Bio-Rad. All other chemicals and reagents used were of analytical grade. *P. americana* were captured at rooms.

Enzyme extract

30 g of *P. americana* washed in distilled water were grinded and homogenized in 100 ml NaCl 0.9% (p/v) with a microcrusher ultraturrax (type TP 10/18). After centrifugation at 4°C for 20 min at 5000 rpm, the supernatant was kept at 4°C and used as crude extract.

Assay of α-amylase activity

The α-amylase activity was routinely assayed as described below. An enzyme solution to be tested was incubated with 1% solution starch on a 20 mM acetate buffer (pH 5.6) at 37°C and the amount of reducing sugars produced was determined by the dinitrosalicylic acid (DNS) (Bernfeld, 1955) method with glucose as the standard. One unit of the enzyme activity was defined as the amount that liberated 1 µmol of glucose equivalent per min per protein mg under the above conditions.

Determination of protein

Protein concentration was determined according to the method of Lowry et al. (1951). Bovine serum albumin was used as the standard.

Purification procedure

To 20 ml of crude extract, ammonium sulphate was added to 80% saturation at 4°C with slow stirring during 12 h. The suspension was centrifuged for 30 min at 5000 trs/min. The precipitate was dissolved in 1 ml of 20 mM acetate (pH 5.6) buffer.

Dissolved precipitate was loaded on a Sephacryl S-100 HR column of pharmacia (1.6 x 64 cm) equilibrated with 20 mM acetate buffer (pH 5.6) and proteins were eluted with the same buffer. The α-amylase fractions were further loaded on a DEAE-sepharose CL-6B column of Sigma (2.4 x 6.5 cm) equilibrated with the same buffer (pH 5.6). Fractions of 1 ml were collected and those containing activity were pooled. Gradient elution was performed with increasing NaCl concentrations (0; 0.2; 0.3; 0.4; 0.6 and 1 M).

The α-amylase fractions, added to solid sodium thiosulphate till a concentration of sodium thiosulphate become 1.7 M were finally loaded on a phenyl-sepharose CL-4B column of pharmacia biotech (1.4 x 5 cm) equilibrated with 20 mM acetate buffer (pH 5.6) containing 1.7 M of sodium thiosulphate. The enzyme was eluted with a graduated gradient of sodium thiosulphate concentration from 1.7 M to 0 M and active fractions were collected.

Estimation of molecular weights

The molecular weights of the purified α-amylase were estimated by SDS-PAGE performed by Laemmli's method (1970) using 12% acrylamide gel and by gel filtration chromatography. Gel filtration chromatography was performed in HPLC system by using TSK (QC-PAK GFC 200) column equilibrated and eluted in 20 mM acetate buffer (pH 5.6) containing sodium azide 0.5% (w/v). Molecular mass values were calculated by using the following proteins as standards: β-amylase (200 Kda), bovine serum albumin (66 Kda), ovalbumin (45 Kda) and cytochrome C (12.4 Kda).

For gel electrophoresis in SDS, the enzyme samples were denatured by a 5 min treatment at 100°C in a 125 mM Tris- HCl buffer (pH 6.8 containing 4% (w/v) SDS, 1% (v/v) β-mercaptoethanol, 20% (v/v) glycerol and 0.025% (v/v) Bromophenol Blue. Electrophoresis was carried out on 1.5 mm thick slap gels (8 x 7 cm). The gel contained 21% acrylamide, 375 mM Tris-HCl buffer (pH 8.8) and 0.1% (w/v) SDS. Electrophoresis was carried out at 10 mA with a 25 mM Tris/192 mM glycine buffer containing 0.1% (w/v) SDS as the electrode buffer (Laemmli, 1970). Proteins were stained with a 0.25% (w/v) Coumassie Brillant Blue R-250 solution containing 40% (v/v) methanol and 10% (v/v) acetic acid. Molecular masses for SDS PAGE were calculated by using the following proteins as standards: phosphorylase B (91 kD), bovine serum albumin (66 kD), ovalbumin (49.9 kD), carbonic anhydrase (35.1 kD), soybean trypsin inhibitor (28.4 kD) and lysozyme (20.8 kD).

Effects of pH and temperature

The effects of pH on this activity were assayed at 37°C in a reaction mixture containing 1% soluble starch and 20 mM acetate buffer (pH 3.6-5.6) and phosphate buffer (pH 5.6-8). The pH stabi-

Table 1. Purification of an α-amylase from *Periplaneta americana*.

Purification step	Volume (ml)	Total activity (UI)	Protein (mg)	Specific activity (UI / mg)	Purification factor	Recovery (%)
Crude extract	20	118.31	62.50	1.89	1	100
Precipitate dialysed	15	84.45	40.62	2.07	1.09	71.38
Sephacryl S-100	17	54.87	3.89	14.08	7.44	46.37
D.E.A.E Sepharose	8	42.04	0.93	45.90	24.28	35.53
Phenyl Sepharose	5	29.24	0.31	94.32	38.42	24.71

Assays were performed under standard conditions described in the text. For procedure, see Materials and Methods. Values given are the averages of at least three experiments.

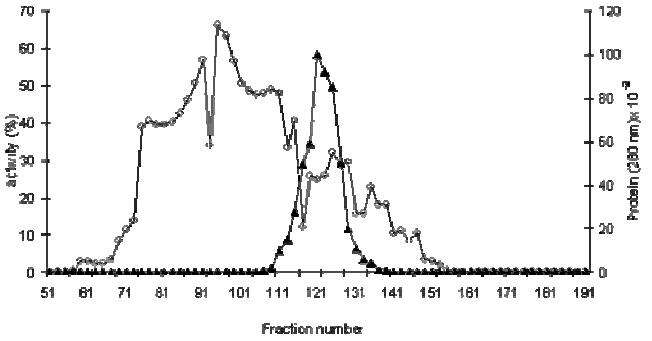

Figure 1. Chromatography on Sephacryl S-100 HR column of *Periplaneta americana* crude extract. The gel-filtration column (1.6 x 64 cm) was previously equilibrated and eluted with 20 mM acetate buffer, pH 5.6, at a flow rate of 0.25 ml/min. Fractions (1 ml/tube) were collected and assayed for amylase activity (A_{540}) and protein content (A_{280}). The fraction volume containing the α-amylase was 17 ml.

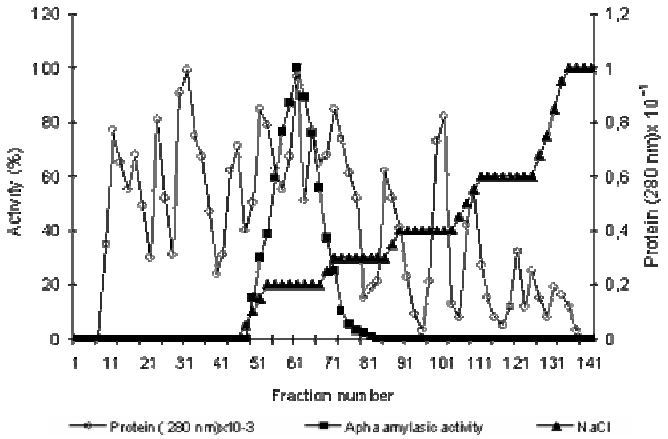

Figure 2. Chromatography on DEAE Sepharose CL-6B column of *Periplaneta americana* crude extract. The sample and column (2.4 x 6.5 cm) were equilibrated with 20 mM acetate buffer, pH 5.6, bound proteins were eluted with a gradient of 0 -1 M NaCl, and fractions of 1 ml/tube were collected. NaCl was estimated using a refractometer. The fraction volume containing the α-amylase was 8 ml.

lity was analysed with enzyme incubated in 20 mM acetate buffer (pH 3.6-5.6) and phosphate buffer (pH 5.6-8) at 37°C for 2 h. After this time, Aliquots were taken and immediately assayed for residual α-amylase activity.

The effects of temperature were assayed at various temperatures in 20 mM acetate buffer (pH 5.6). The thermal denaturation of this enzyme was analysed at various temperatures (35°C to 70°C) for 5 min. The thermal inactivation was analysed with the enzyme incubated at 37°C and optimum temperature (55°C) for 1 h. Aliquots were withdrawn 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.

RESULTS

Enzyme purification

The purification procedure is summarized in Table 1 and shows the results obtained after each step.

Chromatography on sephacryl S-100 HR column: This chromatography step yields several protein peaks. The α-amylase activity presents only one peak (Figure 1). At this step of the purification process, the enzyme preparation has a specific activity of 14.08 UI/mg which corresponds to 7.44 fold purification and a yield of 46.37%. Fractions containing α-amylase activities were pooled to be apply on DEAE sepharose column.

Chromatography on DEAE sepharose CL-6B: α-Amylase activity showed only one peak and is eluted at a 0.2 M NaCl concentration (Figure 2). The enzyme preparation has a specific activity of 45.90 UI/mg, which corresponds to a yield of 35.53%.

Chromatography on phenyl sepharose CL-4B: The active fractions, eluted at about 0.15 M of sodium thiosulphate were collected (5 ml). The specific activity (94.32 UI/mg) was constant in all fractions of the unique protein peak eluted (Figure 3). The active fraction obtained is pooled and kept at 4°C until use.

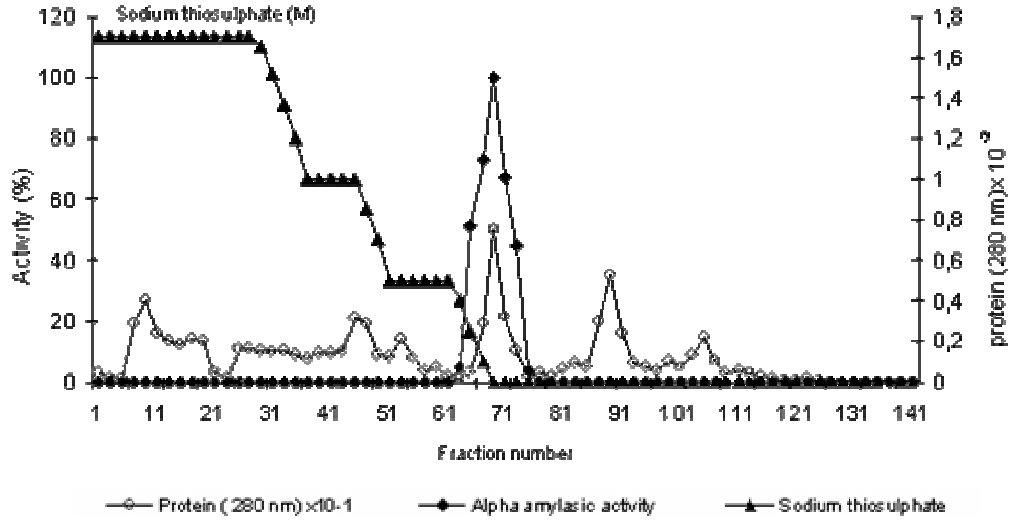

Figure 3. Chromatography on phenyl Sepharose CL-4B column of *Periplaneta americana* crude extract. The sample and column (1.4 x 5 cm) were equilibrated with 20 mM acetate buffer, pH 5.6, containing 1.7 M sodium sulphate. Bound proteins were eluted with a gradient of 1.7 – 0 M sodium sulphate, and fractions of 1 ml/tube were collected. Sodium sulphate was estimated using a refractometer. The fraction volume containing the α-amylase was 5 ml.

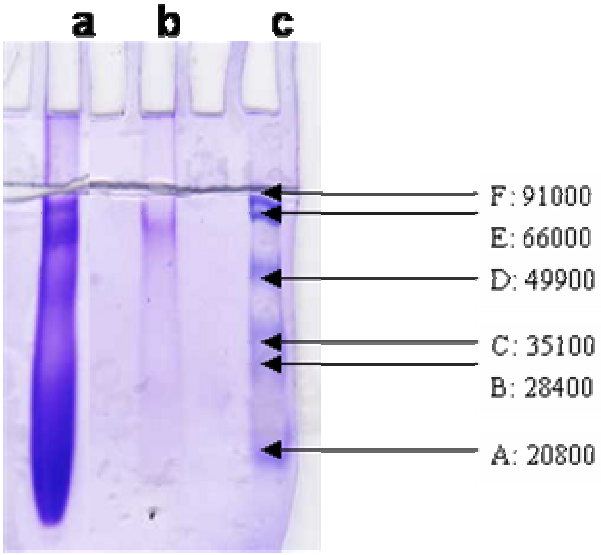

Figure 4. SDS-PAGE of *Periplaneta americana* α-amylase. The samples were loaded onto a 12% gel. **Lane a:** Crude extract of *Periplaneta americana*, **lane b:** SDS-PAGE of the purified α-amylase **lane c:** Molecular weight markers from Bio-Rad (A: Lysozym (Mr = 20.8 KDa); B: trypsic inhibitor (Mr = 28,4 KDa); C: Carbonic anhydrase (35.1 KDa); D: Ovalbumin (49.9 KDa; E: Bovine serum albumin (66 KDa); F: Phosphorylase B (91 KDa)).

Homogeneity of purified α-amylase

The result of Sodium dodecyl sulphate polyacrylamide gel electrophoresis (12%) (SDS-PAGE) of the purified α-amylase is shown in Figure 4. The single band on the gel indicated the homogeneity of the enzyme and no subunit structure.

Molecular weights of the purified α-amylase

The molecular weight of the α-amylase was estimated to be 60,000 Daltons by SDS electrophoresis. The molecular weight determined on gel filtration chromatography on TSK column was 48,000 Daltons.

Optimum pH

As shown in Figure 5, the α-amylase has an optimum pH of 5.6 with soluble starch as substrate.

pH stability

Under the conditions described in material and methods, the enzyme was stable between pH 5.6 and 6.6. While, it was rapidly inactivated at pH below 5.6 and above 6.6 (Figure 6).

Optimum temperature

The α-amylase exhibited the maximum activity at 55°C (Figure 7). An arrhenius plot of the data showed the enzyme to have an activation energy 50.32 Kj/mol with starch (1%) as substrate. The Q^{10} determined between 40°C and 50°C is about 1.82.

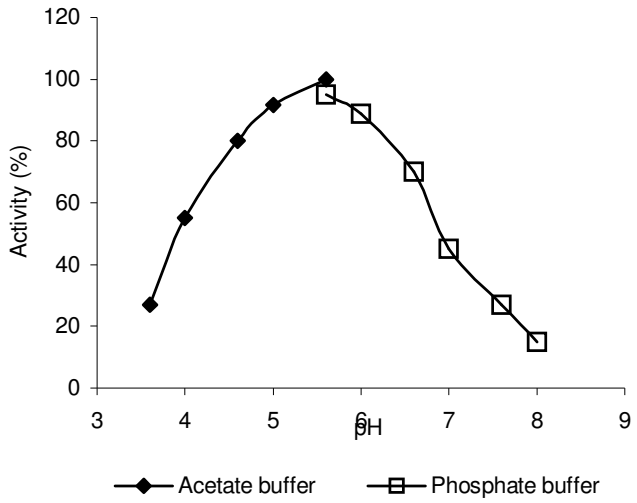

Figure 5. Effects of pH on *Periplaneta americana* α-amylase activity. Reaction mixtures containing 100 µl of enzyme solution, 100 µl of 1% soluble starch and 100 µl of buffers indicated below were incubated at 37°C for 20 min and the amount of reducing sugars produced was determined by the DNS method. pH 3.6 – 5.6, 20 mM acetate buffer; pH 5.6 – 8.0, 20 mM phosphate buffer.

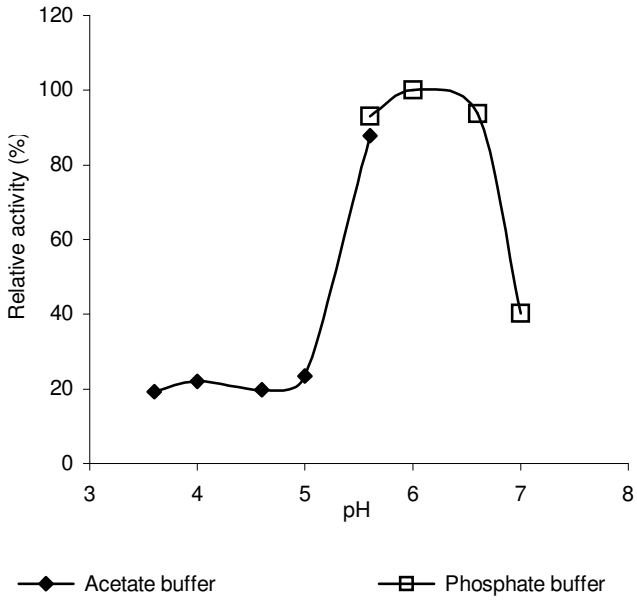

Figure 6. Effect of pH on the stability of *Periplaneta americana* α-amylase. 500 µl of enzyme solution was pre-incubated with 500 µl of buffers indicated below either at 37°C for 2 h. After the pre-incubation, 100 µl of 20 mM acetate buffer, pH 5.6, 100 µl of 1% soluble starch were added to 100 µl of enzyme solution pre-incubated and the remaining activity was assayed under standard conditions described in the text.

Temperature stability

Thermic inactivation study showed that the α-amylase retained 7.6% of its original activity after being incubated

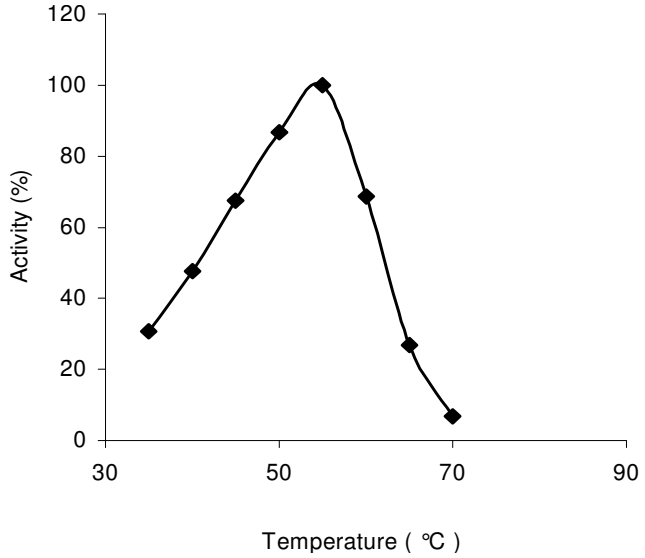

Figure 7. Effect of temperature on *Periplaneta americana* α-amylase. Reaction mixtures containing 100 µl of enzyme solution, 100 µl of 20 mM acetate buffer (pH 5.6) and 100 µl of 1% soluble starch were incubated at various temperatures (35 – 70°C) for 20 min and the amount of reducing sugars produced was determined by the DNS method.

at 55°C for 1 h and 85% of this activity at 37°C (Figure 8). Nevertheless, thermal denaturation study showed that the enzyme was stable for at last 20 min until 55°C. Above this temperature, the enzyme is rapidly inactivated (Figure 9).

Action of several chemical agents

Figure 10 summarizes the effects of various chemical agents on the α-amylase activity. Tris, Fe^{3+}, Ba^{2+}, Mo^{+} and EDTA exhibited the inhibitory effect in that decreasing order at a final concentration of 2.5 and 5 mM. While Ca^{2+}, K^{+}, Cu^{2+}, Mg^{2+} and *p*HMB activated the enzyme, but the effect of K^{+}, Ca^{2+} and *p*HMB appeared more important.

Kinetics parameters of the purified α-amylase

The K_M values for soluble starch, amylopectin and amylose were about 5 mg/ml and 5.55 mg/ml, respectively. Catalytic efficiency was higher with soluble starch than amylopectin or amylose. This α-amylase could not hydrolyse pullulan (Table 2).

Determination of amylase characteristic

The action of the purified amylase on soluble starch was realised at 2 h in 20 mM acetate buffer (pH 5.6) at 37°C. Study of the influence of time on amylase's activity show-

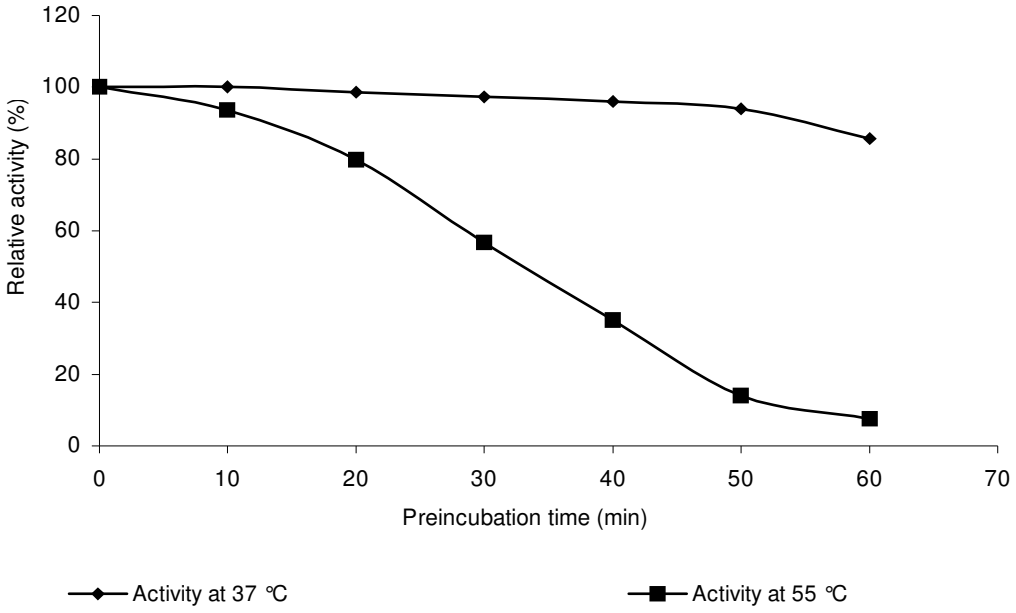

Figure 8. Thermic inactivation at 37°C and 55°C of *Periplaneta americana* α-amylase. Thermal stability of the enzyme was followed for 1 h. The enzyme was incubated in 20 mM acetate buffer pH 5.6. At time intervals, aliquots were withdrawn and immediately cooled in ice-cold water. Residual activities, determined at 37°C under the standard test conditions, are expressed as percentage activity of zero-time control of untreated enzyme. Values given are the averages from at least three experiments.

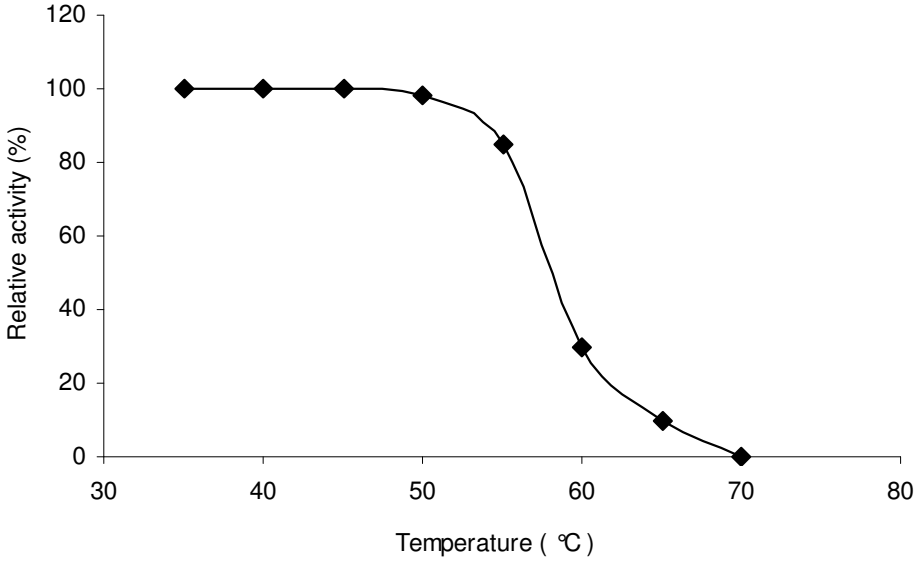

Figure 9. Thermal denaturation of *Periplaneta americana* α-amylase. Thermal stability of the enzyme was followed at various temperatures from 35 to 70°C. The enzyme was incubated in 20 mM acetate buffer pH 5.6. At time intervals, aliquots were withdrawn and immediately cooled in ice-cold water. Residual activities, determined at 37°C under the standard test conditions, are expressed as percentage activity of zero-time control of untreated enzyme. Values given are the averages from at least three experiments.

ed that increase in reaction time leads to higher concentration of maltose (Figure 11). The oligosoccharides produced were determined by HPLC. The analysis of the hydrolysis products revealed maltose and maltodextrin

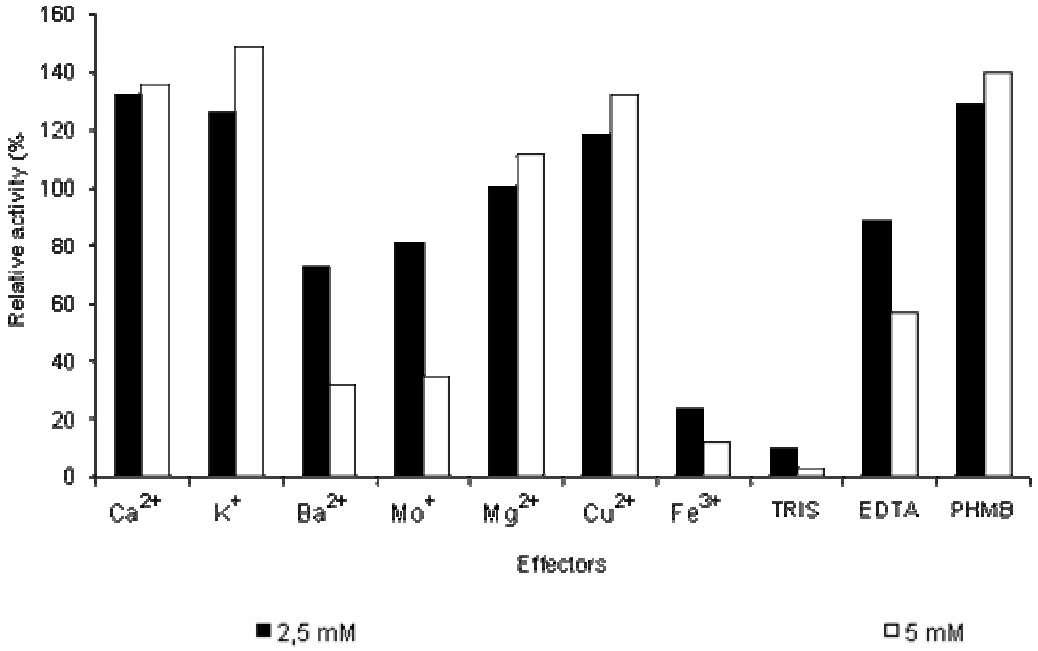

Figure 10. Effect of various compounds on *Periplaneta americana* α-amylase activity. The reaction was performed at 37°C for 25 min in the usual experimental conditions. Values given are the averages of at least three experiments.

Table 2. Kinetic parameters of the purified *Periplaneta americana* α-amylase.

Substrate (conc.)	Km (mg/ml)	Vmax (µmol/min/mg)	Vmax/KM
Soluble starch	5	100	20
Amylopectin	5.55	98.61	17.77
Amylose (DP=17)	5.55	98.61	17.77
Pullulan	0	0	0

Values given are the averages of at least three experiments.

and not glucose in the reaction medium (Figure 12).

Action of the purified α-amylase on maltooligosaccharides

This study showed that the purified α-amylase possessed amylolytic activity in the hydrolysis of maltooligosaccharides (Table 3). This enzyme hydrolysed maltopentaose, maltohexaose, and maltoheptaose. Maltose, maltotriose and maltotetraose were not hydrolysed by the enzyme. The HPLC analysis of the products of the amylolytic reaction showed the presence of maltose and maltotriose when the maltopentose was used as substrate; maltose, maltotriose and maltotetrose when maltohexose was used as substrate; maltose maltotriose, maltotetrose and maltopentose in the maltoheptose hydrolysate (20 min of reaction).

DISCUSSION

The purification of *P. Americana* α-amylase required 3 chromatographic steps; gel filtration chromatography on sephacryl S100-HR, DEAE-sepharose CL-6B chromatography and phenyl-sepharose chromatography. The purified α-amylase has a specific activity of 94.32 UI/mg, which corresponds to 38.42 fold purification, and a yield of 24.41%. The lower purification factor could be explained by an important loss of the enzyme quantity by interaction with the different gels used. The yield the purified α-amylase obtained is near to those of several other sources. Talamond et al. (2002) obtained a yield of 27% with α-amylase from *Lactobacillus fermentum* and Hajime et al. (1983), 25.50% with *Bacillus circulans* α-amylase. The purification of *P. americana* α-amylase was confirmed by the appearance of a single band after SDS/PAGE. The molecular weight of the purified α-

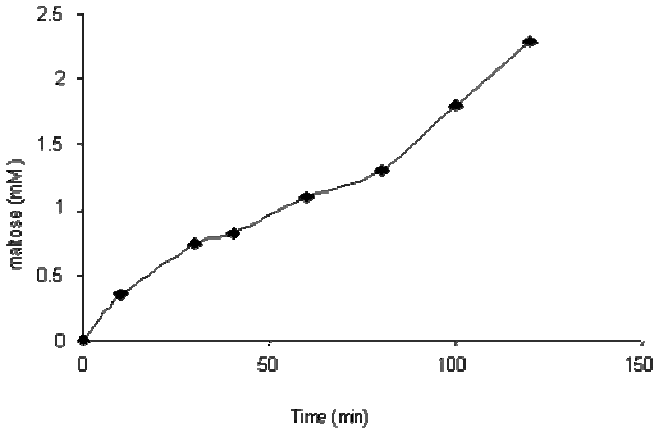

Figure 11. Time course of soluble starch degradation by the purified α-amylase. The experiments were performed with 0.58 U of α-amylase.

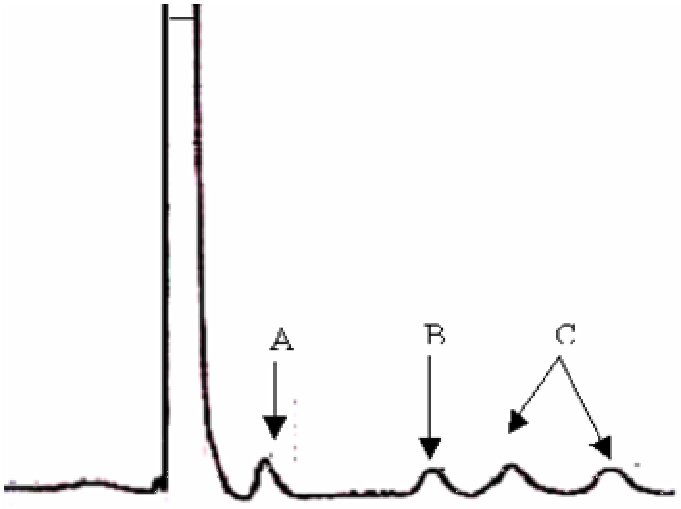

Figure 12. Chromatogram of soluble starch degradation products. Column connected: supercosyl LC-NH_2 5 µm (0.46 x 25 cm); mobile phase: water-acetonitrile (25/75, v/v); flow rate: 0.75 ml/min; peak A: xylose (internal standard); peak B: maltose; peak C: maltodextrin. The experiments were performed with 0.58 U of α-amylase.

amylase, which is a monomeric enzyme, was estimated to be 60,000 (by SDS/PAGE) and 48,000 (by gel filtration on TSK column gel). The difference between the values might be due to the affinity of this amylase to TSK column gel; therefore, the value obtained by SDS electrophoresis might be more reliable. This value was comparable to those reported for the α-amylases from the *Thermus filiformis* ORK A_2 (Egas et al., 1998), the halophilic archaeon *Haloferax mediterranei* (Perez-Pomares et al., 2003), or the *Bacillus stearothermophilus* US-100 (Ben Ali et al., 2001). The molecular mass of the purified α-amylase differ from other α-amylases of *Lactobacillus fermentum* (106 Kda) (Talamond et al., 2002), or from the leaves of *Boscia senega*l*ensis* (36 Kda) (Dicko et al., 2001).

The optimum hydrolysis pH of the purified α-amylase is 5.6. This acidic pH is within the optimum hydrolysis pH of glycosidases that generally spreads from 4.5 to 6.0 (Kelly and Fogarty, 1983). The optimum pH of *P. americana* α-amylase was similar to those reported for other α-amylases from mushroom *Scryta*l*idium thermophilum* (Aquino et al., 2003), or from malted finger millet (Ragi, Eleusine coracana Indaf-15) (Nirmala and Nuralikrishna, 2003). It is, however, different from the α-amylases from archaeon *Hal*o*ferax mediterranei* (pH 7.0 to 8.0) (Perez-Pomares et al., 2003), *Toxoplasma gondii* (pH 8.0) (Ferrer et al., 1999), *Psychrophilic bacterium*TAC 240 B (pH 7.5) (Chessa et al., 1999) and *Trichoderma harzianum* (pH 4.0) (De Azevedo et al., 2000).

The optimum hydrolysis temperature of the purified α-amylase was 55°C. This value was higher than those reported for the α-amylases from *Sulculus diversicolor aquatilis* (45 and 50°C) (Tsao et al, 2003), but lower than those α-amylases obtained from *Bacillus circulans* GRS 313 (60°C) (Dey et al., 2002), extreme thermophilic eubacterium *Rhodothermus marinus* (80°C) (Gomes et al., 2003) and the muscle of *Ascaris suum* (70°C) (Zoltowska, 2001).

Like the amylolytics enzymes characterized from different organisms, the α-amylase from *P. americana* was also stable a wide range of pH and temperature. This enzyme was quite stable in an acidic range between pH 5.6 and 6.6. The purified α-amylase was also stable until 55°C and lost 73% of its original activity after being incubated at 55°C for 1 h. Starches degradation using *P. americana* α-amylase could be done in this zone of pH (5.6 - 6.0) and at temperature lower than 55°C.

The purified α-amylase affinity for starch (0.2 ml/mg) was higher than those of amylopectin and amylose. However, this α-amylase could not attack pullulan (α-(1,6) glucosidic linkage). This behaviour suggests that this enzyme can be a tool of choice for the separation of certain oligosaccharide domains of biological systems containing at the same time α-(1,4) and α-(1,6) glucosidic linkages.

The purified α-amylase affinity for starch was similar to that reported for the α-amylase from *Thermus filiformis* ORK A_2 (Egas et al., 1998). While, this affinity was lower than those reported for the α-amylases from *Lactobacillus manihotivorans* LMG 18010 T (0.29 ml/mg) (Aguilar et al., 2000) and *Trichoderma harzianum* (0.28 ml/mg) (De Azevedo et al., 2000). Nevertheless, Dey et al. (2002), have reported an α-amylase from *Bacillus circulans* GRS 313 with an affinity more lower (0.09 ml/mg).

The effects of effectors were different depending on the ions studied. The activation by Mg^{2+} and the relatively important inhibition by EDTA are in favour of a metallo-protein nature of *P. americana* α-amylase, as already shown in the case of *Ascaris suum* (Nematoda) α-amylases (Zoltowska, 2001). Nevertheless, *P. americana* α-amylase was more activated by Ca^{2+} and K^{+}. There-

Table 3. Hydrolytic activity of *Periplaneta americana* α-amylase on maltooligosaccharides.

Substrate (10 mM)	Hydrolysate product (mM)			
	Maltose	**Maltotriose**	**Maltotetrose**	**Maltopentose**
Maltose	0	0	0	0
Maltotriose	0	0	0	0
Maltotetrose	0	0	0	0
Maltopentose	0.26	0.32	0	0
Maltohexose	0.88	1.38	3.51	0
Maltoheptose	0.82	1.94	5.50	2.07

Reaction is at 37°C, pH 5.6 and incubation time of 20 min.
Values given are the averages of at least three experiments.

fore, these ions can be added in buffers for reactions using this α-amylase for starches saccharification.

The hydrolysis of soluble starch by the purified α-amylase revealed maltose, maltodextrin and maltooligosaccharides and not glucose in the reaction medium (2 h of reaction). This result indicated that the amylolytic enzyme of *P. americana* is an α-amylase (an endogamylase). This indicates that the α-amylase of this insect can be used for starches saccharification without processing glucose. This pattern is similar to those reported for other amylases of mushroom *Scryt*al*idium thermophilium* (Aquino et al., 2003) and *Bacillus claussi* LT 21 (Duedahl-Olesen et al., 2000) but differs from other amylases of *Bacillus subtilis* (Matsuzaki et al., 1974) and *Bacillus staerothermophilus* (Kim et al., 1999). The purified α-amylase was also unable to hydrolyse maltose and maltodextrin to glucose (2 h of reaction) but was more active on the high molecular weight starch. This result was confirmed by the action of the purified α-amylase on maltooligosaccharides. This enzyme efficiently hydrolysed maltopentose, maltohexose and maltoheptose but not maltose, maltotriose and maltotetrose. This indicate that the purified α-amylase hydrolyses only substrates with more than four residues of glucose. We can still suppose that the hydrolysis of starch using *P. americana* α-amylase can produce essentially maltose, maltotriose, maltotetraose and dextrins in the reaction medium.

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