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Full Length Research Paper

Synergism of cockroach (*Periplaneta americana*) α-amylase and α-glucosidase hydrolysis of starches

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Cockroach *Periplaneta americana* α-amylase hydrolysed starch to maltose and maltodextrin. However, this enzyme was not active against maltose and maltotriose (2 h of reaction). α-Glucosidase of the same organism exhibited activities toward maltose and maltodextrin to glucose. Synergism was observed especially between both enzymes to produce glucose for cockroach *P. americana* nutrition.

Key words: α-Amylase, α-glucosidase, cockroach, *Periplaneta americana,* synergism.

INTRODUCTION

Recently, we purified to homogeneity an amylase and α-glucosidase from cockroach, *Periplaneta americana* (unpublished). The α-glucosidase had optimal activity at pH 5.6 and 50°C. This enzyme strongly hydrolyses sucrose, maltose, maltodextrin and *p*NP-α-D-glucopyranoside. However, it was not active against stachyose, pullulan, xylan, cellulose, starch and *p*NP-α-D-glycoside. The yields in transglucosylation reactions at 37°C were very high and could attain 62% with sucrose or maltose as glucosyl donor, and with phenylethanol as glucosyl acceptor. In the presence of phenylethanol, the transglucosylation reaction to form neoglucoconjugates was optimum at pH 5.0.

As for amylase, the hydrolytic activity on starch reached a maximum at 50°C and in a pH 5.0. This enzyme readily hydrolysed soluble starch, amylopectin and amylose(Sigma). In the present paper, we report the synergism between amylase and α-glucosidase from cockroach *P. americana* to emphasize the role of symbiosis in starch degradation. For this study, we utilised starches of yam, potato, cocoyam, cassava and ginger as substrates.

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MATERIALS AND METHODS

Tubers and roots (yam, cassava, cocoyam, potato and ginger) of different botanical origins were obtained from the experimental farm of the Plant Genetic Departement of Abidjan Cocody University (Côte d'Ivoire) and from the National Institute of Agronomical Research (Bouake, Côte d'Ivoire).

Glucose, xylose, soluble starch and maltose were obtained from Sigma-Aldrich. All other chemicals and reagents used were of analytical grade.

Starch extraction

Starches from yam, cassava, cocoyam, potato and ginger were extracted using a modified version of the method of Banks and Greenwood (1975). Tubers or roots were peeled sliced steeped in 0.1% (w/v) sodium bisulphate solution. The slices were ground in a waring blender (Moulinex) and the paste recovered in 4% (w/v) sodium chloride solution and then sieved successively through 500,

250 and 100 µm sieves. The starches were alternatively decanted and washed at least for 4 times. The deposit obtained was spread on an aluminium foil and oven-dried at 45°C for 48 h.

Enzyme assays

Amylase and α-glucosidase were isolated and purified by Sephacryl S100 HR, DEAE Sepharose 4B and Phenyl-sepharose 4B column chromatographies. Each enzyme purified to homogeneity gave a single protein band (coumasse brillant blue) after polyacrylamide gel electrophoresis under non-denaturing conditions (not shown).

Amylase activity was measured at 37°C for 20 min in 0.6 ml of 100 mM acetate buffer (pH 5.0) containing 1% (w/v) of soluble starch (Sigma) and 0.58 U of amylase solutions. The reaction was halted by addition of 2,4-dinitro-salicilic reagent before estimating the reducing sugars released by the Bernfeld method (1951) .

α-Glucosidase activity was assayed by incubating 50 µl of purified enzyme solution with 75 µl of *p*NP-α-D-glucopyranoside (5 mM) and 125 µl of 100 mM acetate buffer (pH 5.0) 50 µl of purified enzyme solution with 75 µl of *p*NP-α-D-glucopyranoside (5 mM) and 125 µl of 100 mM acetate buffer (pH 5.0) at 37°C for 10 min. The reaction were stopped by adding 2 ml of sodium carbonate (1 M). The liberation of *p*-nitrophenoxide anion was followed by measuring the increase in absorbance at 420 nm.

The reference cell contained all reactants except the enzyme. The calibration cuves (*p*NP) were carried out under the same condition of pH and temperature. The unit (U) of activity was defined as the amount of enzyme that catalysed the hydrolysis of 1 µmol of *p*NP-α-D-glucopyranoside or soluble starch (Sigma) used as substrate per min at 37°C under the conditions described above.

Synergism involving amylase and α-glucosidase

Each dried starch (10 mg) was dissolved in 0.6 ml of 100 mM acetate buffer (pH 5.0) and heated for 10 min at 90°C under gentle stirring. After heating, starch dispersed was cooled at room temperature. Then, amylase and α-glucosidase were added. The volume was adjusted with 100 mM acetate buffer (pH 5.0) to have 0.6 ml. To measure only amylase activity, α-glucosidase not be added to the reactional medium. The mixture obtained in each case was incubated at 37°C and the quantities of glucose and maltose were performed at high-performance liquid chromatography (HPLC) by withdrawing aliquots (50 µg) which were heated at 90°C for 5 min. After filtration through a 0.45 µm hydrophilic Durapore membrane (Millipore), 20 µl was analysed quantitatively by HPLC at room temperature. Chromatographic separations of sugars (glucose and maltose generated by the hydrolysis) were performed on a Supelcosyl LC-NH_2 (5 µm) column (0.46 x 25 cm) from Supelco 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 (Kouame et al., 2001).

RESULTS AND DISCUSSION

In vitro various starches hydrolysis by P. americana amylase

Cockroach amylase was able to hydrolyse all the pure starches tested (Figures 1 and 2). The analysis of the hydrolysis products revealed maltose and malto-dextrin

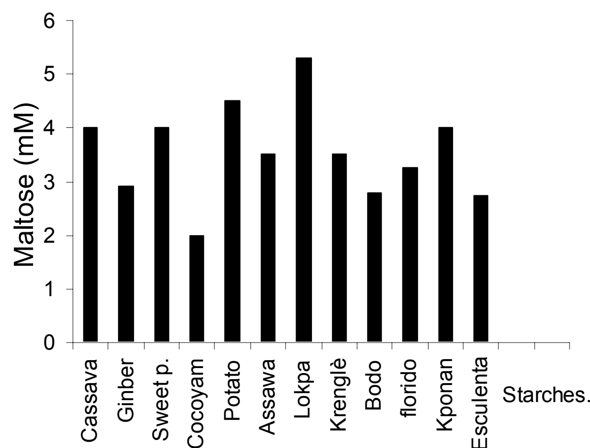

Figure 1: Degradation of various starches by cockroach amylase.

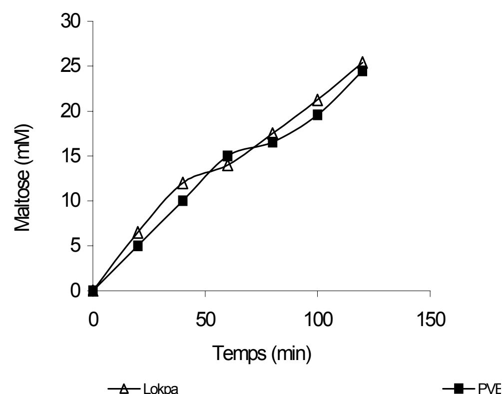

Figure 2: Time course of sweet potato (PVB) and Yam "Lopka" starches degradation.

and not glucose in the reaction medium (Figure 3). This result indicated that the amylolytic enzyme of cockroach *P. americana* may be an α-amylase (i.e an endo-amylase). This pattern is similar to those reported for other amylases of mushroom *Scrytalidium thermophilium* (Aquino et al., 2003) and *Bacillus claussi* LT 21 (Duedahl-Olesen et al., 2000) but differs from other amylase of *Bacillus subtilis* (Matsuzaki et al., 1974) and *Bacillus stearothermophilus* (Kim et al., 1999). We can still suppose that α-amylase was unable to hydrolyse maltose and maltodextrin to liberate glucose (2 h of reaction) but was more active on the high molecular weight starch. The results of the tests on pure starches treated with α-amylase were presented in Figure 1 showing clearly the lower resistance of yam, potato ginber and cassava to enzymatic degradation as compared to the resistance of the starch extracted to cocoyam. Yam "Lopka" starch had the lowest resistance indicating that there are no relation between the enzymatic susceptibility and the external surface area of the granules as described by several workers (Schwimmer, 1945 ; Leach and Schoch, 1961).

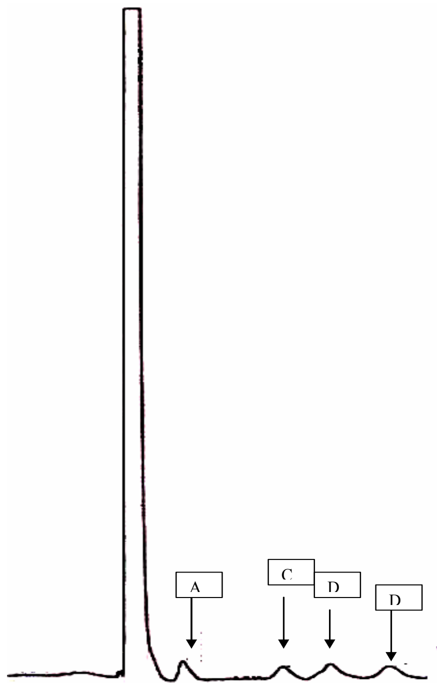

Figure 3. Chromatogram of sweet potato starch degradation products. Column connected: supercosyl LC-NH_2 5 µm (0.46 x 25 cm); mobile phase: water-acetonitrile (25/75, V/V); debit: 0.75 ml/min; peak A: xylose (internal standard); peak C: maltose; peak D: maltodextrin.

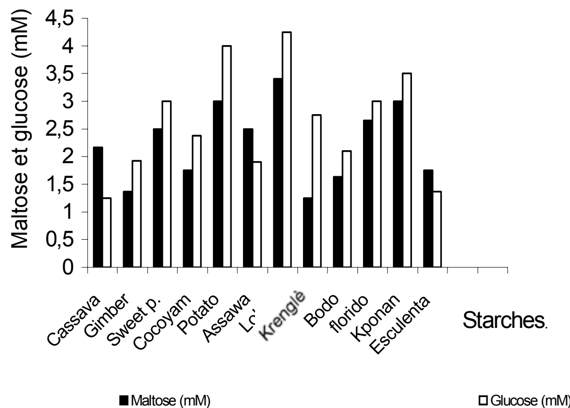

Figure 4 . Degradation of various starches by cockroach amylase and α-glucosidase. The experiments were performed with 0.58 U of amylase and 0.28 U of αglucosidase.

In vitro starches hydrolysis by mixed amylase and α-glucosida

When α-glucosidase and α-amylase were mixed in the same reactional medium, we noted the presence of glucose, maltose and maltodextrin (Figures 4, 5, 6, 7 and 8). The concentration of maltose in this medium was lower than that obtained when this reactional medium did not contain α-glucosidase (Figures 5, 7 and 8). These results showed that the maltose and maltodextrin released by α-amylase were hydrolysed by α-glucosidase. When we studied the influence of time on α-amylase and α-glucosidase, we observed that increase in reaction time leads to higher concentration of glucose and maltose. These results indicated that the combination of these two enzymes for syrup production of glucose and maltose is important because of the high concentrations of glucose and maltose obtained. The both enzymes cooperated to degrade starches to obtain glucose.

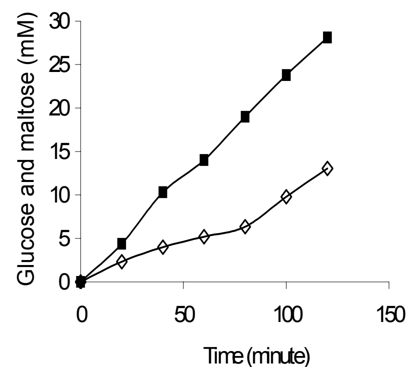

Figure 5. Time course of yam "Lopka" starch degradation by amylase and α-glucosidase. The experiments were performed with 0.58 U of amylase and 0.28 U of αglucosidase.

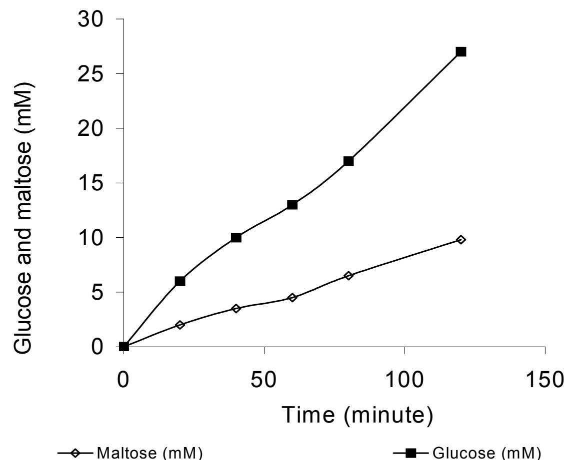

Figure 6. Time course of yam "Lopka" starch degradation by amylase and α-glucosidase. The experiments were performed with 0.58 U of amylase and 0.28 U of α-glucosidase.

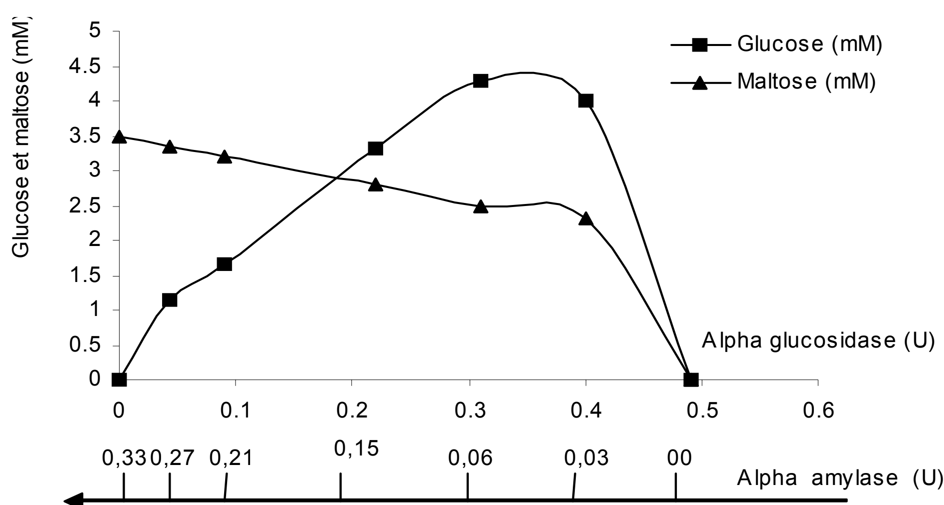

Figure 7. Influence of enzymatic units on sweet potato starch degradation by amylase and α-glucosidase.

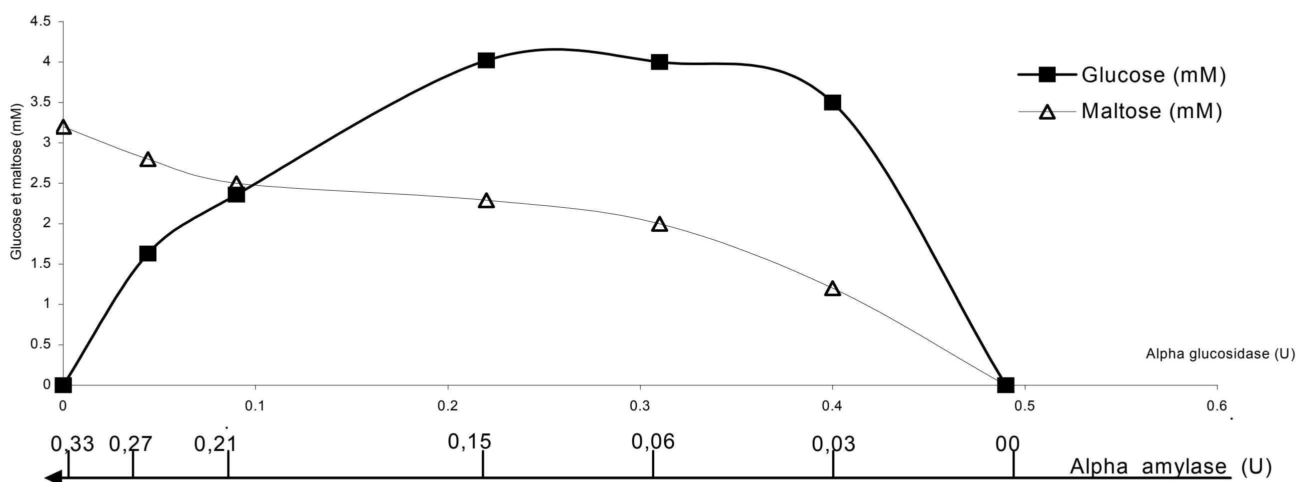

Figure 8. Influence of enzymatic units on yam "Lopka" starch degradation by α-amylase and α-glucosidase.

The maximum activity was achieved with a proportion of 0.3 U α-glucosidase and 0.06 U α-amylase (30:6).

Cockroach *P. americana*, α-amylase exhibited activities toward yam, potato, ginber, cocoyam and cassava starches. These hydrolysis liberated maltose and maltodextrin but not glucose. This enzyme was unable to cleave maltose and maltotriose. α-Glucosidase of the same insect hydrolysed maltose and maltodextrin to glucose.

These enzymes cooperated to degrade starches in order to liberate glucose to achieve an efficient insect nutrition.

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