Effect of Some Compounds on the α- Amylase Isoenzymes Activity purified from Abena-48 Wheat Flour Treated by Tribolium Confusum

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الخلاصة

تم تنقيه أنزيم α - أميليز من طحين الخنطة إبينا - 48 المعامل بالحشرة Tribolium Confusum باستخدام المعاملة الحشرارية، الترسيب بكبريتات الأمونيوم، DEAE سيلسولوز، ثم الفرز الغشائي وكروماتروكرايمنيا التبادل الإيوني. تم فصل خمس متناطرات لأنزيم I، II، III، IV، V و 40 و 20 ميلليتر على التوالي، وفعالية نوعية مقدارها 105120، 97333، 67384، 7503 وحدة إنجازية / ملم بروتين على التوالي مقارنة بالأنزيم الخام.

تم دراسة تأثير بعض المشتقات والمنتجات على فعالية متناطرات - أميليز I، II، III، IV، V، أظهرت بعض المركبات تأثيرات تثبيطية واضحة على فعالية المتناطرات الإنجازية، فقد أظهر فينيل هايبرازين تأثيراً تثبيطياً واضحاً تراوح بين (66.7 - 77.8) %، أما أظهر تأثيراً تثبيطاً تراوح بين (29.6 - 96.5) %، في حين تراوح التأثير التشريطي لحامض الهيدروكسيكربونك (29.6 - 96.5) %، واظهرت أيونات المعادن الثقيلة تأثيرات تثبيطية مختلفة على فعالية متناطرات - أميليز، حيث تراوح التأثير التشريطي لـ α بين (4.8 - 8.5) و (46.1 - 57.6) %، في حين تراوح التأثير التشريطي لـ Cu²⁺، Ca²⁺، Fe²⁺، Cl⁻ بين (85.2 - 37.2) و (91.1 - 31.0) %.

أظهرت تأثيرات تثبيطية على فعالية متناطرات - أميليز أيويد، وأيوديد بيروكسيدايد (I₂ - IO₃⁻) ، ومتانتيترات H₂O₂ تثبيطها تراوح بين (8.5 - 46.1) %، في حين تراوح التأثير التشريطي لـ Cu²⁺، Ca²⁺، Fe²⁺، Cl⁻ بين (85.2 - 37.2) و (91.1 - 31.0) %.

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ABSTRACT

α- amylase was purified from Abena - 48 wheat flour treated by Tribolium confusum using heat treatment, ammonium sulfate precipitation (NH4)2SO4, dialysis and anion exchanger chromatography DEAE-cellulose. Five isoenzymes were obtained I, II, III, IV & V with elution volume of 30, 30, 40, 40, & 20 ml respectively, and with specific activity of 105120, 97333, 67384, 75703 & 85800 μm/mg protein respectively compared with the crude enzyme. Effects of some inhibitors and activators on the α- amylase isoenzymes activity were studied. Phenyl hydrazine showed an inhibitory effect between (66.7-77.8)%, EDTA showed an inhibitory effect between (29.6-96.5)%, while the hydrochloric acid showed and inhibitory effect between (49.6-70.0)%. Heavy metal ions showed a different inhibitory effects on the α- amylase isoenzymes activity Cu^{2+} showed an inhibitory effect between (8.5-46.1)%, while Fe^{2+} showed an inhibitory effect between (4.8-57.6)%. No inhibitory effect was shown for iodoacetamide on the α- amylase isoenzymes activity, and insignificant inhibitory effect was shown with Triglycerate.

Calcium and chloride ions showed activitory effects on the α- amylase isoenzymes activity I, II, III, IV & V. Ca^{2+} showed an activitory effect between (37.2-85.2)%, while Cl showed an activitory effect between (31.0-91.1)%.

INTRODUCTION

The α- amylase (EC: 3.2.1.1.) is one of the hydrolytic enzymes, it has ability to split polysaccharides α-l- 4 linkage and form dextrins, maltose and glucose molecules (1). α- amylase is wide distribution in plants and animals (2), wheat and wheat flour α- amylase is an important enzyme in affecting the quality of wheat (3). α- amylase activity increases as a result of the insects which attacked the stored grains and its product and then cause a high damage of the commercial value (4). Tribolium confusum is one of the important insects that increased the α- amylase activity (5). Special peptides and proteins are used as α- amylase inhibitors. Tendamistat is a proteinaceous inhibitor of α- amylase activity (6,7). Cyclic hexapeptides and cyclic tetrapeptides are another types of α- amylase inhibitors (8), because of the difficulties to obtain such compounds so we study the effect of other compounds that affected α- amylase activity.
MATERIALS AND METHODS

Abena-48 wheat flour obtained from Mosul-flour factory. The adult *Tribolium confusum* had been taken from Mosul-flour factory and incubated at specific conditions of 27°C and 70% humidity for 4 weeks.

**Assay of α- amylase:**

α-amylase activity of the extracts was determined by dinitro- salicylic acid method of Bendelow. The standard curve of maltose was determined by Nelson's colorimetric method of Bendelow (9) using aeries concentrations of maltose. The unit of the activity is (mu) which defined as the number of micromoles of α(1→4) glycosidic bonds hydrolyzed per minute.

**Protein determination:**

Protein in α-amylase extracts and isoenzymes were determined by the modified lowry method (10).

**Purification of α- amylase:**

α-amylase has been extracted and purified as described in (11) with some modification. Sixty grams of treated wheat flour was stirred with 125ml of 0.05M calcium acetate buffer containing 0.1M calcium chloride for two hours at 4°C. The slurry was centrifuged at 9500g for 10 min., the supernatant was filtrated, and heated at 60°C for 15 min at pH 6.6 to inactivate β-amylase, then cooled in an ice bath, and centrifuged at 5000g. The filtrate dialyzed overnight against 0.2% calcium acetate. The dialysate fraction was loaded on DEAE-Cellulose column (2.5 x 40cm) with 0.05M calcium acetate buffer, 10ml fraction collected every 10 min.

**α-amylase effectors:**

Phenyl hydrazine, EDTA, HCl, CuSO₄, FeSO₄, Iodoacetamide, Tristearin, Cl⁻ and Ca²⁺, each at 0.6 g/l concentration, were used for inhibition and activation studies. α-Amylase isoenzymes I,II,III,IV & V were preincubated with one or other of these inhibitors or activators for 30 min. at 37°C using the starch as substrate. The enzymatic activity was assayed using Bendelow method (9).

RESULTS

**α-Amylase purification:**

The results in table (1) showed that the specific activity of crude α-amylase in wheat flour treated with *Tribolium confusum* was 21994mu/mg protein, and the activity after heat treatment was
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27186mu/mg protein. Fig. (1) showed the elution profile obtained by purification of α- amylase from abena -48 wheat flour treated with insect using DEAE-Cellulose. Five peaks were obtained, I, II, III, IV & V with elution volume of (30-50), (70-90),(120-150),(170-200) and (220-230) ml respectively, and with respective specific activity of 105120, 97333, 67384, 75703 and 85800 mu/mg protein. The purification folds were 4.78, 4.43, 3.06, 3.44 and 3.90 respectively compared with the crude enzyme (table 1).

Effect of inhibitors:

Inhibitors in table (2), each with 0.6 g/l concentration, showed a different effects on the α- amylase isoenzymes I, II, III, IV & V. Phenyl hydrazine inhibited the isoenzymes activity by 73.5%, 75.7% 69.1%, 66.7%, and 77.8% respectively. EDTA inhibited the isoenzymes activity by 57.6, 96.5, 33.5, 53.7% and 29.6% respectively. HCl showed an inhibitory effect of 70.0%, 62.3%, 49.6%, 54.4% and 64.2% respectively. The heavy metal Cu\textsuperscript{2+} showed an inhibitory effect of 35.7%, 31.5%, 8.4%, 46.1% and 29.6% for isoenzymes I, II, III, IV&V respectively, (table 2). while Fe\textsuperscript{2+} inhibited the isoenzymes activity I, II, III, IV, V by 57.6%, 37.7%, 4.8%, 38.9% and 49.0% respectively.

On the other hand, Iodoacetamide and Triglycerate showed a slightly inhibitory effect on the isoenzymes activity. Iodoacetamide inhibited the isoenzymes I, IV&V by 7.2%, 23.5%, and 3.5% respectively, while had no effect on the II&III isoenzymes activity Triglycerate inhibited the isoenzymes I, II, III, IV, & V by 7.6%, 19.2%, 4.8%, 12.6% and 26.9% respectively (table 2).

Effect of activators:

The results in table (3) showed that chloride ion activated the α- amylase isoenzymes I, II, III, IV, & V by 50.3%, 56.9%, 91.1%, 31.0% and 75.4% respectively, while calcium ion showed an activitory effect of 56.6%,37.2%, 85.2%, 60.6% and 58.8% respectively.

DISCUSSION

There were evidences that α- amylase had a high activity in abena wheat flour (12,13) and Tribolium Confusum cause an increasing in the α- amylase activity of abena - zero wheat flour (5) Now α- amylase activity was purified from abena -48 using extraction, heat treatment ammonium sulphate precipitation, dialysis, and ion exchange chromatography. In crude extract, α- amylase activity was 21994μu/mg protein (table 1). α- amylase was stable to heat, and heat treatment step was advantageous in removing amounts of contaminating proteins such as β-amylase and
Figure (1): Elution profile of α-amylase alpha-48 wheat flour treated with Triplolium confusum DEAD.
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<td><img src="image-url" alt="Table of Effect of Some Inhibitors on the α-Amylase Isoenzymes Treated with Insect" /></td>
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**Table (2):** Effect of some inhibitors on the α-Amylase Isoenzymes Treated with Insect
<table>
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<th>Effector %</th>
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Table (3): Effect of some activators on the alpha-4-g-amylase isoamylase activity with inscet
REFERENCES