

## Effect of Some Compounds on the $\alpha$ - Amylase Isoenzymes Activity purified from Abena-48 Wheat Flour Treated by *Tribolium Confusum*

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Received  
24/3/2004

Accepted  
15/12/2004

### الخلاصة

تم تنقية أنزيم  $\alpha$  - أميليز من طحين الحنطة ابينا - 48 المعامل بالحشرة *Tribolium Confusum* باستخدام المعاملة الحرارية ، الترسيب بكبريتات الأمونيوم ، الفرز الغشائي وكروماتوكرامينيا التبادل الأيوني DEAE سيليلوز. تم فصل خمس متناظرات للأنزيم I ، II ، III ، IV و V بحجم روغان بلغ 30 ، 30 ، 40 ، 40 و 20 مليلتر على التوالي، وبفعالية نوعية مقدارها 105120 ، 97333 ، 67384 ، 75703 ، 85800 وحدة إنزيمية / ملغم بروتين على التوالي مقارنة بالإنزيم الخام. تم دراسة تأثير بعض المثبطات والمنشطات على فعالية متناظرات  $\alpha$  - أميليز I ، II ، III ، IV و V ، أظهرت بعض المركبات تأثيرات تثبيطية واضحة على فعالية المتناظرات الإنزيمية ، فقد أظهر فينايل هايدرازين تأثيراً تثبيطياً واضحاً تراوح بين (66.7 - 77.8) % ، أما EDTA فقد أظهر تأثيراً تثبيطياً تراوح بين (29.6 - 96.5) % ، في حين تراوح التأثير التثبيطي لحمض الهيدروكلوريك بين (29.6 - 96.5) % . وظهرت أيونات المعادن الثقيلة تأثيرات تثبيطية مختلفة على فعالية متناظرات  $\alpha$  - أميليز ، حيث تراوح التأثير التثبيطي لـ  $\text{Cu}^{+2}$  بين (8.5 - 46.1) % ، في حين تراوح التأثير التثبيطي لـ  $\text{Fe}^{+2}$  بين (4.8 - 57.6) % . لم يلاحظ تأثيراً تثبيطياً للمركب أيودوأسيتاميد على فعالية المتناظرات الإنزيمية ، وكذلك لم يظهر المركب تراي كلبيسيريد تأثيراً تثبيطياً واضحاً على فعالية المتناظرات الإنزيمية .

أظهرت أيونات الكالسيوم والكلور تأثيرات تحفيزية على فعالية متناظرات  $\alpha$  - أميليز I ، II ، III ، IV و V ، فقد تراوح التأثير التحفيزي لـ  $\text{Ca}^{+2}$  بين (37.2 - 85.2) % بينما تراوح التأثير التحفيزي لـ  $\text{Cl}^-$  بين (31.0 - 91.1) % .

## ABSTRACT

$\alpha$ - amylase was purified from Abena - 48 wheat flour treated by *Tribolium confusum* using heat treatment, ammonium sulfate precipitation  $(\text{NH}_4)_2\text{SO}_4$ , 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  $\mu\text{u}/\text{mg}$  protein respectively compared with the crude enzyme. Effects of some inhibitors and activators on the  $\alpha$ - 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 an inhibitory effect between (49.6-70.0)%. Heavy metal ions showed a different inhibitory effects on the  $\alpha$ - amylase isoenzymes activity  $\text{Cu}^{+2}$  showed an inhibitory effect between (8.5-46.1)%, while  $\text{Fe}^{+2}$  showed an inhibitory effect between (4.8-57.6)%. No inhibitory effect was shown for iodoacetamide on the  $\alpha$ - amylase isoenzymes activity, and insignificant inhibitory effect was shown with Triglycerate.

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

## INTRODUCTION

The  $\alpha$ - amylase (EC: 3.2.1.1.) is one of the hydrolytic enzymes, it has ability to split polysaccharides  $\alpha$ -1 $\rightarrow$ 4 linkage and form dextrans, maltose and glucose molecules (1).  $\alpha$ - amylase is wide distribution in plants and animals (2), wheat and wheat flour  $\alpha$ - amylase is an important enzyme in affecting the quality of wheat (3).  $\alpha$ - 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  $\alpha$ - amylase activity (5). Special peptides and proteins are used as  $\alpha$ - amylase inhibitors. Tendamistat is a proteinaceous inhibitor of  $\alpha$ - amylase activity (6,7). Cyclic hexapeptides and cyclic tetrapeptides are another types of  $\alpha$ - amylase inhibitors (8), because of the difficulties to obtain such compounds so we study the effect of other compounds that affected  $\alpha$ - 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 27c° and 70% humidity for 4 weeks.

### Assay of $\alpha$ - amylase:

$\alpha$ -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 a series concentrations of maltose. The unit of the activity is ( $\mu$ ) which defined as the number of micromoles of  $\alpha(1 \rightarrow 4)$  glycosidic bonds hydrolyzed per minute.

### Protein determination:

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

### Purification of $\alpha$ - amylase:

$\alpha$ -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 4c°. The slurry was centrifuged at 9500g for 10 min., the supernatant was filtrated, and heated at 60c° for 15 min at pH 6.6 to inactivate  $\beta$ -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.

### $\alpha$ -amylase effectors:

Phenyl hydrazine, EDTA, HCl, CuSO<sub>4</sub>, FeSO<sub>4</sub>, Iodoacetamide, Tristearin, Cl<sup>-</sup> and Ca<sup>+2</sup>, each at 0.6 g/l concentration, were used for inhibition and activation studies.  $\alpha$ -Amylase isoenzymes I,II,III,IV&V were preincubated with one or other of these inhibitors or activators for 30 min. at 37c° using the starch as substrate. The enzymatic activity was assayed using Bendelow method (9).

## RESULTS

### $\alpha$ -Amylase purification:

The results in table (1) showed that the specific activity of crude  $\alpha$ -amylase in wheat flour treated with *Tribolium confusum* was 21994 $\mu$ /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  $\alpha$ -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  $\alpha$ -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  $\text{Cu}^{+2}$  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  $\text{Fe}^{+2}$  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  $\alpha$ -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  $\alpha$ -amylase had a high activity in abena wheat flour (12,13) and *Tribolium Confusum* cause an increasing in the  $\alpha$ -amylase activity of abena - zero wheat flour (5) Now  $\alpha$ -amylase activity was purified from abena -48 using extraction, heat treatment ammonium sulphate precipitation, dialysis, and ion exchange chromatography. In crude extract,  $\alpha$ -amylase activity was 21994mu/mg protein (table 1).  $\alpha$ -amylase was stable to heat, and heat treatment step was advantageous in removing amounts of contaminating proteins such as  $\beta$ -amylase and

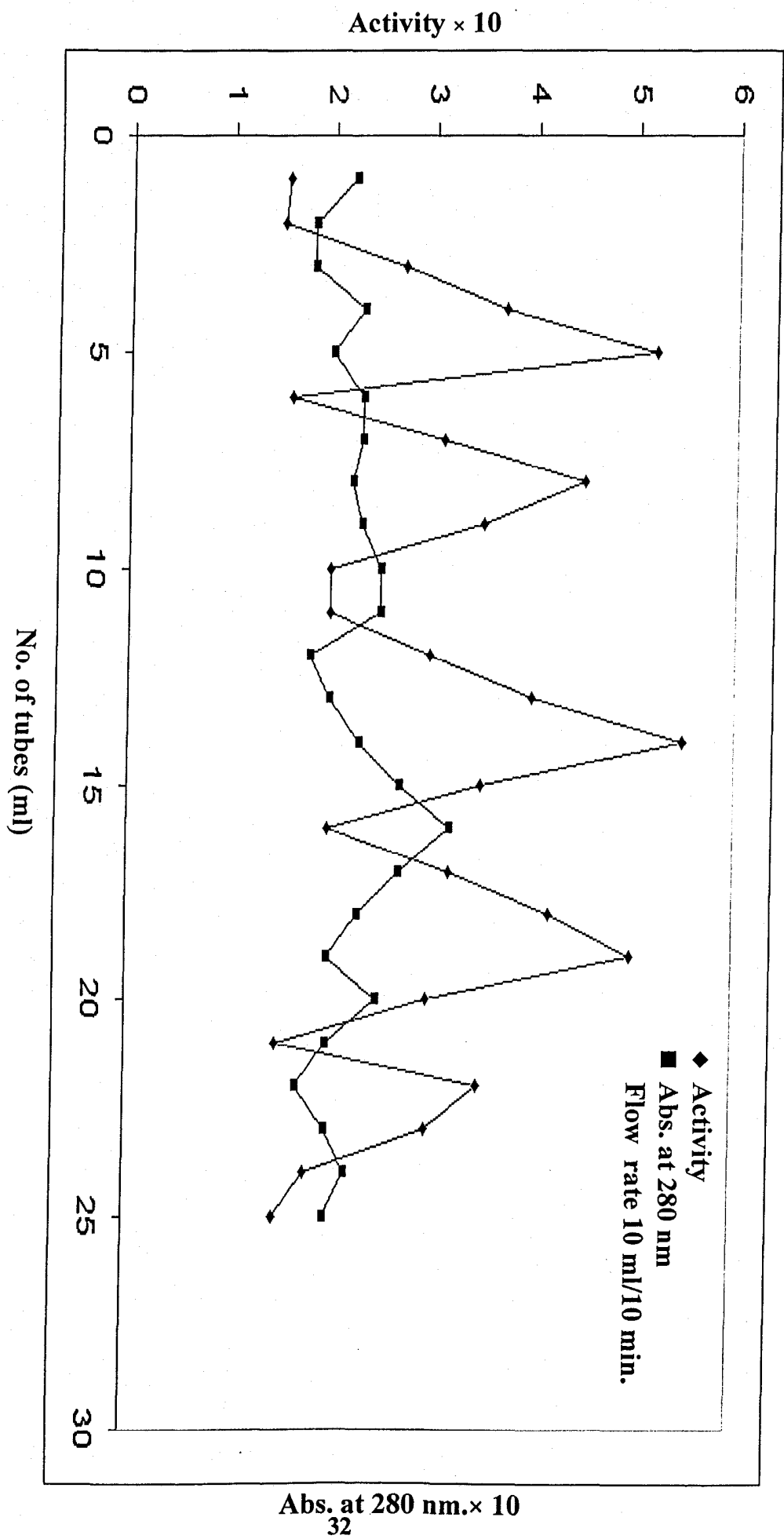


Figure (1): Elution profile of  $\alpha$ -amylase abena-48 wheat flour treated With *Tribolium Confusum* DEAD-Cellulose

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Table (2): Effect of some inhibitors on the abena-48  $\alpha$ -amylas Isoenzymes treated with insect

Inhibitor	PI		PII		PIII		PIV		PV	
	Activity m/ml	Inhibitory Effect%	Activity m/ml	Inhibitory Effect%	Activity m/ml	Inhibitory Effect%	Activity m/ml	Inhibitory Effect %	Activity m/ml	Inhibitory Effect%
Phenylhydrazine	4200	—	4061	—	3038	—	3305	—	4282	—
EDTA	1113	73.5	985	75.7	939	69.1	1100	66.7	950	77.8
Hcl	1780	57.6	139	96.5	2018	33.5	1530	53.7	3014	29.6
CuSo <sub>4</sub>	1252	70.0	1530	62.3	1530	49.6	1507	54.4	1530	64.2
FeSo <sub>4</sub>	2700	35.7	2780	31.5	2780	8.5	1780	46.1	3282	29.6
Iodoacetamide	1780	57.6	2527	37.7	2890	4.8	2018	38.9	2180	49.0
Triglycerate	3898	7.20	4061	Zero	3038	Zero	2890	12.6	4130	3.5
	3880	7.60	3282	19.2	2890	4.8	2527	23.5	3130	26.9

Table (3): Effect of some activators on the abena-48  $\alpha$ -amylase isoenzymes treated with insect

Activator 0.6 g/L	PI		PII		PIII		PIV		PV	
	Activity mu/ml	Activitory Effect %	Activity mu/ml	Activitory Effect %	Activity mu/ml	Activitory Effect %	Activity mu/ml	Activitory Effect %	Activity mu/ml	Activitory Effect %
—	3007	—	3096	—	2261	—	3105	—	3082	—
Cl	4522	50.3	4858	56.9	4322	91.1	4068	31.0	5406	75.4
Ca+2	4710	56.6	4250	37.2	4188	85.2	4989	60.6	4895	58.8

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