

Effects Of Some Compounds on The α -amylase Activity Purified From Abena-24 Wheat Flour Treated By *Tribolium confusum*

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الخلاصة

تم تنقية انزيم α - اميليز من طحين الحنطة ابينا -24 المعامل بالحشرة *Tribolium confusum* باستخدام المعاملة الحرارية ، الترسيب بكبريتات الامونيوم ، الفرز الغشائي وكروماتوكرافيا التبادل الايوني . تم فصل ثلاث متناظرات لانزيم α - اميليز هي على التوالي I ، II و III وبحجم روغان بلغ 90 ، 170 و 220 مليلتر ، وبفعالية نوعية بلغت 73000 ، 185818 و 462333 وحدة انزيمية / ملغم بروتين على التوالي مقارنة بالانزيم الخام.

اظهرت بعض المركبات كل منها بتركيز 0.6g/l تأثيرات مختلفة على فعالية متناظرات α - اميليز I ، II و III فقد اظهر فينيل هايدرازين تأثيراً تثبيطياً واضحاً تراوح بين (70-80%) ، بينما تراوح التأثير التثبيطي لـ EDTA بين (49-53.5%) ، في حين اظهر HCl تأثيراً تثبيطياً تراوح بين (46.4-67.5%). وعند دراسة تأثير ايونات المعادن الثقيلة لوحظ ان Cu^{+2} اظهر تأثيراً تثبيطياً تراوح بين (32.8-42.2%) بينما تراوح التأثير التثبيطي لـ Fe^{+2} بين (26.3-53.5%). من ناحية اخرى لم يلاحظ تأثيراً تثبيطياً واضحاً للمتناظرات الانزيمية بوجود ابودواسيتاميد وتراي كلسيريد (Tristearin). اظهرت ايونات الكلور وايونات الكالسيوم تأثيرات تحفيزية على فعالية المتناظرات الثلاث لانزيم α - اميليز تراوحت بين (27-45.4%) لايون الكلور وبين (15-82%) لايون الكالسيوم.

ABSTRACT

α -Amylase was purified from Abena-24 wheat flour treated by *Tribolium confusum* insect using heat treatment, ammonium sulphate precipitation, dialysis, and anion exchange chromatography.

Three α -amylase isoenzymes were isolated (I, II and III) with elution volume of 90, 170 and 220 ml respectively, with specific activity of 73000, 185818 and 462333 mu/mg protein, respectively compared

with the crude enzyme. Many compounds showed different effects on the α -amylase isoenzymes activity. Phenyl hydrazine, EDTA and HCl showed an inhibitory effect between (70-80%), (49-53.5%) and (46.4-67.5%) respectively. Using heavy metals, it was observed that Cu^{+2} caused an inhibitory effect between (32.8-42.2%), while Fe^{+2} caused an inhibitory effect between (26.3-53.5%). On the other hand, no significant inhibitory effect was shown with iodoacetamide and tristearin. Chloride ion and calcium ion showed an activitory effects on the α -amylase isoenzymes I, II and III ranging between (27-45.4%) for Cl^- and (15-82%) for Ca^{+2} .

INTRODUCTION

α -Amylase (EC3.2.1.1.) is one of the hydrolytic enzymes that hydrolyses the dietary starch and glycogen into glucose and maltose (1). Wheat α -amylase is an important enzyme in affecting the quality of wheat (2). Wheat and wheat flour were attacked by insects, causing an increase in the α -amylase activity and a high damage of the commercial value (3). *Tribolium confusum* is one of the important insects that increased α -amylase activity (4). Many peptides and proteins are affect α -amylase activity. Tendamistat, is a proteinaceous inhibitor of α -amylase (5). Cyclic hexapeptides and cyclic tetrapeptides are used for inhibition α -amylase activity (6). Because of the difficulties to obtain and using such inhibitors, therefore we study the effect of other compounds that affect α -amylase activity.

MATERIALS AND METHODS

The adult (*Tribolium confusum*) had been taken from Mosul-flour factory and incubated at specific condition of (27°C) and (70%) humidity for 4 weeks. Abena-24 flour obtained from Mosul-flour factory and used as source of enzyme.

Assay of α -amylase :

α -amylase activity was determined using dinitro-salicylic acid method (7). The standard curve of maltose was determined by using a series of maltose concentration as standards.

The unit of α -amylase activity is (mu), 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 estimated by modified Lowry method (8).

Extraction and purification of α -amylase enzyme:

This has been done as described in (9) with some modifications. Sixty grams of affected wheat flour was stirred for two hours in 4°C, using 125ml of 0.05M calcium acetate buffer containing 0.1M CaCl₂. The slurry was centrifuged at 9500g for 10min. The supernatant was heated at 60°C for 15min at pH 6.6 then cooled in an ice bath (2-3°C). It is centrifuged at 5000g. The filtrate, then dialyzed over night against 0.2% calcium acetate. The dialysate fraction was loaded on diethyl amino ethyle cellulose (DEAE-cellulose) column (2×50cm) with (0.05M) calcium acetate buffer, 5ml fraction was collected every 5min.

α -Amylase effectors:

Compounds in table (2) and table (3), each with concentration of 0.6g/L, were used to determine their effects on α -amylase isoenzymes activity. The isoenzymes I, II and III were preincubated with each of these compounds for 30min at 37°C using starch as substrat then the activity was assayed using Bendelow method (7).

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 22177mu/mg protein. The activity after heat treatment was 29879mu/mg protein. Fig. (1) showed the elution profile obtained by purification of α -amylase using DEAE-cellulose. Three peaks were obtained (I, II and III) with elution volums of (90), (170) and (220)ml respectively, and with specific activity of 73000, 185818, and 462333 mu/mg protein respectively. The purification folds were 3.3, 8.4 and 20.8 respectively compared with the crude enzyme, table (1).

α -Amylase inhibition:

Examination of the effect of some compounds, each at a concentration of 0.6g/L showed that phenyl hydrazine, EDTA, and HCl had a significant inhibitory effects on the activity of α -amylase isoenzymes I, II and III. Phenyl hydrazine inhibited the enzymatic activity by 70%, 73.3% and 79.5% respectively, table (2). EDTA inhibited the activity by 53.5%, 49.0% and 50.7% respectively, while HCl showed an inhibitory effect by 46.4%, 64.0% and 67.0% respectively.

Heavy metal ions showed different inhibitory effects. The ion Cu⁺² inhibited the activity of α -amylase isoenzymes I, II and III by 3.6%,

25.4% and 37.3% respectively, while Fe^{+2} showed an inhibitory effect by 53.5, 26.3% and 48.5% respectively.

Iodoacetamide inhibited the activity of I and II isoenzymes by 9.0% and 15% but had no effect on the III isoenzyme activity, while tristearin showed an inhibitory effect of 3.6% , 15% and 7.3% to each of I, II and III isoenzymes, table (2).

α -Amylase activation:

Table (3) showed that Cl^- ion activated the α -amylase isoenzymes I, II, and III by 45.4%, 38.0% and 27.4% respectively. Ca^{+2} ion activated I, II, and III isoenzymes by 58.8% ,82.3%, and 15% respectively , table (3).

DISCUSSION

α -Amylase isolated from wheat flour had a high activity (10). *Tribolium confusum* increased wheat flour α -amylase activity (4). The properties of α -amylase enzyme in abena-24 wheat flour was studied (11). In this study the effects of some compounds were studied. Phenyl hydrazine showed a significant inhibitory effect on the isoenzymes activity, indicating that carbonyl group were necessary to maintain the catalytic reactivity of the isoenzymes(12). A metal chelator EDTA, also showed a significant inhibitory effect, this indicated that the cations such as Cu^{+2} and Mg^{+2} if present does participate in the isoenzymes activity (13). HCl treatment caused a significantly reduction in the isoenzymes activity because of the modification of the substrate by the acid treatment and not with a failure to inactivate α -amylase activity (14). The heavy metal ions Cu^{+2} and Fe^{+2} had a different inhibitory effects on the isoenzymes activity table (2). This was attributed to the heavy metal binding effects (15). The results were similar to those in immature hard red spring wheat α -amylase (16). On the other hand, iodoacetamide caused a slight inhibition on the isoenzymes activity, indicating that free sulfhydryl groups if present, it does not participate in the activity of the isoenzymes (15). This was similar to that of the immature hard red spring wheat α -amylase (16). Tristearin also had slightly inhibitory effect on the isoenzymes activity. The activity of α -amylase isoenzymes was increased with calcium and chloride ions table (3). It was reported that α -amylase was a metalloenzyme and its activity increase with Ca^{+2} and Cl^- (17).

Table (1): Partial purification of abena-24 α -amylase treated with insect

purification steps	total volume (ml)	enzyme activity (mu/ml)	protein (mg/ml)	specific activity (mu/mg)	purification fold	recovery (%)
Crude extract	13.5	17520	0.79	22177	1.0	100
Heat treatment	11	12848	0.43	29879	1.4	73.3
(NH ₄) ₂ SO ₄ ppt	16	14016	0.18	77867	3.5	80.0
Dialysis	14	7592	0.15	50613	2.3	43.3
Ion-exchange Chromatography						
Peak I	60	2920	0.040	73000	3.3	16.6
Peak II	20	4088	0.022	185181	8.4	23.3
Peak III	30	5548	0.012	462333	20.8	31.6

Table (2): Inhibition Effect of some compounds on the activity of abena-24 α -amylase isoenzymes treated with insect.

Inhibitor 0.6 g/L	Peak I		Peak II		Peak III	
	Activity mu/ml	Inhibitory effect (%)	Activity mu/ml	Inhibitory effect (%)	Activity mu/ml	Inhibitory effect (%)
.....	3247	3934	4815
Phenylhydrazine	974	70.0	1047	73.3	9817	79.5
EDTA	1509	53.5	2003	49.0	2373	50.7
Hcl	1739	46.4	1415	64.0	1565	67.5
CuSo ₄	2180	32.8	2665	32.2	2780	42.2
FeSo ₄	1509	53.5	2898	26.3	2480	48.5
Iodoacetamide	2955	9.0	3340	15.0	4815	Zero
Tristearin	3130	3.6	3340	15.0	4451	7.5

Table (3): Activation effect of some ions on the activity of abena-24 α -amylase isoenzymes treated with insect.

Activator 0.6g/L	Peak I		Peak II		Peak III	
	Activity mu/ml	Activatory effect (%)	Activity mu/ml	Activatory effect (%)	Activity mu/ml	Activatory effect (%)
.....	3247	3034	4015
Cl ⁻	4722	45.4	4189	38.0	5116	27.4
Ca ⁺²	5159	58.8	51534	82.3	4618	15.0

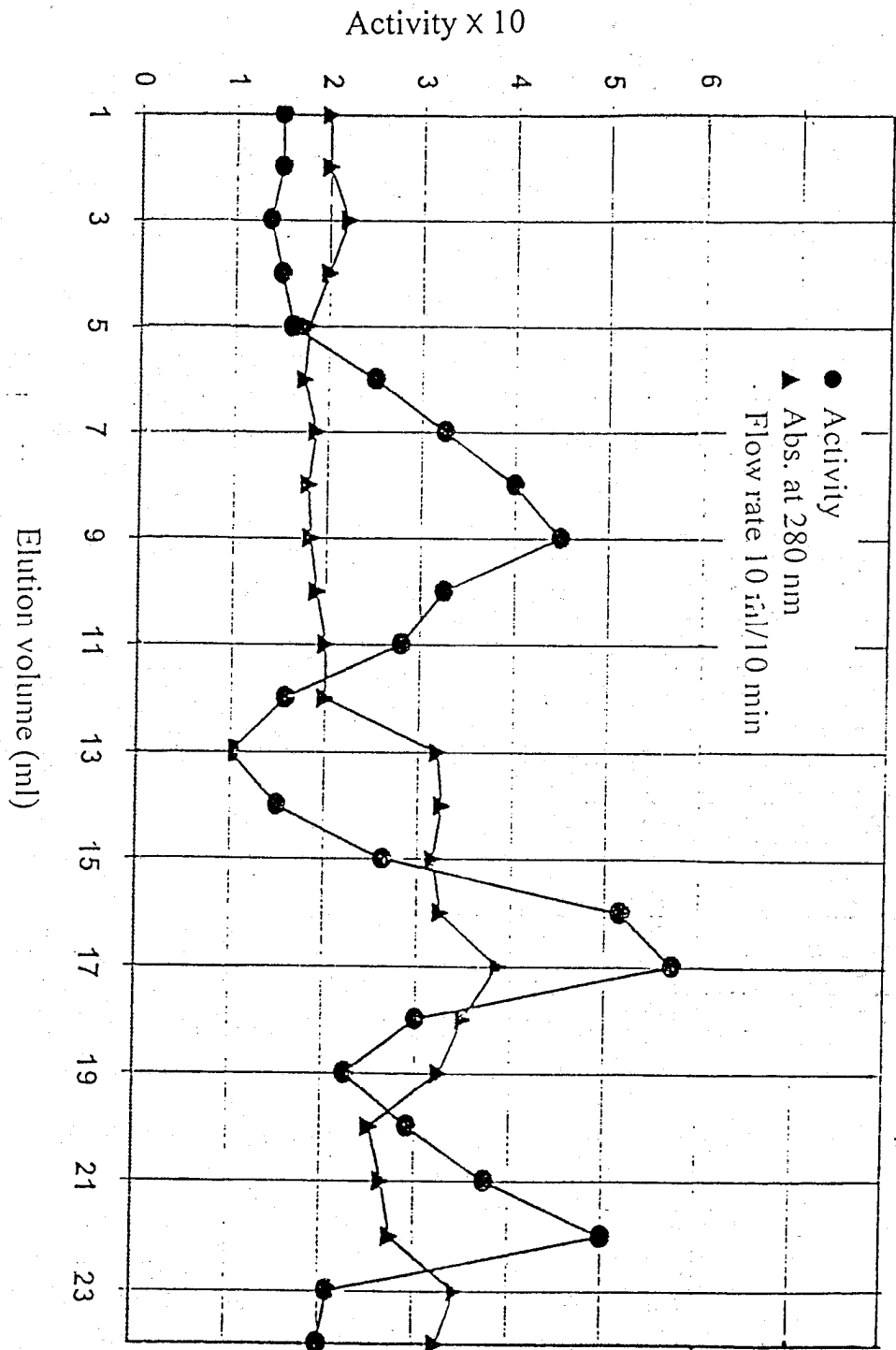


Figure (1): Elution profile of α -amylase abena-24 wheat flour treated with *Tribolium confusum* DEAD-Cellulose

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