J. Plant Production, Mansoura Univ., Vol. 2 (12): 1683 - 1691, 2011

ACTIVITY AND CHARACTERISTICS OF MALTASE FROM COTYLEDONS OF MARROWS (*Cucurbita pepo* L.)

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ABSTRACT

Maltase (EC: 3.2.1.20) was isolated from cotyledons of marrow *(Cucurbita pepo L.).* The enzyme was partially purified by 80% ammonium sulfate and Sephadex G-50 column chromatography. The optimal pH was 6.0. There are two pKa values at 6.0 and 8.0 indicating that there is histidyl residue taking part in enzyme catalysis. The optimal temperature of the enzyme was 50 ^{o}C. The activation energy of maltase was 8.0 KJ/mole. The effect of glucose, lactose, maltose, sucrose, starch and dextrin were tested and the enzyme expressed great specificity for maltose . The enzyme did not show any activity with glucose. The two adenosine compounds adenosine monophosphate (AMP) and adenosine triphosphate (ATP) stimulated maltase activity at both 5mM and 10mM and this may suggest that the maltase-catalyzed reaction is endothermic. Sorbitol, glycerol, sucrose and blue dextran stabilized maltase at 60 ^{o}C with different rates. Sorbitol was the best stabilizer followed by glycerol.

INTRODUCTION

Maltase which is *α*-Glucosidase (*α*-D-glucoside glucohydrolase; EC 3.2.1.20) hydrolyses the α-glycosidic bounds from the non-reducing end of oligosaccharides and polysaccharides with the release of glucose (Silva *et al.,* 2009).

α–Glucosidases form a group of exo-acting glycoside hydrolases with ample specificity , which release *α*-D-glucose from the non-reducing end of *α*-linked substrates (Frandsen and Svensson, 1998).

Glucosidases are widespread in nature, occurring in microorganisms, plants and animals and are classified into three types (Frandsen and Svensson, 1998). Type I hydrolyses sucrose and aryl glucosides better than maltose; type II prefers maltose and isomaltose and has low activity toward aryl glucosides; type III exhibits the same specificity of type II, but also attacks polysaccharides such as amylose and starch (Frandsen and Svensson, 1998). A number of *α*-glucosidases from fungi and from other sources also catalyse reaction of transglycosylation, producing *α*-glucosylated compounds (Kobayash *et al.,* 2003).

However, plant *α*-glucosidases have been reported to hydrolyze soluble starch effectively (Frandsen and Svensson, 1998) and to degrade starch granules present as insoluble polysaccharides in plant seed (Iwata *et al.,* 2003; Yamasaki *et al.,* 2005).

There is no available information about the activity and characteristics of maltase from marrow as C_3-plant. Therefore, the present research aimed to isolate, purify and characterize maltase from the cotyledons of marrow.

MATERIALS AND METHODS

Plant materials

The experimental plant used in this investigation was *Cucurbita pepo L.*(family: Cucurbitaceae). Pure strain of seeds was obtained from Egyptian Ministry of Agriculture.

Germination of seeds

Seeds were germinated and grown according to El-Shora and Ap Rees (1991).

Enzyme extraction

Twenty grams of collected cotyledons from 5-day old seedlings of marrow were homogenized in a blender with 40 ml of 0.1 M acetate buffer (pH 6.0) containing 1.5 mM EDTA disodium salt. The homogenate was filtered through four layers of muslin and centrifuged at 5000 rpm for 20 mins. The supernatant was filtered through a layer of absorbent cotton to get rid of fatty layer. The filtrate was used for biochemical assay of maltase and its purification.

Partial purification of maltase

The enzyme was partially purified by 80% ammonium sulfate and Sephadex G-50 column chromatography.

Enzyme assay

The enzyme was assayed according to Iwata *et al*., (2002).

Effect of pH and temperature on maltase activity

The effect of pH was carried out at pH range from 3 to 10 by using 0.1M acetate buffer (pH 6.0) containing 1.5 mM EDTA disodium salt and the effect of temperature was tested using various temperatures from range 20 to 70 $^{\circ}$C.

Effect of various substrates on maltase activity

Maltose, lactose , sucrose and glucose were tested as a substrate at 10 mM. Starch and dextrin were tested at 10% (w/v) on maltase activity.

Effect of adenosine monophosphate (AMP) and adenosine triphosphate (ATP) on maltase activity.

The effect of AMP and ATP on maltase activity was tested at 5 mM and 10 mM in the assay medium.

Stability of maltase

The stability of maltase was tested in presence of 1 mM sorbitol, 10% (v/v) glycerol, 1 mM sucrose and 1% (w/v) blue dextran.

RESULTS

Effect of pH on maltase activity

The results are illustrated graphically in **Fig. 1**. It appears from these results that maltase activity increased gradually from pH 2.0 to 6.0 after which it is declined. Therefore the optimum pH for maltase seems to be 6.0.

Determination of PKa value of maltase.

Plotting the relation between the pH of maltase and log V (Fig. 2) indicate that there were two pKa values at 6.0 and 8.0.

Effect of temperature on maltase activity

The activity of maltase was studied in a temperature range from 20 to 70 ^{o}C. The results are illusterated graphically in Fig. 3. The results showed that the optimal temperature for maltase was 50 ^{o}C.

Determination of activation energy

Plotting the logarithm of the reaction velocity log (V) for maltase versus the corresponding reciprocals of the absolute temperature (Fig. 4) resulted in straight line for the enzyme. This plot is called Arrhenius plot. The calculated activation energy was 8.0 KJ/mole.

Effect of different substrates on maltase activity

The activity of maltase was measured in the presence of different known substrates for the enzyme. These substrates are glucose, lactose, maltose, sucrose, starch and dextrin. These substrates were tested individually in the reaction mixture of maltase. The obtained results are shown in Fig. 5. These results show that maltose was the best substrate for maltase activity.

Effect of maltose concentration on maltase activity

Maltase was assayed at various concentrations of maltose. This was carried out at the optimal pH and optimal temperature of the enzyme. The results are shown in Fig. 6 which represent the relationship between the velocity (V) of the reaction and the substrate concentration (S). A considerable linear relationship was obtained at lower concentrations after which there was a steady increase in the activity at the higher concentrations.

Determination of V_{max} and K_m of maltase

Plotting the reciprocal of the reaction rate (V^{-1}) against the reciprocal of the concentration (S^{-1}) gave a straight line as shown in Fig. 7. From the plot the values of V_{max} and K_m were calculated and they were 175.4 U/mg protein and 2.2 mM, respectively.

Effect of adenosine monophosphate (AMP) and adenosine triphosphate (ATP) on maltase activity

The activity of maltase was measured in presence of either AMP or ATP at 5 mM and 10 mM. The results in Fig. 8 indicate that the two adenosine compounds stimulated the enzyme activity at tested concentrations. ATP was better stimulator than AMP.

Effect of some compounds on maltase stability

The effect of sorbitol, glycerol, sucrose and blue dextran on maltase stability was measured. The results in Fig. 9 show that blue dextran and sorbitol provided the enzyme with the highest stability compared with the other compounds.

DISCUSSION

The results in the present investigation indicate that the optimal pH value for maltase was 6.0. Other pH values such as pH 5 was reported for the enzyme from *Apis cerana indica* Chanchao *et al*. (2008). Furthermore, the results show that the pH curve of maltase is bell-shaped. Frequently, such curve reflects the deprotonization of an ionizable substrate or amino-acyl side chain in the enzyme. Usually, if the pH profile of the enzyme activity

appeared as bell-shaped, it means the presence of two important amino acid residues in the active site giving a narrow pH optimum. However, if appeared as a plateau it indicates one important amino acid residue in the active site (Price and Stevens, 1982).

Since the characteristics of ionizable side of chains of amino acids depend on pH, the enzyme activity usually varies with pH changes. At extreme pH, the tertiary structure of the protein may be disrupted and the protein is denatured. Even at moderate pH values where the tertiary structure is not disrupted the enzyme activity may depend on the degree of ionization of certain amino acid chains and the pH profile of an enzyme may suggest the identity of those residues (Palmer, 1985).

The enzyme expressed two PKa values 6.0 and 8.0. These values indicate the presence of histidyl group necessary for enzyme catalysis in the enzyme molecule (Bisswanger, 2002).

The optimum temperature of maltase was 50 ^{o}C. This optimal temperature is similar to that reported for maltase from *Apis cerana indica* (Chanchao *et al*., 2008). Increasing the temperature causes an increase in the inherent energy of the system and more molecules obtain the necessary activation of molecules for the reaction to take place. However, there comes a point where the increase of the reaction due to the effect of temperature on the activation of molecules is equal to decrease of reaction rate due to the destruction of tertiary structure Tanaka *et al.* (1998). At this point the activity is a maximum and this temperature is often known as the optimum temperature.

Generally, any chemical reaction whether exothermic or endothermic must overcome the activation energy in order to take place. It is known that the greater the activation energy, the more the heat which supports a successful reaction. An enzyme may lower the overall activation energy of the reaction by binding the reactants in the correct orientation for the reaction to take place (Whnn, 1984).

Under normal conditions, the native catalytically active structure of the enzyme is maintained by a delicate balance of different monovalent ionic forces e.g. hydrogen and hydrophobic interaction. Under an increase in temperature all of these forces except for hydrophobic interactions which are significant up to 60 ^{o}C diminish the protein macromolecule unfolds i.e. acquires a less ordered conformation.

Because active centers of the enzyme usually consist of several amino acid residues brought together only in the native three-dimensional structure of the enzyme such unfolding results in disassembling of the active center and hence is inactivated Aso *et al*. (2001).

The present results showed that maltase has a broad substrate specificity with significant activities for maltase. However, maltose was the best substrate. These results are in harmony with the finding of other workers (Iwata *et al.,* 2003; Chanchao *et al*., 2008).

The K_m and V_{max} values were 2.2 mM and 175.4 U/mg protein, respectively. Other K_m and V_{max} values of 6.8 mM and 120 U/mg protein were reported for the enzyme from sugar beet seed (Chiba *et al*., 1978).

In the present investigation AMP and ATP stimulated maltase activity at the examined concentrations . The stimulation of maltase by AMP and ATP may give the impression that maltase-catalyzed reaction is of endothermic type.

Sorbitol, blue dextran and glycerol stabilized maltase at 60 °C. Sorbitol was the potent stabilizer against inactivation at 60 °C. The thermostability effect of sorbitol and glycerol as polyols have the ability to maintain solvophobic interaction and have the capacity to form hydrogen bonds that play key role in supporting the native conformation of the protein and aid in protein stabilization (Lozano *et al*., 1994; Christiansen and Nielsen, 2002).

Glycerol protected maltase against thermoinactivation. It has been reported by George *et al.* (2001) that addition of small compounds to protein solution and changes its microenvironment provides a simple but practical means of increasing stability of the enzyme. It has been suggested that glycerol excercises its protective effect by inhibiting unfolding (Gekko and Timasheff, 1981). Also, glycerol is known to lead to the preferential hydration of several proteins and increase their transition temperature (Tiwari and Bhat, 2006).

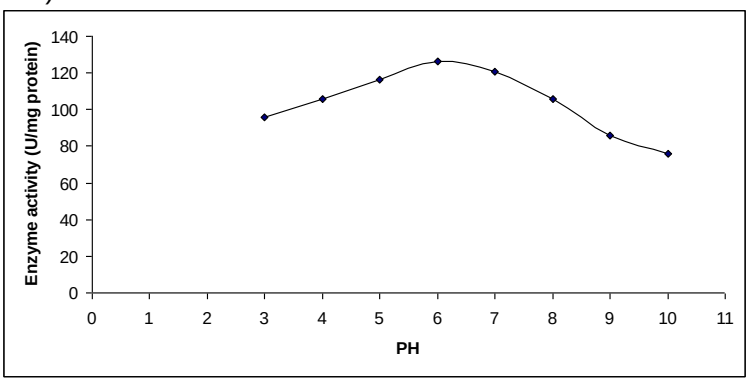

Figure 1: Effect of pH on maltase activity.

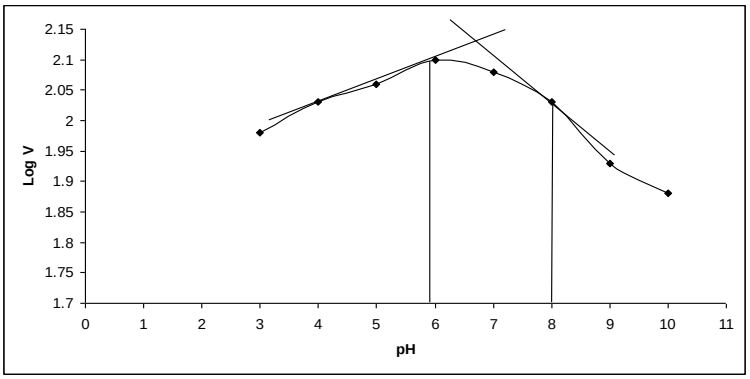

Figure 2: Determination of PK_a of maltase.

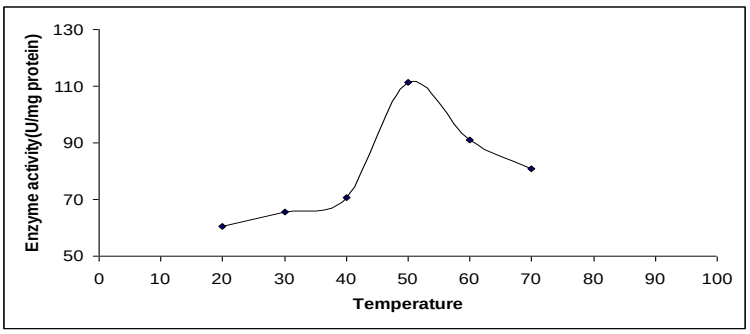

Figure 3: Effect of temperature on maltase activity.

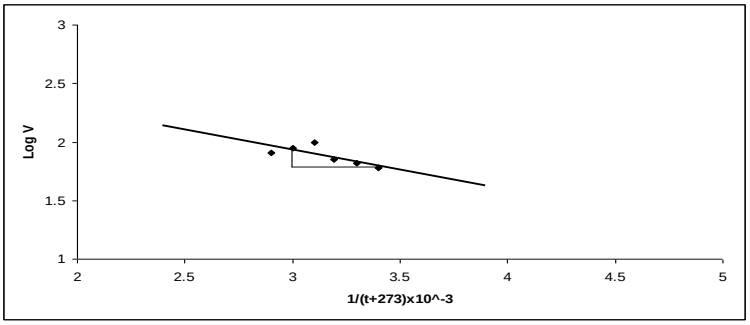

Figure 4: Arrhenius plot of maltase.

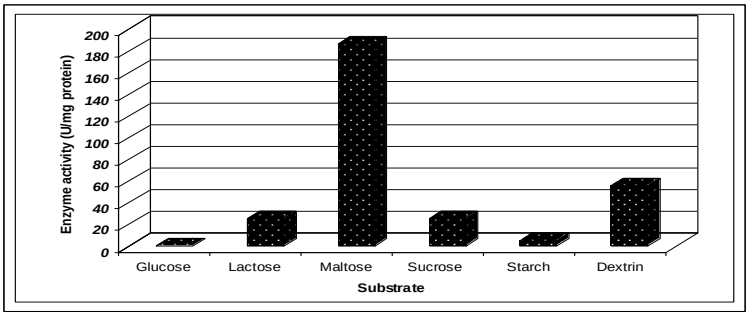

Figure 5: Substrate specificity of maltase.

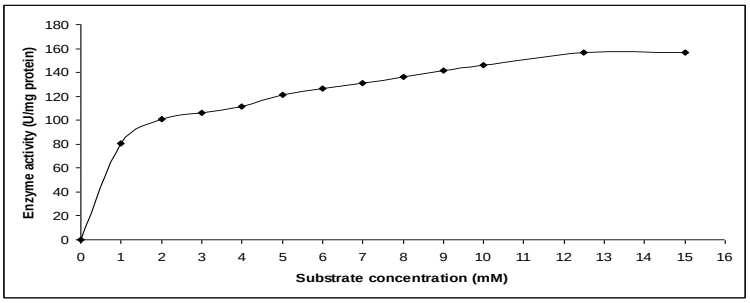

Figure 6: Effect of different concentrations of maltose.

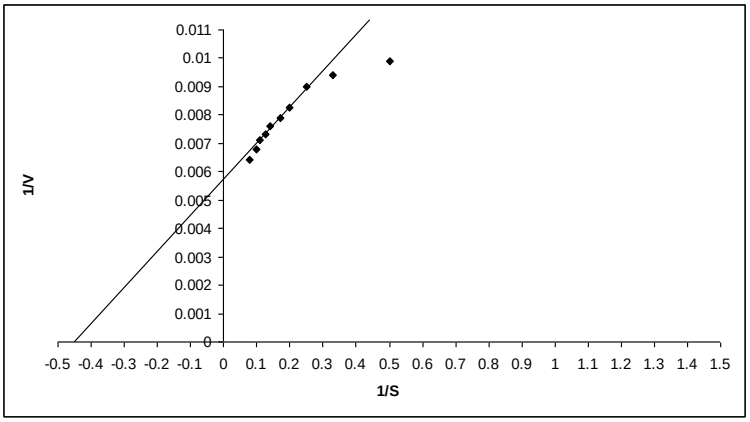

Figure 7: Determination of K_m and V_{max} .

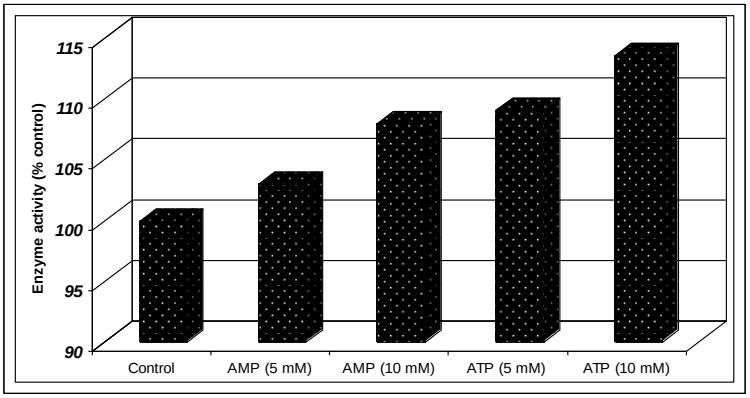

Figure 8: Effect of AMP and ATP on maltase activity.

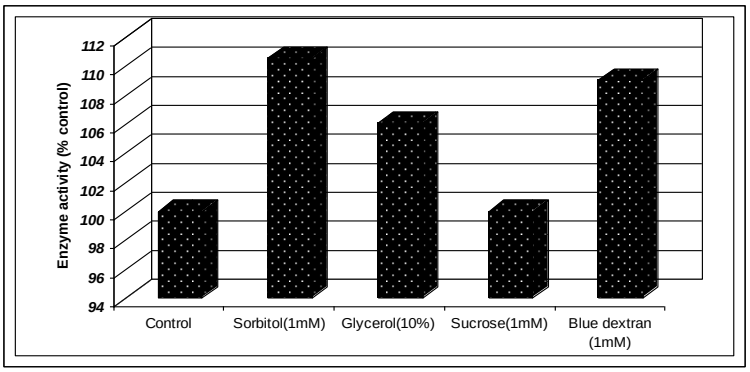

Figure 9: Effect of some compounds on maltase activity at 60 ℃ for 1 hr.

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نشاط وخصائص انزيم المالتيز من فلقات نبات القرع

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****قسم الكيمياء الحيوية بكلية علوم دمياط جامعة المنصورة**

تم عزل انزيم المالتيزمن فلقات نبات القرع .تم تنقية الانزيم جزئيا باستخدام ٨٠% كبريتات أمونيوم كان الرقم الهيدروجينى الأمثل للانزيم ٦ بينما درجة الحرارة المثلى ٥٠ درجة مئوية . G-50 . وسيفادكس

أثبتت الدراسة وجود مجموعة الهيستيديل كمجموعة أساسية لفاعلية الانزيم. تم حساب طاقة التنشيط لانزيم المالتيز ووجد ان قيمتها ٨ كيلو جول/مول. تم دراسة تأثيركل من الجلوكوز واللاكتوز والمالتوز والسكروز والنشا والديكسترين على النشاط الانزيمى ووجد ان المالتوز افضل هذه المواد لعمل الانزيم. اثبتت الدراسة أن الأدينوزين أحادى الفوسفات والأدينوزين ثلاثى الفوسفات قد نشطا الانزيم وكان الادينوزين ثلاثى الفوسفات أكثر فاعلية وخاصة عند تركيز١٠ ميللى مول .تم دراسة ثبات الانزيم عند درجة حرارة ٦٠ درجة مئوية باستخدام كل من السوربيتول والجليسرول والسكروز والديكستران الأزرق حيث كان السوربيتول أكثر هذه المواد فاعلية للثبات الحرارى للانزيم عند ٦٠ درجة مئوية.

قام بتحكيم البحث

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