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Properties of the Alpha Amylase from *Moringa oleifera* Seeds

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Abstract: Crude alpha amylase preparation from *Moringa oleifera* seeds was used to hydrolyze starch. The enzyme has maximum activity at pH 5.0 and stable in a pH range from 4.0 to 8.0. The optimum temperature for the enzyme was 40°C. The enzyme activity was found stable for 20 min. at 90°C. The stability of alpha amylase was increased in the presence of $CoCl_2$. Alpha amylase activity was increased in the presence of $MnCl_2$, $CoCl_2$, $ZnCl_2$ and $CaCl_2$ but decreased with ethylene diamine tetra-acetic acid (EDTA), copper sulphate and silver nitrate. However, glutathione did not show any significant effect on enzyme activity.

Key words: Alpha amylase, seeds, *Moringa oleifera*

Introduction

Historically maltose- producing amylases are divided into two types. First one is ß-amylase (1,4-α-D glucan maltohydrolase EC 3.2.2) which strictly hydrolyzed the α-1,4-glucosidic linkages in amylose, amylopectin and starch related 1,4-α-glucans with the release of ß-maltose from non-reducing ends (Robert and Whelan, 1968). ß-amylase enzyme is present in rye (Daussant *et al.*, 1991), barleys (Sopanen and Lauriere, 1989), maize (Lauriere *et al.*, 1992), wheat (Daussant and Lauriere, 1990), cereals (Daussant *et al.*, 1994), Soybean and Bacillus polymyxa (Fogarty and Griffin, 1975). Second, maltose producing amylase is α-amylase (1,4-α-D- glucan glucanohydrolase EC 3.2.1.1) which yields α-maltose by splitting the α-1,4-glucosidic linkages in starch and glycogen through endo-mechanism. A variety of α-amylases, mostly from microbial (Aguilar *et al.*, 2000, and Sawamy & Seenayya, 1996), mammalian (Qain *et al.*, 1995) and plant (Witt and Sauter, 1996, Songaard *et al.*, 1993 and Memon *et al.*, 1987) sources are well characterized. These enzymes belong to a large family of Ca^{2+}- Protein which have several structural features (Machius *et al.*, 1998 and Svensson,1994). The enzymatic mechanisms of starch degradation in several plant tissues led to the assumption that α-amylase play a major role in the attack on starch granules during germination and developing plant embryo (Rogers and Milliman, 1983). As part of continuing investigation of α-amylase level from the plant tubers, fruits and seeds, this experiment was carried out with a view to define more precisely the nature of the α-amylase in the seeds of *Moringa oleifera* (Sohanjna).

Materials and Methods

The seeds of *Moringa oleifera* were collected during May-June in local areas from University of Sindh Employees colony Jamshoro, Pakistan. Starch and 3,4-dinitrosalisylic acid (DNS) were obtained from E. Merck. All other materials and reagents were of analytical grade and solutions were made in double distilled water.

Preparation of soluble enzyme solution: Seeds of *Moringa oleifera* were defatted in diethyl ether at room temperature. 10.0 g of defatted residue were crushed in 33 ml cold 0.2M Phosphate buffer pH (6.9) containing 1.0% NaCl and centrifuged at 10,000 rpm for 20 min. The supernatant was transferred to 100 ml volumetric flask and this procedure was repeated twicely. The final volume was made upto mark with phosphate buffer. The protein content of enzyme solution was estimated by Bradford method (1976), using bovine serum albumin as standard and it was found to be 1.32 mg ml^{-1}.

Assay of α-amylase: Alpha amylase activity was determined by the method of Bernfeld (1955). Assay system contained 1.0 ml of enzyme solution and 1.0 ml of 1.0% soluble starch solution (pH 6.9 in phosphate buffer) was incubated at 27°C for 3 min. The reaction was terminated by the addition of 2.0 ml DNS (3,4-dinitro salicylic acid) reagent. Colour due to the reducing sugar liberated was developed by heating the reactants in a boiling water bath for 15 minutes and then rapidly cooled to room temperature. After addition of 20.0 ml of double distilled water, the extinction value was determined at 540 nm. Blank was prepared in the same manner (1.0 ml soluble starch was incubated at 27°C for 3 minutes) enzyme solution was added after the addition of DNS.
One unit of α-amylase activity was defined as the amount of enzyme required releasing 1.0 μ mol of reducing sugars.

Effect of pH on α-amylase activity: The optimal pH was determined assaying the enzyme at various pH (3.5 to 7.0) in sodium acetate and phosphate buffer.

Effect of temperature on α- amylase activity and stability: The optimum temperature of the enzyme activity was determined by assaying α-amylase at different temperatures ranging from 25 to 50°C. Thermal stability of α-amylase was investigated by incubating enzyme samples in a buffer (0.02M Sodium acetate, pH 5.0 with and with out 5mM $CoCl_2$) at various temperatures 40, 50, 60, 70, 80, 90 and 98°C for 10 min. Immediately afterwards the enzyme was immersed in ice bath and activity was tested under standard conditions. The stability of the enzyme was also checked by assaying the remaining activity of the enzyme samples in buffer (0.2M Sodium acetate buffer, pH 5.0) incubated at 50 and 60°C for 5 to 30 min.

Effect of various reagents on α-amylase activity: The relative activities of the α-amylase were determined after incubation of 5 mM divalent metal ions and various reagents with enzyme in sodium acetate buffer pH 5.0 for 10 min. at 40°C. Afterwards, α-amylase activity was tested under standard conditions.

Results and Discussion

The occurrence and some properties of α-amylase in the crude sample of *Moringa oleifera* seeds was investigated here. The studies at different level of substrate concentration ranging

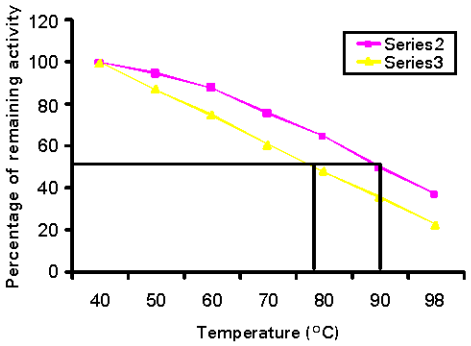

Fig. 7: Thermostability of *Moringa oleifera* seeds alpha amylase activity with (series 2) and with out Co^{2+} (series 3)

from 0.25 to 2.0% of soluble starch (Fig. 2). A positive correlation between substrate concentration and the degree of hydrolysis was observed.

The optimum temperature of α-amylase of *Moringa oleifera* seeds and the optimum temperature was (Fig. 3) found to be 40°C (9 minutes reaction). It thus has higher optimum temperature compared with many α-amylases (Koch *et al.*, 1991 and Chung *et al.*, 1995) but it is lower than sweet potato α-amylase (70°C) (Hagenimana *et al.*, 1992)

The effect of pH on the α-amylase activity was influenced by the kind of substrate and assay conditions. With starch, alpha amylase showed higher activity from pH 4.5 to 5.5 with maximum at 5.0 (Fig. 4). However, it would be considered that the enzyme has a broad pH range of activity.

Thermal stability of α-amylase was monitored by measuring the residual enzymatic activity, on incubation of enzyme for 5 to 30 minutes with out substrate at 50° C and 60°C and then enzyme activity was assayed by standard method (Fig. 5). The half life of alpha amylase of *Moringa oleifera* was noted 22 min. at 60°C and 28 minutes at 50°C.

The alpha amylase activity was measured at pH 5.0 and 40°C in the presence of different metal ions, EDTA and glutathione (5mM concentration)(Fig. 6). The alpha amylase activity of *Moringa oleifera* seeds was significantly increased in the presence of Co^{2+} but Zn^{2+}, Ca^{2+} and Mn^{2+} showed slightly increase in enzyme activity. Glutathione had no effect on α-amylase activity but EDTA, $AgNO_3$, $CuSO_4$ had strong inhibitory effect on α-amylase activity.

Thermal stability of α-amylase activity of *Moringa oleifera* seeds was also checked by monitored residual activity on incubation of enzyme for 10 min. in presence and absence of Co^{2+} (5mM) ions with out substrate at different temperatures. Thermal stability of α-amylase activity was increased and retains more than 35% activity in the presence of Co^{2+} (Fig. 7). It has been observed that the lower concentration of Co^{2+} ions are favourable and enhance enzyme activity whilst higher concentration of Co^{2+} ions inhibited enzyme activity. The increase in enzyme activity may be due binding of divalent cobalt cation on the surface of α-amylase globular macromolecule with greater flexibility decreasing T_m (Saboury *et al.*, 1998).

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