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Effect of modulators on activity of amylase isolated from *Hordeum vulgare*

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ABSTRACT

Amylases [1-6] are enzymes associated with the degradation of starch and are found to exist in different forms. (EC 3.2.1.1) (CAS# 9014-71-5) (Alternate names: 1, 4-α-D-glucan glucanohydrolase; glycogenase) The á-amylases are calcium metalloenzymes, completely unable to function in the absence of calcium. By acting at random locations along the starch chain, α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. The present study focused on the isolation of amylase from plant sources and further to study the effect of various modulators (Inhibitors and Activators) on the activity of the enzyme. Organic compounds and inorganic salts were considered to enumerate the affect of these substances on the activity of crude amylase.

Key words: Amylase, estimation, maltose, modulators, spectrophotometry.

INTRODUCTION

Enzymes are biomolecules that catalyze (*i.e.* increase the rates of) chemical reactions. Enzymes are known to catalyze about 4,000 biochemical reactions. A few RNA molecules called ribozymes catalyze reactions, with an important example being some parts of the ribosome. Synthetic molecules called artificial enzymes also display enzyme-like catalysis.

α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, α-amylase tends to be faster-acting than β-amylase. In animals, it is a major digestive enzyme and its optimum pH is 6.7-7.0. Effects of pH (Introduction to Enzymes) They are widely distributed in microbial, plant and animal kingdoms. The selection of substrate for enzyme production in a SSF process depends upon several factors.Substrates that have been used include sugarcane bagasses,wheat bran, rice bran cassava flour, wheat flour, corn flour, steamed rice, stream pre-treated willow and starch[10-19].

In the present study the authors after surveying the literature came to a conclusion that much work has been done on the isolation, Characterization [7-9], purification and assay of amylases, but very negligible work has been done on the study of modulators and prompted the author to study the effect of various organic and inorganic substances on the activity of amylase

MATERIAL AND METHODS

Barley seeds (Hordeum Vulgare), DNSA, enzyme amylase (0.1%)

EXPERIMENTAL

Amylase enzyme was isolated from Barley seeds (Hordeum Vulgare) using mortar pestle and phosphate buffer and then stored at 4ºC. Standardization by using Di nitro salicylic acid was done by drawing standard graph. Amylase activity in the various modulators with substrate by using the standard curve. Then determination of pure Amylase activity under the same modulators. Then comparison of the results obtained.

Determination of Amylase activity

Aliquots 0.2-2 mL of standard Maltose was transferred to a series of 10 ml graduated tubes. 1ml of crude enzyme was added and the mixture was incubated for 10-15 min in the dark. After 15 min 3 ml of coloring agent DNSA is added. All the tubes were incubated in water bath for 10-15 min at 100°C. The yellow colour developed was measured at 540 nm.

RESULTS AND DISCUSSION

Effect of NaCl on activity of Amylase

After performing the said experiments, to the enzyme sample, varying concentration of the inhibitor NaCl (5-25 W/V) was taken. The setup was incubated in the dark for 15min and 3ml of DNSA was added. Then incubate for 10-15 min in the water bath at 100°C and the activity of the enzyme was studied under specified and defined conditions. Enzyme activity is measured at 540nm.Maximum enzyme activity is shown at 5%, decreased till 20%. The results of analysis are presented in Table 1.

Table 1: Effect of NaCl on activity of Amylase

Conc. of NaCl(W/V)	O.D. (540 nm)	Conc. From standard graph (mg/ml)	IU/ml
Control	0.29	1.18	6.5555
5%	0.32	1.28	7.1111
10%	0.31	1.24	6.8888
15%	0.28	1.12	6.2222
20%	0.26	1.08	6.0000
25%	0.30	1.20	6.6666

Effect of Glycerol on activity of Amylase

To the enzyme sample, varying concentration of the inhibitor Glycerol (5-25 W/V) was taken. The setup was incubated in the dark for 15min and 3ml of DNSA was added. Then incubate for 10-15 min in the water bath at 100°C and the activity of the enzyme was studied under specified and defined conditions. Enzyme activity is measured at 540nm.Maximum enzyme activity is shown at 5%. The results of analysis are presented in Table 2.

Table 2: Effect of Glycerol on activity of Amylase

Conc. of NaCl(W/V)	O.D. (540 nm)	Conc. From standard graph (mg/ml)	IU/ml
Control	0.28	1.12	6.2222
5%	0.29	1.18	6.5555
10%	0.28	1.12	6.2222
15%	0.27	1.08	6.0000
20%	0.26	1.04	5.7777
25%	0.25	1.00	5.5555

Table 3: Activity of amylase in relation to Maltose standard curve

Conc. of Maltose(mg/ml)	O.D. (540 nm)	Enzyme Actvity IU/ml
0.2	0.04	0.8888
0.4	0.07	1.5555
0.6	0.13	2.8888
0.8	0.18	4.0000
1.0	0.22	4.8888
1.6	0.33	7.3333
2.0	0.40	8.8888
2.4	0.41	9.1111
2.8	0.39	8.5555
3.2	0.37	8.2222

CONCLUSION

Temperature fluctuations have significant effect on enzyme activity. Following conclusions are drawn for the enzyme activity and effect of inhibitors on enzyme activity for crude enzyme

source and pure enzyme. Glycerol showed maximum inhibition at 20% concentration for crude enzyme source, and for pure enzyme maximum inhibition is shown at 20%.

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