http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 1 (4): 366-367, 1998 Research Article

Isolation and Characterization of Acid Phosphatase from Wheat (Pirsabak-85) Grains (*Triticum aestivum*)

Naheed Ahmad Shah, Razia Khan, Mohammad Tariq and S.F. Mabood
Department of Chemistry, University of Peshawar, Pakistan

Abstract
Acid phosphatase (Acp., 3.1.3.2) from wheat grain powder was extracted with acetate buffer. Maximum extraction was carried out at pH 5.5. The activity of the extract stored *in vitro* at 4°C was quite stable for upto one week. The enzyme had optimum pH 5.5 and optimum temperature of 30°C. The K_m value was found to be 10 µM for P-introphenyl phosphate as a substrate.

Key words: Wheat grain, acid phosphatase, extraction, acetate buffer

Introduction
Acid phosphatase refers to a group of enzymes which catalyze the hydrolysis of certain monophosphoric esters, a reaction of considerable importance in several body processes, have been isolated from animals and plant tissues as well as from bacterial and fungal cells (Besford, 1979; Herman and Liss, 1963; Fatima *et al*., 1981; Srivastava and Gupta, 1986). Acid phosphatases exhibit optimum activity at pH 4.2-7.4.
Higher acid phosphatase in different wheat varieties are recognized as cold resistance. Acid phosphatase activity is also used in plant breeding as an indicator of cold resistance (Hagima, 1984). So far as we know the detailed kinetics of the enzyme for plant tissues have not been studied as yet, so a study of acid phosphatase isolation and characterization was undertaken.

Materials and Methods
Preparation of the Enzyme: The grains of wheat (Pirsabak-85) were cleaned and ground to a fine powder. The crude enzyme was extracted by shaking powder in 0.1 M acetate buffer (1 g/10 ml) of known pH for 1 h at 30°C. The mixture was then centrifuged at 5000 rpm for 5 min. The supernatant thus obtained was used for further work.

Estimation of Enzyme Activity: Acid phosphatase activity was determined by method of Gutman and Gutman (1940) using P-nitrophenyl phosphate as substrate. P-nitrophenyl phosphate, as the result of hydrolysis was converted into phenolate ions (Yellow Colour) at alkaline pH. The yellow colour was read at 405 nm. 900 µl of ffie substrate (0.1 M) was incubated for 5 min at 30°C. Then 100 µl of enzyme was added to it and mixed. The mixture was further incubated for exactly 5 minutes. The reaction was stopped by adding 4 ml of sodium hydroxide (0.1 M). The yellow product of P-nitrophenolate anions formed was read at 405 nm against the blank, which was prepared by adding 4 ml sodium hydroxide and 100 µl buffer to incubated substrate (900 µl). To increase, precision we recorded the transmittance and then converted it to absorbance according to the formula:

$$A = 2\text{-Log } \% T$$

The activity was expressed as milli mole of substrate hydrolyzed per minutes.

Results and Discussion
Effect of pH on the Activity: Acid phosphatase was extracted from wheat grains at different pH values. The results are given in Fig. 1. which suggest that maximum extraction of the enzyme is achieved at pH 5.5. However, for maize the maximum activity of the enzyme was released at pH 8.5 (Fatima *et al*., 1981). Since these workers used a different extraction medium of different ionic strength, the release of the enzyme seems to depend on the nature as well as on concentration of the medium or on its binding in the tissue. Consequently, a pH 5.5 was used thought, both for extraction as well as for estimating enzyme activity.

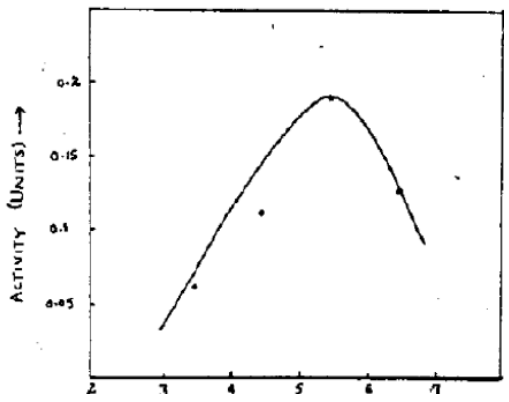

Fig. 1: The effect of pH on activity

Effect of temperature on Enzyme Activity: The enzyme activity was determined at different temperatures varying from 20-70°C. Fig. 2 shows that the enzyme has maximum activity at 30°C. Above this temperature, the activity was decreased sharply which may be due to heat denaturation of the enzyme.

Effect of incubation time on the enzyme activity: Acid phosphatase activity was determined after different incubation times. The results are shown in Fig. 3 which indicate that activity was linear function of time during at least 10 min. After this, activity increased but not proportionately. The deviation may be due to product inhibition.

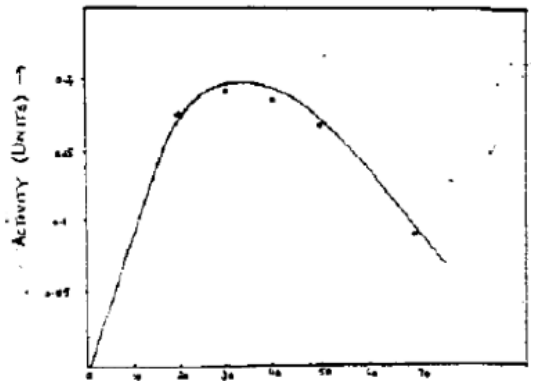

Fig. 2: The effect of temperature on activity

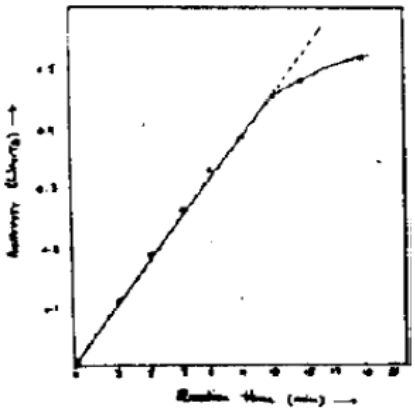

Fig. 3: The effect of incubation time on activity

Effect of substrate concentration on the enzyme activity: The effect of substrate concentration on enzyme activity was studied with P-nitropenyl phosphate as substrate using concentration between 10 pM and 400 pM. With increase in concentration of the substrate the enzyme activity increased for upto 200 pM. Further increase of substrarte concentration slightly decreased the enzyme activity, showing that enzyme was inhibited. The Michaelis-Menten constant Iq (Webb 1963; Mabood *et al.,* 1984) was determined by Hanes plot as showing in Fig. 4, the value was found to be 10 pM.

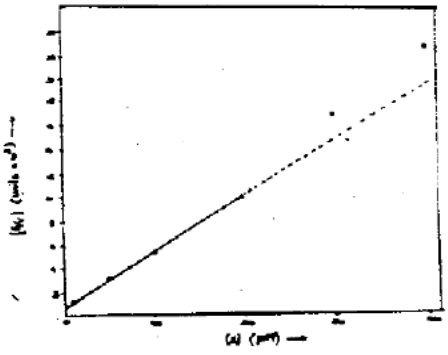

Fig. 4: The effect of substrate concentration based on S/V versus S plot

Effect of enzyme concentration on activity: The effect of enzyme concentration on activity was assayed. The results are shown in Fig. 5. It is evident that the enzyme activity was linear for upto 85 percent of the original enzyme concentration (extract). Hence in further work 85 percent of the enzyme concentration was used for assaying enzyme activity.

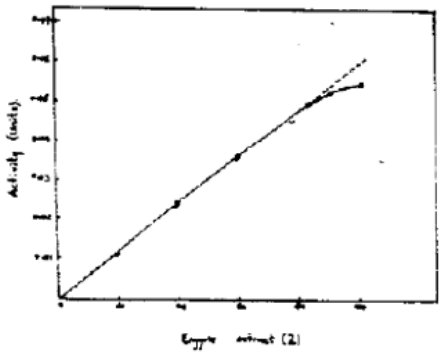

Fig. 5: Effect of enzyme concentration on activity

Effect of *in vitro* storage: To investigate the effect of the *in vitro* storage on the activity of the enzyme, the extract of pH 5.5 was stored at 40°C over a period of one week. The results are given in Table 1. It is noticed that there is no change in activity of the enzyme for upto the period of storage. This sort of ability of the enzyme suggests that it is a suitable enzyme for advanced kinetic. It is concluded that wheat is a good source of acid phosphatase and that the enzyme may be suitable one for advanced kinetic studies. Further purification and characterization as well as detailed kinetic studies of the enzyme are in progress.

Table 1: The effect of storage on activity

Day	aVm (Units)
0	0.202
1	0.206
2	0.202
3	0.202
4	0.201
5	0.207
6	0.206
7	0.206

References

Besford, R.T., 1979. Phosphorus nutrition and acid phosphatase activity in the leaves of seven plant species. J. Sci. Food Agric., 30: 281-285.

Fatima, S., A. Saeed and Z.A. Khan, 1981. Isolation and characterization of acid phosphatase from maize grains (*Zea mays*). Pak. J. Biochem., 14: 56-60.

Gutman, A.B. and B.B. Gutman, 1940. Estimation of acid phosphataes activity from bloodserurn. J. Biocern., 136: 201-209.

Hagima, I., 1984. Study of the activity of some enzymes in relation to the plants resistance to frost. Probleme Genetica Teoretica Aplicata, 16: 111-121.

Herman, M.C. and M. Liss, 1963. Isolation of acid phosphatase from microbes. Biochemistry, 126: 16-16.

Mabood, S.F., P. Khawaja, T.B. Douglas and M.A. Akhatar, 1984. A novel design for precise estimation of Michaelis constants. Pak. J. Biochem., 7: 31-38.

Srivastava, R. and M.N. Gupta, 1986. Acid phosphatase in tubers and triticum-a review. J. Sci. Ind. Res., 45: 402-412.

Webb, J.L., 1963. Enzyme and Metabolic Inhibitors. Vol. 1, Academic Press, New York, USA., pp: 13-48.