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EXTRACTION AND CHARACTERIZATION OF ACID PHOSPHATASE ENZYME FROM GERMINATED PEANUT SEEDS

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ABSTRACT

Peanut (*Arachis hypogaea* L.), is one of the important food crop largely cultivated in China, Africa, USA, Brazil and India. In the present study, acid phosphatase enzyme was isolated and partially purified from germinated peanut seeds. The partial purification process was performed using ammonium sulphate precipitation method. The crude enzyme having a specific activity of 3.5 U/mg was purified using 80% ammonium sulphate precipitation method. The specific activity of the partially purified was found to be 9.7units/mg.The enzyme activity was measured at different incubation time, pH, temperature and substrate concentration. The maximum activity was obtained at 30 minutes of incubation. The enzyme was most active at pH 5.0 and 50^{0}C. There was a corresponding increase in the rate of reaction with the increase in the substrate concentration from 0.25 to 1 mM.

Key words : Acid phosphatase, peanut, *Arachis hypogaea*, enzyme purification, enzyme characterization.

Introduction

Acid phosphatases are group of enzymes that catalyzed the hydrolysis of a number of phosphate esters and have pH optima below 6.0 (Vincent *et al*., 1992; Shahbazkia *et al*., 2009). Acid phosphatases are widely distributed in plants and animals. They are found in yeasts, fungi, seeds of higher plants, fruits as well as in many animal tissues (Nicanuzia dos Prazeres *et al*., 2004; Sambuk *et al*., 2011; Tabaldi *et al*., 2007). They are presumed to convert organic phosphorous into available phosphorous (Eshanpour and Amini, 2003; Amlabu *et al*., 2009) during active cellular growth (Carswell *et al*., 1997). Acid phosphatases are believed to be important for many physiological processes, including the regulation of soluble phosphorous (Pi) (Yan *et al*., 2001). Phosphorus (Pi) is an essential macronutrient for plant growth and development that plays a key role in many processes, including energy metabolism and the synthesis of nucleic acids and membranes (Ehsanpour and Amini, 2003). Acid phosphatases are constitutively expressed in seeds during germination and their activities increase with germination to release the reserve materials for the growing embryo (Biswas and Cundiff, 1991). During germination different phosphate esters of sugars and substrates stored in seed and seedling need to be hydrolyzed for cellular growth (Hoehamer *et al*., 2005).

Although, a number of acid phosphatases from plant source have been reported, detailed studies on the kinetic parameters of only a few acid phosphatases have been explored. Thus, in the present study, acid phosphatase extracted from peanut seedlings was partially purified and different kinetic parameters were examined.

Peanut (*Arachis hypogaea* L.), is a tropical food crop cultivated in large scale in China, Africa, USA, Brazil and India. Peanut seeds are rich in fats and proteins, as well as some vitamins (E and B complex) and several minerals (Castro *et al*., 2011).

Materials and Methods

Mature healthy peanut seeds were washed

thoroughly with distilled water and then surface sterilized. The seeds were soaked in distilled water for 12 hours. The seeds were then allowed to germinate in dark at room temperature. The seedlings were harvested after 3-4 days.

Enzyme extraction : The seedlings were crushed in extraction buffer (Tris-HCl 50mM, EDTA 5mM, NaCl 0.1mM and β-mercaptoethanol 0.04% v/v). The homogenate thus obtained was filtered and centrifuged at 9000 rpm for 15 min at 4^0C. After centrifugation, the clear supernatant was transferred to fresh Eppendorf and treated as crude enzyme preparation.

Partial purification of enzyme : The crude enzyme was partially purified by ammonium sulphate precipitation and the precipitate so obtained were dissolved in citrate buffer and dialysed against citrate buffer (pH 5.0, 50 mM).

Determination of Acid phosphatase activity : Acid phosphatase activity was determined by measuring the amount of p-nitrophenol (pNP) released from substrate p-nitrophenyl phosphate (pNPP) (Campbell *et al.*, 1978). The reaction mixture containing pNPP (2mM), crude enzyme (50 µl) and citrate buffer (50 mM, pH 5.0) was incubated at 30^0C for 30 min, then 2 ml of sodium hydroxide (0.1N) was added to stop the reaction and absorbances were measured at 430 nm using a spectrophotometer. pNP was used as standard. One unit of enzyme (U) is defined as amount of enzyme release 1ìmol of pNP per min under experimental conditions. Specific activity of acid phosphatase is defined as the amount of enzyme that liberates 1.0 µM p-nitrophenol/ minute/mg of protein.

Estimation of protein content : The protein content in crude enzyme preparation was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as a standard.

Effect of incubation time on enzyme activity : The effect of incubation time on enzyme activity was monitored by incubating the enzyme reaction mixture in different time period (10 to 60 min).

Effect of pH and temperature on enzyme activity : The effect of pH on the enzyme activity was determined by measuring the hydrolysis of the substrate pNPP in reaction mixture at various pH values ranging from pH 3.0-8.0. Buffers used were acetate buffer (100 mM, pH 3.0–5.0) and sodium phosphate buffer (100 mM, pH, 6.0–8.0). The effect of temperature on acid phosphatase activity was performed in 20 mM citrate buffer pH 5.0 over a temperature range of 10-90^0C using pNPP under standard test conditions.

Optimum pH for enzyme activity was determined by incubating the enzyme substrate mixture in 0.1 M citrate buffer of different pH; 3.5 to 6.0. Temperature stability was determined by incubating the enzyme substrate mixture in 30 to 90°C under assay condition.

Effect of substrate concentration on enzyme activity : The rate of acid phosphatase activity was determined at different substrate concentrations (0.25–2 mM) in a reaction mixture containing 100 µl of enzyme, 200 µl of citrate buffer (pH 5.0, 100 mM) and 200 µl of substrate.

Results and Discussion

Acid phosphatase activity in germinating peanut seedlings : The enzyme activity and specific activity of crude enzyme was found to be 0.567 units/L and 3.5units/ mg, respectively. The specific activity was found to be 9.7 units/mg after 80% $(NH_4)_2$ SO_4 saturation.

Effect of incubation time on acid phosphatase activity : Acid phosphatase activity was measured at different periods of incubation (10 to 60 min). The activity increased slowly from 10 to 30 minutes and then reduces gradually (Fig. 1).

Similar results were reported by Okuda *et al.* (1987) in tobacco cell and by Mohammed (2010) in *Vigna aconifolia*.

Effect of temperature on acid phosphatase activity : Acid phosphatase was found to be active over a wide range of temperature (30 to 70°C). Maximum enzyme activity was observed at 50°C (Fig. 2) and thereafter the enzyme activity decreased noticeably but not inactivated completely even at 80°C. The results indicate the thermo stable nature of acid phosphatase isolated from germinated peanut seeds. Similar observations have been reported for the acid phosphatases from cotyledons of *Psoralea corylifolia* L., from garlic seedling which also shows optimum enzyme

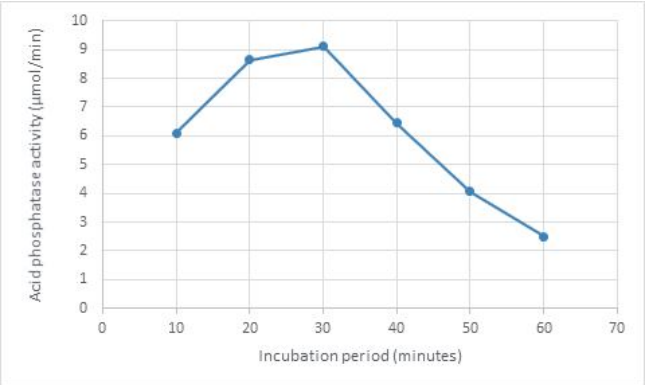

Fig. 1 : The effect of incubation time on acid phosphatase activity extracted from germinated peanut seeds.

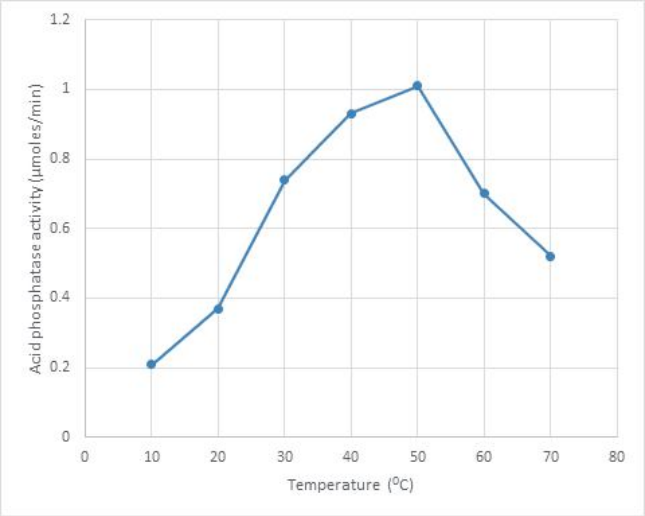

Fig. 2 : The effect of temperature on acid phosphatase activity extracted from germinated peanut seeds.

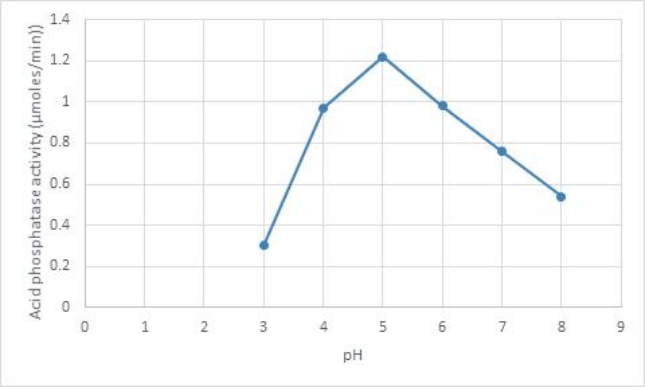

Fig. 3 : The effect of pH on acid phosphatase activity extracted from germinated peanut seeds.

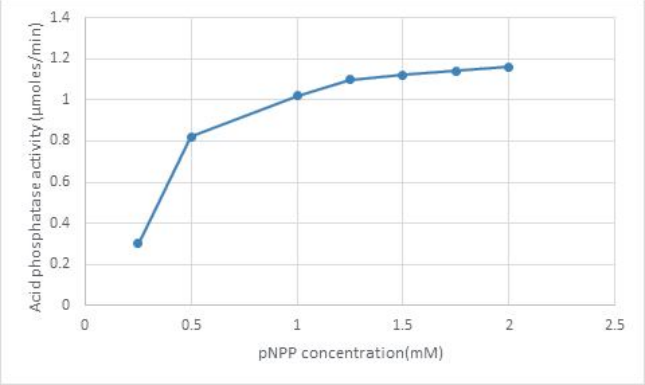

Fig. 4 : The effect of substrate concentration on acid phosphatase activity extracted from germinated.

activity at 50°C (Yenigün and Güvenilir 2003). Acid phosphatase activity from *Vigna mungo* L. seedlings showed highest activity at 55°C (Asaduzzaman *et al*., 2011).

Effect of pH on acid phosphatase activity : Acid phosphatase activity was recorded at different pH ranging from 3.5 to 6.0. The enzyme activity increased from pH 3.5 to 5.2 and optimal activity was recorded at pH 5.2 (Fig. 3). Similar observations have been reported for the acid phosphatases from different plant sources. Most of the acid phosphatases require acidic pH for their catalytic activity. Chickpea acid phosphatase was found to have maximum enzyme activity at pH 5.0 (Asaduzzaman *et al*., 2011).

Effect of substrate concentration on acid phosphatase activity : Acid phosphatase activity was measured at different substrate (p-NPP) concentration from 0.25-2mM. The result (Fig. 4) showed that for increase in the substrate concentration from 0.25 to 1.0 mM, there was a corresponding increase in the rate of reaction and then a slight increase was obtained from1 to 2 mM. The values of the present study agree somewhat well with those of Surchandra *et al*. (2012), Nadir *et al*. (2012), Saeed *et al*. (2014).

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