STUDIES ON LIPASE [EC 3.1.1.3] FROM GERMINATING *Hura crepitans* SEEDS

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ABSTRACT

Lipase activity was monitored on germinating Hura crepitans seeds for 16 days in the dark with olive oil as substrate. The enzyme activity increases from 4.5 units on day zero to 12.0 units on the 12th day and later fell to 10.5 units on the 16th day of germination. The effect of pH on lipase activity was determined with two different peaks at 6.0 and 8.0 respectively. This suggests that there are both acidic and alkaline lipases in the seeds of Hura crepitans. Inhibition showed that EDTA is an activator at concentration below 0.18M. It is also an activator at concentration above 0.5M. The enzyme has a Km of 0.18M with a Vmax of 0.69.

Keywords: *Hura crepitans*, Lipase, Inhibition, Activation, Km and Vmax

INTRODUCTION

Hura crepitans (Sandbox tree) is a member of the Euphorbiaceae (Spurge family) (Thumm *et al.,* 2002). This dicotyledonous plant can be used as antifungal agents (Van Custen *et al.,* 1994), it is known to poses antiviral activity (Semple *et al.,* 1998), antidiarrhoeal activity (Tona *et al*., 2000), antimalarial activity (Tona *et al.,* 1999), and plays a role in the inhibition of phycomycosis and dermatophytosis (Nwosu and Okafor, 1995). Its bark is used in the management and treatment of leprosy (Lewis and Lewis, 1977) and the leaves for treatment of ringworm in India (Ghandi, 1973). The milky latex is used in the removal of decayed tooth and its oil as purgative (Trease and Evans, 1983). *H. crepitans* oil is used for manufacturing soap, body lotion, polish and paint (Crueger and Crueger, 1990).

H. crepitans contains carbohydrates, proteins, palmitoleic acid, oleic acid as well as stearic, arachidonic, and myristic acids. A protease has been isolated and purified from the latex of *Euphobia supine* (Arima *et al.,* 2000). Plants of the family Euphobiaceae are known to contain tyrosine kinases (Zheng and Ramirez, 1999) and DNA topoisomerase II (Wada *et al.,* 1998).

Lipases or acylglycerols acylhydrolases (EC 3.1.1.3) are enzymes which hydrolyze ester of long chain aliphatic acid from glycerol at oil /water interface (Jensen, 1983). This enzyme has been detected in the food reserve tissues of growing seedling of many plants and especially in those that contains large amount of triacylglycerols (Beevers and Hills, 1987). In view of the role this enzyme plays in the energy support of plants and its great applications in various industrial processes, and in furtherance of our effort to characterize lipase from different plants of pharmacological importance, there exist the need to isolate and further characterize lipase from this plant with respect to finding more comparative properties with lipase from other plants.

MATERIALS AND METHODS

Materials: *H. crepitans* seeds were collected from different parts of the University of Nigeria and identified by the curator of Botany Department, University of Nigeria, where voucher specimens were deposited.

Reagents: Folin ciocalteau was obtained from BDH England, β-mecapthoethanol was from Sigma, and USA while Bovine Serum Albumin was from Merk, Darmsar, Germany. All other reagents were of analytical grade and were obtained from Sigma USA.

Seed Germination: 100 viable seeds were placed in a clean 500 ml beaker filled with water. The set up was left for 24 hr for imbibitions to occur. At the end of imbibitions, the seeds were placed in a box filled with humus soil and watered. This was allowed to germinate in the dark for 16 days.

Enzyme Isolation: At the end of every 24 hr during germination, 10 seeds were removed, washed in distilled water and used for enzyme extraction. The cleaned seedlings were crushed with mortar and pestle in cold acetone for about 10 min. The crude powder was then filtered with cheesecloth and washed with cold acetone until free of oil. The residual finely crushed plant materials were dried in a vacuum dedicator, weighed and used for lipase assay (Vyas and Petal, 1968).

Enzyme Assay: The assay procedure was basically that of Vyas and Petal (1968), with slight modifications. 1g of groundnut oil with 5 ml of 0.2M-phosphate buffer (pH 8.0) and 5ml-distilled water were mixed and stirred on a mechanical shaker for 5 min. Then 0.1g of test material (enzyme) was added and vigorously stirred. This was allowed for 24 hr at ambient temperature in an electric shaker. Free fatty acid liberated was determined through titration with 0.1M NaOH solutions.

Blank consists of the same cocktail above with the test material (enzyme) being added at the end of 24 hr of incubation. Activity was expressed in terms of volume (ml) of 0.1M NaOH used during titration after subtraction of the blank.

Inhibition Studies: Enzyme activity was assayed as earlier described in the presence and absence of different concentrations of EDTA (0.1, 0.3, 0.5, 1 and 2 M) respectively.

pH Optimum: The optimum pH of the enzyme was determined using the assay method earlier described though with phosphate buffer of different pH (5.0 - 9.0).

RESULTS AND DISCUSSION

Enzyme Activity: The activity of the enzyme increased from day zero and peaked at the 12th day of germination (Figure 1). Lipase activity has been reported in the endosperm of most seeds during germination (Eze *et al.,* 2000; Eze *et al.,* 2005). The result obtained from these studies is in agreement with other studies done using oil seeds; corn (Lin *et al.,* 1983) and wheat (Pacholy, 1972). The increase in activity may not be unconnected with intense metabolic activity taking place in the endosperm of seeds during germination in which there was increased lipolysis and most of the triacylglycerols were converted to free fatty acids and glycerols and subsequently to sucrose through the glyoxylate pathway for the support of the plant growth.

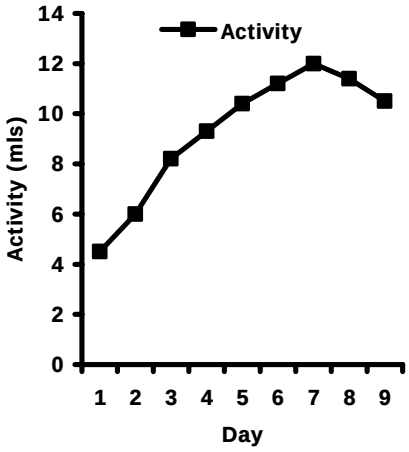

Figure 1: Variation of lipase activity with period of germination (days)

Optimum pH: The enzyme has pH peaks at 6 and 8.0 (Figure 2). The pH optimum had a major peak at 8.0 and a minor one at 6.0. This showed that there were two types of lipase in the crude extract, the slightly acidic and slightly alkaline lipases. Similar results were obtained by Rosnitschek and Theimer (1980) on rape seed and Lin et al. (1983) on soybean seedlings.

Figure 3 is a histogram showing the effect of different concentration (0.1-2M) of EDTA on lipase

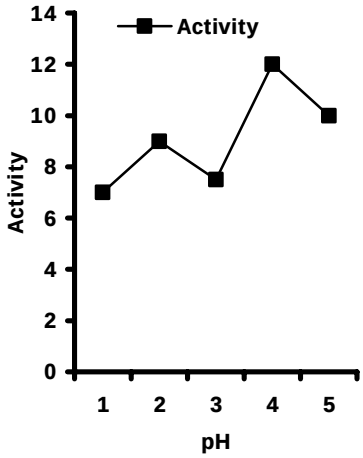

Figure 2: pH optimum of lipase activity

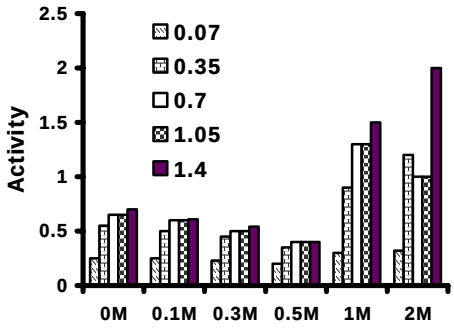

Figure 3: Effect of different concentration of EDTA on lipase activity

activity. 0.1M, 0.3 and 0.5M showed a decrease in activity with 0.5 M having the highest activity. At concentrations higher than 0.5M (1M and 2M), the inhibitor showed no inhibitions, therefore the activity was higher, suggesting that EDTA becomes an activator at concentrations higher than 0.5 M. EDTA thus attained a threshold of inhibition so that above 0.5M level, it became unable to bind the divalent cations, and may have initiated some intermolecular interactions above the critical concentrations.

The intermolecular reactions made the solution more acidic and changed the pH towards 6.0 in which case it could imply that the substrate (olive oil) may yield better product that can be acted upon at pH 6.0 and 8.0. This gives room for further research into the substrate-pH interactions.

Substrate concentrations: EDTA is an uncompetitive inhibitor as shown by the double reciprocal plot (Figure 4). When lipase activity was plotted with respect to the substrate concentration in the presence and absence of inhibitor (Figure 3), the result prove EDTA to be an activator with increasing substrate concentration. Activity decreases in the presence of low concentration of EDTA.

EDTA could be an activator above a certain concentration. The result of the Lineweaver-Burk plot of 1/v against 1/s shows a decreasing inhibition at

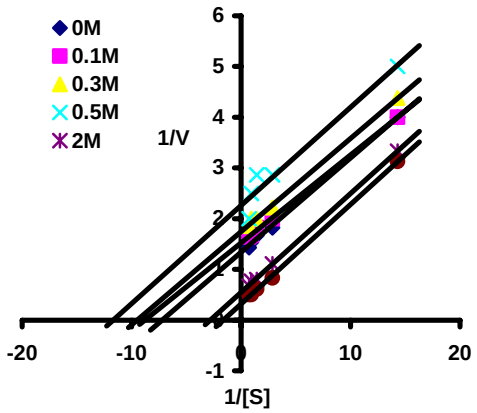

Figure 4: Lineweaver-Burk plot of the activity of lipase from Hura crepitans in the presence and absence of EDTA at various concentrations of substrate

0.1M, 0.3M and 0.5M. Also the result of the plot of activity against the concentration of the inhibitors showed the highest activities at 2M followed by 1M, 0M, 0.1M, 0.3M and 0.5M. From this result, at high concentration, EDTA acted as an activator. Investigation into the mechanism of activation and inhibition at different concentrations should be researched into for proper understanding of this enzyme.

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