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ACID PHOSPHATASE AND ADENOSINE TRIPHOSPHATASE FROM PLANT LATICES

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Abstract

Fifteen plant latices of the families Apocynaceae, Asclepiadaceae and Sapotaceae have been investigated for the presence of acid phosphatase and adenosine triphosphatase enzymes. The latices studied possess both acid phosphatase and adenosine triphosphatase enzymes. One of the richest sources of enzymes was from the latex of *Vallaris solanacea* for acid phosphatase and *Plumeria rubra* for adenosine triphosphatase. Significance of these enzymes in latices is discussed.

Introduction

Latex, contained in specialized cells called laticifers, is an emulsion of a liquid serum which holds a mixture of variety of substances. Number of enzymes carrying out varied functions are known to occur at high concentrations in various latices (Shukla & Krishnamurthi, 1971). Among them, acid phosphatase and adenosine triphosphatase are important for the phosphorous metabolism in latices. Giordani *et al.*, (1986) studied the purification of acid phosphatase in the latex of *Asclepias curassavica*. Similarly, Lynn & Clevette-Radford (1987 a,b) purified the acid phosphatase enzyme of latices from Euphorbiaceae members. Involvement of ATP produced by ATPase in *Hevea* latex is great (Park & Bonner, 1958; Sethuraj, 1968; Archer, 1980). Since these two enzymes closely relate to the phosphorous metabolism in the latices, 15 latices belonging to the families Apocynaceae, Asclepiadaceae and Sapotaceae have been examined for the presence of acid phosphatase and adenosine triphosphatase enzymes.

Materials and Methods

For ATPase and AcPase enzyme studies, latex from incised stem and leaf removed along the stem was collected in vials containing respective ice cold-buffer. The volume collected in the ratio of 1:1 latex-buffer was centrifuged at 2000X g for 30 min in MB refrigerated centrifuge. The supernatant, referred to as "Latex Sample-S" was utilized for enzyme studies. For acid phosphatase, the supernatant was dialyzed overnight against distilled water to remove inorganic phosphate.

Acid Phosphatase (AcPase): The assay mixture contained, 0.5 ml of 1.1 mM P-nitrophenyl phosphate, 3.4 ml of citric acid – sodium hydroxide buffer (pH 4.8-5.2) and 0.1

ml of Latex Sample-S. The reaction was stopped after 30 min., of incubation period with 0.4 ml of 1M NaOH. Total volume was made to 5 ml with distilled water. The activity was determined by measuring the change in absorbance at 400 nm due to P-nitrophenol formation. In control samples, the latex Sample-S was added after the addition of 1M NaOH (Bessey *et al.*, 1946). The enzyme activity was expressed as µg of P-nitrophenol released/h/mg of protein.

Adenosine Triphosphatase (ATPase): The assay mixture contained 0.25 ml of 0.02 M ATP solution, 0.25 ml of 0.2 M histidine buffer (pH 7.0), 0.1 ml of 0.05M magnesium chloride, 0.3 ml of distilled water and 0.1 ml of Latex Sample-S. The tubes were incubated at 28°C for 0 and 5 min. The reaction was stopped with 1 ml of perchloric acid. Mixture was centrifuged at 1000 g for 15 min., (Wayney Kielly, 1955). After centrifugation the supernatant was analyzed for released inorganic phosphorous (Fiske & Subbarow, 1925). The activity was expressed as µmole of phosphorous released/h/mg of protein. The method of Lowry *et al.*, (1951) was used for the determination of protein. The assay conditions for the enzymes were standardized.

Table 1. Changes in AcPase and ATPase in latices of various plant species.

Name of the plant species	AcPase	ATPase
Apocynaceae		
Aganosma caryophyllata	0.10 ± 0.007	0.09 ± 0.06
Allamanda cathartica	0.12 ± 0.02	0.20 ± 0.03
A. violacea	0.14 ± 0.03	0.06 ± 0.01
Nerium indicum (pink flowered var.)	0.40 ± 0.08	0.06 ± 0.04
N. indicum (white flowered var.)	0.60 ± 0.04	0.08 ± 0.03
Plumeria alba	1.13 ± 0.10	0.28 ± 0.05
P. rubra	1.17 ± 0.10	0.61 ± 0.01
Tabernaemontana divaricata (single flowered var.)	0.19 ± 0.03	0.08 ± 0.01
T. divaricata (double flowered var.)	0.59 ± 0.09	0.04 ± 0.02
Thevetia peruviana (yellow flowered var.)	0.06 ± 0.04	0.08 ± 0.03
T. peruviana (white flowered var.)	0.05 ± 0.01	0.06 ± 0.01
Vallaris solanacea	1.42 ± 0.28	0.22 ± 0.04
Asclepiadaceae		
Calotropis gigantea	0.50 ± 0.08	0.21 ± 0.03
C. procera	0.95 ± 0.26	0.07 ± 0.01
Sapotaceae		
Manilkara zapota	0.91 ± 0.05	0.14 ± 0.05

*Values are the mean ± SD based on 3 readings for each plant.

Results

All the values are completely free from other ground tissues since turgour pressure in the laticifers is greater than the ground tissues whenever injury is made to plant. Differences in the activities in the selected plant species was observed (Table 1). Such enzyme differences may be used as a physiological marker. The latices studied possess both acid phosphatase and adenosine triphosphatase activities. Latex of *V. solanacea* exhibited the highest AcPase activity as evidenced from the concentration of P-nitrophenol followed by two species of *Plumeria* with least in 2 varieties of *T. peruviana*. Double flowered var. of *T. divaricata* and white flowered variety of *N. indicum* have almost two fold higher AcPase activity than that of their single flowered variety and pink flowered variety, respectively. Though there was no significant difference in ATPase activity of all the species studied, the highest and the least activities were recorded in *P. rubra* and double flowered variety of *T. divaricata*, respectively. *A. cathartica*, *P. alba* and *C. gigantea* showed almost three times more ATPase activity than that in *A. violacea*, *P. rubra* and *C. procera*, respectively.

Discussion

The data suggests that these enzymes are likely to be associated with normal cellular metabolism in laticifers. Pasternark (1970), Shukla & Krishnamurthi (1971) and Jayabalan (1987) reported that each enzyme assayed in a particular cell or tissue or latex has a specific role and its difference in distribution reflects the biochemical changes at specific sites in the cells which are related to the structure and function of such cells.

The presence of Acid phosphatase activity in all the latices studied would indicate the presence of lysosomal enzyme since it is characteristically associated with lysosome (Matile, 1968). It is also reported in latex of *Euphorbia lathyris*, *E. trigona*, *Elaeophorbia drupiflora* (Lynn & Clevette-Radford, 1987b), *Papaver somniferum* (Antoun & Roberts, 1975), *Manilkara zapota* (Selvaraj & Pal, 1985) and *Hevea* (Jacob & Sontag, 1974). Generally, phosphatases are involved in hydrolysis of organic phosphate esters and in regulation of the plant cell metabolism through inorganic phosphorous level (Tsubol *et al.*, 1957; Murray, 1980). The AcPase activity helps to accumulate phosphate in the vacuole and afterwards the phosphate is utilized again in the cytoplasm as suggested by Giordani *et al.*, (1986).

All the latices investigated showed the presence of ATPase activity. ATP required for the intercellular transport of metabolites directly depends on the ATPase activity (Van-Steveninck, 1976). Rubber biosynthesis in *Hevea* depends on the availability of ATP and optimal concentration of ion in latex (Sethuraj, 1968; Archer, 1980). The presence of ATPase enzyme in latex indicates that laticiferous tissue is metabolically an active tissue and may be the reservoir of biochemical reactions.

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