Applied Biological Research 14 (1): 100-103, 2012 **Short Communication**

FIRST REPORT OF DPP-III IN PLANTS: ITS SUBCELLULAR LOCALIZATION IN GERMINATING MUNG BEAN (*Vigna radiata)* SEEDS AND DISTRIBUTION IN PLANT PARTS

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ABSTRACT

Arginyl-arginyl-4-methoxy-β-naphthylamide (Arg-Arg-4mβNA) hydrolyzeing activity is known as dipeptidylpeptidase-III (DPP-III). DPP-III enzyme hydrolyzes various bioactive peptides, including enkephalins, angiotensins, gastric peptides and Arg-Arg-4mβNA. We report the presence of DPP-III enzyme in plants for the first time. The enzyme was noticed in mung bean [*Vigna radiata*] seeds germinated at 25ºC for 48 h. The crude enzyme preparation had optimum activity at alkaline pH of 8.0. Sub-cellular distribution of this enzyme revealed its cytosolic localization. Enzyme activity when investigated in different parts of mung bean plant revealed maximum activity in seeds followed by roots, stem and leaves. The ubiquitous distribution of this enzyme signifies housekeeping role of this enzyme in plant physiology. Maximum activity in seeds indicates its possible role in germination.

Keywords: Arg-Arg-4-mβNA, cytosolic, DPP-III, germinating seeds *Vigna radiata*

INTRODUCTION

Dipeptidyl peptidases (DPPs) are the enzymes which cleave dipeptides from amino-termini of polypeptide chains. These enzymes are classified into four types, viz., I, II, III and IV, on the basis of their substrate specificity, subcellular localization and physiochemical parameters (MacDonald and Barret, 1986). Among these enzymes DPP-III (E.C. 3.4.14.4) hydrolyses Arg-Arg-4mβNA and has also been named dipeptidyl aminopeptidase III. It was first identified in bovine pituitary (Ellis and Nuenke, 1967). Later, this enzyme was purified from different mammalian species (MacDonald and Barrett, 1986), slime mold (Chan *et al*., 1985), *Drosophila melanogaster* (Mazzocco *et al*., 2003) and goat brain (Dhanda *et al*., 2007). Studies of DPP-III in different species suggest the important role of DPP III in enkephalin and angiotensin metabolism. It is also known to participate in the processing of peptide hormones and metabolism of bioactive peptides (Dhanda *et al*., 2007). However, in plants DPP studies are fragmentary and sporadic. Only DPP-IV is reported in barley seeds which degrade proline-containing peptides transported from the endosperm to the embryo of the germinating barley grain (Davy *et al.*, 2000). Seed storage proteins are mobilized in leguminous plants by enzymatic hydrolysis during seed germination and thus provide amino acids for growth of embryonic plants (Caldwell and Sparrow, 1976). DPP-III has been explored for first time in plants in germinating *Vigna radiata* seeds along with its distribution in different parts of this plant has been described in this communication.

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MATERIALS AND METHODS

Mung bean [*Vigna radiata* var. Pusa (P) 9531] was procured from Indian Agriculture Research Institute, Regional Station, Karnal (India). Arg-Arg-4mβNA and Fast Garnet GBC (o-aminoazotoluene diazonium salt) were obtained from Sigma-Aldrich USA. Tris, sucrose, NaCl and other ingredients used were purchased from Himedia, India.

Assay of DPP-III

Assay of DPP-III was done with Arg-Arg-4mβNA as substrate at pH 8.0 (50 mM tris-HCl as assay buffer containing 100 mM NaCl and 1 mM β-mercaptoethanol (β-ME). One unit of enzyme activity is defined as the amount of enzyme that liberates 1 nanomole of 4mβNA at 37°C under assay conditions (Dhanda *et al.*, 2007). The seeds were sterilized with 70% ethanol and 0.2% mercuric chloride for 3 min. and then rinsed in distilled water. These seeds were germinated for 48 h at 25°C and used as a source of enzyme.

Determination of pH optima in crude homogenate

A 10% homogenate was prepared in tris-HCl (50 mM, pH 7.4 containing 1mM β-ME). The pH dependence of the enzyme in homogenate was determined by using 50 mM sodium acetate (pH 4.0-5.5), sodium-phosphate (pH 6.0-7.5), tris-HCl (pH 8.0-9.0), glycine-NaOH (9.5-10.5), sodium phosphate-NaOH buffer (11.0-11.5) and KCl-NaOH (12.0-13.2) as assay buffers containing 100 mM NaCl and 1 mM β-ME.

Sub-cellular localization of DPP-III in plant cell

A 10% homogenate was prepared in chilled, tris (25 mM) and sucrose (250 mM) buffer, pH 7.4 using pestle-mortar. The homogenate was centrifuged at 100 *g* for 10 min., nuclear pellet collected and supernatant sub-fractionated into chloroplast (2000 *g* for 10 min.), mitochondria and peroxisomes (10,000 *g* for 10 min.), microsomal (pellet) and cytosolic (supernatant) fractions by ultracentrifugation (1,00,000 *g* for 60 min.). The pellets were washed twice and each pellet further homogenized in presence of 0.1% triton X-100 to rupture the subcellular organelles. The fractions were dialyzed overnight against extraction buffer to remove Triton X-100. Different markers were used for the assay of different organelles.

Distribution of DPP-III in different parts of mung bean plant

Seeds of mung bean (*Vigna radiata*) were sown in soil in summer and spring seasons. The plants were uprooted when they attained the height of 30 cm and different parts (stem, leaves and roots) were taken. Each part was cleaned and 10% homogenate prepared in tris (25 mM) and sucrose (250 mM) buffer of pH 7.4. The homogenate was centrifuged and supernatant collected. DPP-III activity was determined in each homogenate. The total activity with reference to seed and activity per gram tissue weight was calculated.

RESULTS AND DISCUSSION

Sub-cellular localization of DPP-III

The pH dependence of DPP-III in homogenate is shown in Fig. 1. The enzyme works optimally at slightly alkaline of pH 8.0. All the fractions were evaluated for DPP-III, DNA (diphenylamine method), chlorophyll, catalase and RNA (orcinol) as markers of nuclear, chloroplast, peroxisomal and endoplasmic reticulum, respectively. Negligible cross activity was observed in different fractions. This may be due to the presence of organelles at different developmental stages (as seeds are germinating and are in the stage of rapid metabolism) which sediment at different speed. No specific cytosolic marker was studied but cytosolic fraction also had negligible activity of other examined biomolecules

Table 1: DPP (III) activity in parts of mung bean plant

Sources	Activity		
	n moles min^{-1} ml^{-1}	%	g^{-1} tissue weight
Leaves	10.65	30.0	53.25[a] ± 0.83
Stem (chl)[b]	19.07	53.7	95.35 ± 1.17
Stem	21.42	60.3	107.10 ± 1.06
Roots	23.44	66.0	117.20 ± 1.70
Seeds	35.50	100.0	177.50 ± 1.95

[a] The values are mean ± standard error of 3 different experiments; [b] chl = Chlorophyll

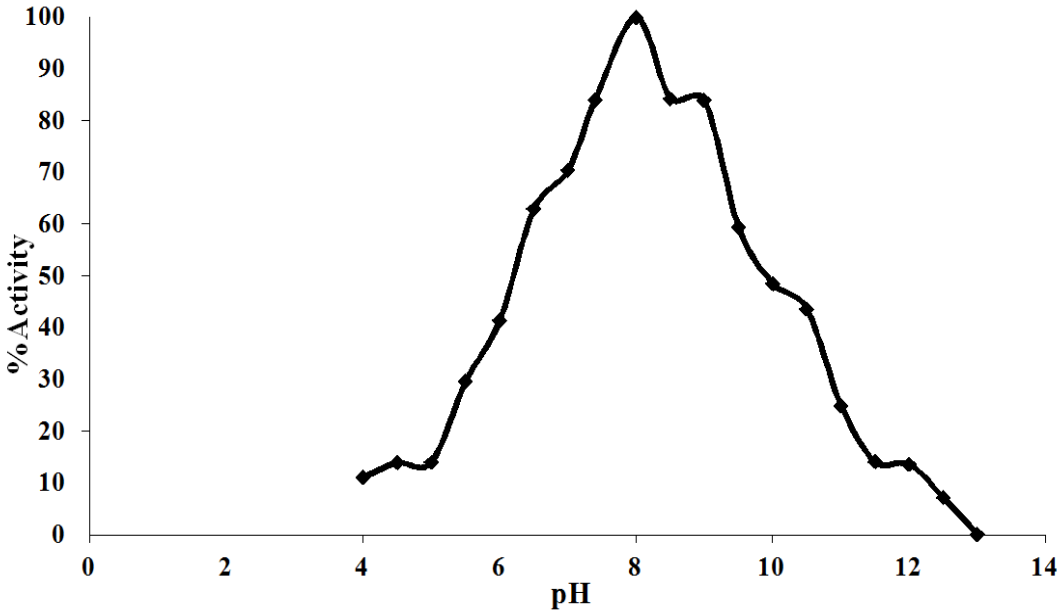

Fig. 1: pH optima of DPP-III in crude mung bean seeds extract

thereby suggesting a pure cytosolic preparation (Table 2). Subcellular localization studies of enzyme revealed that DPP-III is cytosolic (81.34%). The cytosolic nature of DPP-III has also been reported in different mammalian tissues (Ellis *et al*., 1967; Ohkubo *et al*., 2000; Dhanda and Singh, 2010). DPP-III is thought to play important role in the terminal stages of metabolism of proteins and peptides thereby delivering dipeptide moieties for further catabolism by cytosolic peptidases.

Table 2: Sub-cellular localization of Arg-Arg-4mβNA hydrolyzing activity in germinating mung bean seeds

Fractions	Total volume (ml)	Activity		
		(n mol min^{-1} ml^{-1})	Total (n mol min^{-1} ml^{-1})	Per cent Activity
Homogenate (10%)	235.0	-	-	-
Homogenate supernatant	210.0	14.73[a]±0.55	3093	100.00
Nuclear fraction	29.0	8.18 ±0.21	237	7.67
Chloroplast fraction	25.0	3.32 ±0.47	83	2.68
Mitochondria fraction	22.0	6.73 ±0.39	148	4.78
Microsomal fraction	19.5	5.20 ±0.70	101	3.27
Cytosolic fraction	185.0	13.60 ±0.26	2516	81.34

[a] Values are mean ± standard error of 3 different experiments

Distribution of DPP-III in different parts of mung bean plant

Amongst the different studied plant parts seeds had maximum activity per gram wet weight followed by roots, stem and leaves (Table 1). Green stem (with chlorophyll) had less activity as compared to stem without chlorophyll. The higher activity of DPP-III in seed is indicator of its possible role in germination. No functional role is assigned to DPP-III enzyme at this stage but its indicator of universal distribution in all the parts of the plant suggests its role in some housekeeping activity of the cell.

Conclusion: DPP-III of mung bean (*Vigna radiate*) is cytosolic in nature. The seeds have maximum activity amongst the various parts of mung bean plant. Ubiquitous distribution of this enzyme in different plant parts o suggests its role in protein metabolism. This is the first report of DPP-III in plants.

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