Indian Journal of Animal Sciences **79** (9): 715–718, August 2010

Distribution of dipeptidylpeptidase III homologue in brain and vital organs in goat (*Capra hircus*)

SUMAN DHANDA[1] and HARI SINGH[2]

Kurukshetra University, Kurukshetra, Haryana 136 119 India

Received: 14 October 2009; Accepted: 5 May 2010

ABSTRACT

Goat brain dipeptidyl peptidase III (DPP-III) was recently purified and characterized. The crude enzyme preparation had optimum activity at alkaline pH of 8.5. The regional and subcellular distribution of this protease in brain along with its distribution in different goat tissues was investigated. The enzyme activity was associated with the cytosolic fraction when assayed using synthetic substrate Arginyl-Arginyl-4-methoxy-β-napthylamide (Arg-Arg-4mβNA). The pituitary body had the highest density of the enzyme and thalamus had the least when expressed on the basis of per gram wet tissue weight. Other regions of brain tend to have similar activtiy. Tissue distribution studies revealed that pancreas was the most enriched with DPP followed by liver, thymus, brain and parotid. Kidney, spleen, thyroid, paratyroid had similar activity whereas lung, heart, skeletal muscle, skin, eye lens and adrenal had comparatively lower activity. Prostate gland and testis were enriched in DPP III activty. In female goat fallopian tubes and ovary had higher activity as compared to the uterus. The enzyme is present in all the tested tissues. This indicates that the enzyme is constitutive thereby suggesting its role in some vitalcullular function.

Key words: Arg-Arg-4-methoxy-β-naphthylamide, Cytosolic, DPP-III, Goat brain, Pancreas, Pituitary

Dipeptidyl peptidases (DPPs) are the group of enzymes which cleave dipeptides from amino-termini of the polypepetide chains. Recently, we purified goat brain dipeptidyl peptidase III (DPP-III) (Dhanda *et al*. 2007) which has a molecular weight of 69 kDa and specifically hydrolyses Arg-Arg-4mβna at optimum pH of 8.5. The enzyme is a metalloprotease having role of thiol in regulation (Dhanda *et al*. 2008a). DPP-III was first reported in bovine pituitary (Ellis and Nuenke 1967) and later purified from several mammalian tissues. The exact role of this enzyme in physiological processes is not well understood but as this enzyme hydrolyses enkephalins and angiotensin II and III (Dhanda *et al*. 2008b), thereby suggest its role in their metabolism. Goat brain DPP-III exhibits affinity for enkephalins in micromolar range (Dhanda *et al*. 2008a). The enzyme might be involved in pain modulation. DPP-III is less studied as compared to other DPPs. Therefore, in the present communication, we describe the subcelluar localization, regional distribution in brain alongwith its distribution in different goat tissues.

MATERIALS AND METHODS

Fresh goat brain and other tissues were used in all the experiments and were obtained from a local slaughterhouse in Kurukshetra. The tissue homogenates for subcellular fractionation, regional and tissue distribution were prepared using a Remi tissue homogeniser equipped with a glass pestle. Other substrates viz. Arg-Arg-4mβNA, Arg-4mβNA, were procured from Bachem Feinchemikalein AG, Bubendorf, Switzerland.

Present address: [1,2]Department of Biochemistry, Kurukshetra University, Kurukshetra 136 119 ([1]email: dhandasuman @rediffmail.com).

Enzyme assays

Assay of DPP-III. DPP-III was assayed with Arg-Arg-4mβNA as substrate as described by Dhanda *et al*. (2007). One unit of enzyme activity was defined as the amount of enzyme that liberated one nanomole of 4mβNA from the substrate per minute under assay conditions.

Assay for aminopeptidase B. Aminopeptidase B activity was determined spectrophometrically using Arg-4mβNA as a substrate in 50 mM Tris-HCI, pH 7.5 as described by Bogra *et al*. (2007). Hundred microlitres of enzyme homogenate were used and assay was carried out at 37°C for 30 min. One unit of enzyme activity is expressed as that amount of enzyme which releases one nanomole of 4mβNA per min from substrate under assay conditions.

Enzyme extraction and determination of pH optima. Goat brain homogenate (20%) was prepared in 50mM Tris buffer of pH 7.4. The homogenate was acid precipitated to pH 4.5 as described by Dhanda et al. (2007). The pH dependence of

the enzyme activity in homogenate as well acid precipitated sample was determined in 50mM sodium acetate (4.5–5.9), sodium phosphate (6.0–7.0), Tris-HCl (7.0–9.0), glycine-NaOH (9.0–10.5), Na_2HPO_4-NaOH (11–11.5), KCI-NaOH (12.0–13.5) buffers.

Subcellulcar localization: Fresh goat brain was washed with chilled saline (0.9% NaCl) and 20% homogenate was prepared in chillded Tris (25mM)-sucrose (2.50 mM) buffer, pH 7.4 using a Remi glass homogenizer. The homogenate was centrifuged at 200g for 5 min to remove the cell debris. The supernatant was subfractionated into nuclear (800 g), mitochondril (8 000 g), lysosomal (16 000 g), microsomal (105 000 g) and cytosolic (supernatant) fractions by differential centrifugation method (Koenig 1974). All these pellets were sonicated thrice for 15 sec each separately in the presence of 0.1% Triton X-100 to rupture the subcelluar organelles. All these samples were acid precipitated to pH 4.5 by drop-wise addition of chilled 1 N HCl and kept overnight at 4°C and centrifuged at 13 000 g in refrigerated centrifuge for 20 min. The pH of the supernatant was again raised to 7.4 by drop-wise addition of chillded 1 N NaOH before estimating the DPP-III enzyme activity.

Regional distribution of DPP in goat brain and vital organs: Goat brain was separated into different parts, viz. cerebrum, cerebellum, pans varoli, medulla oblongata, pituitary body, olfactory bulb, subthalamic nucleus, hypothalamus, thalamus, globus pallidus and caudate nucleus. Different tissues obtained from freshly slaughtered goat were taken. Each part of brain and each tissue was thoroughly cleaned and washed with 0.9% saline, weighed, chopped in small pieces and 20% homogenate was prepared in Tris (25mM)-sucrose (250 mM) buffer, pH 7.4 by homogenizing thrice for 15 sec each in a homogenizer. Each homogenate was acid precipitated to pH 4.5 and treated as described above. The activity of DPP-III for each part was quantitated in supernatant and expressed as per gram tissue weight. The percentage of total activity and activity per gram tissue weight was calculated in each part.

RESULTS AND DISCUSSION

pH optima studies with crude homogenate and inactivation of aminopeptidase β: Before carrying out the studies of DPP-III, special attention was paid to remove aminopeptidase B which might interfere with the estimation of Arg-Arg-4mβNA hydrolyzing activity by sequentially removing Arg from the substrate. Crude homogenate with Arg-Arg-4mβNA revealed 2 pH optima, one at pH 7.5 (corresponding to aminopeptidase B) and other at pH 8.5 (corresponding to DPP-III) (Fig. 1a). Aminopeptidase B activity is relatively high in brain tissue (Marks and Lajtha 1971) and was also detected in goat brain using Arg-4mβNA

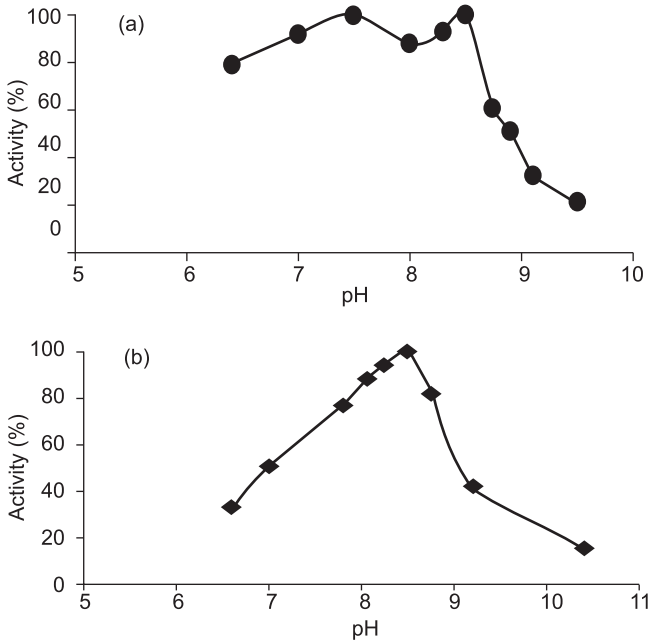

Fig. 1. pH optima of DPP-III before (a) after (b) acid precipitation of crude homogenate.

Table 1. DPP-III and aminopeptidase B activities of homogenate and acid precipitated samples

Sample	DPP-III		Aminopeptidase B	
	Activity (nmol/min/ml)	% activity	Activity (nmol/min/ml)	% activity
Brain homogenate	2.78*±0.44	83	6.49±0.89	100
Acid precipitated sample	3.36±0.40	100	0.030±0.93	0.46

*Values are mean±standard error of three different experiments.

Table 2. Subcellular localization of DPP-III in goat brain

Fraction	Total volume (ml)	Activity (nmol/ min/ml)	Total activity (nmol/min)	% activity
Homogenate (20%)	260	–	–	–
Homogenate (supernatant)	247	1.612*±0.43	398.16	–
Nuclear fraction	10	0.344±0.74	3.34	0.860
Mitochondrial fraction	10	0.117±0.67	1.17	0.293
Lysosomal fraction	12	0.095±0.23	1.14	0.285
Microsomal fraction	14	0.284±0.10	3.976	0.99
Cytosolic fraction	200	1.945±0.35	389	97.5

The percentage of activity in each fraction was calculated by taking sum of activities in different fractions as 100.

*Values are mean±standard error of 3 different experiments.

(Table 1). Since aminopeptidase B is acid labile (Lee and Snyder 1982), we included acid precipitation of crude extract to a pH of 4.5 to exclude and hydrolytic activity of aminopeptidase B towards Arg-Arg-4mβNA. This completely inactivated the aminopeptidase B activity. After cid precipitation, the pH optimum studies revealed only single peak at 8.5 (Fig.1b) in addition to the resulting increase in activity of DPP-III (Table 1). Thus all the samples were cid precipitated to pH 4.5 before they were studied fro DPP-III activity.

Subcellular localization of goat brain DPP: Subcellular localization of this enzyme revealed maximum enzyme activity (97.5%) in cytosolic fraction (Table 2). The studies were carried out in comparison to cathepsin B (EC-3.4.22.1) which is a well established lysosomal cysteine proteinase (Kamboj *et al*. 1990). DPP-III was also reported to be of cytosolic origin in different tissues (Ellis and Nuenke 1967, Grdisa and Vitale 1991, Ohkubo *et al.* 2000) whereas in rat brain it was localized in membrane (Lee and Snyder 1982). Two DPP-III activities one in cytoplasm ad other associated with synaptosomal membranes of crude mitochondrial pellet was reported in guinea pig brain (Smyth and O'Cuinn, 1994). DPP-III, being a cytosolic enzyme is though to be an important in the terminal stages of protein and peptide metabolism delivering dipeptide moieties for further catabolism by cytoplasmic dipeptidases.

Regional distribution of DPP in goat brain: Regional distribution studies revealed about 3-fold variation between lowest and highest activity within the brain. The pituitary body had the highest enzyme density (activity/gram wet tissue) followed by olfactory bulb, subthalamic nucleus and cerebrum. Other regions had similar enzyme activity. Thalamus had the least activity (Fig. 2). Pituitary body, beign the master gland of brain tissue, controls and regulates the secretion of hormones to coordinate functions of different parts of the body. The high density of enzyme in pituitary gland suggests its importance in peptide processing. The highest activity of DPP-III was also reported in pituitary of rat (Lee and Snyder 1982, Imai and Kato 1984).

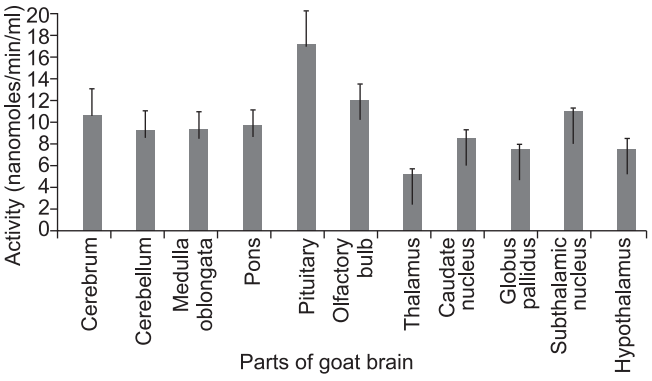

Fig. 2. Regional distribution of DPP-III activity in different parts of goat brain (per g wet weight).

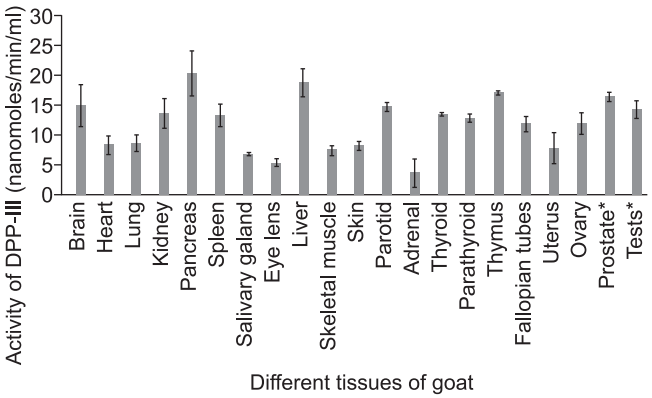

Fig. 3. Tissue distribution of DPP-III in goat (per g wet weight). All the tissues were obtained from female (*from male goat).

DPP-III disribution in different tissues of goat: Among different screened tissues, pancreas was found to have the highest activity per gram wet tissue folowed by liver, thymus, brain and parotid. Kidney, thyroid, parathyroid and spleen had the similar activity (Fig. 3). In rat, highest activity was reported in liver followed by pancreas (Lee and Snyder, 1982), whereas the reverse is in goat. McDonalf and Schwabe (1977) also reported the maximum DPP-III activity in pancreas. In pancreas, the enzyme might be participating in hormonal peptide biosynthesis. Lung, heart, skeletal muscle, skin, adrenal, salivary gland and eye lens of goat had comparatively lower DPP-III activity. This is in contrast to observations in bovine lens where DPP-III was found in abundance which varied with the structural changes of lens proteins (Swanson *et al*. 1978) and also induced lens opacification in cooperation with other proteolytic enzymes during cataract formation in Shumiya rats through the intracellular turnover of lens proteins (Zhan *et al*. 2001). This enzyme was also reported in reproductive organs of male and female goat. Prostate and testis both reveal the higher activity as compared to other organs. Uterus had low activity as compared to other reproductive organs of female goat. Increased DPP-III activity was reported in the endometrial carcinomas of various histological types, grade and depth of myometrial role of DPP-III is associated with processes common for the particular cell compartments, rather than specific function of one or the other cell type. The present distribution shows this protein to be housekeeping enzyme. However, the physiological role and pathological significance of this enzyme remains to be resolved.

DPP-III of goat brain is cytosolic in nature. The pituitary body has the highest enzyme density amongst various brain parts. The enzyme is widely distributed in different goat tissues thereby might be involved in protein metabolism.

ACKNOWLEDGEMENTS

We are thankful to Dr Rajvir Dahiya, Director, Urology lab, San Francisco, USA, for providing generous gift of

substrate. Suman Dhanda is thankful to UGC for providing the financial help in the form of JRF for carrying out the work.

REFERENCES

Bogra P, Singh J and Singh H. 2007. Aminopeptidase V: regional and subcellular distribution in goat brain tissue. *Journal of Indian Chemical Society* **84**: 596–602.

Dhanda S, Singh H, Singh J and Singh T P. 2007. Isolation, purification and characterization of DPP-III homologue from goat brain. *Protein Expression and Purification* **52**(2): 297–305.

Dhanda S, Singh H, Singh J and Singh T P. 2008a. Functional characterization and specific effects of various peptides on enzyme activity of DPP-III homologue from goat brain. *Journal of Enzyme Inhibition and Medical Chemistry* **23**(2): 174–81.

Dhanda S, Singh J and Singh H. 2008b. Hydrolysis of various bioactive peptides by goat brain dipeptidylpeptidase-III. *Cell Biochemistry and Function* **26** (3): 339–45.

Ellis S and Nuenke J M. 1967. Dipeptidyl arylamidase III of pituitary, purification and characterization. *Journal of Biological Chemistry* **242** (20): 4623–29.

Grdisa M and Vitale L. 1991. Types and localization of aminopeptidases in different human blood cells. *International Journal of Biochemistry* **23**: 339–45.

Imai K and Kato T. 1984. Dipeptidyl aminopeptidase in neonatal rat brain regions. *Experimentia* **40**(3): 263–64.

Kamboj R C, Pal S and Singh H. 1990. Purification and characterization of cathepin B from goat brain. *Journal of Bioscience* **15**: 39–408.

Koenig H. 1974. The isolation of lysosomes from brain. *Methods of Enzymology* **31**: 457–77.

Lee C M and Snyder S H. 1982. Dipeptidyl aminopeptidase III of rat brain. Selective affinity for enkephalin and angiotensin. *Journal of Biological Chemistry* **257**: 12043–50.

Marks N and Lajtha A. 1971. *Handbook of Neurochemistry*. pp. 49–139 Plenum Press, New York.

McDonald J K and Schewabe C. 1977. *Proteinases in Mammalian Cells and Tissues*. pp. 311–91. Elsevier/North Holland Biomedical press, Amsterdam.

Ohkubo I, Li Y H, Maeda T, Yamamoto Y, Yamane T, Du P G and Neishi K. 2000. Molecular cloning and immunohistochemical localization of rat dipeptidyl peptidase III. *Forensic Science International* **113**: 147–51.

Simaga S, Babic D, Osmak M, Ilic-Forko J, Vitale L, Milicic D and Abramic M. 1998. DPP-III in malignant and non-Malignant gynaecological tissues. *European Journal of Cancer* **34** (3) 399–405.

Smyth M and O'Cuinn G. 1994. Dipeptidylaminopeptides of guineapig brain: specificity for short ogliopeptide sequences. *International Journal of Biochemistry* **26**(7) 913–21.

Swanson A A, Albers-Jackson B and McDonald J K. 1978. Mammalian lens Dipeptidyl Aminopeptidase 3. *Biochemistry Biophysics Research Communication* **84**: 1151–59.

Zhan H, Yamamoto Y, Shumiya S, Kunimatsu M, Nishi K, Ohkubo I and Kani K. 2001. Peptidases play an important role in cataractogenesis: an immunohistochemical study on lenses derived from Shumiya cataract rats. *Histochemistry Journal* **33** (9–10): 511–21.