Dipeptidyl Peptidase II : Regional and Subcellular Distribution in Goat Brain

PREETI GARG, SURESH PAL, NEERA RAGHAV,
RAMESH C. KAMBOJ and HARI SINGH*
Biochemistry Laboratory, Department of Chemistry, Kurukshetra University, Kurukshetra-132 119

Manuscript received 26 April 1991, revised 4 October 1991, accepted 28 October 1991

THE capacity of dipeptidyl peptidase II (EC 3.4.14.2) to hydrolyse peptides with penultimate prolyl residues[1] suggested its involvement in the breakdown of collagen connective tissue protein[2]. This

protease also plays a significant role in various diseased conditions like thromboembolism, myocardial infarctions, diabetes mellitus and alcoholism[3], human carcinoma cells[4] and in lung inflammation[5]. These two factors prompted us to study the regional and subcellular distribution of this protease in goat brain because bioactive peptides present in brain like substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) having penultimate prolyl residues may be the special targets of DPP II. In addition to its distributions in brain parts, the ability of certain cations and anions to modify the activity of DPP II has also been evaluated.

Abbreviations used are 4mβNA, -4-methoxy-β-naphthylamide ; βNA, -β-naphthylamide ; DPP II, dipeptidyl peptidase II.

Experimental

Fresh goat brain tissue removed from the freshly sacrificed goat was used. Lys-Ala-4mβNA (Bachem, Switzerland), Fast Garnet GBC (*o*-aminozotoluene diazonium salt) and 4-methoxy-β-naphthylamine (both Sigma) were used. A refrigerated Remi C-24 centrifuge machine was used for subcellular fractionation. The absorbance was recorded by using an EC spectrophotometer (350−950 nm range).

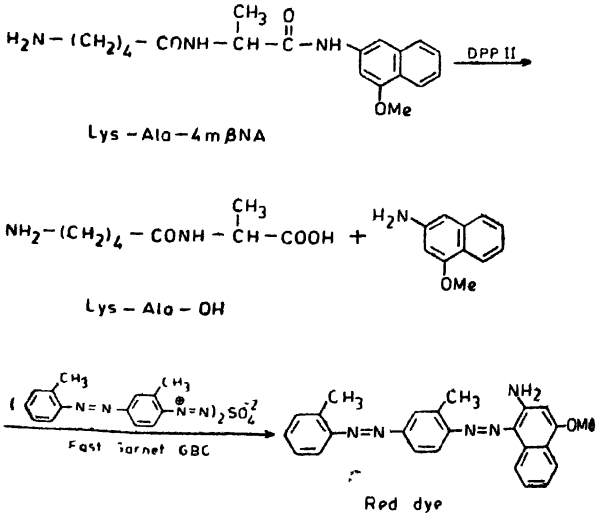
Enzyme homogenate : Fresh goat brain was homogenised (10%, w/v) in glass-distilled cold water containing 0.1% Triton X-100 three times for a period of 15 s each time. The homogenate was stored at 4° and was used as source of enzyme for the experiments.

Assay of DPP II : A simple colorimetric method for estimating DPP II activity in crude brain extract was used. The method adopted excluded the use of diisopropyl fluorophosphate (DFP), an inhibitor of serine proteases to stop the enzymatic reaction[6], which was done by lowering the pH of the assay mixture from 5.5 to 4.0. It was separately shown that no enzyme activity survived at pH 4.0 even without the addition of an inhibitor. The free serine $-CH_2OH$ group(s) at the enzyme active site did not interfere in the coupling reaction of the liberated 4-methoxy-β-naphthylamine with Fast Garnet GBC dye and hence these serine residues needed no modification. The detailed procedure of the colorimetric estimation of DPP II in crude brain extracts is as follows. The enzyme homogenate (0.5 ml) was added to the assay buffer (1.5 ml ; 10 m*M* sodium acetate, pH 5.5) and preincubated for 5 min at 40°. The enzymatic reaction was started by adding the substrate stock solution (50 μl ; Lys-Ala-4mβNA, 2 mg ml^{-1} DMSO). After 30 min, the reaction was stopped with the colouring reagent (2.0 ml ; prepared by mixing equal volumes of 1.0 *M* sodium acetate buffer of pH 4.0 and Fast Garnet GBC dye, 1 mg ml^{-1} in water). After 10 min, the red coloured dye was extracted with n-butanol (4.0 ml) and estimated at 520 nm. Both enzyme and substrate blanks were also included. The absorbance at 520 nm was then converted to activity in nanomoles of 4-methoxy-β-naphthylamine liberated per min per ml enzyme homogenate.

For subcellular localisation of DPP II, the goat brain was homogenised in cold 0.25% sucrose solution in a Remi glass homogeniser operating at moderately high speeds. This homogenate was subfractionated into nuclear (800×g pellet), mitochondrial-lysosomal (16000×g, pellet) and soluble (16000×g, supernatant) fractions by differential centifugation method[7]. Each fraction was then separately homogenised in presence of 0.1% Triton X-100 before measuring the activity of DPP II. For estimating distribution of DPP II in various regions of brain tissue, different parts of brain like cerebellum, medulla oblongata, thalamus, hypothalamus, pons-varolli, cerebrum and pituitary body were separately homogenised and processed for DPP II activity.

Results and Discussion

The DPP II activity has traditionally been assayed with a synthetic substrate Lys-Ala-4mβNA where the released 4-methoxy-β-naphthylamine is quantitated fluorometrically[6]. Due to the non-availability of a fluorometer, a simple colorimetric assay was used for DPP II where the released 4-methoxy-β-naphthylamine was coupled with Fast Garnet GBC dye (Scheme 1). Randell and Sannes[6] used diisopropyl fluorophosphate (DFP), a serine protease inhibitor to stop the enzyme reaction. DFP, besides being costly, is highly poisonous and its use has been drastically curtailed because of its hazardous nature. In the present method, the enzymatic reaction was stopped by lowering the pH to 4.0 where DPP II has no activity and this low pH does not interfere with the coupling reaction of 4-methoxy-β-naphthylamine with Fast Garnet GBC to yield a red dye to be estimated at 520 mn. A 1.0 *M* sodium acetate



Scheme 1

buffer (pH 4.0) was therefore used as stopping reagent. The high molarity of Na^+ served a dual purpose of providing and maintaining the pH at 4.0 and for inhibiting the enzyme DPP II. The free serine residue(s) at the active site did not interfere with the coupling reaction unlike thiol, $-CH_2SH$ group[8].

This colourimetric assay when used for assaying the DPP II activity of goat brain in the pH range 3.0–9.0, it gave a pH optimum 5.5 for DPP II[9]. Goat brain DPP II was inhibited by Na^+ ions. At 20 m*M* Na^+ ion concentration, the DPP II activity was reduced to half of the normal value while at 80 m*M* there was about 75% inhibition. In addition to Na^+ ions, other cations like Li^+, K^+ and $tris^+$ were also inhibitory to the activity of DPP II. The inhibition was seen to increase with the increase in size/atomic weight of the inhibitory cation (data not shown).

The enzyme DPP II was found to reside in the mitochondrial-lysosomal fraction of goat brain which contained 50% of the total activity (Table 1). A considerable amount of DPP II activity was also observed in nuclear (21%) and soluble (29%) fractions. In comparison to the known lysosomal enzyme cathepsin B (EC 3.4.22.1)[10], the activity of DPP II was low in lysosomal fractions. This lysosomal nature of DPP II has also been shown in other tissues[11].

TABLE 1—SUBCELLULAR LOCALISATION OF DPP II IN GOAT BRAIN*

Fraction	Total activity	% Activity
Homogenate	738.50	—
Nuclear (N)	195.50	20.77
Mitochondrial-Lysosomal (M-L)	466.70	49.60
Soluble (S)	298.85	29.63

*Each fraction was separately homogenised in presence of 0.1% Triton X-100. The activity in each fraction was quantitated using Lys-Ala-4mβNA as substrate at pH 5.5 and expressed as nmoles of 4-methoxy-β-naphthylamine released per min at 40°. The per cent activity has been calculated by comparing the activity of each fraction with the sum of activities (N+M-L+S) takan as 100.

Further, the localisation of the protease in different regions of the brain was also determined by separating it into its seven parts : cerebrum (CE), cerebellum (CL), pons-varolli (PV), thalamus (TH), hypothalamus (HT), medulla oblongata (MO) and pituitary gland (PG). DPP II activity was separately determined in each part after homogenisation. The cerebrum was found to contain maximum activity (64.9%) followed by cerebellum (12.7%), thalamus (7.32%), medulla oblongata (4.56%), pons-varolli (3.83%), hypothalamus (3.79%) and pituitary gland (3.42%). Though cerebrum contains the greatest percentage of total DPP II activity because of its largest size, pituitary body had the highest specific activity followed by medulla oblongata, pons-varolli, cerebellum, hypothalamus, cerebrum and thalamus in that order. Pituitary being the master gland of the brain tissue, controls and regulates the secretion of hormones to coordinate functions of different parts of the body. The heavy concentration of DPP II in terms of specific activity in pituitary gland may be suggestive of a major role for this protease in peptide processing.

Acknowledgement

Two of the authors (S.P. and N.R.) thank C.S.I.R., New Delhi, for financial assistance in the form of N.E.T. fellowships. Thanks are due to Prof. S. P. Singh, Chairman, Chemistry Department, for facilities.

References

1. D. A. EISENHAUER and J. K. MCDONALD, *J. Biol. Chem.*, 1986, **261**, 8859.
2. J. K. MCDONALD, A. R. HOISINGTON and D. A. EISENHAUER, *Biochem. Biophys. Res. Commun.*, 1985, **126**, 63.
3. T. VANHA-PERTTULA and J. K. KALLIOMAKI, *Clin. Chim. Acta*, 1972, **44**, 249.
4. M. KOMATSU, M. URADE, M. YAMAOKA, K. FUKUSAWA and S. HARADA, *J. Natl. Cancer Inst.*, 1987, **68**, 863.
5. S. H. RANDELL and P. L. SANNES, *Inflammation (NY)*, 1988, **12**, 67.
6. S. H. RANDELL and P. L. SANNES, *J. Histochem. Cytochem.*, 1985, **33**, 677.
7. H. KOENIG, *Methods Enzymol.*, 1974, **31**, 460.
8. A. J. BARRETT, *Anal. Biochem.*, 1972, **47**, 280.
9. J. K. MCDONALD, F. H. LEIBACH, R. E. GRINDELAND and S. ELLIS, *J. Biol. Chem.*, 1968, **243**, 4143.
10. R. C. KAMBOJ, S. PAL and H. SINGH, *J. Biosci.*, 1990, **15**, 397.
11. G. KRIEL, L. HAIML and G. SUCHANEK, *Eur. J. Biochem.*, 1980, **111**, 49 ; J. K. MCDONALD, T. J. REILLY, B. B. ZEITMAN and S. ELLIS, *J. Biol. Chem.*, 1968, **243**, 2028 ; J. K. MCDONALD and C. SCHWABE, *Biochim. Biophys. Acta*, 1980, **616**, 68.