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## Functional characterization and specific effects of various peptides on enzymatic activity of a DPP-III homologue from goat brain

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### Abstract

The purified dipeptidyl aminopeptidase from goat brain showed several characteristics similar to DPP-III although it possesses a dissimilar molecular weight and different inhibition behavior. The enzyme was found to be inhibited by metallochelators and thiol inhibitors which could be reversed by introducing metals and thiols, respectively. The enzyme activity is also significantly affected by DMSO and ethanol. It was found to be highly sensitive to even very low concentration of urea. The inhibitory potency of several dipeptides and bioactive peptides on this enzyme was investigated to characterize its active site. The highest potency was observed for the dipeptides having aromatic and bulky side chains such as Phe-Met, Leu-Arg, Met-Arg, Trp-Met and Leu-Trp.

**Keywords:** Dipeptidyl aminopeptidase, metallo enzyme, goat brain, Arg-Arg-4-methoxy- $\beta$ -naphthylamide, DPP, thiol enzyme, inhibition

### Introduction

Dipeptidylpeptidase III (DPP-III) is an aminopeptidase which was first reported in bovine pituitary gland [1] and later purified from several mammalian tissues [2]. The enzyme is a metallo protease having an optimum pH of 8–10 and specifically hydrolyzes Arg-Arg from the N-termini of dipeptidyl arylamides and oligopeptides. Though involvement of DPP-III in physiological processes is not well understood it is thought to be involved in the breakdown of oligopeptides like Leu and Met-enkephalin [3], angiotensin II or  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) [4].

Recently, we purified and characterized DPP from goat brain, which like DPP-III (EC 3.4.14.4) removes N-terminal dipeptides from Arginyl-Arginyl-4-methoxy- $\beta$ -naphthylamide (Arg-Arg-4m $\beta$ NA) at pH 8.5. The enzyme has a pI of 4.5 and a calculated molecular weight of 70 kDa. Our studies revealed enzyme inhibition by metallochelators and thiol inhibitors [2]. In the present study, we have further

characterized the DPP-III from goat brain. We found that the enzyme activity is affected by metal ions and thiol reagents. The effects of several peptides have been studied and it has been found that the enzyme displayed micromolar affinity for enkephalins. It appears that the enzyme might play a physiological role in regulating enkephalin disposition.

### Materials and methods

#### Materials

Phe-Met, Pro-Arg, His-Lys, Ser-Met, Pro-Phe, Pro-Glu, Leu-Trp, Leu-Arg, Asp-Ala, Phe-Leu, His-Leu, Arg-Phe, Trp-Met, His-Phe, Arg-Val, Gly-Glu, Phe-Ala, Met-Arg, Lys-Ala, Val-Tyr, Tyr-Ile, Tyr-Gly, His-Pro, Gly-Phe, Phe-Arg, Arg-Trp, Trp-Met, Asp-Phe-NH<sub>2</sub>, enkephalin, Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu), Gly-Phe-Leu, Met enkephalin (Tyr-Gly-Gly-Phe-Met), Gly-Arg and Arginyl-Arginyl-4methoxy- $\beta$ -naphthylamide (Arg-Arg-4m $\beta$ NA) were from

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Bachem Feinchemikalien Co., Budendorf, Germany. Dithioerythreitol (DTE), dithiothreitol (DTT), glutathione, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), *p*-aminophenyl mercuric acetate and *o*-phenanthroline were all from Sigma Chemical Co., St. Louis, MO, USA. Ethylene diaminetetraacetic acid (EDTA) and Tris-HCl were from HiMedia Chemical Company, Bombay.

### Methods

**Purification and assay of DPP.** DPP was purified and assayed as described by Dhanda et al [2]. Arg-Arg-4m $\beta$ NA was used as substrate in assay buffer (Tris-HCl, 50 mM, pH 8.5, containing 100 mM NaCl and 1 mM  $\beta$ -mercaptoethanol ( $\beta$ -ME)). One unit of enzyme activity was defined as the amount of enzyme that liberated one nanomole of 4m $\beta$ NA from the substrate per minute under assay conditions.

**Effect of different thiol compounds.** In order to explore the role of thiol in enzyme catalysis, the effect of different thiol compounds (DTE, DTT, cysteine, glutathione, thioglycolic acid and  $\beta$ -ME) on enzyme activity was studied. The enzyme was preincubated with each thiol compound in assay buffer ( $\beta$ -ME was not added to the assay buffer for these experiments) at 37°C for 10 min. The reaction was initiated by addition of 150  $\mu$ M of substrate. The activity is expressed as the percent activity compared to the control.

**Reversal of DTNB inhibition by thiol compounds.** As earlier reported [2], DPP-III from goat brain is inactivated by reaction with 2.5 mM DTNB. After extensive dialysis for 24 h against Tris-HCl buffer (50 mM, pH 7.0), the reversibility of inhibition was measured in the presence of reducing agents as done for thiol compounds.

**Effect of different metal ions.** The influence of various divalent metal ions ( $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Mg}^{2+}$ ) on enzyme activity was studied by addition of the appropriate chloride salts except  $\text{FeSO}_4$  for  $\text{Fe}^{2+}$ . The enzyme was preincubated in all cases with the appropriate salts in assay buffer at 37°C for 10 min. The reaction was started by addition of 150  $\mu$ M of substrate and the activity is expressed as the percent activity of the control.

**Reversal of *o*-phenanthroline inhibition by different metal ions.** Reactivation of enzyme activity from *o*-phenanthroline-inactivated DPP-III was measured under the following conditions. Enzyme treated with 2.5 mM *o*-phenanthroline for 10 min was extensively dialysed against Tris-HCl buffer (50 mM, pH 7.0) and

then assayed in the presence of different metal ions as done previously to study the effect of different metal ions and activity was expressed as percentage of control.  $\text{Li}^+$ ,  $\text{K}^+$  and  $\text{Hg}^{2+}$  were studied at one concentration and the effect of some metal ions was studied in the presence of another metal and also in the presence of EDTA.

**Effect of DMSO and ethanol.** The enzyme was separately incubated with different concentrations of DMSO and ethanol (1–15%; v/v) at 37°C for 10 min and the activity is expressed as percentage of the maximum.

**Stability of DPP-III under storage conditions.** The purified enzyme was stored at –20°C in 50 mM Tris-HCl buffer, pH 7.5 containing 10% glycerol. The enzyme was also stored without glycerol. Aliquots were drawn at regular intervals of time and the residual activity of the stored enzyme was estimated by standard assay conditions.

**Effect of urea.** The enzyme was incubated with different urea concentrations (0.1–2.0 M) at 37°C for 10 min and the residual enzyme activity was calculated and expressed as percentage of control. The renaturation of urea-treated enzyme was investigated under the following conditions: The enzyme treated with 1.0 M and 1.5 M urea for 10 min was extensively dialysed against Tris-HCl buffer (50 mM, pH 7.0) and assayed for the enzyme activity. The residual activity of the enzyme is expressed as percent activity as compared to control.

**Effect of different peptides,  $K_i$  determination and mode of inhibition by some potent peptide inhibitors.** To characterize the active site of the enzyme, a number of di-, tri- and oligopeptides were screened for their inhibitory potency. The enzyme was incubated with each peptide in assay buffer at 37°C for 10 min before assaying. The reaction was initiated by addition of 150  $\mu$ M of substrate. The activity is expressed as the percentage of the control. For more potent peptides such as Leu-Trp-Met-Arg-Phe-Ala, Arg-Phe-Ala, Gly-Phe-Leu, Leu-Trp-Met, Leu-Trp, Trp-Met-Asp-Phe-NH<sub>2</sub> (Gastrin tetrapeptide), Tyr-Gly-Gly-Phe-Leu, Tyr-Gly-Gly-Phe-Met, Val-Tyr and Trp-Met, their  $K_i$  value and mode of inhibition was determined. For these experiments a constant enzyme concentration was incubated with different peptide concentrations (from 10  $\mu$ M–500  $\mu$ M) and the enzyme activity was assayed. The experiment was carried out in triplicates for 3 batches. To the first batch, 40  $\mu$ M substrate was added. Similarly, in second and third batches, 100  $\mu$ M and 150  $\mu$ M substrate was added respectively. The reaction rates

were calculated in terms of nanomoles of 4m $\beta$ NA liberated per mL of enzyme.

## Results and discussion

### Effect of different thiol reagents

It has been shown earlier that sulphydryl (DTNB, PCMB) and metal chelating agents (EDTA and *o*-phenanthroline) reduce DPP-III activity from goat brain [2]. Further evidence of involvement of thiol groups in the catalysis was provided by the use of thiol compounds. The results of these studies are presented in Figure 1a. All the thiol reagents, except  $\beta$ -ME, inhibit DPP-III, which indicates involvement of thiol group in enzyme catalysis. The results are similar to those also reported for human placental DPP-III [5] except for  $\beta$ -ME which also inhibited the placental enzyme.

### Reversal of DTNB inhibition by different thiol compounds

DTNB (2.5 mM) inhibited the native enzyme as the enzyme had only 13% of its initial activity and the inhibition was irreversible on dialysis. This inhibition could be partially reversed by the addition of thiol compounds such as cysteine, glutathione, thioglycolic acid and DTT.  $\beta$ -ME and DTT completely restored

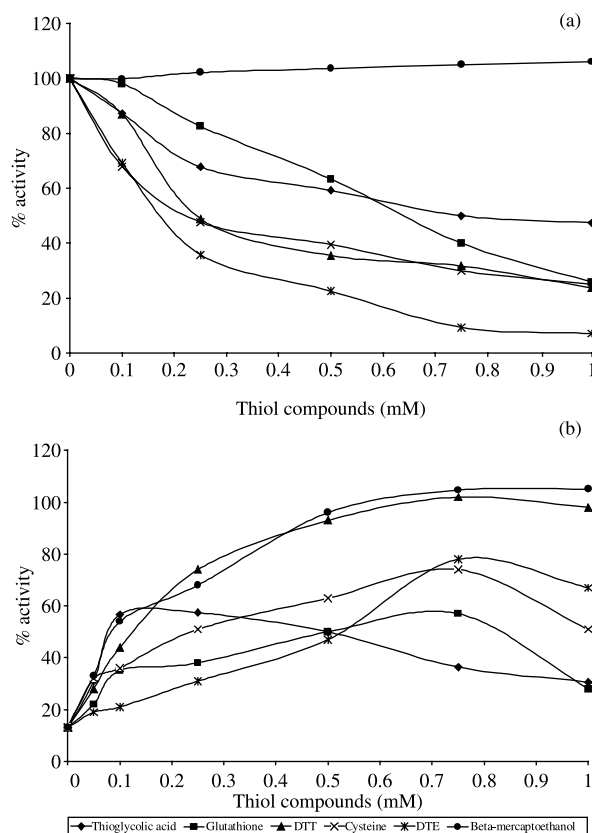


Figure 1. Effect of thiol compounds on (a) untreated and (b) DTNB-pretreated DPP-III.

the enzyme activity (Figure 1b). The results indicated that there is some involvement of a  $-SH$  group in the enzyme catalysis. However  $\beta$ -ME caused 50% inhibition of lens DPP-III activity. But PCMB-inhibited enzyme activity could be partially restored by a very high concentration of 7 mM  $\beta$ -ME [6].

### Effect of metal ions

As chelating agents were found inhibitory, the role of metal ions was studied in enzyme catalysis. The results presented show significant activation with  $Co^{2+}$  and inhibition with  $Zn^{2+}$ ,  $Ni^{2+}$  and  $Cu^{2+}$ . Other ions like  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Mn^{2+}$  and  $Fe^{2+}$  were slightly inhibitory (Figure 2a). Human RBC's DPP-III has been reported to be slightly activated by 10  $\mu$ M  $Zn^{2+}$  [7] whereas  $Co^{2+}$  only activated human placental DPP-III with enkephalin substrate [8] and not with Arg-Arg-4m $\beta$ NA [5].

### Reversal of *o*-phenanthroline inhibition by different metal ions

Incubation of DPP-III with *o*-phenanthroline (2.5 mM) resulted in 85% inhibition of enzyme activity. The suppressed activity could be partially recovered by addition of some metal ions like  $Zn^{2+}$ ,  $Ni^{2+}$ ,  $Co^{2+}$ ,  $Fe^{2+}$ ,  $Mn^{2+}$  and  $Cu^{2+}$  but none could completely restore the activity (Figure 2b).  $Mg^{2+}$  and  $Ca^{2+}$  did not exert any effect.  $Zn^{2+}$  at a concentration of 50  $\mu$ M was most effective for restoring the

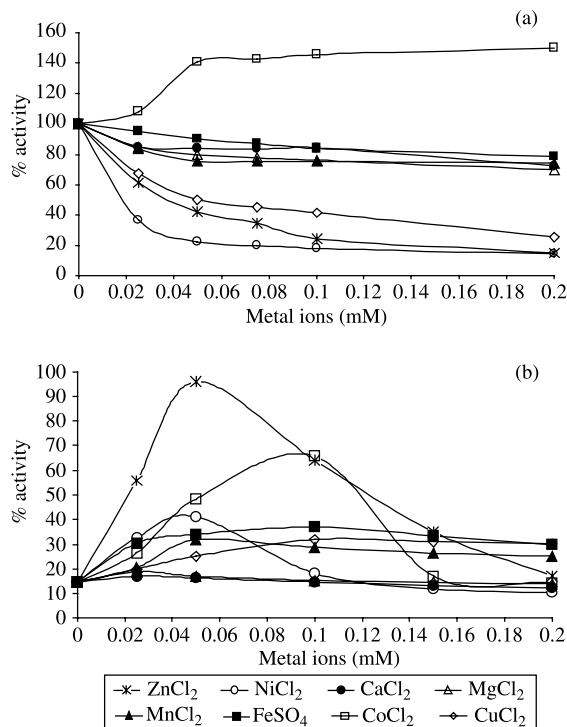


Figure 2. Effect of different divalent metal ions on (a) untreated and (b) *o*-phenanthroline pretreated DPP-III.

activity (~96%). The results indicated that it is a metalloprotease. On the basis of these studies, goat brain enzyme can be considered as a  $\text{Zn}^{2+}$ -containing metalloenzyme.

Our studies are in agreement to that of human placental DPP-III [5] but  $\text{Co}^{2+}$  was most effective in restoring the activity of EDTA-treated DPP-III from human red blood corpuscle enzyme [7] whereas  $\text{Ca}^{2+}$  and  $\text{Co}^{2+}$  were reported most effective in restoring the activity of EDTA-pretreated guinea pig brain DPP-III [9]. Metal ion activation is common in aminopeptidases. Though the reasons for  $\text{Co}^{2+}$ -activation for metallo-enzymes is not clear but like goat brain DPP,  $\text{Co}^{2+}$  is also reported to be an activator of a metallo-endopeptidase [10] and enkephalinase B [11], leucine aminopeptidase of bovine lens [12,13], human and porcine liver aminopeptidases [14,15], some microbial peptidases and leukocyte and erythrocyte aminopeptidases [16,17].

Additional metal ions were studied at a concentration of 0.1 mM. Effect of metals studied indicated that  $\text{Li}^+$  and  $\text{K}^+$  were slightly inhibitory whereas  $\text{Hg}^{2+}$  caused loss of ~90% activity which may be due to formation of a covalent mercaptide bond between the active thiol group and  $\text{Hg}^{2+}$ .  $\text{Hg}^{2+}$  was also reported as inhibitory by Lynn [18] and Lee and Snyder [3]. When metals were studied in combination, addition of  $\text{Co}^{2+}$  to  $\text{Ni}^{2+}$ -inhibited enzyme partially recovered the activity and the addition of  $\text{Zn}^{2+}$  to  $\text{Co}^{2+}$ -activated enzyme abolished the first metal effect (Table I), which indicate metal competition for the same binding site. This observation that the enzyme is active without metal ions and *o*-phenanthroline-treated enzyme had residual activity indicates the presence of slowly exchanging sites on goat brain aminopeptidase and this measured metal ion effect may be due to a regulatory site.

DPP-III was earlier classified as a serine protease [18,19]. However, unlike enzymes from other sources,

goat brain enzyme is apparently a metalloenzyme with cysteine residues either located on the catalytic site or involved in regulation. The differential sensitivity to the reagents cannot be explained at present. Several other peptidases of this type have been reported which are metalloenzymes having sulphhydryl groups at the active site of the enzyme [15,16]. Bovine lens aminopeptidase is inhibited by metal chelators, heavy metals and sulphhydryl reagents [20] and it was suggested that the metal ion is bound through a sulphhydryl group at the active site of enzyme [21]. The role of cysteine residues in regulation of rat DPP-III [22] and mammalian DPP-III [23] activity has been established.

**Effect of DMSO and ethanol.** Goat brain DPP is assayed with Arg-Arg-4mβNA dissolved in DMSO. Therefore, the effect of different DMSO concentrations was studied on enzyme activity. The activity progressively increased with increase in DMSO concentration up to 5% (Figure 3), followed by a gradual fall in enzyme activity with further increase in DMSO concentration. The reason for this behavior is not known, it might be due to the increase in substrate solubility. Unlike DMSO, the effect of ethanol on DPP was inhibitory, the enzyme losing about half of its activity at 5% ethanol concentration (Figure 3).

Table I. Effect of metal ions in combination with other metal ions and EDTA on the activity of DPP-III.

Inhibitor	% activity
Control (No inhibitor)	100
1 mM EDTA + 0.1 mM $\text{ZnCl}_2$	50.3
0.1 mM $\text{ZnCl}_2$	46.8
1 mM EDTA	49.2
0.1 mM $\text{CoCl}_2$	174.4
0.1 mM $\text{NiCl}_2$	12.4
0.01 mM $\text{ZnCl}_2$	62.6
0.1 mM $\text{CoCl}_2$ + 0.01 mM $\text{ZnCl}_2$	107.7
0.1 mM $\text{CoCl}_2$ + 0.1 mM $\text{ZnCl}_2$	54.5
0.1 mM $\text{NiCl}_2$ + 0.1 mM $\text{CoCl}_2$	53.6
0.1 mM $\text{HgCl}_2$	11.1
0.1 mM KCl	82.0
0.1 mM LiCl	73.0

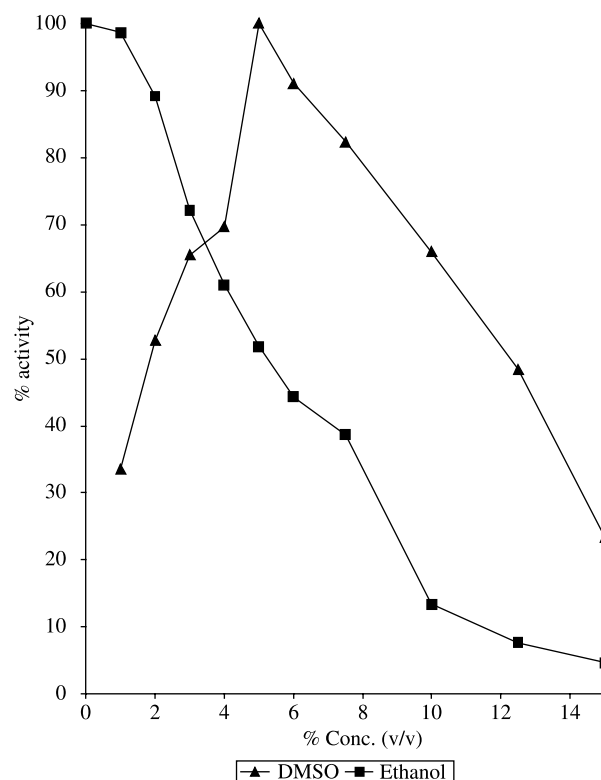


Figure 3. Effect of DMSO and ethanol on DPP-III activity.



Table II. Stability of DPP-III under storage conditions.

Time (in months)	% residual activity in the presence (or absence) of glycerol
Control	100
After 1 month	73.5 (61.85)
After 2 months	59 (21.97)
After 3 months	50 (13.32)
After 4 months	40.3 (7.01)
After 5 months	18 (1.72)
After 6 months	8 (0.25)

The purified DPP-III was stored in 50 mM Tris-HCl buffer pH 7.5 at  $-20^{\circ}\text{C}$  in the presence of 10% glycerol. The table shows % relative activity compared with the activity at the time of purification as 100. Values in parentheses show the activity in the absence of glycerol.

**Stability of DPP-III.** To find out the storage stability, enzyme activity was monitored at an interval of 1 month. It was found that the enzyme was sensitive to freezing. There was loss of about 25% activity in one month, 40% in 2 months and 50% in 3 months and almost total loss was seen after 6 months. Glycerol had some stabilizing effect as without glycerol there was loss of about 40% activity in one month and 70% in 2 months (Table II).

#### Effect of urea

The enzyme is very sensitive to even very low urea concentration. About 85% activity was lost at 1.0 M and almost complete loss of activity occurred at 1.5 M urea concentration (Figure 4). The enzyme inhibition was found to be irreversible as shown by dialysis.

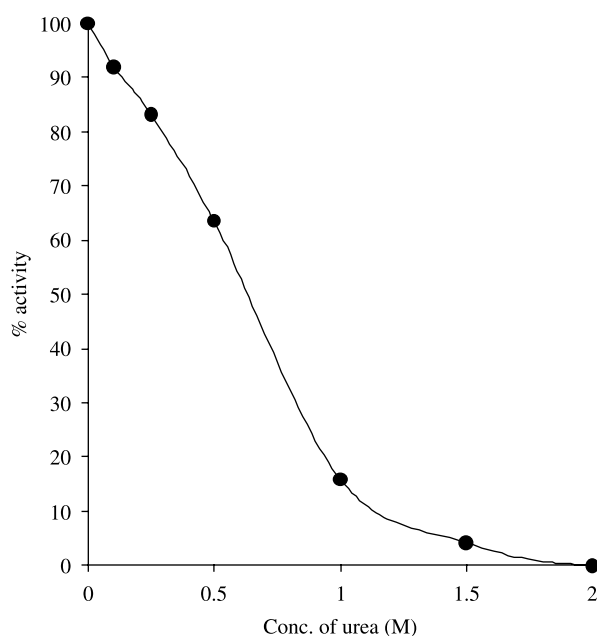


Figure 4. Effect of urea on DPP-III activity.

#### Effect of various peptides

The inhibitory potency of several dipeptides, tripeptides and a few oligopeptides on the rate of hydrolysis of Arg-Arg-4m $\beta$ NA by this dipeptidyl peptidase was investigated to explore the characteristics of its active site. Studies with peptides and small organic compounds are a common approach to identify potential substrates and first lead structures for drug design [24–25]. The results with the dipeptides used are presented in Table III and the tri-/tetra- and oligopeptides are summarized in Table IV.

Among the screened dipeptides, Leu-Arg, Met-Arg are potent inhibitors causing more than 95% inhibition at 0.5 mM concentration. Other potent inhibitors like Phe-Met, Leu-Trp, Tyr-Met, Val-Tyr, Phe-Arg were inhibitory in the range of 63%–85% at this concentration (Table III). For tri-/tetra- and oligopeptides, the highest inhibitory potency (more than 90%) was noted for Arg-Phe-Ala, Leu-Trp-Met-Arg-Phe-Ala and Leu-Trp-Met even at 0.5 mM concentration. At the same concentration, Trp-Met-Asp-Phe, Gly-Phe-Leu and Met-enkephalin inhibited enzyme activity to 80, 79 and 61%, respectively, while inhibition by other peptides was less than 60% (Table IV). Among the different screened peptides, it is evident that peptides with aromatic and bulky side chains on one/both amino acids were potent inhibitors. Similar studies have also been conducted for rat and guinea pig brain DPP-III [3,9] and enkephalinase B from calf brain [26].

The  $K_i$  values were determined with a large number of peptide inhibitors by assay where Arg-Arg-4m $\beta$ NA was used as substrate.  $K_m$  value for this substrate was 39  $\mu\text{M}$  [2]. The dose-response data was analysed graphically by the method of Dixon [27] (Figure 5a) to determine  $K_i$  values and also by the method of Cornish-Bowden [28] (Figure 5b) to determine the mechanism of inhibition. The  $K_i$  values calculated from graphs along with the type of inhibition for different peptides are listed in Table V. Different peptides inhibited the enzyme to different extents and  $K_i$  varied from 4–380  $\mu\text{M}$ . When competing substrate is used as an inhibitor, its  $K_i$  value is very close to that of the  $K_m$  [29]. Our studies reveal micromolar affinities for enkephalins ( $K_i$  90 and 130  $\mu\text{M}$  for Leu and Met-enkephalins, respectively; Table V), which is close to the observed value of 115  $\mu\text{M}$  for guinea pig brain DPP-III [9] but this is much higher as compared to that obtained for the rat brain DPP-III [19].

Differences in the  $K_i$  values of peptides of the same size indicate that DPP-III prefers peptides of a particular structure. The importances of interactions at  $S'_2$  and  $S'_3$  subsites is illustrated by differences in their affinities for Leu-Trp < Leu-Trp-Met < Leu-Trp-Met-Arg-Phe-Ala as indicated by their  $K_i$  values (Table V). The  $K_i$  decreased with increasing length on

Table III. Effect of different dipeptides on the activity of goat brain DPP-III.

Dipeptide	Concentration (mM)	% inhibition
Phe-Met	0.5	78
	1.0	85
Pro-Arg-OH	0.5	41
	1.0	54
His-Lys	0.5	36
	1.0	41
Ser-Met	0.5	32
	1.0	32
Pro-Phe	0.5	40
	1.0	56
Pro-Glu	0.5	35
	1.0	34
Leu-Trp	0.5	85
	1.0	90
Leu-Arg	0.5	100
	1.0	100
Asp-Ala	0.5	25
	1.0	25
Phe-Leu	0.5	40
	1.0	58
His-Leu	0.5	57
	1.0	76
Arg-Phe	0.5	46
	1.0	56
Trp-Met	0.5	76
	1.0	94
His-Phe	0.5	42
	1.0	46
Arg-Val	0.5	33
	1.0	49
Gly-Glu	0.5	39
	1.0	36
Phe-Ala	0.5	30
	1.0	41
Met-Arg	0.5	95
	1.0	99
Ile-His	0.5	37
	1.0	34
Lys-Ala	0.5	20
	1.0	23
Val-Tyr	0.5	63
	1.0	84
Tyr-Gly	0.5	35
	1.0	31
Tyr-Ile	0.5	60
	1.0	72
His-Pro	0.5	18
	1.0	16
Gly-Phe	0.5	32
	1.0	46
Phe-Arg	0.5	68
	1.0	75
Arg-Trp	0.5	40
	1.0	46

The enzyme was incubated at 37°C in the standard reaction mixture with the substrates at a final concentration of 0.15 mM. The relative activity was calculated in relation to the most hydrolyzed substrate, Arg-Arg-4mβNA (100%).

the C-terminus for the above 3 peptides. Multiple bindings within the active site have been reported for the enkephalin-degrading DPP of porcine brain [30] and human liver alanine aminopeptidase [31].

Table IV. The effect of different tripeptides/tetrapeptides/oligo-peptides on the activity of goat brain DPP-III.

Peptide	Concentration (mM)	% inhibition
Arg-Phe-Ala.	0.5	93
	1.0	99
Leu-Trp-Met-Arg-Phe-Ala	0.5	99
	1.0	100
Leu-Trp-Met	0.5	90
	1.0	92
Ala-Ala-Ala-Ala	0.5	58
	1.0	49
Tyr-Gly-Gly-Phe	0.5	40
	1.0	53
Trp-Met-Asp-Phe	0.5	80
	1.0	82
Ala-Ala-Ala	0.5	No inhibition
	1.0	4
Gly-Phe-Leu	0.5	79
	1.0	82
Tyr-Gly-Gly-Phe-Leu (Leu-enkephalin)	0.5	56
	1.0	92
Tyr-Gly-Gly-Phe-Met (Met-enkephalin)	0.5	61

The enzyme was incubated at 37°C in the standard reaction mixture with the substrates at final concentration of 0.15 mM. The relative activity was calculated in relation to the most hydrolyzed substrate, Arg-Arg-4mβNA (100%).

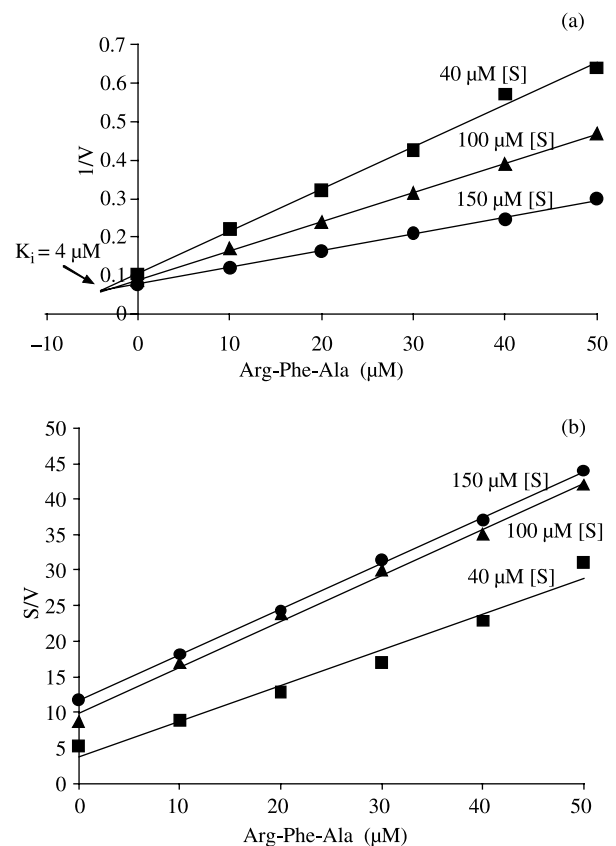


Figure 5. Dixon plot for determination of (a) apparent inhibition constant  $K_i$  and (b) Cornish-Bowden plot for DPP-III with Arg-Phe-Ala.

Table V. Inhibition of DPP-III from goat brain with different peptides.

Peptide	K <sub>i</sub> ((M)	Type of inhibition
Leu-Trp-Met-Arg-Phe-Ala	7	Competitive
Arg-Phe-Ala	4	Competitive
Gly-Phe-Leu	15	Non-competitive
Leu-Trp-Met	31	Competitive
Leu-Trp	39	Competitive
Gastrin tetra peptide-NH <sub>2</sub>	55	Competitive
Leu-enkephalin	90	Competitive
Met-enkephalin	130	Competitive
Val-Tyr	250	Competitive
Trp-Met	380	Competitive

Analysis of kinetics of the reaction between a proteinase and a potential inhibitor delineates the likely control point in a complex biological system and is of utmost importance for the therapeutic intervention [32]. The present studies on diverse peptides may help in designing some new and more effective inhibitors for this enzyme. Though no specific function can be assigned to goat brain aminopeptidase, affinities in the micromolar range for enkephalins show that the enzyme may hydrolyze these bioactive peptides and has functional homology with DPP-III. The molecular structure and nature of the enzyme is still obscure and is presently the subject of investigation.

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### References

- [1] Ellis S, Nuenke JM. Dipeptidyl arylamidase III of pituitary, purification and characterization. *J Biol Chem* 1967;242:4623–4629.
- [2] Dhanda S, Singh H, Singh J, Singh TP. Isolation, purification and characterization of a DPP-III homologue from goat brain. *Protein Express Purif* 2007;52(2):297–305.
- [3] Lee M, Snyder SH. Dipeptidyl aminopeptidase III of rat brain. Selective affinity for enkephalin and angiotensin. *J Biol Chem* 1982;257:12043–12050.
- [4] Grdisa M, Vitale L. Types and localization of aminopeptidases in different human blood cells. *Int J Biochem* 1991;23:339–345.
- [5] Shimamori Y, Watanabe Y, Fujimoto Y. Purification and characterization of dipeptidyl aminopeptidase III from human placenta. *Chem Pharm Bull* 1986;34(8):3333–3340.
- [6] Swanson AA, Albers-Jackson B, McDonald JK. Mammalian lens dipeptidyl aminopeptidase III. *Biochem Biophys Res Commun* 1978;84:1151–1159.
- [7] Abramic M, Zubanovic M, Vitale L. Dipeptidyl peptidase III from human erythrocytes. *Biol Chem* 1988;369:29–38.
- [8] Shimamori Y, Watanabe Y, Fujimoto Y. Human placental dipeptidyl aminopeptidase III: Hydrolysis of enkephalins and its stimulation by cobaltous ion. *Biochem Med Metb Biol* 1988;40:305–310.
- [9] Smyth M, O'Cuinn G. Dipeptidyl aminopeptidase III of guinea pig brain: Specificity for short oligopeptide sequences. *J Neurochem* 1994;63:1439–1445.
- [10] Orlowski M, Michaud C, Chu TG. A soluble metalloendopeptidase from rat brain. Purification of the enzyme and determination of specificity with synthetic and natural peptides. *Eur J Biochem* 1983;135:81–88.
- [11] Inaoka Y, Tamaoki H. Purification and characterization of enkephalinase B from rat brain membrane. *Biochim Biophys Acta* 1987;25:27–35.
- [12] Lasch J, Kudernatsch W, Hanson H. Bovine-Lens leucine aminopeptidase kinetic studies with activators and competitive inhibitors. *Eur J Biochem* 1973;34:53–57.
- [13] Thompson GA, Carpenter FH. Leucine aminopeptidase (bovine lens). The relative binding of cobalt and zinc to leucine aminopeptidase and the effect of cobalt substitution on specific activity. *J Biochem* 1976;251:1618–1624.
- [14] Garner CW, Behal FJ. Human liver aminopeptidase. Role of metal ions in mechanism of action. *Biochemistry* 1974;13:3227–3233.
- [15] Imamura T, Kawata S, Ninomiya K, Makisuma S. Porcine liver aminopeptidase. Further characterization of its sulfhydryl groups. *J Biochem* 1983;94:267–273.
- [16] Aratani H, Kawata S, Tsuruyama S, Yoshida N, Makisumi S. Purification and characterization of an aminopeptidase from bovine leukocytes. *J Biochem* 1984;96:107–115.
- [17] Vitale LJ, Zubanovic M, Abramic A. Properties and distribution of aminopeptidase and dipeptidylaminopeptidase III of human erythrocytes. *Acta Biol Med Germ* 1981;40:1489–1495.
- [18] Lynn KR. Isolation and some properties of dipeptidyl peptidase II and III from porcine spleen. *Int J Biochemistry* 1991;23:47–50.
- [19] McDonald JK, Schwabe C. Proteinases in mammalian cells and Tissues, Amsterdam: Elsevier / North Holland Biomedical Press; p 311–391.
- [20] Wolff JB, Resnik RA. Aminopeptidase of the ocular lens I. Metal-ion requirements and synergistic activation. *Biochim Biophys Acta* 1963;588:612.
- [21] Cuypers HT, Van Loon-Klassen L, Vree Egberts WTM, De Jong WW, Blomendal H. Sulfhydryl content of bovine eye lens leucine aminopeptidase. Determination of the reactivity of the sulfhydryl groups of the zinc metalloenzyme, of the enzyme activated by Mg<sup>2+</sup>, Mn<sup>2+</sup>, and Co<sup>2+</sup> and of the metal-free apoenzyme. *J Biol Chem* 1982;257:7086–7091.
- [22] Li YH, Maeda T, Tamane T, Ohturbo I. Alteraction of rat dipeptidyl peptidase III by site-directed mutagenesis: Cysteine (176) is at regulatory residue for the enzyme activity. *Biochem Biophys Res Commun* 2000;276:553–558.
- [23] Abramic M, Simaga S, Osmak M, Cicin-Sain L, Vukelic B, Vlahovick K, Doloveak L. Highly reactive cysteine residues are part of the substrate binding site of mammalian dipeptidyl peptidase III. *Int J Biochem Cell Biol* 2004;36(3):243–446.
- [24] Moos WH, Green GD, Pavia MR. Recent advances in the generation of molecular diversity. *Annu Rep Med Chem* 1993;28:315.
- [25] Thompson LA, Ellman JA. Synthesis and applications of small molecule libraries. *Chem Rev* 1996;96:555.
- [26] Van Amsterdam JG, Van Buuren KJ, Koomen KJKP, Soudijn W. Effect of various enkephalin analogs and dipeptides on the enzymatic activity of enkephalinase B isolated from calf-brain striatum. *Life Sci* 1986;38(12):1111–1118.



- [27] Dixon M. The graphical determination of  $K_m$  and  $K_i$ . *Biochem J* 1953;55:170–171.
- [28] Cornish-Bowden A. simple graphical method for determining the inhibition constants of mixed, uncompetitive and non-competitive inhibitors. *Biochem J* 1974;137:143–144.
- [29] Chu TG, Orlowski M. Soluble metalloendopeptidase from rat brain: Action on enkephalin-containing peptides and other bioactive peptides. *Endocrinol* 1985;116:1418–1425.
- [30] Cherot P, Fournie MC, Laval J. Purification and characterization of an enkephalin-degrading dipeptidyl-aminopeptidase from porcine brain. *Biochemistry* 1986;25:8184–8191.
- [31] Garner CW, Behal FJ. Human liver alanine aminopeptidase. Inhibition by amino acids. *Biochemistry* 1975;14:3208–3212.
- [32] Travis J, Salvesen GS. Human plasma proteinase inhibitors. *Annu Rev Biochem* 1983;52:655.