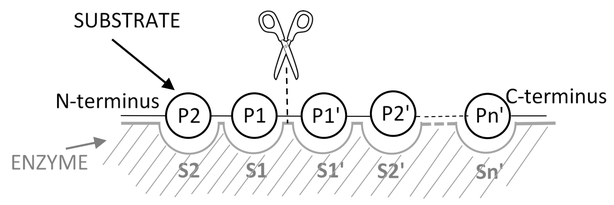
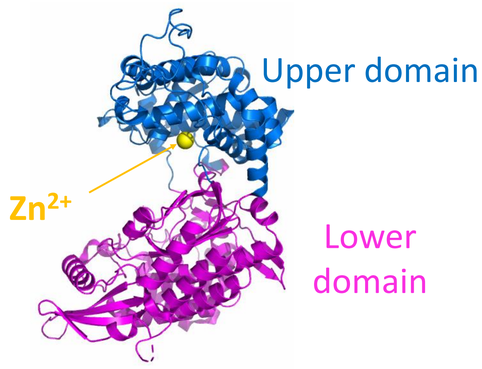
NEUROPEPTIDES - SUPSTRATES OF DIPEPTIDYL PEPTIDASE III

Sanja Tomić[1], Zrinka Karačić[1], Antonija Tomić[1], Lidija Brkljačić[1], Filip Šupljika[2]

[1]*Institute Ruđer Bošković, Bijenička cesta 54, 10 000 Zagreb, Croatia*
[2]*University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb , Croatia*

Dipeptidyl-peptidase III (**DPP III**) is a monozinc peptidase that catalyzes the hydrolytic cleavage of dipeptides sequentially from the N-terminus of peptides of three or more amino acids.





It is widely distributed in mammalian tissues and is thought to be involved in the final steps of normal intracellular protein degradation. However, its marked affinity for some bioactive peptides (angiotensins and opioid peptides) suggests more specific functions, such as its role in blood pressure regulation and its involvement in the mammalian pain regulatory system.

EXPERIMENTAL AND COMPUTATIONAL STUDY

HPLC-MS, ITC and fluorimetric measurements

peptide	sequence	HPLC-MS (cleaved)[a]	K_d / μM[b]	K_i / μM[c]
I-tynorphin	IVYPW	Y	0.0973 ± 0.0091	0.00045 ± 0.00005
S-tynorphin	SVYPW	Y	0.298 ± 0.061	0.0077 ± 0.0007
tynorphin	VVYPW	Y	0.386 ± 0.127	0.0112 ± 0.0008
valorphin	VVYPWTQ	Y	1.78 ± 0.21	0.0365 ± 0.0029
angiotensin II	DRVYIHPF	Y	2.22 ± 0.24	4.4 ± 0.5
Leu-valorphin-Arg	LVVYPWTQR	Y	2.50 ± 1.92	5.2 ± 0.5
hemorphin-4	YPWT	Y	39.4 ± 14.6	6.5 ± 0.7
endomorphin-2	YPFF	Y	40.1 ± 4.8	10.4 ± 1.0
Leu-enkephalin	YGGFL	Y	118 ± 39	10.4 ± 1.4
β-casomorphin	YPFVEPI	Y	130 ± 87	1.0 ± 0.1
Arg-vasopressin	C*YFQNC*PRG	N	n. d.	n. d.[d]
hemopressin	PVNFKFLSH	N	n. d.	n. d.
β-neoendorphin	YGGFLRKYP	N	n. d.	n. d.
dynorphin A (1-8)	YGGFLRRI	50% ?	n. d.	n. d.

* disulfide bridge
[a]cleavage (**Y**es/**N**o) HPLC-MS - peptide degradation (1 mM peptide & 0.18 μM enzyme) after 24 h (25°C ammonium bicarbonate buffer pH = 7.4)
[b]Thermodynamic parameters of peptide binding to human DPP III at 25 °C and pH = 7.5 in 20 mM TrisHCl buffer
[c] inhibition constant (inhibition of enzyme-catalyzed cleavage of artificial substrate Arg_2-2NA at 25 °C in 20 mM TrisHCl buffer pH = 7.5)
[d] no inhibition trend detected with peptide in the range of 1-50 μM

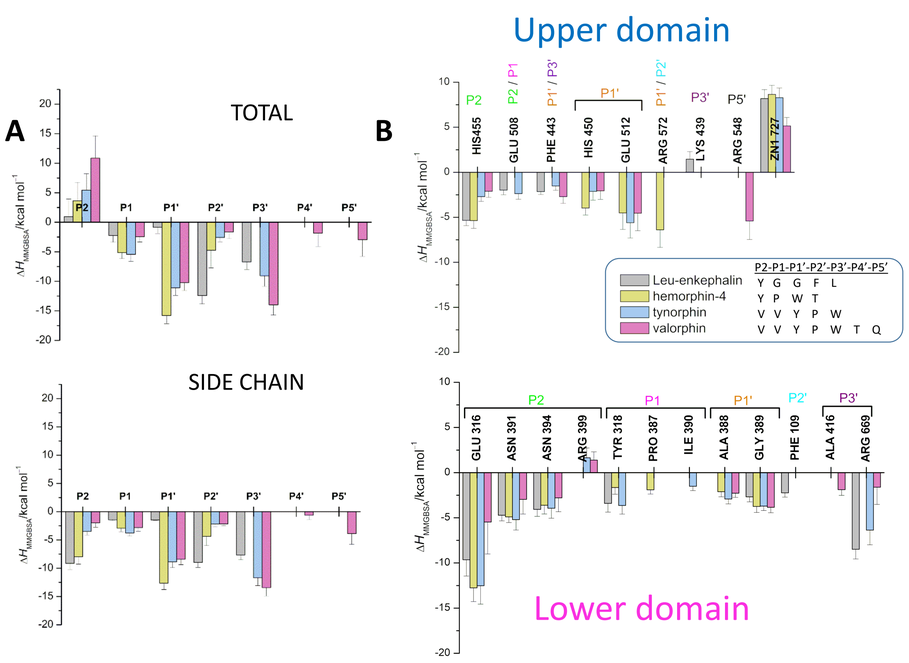
Kinetic parameters of peptide degradation as measured by ITC using SIM at 25°C in 50 mM TrisHCl buffer with 100 mM NaCl and pH 7.5

	$\Delta_r H$ (kJ/mol)	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($s^{-1}\,M^{-1}$)
Leu-valorphin-Arg	-6.4 ± 0.3	33.9 ± 6.4	0.35 ± 0.09	$1.03 \cdot 10^4$
Leu-enkephalin	-6.55 ± 0.07	34.7 ± 5.7	1.08 ± 0.12	$3.11 \cdot 10^4$
hemorphin-4	-7.5 ± 0.7	55.1 ± 13.1	6.11 ± 0.96	$1.11 \cdot 10^5$

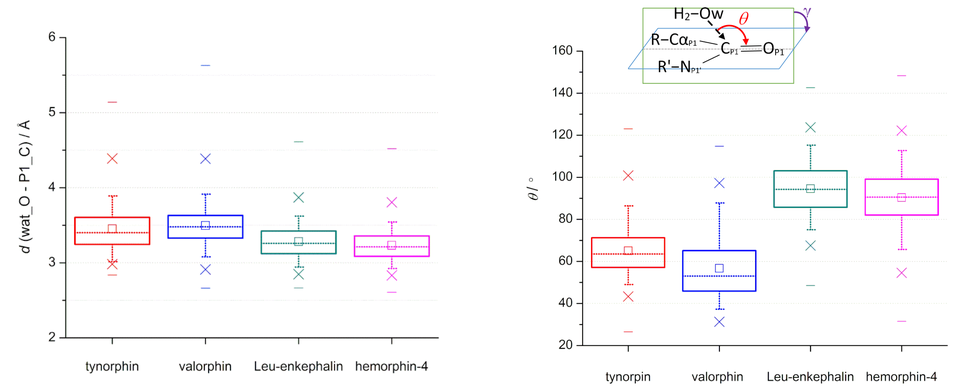
Ligand binding (MD simulations)

(*AMBER20, 2 x 1μs, NpT ensamble, ff19SB force field, OPC water*)

Residue based MM/GBSA binding enthalpies calculated for amino acid residues of ligand (A) and protein (B) during the last 0.6 μs of MD simulations of DPP III – peptide complexes



Box plots of the distance between the oxygen atom of the water molecule (Ow) and carbonyl carbon atom at ligand P1 position (C), and an angle defining the direction of OH^- attack calculated between O-C-Ow atoms



CONCLUSION: The neuropeptide hemorphin-4 was identified as a new substrate of DPP III, whereas valorphin, Leu-valorfin-Arg, and β-casomorfin, previously identified as inhibitors of DPP III, were found to be its substrates. The computational data helped explain the differences between substrates that are effectively hydrolyzed and those that are slowly hydrolyzed by DPP III. Molecular modeling of selected peptides revealed uniform binding to the lower domain β-strand residues of DPP III *via* peptide backbone atoms, but also previously unrecognized stabilizing interactions with conserved residues of the metal-binding site and catalytic machinery in the upper domain. In addition, the position of the water molecule performing the nucleophilic attack was found to be more favorable in the complexes with good substrates than with slow substrates.

REFERENCE: Karačić, Zrinka; Šupljika, Filip; Tomić, Antonija; Brkljačić, Lidija; Tomašić Paić, Ana; Ćehić, Mirsad; Tomić, Sanja, Neuropeptides, substrates and inhibitors of human dipeptidyl peptides III, experimental and computational study - a new substrate identified. *International Journal of Biological Macromolecules*, **220** (2022), 1390-1401.

ACKNOWLEDGEMENTS: This work has been fully supported by Croatian Science Foundation under the project IP-2018-01-2936 and by Foundation of the Croatian Academy of Sciences and Arts.