Computational modelling an aid to understand binding and hydrolysis of neuropeptides in the active site of human DPP III

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

[1]*Institute Ruđer Bošković, Bijenička cesta 54, 10 000 Zagreb, Croatia*
[2]*Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10 000 Zagreb, Croatia*

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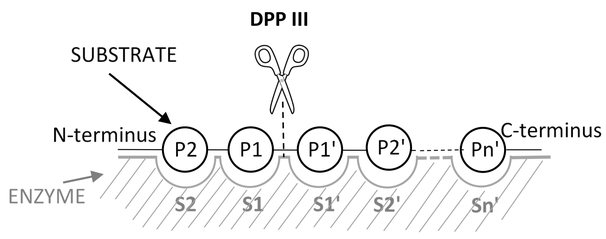
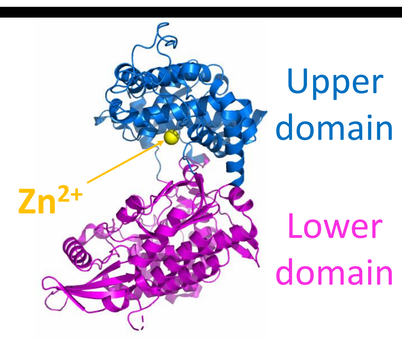
Dipeptidyl-peptidase III (**DPP III**; EC 3.4.14.4) is a two-domain monozinc exopeptidase of the peptidase family M49 that hydrolyzes dipeptides from the unsubstituted N-terminus of its substrates.





BROAD SUBSTRATE SPECIFICITY

DPP III is considered to be an enzyme with broad substrate specificity with studies showing that tetrapeptides to octapeptides are the best substrates. As it is located in the cytosol, it is presumed to have a role in the final stages of protein turnover. It has also been found in extracellular fluids, where it is involved in the regulation of blood pressure through its activity on angiotensin II and its derivatives, and, due to its affinity and activity towards various neuropeptides, among them enkephalins and endomorphins, it is thought to play a role in pain regulation.

To deepen the understanding of natural substrates of human DPP III various neuropeptides were tested as its substrates and inhibitors.

CALORIMETRIC MEASUREMENTS

Table 1. Thermodynamic parameters of peptide binding to human DPP III at 25 °C and pH = 7.5 in 20 mM TrisHCl buffer.

peptide	K_d / μM	$\Delta_r H$ /kcal mol^{-1}	$\Delta_r G$ /kcal mol^{-1}	$-T^*\Delta_r S$ /kcal mol^{-1}
I-tynorphin	0.0973 ± 0.0091	8.01 ± 0.24	-9.58 ± 0.05	-17.6 ± 0.2
S-tynorphin	0.298 ± 0.061	5.69 ± 0.24	-8.91 ± 0.12	-14.7 ± 0.4
tynorphin	0.386 ± 0.127	6.19 ± 0.29	-8.77 ± 0.19	-15.0 ± 0.1
valorphin	1.78 ± 0.21	4.64 ± 0.19	-7.86 ± 0.07	-12.5 ± 0.1
angiotensin II	2.22 ± 0.24	6.17 ± 0.72	-7.72 ± 0.07	-13.9 ± 0.6
Leu-valorphin-Arg	2.50 ± 1.92	4.61 ± 2.15	-7.77 ± 0.45	-12.4 ± 1.8
hemorphin-4	39.4 ± 14.6	8.70 ± 1.82	-6.05 ± 0.24	-14.7 ± 1.6
endomorphin-2	40.1 ± 4.8			
Leu-enkephalin	118 ± 39			
β-casomorphin	130 ± 87			
Arg-vasopressin	n. d.			
hemopressin	n. d.			
β-neoendorphin	n. d.			

Table 2. 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.

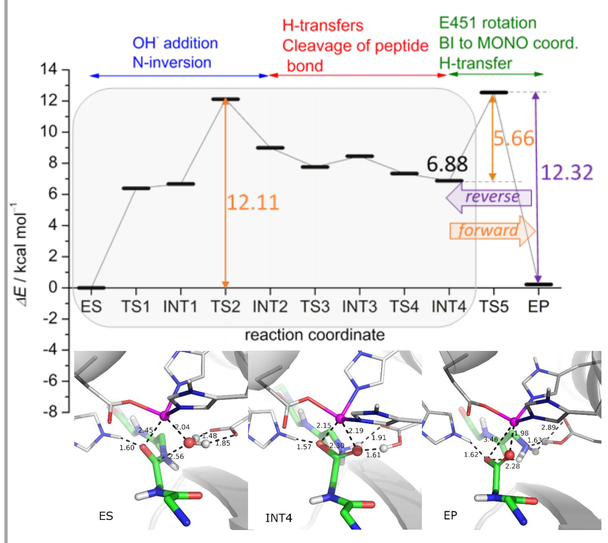
peptide	$\Delta_r H$ / kcal mol^{-1}	K_M / μM	k_{cat} / s^{-1}	(k_{cat}/K_M) / s^{-1} M^{-1}
Leu-valorphin-Arg	-1.53 ± 0.07	33.9 ± 6.4	0.35 ± 0.09	$1.03 \cdot 10^4$
Leu-enkephalin	-1.57 ± 0.02	34.7 ± 5.7	1.08 ± 0.12	$3.11 \cdot 10^4$
hemorphin-4	-1.79 ± 0.17	55.1 ± 13.1	6.11 ± 0.96	$1.11 \cdot 10^5$

COMPUTATIONAL MODELLING

Mechanism of hydrolysis
(QM/MM calculations)[1,2]

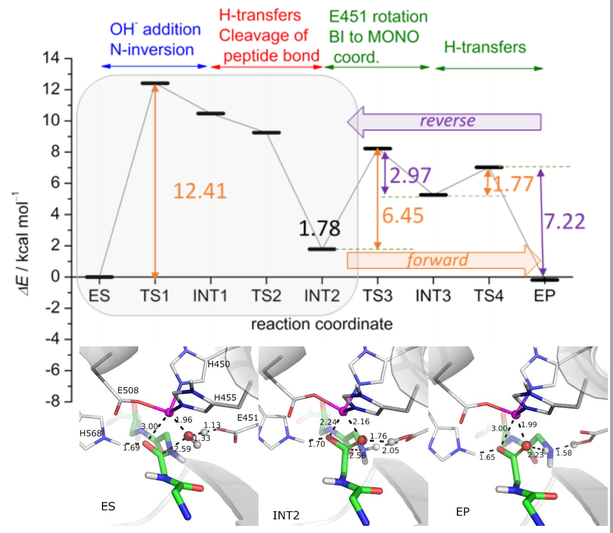
„GOOD" substrate

Tyr – Gly ÷ Gly – Phe – Leu
[Leu-enkephalin]



„SLOW" substrate

Val – Val ÷ Tyr – Pro – Trp
[tynorphin]



➢ *ONIOM calculations: B97D/[6-31G(d)+LanL2DZ-ECP] + ZPVE$_{B97D/[6\text{-}31G(d)+LanL2DZ\text{-}ECP]}$*

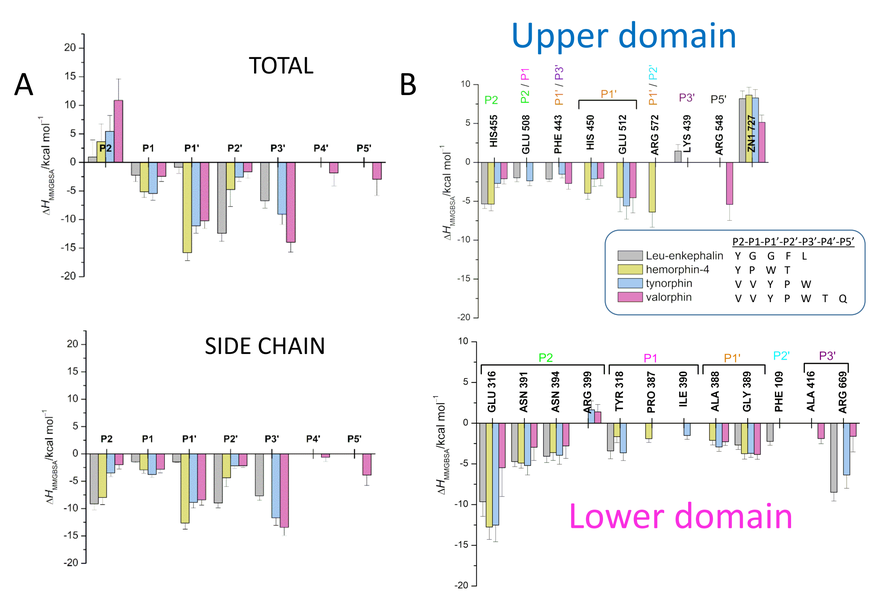


Figure 1. MM/GBSA binding enthalpies of DPP III – peptide complexes calculated for amino acid residues of the ligand (A) and the protein (B) during last 0.6 μs of MD simulations.

Ligand binding
(MD simulations)

➢ *AMBER 20, 2 x 1μs, NpT ensamble, ff19SB force field, OPC water molecules, extended 4-ligand hybrid bonded/non-bonded parameters for Zn(II)[3]*

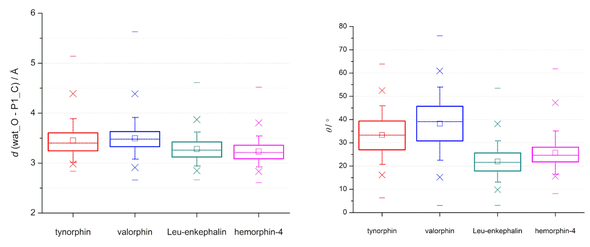


Figure 2. Box plots of the distance between the oxygen atom of the water molecule and carbonyl carbon atom at ligand P1 position, and angle between a normal vector to the plane of the 2nd peptide bond and a vector defined by the direction of the OH^- attack.

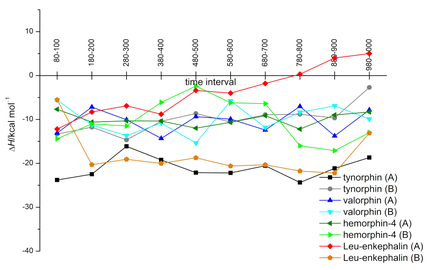


Figure 3. MM/PBSA energies for the DPP III – peptide complexes calculated on a set of 10 equally distributed intervals from 1 μs long MD simulations.

CONCLUSION: We identified the opioid peptide hemophin-4 as possible physiological human DPP III substrate. Additionally, we showed that valorphin- and tynorphin-derived peptides are substrates of this enzyme. QM/MM calculations and MD simulations in combination with free energy calculations uncovered differences in the catalytic efficiency of the enzyme during peptide hydrolysis of good and slow substrates.

REFERENCES: 1. A. Tomić et. al *Phys. Chem. Chem. Phys.* 18 (**2016**)
2. A. Tomić, S. Tomić *Int. J. Mol. Sci.* 23 (**2022**)
3. A. Tomić et. al *J. Chem. Inf. Model.* 59 (**2019**)

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