Role of dipeptidyl peptidase III in pain regulation through cleavage of neuropeptides

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Dipeptidyl peptidase III (DPP III) is a unique zinc-dependent exopeptidase that hydrolyses dipeptides from the N-terminus of short peptides, typically 3 to 10 peptides in length [1]. It is a cytosolic protein with a presumed role in intracellular protein catabolism and turnover. By *in vitro* assays it has been shown to act towards various endogenous peptides, such as angiotensin II and its derivatives, Leu- and Met-enkephalins and endomorphins 1 and 2. This enzyme is widely expressed in human tissues, but its colocalization with opioid peptides in the central nervous system suggest a role for this enzyme in the mammalian endogenous pain regulation system [2]. It has also been shown to have a role in blood pressure regulation by affecting the renin-angiotensin system in *in vivo* experiments on *knock-out* mice [3]. We set up to test various neuropeptides as potential substrates and inhibitiors of human DPP III, and we chose them keeping in mind the sequence previously confirmed DPP III substrates.

METHODS

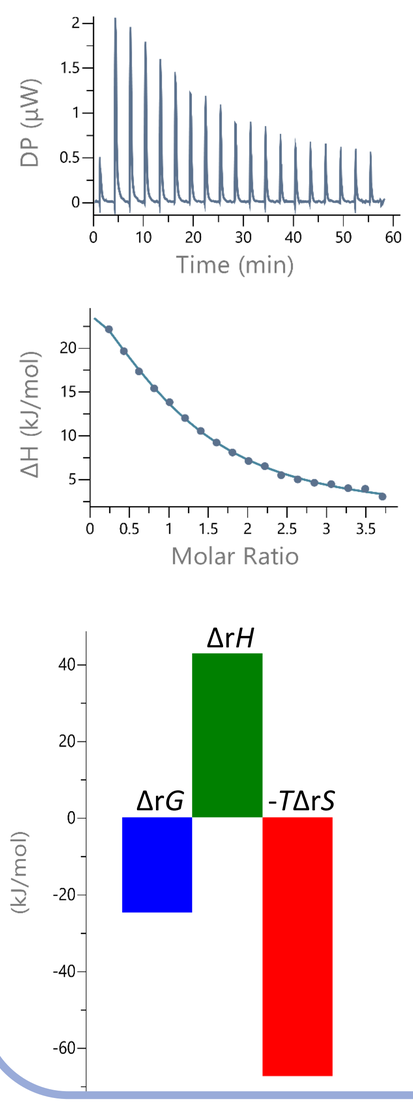
To identify new peptide substrates of DPP III, we used HPLC-MS to detect enzymatic activity of human DPP III towards a selected list of untested neuropeptides, as well as known DPP III substrates. Peptide degradation was monitored by quantification of remaining peptide in the reaction mixture. Peptide binding affinity was then analysed using isothermal titration calorimetry (ITC), where an inactive variant of human DPP III, E451A, was incubated with an excess of peptide.

RESULTS

SUBSTRATES :
hemorphin-4
valorphin
Leu-valorphin-Arg
β-casomorphin

Not substrates nor inhibitors:
vasopressin
hemopressin
β-neoendorphin

ITC titration and signature plot of inactive human DPP III variant E451A with hemorphin-4



Thermodynamic parameters for DPP III substrates:

peptide	sequence	K_D / µM	$\Delta_r H$ / kJ mol^{-1}	$\Delta_r G$ / kJ mol^{-1}	$-T^*\Delta_r S$ / kJ mol^{-1}
I-tynorphin	IVYPW	0.0973 ± 0.0091	33.5 ± 1.0	-40.1± 0.2	-73.6 ± 0.8
S-tynorphin	SVYPW	0.248 ± 0.061	23.8 ± 1.0	-37.3 ± 0.5	-61.6 ± 1.6
tynorphin	VVYPW	0.386 ± 0.127	25.9 ± 1.2	-36.7 ± 0.8	-62.6 ± 0.4
valorphin	VVYPWTQ	1.78 ± 0.21	19.4 ± 0.8	-32.9 ± 0.3	-52.3 ± 0.5
angiotensin II	DRVYIHPF	2.22 ± 0.24	25.8 ± 3.0	-32.3 ± 0.3	-58.1 ± 2.7
Leu-valorphin-Arg	LVVYPWTQR	2.50 ± 1.92	19.3 ± 9.0	-32.5 ± 1.9	-51.7 ± 7.6
Leu-enkephalin	YGGFL	11.8 ± 3.9			
hemorphin-4	YPWT	39.4 ± 14.6	36.4 ± 7.6	-25.3 ± 1.0	-61.7 ± 6.6
endomorphin-2	YPFF	40.1 ± 4.8			
ß-casomorphin	YPFVEPI	130 ± 87			

We determined a significant entropic contribution to the binding free energy, characteristic of DPP III, for all peptides cleaved by the enzyme. Among newly identified enzyme substrates, hemorphin-4 and valorphin are opioid peptides with antinociceptive properties, derived from proteolytic cleavage of hemoglobin [4].

FURTHER EXPERIMENTS

We want to directly measure the kinetic parameters for peptide cleavage. We will attempt to achieve this using HPLC-MS/MS. In order to do that, we need to precisely quantify very small changes in peptide amounts (less that 5% difference), which may turn out to be challenging. An alternative approach using ITC in single-turnover experiments might also be applicable. Our results should help to clarify the role of DPP III in pain regulation.

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