Microscale thermophoresis measurements of the interaction between human dipeptidyl peptidase 3 and Kelch domain of Keap1 protein

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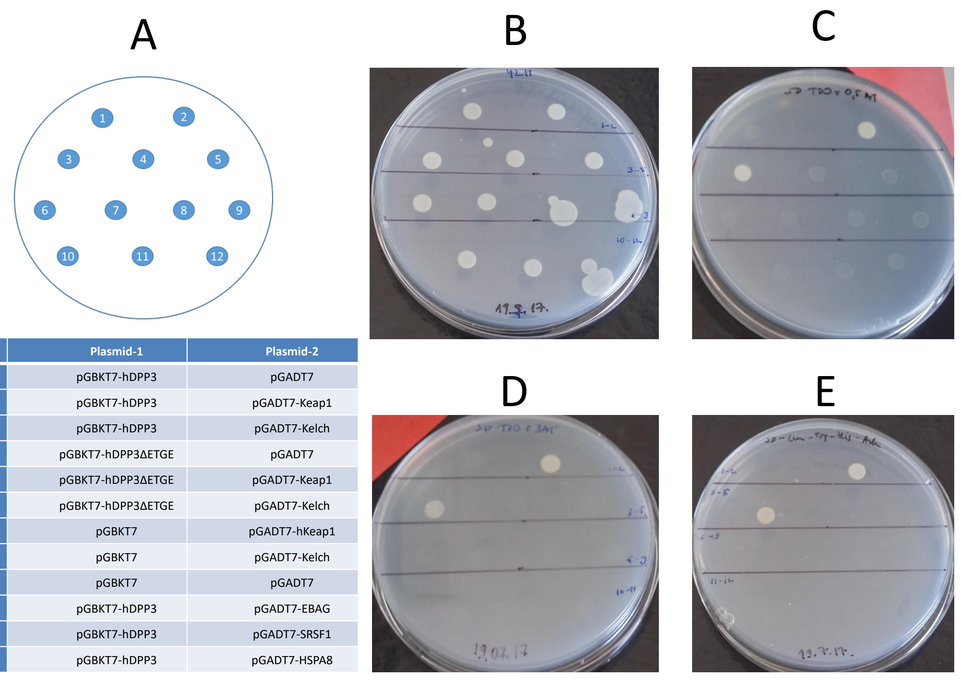
Introduction

Human dipeptidyl peptidase 3 (hDPP3) is a metallopeptidase from the M49 family of peptidases that cleaves dipeptides from the amino-termini of 3-10 long peptides in vitro. Apart from its proposed role in the final stages of proteolysis in the cell, and potential role in the regulation of blood-pressure, pain and infection through cleavage of bioactive peptides, it has been confirmed that hDPP3 is involved in the regulation of Nrf2-Keap1 signaling pathway through its interaction with Keap1 protein. Nrf2-Keap1 pathway is the main regulator of the oxidative stress response. It has been shown that hDPP3 binds Keap1, which consequently releases Nrf2 transcription factor from the complex with Keap1, and enables its translocation to the nucleus and the activation of genes coding for a series of proteins that protect the cell from the damage caused by xenobiotics and oxidative stress. Keap1 is interacting with hDPP3 through its Kelch domain. The interaction between hDPP3 and Keap1, and hDPP3 and Kelch domain of Keap1 protein has been confirmed with several methods, however, the thermodynamic parameters of the interaction have not been measured. We confirmed the interaction between wt hDPP3 and both full length Keap1 protein, and Kelch domain of Keap1 protein by yeast two-hybrid (Y2H) assay, and used microscale thermophoresis (MST) to measure the dissociation constant (K_d) of the hDPP3-Kelch domain complex for the wild type hDPP3, hDPP3 variant E690K, and two hDPP3 mutants found in cancer, R703C and R703H to check if the hDPP3 variant and mutants bind Kelch domain with different affinities than the wt. We performed only preliminary measurements, however, we established that it is possible to use MST to determine the thermodynamic parameters of the hDPP3-Kelch interaction.

Methods and Results

Figure 1: **Y2H experiment to test the interaction between wt hDPP3 and Keap1 protein, Kelch domain of Keap1 protein, and several putative interactors of hDPP3 confirmed that hDPP3 binds the Kelch domain of Keap1 protein through its ETGE motif;** yeast *S. cerevisiae* strain AH109 was transformed with 2 plasmids containing DNA binding domain of yeast transcription factor Gal4 fused to hDPP3 or hDPP3ΔETGE and activating domain (AD) of Gal4 fused to either Keap1, Kelch domain of Keap1 or one of the 3 proteins considered as putative interactors of hDPP3 – only yeast transformed with pGBKT7-hDPP3 and pGADT7-Keap1, and pGADT7-Kelch, respectively, grow on selection plates
A: schematic of the plate
B: SD/-Leu -Trp – control plate for the transformation of the yeast
C: SD/-Leu -Trp -His + 0,5 mM 3-AT – selection plate
D: SD/-Leu -Trp -His + 3 mM 3-AT – selection plate
E: SD/-Leu -Trp -His -Ade – selection plate



Y2H	Plasmid-1	Plasmid-2
1	pGBKT7-hDPP3	pGADT7
2	pGBKT7-hDPP3	pGADT7-Keap1
3	pGBKT7-hDPP3	pGADT7-Kelch
4	pGBKT7-hDPP3ΔETGE	pGADT7
5	pGBKT7-hDPP3ΔETGE	pGADT7-Keap1
6	pGBKT7-hDPP3ΔETGE	pGADT7-Kelch
7	pGBKT7	pGADT7-hKeap1
8	pGBKT7	pGADT7-Kelch
9	pGBKT7	pGADT7
10	pGBKT7-hDPP3	pGADT7-EBAG
11	pGBKT7-hDPP3	pGADT7-SRSF1
12	pGBKT7-hDPP3	pGADT7-HSPA8

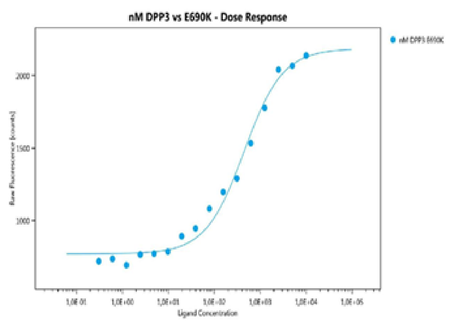


Figure 2: The hDPP3 was labelled with NT-647 dye (Maleimide coupling). In the MST experiment we have kept the concentration of labelled hDPP3 constant (60 nM), while the concentration of the nonlabelled E690K was varied between 10 µM – 0,3 nM.

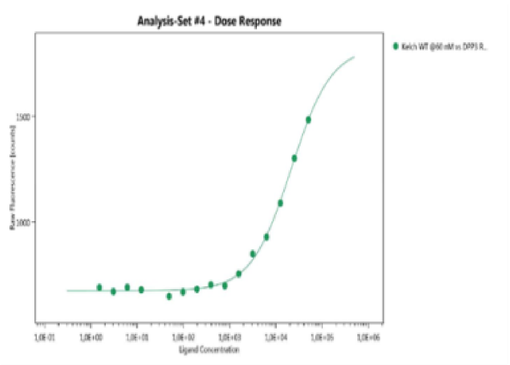


Figure 3: The hDPP3 was labelled with NT-647 dye (Maleimide coupling). In the MST experiment we have kept the concentration of labelled hDPP3 constant (60 nM), while the concentration of the nonlabelled R703C was varied between 50 µM – 1,5 nM

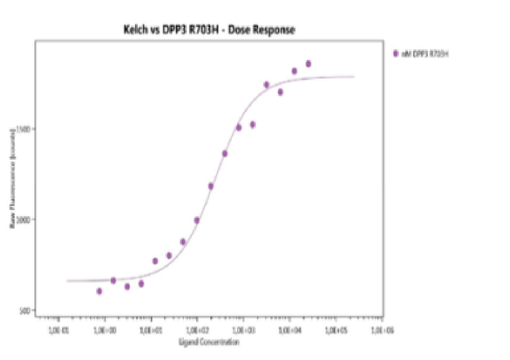


Figure 4: The hDPP3 was labelled with NT-647 dye (Maleimide coupling). In the MST experiment we have kept the concentration of labelled hDPP3 constant (60 nM), while the concentration of the nonlabelled E703H was varied between 25 µM – 0,7 nM.

Table: MST measurement of the interaction between Kelch domain of Keap1 protein and hDPP3

Fluorescent Molecule	Source of Fluorescence	Titrant	Expected K_d	MST measured K_d
Kelch	NT-647	DPP3 E690K	NA	$K_d \geq 420$ nM
Kelch	NT-647	DPP3 R703C	NA	$K_d \leq 21$ µM
Kelch	NT-647	DPP3 R703H	NA	$K_d = 211$ nM ± 35 nM
Kelch	NT-647	DPP3 WT	NA	ND

Summary

We confirmed the interaction of hDPP3 with the Keap1 protein, mediated through its Kelch domain, in yeast two-hybrid system, and preformed preliminary MST measurements of the affinity of the interaction between Kelch domain, wt hDPP3, hDPP3 variant E690K, and two hDPP3 mutants found in cancer, R703C and R703H. MST measurements did not give us conclusive results about the affinity, since the method needs to be optimized. However, we established that it is possible to determine the affinity of this interaction by MST, and we plan to optimize the method and perform further measurements in the future. Based on the results of MST, we also plan to measure the interaction parameters by isothermal titration calorimetry (ITC).

References

1 Hast et al., Cancer res 2013;73:2199-2210
2 Wienken et al., Nat Commun 2010;1:100
3 Gundic et al., Croat Chem Acta 2016; 89:217-228