Molecular interaction analysis of peptidases: from small inhibitors to large macromolecules

Pieter VAN WIELENDAELE, Emilie DE HERT, Kaat KEHOE, Gwendolyn VLIEGEN, Yentl VAN RYMENANT, Yann STERCKX, Anne-Marie LAMBEIR, Ingrid DE MEESTER

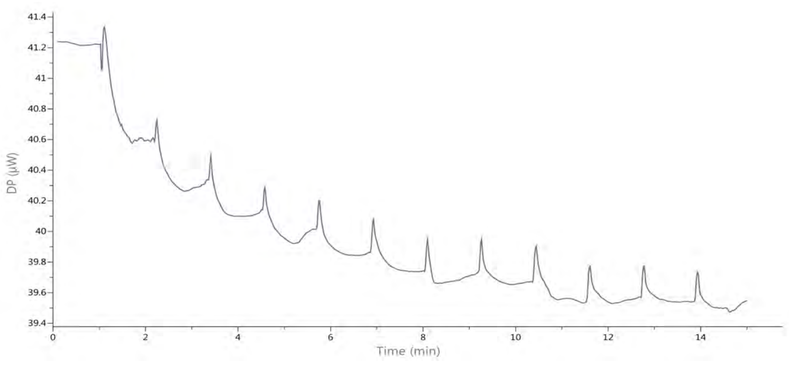
Laboratory of Medical Biochemistry, Department of Pharmaceutical Sciences, University of Antwerp, Antwerp, Belgium

Introduction

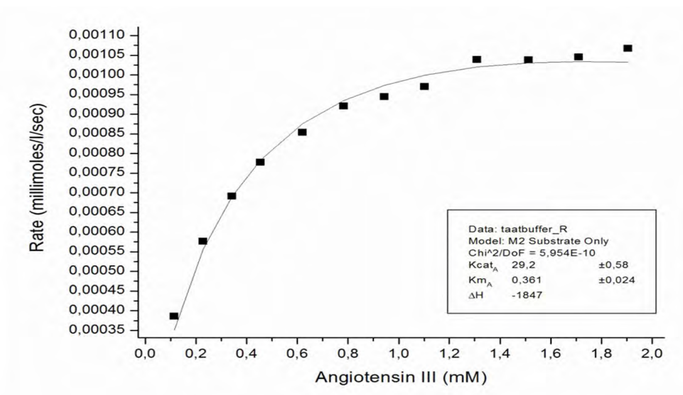
Peptidases play vital roles in the body's metabolism and its regulation by diverse signaling pathways. The development of new biophysical methodologies in the field of molecular interactions allows to study interactions of peptidases with diverse binding partners. Using Isothermal Titration Calorimetry (ITC, Microcal Peaq-ITC, Malvern) and Grating-coupled Interferometry (GCI, Creoptix WAVEdelta, Creoptix AG), we aim at analyzing molecular interactions of certain proline-specific peptidases, all members of the S28 and S9 families of serine proteases. Since these detection methods rely on basic aspects of the interactions (ITC: heat exchange, GCI: mass accumulation upon binding), no detection labels are needed on the molecules under study. By combining ITC and GCI, we determine the affinity (K_d), kinetic aspects (k_{on} and k_{off}), as well as the thermodynamic aspects (stoichiometry, entropy and enthalpy) of interactions of the peptidases with diverse types of molecules, ranging from 'small molecules' to large macromolecules (e.g. antibodies). Moreover, ITC also allows to directly study the kinetic aspects of the enzymatic conversion of naturally occurring unmodified substrates. In this way, we aim at further uncovering the physiological and molecular role of the peptidases, and characterizing peptidase-binding agents for several applications (e.g. development of inhibitors, characterization of antibodies for immune-assays), Some (preliminary) results are shown.

Results

1) Angiotensin III as substrate for Prolyl carboxypeptidase (PRCP) studied using ITC[1]



ITC thermogram for multiple injection run with 12 mM Angiotensin injected in 50 nM recombinant PRCP. Instrument: Microcal Peaq-ITC (Malvern).



Michaelis-Menten plot for the ITC data with PRCP and Angiotensin III, obtained from Origin 5 software.

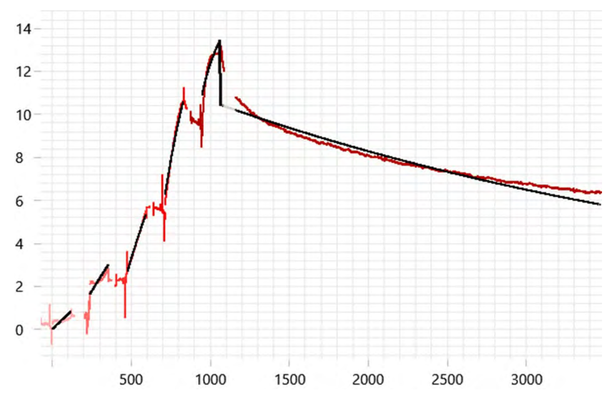
Parameter	Km (mM)	kcat (s-1)	kcat/Km (10^3 M^{-1} s^{-1})
RP-HPLC	0,33 +/- 0,04	39 +/- 1,9	118,18
ITC	0,351 +/- 0,024	29,9 +/- 0,6	90,33

Comparison of fitted ITC results with RP-HPLC data (obtained using Shimadzu HPLC, based on quantification of cleaved-off Phe residues).

2) Interaction between PRCP and compound 8o[2] (687 Da) (preliminary)

- Grating-coupled interferometry: Creoptix WAVEdelta
- Regeneration-free kinetics set-up
- Amine coupling of PRCP (150 nM, 300 µl) on polycarboxylate surface

  => level of +/- 3200 pg/mm^2
- Injection of compound 8o: from 0,586 nM to 37,5 nM (300 µl)

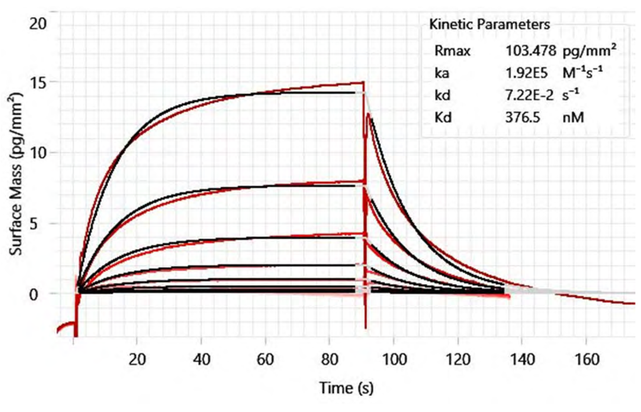


Parameter	Fitted value
Rmax	17,540 pg/mm^2
k_{on}	1,84E5 $M^{-1}s^{-1}$
k_{off}	2,65E-4 s^{-1}
Kd	1,4 nM

3) Interaction of Fibroblast activation protein α (FAP) and F-19 mAb (preliminary)

- Grating-coupled interferometry: Creoptix WAVEdelta
- Multiple-cycle kinetics set-up
- Immobilization of F-19 mAb on protein A/G surface

  => 150 µl of 63 nM for each cycle: / IL = 150 pg/mm^2
- Injection of rh_FAP: from 0,3 to 60 nM (250 µl; limited sample):



Parameter	Fitted value
Rmax	103,48 pg/mm^2
k_{on}	1,92E5 $M^{-1}s^{-1}$
k_{off}	7,22E-4 s^{-1}
Kd	376,5 nM

References.: 1) Kehoe *et al.,* (2016) *BBA Proteins & Proteomics;* 1864:11, 1481-88 2) Zhou *et al.* (2010) *J Med Chem* , 53(19):7251-63

This work received support from the UA research fund (GOA 150163) & Flanders Research Foundation (FWO, predoctoral fellowship Emilie De Hert & Kaat Kehoe).