GE Healthcare

Binding kinetics by Surface Plasmon Resonance: Insight into Structure-kinetics/thermodynamics relationships

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Introduction

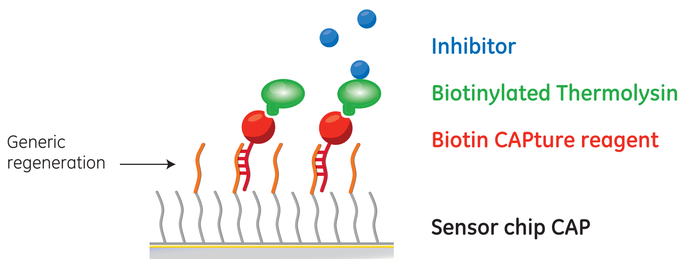
Biacore™ systems are highly sensitive and robust instruments capable of characterizing binding kinetics of biochemical interactions over a wide dynamic range and at very low response levels. This is a prerequisite when studying structure-kinetics relationships for small molecule series with large variation in their kinetic behaviors and when working with difficult protein targets.

In an on-going collaboration with Philipps-University Marburg we have been determining the binding kinetics between Thermolysin and a range of inhibitors with known thermodynamic and structural properties. These inhibitors are part of congeneric, systematic series initially designed for Structure-Activity Relationships by ITC and crystallography. However, Structure-Kinetics-Thermodynamic relationship of such a set of closely related compounds has not been reported so far. By thorough analysis of this system we aim to deepening our understanding about the importance of binding kinetics in small molecule-protein interactions.

Here we present novel data from the kinetic characterization of the thermolysin inhibitors using Biacore T200 and some early conclusions around the relation between kinetics, thermodynamic and structure.

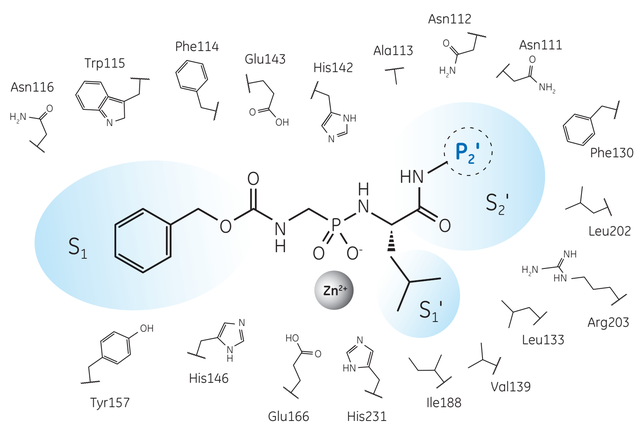
Assay overview

Thermolysin (M_w = 37.5 kDa, Calbiochem) was biotinylated and immobilized using the Biotin CAPture Kit (GE Healthcare) enabling reversible capture. Inhibitors (M_w average 454 Da) were analyzed using Single Cycle Kinetics. Experiments were performed on Biacore T200 and data was fitted to a 1:1 binding model.



Thermolysin as a model system for SAR and SKR

- Thermolysin, a zink metalloprotease, is a proven model system for Structure Activity Relationships (SAR) based on thermodynamic and crystallographic studies (1,2)
- The binding site consists of three specificity pockets; S_1, S_1' and S_2'
- Inhibitor series were designed by varying the P_2' position in the S_2' site



The binding pocket of Thermolysin. Highly variable water patterns characterize the binding of molecular portions to the least buried site, the S_2' site.

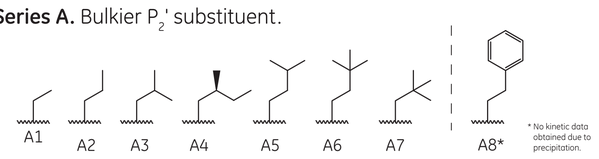
References

1. A. Biela, N. N. Nasief, M. Betz, A. Heine, D. Hangauer, G. Klebe, Angew. Chem. Int. Ed. 2013, 52, 1822 –1828; Angew. Chem. 2013, 125, 1868 –1876.
2. Krimmer SG, Betz M, Heine A, Klebe G. ChemMedChem. 2014 May;9(5):877

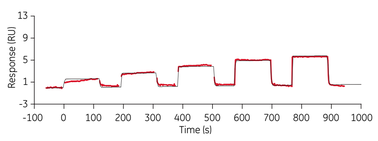
Results SPR analysis

20 available thermolysin inhibitors from the congeneric, systematic series provided by the group of Prof Klebe, were analyzed by SPR. The sensorgrams showed clear differences in binding characteristics within and/or between series. Representative sensorgrams are shown below.

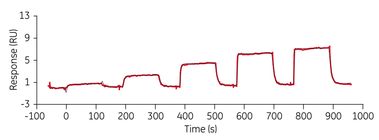
Series A. Bulkier P_2' substituent.



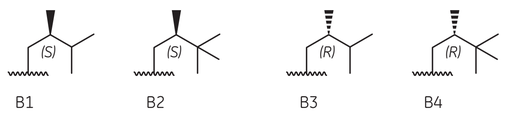
A1



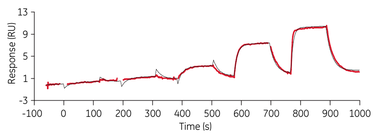
A5



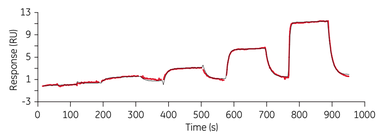
Series B. Steroisomers from A4.



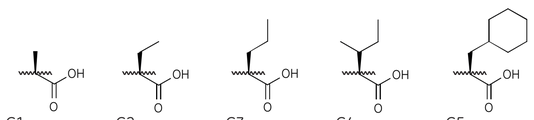
B2



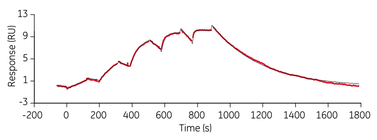
B3



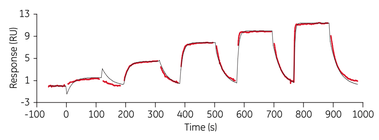
Series C. Addition of carboxy group + bulkier P_2' substituent.



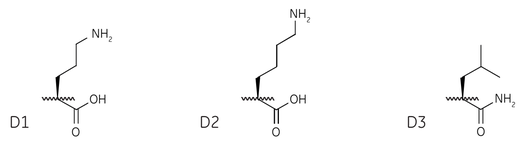
C2



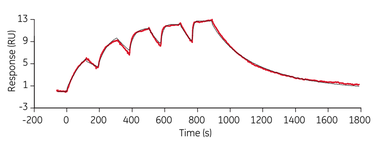
C5



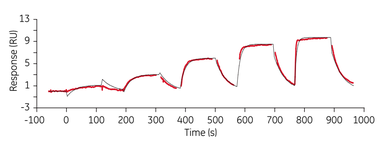
Series D. Addition of an amine or amide.



D1



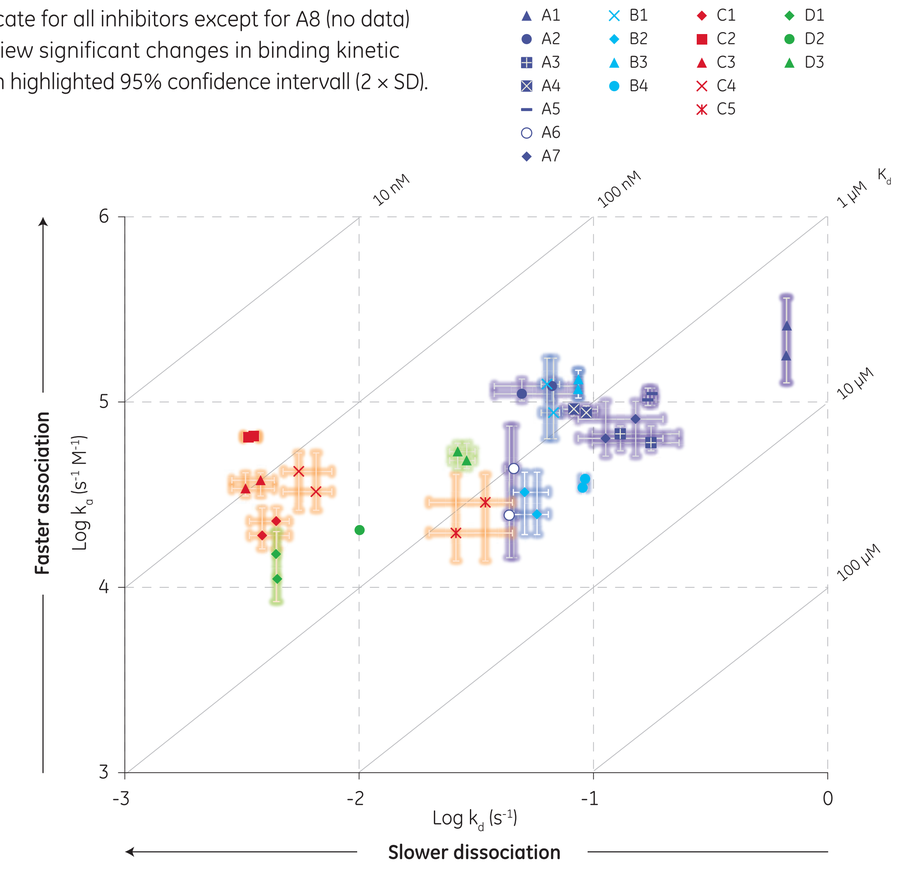
D3



Structure-kinetic relationship

Binding kinetic parameters were determined in duplicate for all inhibitors except for A8 (no data) and D2 (single data) due to precipitation issues. To view significant changes in binding kinetic properties, k_d values were plotted agains k_a values with highlighted 95% confidence intervall (2 × SD).

- Modifications in P_2' resulted in k_d values from 6.7×10^{-1} to 6.0×10^{-3} s^{-1} and k_a values from 1.3×10^{4} to 2.2×10^{5} $s^{-1}M^{-1}$.
- Most compounds within the same series distribute along the same isoaffinity lines; a structural change that affects k_d seem to be compensated for by a change in k_a.
- Series C, with added carboxy group, displayed significantly more stable complex formations compared to series A, without the carboxy group.
- The exception is C5, the bulkiest inhibitor in series C, indicating that large, apolar residues may affect dissociation.
- Highest affinity was seen for C2 but further increase of the P_2' in series C does not favour the k_a.
- B1 and B3 have similar kinetics to A4. Further addition of a methyl group (B2 and B4) results in significantly slower k_a. Notably, this pairing is on contrast to the thermodynamic/crystal structure data where the compounds paired according to stereochemistry:
  B1 and B2 (ΔΔH = 1.0 kJ/mol; -TΔΔS = 2.1 kJ/mol);
  B3 and B4 (ΔΔH = 0.5 kJ/mol; -TΔΔS = 0.2 kJ/mol).



Conclusions

- Kinetic parameters for thermolysin inhibitors from closely related series, were determined in Biacore T200 using reversible capture of biotinylated Thermolysin
- The small modifications of the inhibitor structures resulted in a variation in dissociation with 2 orders of magnitude and a variation in association with 1 order of magnitude
- The most pronounced kinetic effect was achieved by altering the electrostatics of the compounds as seen by the addition of a carboxy group (series C) resulting in a significant decrease in k_d
- Ongoing analysis of the Biacore results combined with existing thermodynamic and high resolution crystallographic data aim to further our understanding of Structure-Kinetics-Thermodynamic relationships in general

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