Peptide cleavage patterns as an approach to investigate serine protease activity

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Background

Literature suggests a role for serine protease activities in pathologies of different origin. As the selectivity of the synthetic substrates generally used to measure their enzyme activity is questioned, a more advanced profiling of the proteolytic activity in biological samples is still lacking. As protease activated receptors (PARs) and substance P are involved in various diseases, a set of synthetic peptides based on their sequences were used for proteolytic profiling of trypsin 1, 2, 3, trypatase, thrombin, chymotrypsin, cathepsin G, neutrophil elastase and pancreatic elastase.

Materials and Methods

Equimolar concentrations of pure enzyme (20 – 25 nM)1 were incubated with 10 µM peptide (PAR-based or substance P) at 37°C for 30 seconds, 10/30 minutes and 1 hour. Fragments were identified using MALDI-TOF/TOF and https://biot.shinyapps.io/PARs/.2

Results

Cleavage patterns of the synthetic peptides after incubation with pure enzymes are depicted below. On top, the amino acid sequence is shown and for each time point the observed cleavage sites are indicated. The identified fragments are presented as colored rectangles, a more intense blue color represents a more abundant fragment.

Conclusion

Distinct cleavage patterns of PAR-based peptides allow to distinguish between trypsin 1, 2, 3, thrombin and trypatase

Trypsin 3 and thrombin
No or very limited cleavage of PAR2 after 1 hour
Thrombin can be distinguished from trypsin 3 using the thrombin selective inhibitor PPACK

Trypsin
No or very limited cleavage of PAR2 after 1 hour

Trypsin 1
No efficient cleavage of PAR2 and PAR3

Trypsin 2
Efficient cleavage of PAR2 and PAR3

Cleavage pattern of PAR1-based peptide confirms trypsin- and chymotrypsin-like activity of cathepsin G

Trypsin-like activity
Chymotrypsin-like activity

ARTRARPSK | ATNLDP | SF | LLRNPNDK

Cleavage patterns of PAR3-based peptide allow to distinguish between neutrophil and pancreatic elastase

Neutrophil elastase
No efficient cleavage of PAR3 and substance P

Pancreatic elastase
Efficient cleavage of PAR3 and substance P

The biggest challenge of this project was to distinguish between the trypsin-like enzymes. The experiments resulted in distinct cleavage patterns of synthetic peptides for each serine protease and allowed to distinguish between enzymes with closely related proteolytic specificities. Furthermore, the setup described here can be used to investigate serine protease activity in large numbers of biological samples. We aim to study these cleavage patterns using biological samples as enzyme source. Depending on the sample or pathology of interest, different peptides can be included in the protocol. In case of value as a biomarker, the protocol can easily be used in clinical settings since MALDI is an uncomplicated technique which is available in many clinical laboratories.

References and acknowledgements

1Loew et al. (2000) Biochemistry

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