High-throughput epitope identification for snakebite antivenom

Engmark, Mikael; De Masi, Federico; Laustsen, Andreas Hougaard; Gutiérrez, José María; Lomonte, Bruno; Andersen, Mikael Rørdam; Lund, Ole

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
High-throughput epitope identification for snakebite antivenom

Mikael Engmark¹, Federico De Masi¹, Andreas Hougaard Laustsen², José María Gutiérrez³, Bruno Lomonte³, Mikael Rørdam Andersen¹, and Ole Lund¹

(1) Department of Systems Biology, Technical University of Denmark
(2) Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen
(3) Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica

Correspondence: mikael@bs.dtu.dk

Introduction
Insight into the epitopic recognition pattern for polyclonal antivenoms is a strong tool for accurate prediction of antivenom cross-reactivity and provides a basis for design of novel antivenoms. In this work, a high-throughput approach was applied to characterize linear epitopes in 966 individual toxins from pit vipers (Crotalidae) using the ICP Crotalidae antivenom. Due to an abundance of snake venom metalloproteinases and phospholipase A₂ in the venoms used for production of the investigated antivenom, this study focuses on these toxin families.

Objectives
- Identify epitopes in toxins used in immunization
- Characterize tolerated amino acid substitutions in identified epitopes
- Predict cross-reactivity of antivenom

Immunization mixture

To identify epitopes observed in toxigenic sequences, 15-mer peptides with median signals above 20 AU were tested for compatibility with the antivenom. Epitope core sequences were localized to the amino acid sequence of each pit viper toxin. Using two or more overlapping 15-mer peptides, we find that flanking conservation in toxin epitopes and flanking residues is maintained. This enables parallel automated identification of epitopes in hundreds of toxins.

Studying linear epitopes using peptide microarrays

Mean AU overlapping peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Mean AU</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 metalloproteinase</td>
<td>200.0</td>
</tr>
<tr>
<td>Lyso-phospholipase A₁</td>
<td>180.0</td>
</tr>
</tbody>
</table>

The α-helical vector diagram of the B. asper metalloproteinase is found to be highly conserved among viper metalloproteinases. Based on multiple sequence alignment of pit viper toxins sharing at least seven of the eight epitope residues and mean signal intensity at the high signal threshold, we find that flanking residues outside the core epitope have small effect on antivenom recognition. Expanding the analysis to the 42 toxins that share at least five of the epitope residues, further enrichment of the epitopes is observed in all of the corresponding eight 15-mer peptides, although the microarray signals are reduced up to seven times (data not shown).

These results suggest that ICP Crotalidae polyvalent antivenom might offer protection against the investigated metalloproteinases, including the toxins from the Asian Gloydius species if these in vitro experiments translate to the in vivo situation.

Conclusions
- Custom-designed high density peptide microarray technology enables parallel automated identification of epitopes in hundreds of toxins.
- Integrating multiple sequence alignment allows investigation of the effect of epitope variation on antivenom recognition.
- Cross-reactivity of antivenom is correlated to the degree of conservation in toxin epitopes and flanking residues.

Acknowledgement
The peptide microarray experiments were performed at Schafer-N, Copenhagen. We would like to thank Claus Schafer, Christian Jakob Hansen, and Jens Krogsgaard for experimental setup and support. We further thank the Novo Nordisk Foundation for financial support (grant number: NNF13OC00061311).

References