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

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

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

Epitopes locate to surface regions

To identify epitopes, the observed peptide-specific signal intensities were mapped back to amino acid sequence of each pit viper toxin. Using two or more overlapping 15-mer peptides with median signals above 20 AU, epitope core sequences were localized to the amino acid sequence of each pit viper toxin. Using two or more overlapping 15-mer peptides, epitope core sequences were localized to the amino acid sequence of each pit viper toxin. 

Studies on microarray

CLUSTAL O(1.2.1) multiple sequence alignment

<table>
<thead>
<tr>
<th>QUERY</th>
<th>Mean AU overlapping peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>YIELVIVADHGMFTKYDSNLDTI</td>
<td>146.4</td>
</tr>
<tr>
<td>YIELVIVADHGMFTKYNGDSDKI</td>
<td>200.3</td>
</tr>
<tr>
<td>YVELVIVADHGMFTKYNRNLTEV</td>
<td>174.2</td>
</tr>
<tr>
<td>YVELVIVADHGMFTKYNGNLKKI</td>
<td>187.5</td>
</tr>
<tr>
<td>YIELVVVADHGMFTKYNGNLNTI</td>
<td>191.1</td>
</tr>
<tr>
<td>YIELAVVADHGMFTKYNSNIDTI</td>
<td>187.1</td>
</tr>
<tr>
<td>YIELAVVADHGMFTKYNSNVNTI</td>
<td>232.8</td>
</tr>
<tr>
<td>YIELAVVADHGIFTKYNSNLNTI</td>
<td>249.0</td>
</tr>
<tr>
<td>YIELAVVADHGIFTKYNSNLNTI</td>
<td>249.0</td>
</tr>
<tr>
<td>ADHGIFTK</td>
<td>150.0</td>
</tr>
</tbody>
</table>

The α-helical red epitope in the Crotalus atrox metalloproteinase is found to be highly conserved among many venom metalloproteinases. Based on multiple sequence alignment of pit viper toxins sharing at least seven of the epitope residues and mean signal intensity of the top 15-mer peptides harboring the epitope, we find that flanking residues outside of the core epitope has small effect on antivenom recognition. Expanding the analysis to the 42 toxins that share at least five of the epitope residues, the effect of epitope variation on cross-recognition becomes more profound. The intensity of the eight 15-mer peptides harboring the epitope, we find that flanking residues outside of the core epitope has small effect on antivenom recognition.

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 epitope conservation in toxin epitopes and flanking residues

Acknowledgement

The peptide microarray experiments were performed at Schaefer-N Copenhagen. We would like to thank Claus Schaefer, Christian Åkild Hansen, and Jens Kringelum for experimental setup and support. We further thank the Novo Nordisk Foundation for financial support (grant number: NNF15OC0015613).

References