High-throughput epitope identification for snakebite antivenom

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Publication date:
2015

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

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

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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 A\textsubscript{2} 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 specific signal intensities were mapped back to the 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 and subsequently mapped to crystal structures or homology models. As examples, P4 metalloproteinase and Lys49-phospholipase A\textsubscript{2} from Bothrops asper ( venom used in antivenom production) are presented here.

Immunization mixture\textsuperscript{1}

Major venom components

Studies linear epitopes using peptide microarrays

To in silico generation of peptide library

Antibody binding and detection

Effect on cross-recognition

\textbf{CLUSTAL O (1.2.1) multiple sequence alignment}

\begin{tabular}{lcc}
\hline
QUERY & ---ADHGIFTK--- & MEAN AU overlapping peptides  \\
Q07155 & Buthrotox asper & 249.0 \\
P45312 & Buthrotox asper & 249.0 \\
PQ0121 & Buthrotox asper & 189.9 \\
Q9H986 & Bothrops asper & 189.9 \\
E3X121 & Bothrops moojeni & 232.8 \\
Q9J460 & Bothrops pauloensis & 187.3 \\
Q9J240 & Bothrops pauloensis & 187.3 \\
Q9J226 & Bothrops pauloensis & 187.3 \\
P2794 & Lachesis muta & 191.3 \\
T1D75 & Crotalidae armoricis & 187.5 \\
J3B6Q & Crotalidae adamanteis & 176.2 \\
J3B6Q & Crotalidae adamanteis & 174.2 \\
J3B6Q & Crotalidae adamanteis & 174.2 \\
F8S112 & Crotalidae armoricis & 176.2 \\
Q9R80 & Crotalidae armoricis & 201.3 \\
Q9R80 & Crotalidae armoricis & 201.3 \\
Q9F94 & Crotalidae armoricis & 154.9 \\
J3B6Q & Crotalidae adamanteis & 176.2 \\
Q9F94 & Crotalidae armoricis & 164.4 \\
J3B6Q & Crotalidae adamanteis & 164.4 \\
\hline
\end{tabular}

The \( \alpha \) helix shaped red epitope in the B. asper metalloproteinase is found to be highly conserved among pit viper metalloproteinases. Based on multiple sequence alignment of pit viper toxins sharing at least seven of the eight epitope residues and mean signal 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. Expanding the analysis to the 42 toxins that share at least five of the epitope residues, mean signal conservation in toxin epitopes and flanking residues is still 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 polyclonal antivenom might offer protection from the investigated metalloproteinases, including the toxins from the Asian Crotalidae 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 Aajeb Hansen, and Jens Skjødt Hansen, and Jens Andersen. We would like to thank Claus Schafer, Christian Aajeb Hansen, and Jens Skjødt Hansen, and Jens Andersen. We would like to thank Claus Schafer, Christian Aajeb Hansen, and Jens Skjødt Hansen, and Jens Andersen. We would like to thank Claus Schafer, Christian Aajeb Hansen, and Jens Skjødt Hansen, and Jens Andersen.

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