High-throughput epitope identification for snakebite antivenom

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DTU Systems Biology
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High-throughput epitope identification for snakebite antivenom

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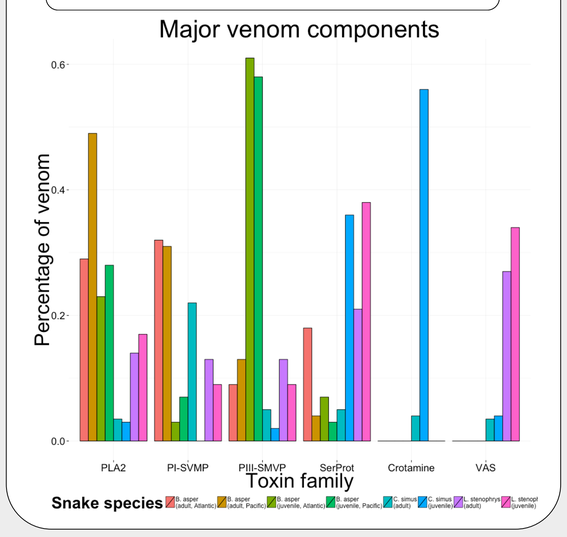
Introduction

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_2s in the venoms used for production of the investigated antivenom, this study focuses on these toxin families.

Objectives

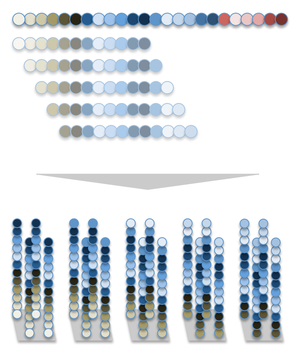
- Identify epitopes in toxins used in immunization
- Characterize tolerated amino acid substitutions in identified epitopes
- Predict cross-reactivity ofof antivenom

Immunization mixture[1]



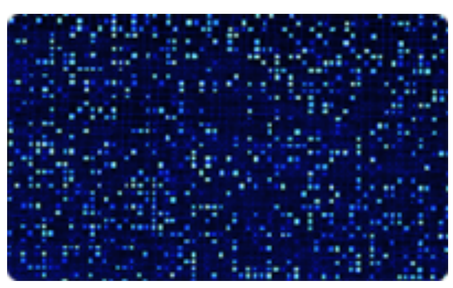
Studying linear epitopes using peptide microarrays

In silico generation of peptide library



Synthesis on microarray

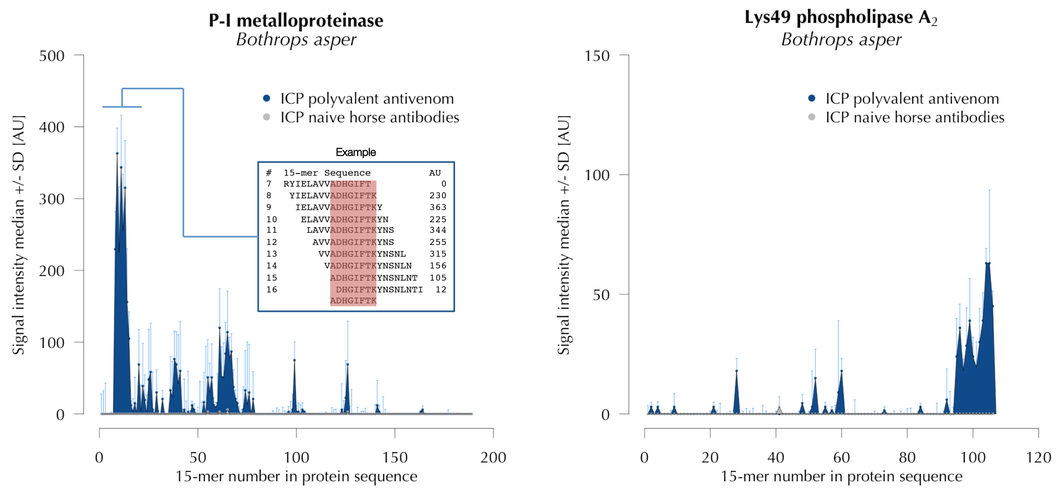
Antibody binding and detection

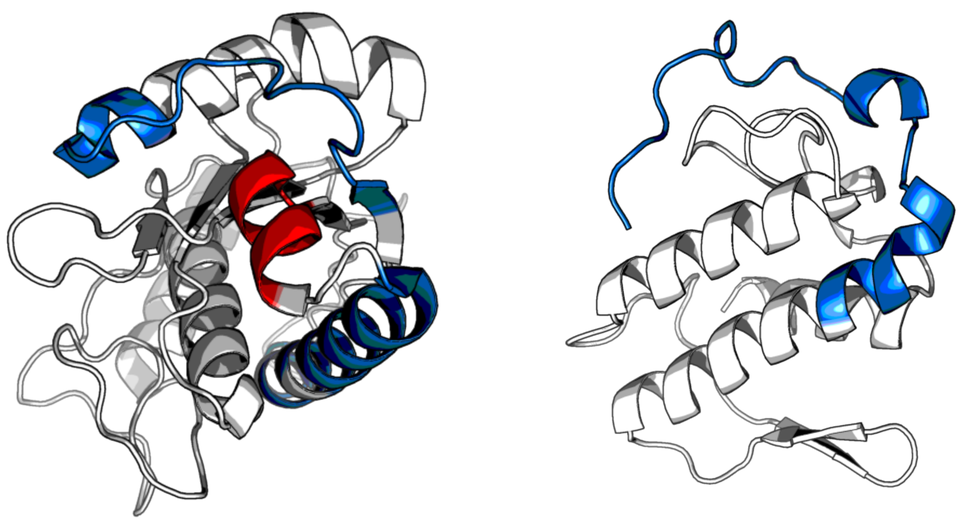


Data analysis and protein modeling

Epitopes locate to surface regions

To identify epitopes the observed peptide 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, P-I metalloproteinase and Lys49-phospholipase A_2 from *Bothrops asper* (venom used in antivenom production) are presented here.





The epitope core sequences are highlighted in blue except for the high-signal epitope in *Bothrops asper* P-I metalloproteinase that is highlighted in red. All epitopes are found to be exposed on the protein surface. The structure of the metalloproteinase (PDB: 2W13)[2] was obtained from the Protein Data Bank (pdb.org), and the homology model of the phospholipase A_2 was built using CPHmodels[3] based on a crystal structure of the Lys49-phospholipase from *B. moojeni* (PDB: 4KF3)[4] with 87.7% identity.

Effect on cross-recognition

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CLUSTAL O(1.2.1) multiple sequence alignment
                                                    Mean AU
                                                    overlapping
                                                    peptides

QUERY                          -------ADHGIFTK--------
Q072L5|Bothrops asper          YIELAVVADHGIFTKYNSNLNTI    249.0
P83512|Bothrops asper          YIELAVVADHGIFTKYNSNLNTI    249.0
P0DJE1|Bothrops asper          YIELAVVADHGIFTKYNSNLNTI    249.0
Q5XUW8|Bothrops insularis      YIELAVVADHGMFTKYNSNLNTI    189.9
E3UJL4|Bothrops neuwiedi       YIELAVVADHGMFTKYNSNVNTI    232.8
P0C6S0|Bothrops pauloensis     YIELAVVADHGMFTKYNSNINTI    188.3
C0HJU2|Bothrops pauloensis     YIELAVVADHGMFTKYNSNVNTI    232.8
P0C6S1|Bothrops pauloensis     YIELAVVADHGMFTKYNSNIDTI    187.1
P22796|Lachesis muta           YIELVVVADHGMFTKYNGNLNTI    191.1
T1DJY5|Crotalus horridus       YVELVIVADHGMFTKYNGNLKKI    187.5
J3SBQ2|Crotalus adamanteus     YVELVIVADHGMFTKYNRNLTEV    174.2
J3SBQ1|Crotalus adamanteus     YVELVIVADHGMFTKYNRNLTEV    174.2
J3RY86|Crotalus adamanteus     YVELVIVADHGMFTKYNRNLTEV    174.2
F8S112|Crotalus adamanteus     YVELVIVADHGMFTKYNRNLTEV    174.2
O73795|Gloydius brevicaudus    YIELVIVADHGMFTKYNGDSDKI    200.3
Q90WC0|Gloydius brevicaudus    YIELVIVADHGMFTKYNGDSDKI    200.3
Q698K8|Gloydius brevicaudus    YIELVIVADHGMFTKYNGDSDKI    200.3
Q1PBD1|Gloydius halys          YIELVIVADHGMFTKYDSNLDTI    154.9
J3S830|Crotalus adamanteus     YVELVIVADHGMFTKYNRNLTEV    174.2
Q9YI19|Gloydius brevicaudus    YIELVVVADHGMFTKYDSNLDTI    164.4
Q9PVK9|Gloydius brevicaudus    YIELVVVADHGMFTKYDSNLDTI    164.4
                               *:**::*****.***** :
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The α-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 piper 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, binding 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 polyvalent antivenom might offer protection from 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

References

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