High-throughput epitope identification for snakebite antivenom

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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 A₂ in the venomus 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 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 A₂ from Bothrops asper (venom used in antivenom production) are presented here.

Studying linear epitopes using peptide microarrays

In situ generation of peptide library
Antibody binding and detection

Effect on cross-recognition

CLUSTAL O(1.2.1) multiple sequence alignment

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The calculated values (AU) in 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) was obtained from the Protein Data Bank (pdb.pdb), and the homology model of the phospholipase A₂ was built using CPHmodels¹ based on a crystal structure of the lys49-phospholipase A₂ from B. maurus (PDB: 4KF3) with 87.7% identity.

The peptide microarray experiments were performed at Schafer-N, Copenhagen. We would like to thank Claus Schafer, Kenta Iwamoto, and Jens Laustsen for technical assistance, and Jannik Søndergaard for data analysis.

Mean AU overlapping peptides

The peptides with the highest signal were selected for synthesis on microarray. A microarray (MWG Biotech) with 768 spots was prepared of which 384 spots were used in the experiments, and 384 spots were left empty.

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Acknowledgements

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References