Production of Monoclonal Antibodies (mAbs) Purified Anti-metalloprotease from the Venom of the Serpent *Bitis arietans*

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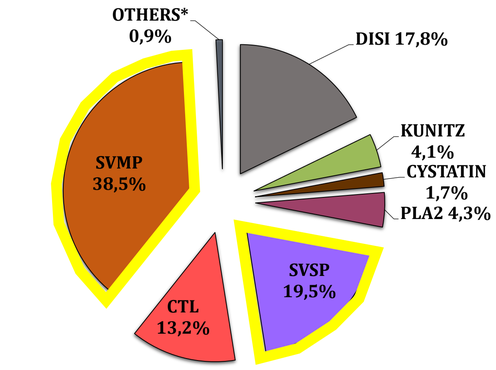
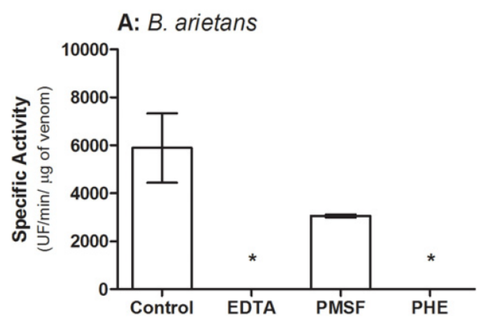
Introduction

The snake *Bitis arietans* is one of the most important medicinal snakes on the African continent. Its venom causes local effects, severe pain, swelling, blisters, bruises, local bleeding and coagulation disorders, necrosis, enlarged lymph nodes in the affected region, severe hypotension and death. Proteomic and transcriptomic analyzes reveal that the venom is composed of a large number of proteins, including metalloproteases (SVMP) and serinoproteases (SVSP). Tests of the biological activity of the venom by selective inhibition with EDTA, PMSF and PHE, corroborate the results of proteomics and transcriptomics.

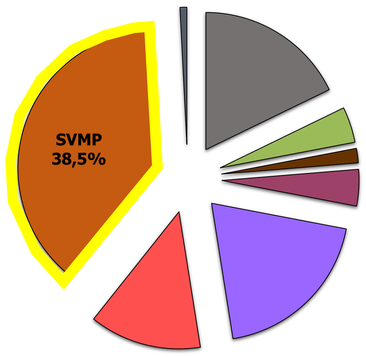


Proteomic and transcriptomic analyzes



Biological activite

SVMPs represent almost 40% of the venom They are classified into three classes, according to the composition of their domains.



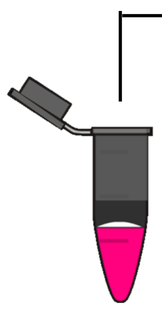
P-I → Pre | Pro | Catalytic

P-II → Pre | Pro | Catalytic | Disintegrin

P-III → Pre | Pro | Catalytic | Disintegrin | Cysteine

Methodology

1. Two steps of molecular exclusion chromatography
2. Proteolytic activity - FRET substrates
3. Characterization of the enzymatic class - EDTA, 1.10 PHE and PMSF
4. Mass spectrometry identification

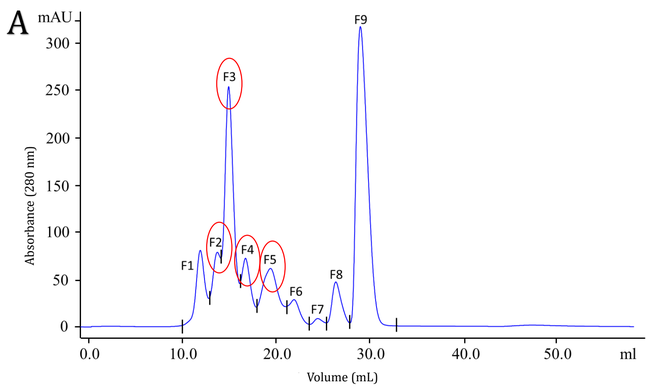


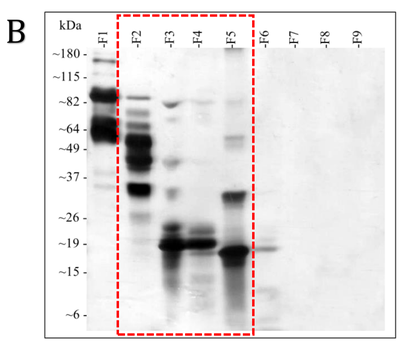
Crude venom

Next steps

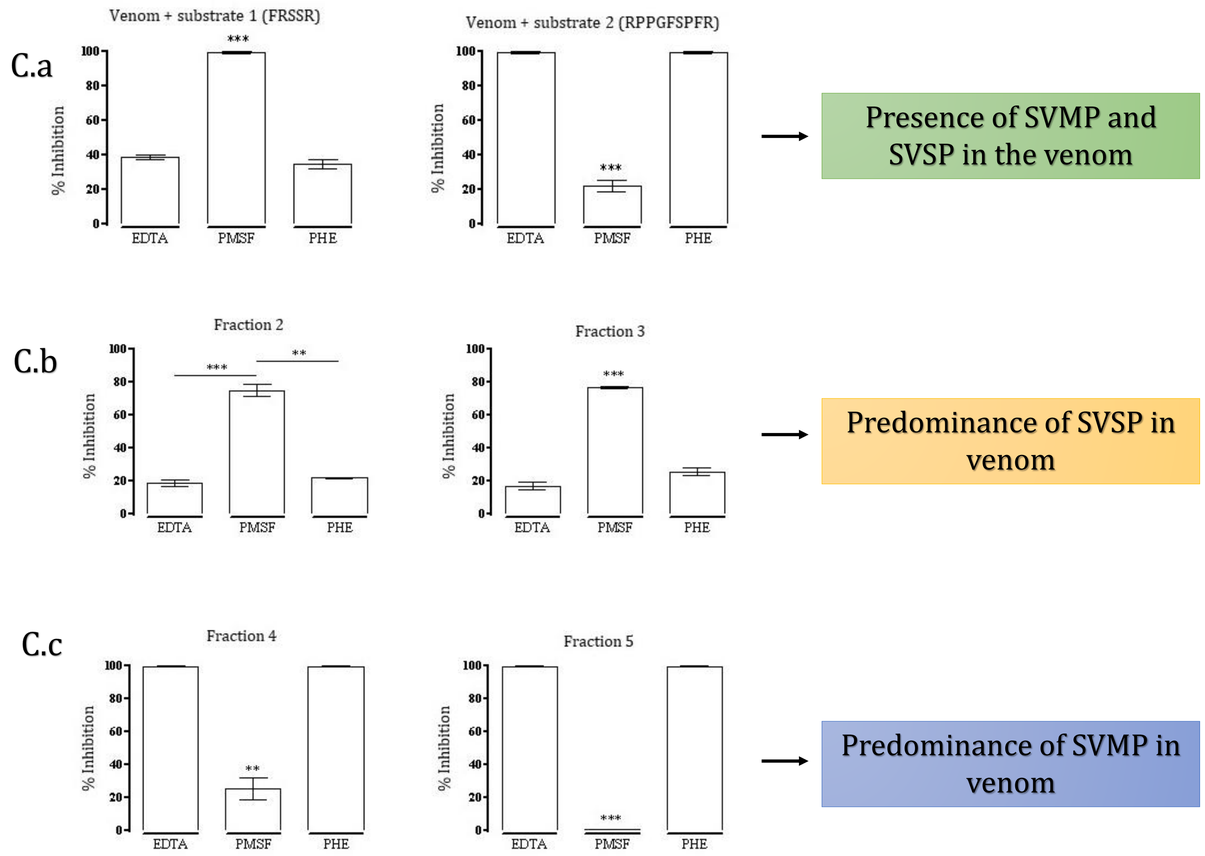
- Immunization
- Peritoneal cells
- Identification of anti-SVMP mAbs
- Fusion – myelomas + activated B cells
- Identification and expansion
- Evaluation of antitoxic activity
- Quantification of proteins
- Titration of avidity and affinity

Results

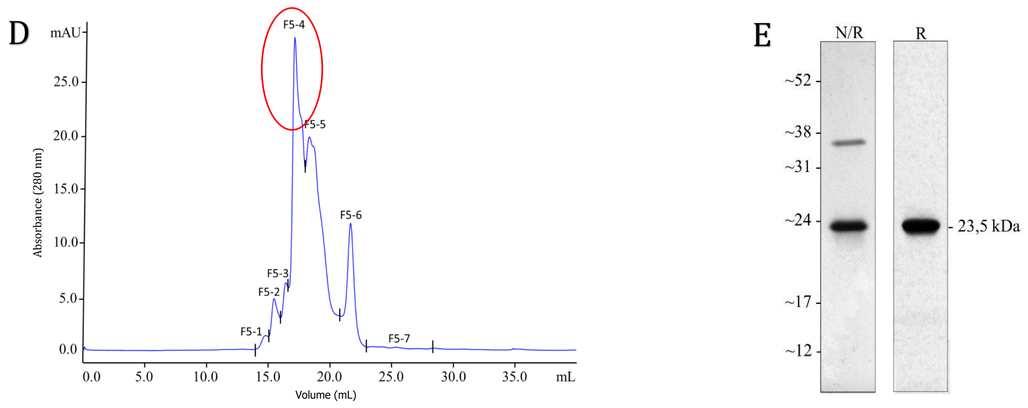




20 mg of lyophilized venom were subjected to a phase of molecular exclusion chromatography in a Superose 12 HR column, balanced and eluted with ammonium acetate (50 mM) in a climate-controlled environment (22 ± 2ºC) (Figure A). The electrophoretic profile was evaluated on gel with a gradient of 8-16% acrylamide and the bands revealed by impregnation with silver nitrate (Figure B).



Using selective inhibitors, the presence of serinoproteases and metalloproteases in BaV were indicated by inhibition with PMSF or with EDTA and phenanthroline, respectively (Figure C.a). In fractions 2 and 3 the majority presence of serinoproteases was revealed (Figure C.b) and in fractions 4 and 5 the majority presence of metalloproteases (Figure C.c).



The fifth chromatographic peak (F5), obtained from the first purification step, was subjected to a second cycle of molecular exclusion chromatography on a Superdex 75 column, equilibrated and eluted with ammonium acetate (50 mM) in an air-conditioned environment (22 ± 2ºC) (Figure D). The fraction 5-4 (4 ug/well), corresponding to the metalloprotease isolated from fraction 5, was submitted to 12% polyacrylamide gel under reducing (R) and non-reducing (N/R) conditions (Figure E).

Conclusion

- SVMPs are classified into three classes, according to the composition of their domains
- First stage of the *B. arietans* venom fractionation originated 9 subfractions – where fraction 2, 3, 4 and 5 was selected
- SVMP is inhibited by EDTA and phenanthroline
- Fractions 4 and 5 have a majority presence of SVMPs
- Second stage of fraction 5 molecular exclusion chromatography gave rise to 5 subfractions where the F5-4 fraction was selected to continue the experiments

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Acknowledgments

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