IOCT 2025 Conference
The 3rd International Online Conference on Toxins
10–12 September 2025 | Online
MDPI

Refolding of recombinant Tityus toxins improves antigen quality for use as immunogens in Antivenom production

Belen Gonzalez Viacava[1,3], Christian L. Macoretta[1], Carla M. Falcon[1], Adolfo R. de Roodt[1] ,Leonardo G. Alonso[2], Matías Fingermann[1]
[1]National Institute of Biological Production, Dr. Carlos G. Malbrán – ANLIS.
[2]Protein Chemistry Laboratory, NANOBIOTEC, UBA–CONICET, [3]bgonzalez@anlis.gob.ar

INTRODUCTION & AIM

Approximately 80% of the 8,000 annual envenomation cases reported in Argentina are caused by scorpion stings, with *Tityus carrilloi* being the most medically relevant species. Antivenom, the only specific treatment for severe cases, is produced from the plasma of horses hyperimmunized with *T. carrilloi* venom. However, venom availability often represents a major bottleneck for antivenom production. Recombinant toxins have been explored in the closely related species *Tityus serrulatus* as potential complements or alternatives to native venom. A similar strategy could be applied to *T. carrilloi* by identifying key toxin candidates and optimizing expression systems to enhance antigenicity. In this study, we focused on two sodium channel–targeting candidates: a non-toxic protein, TsNtxP (6.8 kDa) from *T. serrulatus*, and Tt1g (6.9 kDa) from *T. carrilloi*, which is the only toxin described so far in this species. Both proteins possess complex structures stabilized by four disulfide bonds. We evaluated how their antigenicity is affected by refolding conditions that promote native-like conformations.

METHODS

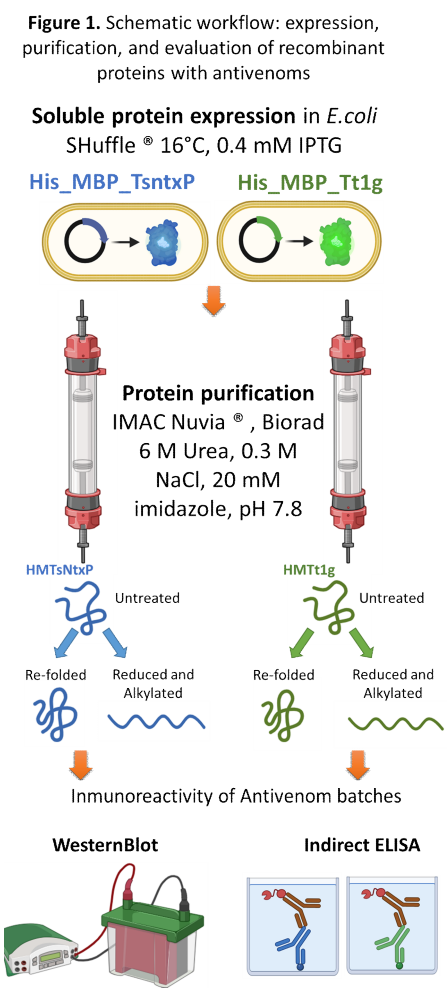
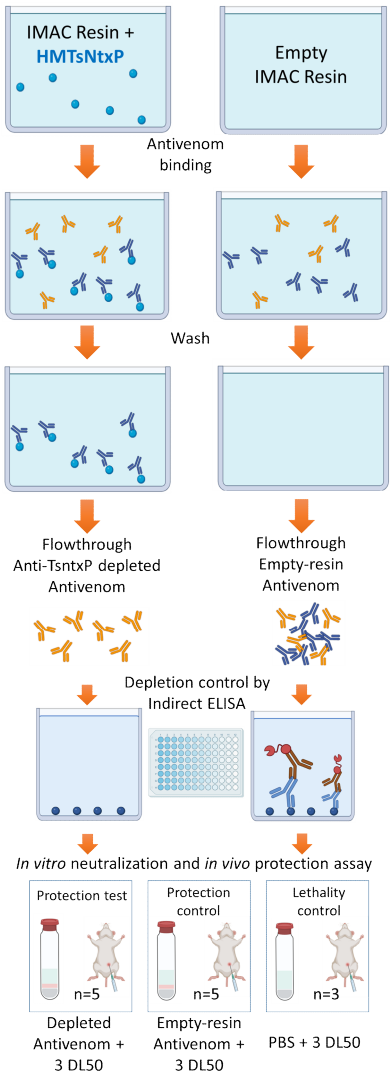


Figure 1. Schematic workflow: expression, purification, and evaluation of recombinant proteins with antivenoms

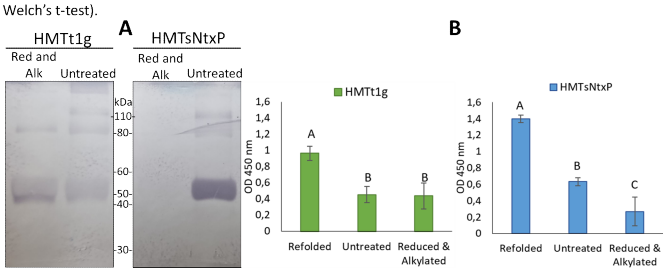
Figure 2. Anti-TsntxP capture assay.



RESULTS & DISCUSSION

Soluble recombinant TsNTxP and Tt1g fused to MBP were successfully expressed in *E. coli* Shuffle ®. Both chimeric proteins were specifically recognized by scorpion antivenom batches. While disulfide-bridges reduction significantly decreased immune-specific recognition of the originally purified proteins, this recognition was strongly increased by refolding conditions. This suggests a relevant role of conformational epitopes (Figure 3).

Figure 3. A. Western Blot analysis. **B.** Indirect Elisa analysis with 6 Antivenom batches coating plates with HMTt1g or HMTsNtxP. Groups sharing the same letter are not significantly different ($p > 0.05$, Welch's t-test).



As TsNTxP exhibited stronger reactivity than Tt1g, its antigenic potential was further evaluated. Refolded HMTsNTxP molecules were immobilized on an IMAC column, and specific antibodies were depleted from scorpion antivenom after resin elution (Figure 2). Indirect ELISA confirmed a marked reduction in recognition by the depleted antivenom compared to untreated antivenom and control antivenom exposed to empty IMAC column (Figure 4). The depleted antivenom was then tested *in vivo* using a mouse neutralization assay (3 × LD_{50} scorpion venom). All animals receiving the depleted antivenom did not survive, whereas all mice treated with control antivenom were fully protected (Table 1).

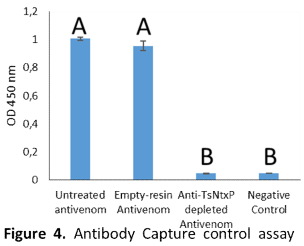


Figure 4. Antibody Capture control assay by Indirect Elisa coating plates with HMTsNtxP. Groups sharing the same letter are not significantly different ($p > 0.05$, Kruskal Wallis)

Table 1. *In vitro* Neutralization and *in vivo* protection in mice. Each assay was conducted in duplicate following the Guide for the Care and Use of Laboratory Animals (8th Ed.) and Institutional POEs.

	Neutralization control	Neutralization Test	Lethality control
Sample	Empy-resin Antivenom	Anti-TsNtxP depleted Antivenom	PBS
Escorpion Venom	3 DL50	3 DL50	3 DL50
Survival mice/Total mice	5/5 (100%)	0/5 (0%)	0/3 (0%)

CONCLUSION

In this work, a significant role of conformational epitopes in immune recognition by *T. carrilloi* antivenom was observed for both TsNTxP and Tt1g. Refolded TsNTxP not only exhibited the highest antigen-specific recognition across six different antivenom batches, but also depleted most of the antivenom's neutralizing capacity. These results suggest that this protein is a promising candidate antigen for antivenom development.

FUTURE WORK

Evaluation of the protective capacity induced by passive and/or active immunization with the refolded antigens in murine models of *Tityus carrilloi* envenomation.