Optimizing SDS-Induced Hexamer Formation of Myotoxin-II from *Bothrops asper* for Structural Characterization

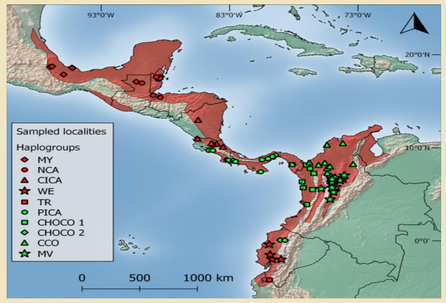
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Introduction

More than 57,000 snakebites occur annually in the Americas, with a reported fatality rate of 0.6% and serious sequelae in over 3% of victims [1]. Bothrops asper is a major cause of severe envenoming, particularly among rural agricultural workers. Envenoming can cause swelling, hemorrhage, skeletal muscle necrosis, permanent tissue damage, and amputation [2]. Its venom contains myotoxic phospholipase A_2 (PLA_2) and PLA_2-like toxins, including Myotoxin-II (Mt-II) [3].



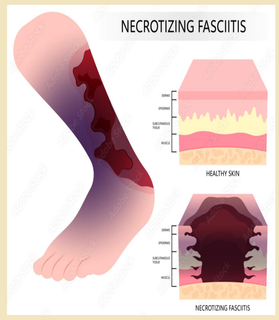


Figure 1. Geographic distribution of Bothrops asper across Middle America and northwestern South America. Red shading indicates the species range, while symbols mark sampled localities and phylogeographic groups. Adapted from Saldarriaga-Córdoba et al. [4].

Mt-II is strongly myotoxic despite minimal catalytic activity [3] and remains soluble and monomeric in SDS-free aqueous solution at concentrations up to 3 mg/mL [5].



Figure 2. Ribbon representation of one Bothrops asper Mt-II subunit. The available structure defines the protein fold but does not reveal the SDS-induced hexameric arrangement proposed to contribute to membrane damage. Source: RCSB Protein Data Bank, PDB ID 1CLP [6].

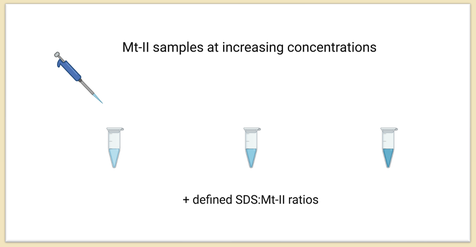
Recent biophysical studies using sedimentation-velocity analytical ultracentrifugation and nuclear magnetic resonance showed that low SDS concentrations induce a discrete Mt-II hexamer [5]. This oligomer may represent a pore-forming assembly that contributes to membrane disruption, although its physiological structure and role remain unresolved. As a hexamer, MT-II could form a pore that could span the membrane of cells and lead to cell apoptosis by allowing ions and nutrients to cross the membrane and eliminate important gradients like Na+, Ca++, K+ and pH.

Goals

- Gather additional evidence supporting the proposed Mt-II pore-formation hypothesis.
- Corroborate the previously reported SDS-induced Mt-II hexamer using sedimentation-velocity analytical ultracentrifugation.
- Identify the Mt-II concentration and SDS:Mt-II ratio that maximize formation of a homogeneous hexamer-like species while minimizing larger aggregates.
- Produce optimized samples suitable for structural characterization by:
- X-ray crystallography, in collaboration with Prof. Omid Haji-Ghassemi at the University of Calgary.
- Cryogenic electron microscopy, in collaboration with Prof. Howard Young at the University of Alberta.

Methods

Mt-II samples were prepared at increasing protein concentrations, mixed with defined SDS:Mt-II ratios, and analyzed by sedimentation-velocity AUC. Data were processed using UltraScan-III [7] to identify monomeric (~2 S), hexamer-like (~6 S), and higher-order or aggregated (>6 S) species.



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Results

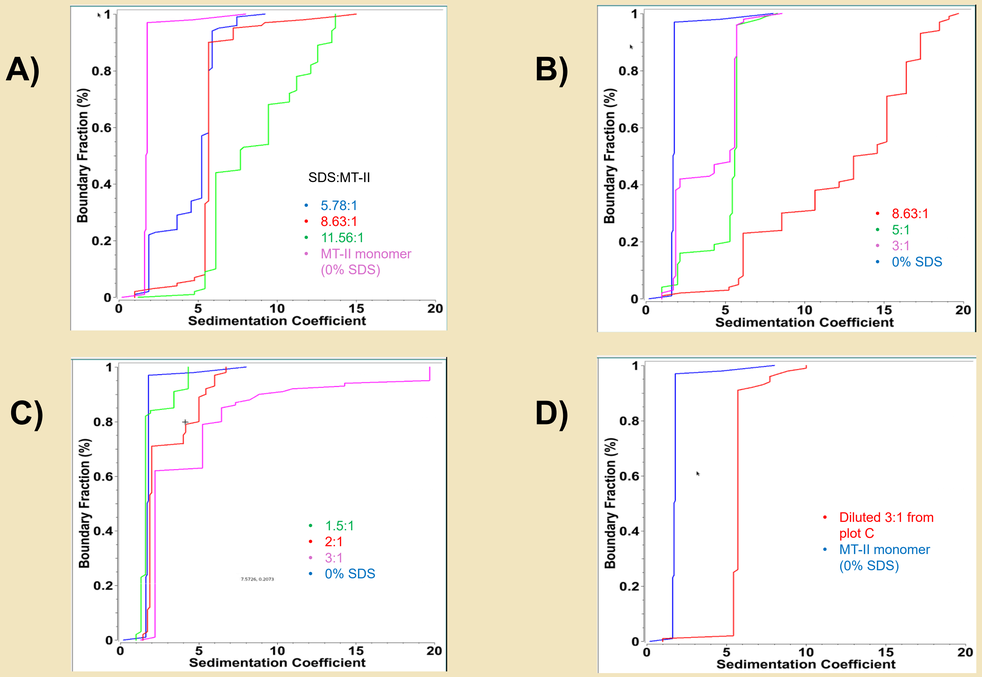


Figure 3. Integral sedimentation coefficient distributions of Mt-II screened across SDS:Mt-II ratios at protein concentrations of 0.33 mg/mL **(A)**, 1.0 mg/mL **(B)**, 6.8 mg/mL **(C)**, and the clarified, diluted 0.3 mg/mL sample **(D)**. AUC analysis prepared by Sigang Yu.

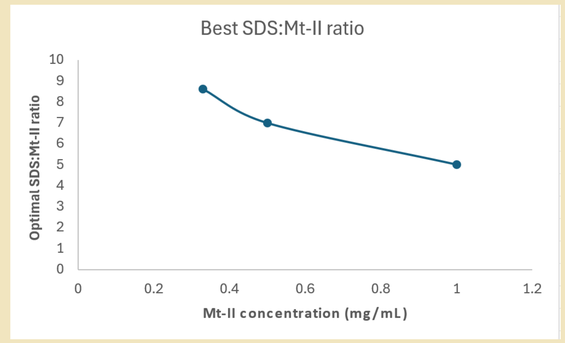


Figure 4. Optimal SDS:Mt-II ratio plotted against Mt-II concentration for conditions that produced the strongest ~6 S hexamer-like signal. The required SDS:Mt-II ratio decreased from 8.63:1 at 0.33 mg/mL to 7:1 at 0.5 mg/mL and 5:1 at 1.0 mg/mL.

Discussion and Conclusions

- At lower Mt-II concentrations, a higher SDS:Mt-II ratio was required to promote formation of the ~6 S hexamer-like species.
- As Mt-II concentration increased, the optimal SDS:Mt-II ratio shifted lower.
- Excess SDS may form micelles that bind one or several Mt-II molecules, producing heterogeneous species larger than the hexamer.
- These higher-sedimenting species likely result from non-specific detergent-mediated association, SDS-micelle formation, or aggregation
- Overall, the discrete Mt-II hexamer remains the strongest candidate for a physiologically relevant oligomer, whereas larger assemblies are more likely experimental artifacts.

Future Directions

- Further refine Mt-II concentration and SDS:Mt-II ratio conditions to maximize homogeneous hexamer formation while minimizing larger aggregates.
- Evaluate optimized samples by X-ray crystallography in collaboration with Prof. Omid Haji-Ghassemi at the University of Calgary.
- Evaluate optimized samples by cryogenic electron microscopy (cryo-EM) in collaboration with Prof. Howard Young at the University of Alberta.

References

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