Cloning & Characterization of CRISP Transcripts from Snake Venom

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Abstract

The CAP (CRISP, antigen 5 and Pr-1) protein superfamily occur in a great variety of species. Cysteine-rich secretory proteins (CRISPs) are a subgroup of this superfamily that, like many other toxins, show potential in their biological and pharmaceutical functions. CRISPs are found in a wide variety of animal tissue and snake venoms, where they inhibit both potassium-induced smooth muscle contraction and cyclic nucleotide-gated channels. From the CRISPs studied, the few that have been functionally characterized were reported to exhibit a multitude of activities. However, many venom CRISPs have yet to be assigned specific functions.

Therefore, to explore the biological and pharmaceutical functions of venom CRISP, we cloned and characterized CRISP transcripts in the venom extracted from a Western diamondback rattlesnake (*Crotalus atrox*). The results obtained from this research would be supplemental for future attempts at unlocking the full potential of CRISPs.

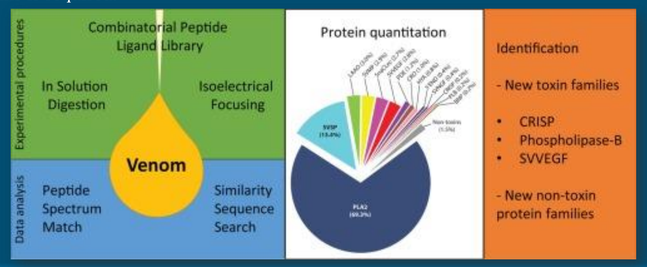
1. Introduction

Despite the abundance of resources, thousands of people in both developed and underdeveloped countries are killed by venomous species every year. The production of antivenom is complicated by the intraspecific variation in venom composition, accentuating the importance of venom research. Most venom consists of a mixture of polypeptides and other molecules with diverse biological activities designed to attack several homeostatic structures within the prey in a precise manner. Thus, introspection of these factors may potentially provide useful information for the structural design of drugs and for the development of life-saving antidotes of greater specificity.

Cysteine-rich secretory proteins (CRISPs) are a broadly distributed family of proteins that have been isolated from multiple different animal tissues, including reptile venom, a large array of vertebrate tissue, and several other invertebrate tissue. Traditionally, CRISPs have been categorized into one of three primary types: CRISP-1, CRISP-2, and CRISP-3, based on their expression, location and roles.

Although many of the venom CRISPs currently are not known to have an identifiable function, some have shown a diverse array of biological activities. The most common svCRISP activity has been non-enzymatic inhibition of several types of membrane channels, but they have exhibited many other activities such as induction of hypothermia in prey animals and specific proteolysis. Target binding to modify cellular signaling cascades is a typical function of CRISPs, along with other proteins of the CAP superfamily.

Despite the discovery of several snake venom CRISPs (svCRISPS), most of these proteins have yet to be isolated and characterized experimentally. In turn, the function and biological role of the majority of CRISPs isolated from snake venom remain unknown. In order to dissect their potential functions in venoms and therefore, evaluate and asses their roles into pharmaceutical applications, further analysis of this subgroup of proteins is required.



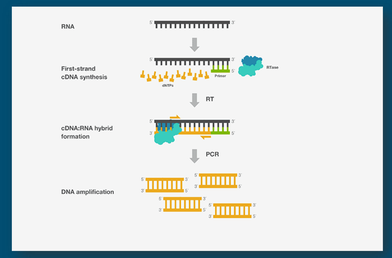
2. Materials and Methods

The unique venom CRISP transcripts were obtained by performing the following experiments:

1) *Crotalus atrox* crude venom was purchased from Miami Serpentarium Laboratories, Florida.
2) The total RNA in fifty micrograms of crude venom was reverse transcribed into cDNA.
3) RT-PCR was performed by using venom cDNA as template and a pair of CRISP specific PCR primers.
4) The PCR amplicon was purified from the agarose gel and ligated into pJET 1.2 vector.
5) The recombinant DNA was further transformed into *E. coli* competent cells.
6) Bacterial clones containing unique CRISP transcripts were screened by PCR for different molecular size and by restriction enzyme for different DNA digestion patterns.
7) The unique CRISP clones were sequenced by Sanger sequencing.

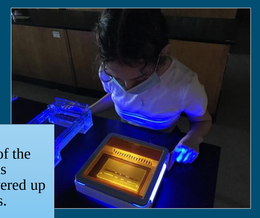
The most common CRISP transcript was characterized by bioinformatics.

- Amino acid sequence alignment.
- Phylogenetic tree construction.
- Modeling protein three-dimensional (3D) structure.

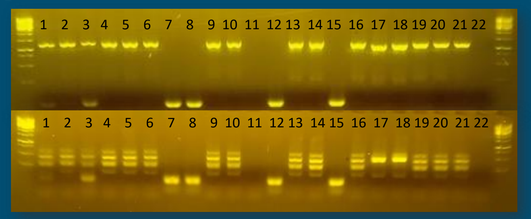


3. Results and Discussion

1) Screening unique CRISP transcripts



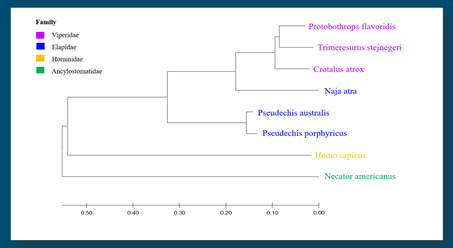
Upon inspection of the gel electrophoresis results, we discovered up to 3 unique clones.



As the results show, clones numbered 1, 2 and 17 are unique amongst the rest. Some clones did not have an insert, and therefore we could not determine if they were unique. The most common CRISP is cone 2.

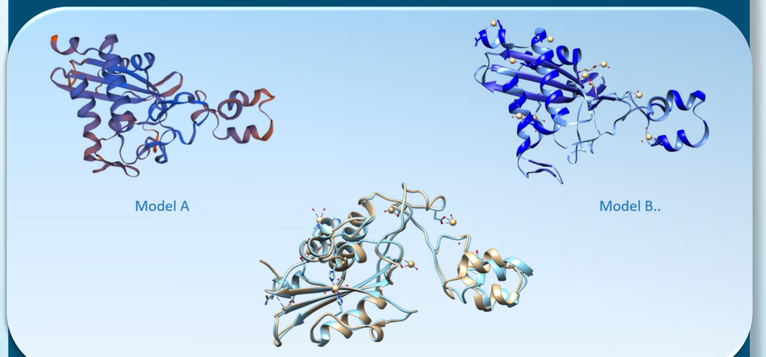
2) Characterizing CRISP amino acid sequences

After studying the characteristics of svCRISP from the snake venom of the Western Diamondback rattlesnake, comparisons were drawn to other families of snakes, along with the CRISPs found in different animals in order to further investigate the transcripts and their relatedness.



These results were drawn using the cDNA derived directly drawn from the svCRISP that was studied (*Crotalus atrox*), while the others were selected based on the abundance of CRISP they have been discovered to possess.
The alignment was made using Clustal Omega, while the phylogenetic tree was built using MEGA X.

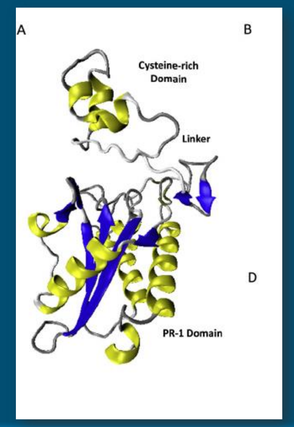
3) Modeling CRISP 3D Structure



Above, two models of CRISP protein structures are displayed. The left (labeled A) was derived using SWISS-MODEL, an online program used for protein structure predictions. On the right, (labeled B), is a protein structure built using the program Chimera 1.14

The bottom figure displays both Model A and Model B layered together. The light blue one portrays Model B whereas the gold one portrays Model A, the template model. Between the two models, there was calculated to be only 0.425 angstroms of a difference. The template was very close to the yielded model based off the results because this strand has been discovered and published on the Protein Data Base.

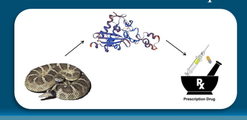
The structure of CRISPs are comprised of two well defined domains named the PR-1 domain and cysteine rich domain. 16 conserved cysteines fold into two domains. The PR-1 domain that contains six conserved cysteine residues, and the smaller CRD that contains 10 conserved cysteine residues.



4. Conclusion

In order to help in the functional characterization of these family of proteins, we cloned and characterized a transcript of CRISP extracted from the snake venom of a Western Diamondback Rattlesnake.

1) Three unique CRISP transcripts were identified from the crude venom of *C. atrox*.
2) The translated amino acid sequence of the most common CRISP transcript was analyzed by multiple sequence alignment and phylogenic tree.
3) CRISP 3D structure was modeled based on the translated amino acid sequence of the most common CRISP, and web server.



Acknowledgments

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Image citations

- Fig 1: Melani, R. D. In-Depth Venome of the Brazilian Rattlesnake Crotalus durissus terrificus: An Integrative Approach Combining Its Venom Gland Transcriptome and Venom Proteome.
- Reverse Transcription Applications: Thermo Fisher Scientific - US. (n.d.). Retrieved from https://www.thermofisher.com/us/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/rt-education/reverse-transcription-applications.html