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SNAKE VENOM COMPONENTS AND THEIR CROSS REACTIVITY: A SHORT REVIEW (U)

by

Bradley J. Berger* and Abdul R. Bhatti

DRDHP-11

October 1988

* Summer Student – May/August 1988

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SUMMARY

Snake venoms are complex mixtures of organic and inorganic compounds, many of which exhibit biological activity. It has been demonstrated that antisera raised against a single purified venom protein from one species of snake will react with proteins in the venom of other species. This cross-reactivity between species may have evolutionary applications, but recent studies on the variability of venom components within a species make these evolutionary conclusions questionable.

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INTRODUCTION

Venomous snakes belong to three families within the suborder Serpentes: the Uiperidae (old world and pit vipers), the Elapidae (a large and varied group which includes cobras, coral snakes, and sea snakes), and the Colubridae (an artificial group containing at least 80 venomous genera) (59). An alternate classification is to place the sea snakes into their own family, the Hydrophiidae. Research into the contents of snake venom has its roots in the mid-nineteenth century when Lucien Bonaparte precipitated a toxic powder out of *Vipera berus* venom (see Table 1 for common names of snakes) and S.W. Mitchell precipitated toxin out of *Crotalus* venom (cited in 5). Since that time the work on defining the composition of snake venom has continued, with the majority of the studies involving four subfamilies: Viperinae (old world vipers), Crotolinae (pit vipers), Elapinae (cobras and coral snakes), and Hydrophiini (sea snakes) (59). Recent advances in purifying and characterizing snake venom toxins have made it clear that there is a potential for their use as biological warfare agents.

The toxic components, especially the neurotoxins, are extremely potent and quite stable, and, with present day recombinant DNA technology, it is theoretically possible to produce these factors in large quantities (58).

As early as 1872, it was observed that there were distinctive differences among the venoms isolated from different species of snake, and by 1902 experiments were being performed to identify antigenic similarities between the venoms of various species (cited in 40). Since that time, there have been many studies on the identification of both the unique and conservative components present in snake venoms. The principal aim of this communication is to review the recent advances in defining the cross-reativity of snake venom components, with a short discussion of evolutionary implications.

The Components of Snake Venom

As evidenced by many authors, snake venoms are complex mixtures of organic and inorganic compounds (4, 7, 14, 45). While not as well studied, the non-protein fraction of snake venom has been characterized and reviewed by Bieber (3). In general, snake venoms were found to contain sodium, potassium, phosphorus and chloride, as well as trace amounts of calcium, zinc, magnesium, copper and manganese (10, 15, 19). As well, riboflavin, nuceosides (especially guanosine), peptides and amides (including serotonin and bufotonin) have been detected in some snake venoms (13, 24, 51, 61). While carbohydrates and lipids (such as the procoagulant glycoprotein in *Vipera russelli*) have been detected in snake venom, little has been published on this aspect (57).

Unlike the non-protein components, proteins in snake venom have

been extensively studied, and intensive research into their structure and function continues. In fact, due to the volume of research into this area, it is beyond the scope of this paper to review more than a few examples of relevant proteins in snake venoms. In general, snake venom proteins are divided into three groups: nerve growth factors, enzymes, and toxins. Nerve growth factors are agents which cause differentiation of sympathetic or sensory neurons (32) and, as of 1979, had been discovered in at least twenty different venomous snakes (23).

Enzymes

The enzyme components of snake venoms are responsible for much of the visible physiological damage, such as tissue necrosis, coagulant or anticoagulant activity, and pain (36, 53). Ammong the most important enzymes found in snake venoms are phospholipase A_2 (PLA_2), L-amino acid oxidase, and phosphodiesterase.

PLA_2 is a protein of approximately 14000 molecular weight, which hydrolizes phosphatidylcholine to lysophosphatidylcholine and a fatty acid (22). This activity causes the destruction of cell membranes, leading to hemolysis. In the last decade, PLA_2 has been purified from many snakes, including *Crotalus adamanteus* and *Bothrops atrox* (16), and it has been purified and sequenced from *Bitis gabonica* (6) and from *Naja melanoleuca* (26). More recently, PLA_2 has been purified from *Trimeresurus gramineus*, *Angkistrodon contortix contortix*, *T. flavoviridis*, *Bothrops asper*, *Pseudoechis australis*, and *Enhydrina schistosa* (20, 33, 42, 43, 54 56).

L-amino acid oxidase is detected in venomous snakes, except sea snakes, and is a protein of approximately 135,000 molecular weight that

contains FAD (flavin adenosine dinucleotide) as a cofactor and about five percent carbohydrate (25). Studies have shown that this enzyme exists as three isozymes of equal specific activity (50). Phosphodiesterase, which hydrolyzes the phosphodiester bonds of polynucleotide chains at the 3' end, is found in snakes from all venomous families (37). The enzyme is approximately 110,000 molecular weight and has been recently purified and characterized from *Cerastes cerastes* venom (21). As well as the three major enzymes mentioned, there are at least 23 other types of enzyme in snake venom (according to Russell (48). Among these are proteinases, ATPases, coagulants, arginine esterase and anti-coagulants (1, 29, 31, 44, 62).

Toxins

Toxins are proteins found in snake venom which cause disruption of vital functions, such as nerve transmission and autonomic activities (27). The distinction between enzymes and toxins lies in the fact that many toxins have no enzymatic activity (such as short chain neurotoxins), and many enzymes do not have lethal activity (though they may cause physiological damage). However, this division is somewhat arbitrary as crotoxin, for example, has both neurotoxic and PLA_2 activity (14). The main toxins in snake venom are grouped into four types: neurotoxins (which block the transmission of nerve impulses), myonecrotic toxins (which degrade muscle tissue), membrane toxins (which disrupt cellular membranes), and "other" toxins (those whose actions have not yet been characterized, or which do not fit in the other catagories) (27). There has been a great deal of work on the purification and characterization of toxins from the venom of diverse snakes, such as neurotoxins from *Trimeresurus mucosquamatus, Enhydrina schistosa, Acanthophis antarcticus,* and *Naja ssp,* myonecrotic toxin from *Bungarus fasciatus,* and "other" toxins in

Atractaspis spp, and *Agkistrodon piscivorus* (7, 17, 28, 30, 35, 46, 52).

Antigenic Similarity of Proteins from Different Snake Venoms

Given that venoms from unrelated snakes often contain many common enzymes, it is not surprising that there can be antigenic cross-reactivity (ie: where antisera generated against one snake venom partially neutralizes venom from another species of snake). For example, PLA_2 activity is detected in virtually all snake venoms and has been purified and sequenced from many (11, 25). A comparison of the sequences of PLA_2 from 29 different snakes shows that all Elapids have a PLA_2 with the same overall configuration, but Asian Elapids and Australasian Elapids have a slightly different configuration around the active site (11). Thus, there should be a great deal of antigenic similarity between the PLA_2's of Elapid snakes, especially within a particular geographic subgroup.

More importantly, many researchers have raised antibodies against specific toxins from a single species of snake and detected cross-reacting proteins in other species. Lomonte *et al.* (34), using antisera against *Bothrops asper* myotoxin, detected a cross-reacting protein in *B. nummifer, B. schlegeli, B. godmani, B. picadoi,* and *Agkistrodon bilineatus.* The detected proteins had the same molecular weight (16,000) in all species except *B. picadoi*, which had a molecular weight of 24,000, but the isoelectric point was quite variable from species to species. Therefore the cross-reactive component was similar, but not identical to the original myotoxin. Monoclonal antibodies raised by Arumae et al. (2) against the *Vipera lebentina* nerve growth factor (molecular weight of 32,500) cross-reacted with the factors from

Vipera ursini (molecular weight of 37,000), *V. berus berus*, *Echis carinatus* (molecular weights of 36,000 and 44,000, respectively), *Bungarus caeruleus*, *Agkistrodon halys*, *Naja naja oxiana*, *Naja naja atra*, *Naja naja*, and the mouse salivary gland nerve growth factor. Cross-reactivity over such a wide range of species would suggest that the antigenic site on the nerve growth factor must be quite conservative.

Antibodies against a toxin from *Crotalus scutulatus scutulatus* (molecular weight of 20,000-22,000) strongly cross-reacted with a similar protein from *C. durissus*, *C. viridis concolor*, and *C. scutulatus* samples; and weakly with *C. atrox* and *Trimeresurus flavoviridis* (60). Antibodies against crotoxin from *Crotalus durissus terrificus* also reacted with *C. horridus atricaudatus* and *C. basiliscus*, suggesting that these latter two species have a similar toxin (18). Rael *et al.* (47) generated monoclonal antibodies against Mojave toxin from *Crotalus scutulatus scutulatus*, and found that there was cross-reactivity with other proteins, such as those in the venom from *C. basiliscus*, *C. durissus durissus*, *C. d. terrificus*, *C. horridus horridus*, and *C. viridis concolor*.

Evolutionary Implications

The most popular application of the results obtained from serological cross-reactivity studies is snake phylogeny and evolution. It is well known that the evolutionary relationship of venomous snakes is not clearly characterized (12, 39, 59). There have been many attempts at determining snake phylogeny on the basis of the sequence changes

found in conserved proteins, such as PLA_2, neurotoxins and cytotoxins (2, 8, 12). In this type of analysis, the number of sequence changes is directly related to the evolutionary distance between the species studied. However, since it has been pointed out that different phylogenies arise when different proteins are the basis for sequence analysis (55), researchers are starting to draw evolutionary conclusions from electrophoretic and immunological studies. Mengden *et al.* (39) determined the phylogenetic relationship of *Pseudoechis* species using electrophoretic fractionation patterns of proteins. Weinstein *et al.* (60), and Detrait and Girons (9) have used immunological cross-reactivity as a basis for evolutionary speculation. Since snakes that are closely related have similar components in their venom, it is assumed that the degree of cross-reactivity directly correlates to the evolutionary distance between the snakes studied.

However, there have been recent studies which show that these types of evolutionary conclusions are not valid. Schaeffer (49) has found that individual lots of commercial venom (from which almost all immunological and biochemical studies are performed) are unique in their composition, even though obtained from the same species (either *Echis carinatus* or *Echis coloratus*). It has been found that the composition of *Bothrops atrox* venom varies from individual to individual on the basis of age, and that *Crotalus atrox* venom composition can vary from one geographical area to another (38, 41). These results seem to indicate that the variability in protein composition makes it difficult to draw strong evolutionary conclusions from the biochemical and immunological analyses of venom components. As well, the fact that antibodies against the nerve growth factor from *Vipera lebentina* cross-reacted with the nerve growth factor from mouse salivary gland tends to show how little antigenic similarity may relate to phylogeny in some cases. This

variability means that a protein containing a cross-reactive epitope may not always be detected in a given snake, and, thus, the epitope's presence or absence cannot be used as an indication of the evolutionary distance between species of snakes.

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TABLE 1: COMMON NAMES OF RELEVANT SNAKES

SUBFAMILY	SNAKE	COMMON NAME
Viperinae		
	Vipera berus	European Viper
	Vipera russelli	Russell's Viper
	Bitis gabonica	Gaboon Viper
	Cerastes cerastes	Mojave Desert Sidewinder
	Atractaspis spp.	Burrowing Vipers
	Vipera lebetina	Leventine Viper
	Vipera ursini	Meadow Adder
	Echis carinatus	Saw-scaled Viper
	Echis coloratus	Carpet Viper
Crotolinae		
	Crotalus adamanteus	Eastern Diamondback Rattlesnake
	Bothrops atrox	Fer-de-Lance
	Trimeresurus gramineus	Indian Green Tree Viper
	Agkistrodon contortix contortix	Southern Copperhead
	Trimeresurus flavoviridis	Okinawa Habu
	Bothrops asper	Barba Amarilla
	Trimeresurus mucosquamatus	Taiwan Habu
	Agkistrodon piscivorus	Eastern Cottonmouth
	Bothrops nummifer	Jumping Pit Viper
	Bothrops schlegeli	Schlegel's Palm Viper
	Bothrops godmani	Godman's Pit Viper
	Bothrops picadoi	Pit Viper
	Agkistrodon bilineatus	Mexican Moccasin
	Agkistrodon halys	Pit Viper
	Crotalus scutulatus scutulatus	Mojave Diamondback Rattlesnake
	Crotalus durissus durissus	Central American Rattlesnake
	Crotalus atrox	Western Diamondback Rattlesnake
	Crotalus durissus terrificus	South American Rattlesnake
	Crotalus horridus atricaudatus	Cranebrake Rattler
	Crotalus basiliscus	Mexican West-Coast Rattlesnake
	Crotalus horridus horridus	Timber Rattler
	Crotalus viridis concolor	Prairie Rattlesnake
Elapinae		
	Naja melanoleuca	Black-Lipped Cobra
	Pseudechis australis	King Brown Snake
	Acanthopsis antarcticus	Death Adder
	Bungarus caeruleus	Common Indian Krait
	Naja najua oxiana	Ochkovayazmeya
	Naja naja atra	Taiwan Cobra
	Naja naja	Indian Cobra
Hydrophiini		
	Enhydrina schistosa	Common Sea Snake

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Snake venoms are complex mixtures of organic and inorganic compounds, many of which exhibit biological activity. It has been demonstrated that antisera raised against a single purified venom protein from one species of snake will react with proteins in the venom of other species. This cross-reactivity between species may have evolutionary applications, but recent studies on the variability of venom components within a species makes these evolutionary conclusions questionable.

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Key words: snake venoms, enzymes, toxins, evolution