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Electrophoretic Characterization of Elapid, Viperid and Crotalid Snake Venoms

by

C.E. Connolley-Mendoza, T. Bhatti and A.R. Bhatti

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ELECTROPHORETIC CHARACTERIZATION OF ELAPID, VIPERID AND CROTALID SNAKE VENOMS

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ABSTRACT

This report deals with comparative studies of snake venoms from 21 species representing Elapidae, Crotalidae and Viperidae. Both native and denatured venoms were analyzed by polyacrylamide gel electrophoretic methods with or without sodium dodecyl sulfate. Electrophoreses showed qualitatively the commonality of protein and polypeptide components in venoms from various snake species. Electropherograms also showed the characteristic protein and polypeptide profiles which differentiate one species from another. These profiles, consisting of a combination of protein or polypeptide bands, suggested that each venom is unique for each species, although similarity abounds among subspecies or related species.

INTRODUCTION

The pharmacology and toxicology of snake venoms, in general, and rattlesnake venom toxicity in particular, have been reviewed by Henriques and Henriques (1971) and Facklam (1983), respectively. The major actions and active principles of snake venoms have been classified by Tu (1986a and 1986b) as highly lethal, relatively non-lethal and autopharmacological. The presynaptic and/or postsnyaptic toxins trigger the highly lethal neurotoxic action (Chang, 1979); cardiotoxin triggers the cardiotoxic action (Lee and Lee, 1979); whereas myotoxin triggers the mycotoxoic and hemorrhagic actions (Ohsaka, 1979). Snake venoms have also been reported to cause serious renal lesions (Sitprija and Boonpucknavig, 1979).

Relatively non-lethal venom activities include hemolysis (Condrea, 1979), blood coagulation (Seegers and Ouyang, 1979), increased vascular permeability (Somani, 1962), anticomplementary effects (Alper, 1979), and the action of the nerve growth factor (NGF) (Hogue-Angelletti and Bradshaw, 1979). NGF potentiates the poisonous effects of venoms by stimulating the responsive cells, and thus, rendering them vulnerable. NGF found in venom may also represent the manner in which the excess material, otherwise synthesized for endocrine functions, is removed from the venom or submaxillary gland. NGF has also been reported to regulate nerve growth and differentiation.

Autopharmacological actions, produced by some venoms are mediated by bradykinin-, histamine-, and serotonin-releasing enzymes (proteases) (Lee and Lee, 1979); Ohsaka, 1979; Rosenberg, 1979; Rothschild and Rothschild, 1979; Tu, 19865a and 1986b). Snake venoms also contain other enzymes (Henriques and Henriques, 1971; Iwanaga and

Susuki, 1979; Ramachandran et al., 1984; Tu, 1986a). Ramachandran et el., (1984) have detected different enzyme activities in a protein fraction derived from a cobra venom. The importance of an enzyme in the mechanism of neurotoxicity has been demonstrated by Hendon and Tu (1979), who have shown that a combination of events must happen before a certain toxic factor can act. For example, the action of phospholipase A (PLA) on membranes and the release of crotoxin-a must occur before crotoxin-b (a toxic component of the rattlesnake venom) acts on the receptor to effect neurotoxicity. Crotoxin-b neurotoxicity can be inhibited without the loss of PLA activity. This report clearly suggests that studies of a toxic factor, in insolation from other venom components, may give misleading results.

Thorough knowledge of the biological and toxic nature of venoms from different species is paramount to avoid pitfalls that may be encountered when a single specific toxic factor is selected for toxicity assessment. Isolation and comparative characterization of toxic components are essential in understanding the mechanism and potential hazard of snake venom components, either singly or in combination, as BW agents. Knowledge of the commonality of a toxic factor, or a combination of toxic factors, in venoms from various snake species is also crucial. This knowledge is fundamental in development of a more comprehensive type of protection and therapy against many, if not all, types of venoms and their constituents.

Obviously, there are several active factors in a particular venom sample. Adequate knowledge of the properties of different venoms is necessary before pursuing studies of a specific toxic factor and before developing a specific prevention/therapeutic method or material against a specific toxin. Likewise, developing a preventive/therapeutic method or material against venom for each snake species is

impractical.

The advent of biotechnology and genetic engineering magnifies the potential hazard of snake venom components as biological warfare (BW) agents. Some venom components are sufficiently lethal and debilitating to be considered as part of a "new biotechnological generation" of BW agents. With biotechnology, obtaining large amounts of the lethal factors present in venoms is no longer a remote possibility.

The objective of the present study was to obtain basic understanding of the properties inherent to native and denatured snake venoms. This report discusses the results of comparative studies using electrophoretic methods to characterize venoms from 21 snake species.

MATERIALS

Chemicals

The chemicals and reagents used in this study were as follows: acrylamide, bis-acrylamide, 2-mercaptoethanol, Coomassie blue (N,N,N'N'-tetramethylethylenediamine), silver stain kit (Sigma Chemical Co., St. Louis, MO); sodium dodecyl sulfate (SDS), tris(hydroxymethyl)-aminomethane (tris) and molecular markers (Bio-Rad Laboratories, Richmond, CA); bromphenol blue (J.T. Baker Chemical Co., Phillipsburg, NJ); glycerol (Fisher Scientific Co., Fair Lawn, NJ); acetic acid, ACS analytical reagent (BDH Chemicals Canada Ltd., Toronto); methanol and prestained SDS-PAGE protein standards (Bio-Rad Laboratories).

Venom Samples

Table I shows the identification numbers, scientific and common names of snakes producing the venoms studied. All venom samples were purchased from Sigma Chemical Co.

METHODS

Sample Preparative Solution

For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the solution used for sample preparation was composed of the following: 1 part of 0.5 M of tris, pH 6.8; 1 part of 10% SDS; 0.1 part of 2-mercaptoethanol; 1 part of glycerol; 6.9 parts of distilled water and enough bromphenol blue to make the solution deep blue. For non-denaturating electrophoresis, the same preparative solution without SDS was used.

Buffer and Acrylamide Stock Solutions

Appendix 1 shows the compositions of the buffer and acrylamide stock solutions. It also shows the final concentration of each ingredient used in the electrophoretic gels.

Sample Preparation and Application

To prepare the stock solution containing 10 mg/mL of each sample, the venom was weighed and dissolved in the same tris preparative solution, pH 6.8, without SDS and bromphenol blue. The resulting solution was further diluted with the same preparative solution to obtain the desired amount in a sample volume of 10-20 uL. Using a

Hamilton microliter syringe, aliquots were placed in electrophoretic wells.

Electrophoretic Method

The SDS-treated proteins were separated in an electrophoresis unit (Bio-Rad Laboratories, Model 220, Technical Marketing Assoc. Ltd., Mississauga, Ontario) according to the Laemmli SDS-PAGE method (1970). The native venoms were resolved in gels without SDS. Prestained molecular markers (17,000 to 135,000 apparent M.W.) were used as references and as indicators of the quality of the electrophoretic gel.

Coomassie Blue Staining

After electrophoresis, gels were stained for ~1 h in a solution containing 0.2% of Coomassie Brilliant Blue R and 7% acetic acid in a 1:1 volume of distilled water and methanol. The excess stain was removed by a destaining solution containing 7% acetic acid and 5% methanol in distilled water. The destaining solution was changed until satisfactory definition of bands was achieved. To ensure even staining or destaining, dishes containing the gels, were gently agitated. The gels were photographed soon after staining and stored in plastic wraps for future reference.

Silver Staining

The gels were also stained with a silver stain by using a standard method provided by the Sigma Chemical Co., with the silver stain kit. To achieve proper reduction of silver, it required 10-30 sec and careful attention. Soon after fixation, gels were photographed and stored in plastic wraps.

RESULTS

Analysis of the Native Venoms by Non-SDS-PAGE

Figure 1 shows typical electrophoretic profiles of native venoms from three snake families as obtained in non-SDS gels. These electrophoretic profiles demonstrate both bands with similar and dissimilar electrophoretic mobilities between species. The black dots between adjacent lanes mark some of the protein bands with similar electrophoretic mobilities. Cobra venoms have fewer distinct protein bands than those found in the viper and rattlesnake venoms [Figure 1, venoms 14, 15, 16 and 17 (cobras) versus venoms 5, 6, 9 and 12 (rattlesnakes) or venoms 4, 8, 13 and 18 (vipers)]. Protein bands, with similar electrophoretic mobilities, occur more frequently in venoms derived from snakes belonging to the same family [Figure 1, venoms 15 and 16 (cobras); venoms 13, 18, 19 and 21 (vipers); venoms 2, 6 and 9 (rattlesnakes)]. These proteins with similar electrophoretic mobilities are not necessarily present in the same concentrations in the different species.

Table II shows the protein bands present in the different venoms as determined by non-SDS-PAGE and the four reference proteins selected randomly from the native venom of Atheris squamigera (Figure 1, Lane A). They are labelled "a", "b", "c", and "d" ["d" has a mobility similar to that of lysozyme standard (not shown)]. The majority of these venoms have bands with similar electrophoretic mobilities to those selected from A. squamigera. These proteins frequently migrate in the first half of the gel proximal to the origin, where proteins with slow electrophoretic mobility are found. Protein bands are rarely present in the second half of the gel, distal from the origin.

Analysis of Venoms by SDS-PAGE

The typical SDS-electrophoretic profiles of the SDS-treated venoms are shown in Figure 2. Polypeptides with similar electrophoretic mobilities are marked with black dots in this figure as well. The SDS gels also show the majority of polypeptides are in the ≤17,000 M.W. region. Furthermore, species from the same phylogenetic family have similar polypeptides, with occur frequently in the ≤17,000 M.W. region [Figure 2, venoms 3 and 21 (viperids); venoms 11 and 12 (crotalids); venoms 16 and 17 (elapids)]. The polypeptides in the 130,000 M.W. region have been observed only in the crotalids (Figure 2, No. 1 and 7) and viperids (Figure 2 Nos. 19 and 20), often appearing as very faint bands.

To describe and compare the different venoms, the polypeptides present in each venom are classified into 6 groups based on molecular weight (Table III). The data show that more polypeptides migrate in the ≤17,000 M.W. region than in the ≥17,000 - 30,000 M.W. region. Polypeptides with similar electrophoretic mobilities occur most frequently in the ≤17,000 region. Table III also shows the total number of protein bands present in the native venoms. Statistical analyses indicate that the viper and rattlesnake venoms have comparable total number of polypeptide bands, which are significantly greater ($p \leq 0.05$) than those present in the cobra venoms.

Table IV shows the presence of polypeptide bands in different venoms that have similar electrophoretic mobilities as the 4 selected molecular markers. Polypeptide bands with mobilities similar to the 130,000 M.W. marker are absent, while those similar to the 50,000, 39,000 and 17,000 M.W. markers occur more frequently. Typical electropherograms of SDS-treated venoms in SDS gels show that the majority of

polypeptide bands occur in the ≤17,000 M.W. region (Figure 2, I and II). The electrophoretic profiles show that different venoms from the different snake species have several polypeptide bands with similar electrophretic mobilities. These bands are marked with black dots. Furthermore, polypeptides present in venoms from the 4 Naja species studied (Figure 2, II, Venoms 14, 15, 16 and 17) predominantly occur in the ≤17,000 M.W. region.

Comparison of Native and SDS-Treated Proteins

Figure 3 shows the marked difference between the typical electrophoretic profiles of the native and SDS-treated venoms. Cobra venoms (Figure 3, 1) treated with SDS, give mostly low molecular weight polypeptides, unlike the viper (Figure 3, 11) and the crotalid (Figure 3, III) venoms. The rates of electrophoretic mobility of the polypeptide subunits and proteins indicate the similarities as well as the differences among venoms from various species belonging to the same, or different, phylogenetic families. The SDS-treated venoms are markedly more sensitive to detection than the native venoms. For example, the native venom (Figure 3, I, N. naja, left lane) requires at least 300 µg of the sample, whereas the SDS-treated venom requires less than 100 µg to obtain adequate detection.

Comparison of Silver and Coomassie Blue Stains

Figure 4 shows a pair of typical non-SDS electropherograms of native venom proteins from representative species of Elapidae, Viperidae and Crotalidae. Some proteins detectable by the Coomassie stain are undetectable by the silver stain and vice versa. For example, the fastest-migrating bands in the A. piscivorus piscivorus venom (Figure 4, Lane B) were detected with the silver stain but not

with the Coomassie blue stain. The opposite was observed with the faster migrating bands in venoms from Cerastes cerastes (Lane D) and V. palaestinae (Lane H). With N. naja venom (Lane F), only 2 bands were detected with the silver stain whereas 8 bands were detected with the Coomassie blue stain.

Similar staining properties were observed using the silver and Coommassie blue staining methods for SDS-PAGE gels (Figure 5). Some protein bands reacted better with the Coomassie blue stain than with the silver stain, and vice versa (see Lanes A to H). In addition, the polypeptides stained more intensely with Coomassie blue than with silver stain, except for the faster moving polypeptides in the N. naja venom (Lane H).

DISCUSSION AND CONCLUSION

The results obtained from SDS-PAGE and non-SDS-PAGE of venoms from different species, genera and families indicate similar protein and polypeptide electrophoetic profiles. Similar electrophoretic mobilities of polypeptides with ≤17,000 M.W. are more evident in those species belonging to the same genus or family. Iwanaga and Suzuki (1979) have shown the presence of polypeptides with similar electrophoretic mobilities in the venoms of Crotalus spp. Our study, showing the striking similarity of the profiles of proteins or polypeptides present in the venoms from crotalids and viperids, suggests a somwehat closer phylogenetic relationship than generally accepted. The similarity of the electrophoretic profiles may also support classification of crotalids under the subfamily Crotalinae, family Viperidae, as suggested by Underwood (1979). On the other hand, Boquet (1979) suggested that evolutionary changes that occurred in the composition and structure of a protein could be very extensive without affecting

its catalytic or toxic activities. He studied the primary structure of homologous proteins in toxins from various snake species to establish a hierarchy based on their structural and antigenic properties.

As an identification method, electrophoretic profiles obtained from both SDS and non-SDS-PAGE, would show the similarities between species as well as their unique differences. This similarity, or disparity, of components present in V. russelli with venoms from different species has been demonstrated by immunogenic cross reactivity (Berger, B.J. and Bhatti, A.R. 1988). Some proteins or polypeptides detected by these methods in venom samples may be toxic and/or non-toxic components. Thus, adequate knowledge of the presence of these components is paramount to understanding the overall toxicity and in the development of protection strategies against the toxic effects of these venom moeities.

Switzer et al., (1979) and Oakley et al., (1980) have reported that the silver stain is more sensitive for protein detection than the Coomassie blue stain. Our study suggests that the silver stain is not necessarily the most highly sensitive for detection of proteins of the types present in snake venoms. Certain proteins from native venoms, and polypeptides from SDS-treated venoms, react poorly with the silver stain, but are readily detectable with the Coomassie stain. We, therefore, conclude that the general usefulness and reliability of this method remains uncertain (see also Hames, 1981).

Furthermore, we conclude that several proteins or polypeptides are common in the venom from different snake species belonging to different genera and families. At the same time, other proteins or polypeptides appear to be unique and characteristic only to species belonging to the same family. Knowledge of the roles of the enzymatic

and non-enzymatic components in the overall mechanism of venom toxicity is crucial to the development of prophylaxis and therapy against venoms. This knowledge would provide a basis for formulating a 'synthetic antigen' (a combination or cocktail of toxic and adjuvant or synergistic factors present in snake venoms). Hopefully, this 'synthetic antigen' would produce effective immunity against a wide range of toxins present in venoms of different snake species.

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TABLE I

SOURCES OF THE SNAKE VENOMS STUDIED

VENOM NO.	SPECIES*	COMMON NAME
	Elapidae:	
14.	Naja melanoleuca	black cobra
15.	Naja haje	Egyptian cobra
16.	Naja naja	Common Indian cobra
17.	Naja naja kaouthia	Thailand cobra
	Viperidae:	
4.	Atheris squamigera	green bush viper
3.	Bitis gabonica	Gaboon viper
8.	Cerastes cerastes	desert horned viper
13.	Echis carinatus	saw-scaled viper
18.	Vipera ammodytes	Southern European sand viper
19.	Vipera lebetina	Levantine viper
20.	Vipera palaestinae	Palestinian viper
21.	Vipera russelli	Russell's viper
	Crotalidae:	
1.	Agkistrodon rhodostoma	Malayan pit viper
2.	Agkistrodon p. piscivorus	Eastern cottonmouth moccasin
5.	Bothrops jararaca	South American pit viper
6.	Bothrops lansbergii	South American hognose viper
7.	Bothrops nummifer	jumping viper
12.	Crotalus basiliscus	Mexican West-Coast rattlesnake
9.	Crotalus viridis viridis	prairie rattlesnake
10.	Crotalus viridis oreganus	Pacific rattlesnake
11.	Crotalus molossus molossus	black-tailed rattlesnake

*Crotalids have also been classified under family Viperidae, subfamily Crotalinae (Underwood, 1979).

TABLE II

PRESENCE OF PROTEINS WITH SIMILAR ELECTROPHORETIC MOBILITIES*
IN NATIVE VENOMS OF VARIOUS SNAKE SPECIES

VENOM NO.	SPECIES	PROTEIN BAND "a"	"b"	"c"	"d"
Elapidae:					
14.	Naja melanoleuca	+(d)	+	-	-
15.	N. haje	-	-	-	-
16.	N. naja	-	+(f)	+(f)	-
17.	N. n. kaouthia	+	-	-	-
Viperidae:					
4.	Antheris squamigera	+	+	+	+
3.	Bitis gabonica	+(f)	+(d)	+	+(f)
8.	Cerastes cerastes	+	+	+	-
13.	Echis carinatus	-	+(d)	+	-
18.	Vipera ammodytes	-	-	-	-
19.	V. lebentina	+	+	-	-
20.	V. palaestina	-	-	+(d,f)	-
21.	V. russelli	+(d)	-	-	-
Crotalidae:					
1.	Agkistrodon rhodostoma	-	-	-	-
2.	A. piscivorus piscivorus	+	+(d)	+(f)	-
5.	Bothrops jararaca	+(d)	+(f)	-	-
6.	B. lansbergii	+(d)	+	-	-
7.	B. nummifer	-	+(f)	+	-
12.	Crotalus basiliscus	+(d)	+	-	-
9.	C. viridis viridis	+	-	+	-
10.	C. viridis oreganus	-	+(d,f)	+(f)	-
11.	C. molossus molossus	+	-	+(f)	-

* Venoms were separated in non-SDS acrylamide gels. Venom No. 12 is used as a reference, with polypeptide bands labelled "d" (similar mobility to lysozyme of ≤17,000 M.W.), "a", "b" and "c" randomly selected. A plus (+) or a minus (-) sign indicates the presence or absence, in various species, of polypeptide bands that have similar electrophoretic mobilities as those labelled in No. 12. Parenthetic (d) indicates diffused band and (f) faint band.

TABLE III

NUMBER OF BANDS OBSERVED IN NATIVE AND SDS-TREATED VENOMS

VENOM NO.	SPECIES	NUMBER OF BANDS						TOTAL	
		17K	27K	39K	50K	75K	130K	SDS	NATIVE*
Elapidae									
14.	Naja melanoleuca	5	2	1	1	2	0	11	10
15.	N. haje	5	3	2	1	1	0	12	11
16.	N. naja	7	0	0	0	0	0	7	12
17.	N. naja kaouthia	5	2	0	0	1	0	8	7
Crotalidae									
1.	Agkistrodon rhodostoma	9	3	2	1	0	1	16	15
2.	A. piscivorus piscivorus	7	3	1	1	0	0	12	15
5.	Bothrops jararaca	5	1	1	1	0	0	8	12
6.	B. lansbergii	4	3	1	2	1	0	11	15
7.	B. nummifer	7	0	2	2	0	1	12	12
12.	Crotalus basiliscus	7	4	2	0	0	0	13	13
11.	C. molossus molossus	11	3	1	1	0	0	16	15
10.	C. viridis oreganus	7	3	2	3	0	0	15	16
9.	C. viridis viridis	7	3	1	2	0	0	13	14
Viperidae									
4.	Antheris squamigera	8	3	2	3	0	0	16	15
3.	Bitis gabonica	4	4	2	1	1	1	13	14
8.	Cerastes cerastes	4	4	2	3	1	0	14	15
13.	Echis carinatus	7	3	3	3	0	0	16	12
18.	Vipera ammodytes	5	3	2	2	1	0	13	13
19.	V. lebetina	10	2	1	1	0	1	15	11
20.	V. palaestinae	5	4	2	2	2	1	16	12
21.	V. russelli	9	3	1	2	1	0	16	14

*On electropherograms for the same species of snakes, native (non-SDS treated) venom generally showed larger protein molecules than those treated with SDS.

TABLE IV

POLYPEPTIDES WITH SIMILAR ELECTROPHORETIC MOBILITY FROM SNAKE VENOMS TREATED WITH SDS AND SEPARATED IN SDS ACRYLAMIDE GELS

VENOM NO.	SPECIES	POLYPEPTIDE BAND* (M.W.) 17K	39K	50K	130K
Elapidae:					
14.	Naja melanoleuca	+	+(f,d)	-	-
15.	N. haje	-	+(f)	-	-
16.	N. naja	-	-	-	-
17.	N. n. kaouthia	-	-	-	-
Viperidae:					
4.	Antheris squamigera	-	-	-	-
3.	Bitis gabonica	+(f)	-	+(f)	-
8.	Cerastes cerastes	+(d)	-	+	-
13.	Echis carinatus	-	+	+	-
18.	Vipera ammodytes	+	-	-	-
19.	V. lebetina	+	-	-	-
20.	V. palaestinae	+(f)	-	+	-
21.	V. russelli	-	+.(d)	-	-
Crotalidae:					
1.	Agkistrodon rhodostoma	+	-	-	-
2.	A. piscivorus piscivorus	-	-	-	-
5.	Bothrops jararaca	-	-	-	-
6.	B. lansbergii	-	+(d)	+	-
7.	B. nummifer	-	-	+	-
9.	Crotalus viridis viridis	-	-	+	-
10.	C. viridis oreganus	+(d)	+(d)	-	-
11.	C. molossus molossus	+	+(f,d)	-	-
12.	C. basiliscus	+	-	-	-

* Prestained molecular standards (Bio-Rad Lab.) are used as a reference, 17K = lysozyme, 39K = carbonic anhydrase, 50K = ovalbumin, and 130K = phosphorylase b. A plus (+) sign indicates the presence of a polypeptide band with similar electrophoretic mobility as that of the standard. Parenthetic (d) indicates diffused band and (f) faint band.

APPENDIX A
TO SUFFIELD MEMORANDUM NO. 1280
DATED 2 MAY 1988

PREPARATION OF ELECTROPHORESIS STOCKS AND OTHER SOLUTIONS

	ITEM	CONCENTRATION
A.	Resolving Tris buffer, 10 X (stocks):	0.05 M
B.	Tris buffer pH 8.9 (stocks) for stacking or resolving (upper) gel:	3.0 M
C.	Acrylamide/bis-acrylamide (stocks):	
	a. For resolving gel	30% : 0.8%
	b. For stacking gel	30% : 0.24%
D.	Resolving gel preparation (60 mL) (2 slabs):	
	a. 25 mL acrylamide : bis-acrylamide (C.a)	12.5% : 0.24%
	b. 7.5 mL Tris (B)	0.375M
	c. 0.6 mL SDS	0.1%
	d 0.02 mL TEMED	0.0003%
	d. 26.6 mL distilled water	
	c. 0.3 mL ammonium persulfate*	0.0005%
F.	Stacking gel preparation (20 mL) (for 2 gels):	
	a. 3.34 mL acrylamide : bis-acrylamide (C.b)	5.01% : 0.13%
	b. 2.5 mL Tris (B)	0.375M
	c. 0.2 mL 10% SDS	0.1%
	d. 0.01 mL TEMED	0.0005%
	e. 0.2 mL ammonium persulfate*	0.001%
G.	Resolving solution:	
	a. Diluted (A) 1:10 before use	0.005M

* Use a fresh solution

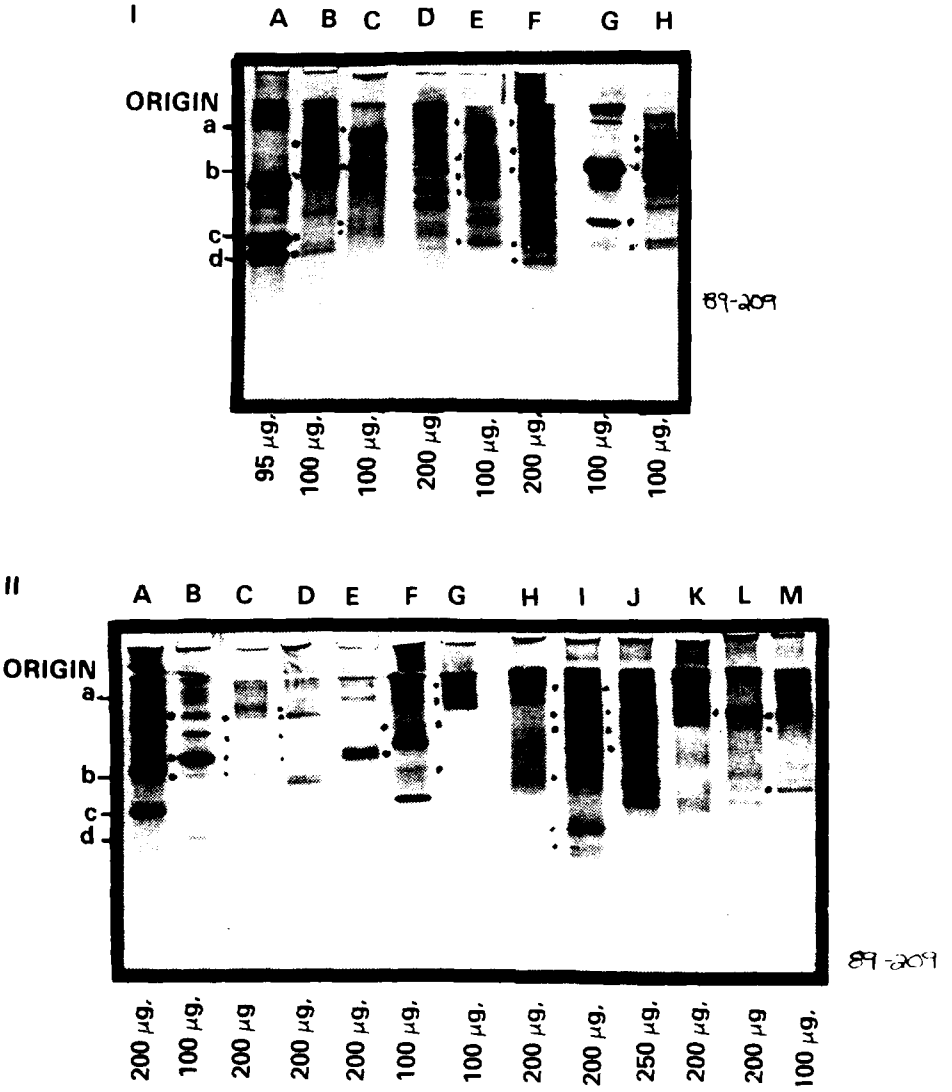

Figure 1

Composites of typical non-SDS electropherograms of native venoms. The dots between adjacent lanes indicate protein bands with similar electrophorectic migration rates. I. Lane A, *Atheris squamigera;* **B,** *Bothrops jararaca;* **C,** *Crotalus viridis oreganus* **D,** *Crotalus basiliscus;* **E,** *Bitis gabonica;* **F,** *Agkistrodon rhodostoma;* **G,** *Vipera lebetina;* **H,** *Echis carinatus.* **II. Lane A,** *Cerastes cerastes;* **B,** *Naja melanoleuca;* **C,** *N. haje;* **D,** *N. naja;* **E,** *N. naja kaouthia;* **F,** *V. ammodytes;* **G,** *V. russelli;* **H,** *Agkistrodon piscivorus piscivorus;* **I,** *Bothrops lansbergii;* **J,** *Crotalus viridis viridis;* **K,** *V. palaestinae;* **L,** *Bothrops nummifer;* **M,** *Crotalus molossus molossus.* **The bands labeled "a", "b", "c", and "d" were randomly selected and used as references to compare different venoms. The number below each lane is the amount (µg) of each venom sample used.**

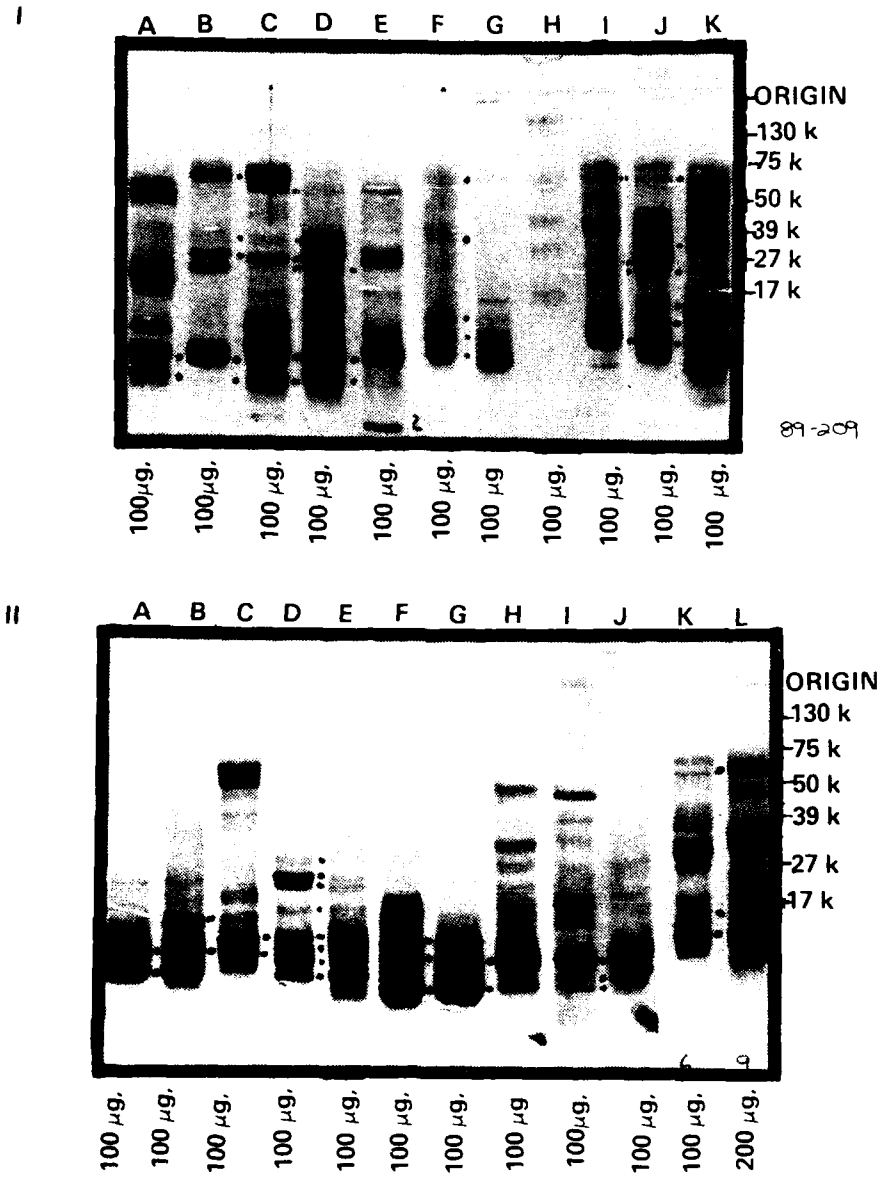

Figure 2

Composites of typical SDS electropherograms of venoms from the 21 snake species. The dots between adjacent lanes indicate the polypeptide bands with similar electrophoretic migration rates. I. Lane A, *Vipera lebetina;* **B,** *V. palaestinae;* **C,** *V. russelli;* **D,** *Bitis gabonica,* **E,** *Atheris squamigera,* **F,** *Bothrops jararaca,* **G,** *Bothrops nummifer;* **H, SDS-PAGE standard; I,** *Cerastes cerastes;* **J,** *Bothrops lansbergii;* **K,** *Crotalus viridis oreganus.* **II. Lane A** *Naja haje;* **B,** *N. melanoleuca;* **C.** *Echis carinatus;* **D,** *Crotalus basiliscus;* **E,** *Crotalus molossus molossus;* **F,** *Naja naja;* **G,** *N. naja kaouthia;* **H,** *V. ammodytes;* **I,** *Agkistrodon rhodostoma;* **J,** *Agkistrodon piscivorus piscivorus;* **K,** *Bothrops lansbergii;* **L,** *Crotalus viridis viridis.* **The number below each lane is the amount (µg) of each venom sample used.**

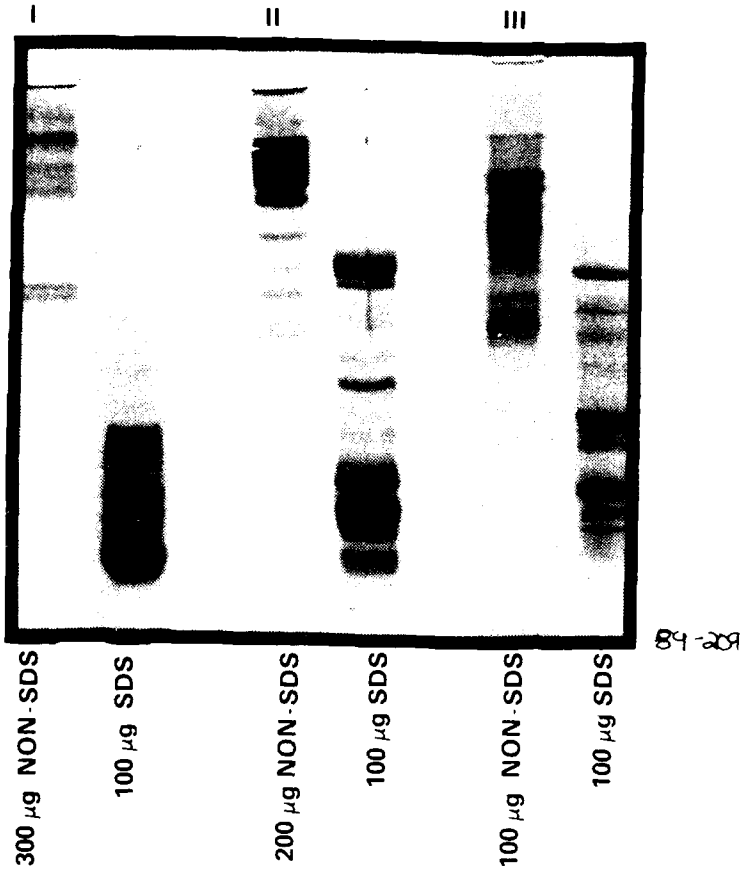

Figure 3

Pairs of representative polyacrylamide electropherograms, with SDS and without SDS, of venoms (stained with Commassie blue); I. *Naja naja* **(Elapidae), II.** *Vipera russelli* **(Viperidae), III.** *Agkistrodon rhodostoma* **(Crotalidae).**

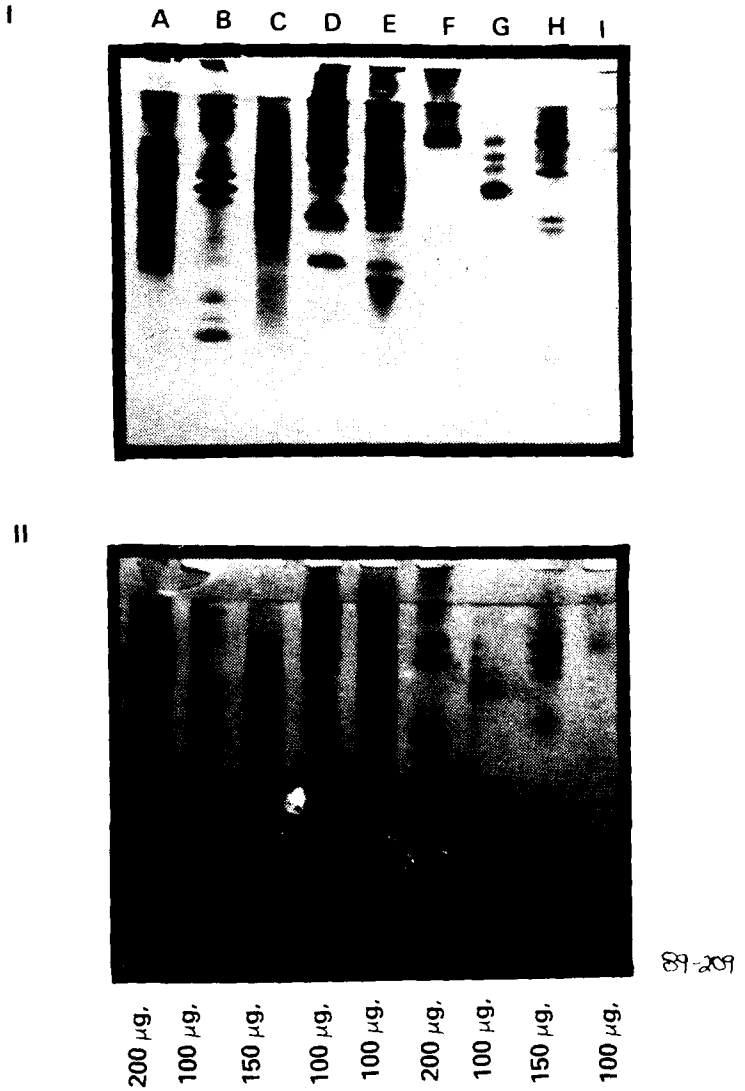

Figure 4

A pair of typical electropherograms of the native venoms, representing three snake families, detected with (I) silver stain and (II) Coomassie blue stain. Lanes A, *Agkistrodon rhodostoma;* **B,** *A. piscivorus piscivorus;* **C,** *Bothrops jararaca:* **D,** *Echis carinatus:* **H,** *Vipera palaestinae:* **I,** *Vipera russelli.* **The numbers below each lane correspond to the amount (µg) of venom sample applied.**

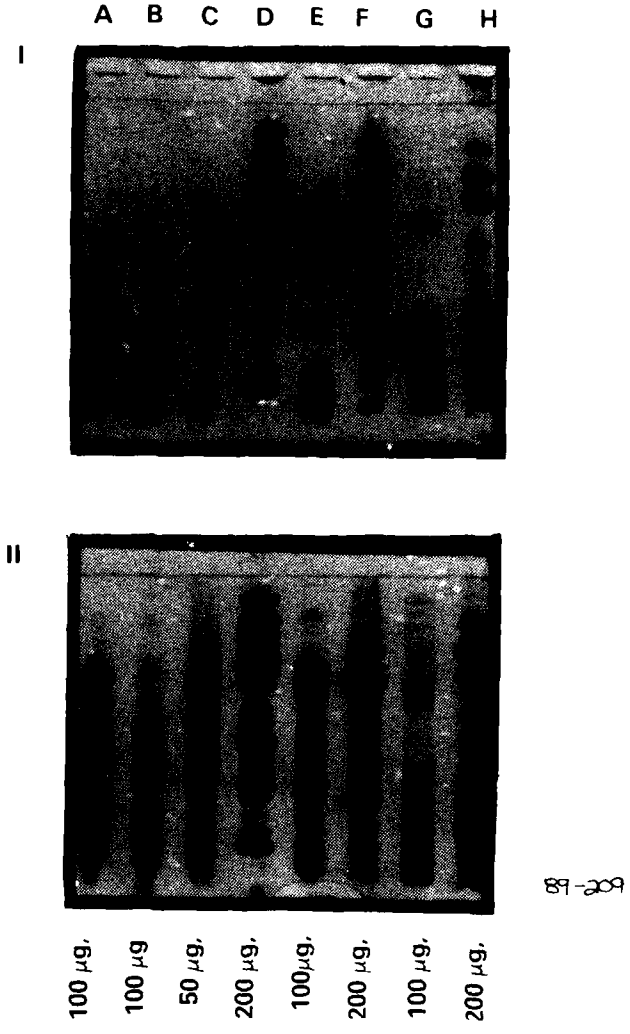

Figure 5

A pair of typical SDS-electropherograms of the polypeptides, from venoms representing three snake families, detected with (I) silver stain and (II) Coomassie blue stain. Lanes A, *Akistrodon rhodostoma;* **B,** *A. piscivorus piscivorus;* **C,** *Bothrops jararaca;* **D,** *Cerastes cerastes;* **E,** *Crotalus viridis oreganus;* **F,** *Echis carinatus;* **G,** *Naja naja;* **H,** *Vipera russelli.* **The number below each lane corresponds to the amount (µg) of venom sample applied.**

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This report deals with comparative studies of snake venoms from 21 species representing Elapidae, Crotalidae and Viperidae. Both native and denatured venoms have been analyzed by polyacrylamide gel electrophoretic methods with or without sodium dodecyl sulfate. Electrophoreses showed qualitatively the commonality of protein and polypeptide components in venoms from various snake species. Electropherograms also showed the characteristic protein or polypeptide profiles which differentiate one species from another. These profiles, consisting of a combination of protein or polypeptide bands, suggested that each venom is unique for each species, although similarity abounds amoung subspecies or related species.

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Snake venoms

Elapidae

Viperidae

Crotalidae

Characterization

Proteins

Polypeptides

Electrophoresis

SDS-PAGE