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Electrophoretic analysis of snake venoms

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ABSTRACT

Electrophoretic analyses were conducted on snake venoms from 21 species representing Elapidae, Crotalidae and Viperidae. Denatured and native venoms were analyzed by polyacrylamide gel electrophoretic (PAGE) methods with sodium dodecyl sulfate (SDS) and without SDS. Both SDS-PAGE and PAGE profiles of venoms from different snake species indicate that some proteins and polypeptide components of these venoms have common electrophoretic characteristics suggesting a genetic relationship. Conversely, the electropherograms also showed the characteristic protein and polypeptide profiles that could differentiate one snake species from another. Therefore, both SDS-PAGE and PAGE profiles suggest that proteins and polypeptides with similar characteristics abound among subspecies or related species, although each venom has a unique profile that differentiates one species from the other.

INTRODUCTION

Snake venom toxicity differs between species because of its composition. It is a mixture of organic and inorganic compounds [1–3]. Mainly the protein components display pharmacological activity and lethal and injurious effects and they are also immunogenic [4,5]. It is important to understand the composition of the snake venom because of its diverse pharmacological and toxicological actions. Venom components vary from highly lethal active principles [6–12] to relatively non-lethal but deleterious actions [13–18]. Immunoelectrophoresis, immunodiffusion and, more recently, Western immunoblot methods have been employed in the study of snake venoms [19,20]. A review of snake venom components and their cross-reactivity [21] has been published.

Electrophoresis has been used extensively to analyze components of snake venoms. At present, comparative electrophoresis has been limited to studies of snake venoms from a few species, following sodium dodecyl sulfate (SDS) treatment [5,22–24]. SDS depolymerizes proteins into subunits. In SDS polyacrylamide gel electrophoresis (SDS-PAGE), polypeptides with the same molecular mass but derived from two different protein molecules may co-migrate. In PAGE, native protein molecules remain intact and migrate independently based on their characteristic charges. Hence PAGE gives a better assessment of how many different types of native protein molecules are present in a given venom sample.

In order to obtain a basic understanding of the electrophoretic properties inherent to native and

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denatured snake venoms, analyses of venoms from 21 snake species were conducted by using both SDS-PAGE and PAGE methods.

EXPERIMENTAL

Sample preparative solution

The venom samples (Sigma, St. Louis, MO, USA), in amounts indicated in the figure captions, were dissolved in a solution composed of the following: 1 part of 0.5 *M* Tris [(trishydroxymethyl)aminomethane] (Bio-Rad Labs., Richmond, CA, USA) at pH 6.8, 1 part of 10% SDS (Bio-Rad Labs., Mississauga, Canada); 0.1 part of 2-mercaptoethanol (Sigma), 1 part of glycerol (Fisher Scientific, Fair Lawn, NJ, USA), 6.9 parts of distilled water and 1 part of 0.01% bromophenol (J. T. Baker, Phillipsburg, NJ, USA) to serve as a marker during SDS-PAGE. For PAGE, the same preparative solution without SDS was used to dissolve the venoms.

Electrophoresis

The SDS-treated venoms were separated in duplicate by SDS-PAGE in an electrophoresis unit (Bio-Rad Labs., Model 220; Technical Marketing Associated, Mississauga, Canada) according to Laemmli [25]. The native venoms were resolved in PAGE without SDS. Prestained molecular markers (Bio-Rad Labs.) used as references have apparent relative molecular masses of $17 \cdot 10^3$ (lysozyme), $27 \cdot 10^3$ (soybean trypsin inhibitor), $39 \cdot 10^3$ (carbonic anhydrase), $50 \cdot 10^3$ (ovalbumin), $75 \cdot 10^3$ (bovine serum albumin) and $130 \cdot 10^3$ (phosphorylase C).

After electrophoresis, the gels were stained for 1 h with Coomassie Brilliant Blue R (N,N,N′,N′-tetramethylethylenediamine) (Sigma) in 7% aqueous acetic acid. The duplicate gels were stained with a silver nitrate stain following a standard method supplied with the silver stain kit (Sigma).

RESULTS

A typical PAGE profile of the native venoms from three snake families is given in Fig. 1. These profiles show protein bands of venoms from different species with similar electrophoretic mobilities (marked with black dots) and those bands which are unique to each specific venom. Fig. 1 also shows that the cobra venoms (II, lanes B, C, D and E) have fewer distinct protein bands than those found in the viper venoms (I, lanes A and H; II, lanes A and F) and rattlesnake venoms (I, lanes B and D; II lanes I and J). Fig. 1 further illustrates that protein bands with similar electrophoretic mobilities occur more frequently in venoms of snakes belonging to the same family. Examples of bands with similar mobilities obtained form venoms of snake species all belonging to the cobra family (II, lanes C and D), vipers (I, lanes H and G; II, lanes F and G) and rattlesnakes (II, lanes H, I and J) are depicted in Fig. 1. However, venoms from different species do not necessarily have the same relative proportions of proteins with similar mobilites.

Fig. 1 also shows four randomly selected protein bands, labelled a–d, from a native venom of *Atheris squamigera* (lane A). Band d has a mobility similar to that of the lysozyme standard (not shown). Most of the venoms examined have protein bands that have similar electrophoretic mobilities to these selected proteins bands of *A. squamigera*.

Typical SDS-PAGE profiles of the SDS-treated venoms (Fig. 2) show polypeptides with similar electrophoretic mobilities (marked with black dots). Polypeptides with similar mobilities occur more frequently in the $17 \cdot 10^3$ relative molecular mass region (I, lanes C and D, viperids; II, lanes D and E, crotalids; II, lanes F and G, elapids). Fig. 2 also shows that polypeptides in the $130 \cdot 10^3$ relative molecular mass region are prominent in the crotalid venoms (I, lane G; II, lane J) and very faint in the viperid venoms (I, lanes A and B).

Polypeptide bands with mobilities similar to the $17 \cdot 10^3$, $39 \cdot 10^3$ and $50 \cdot 10^3$ relative molecular mass markers (marked with black dots) are present more frequently than those similar to the $130 \cdot 10^3$ relative molecular mass marker in venoms of different snake species (Fig. 2, I and II). Further, Fig. 2 indicates that the venoms from

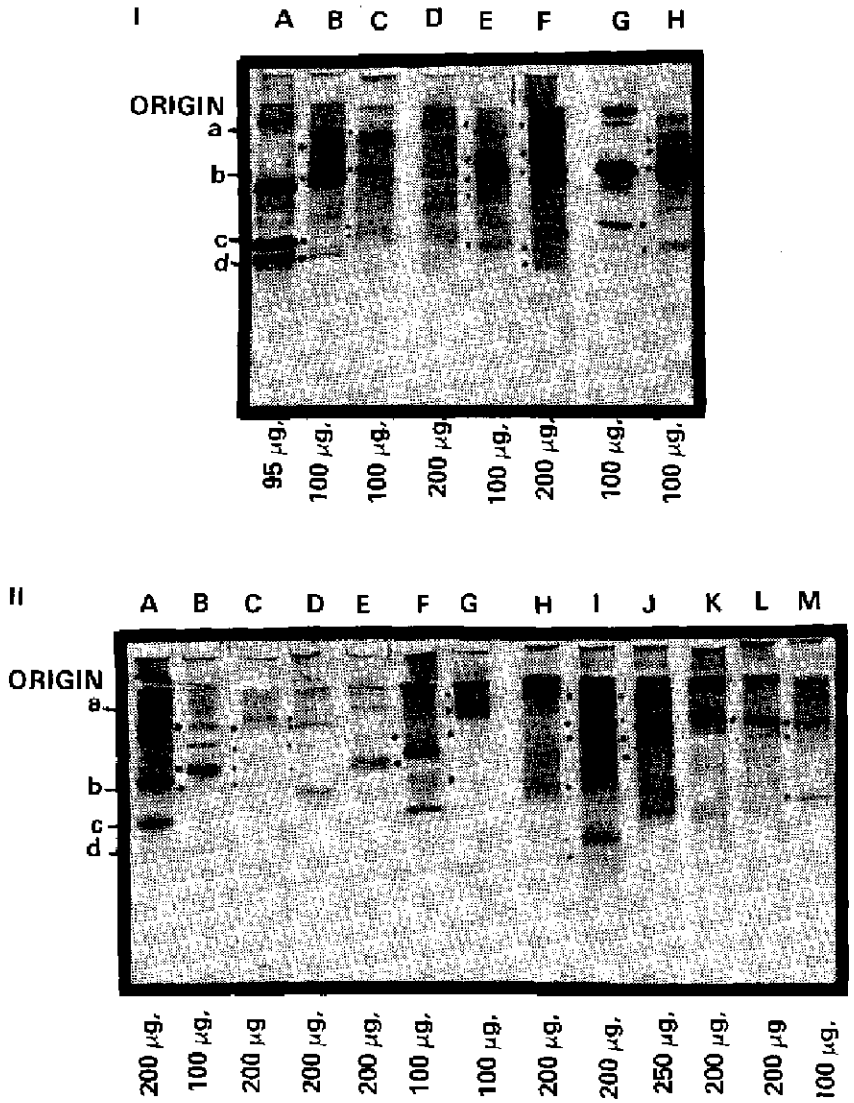

Fig. 1. Composites of typical non-SDS electropherograms of native venoms. The dots between adjacent lanes indicate protein bands with similar electrophoretic migration rates. I: Lane A = *Atheris squamigera*; B = *Bothrops jararaca*; C = *Crotalus viridis oreganus*; D = *Crotalus basiliscus*; E = *Bitis gabonica*; F = *Agkistrodon rhodostoma*; G = *Vipera lebetina*; H = *Echis carinatus*. II: Lane A = *Cerastes cerastes*; B = *Naja melanoleuca*; C = *N. haja*; 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 labelled a–d were randomly selected and used as references to compare different venoms. Below each electropherogram is the amount of each venom sample used.

the four *Naja* species (II, lanes A, B, F and G) predominantly have polypeptides with relative molecular mass less than $17 \cdot 10^3$.

In order to describe and compare the different venoms, the polypeptides present in each venom are classified into six groups based on relative molecular mass (Table 1). The data show that most of the polypeptides have relative molecular mass less than $17 \cdot 10^3$ and rarely larger than $130 \cdot 10^3$. In addition, polypeptides, with similar elec-

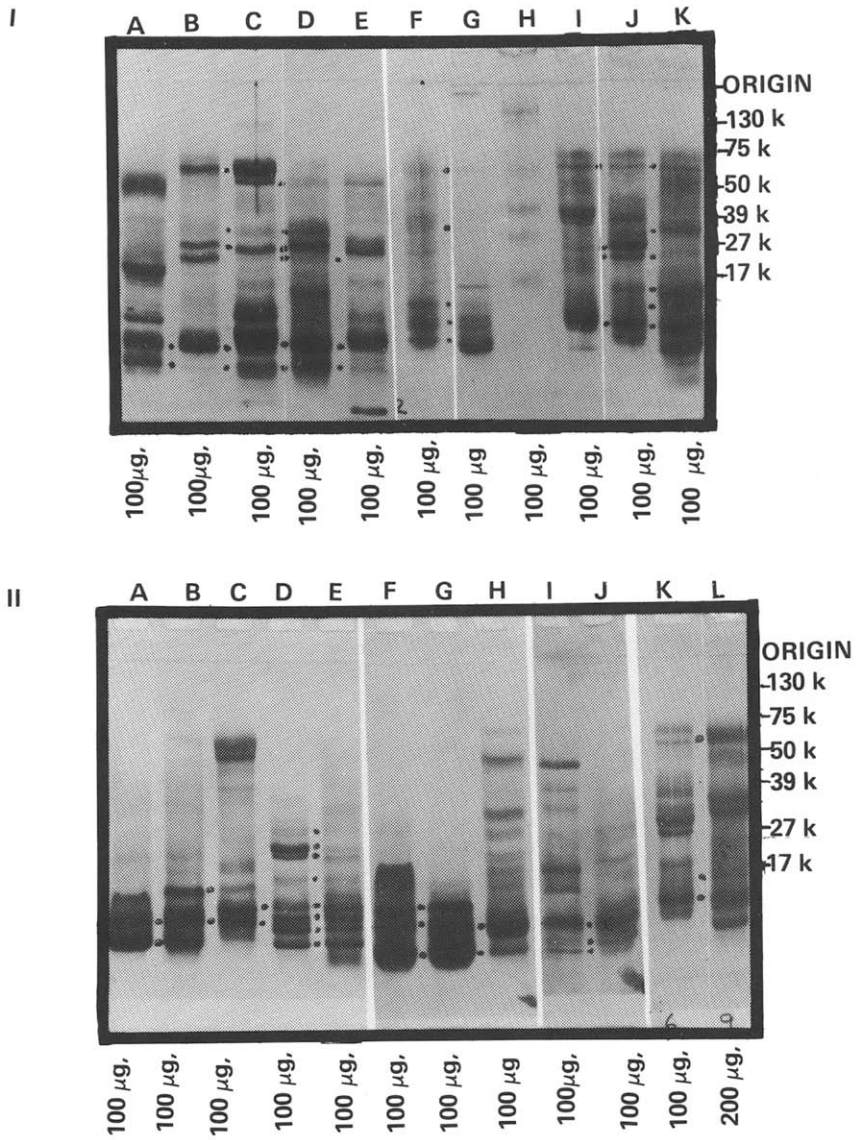

Fig. 2. Composites of typical SDS electropherograms of venoms from 21 snake species. The dots between adjacent lanes indicate the polypeptide bands with similar electrophoretic migration rates. I: Lane A = *Vipera lebetina*; B = *V. palaestinae*; C = *V. russelli*; D = *Bitis gabonica*; E = *Atheris 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 haja*; B = *N. melanoleuca*; C = *Echis carinatus*; D = *Crotalus basiliscus*; E = *Crotalus 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*. Below each electropherogram is the amount of each venom sample used. Numbers on the right are relative molecular masses (k indicates $\cdot 10^3$).

trophoretic mobility occur more frequently in the relative molecular mass $< 17 \cdot 10^3$ region. Table I also shows the relative total number of protein bands present in native and SDS-treated venoms. Statistical analyses indicate that the viper and the rattlesnake venoms have comparable total numbers of polypeptide bands. Both have significantly more ($p \leq 0.05$) polypeptide bands than the cobra venoms.

Fig. 3 shows the marked difference between

TABLE I

NUMBER OF BANDS OBSERVED IN NATIVE AND SDS TREATED VENOMS

Species	Number of bands in specified relative molecular mass region						Total	
	$\leq 17 \cdot 10^3$	$(17–27) \cdot 10^3$	$(28–39) \cdot 10^3$	$(40–50) \cdot 10^3$	$(51–75) \cdot 10^3$	$(76–130) \cdot 10^3$	SDS	Native[a]
Elapidae								
Naja melanoleuca	5	2	1	1	2	0	11	10
N. haje	5	3	2	1	1	0	12	11
N. naja	7	0	0	0	0	0	7	12
N. naja kaouthia	5	2	0	0	1	0	8	7
Crotalidae								
Agkistrodon rhodostoma	9	3	2	1	0	1	16	15
A. piscivorus piscivorus	7	3	1	1	0	0	12	15
Bothrops jararaca	5	1	1	1	0	0	8	12
B. lansbergii	4	3	1	2	1	0	11	15
B. nummifer	7	0	2	2	0	1	12	12
Crotalus basiliscus	7	4	2	0	0	0	13	13
C. molossus molossus	11	3	1	1	0	0	16	15
C. viridis oreganus	7	3	2	3	0	0	15	16
C. viridis viridis	7	3	1	2	0	0	13	14
Viperidae								
Antheris squamigera	8	3	2	3	0	0	16	15
Bitis gabonica	4	4	2	1	1	1	13	14
Cerastes cerastes	4	4	2	3	1	0	14	15
Echis carinatus	7	3	3	3	0	0	16	12
Vipera ammodytes	5	3	2	2	1	0	13	13
V. lebetina	10	2	1	1	0	1	15	11
V. palaestinae	5	4	2	2	2	1	16	12
V. russelli	9	3	1	2	1	0	16	14

[a] On non-SDS electropherograms.

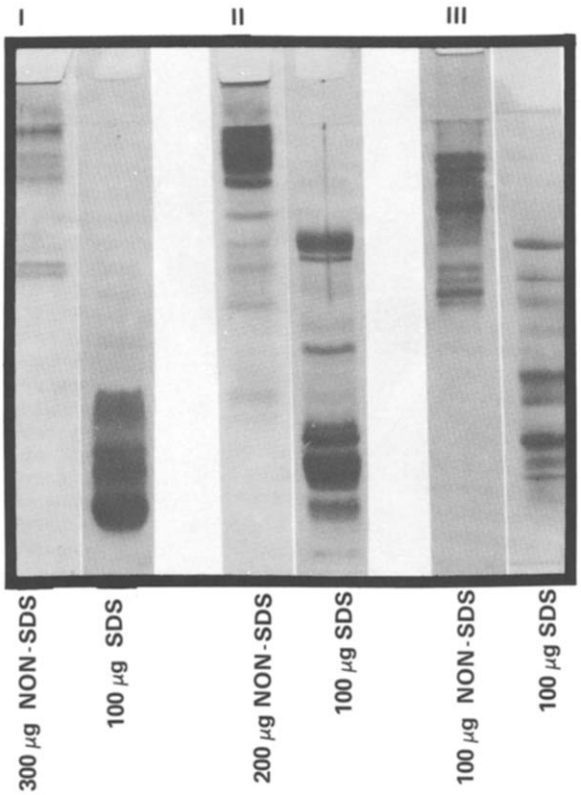

Fig. 3. Pairs of representative polyacrylamide electropherograms, with and without SDS, of venoms (stained with Coomassie Brilliant Blue). I = *Naja naja* (Elipidae); II = *Vipera russelli* (Viperidae); III = *Agkistrodon rhodostomae* (Crotalidae).

typical electrophoretic profiles of the native and SDS-treated venoms. SDS-treated venoms of cobras (lane I) have polypeptides with mostly low relative molecular mass, unlike the SDS-treated venoms of vipers (lane II) and crotalids (lane III). The rates of electrophoretic mobility of the proteins and their polypeptide subunits indicate both the similarities and the difference among venoms from various species belonging to the same, or different, phylogenetic families.

It was further observed (Fig. 3) that the SDS-treated venoms are markedly more sensitive to detection than the native venoms. For example, with *N. naja*, in order to obtain adequate and comparable quality of detection, *ca.* 100 μg of the SDS-treated venom (I, right lane) were needed compared with *ca.* 300 μg of the native venom (I, left lane).

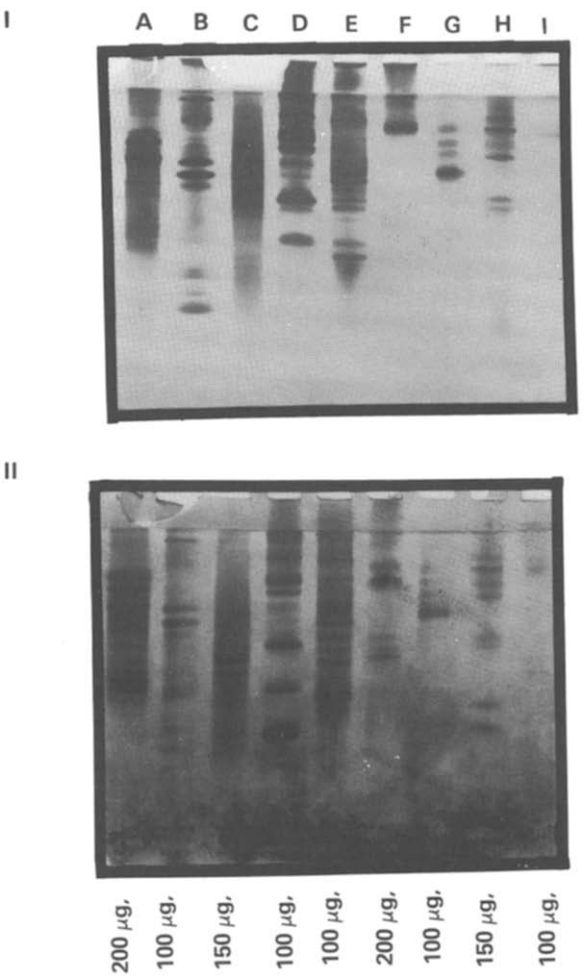

Fig. 4. Pair of typical electropherograms of the native venoms, representing three snake families, detected with (I) silver stain and (II) Coomassie Brilliant Blue stain. Lanes: A = *Agkistrodon rhodostoma*; B = *A. piscivorus piscivorus*; C = *Bothrops jararaca*; D = *Cerastes cerastes*; E = *Crotalus viridis oregonus*; F = *Naja naja*; G = *Echis carinatus*; H = *Vipera palaestinae*; I = *Vipera russelli*. The numbers below each lane are the amounts of venom sample applied.

Fig. 4 shows a pair of typical electropherograms of native venom proteins from representative species Elapidae, Viperidae and Crotalidae, indicating that some proteins detected with the Coomassie Brilliant Blue stain are undetectable with the silver stain and *vice versa*. For example, the fastest migrating bands in the *A. piscivorus piscivorus* venom (lane B) were detectable with the silver stain but not with the Coomassie Brilliant Blue stain. The opposite was observed with

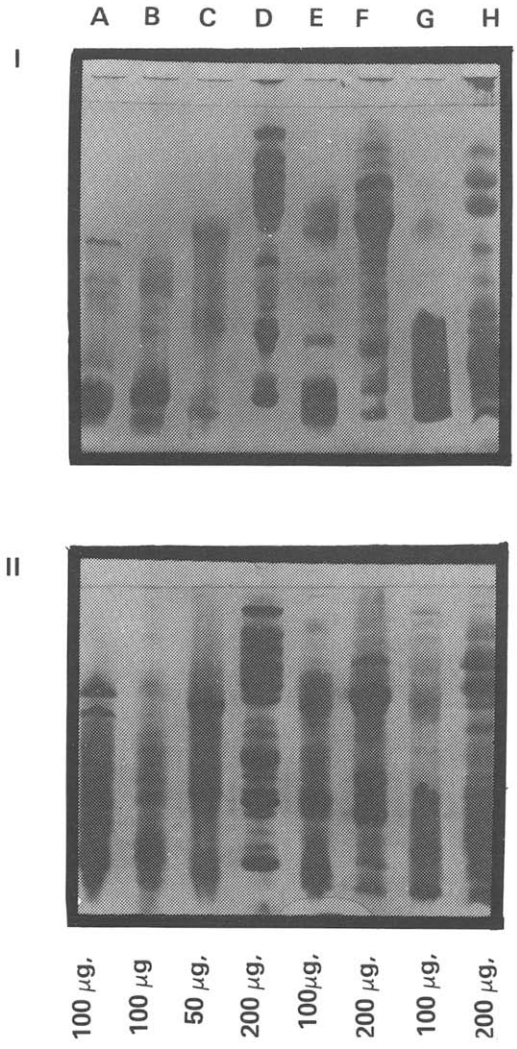

Fig. 5. Pair of typical SDS electropherograms of the polypeptides from venoms representing three snake families, detected with (I) silver stain and (II) Coomassie Brilliant Blue stain. Lane: A = *Agkistrodon rhodostoma*; B = *A. piscivorus piscivorus*; C = *Bothrops jararaca*; D = *Cerastes cerastes*; E = *Crotalus viridis oreganus*; F = *Echis carinatus*; G = *Naja naja*; H = *Vipera russelli*. The numbers below each lane are the amounts of venom sample applied.

the faster migrating bands of the venoms of *Cerastes cerastes* (lane D) and *V. palaestinae* (lane H). With the *N. naja* venom (lane F), the silver stain detected only two protein bands whereas the Coomassie Brilliant Blue stain detected eight bands. Similar staining properties were observed when silver and Coomassie Brilliant Blue staining methods were used on SDS-PAGE gels (Fig. 5).

DISCUSSION

Previous studies of protein components of venoms from elapid and crotalid snakes have been accomplished by electrophoretic methods using starch gel [26,27] or polyacrylamide gel [5,22,28–30]. By using PAGE with 12% polyacrylamide gels in 6 *M* urea [30], more than ten protein bands have been consistently detected for venoms from various species of North American snakes [5]. Protein components with similar migration rates appeared to be more prevalent among venoms from snakes belonging to the same genus.

Likewise, a study [22] using SDS-PAGE according to Laemmli [25] demonstrated a striking similarity of some protein components of venoms from five rattlesnake species. The separating gel used also contained 12% polyacrylamide gels in addition to 0.1% SDS.

Electrophoretic profiles obtained for venoms from different species, genera and families by PAGE and SDS-PAGE indicate similar electrophoretic mobilities of some proteins and polypeptides. The similarity of the electrophoretic profiles of proteins or polypeptides present in the venoms of Crotalidae species observed in this study concurred with the earlier reports [5,22]. The similarity of, or the difference in, protein components present in *V. russelli* venoms compared with those from different species has also been demonstrated by immunogenic cross-reactivity [20]. The primary structures of homologous proteins in toxins of various snake species have been studied to establish a hierarchy [31]. It was reported that evolutionary changes in the composition and structure of a protein could be very extensive without affecting its catalytic or toxic activity.

Previous reports have shown that several snake venoms contain enzymes [5,6,8,32]. Bradykinin-, histamine- and serotonin-releasing proteases have been shown to mediate autopharmacological actions produced by some venoms [8,10,11,33–35]. In addition, Hendon and Tu [36] have demonstrated the importance of an enzyme in the mechanism of neurotoxicity in that a combination of events must happen before a certain

toxic factor can exert its action. It is necessary, therefore, to establish the toxicity of the proteins or polypeptides present in snake venoms investigated. A knowledge of individual or combinations of toxic factors is crucial and fundamental in developing a more comprehensive type of protection and therapy against many, if not all, types of snake venoms and constituents. Therefore, it is impractical to develop preventive and/or therapeutic measures against venom of each snake species or individual toxic component of snake venoms.

This study suggests that certain proteins from native venoms and polypeptides derived from SDS-treated venoms do not react strongly with the silver nitrate stain and are not readily detectable. In contrast, they produce an intense reaction with the Coomassie Brilliant Blue stain and are readily detectable. These findings concurred with a previous report [37] that the general usefulness and reliability of the silver stain method [38,39] remain uncertain.

In conclusion, the characterization of proteins and polypeptides present in venoms of 21 snake species by PAGE and SDS-PAGE has been achieved. The electrophoretic profiles show that several polypeptides or proteins with similar electrophoretic mobilities are commonly present in venoms of different but related snake species. Similarly, certain proteins, or polypeptides from these venoms, appear typical only of species belonging to the same genus or family. The study further suggests the usefulness of both PAGE and SDS-PAGE in characterizing the relationship and the uniqueness of each snake family or subspecies between related species.

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