

COMPARATIVE STUDY OF THE VENOMS OF CROTALIDAE, ELAPIDAE, VIPERIDAE
AND BOIGINAE

Robert A. Young and Donald M. Miller

Department of Physiology, Southern Illinois University, Carbondale 62901

ABSTRACT. Venoms of rear-fanged species were compared with other venoms by cellulose-acetate electrophoresis. The venoms of rear-fanged species and subspecies are as complex as the venoms of Crotalidae, Elapidae and Viperidae in regards to numbers of protein components and spectrum of electrophoretic mobilities. Similarities and differences exist between venoms of families genera, species and subspecies. The electrophoretic pattern for a given rear-fanged species' or subspecies' venom is characteristic for that species or subspecies. At pH 8.8 all proteins except three were cationic. There was no correlation between venom complexity and complexity of venom delivery apparatus.

Considerable work has been done on analyzing and comparing the venom proteins of various species of Crotalidae, Elapidae and Viperidae. Bertke, et al. (1966), presented comparisons of Crotalidae and Elapidae by use of starch-gel electrophoresis. A qualitative analysis of Crotalidae and Elapidae venoms by use of disc electrophoresis was published by Basu, et al. (1969). An intra-specific comparison of cobra (Naja) venom was reported by Tu and Ganthavorn (1968), using immunological methods and polyacetate electrophoresis. Slotta, et al. (1966), obtained excellent results with cobra (Naja naja) venom using cellulose-polyacetate electrophoresis. Extensive work on Crotalidae venom has been presented by Fiero, et al. (1972); Basu, et al. (1969); Bonilla and Horner (1969); and Dubnoff and Russell (1970). Viperidae venom has been studied by Dimitrov (1971); Devi, et al. (1970); Ohsaka, et al. (1970); and Dimitrov (1971).

Notwithstanding, little or no work has been done on the venomous secretion of the rear-fanged (opisthoglypha) snakes of the subfamily Boiginae. With the exception of the boomslang, Dispholidus typus (Pope, 1958) and the African twig snake, Thelotornis kirtlandi (Fitzsimons, 1962), these snakes are not considered dangerous to humans, but they do possess toxic secretions which are at least capable of killing or immobilizing small prey.

Previous workers, Bertke, et al. (1966), Tu and Ganthavorn (1968), Bonilla and Horner (1969), and Basu, et al. (1969) suggested that venom constituents were characteristic for a given species or subspecies, and could be used as a method for classification. It was the purpose of this comparative study to determine if the uniqueness of venom components were not only true for front-fanged species of the families Crotalidae, Elapidae and Viperidae, but also for the rear-fanged species and subspecies of the subfamily Boiginae.

MATERIALS AND METHODS

Lyophilized venoms of the following species were obtained commercially from the Miami Serpentarium Laboratories: Boiginae: Boiga irregularis, Dispholidus typus, Leptodiera annulata annulata; Leptodiera annulata rhombifera; Crotalidae: Crotalus adamanteus; Elapidae: Naja naja naja; and Viperidae: Causus rhombeatus. Also incorporated into the study was venom from a specimen of Naja nigricollis collected near Nakuru, Kenya, Africa. The venom was obtained from the glass front of its cage and air dried. Air dried venom from a specimen of Agkistrodon piscivorus leucostoma collected near Pine Hills, Illinois, USA was also used to represent Crotalidae. All lyophilized and air dried venoms were reconstituted with double distilled water to yield 1 mg/ml solutions with the exception of that from Dispholidus typus (used as a 200 mg/ml solution), and Boiga irregularis and Agkistrodon piscivorus leucostoma which were represented by only trace amounts.

Cellulose polyacetate strips (Senraphore III) were used as the supporting media in conjunction with the Gelman electrophoresis apparatus. All electrophoresis was carried out at 280 V for forty-five minutes using a 0.05M Tris-barbital sodium buffer, pH 8.8. Four strips were placed in the electrophoresis chamber simultaneously, thus giving a current of 3 mA through each strip. After electrophoresis was complete, the strips were stained for ten minutes in Ponceau S stain for protein, and decolorized in three successive baths of 5.0% acetic acid. The strips were then dehydrated in two baths of absolute methanol and cleared in 15.0% alcoholic-acetic acid. The clear polyacetate strips, mounted on glass slides, were then placed in a scanning densitometer (Gelman Instrument Company) in order to obtain accurate graphic representation of the protein fractions that were present on the strips. The scanning densitometer was equipped with an integrator which allowed the determination of the per cent protein component of the total protein. Each protein fraction of a venom was then plotted on a per cent scale of migration to allow for comparison of the venom samples.

In order to ascertain the extent to which the mobility patterns can be compared to one another, venoms of two species were combined and then separated by electrophoresis. The resulting patterns were then compared to the individual patterns.

RESULTS

Most of the protein components of the venoms migrated to the cathode at pH 8.8, with the exception of an anionic fraction in A.p. leucostoma venom and two immobile proteins. Variations in the concentration of each component (i.e. intensity of stain) are reflected in the width of the black bands in Fig. 1.

The densitometric scan of N. n. naja venom exhibited nine distinct components. The sixth component consistently had the highest concentration (36%) and the leading (ninth) component was unique among the venoms studied. All thirteen samples of this venom produced similar electrophoretic patterns.

Electrophoresis of N. nigricollis venom resulted in ten components being separated. A total of eight samples were electrophoretically separated. The leading fraction was always the most concentrated (25%) and consistently pro-

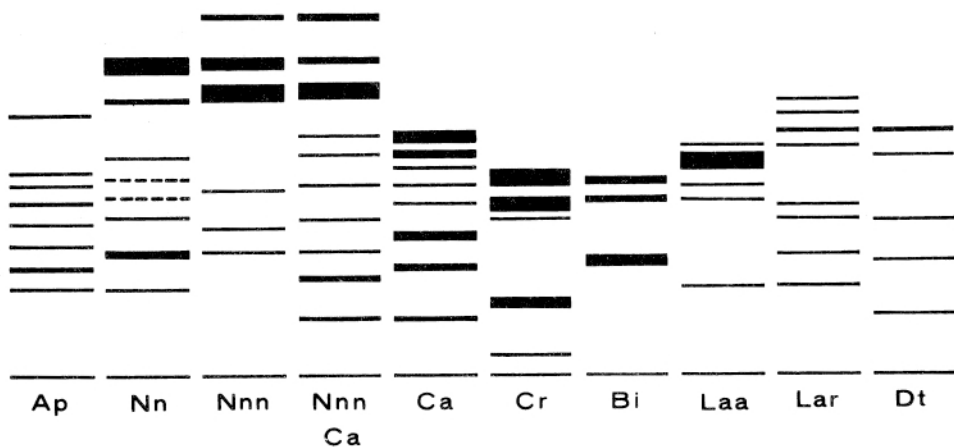


Figure 1. Diagram of electrophoretic patterns of venoms. Electrophoresed strips were lined up, overlain with tracing paper, and lines drawn to correspond to protein fractions. Densitometer scans were used to increase width of line to reflect actual concentration. Patterns are labeled at origin to correspond to following: Ap=Agkistrodon piscivorus leucostoma; Nn=Naja nigricollis; Nnn=Naja naja naja; Ca=Crotalis adamanteus; Cr=Causus rhombeatus; Bi=Boiga irregularis; Laa=Leptodiera annulata annulata; Lar=Leptodiera annulata rhombifera; and Dt=Dispholidus typus.

duced an irregular pattern on the cellulose polyacetate strip. A very broad low concentrated (11.0%) trailing fraction was present.

Ten samples of C. adamanteus were electrophoresed with all of them exhibiting nine components of relatively uniform width. The first and fifth bands were the most concentrated fractions representing 16.5% and 19.6% of the total proteins. The patterns of C. adamanteus at no time produced the three leading components found on the N. n. naja strips. Also, the wide, low concentrated trailing fraction found in N. nigricollis, C. rhombeatus and D. typus was never present.

Venom proteins of A. p. leucostoma were separated and resulted in the only anionic fraction found among the venoms studied. A total of nine protein components were distinguished in all four samples, with the second cationic fraction having the greatest concentration (22.4%).

Several fractions were separated from each of ten samples of C. rhombeatus venom. The second component was the most concentrated (33.3%) in all cases. The broad, low concentrated trailing band formed in N. nigricollis and D. typus was consistently present, but comprised of three minor components when scanned by the densitometer.

The venom of D. typus produced an electrophoretic pattern with all components staining very lightly. Fourteen samples were analyzed with seven com-

ponents being detected by the densitometer. A broad, third band was always the most concentrated fraction, usually approximating fifty per cent of the total protein. A broad trailing component was visualized on the polyacetate strip, but upon densitometric scan it was recorded as two components.

The electrophoretic pattern of all four B. irregularis venom samples exhibited three fractions. The third fraction was consistently the most concentrated component, making up 41.8% of the total venom protein.

Electrophoresis of L. a. annulata venom resulted in six protein fractions. In all three samples, the fourth component was most concentrated (24.6%), although the leading (sixth) fraction also approached this value (22.6%).

The venom of L. a. rhombifers separated into nine components. In the three samples analyzed, the fourth and eighth components were the most concentrated with values of 17.4% and 16.1% respectively.

Electrophoretic results of the composite N. n. naja and C. adamanteus venom exhibited sixteen fractions. All fractions, except the twelfth, individually made up only small portions of the total protein content of the venom. The twelfth component with a value of 17.4% was the only protein fraction having a large percentage of total protein value. All protein fractions were cationic. An interspecific comparison was then made. In comparing all of the venoms, there was a total of seventy proteins which were determined by their respective distances of migration on the polyacetate strips. Twenty-one of the seventy proteins had migration distances that were common among two or more species.

DISCUSSION

Other workers, Bau, et al. (1969), Bonilla, et al. (1969), Bertke, et al. (1966), and Tu and Ganthavorn (1968) have found that the electrophoretic patterns of snake venoms are characteristic for other than rear-fanged species. The results of this study suggest that rear-fanged species also have characteristic electrophoretic patterns and are of equal complexity. Most of the proteins of the venoms in this study migrated to the cathode, thus their isoelectric points must be higher than pH 8.8. Two proteins did not migrate, consequently their isoelectric points were approximately pH 8.8, and one protein was anionic in its migration, therefore its isoelectric point was lower than pH 8.8.

The electrophoretic pattern obtained for N. n. naja venom closely agreed with that reported by Slotta, et al. (1966), in that nine cationic protein components were produced. Basu, et al. (1969) separated thirteen cationic fractions using disc gel electrophoresis at pH 4.4. Upon comparing venom of N. n. naja with other venoms, it appeared that the leading (ninth) component was not present in any of the other venoms analyzed and that the protein fractions of N. n. naja were more mobile than most protein of other venoms. Tu and Ganthavorn (1968), using cellulose polyacetate electrophoresis, compared venoms of N. n. atra and N. n. siamensis and found them to be similar but not identical. In comparing the patterns of N. n. naja obtained in this study to N. n. atra and N. n. siamensis it was found that there were close similarities, especially

between N. n. naja and N. n. atra. The venom of N. n. naja had one component similar to A. p. leucostoma, one fraction in common with C. adamanteus, one in common with C. rhombeatus, one component similar to a component in N. nigricollis venom.

The pattern for N. nigricollis venom was somewhat similar to N. n. naja. The highly concentrated sixth component of N. n. naja did not appear to be present in the N. nigricollis venom. Similarly, the highly concentrated leading fraction of N. nigricollis was not present on N. n. naja venom. However, the seventh N. nigricollis fraction seemed similar in migration distance to the sixth N. n. naja fraction. The N. nigricollis venom did not appear to have any fractions in common with the C. adamanteus venom. The seventh component of N. nigricollis appeared as the fourth fraction in C. rhombeatus venom. In general, the electrophoretic pattern of N. nigricollis venom exhibited a wide spectrum of mobilities with the majority of the protein fraction being in the more mobile portion.

The electrophoretic pattern for C. adamanteus venom revealed ten cationic protein fractions. Basu, et al. (1969), using disc gel electrophoresis, obtained thirteen fractions most of which were anionic at pH 8.3. The pattern for C. adamanteus venom showed that none of the more highly charged fractions of the Naja species were present. Two protein fractions of the C. adamanteus venom were similar in mobilities to two fractions of C. rhombeatus venom. The fourth, seventh and ninth C. adamanteus fractions were similar in migration distances to the second, fifth and seventh cationic fractions of A. p. leucostoma venom, respectively. The proteins of C. adamanteus venom were of intermediate mobilities with the bulk of the fractions being slightly toward the lighter end of the range.

The electrophoresis of A. p. leucostoma venom resulted in one anionic fraction, and eight cationic fractions. Although Agkistrodon is a Crotalidae like Crotalus, the venoms were not noticeably similar and had only three components in common. In comparing the Agkistrodon venom with the venom of the viper, C. rhombeatus, it was found that the sixth cationic Agkistrodon component had an equal migration distance to the fourth component of C. rhombeatus venom.

The venom of C. rhombeatus produced seven cationic fractions with intermediate mobilities, but most occurred at a heavier end of the range than those of Crotalus.

In analyzing the venoms of the rear-fanged species one finds that, except for the venom of Boiga irregularis, they are like those of other venomous species in that all the venoms have complex protein compositions. The protein constituents of the rear-fanged species, for the most part, covered nearly as wide a spectrum of electrophoretic mobilities as did the venoms of the major groups of poisonous snakes.

The three protein fractions of Boiga irregularis venom had intermediate electrophoretic mobilities with the leading fraction accounting for 41.8% of the total venom protein. By analysis of migration distances the Boiga venom and the venom of A. p. leucostoma had one component with similar migration distances as did the Boiga and N. nigricollis venom. The first and third Boiga fractions and the third and fourth L. a. annulata fractions, respectively

had corresponding migration distances.

Analysis of the two Leptodiera subspecies revealed few similarities in venom proteins at the subspecific level as was noted by Tu and Ganthavorn (1968). The venom of L. a. rhombifera contained the second highest number (nine) of protein components of all venoms studied. The nine components represented a wide spectrum of mobilities with the fourth and eighth fractions making up 17.4% and 16.1% of the total venom protein. The venom of L. a. annulata separated into six fractions. The most concentrated fractions were the fourth and sixth components with 24.6% and 22.6% respectively. Both Leptodiera subspecies had a non-mobile fraction with a migration distance similar to a component of N. n. naja, while the eighth C. adamanteus fraction was equal in migration distance to the sixth fraction of L. a. annulata. The fourth L. a. annulata component apparently migrated as did the fourth of A. p. leucostoma. In comparing L. a. annulata to D. typus, one common fraction was found.

The venom of L. a. rhombifera contained three protein fractions that were similar to ones in the N. n. naja venom, and contained one which appeared common to the N. nigricollis venom. L. a. rhombifera and C. rhombeatus venoms had one common fraction. The leading protein component of C. adamanteus coincided with the fifth fraction of the L. a. rhombifera venom. Two common components were found between L. a. rhombifera and D. typus venoms.

The venom of D. typus evidently contained less total protein than the other venoms as was determined by the faint staining of the cellulose polyacetate strips. Seven protein fractions were discerned by densitometric scanning, with a very wide third band representing forty-eight per cent of the total protein. The densitometer registered the first side band on the cellulose polyacetate strip as two separate fractions. The separation of this region into two separate bands may be due to the high sensitivity to which the scanner was adjusted. Nevertheless these two bands correspond to two bands previously noted in N. nigricollis and C. rhombeatus. The venom of D. typus had one protein fraction which corresponded to one of the C. rhombeatus protein fractions. The venom of C. adamanteus and D. typus had two fractions on common and D. typus and A. p. leucostoma venoms had one common fraction. D. typus and N. nigricollis had one common component.

The analysis of the N. n. naja - C. adamanteus venom pattern provided evidence to support the validity of the methods used to compare the venom samples. The N. n. naja - C. adamanteus pattern showed fourteen components, most of which had corresponding counterparts in the individual N. n. naja and C. adamanteus patterns. Five of the eight C. adamanteus fractions appeared in the composite patterns, and the five of the eight N. n. naja fractions were found in the composite pattern. Four of the components of the composite separation did not have components with similar migration distances in the individual patterns. With the exception of the two heavy fractions, these unaccounted for fractions had migration distances that were fairly close to fractions of the individual venom patterns. Interactions between venom constituents may have caused changes in weight and charge of the protein molecules and thus changed the electrophoretic mobilities of the protein and, hence, the inability to match them exactly with a fraction in the individual patterns.

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