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Venom protein of the haematotoxic snakes *Cryptelytrops albolabris*, *Calloselasma rhodostoma*, and *Daboia russelii siamensis*

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ABSTRACT: The protein concentration and protein pattern of crude venoms of three major haematotoxic snakes of Thailand, *Cryptelytrops albolabris* (green pit viper), *Calloselasma rhodostoma* (Malayan pit viper), and *Daboia russelii siamensis* (Russell's viper), were studied. The protein concentrations of all lots of venoms studied were comparable. The chromatograms, from reversed phase high performance liquid chromatography, of *C. albolabris* venom and *C. rhodostoma* venom were similar but they were different from the chromatogram of *D. r. siamensis* venom. *C. rhodostoma* venom showed the highest number of protein spots on 2-dimensional gel electrophoresis (pH gradient 3–10), followed by *C. albolabris* venom and *D. r. siamensis* venom, respectively. The protein spots of *C. rhodostoma* venom were used as reference proteins in matching for similar proteins of haematotoxic snakes. *C. albolabris* venom showed more similar protein spots to *C. rhodostoma* venom than *D. r. siamensis* venom. The minimum coagulant dose could not be determined in *D. r. siamensis* venom.

KEYWORDS: 2-dimensional gel electrophoresis, reverse phase high performance liquid chromatography, minimum coagulant dose

INTRODUCTION

In Thailand there are 163 snake species, 48 of which are venomous snakes. The venomous snakes can be classified, according to the physiological effects of the venoms, as neurotoxic snakes, haematotoxic snakes, and myotoxic snakes. Neurotoxic snake venom affects the neuromuscular junctions, both pre- and post-synaptic. Haematotoxic snake venom affects haemostasis and myotoxic snake venom affects the muscles[1]. *Cryptelytrops albolabris* (green pit viper), *Calloselasma rhodostoma* (Malayan pit viper), and *Daboia russelii siamensis* (Russell's viper) are three major haematotoxic snakes of Thailand. They were responsible for 70% of snakebite cases where the culprit snakes were identifiable in 2008. *C. albolabris* and *C. rhodostoma* are widely distributed throughout the country while *D. r. siamensis* has its major habitat in the central region[1–3]. *C. albolabris* bites usually do not lead to death unlike *C. rhodostoma* or *D. r. siamensis* bites whose delayed treatment can be life threatening[3,4]. The venoms of these three haematotoxic snakes affect blood coagulation through different pathways. The venoms of *C. albolabris* and *C. rhodostoma* possess a thrombin-like activity thus inducing defibrination[5–7]. The venom of *D. r. siamensis* directly affects factor X and factor V of the haemostatic system[8,9]. Among several components of the venom, protein, enzymatic and non-enzymatic, is the major component. It is interesting to study the similarity and the difference of venom proteins of these snakes.

MATERIALS AND METHODS

Venoms

The venoms used in this study consisted of pooled venoms from snakes in the snake farm of Queen Saovabha Memorial Institute. Two lots of venom of each haematotoxic snake were studied, TA 1/02/44 and TA 1/45 of *C. albolabris*, Std A/42 and CR 1/47 of *C. rhodostoma*, and V/42 and VS 1/45 of *D. r. siamensis*.

Protein concentration

The protein concentration of 0.1 mg/ml (w/v) venom preparation was analysed by Folin-Lowry method using Bovine serum albumin (Sigma) as standard protein[10].

Table 1 Venom protein concentration, MCD-F, MCD-P, and LD_{50}.

Venom lots	Protein conc. (mg/ml)[a]	MCD-F (mg/l)	MCD-P (mg/l)	LD_{50} (µg/mouse)
TA 1/02/44	0.18	498.4 (77.0)[b]	446.7 (58.0)[b]	14.2 (12.5)[b]
TA 1/45	0.18	494.1	439.5	15.0
Std A/42	0.23	49.9 (32.6)[b]	49.9 (18.4)[b]	3.3 (2.7)[b]
CR 1/47	0.19	55.6	71.3	3.6
V/42	0.20	–	–	124.8 (107.5)[b]
VS 1/45	0.19	–	–	82.4

[a] Bovine serum albumin equivalent.
[b] Data from Theakston & Reid[14].

Protein pattern

Protein patterns were studied by SDS-PAGE (Laemmli)[11], reversed-phase-high performance liquid chromatography (RP-HPLC 1100 series, Agilent Technology), and 2-dimensional gel electrophoresis (Multiphor II, Pharmacia).

RP-HPLC analysis of venom was performed using Hypersil ODS 4.0×125 mm column (Agilent Technology) and two mobile phases, A 0.1% TFA, 5% ACN and B 0.1% TFA, 95% ACN. All solvents used were HPLC grade. Venom sample was prepared as 1 mg/ml in the mobile phase A. Analytical protocol was 20 µl sample, linear gradient of 5% to 95% ACN (0–100% mobile phase B) at 1 ml/min flow rate and 60 min running time. Protein absorption was monitored at 280 nm[12].

The first dimension (isoelectric focusing, IEF) of 2-dimensional gel electrophoresis was performed on 7 cm Immobiline dry strip gel pH 3–10 (GE Healthcare) using 230 µg of venom. Running protocol was as recommended in the system instructions[13]. Second dimension was performed on 12.5% SDS-PAGE. All chemicals used were analytical grade. Matching for similar protein spots was done by IMAGEMASTER 2D ELITE version 4.01 (Amersham Biosciences).

Minimum coagulant dose

The minimum coagulant dose (MCD) was studied as recommended by WHO Collaborating Centre for the Control of Antivenoms, Liverpool School of Medicine, UK[14]. Fibrinogen from bovine plasma Type IV (Sigma) was used for the MCD-F testing and fibrinogen from human plasma (Sigma) was used for the MCD-P testing.

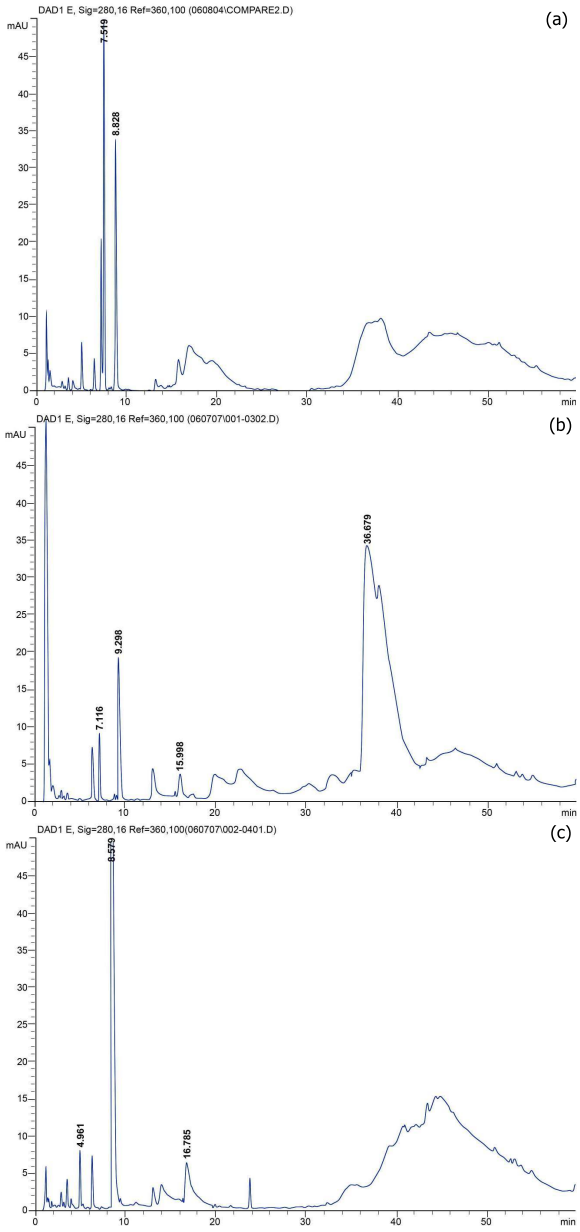

Fig. 1 The venom chromatograms from RP-HPLC. (a) *C. albolabris* venom lot TA 1/02/44, (b) *D. r. siamensis* venom lot V/42, and (c) *C. rhodostoma* venom lot Std A/42.

RESULTS AND DISCUSSION

The protein concentrations of six lots of studied venoms (0.1 mg/ml) were comparable (Table 1). The MCD-F and MCD-P of the venoms were not correlated with the venom toxicity. The LD_{50} values of all lots of studied venom were comparable with the study of Theakston and Reid[14] but that of the MCD-F and MCD-P were very different (Table 1).

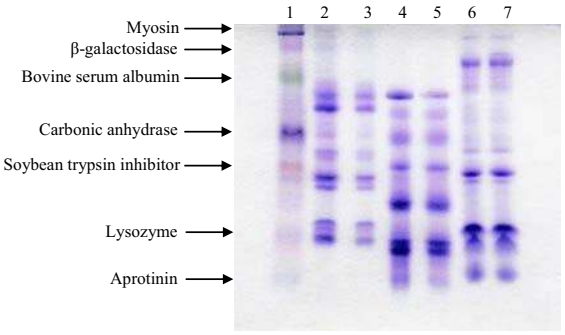

Fig. 2 SDS-PAGE profile of the venoms. Lane 1: molecular weight marker; lane 2: *Cryptelytrops albolabris* venom lot TA 1/02/44; lane 3: *C. albolabris* venom lot TA 1/45; lane 4: *Calloselasma rhodostoma* venom lot Std A/42; lane 5: *C. rhodostoma* venom lot CR 1/4; lane 6: *Daboia russelii siamensis* venom lot V/42; lane 7: *D. r. siamensis* venom lot VS 1/45.

The MCD-F and MCD-P could not be determined in *D. r. siamensis* venom because *D. r. siamensis* venom could not induce coagulation within the time limit of the testing protocol, 60 s. This is because *D. r. siamensis* venom indirectly induces coagulation through a procoagulant enzyme while the thrombin-like activity of *C. albolabris* and *C. rhodostoma* venoms act directly on fibrinogen.

From RP-HPLC, the chromatograms of *C. albolabris* and *C. rhodostoma* venoms were similar but they were different from the chromatogram of *D. r. siamensis* venom (Fig. 1).

On SDS-PAGE, the venom protein patterns of two lots of each snake species were identical but they were different from the venom protein patterns of other snake species studied (Fig. 2).

On 2-dimensional gel electrophoresis (Fig. 3), *C. rhodostoma* venom showed the highest number of protein spots (191 spots) followed by *C. albolabris* venom (159 spots) and *D. r. siamensis* venom (125 spots). When the protein spots were matched for similar proteins, using *C. rhodostoma* venom protein spots as references, *C. albolabris* venom revealed more similar protein spots to *C. rhodostoma* venom than *D. r. siamensis* venom (Fig. 4).

All haematotoxic snakes studied are members of Viperidae family but they are different in subfamily and genus. *C. albolabris* is a member of genus Cryptelytrops and *C. rhodostoma* is a member of genus Calloselasma; both genera are within the subfamily Crotalinae. *D. r. siamensis* is a member of genus Daboia of the subfamily Viperinae[15,16]. Venom protein patterns from this study revealed that snakes

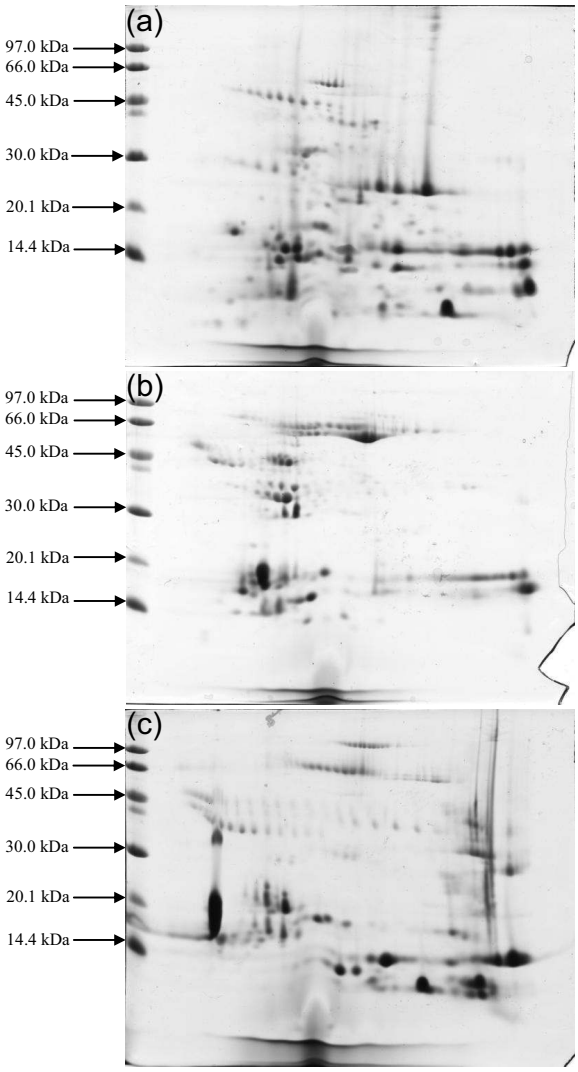

Fig. 3 Venom protein spots from 2-dimensional gel electrophoresis. (a) *C. rhodostoma* venom (191 protein spots), (b) *C. albolabris* venom (159 protein spots), and (c) *D. r. siamensis* venom (125 protein spots).

of the same subfamily, although of different genera, possessed more similar venom proteins than snakes of different subfamilies. The thrombin-like proteins of *C. albolabris* and *C. rhodostoma* should be explored among the matched protein spots of these two venoms while the factor X and factor V activator proteins of *D. r. siamensis* should be investigated among the unmatched protein spots of *D. r. siamensis* venom to *C. rhodostoma* venom.

As venom proteins are abundant, the 7-cm gel strip used for isoelectric focusing in this study could not separate all venom proteins into single protein

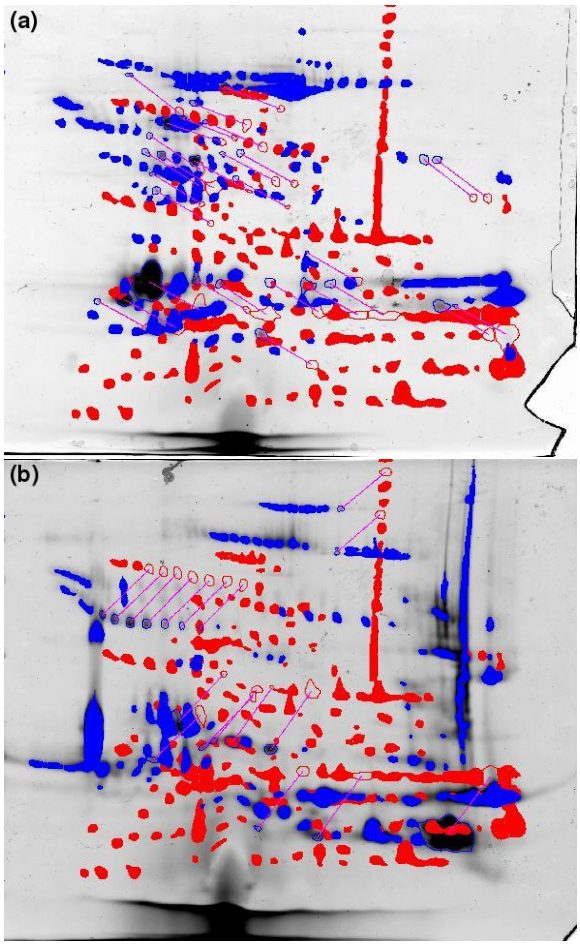

Fig. 4 (a) *C. albolabris* venom. 111 protein spots were matched with *C. rhodostoma* venom. Purple rings with connecting lines indicate the matched protein spots of *C. albolabris* venom and *C. rhodostoma* venom. Red spots indicate the unmatched protein spots of *C. rhodostoma* venom. Blue spots indicate the unmatched protein spots of *C. albolabris* venom. (b) *D. r. siamensis* venom. 88 protein spots were matched with *C. rhodostoma* venom. Purple rings with connecting lines indicate the matched protein spots of *D. r. siamensis* venom and *C. rhodostoma* venom. Red spots indicate the unmatched protein spots of *C. rhodostoma* venom. Blue spots indicate the unmatched protein spots of *D. r. siamensis* venom.

spots. Some spots observed could be spots of protein complex. At present, there are many pharmaceutical products, for diagnostic or therapeutic purposes, that were developed from snake venom proteins[17]. Purification of crude venom for further study would also be interesting because, beside the toxic proteins, other proteins with beneficial applications might be discovered.

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REFERENCES

1. Jintakune P, Chanhome L (1997) *Venomous Snakes in Thailand*. Queen Saovabha Memorial Institute. Bangkok, Thailand.
2. Chanhome L, Cox MJ, Wilde H, Jintakoon P, Chaiyabutr N, Sitprija V (1998) Venomous snakebite in Thailand I: Medically important snakes. *Mil Med* **163**, 310–7.
3. Viravan C, Looareesuwan S, Kosakarn W, Wuthiekanun V, McCarthy CJ, Stimson AF, Bunnag D, Harinaurta T, Warrell DA (1992) A national hospital-based survey of snakes responsible for bites in Thailand. *Trans Roy Soc Trop Med Hyg* **86**, 100–6.
4. Looareesuwan S, Viravan C, Warrell DA (1988) Factors contributing to fatal snake bite in the rural tropics: Analysis of 46 cases in Thailand. *Trans Roy Soc Trop Med Hyg* **82**, 930–4.
5. Mitrakul C (1973) Effects of green pit viper (*Trimeresurus erythrurus* and *Trimeresurus popeorum*) venoms on blood coagulation, platelets and the fibrinolytic enzyme systems: Studies in vivo and in vitro. *Am J Clin Pathol* **60**, 654–62.
6. Rojnuckarin P, Intragumtornchai T, Sattapiboon R, Muanpasitporn C, Pakmanee N, Khow O, Swasdikul D (1999) The effects of green pit viper (*Trimeresurus albolabris* and *Trimeresurus macrops*) venom on the fibrinolytic system in human. *Toxicon* **37**, 743–55.
7. Gaebert AK (1977) Isolation of thrombin-like factors from Malayan pit viper venom by affinity chromatography. *Toxicon* **15**, 217–24.
8. Di Scipio RG, Hermodson MA, Davie EW (1977) Activation of human factor X (Stuart factor) by a protease from Russell's viper venom. *Biochemistry* **16**, 5253–60.
9. Kisiel W (1979) Molecular properties of the Factor V-activating enzyme from Russell's viper venom. *J Biol Chem* **254**, 12230–4.
10. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**, 265–75.
11. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–5.
12. Nawarak J, Sinchaikul S, Wu CY, Liau MY, Phutrakul S, Chen ST (2003) Proteomics of snake venoms from Elapidae and Viperidae families by multidimensional chromatographic methods. *Electrophoresis* **24**, 2838–54.
13. Berkelman T, Stenstedt T (1998) *2 Dimensional Elec-*

trophoresis Using Immobilized pH Gradient: Principles and Methods, Amersham Biosciences, Uppsala.
14. Theakston RDG, Reid HA (1983) Development of simple standard assay procedures for the characterization of snake venom. *Bull World Health Organ* **61**, 949–56.
15. Gumprecht A, Tillack F, Orlov NL, Captain A, Ryabov S (2004) *Asian Pitvipers*, GeitjeBooks, Berlin.
16. Malhotra A, Thorpe RS (2004) A phylogeny of four mitochondrial gene regions suggests a revised taxonomy for Asian pitvipers (*Trimeresurus* and *Ovophis*). *Mol Phylogenet Evol* **32**, 83–100.
17. Fox JW, Serrano SMT (2007) Approaching the golden age of natural product pharmaceuticals from venom libraries: An overview of toxins and toxin-derivatives currently involved in therapeutic or diagnostic applications. *Curr Pharmaceut Des* **13**, 2927–34.