CHAPTER I

INTRODUCTION

1. Background and Rationale

Venomous snakes are considered to be dangerous to humans and still pose public health concerns. Almost 300 species of venomous snake from 5 families were found worldwide (Barry *et al.*, 2002). In Thailand, 48 species of venomous snake are members of Elapidae and Viperidae families. Both of them are the most two dangerous venomous snakes. The incidence rate of bites by venomous snake in Thailand is probably 7,000 cases per year. Most of the snakebites are due to Malayan pit viper, green pit viper, cobras, and Russell's viper, respectively. However, most fatal cases of venomous snake bites in Thai victims are cobras and Russell's viper, envenoming (Jintanukul and Janhome, 2539).

Daboia russellii siamensis is a member of Russell's viper (*Daboia russellii*). The habitats of these species are mainly in grassy areas in Eastern Asia and Southeast Asia (Warrell, 1989). Russell's viper is classifying of genus *Daboia* (*Vipera*), species *russellii*. Russell's viper can be classified to 5 subspecies based on the difference of coloration and markings, including *Daboia russellii russellii* (India and Pakistan), *Daboia russellii pulchella* (Sri Lanka), *Daboia russellii siamensis* (Thailand, Myanmar and China), *Daboia russellii formosensis* (Taiwan), and *Daboia russellii limitis* (Indonesia) (**Figure 1**). The incidence of the snakebites *Daboia russellii siamensis* are increased two times during the plant and harvest of rice fields. Because the snakes live in the rice fields, many farmers are bitten during working in the rice fields. Moreover, their rural location hinders them from seeking immediate medical attention. In fact, it is often as long as five hours before the victim can reach a medical facility. The most devastating effects of the venom, however, act on the blood clotting mechanism, which has serious implications for kidney function. Fourteen of 45 people die after receive dialysis Of 45 people who received dialysis, 14 die (Nuchtaphan, 2525; Pochanukul, 2531). Russell's viper is responsible for the snake bite morbidity and mortality in the central rice growing area of Thailand (Warrell, 1989).

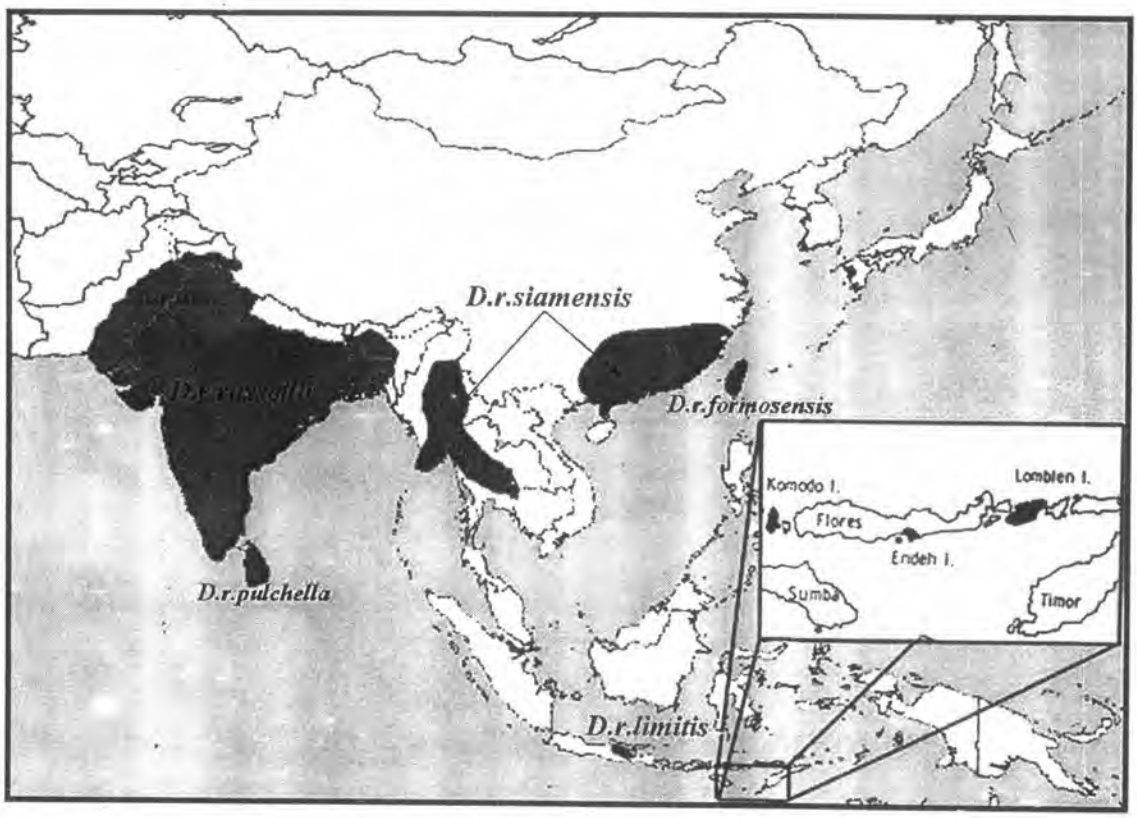


Figure 1 Distribution of *Daboia russellii* subspecies (Warrell, 1989).

Russell's viper venom can cause the damage of vascular endothelium and cause internal hemorrhage (Warrell, 1989). The venom induce disseminated intravascular coagulation (DIC) due to stimulation of coagulation factor X, and induce edema, paralysis, wound necrosis and hypotensive effects (Kini and Iwanaga, 1986; Warrell, 1989). Deaths usually occur between 15 minutes and 9 days after bite and have been attributed to acute renal failure, vasodilatation, intracranial or massive intra-abdominal haemorrhage, shock caused by increased vascular permeability (Kini and Iwanaga, 1986; Warrell, 1989). In addition, the blood is usually incoaglulable in patients with systemic envenoming. There is intense hypofibrinogenaemia associated

with a relatively severe reduction of factor V, X and XIIA compared with VIII, of blood coagulation, protein C and ATIII are depleted. Levels of fibrin/fibrinogen degradation products (FDP) are very high, while plasminogen and antiplasmin levels are reduced. About two-thirds of the FDP are of the cross-linked type. Thrombocytopenia is usual and there is poor platelet aggregation and evidence of massive platelet activation (elevated plasma level of β-thromboglobulin and platelet factor 4) (Than *et al.*, 1988). Early evidence of envenoming, before the blood becomes incoagulable, includes depletion of factor V, elevation of FDP above 80 μg/ml, and moderate decrease in fibrinogen and factor X concentration (Warrell, 1989). Therefore, the causes of haemostatic abnormalities are found to be hypofibrinogenaemia effects of venom, the pathogenesis of hypofibrinogenaemia effects remains to be determined. Fribrinogenase agents in the venom are proposed to acting fibrinogen *in vivo* resulting in fibrinogen depletion and hypofibrinogenaemia. Although these proteins have not been purified from crude venom and partially sequenced at the protein level, cDNA sequences and complete protein sequences have not been reported in Russell's viper. Obtaining the complete cDNA sequences of these proteins will give us more accurate amino acid sequences of fibrinogenase from Thai in Russell's viper venom will also determined.

1.1 Russell's Viper

Venomous snake in family Viperidae can be divided in to 2 subfamilies, viperinae and crotalinae. Although viperinae genus does not have the heat-sensitive pit organs common to the Crotalinae. In Thailand, *Daboia russellii siamensis* is viperinae snake found in northern and central area. The snakes are about 90-120 cm in body length (Pochanukul, 2531), portly and short shape, triangle-shape head, narrowed neck, short tail, light brown body and heavy brown oval marks (**Figure 2**) (Jintanukul and Janhome, 2539). They also have large retractable fangs. The average length of fangs in adult is 16 mm (Warrell, 1989). They are a very muscular snake and can move rapidly and convulsively by lunging movements to attack the aggressor or, more commonly, to attempt escape. The natural prey includes small vertebrates, especially rodents, frogs, lizards, snakes and birds. They are nocturnal and ground dwelling but have occasionally been found swimming and climbing trees (Warrell, 1989).

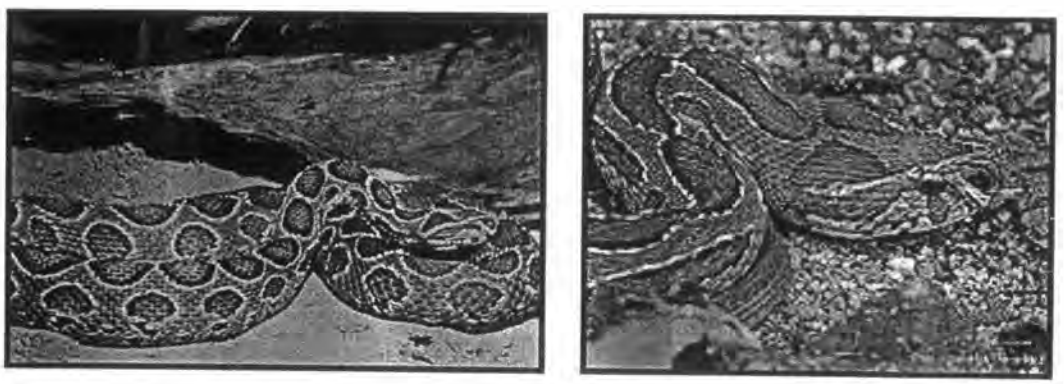


Figure 2 Morphology of *Daboia russellii* (http://en.wikipedia.org/wiki/Daboia)

1.2 Clinical signs and symptoms of Russell's viper bite

The Russell's viper venom exhibits a striking geographical variation in the composition and clinical effects of venom even within same subspecies (Warrell, 1989) (**Table 1**). Haemostatic abnormalities are described from all countries but the coagulopathy is less marked in Sri Lanka than in Myanmar (Phillips *et al.*, 1988; Than-Than *et al.*, 1988). Pituitary haemorrhage has so far been described only in Myanmar and southern India. Intravascular haemolysis was most marked in Sri Lanka but has been reported from India and Thailand. Neuromyotoxicity is the dominant clinical feature in Sri Lanka and may well occur in India. Chemosis and facial edema, as evidence of increased capillary permeability, have so far been described only in Myanmar where they are common features of severe envenoming. Primary shock and hypotension are most commonly described in Myanmar but have also been mentioned in reports from the other countries (Warrell, 1989).

Table 1 Geographical variation in the clinical manifestations of *Daboia russellii* bite (Warrell, 1989).

Symptoms	Sri Lanka	India	Myanmar	Thailand	Taiwan
Coagulopathy	+	++	++	++	?
Renal failure	++	+	++	+	+
Pituitary infarction	-	+	++	-	?
Intravascular haemolysis	++	+	-	+	?
Neuro-myotoxicity	++	+	-	-	?
Generalized capillary permeability	-	-	++	-	?
Primary shock/hypotension	-	+	++	-	?

1.3 Components of Russell's viper venom

Over 95% of the dry weight of most venom is polypeptide which includes enzymes, toxins and small peptide. Each class can modulate the physiological response of envenomed animals (Karalliedde, 1995). Its components include neurotoxins, cytotoxins, cardiotoxins, nerve growth factors, lectins, factor IX/X-binding protein, disintegrins, various enzymes and enzyme inhibitors (Matsui *et al.*, 2000). Several of proteins are also found in most venomous snakes, including hyaluronidase, phospholipase A_2 (PLA_2), collagenase, nucleotidase, amino acid oxidase, ribonuclease, lactate dehydrogenase, deoxyribonuclease, arginine esterhydrolase, fibrinogenolytic enzyme, serine protease etc (Warrell, 1989; Tsai *et al.*, 1996; Matsui *et al.*, 2000).

Fibrinolytic and fibrinogenolytic activity has been described in venoms of a number of snake species, including member of the Crotalidae, Viperidae, and Elapidae families. Although the enzymes described here are direct-acting proteinases, there are scattered reports of plasminogen activator or plasminogen activator-releasing activities in snake venom. There are also numerous reports of fibrinolytic activity in a variety of bloodsucking animals and insects. Presumably, the purpose of fibrinolytic activity in these creatures is to prevent the host blood from clotting, thereby facilitating feeding and ingestion. Additionally, there are a number of reports describing proteinases with fibrinolytic activity from different organism, including brinase, the fibrinolytic enzyme from the mold *Aspergillus oryzae* (Eie *et al.*, 1972; Frisch, 1972).

Fibrinogenolytic enzymes has been carried out covering Aα- chain and Bβ- chain degrading fibrinogenase found in snake venoms. The Aα- chain fibrinogenase (classify in metalloproteinase) and Bβ- chain fibrinogenase (classify in serine proteinase) can be defined operationally as venom enzyme degrading preferentially (although not exclusively) either the Aα- or Bβ- chain of fibrinogen,

respectively. These endoproteinase are direct-acting and do not require any other factors for activity. They do not release fibrinopeptides A or B and do not induce fibrin clot formation. They are neither plasmin-like nor thrombin-like.

In addition, it is possible that serine beta-fibrinogenase in the venom may prevent blood clotting in the patient, result in the spread of the other components of the venom throughout the blood circulation. Moreover, this enzyme may useful for the dissolution of blood clots formed in pathological conditions, and beta-fibrinogenase did not possess a hemorrhagic effect and had a stronger hydrolytic effect toward human fibrinogen and less suppressed by the protease inhibitors of human plasma. It is worthwhile evaluating the possible application of β- fibrinogenase as an antithrombotic agent. Furthermore β- fibrinogenase will be develop to specific antivenom for reduce symptomatic adverse drug reaction of patients when use whole antivenom.

Serine beta-fibrinogenase has been shown to correlate with arginine ester hydrolase, that specific cleavage Bβ- chains of fibrinogen by hydrolyze ester bond and amides in fibrinogen. Serine beta-fibrinogenase has been found in *Crotalidae*, *Viperidae* and *Elapidae* families of snake venom. However, it has not been reported in *Daboia russellii siamensis* venom of Thailand. Therefore, study on the serine beta-fibrinogenase in *Daboia russellii siamensis* venom of Thailand, as well as the genetic information and evolutionary relationship to other proteolytic enzymes will be useful for new knowledge to elucidate the role of serine beta-fibrinogenase in fibrin polymerization and remove fibrinogen from blood.

2. Research Questions

What is the complete cDNA sequence of serine beta-fibrinogenase homolog gene from *Daboia russellii siamensis* in Thailand?

3. Objective of the study

Molecular cloning and characterize the cDNA sequence of serine beta-fibrinogenase homolog of *Daboia russellii siamensis* in Thailand.

4. Keywords

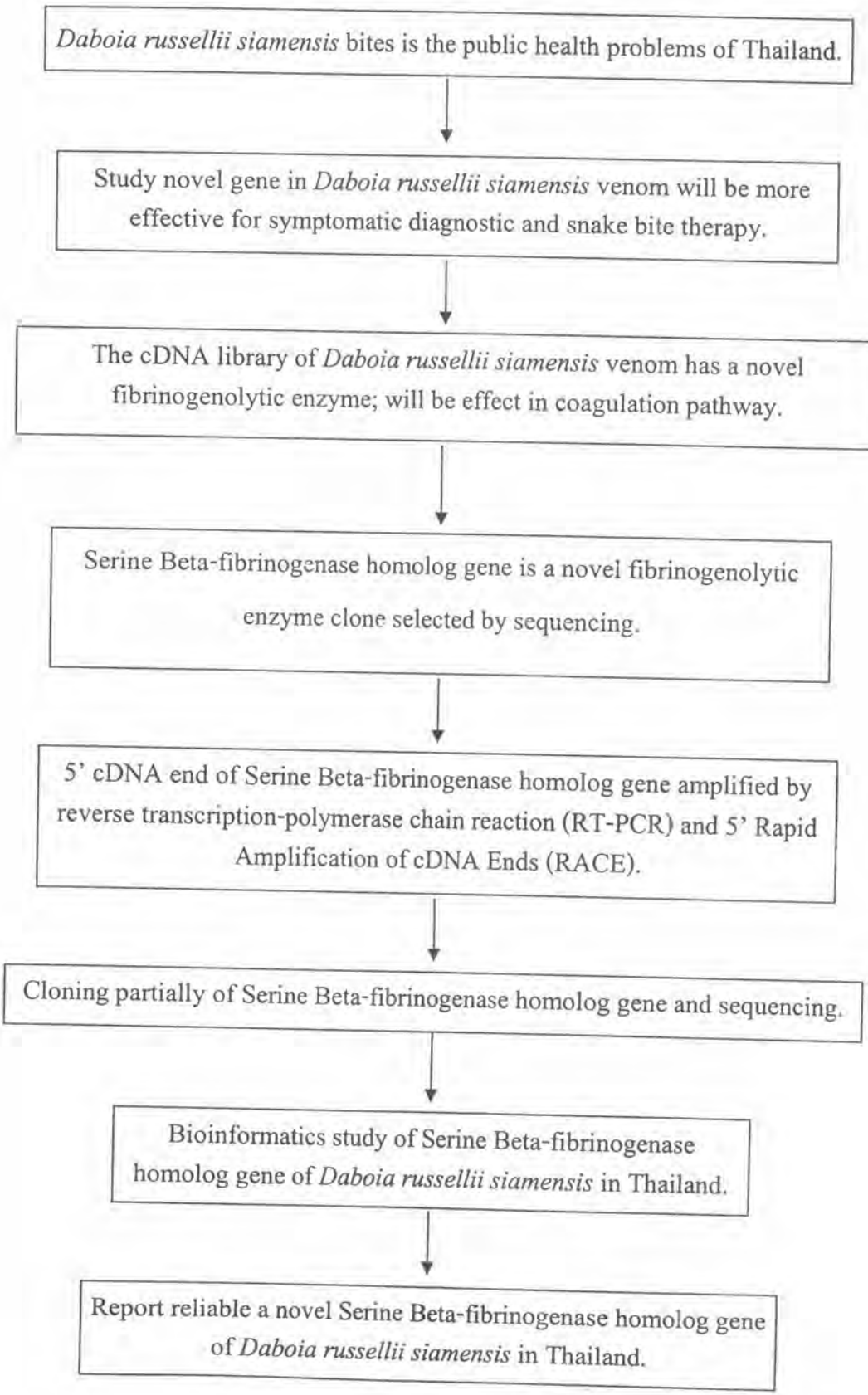
Molecular cloning

Russell's viper

Serine beta-fibrinogenase homolog

Daboia russellii siamensis

5. Conceptual Framework



6. Expected Benefit & Application

1. Genetic information on Serine beta-fibrinogenase homolog of *Daboia russellii siamensis* in Thailand will be useful for new knowledge of Russell's viper.

2. The cDNA can be used to produce recombinant proteins. These proteins are potentially useful as diagnostic agents or reagents to produce specific antivenom.