CHAPTER II

LITERATURE REVIEWS

2.1 Toxicology of snake

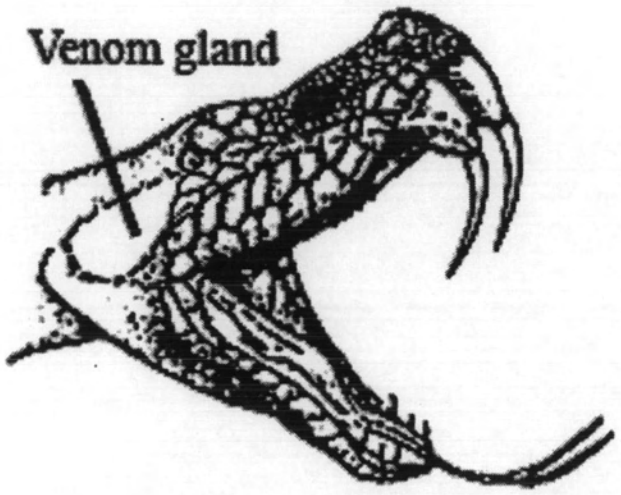
There are more than 200 known species of venomous snakes classified into several families such as Elapidae, Viperidae, Crotalidae, Hydrophidae and Colubidae. Snake lives in several and difference climate such as tropical rain forest, desert, sea and city park. Snake is a carnivore, it knows as the good predator. Snake eats various types of animals example rat, flog, bird, cat or dog. Interesting, King cobra eats the other snake. The snake using venom to kills, help to digest prey and for defence. Toxicology of snake will describe on topic.

2.1.1 Snake venom

Venoms affect key elements of almost every animal physiological pathway. Neurotoxin blocks the nerve-muscle junction, cytotoxins and phospholipases induce muscle necrosis, CVF suppress circulating component and various proteins including enzyme cause systematic hemostatic disorder. Venom component seem to be fairly common and related within each snake family. Neurotoxin are typical of Hydrophidae and Elapidae venoms while hemostatically active component are generally found in Viperidae, Crotalidae and Elapidae snake (Lu *et al.*, 2005).

2.1.2 Venom gland

Venoms are secreted and stored in venom gland. The gland which secretes the zootoxin is a modification of the parotid salivary gland of other vertebrates. Venom gland is situated on each side of the head below and behind the eye, invested in a muscular sheath (Fig. 2.1)

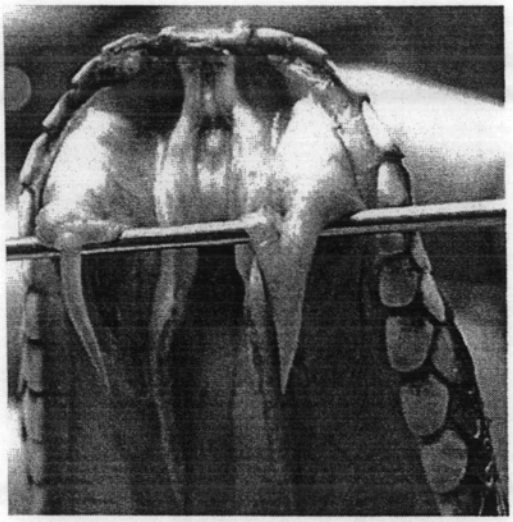


(http://www.calpoison.org/public/snake-gland.gif)

Fig. 2.1 Venom gland

2.1.3 Snake fangs

The venom is injects into the victim via the sharp fangs which causes damage in several ways. Fangs are sharp, long, hollow teeth that are hooked up to small sacs in the snake's head behind their eyes (Fig. 2.2). Anyway, some species of cobra using fangs spray its venom into the eyes of victims.



http://www.kingsnake.com/snakegetters/slides/18fangsheath.html

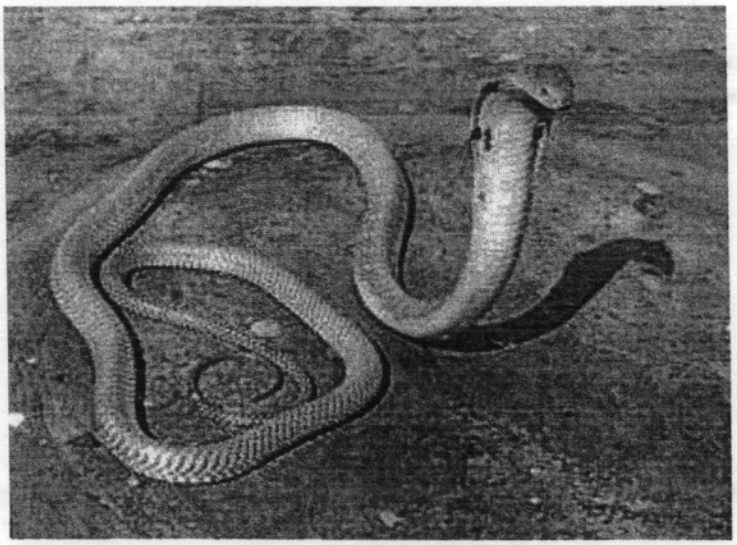
Fig. 2.2 Snake fangs

2.2 Snake in Thailand

Thailand is situated on south East Asia whereas tropical rain forest climate. Many species of wildlife are live in this country, especially snakes. It was estimated that 165 snakes are found in Thailand inwhich 46 species are venomous snakes. The information of dangerous snake in Thailand was also available.

2.2.1 King cobra (*Ophiophagus hannah*)

The king cobra is the world's longest venomous snake. It averages 3.7 m in length but is known to grow to 5.5 m. The color usually yellow to brown, with a black and white spectacle pattern on top and two black and white spots on the lower surface. It eats primarily other snakes.



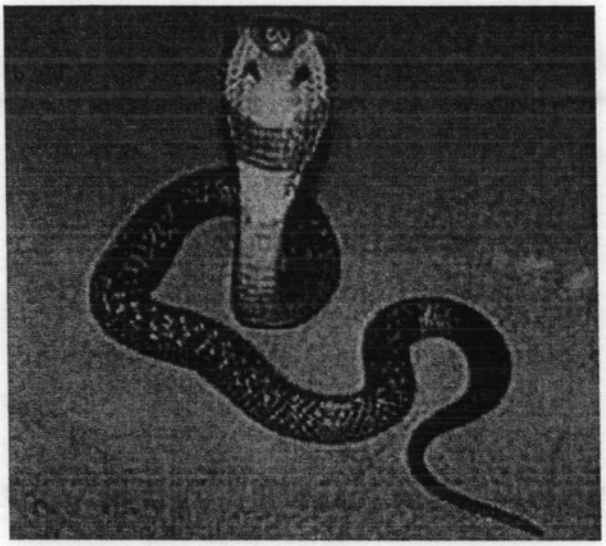
(http://www.kanchanapisek.or.th/kp7/science/Snake.html)

Fig. 2.3 King cobra

Habitat: Philippines, Malaysia, southern China, Myanmar, India, Thailand and Malaysia.

2.2.2 Cobra (Naja sp.)

The color is dark brown or black with ridged or keeled scales and pale rings on the neck. Some species can spray its venom from a distance of about 2.4 m into the eyes of its victims causing temporary blindness and great pain. The venom of cobras often contains a powerful neurotoxin and acts on the nervous system. Cobra venom has been used for many years in medical research because it has an enzyme lecithinase that dissolves cell walls as well as membranes surrounding viruses.



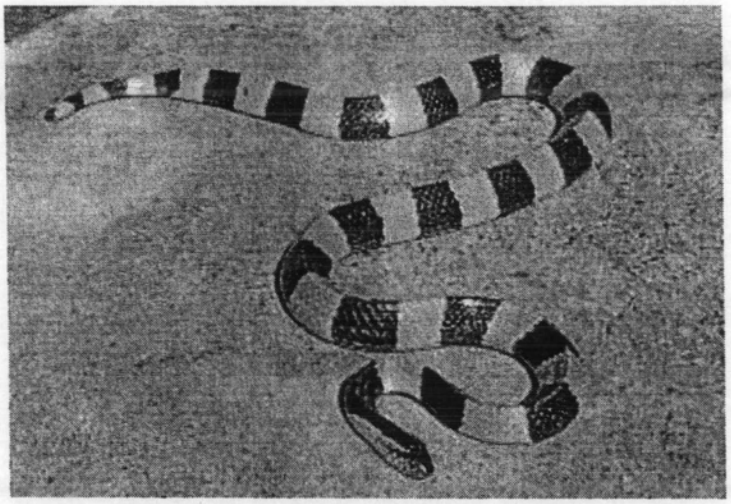
(http://www.kanchanapisek.or.th/kp7/science/Snake.html)

Fig. 2.4 Cobra

Habitat: Philippines, Malaysia, southern China, Myanmar, India, Thailand and Malaysia

2.2.3 Banded Krait (*Bungarus fasciatus*)

The black and yellow bands its triangular body cross-section and the marked vertebral ridge consisting of enlarged vertebral shields along its body. The head is broad and depressed. The eye is black. It has arrow-head like yellow markings on its otherwise black head and has yellow lips, lore, chin and throat. The banded krait has been recorded to grow up to a length of 2.2 m normally the maximum length encountered is 1.8 m or less. The snake has an entire anal scale and single subcaudals. The tail is small and ends like a finger-tip generally being one tenth the length of the snake.



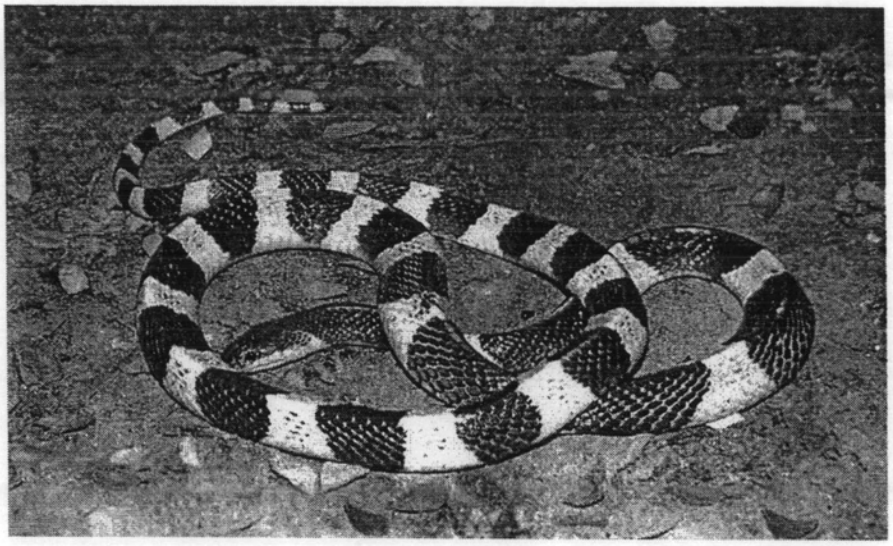
(http://www.kanchanapisek.or.th/kp7/science/Snake.html)

Fig. 2.5 Banded Krait

Habitat: Thailand and Myanmar

2.2.4 Malayan krait (*Bungarus candidus*)

Malayan kraits usually range between 1 to 1.5 m in length, specimens as large as 2.0 m have been observe. Most species of krait are covered in smooth glossy scales that are arranged in bold striped patterns of alternating black and light-colored areas. This gives the snake camouflage in its habitat of grassland and scrub jungle. The scales along the dorsal ridge of the back are hexagonal. The head is slender and the eyes have round pupils. Kraits have a pronounced dorso-lateral flattening and are triangular in cross-section. The tail tapers to a thin point.



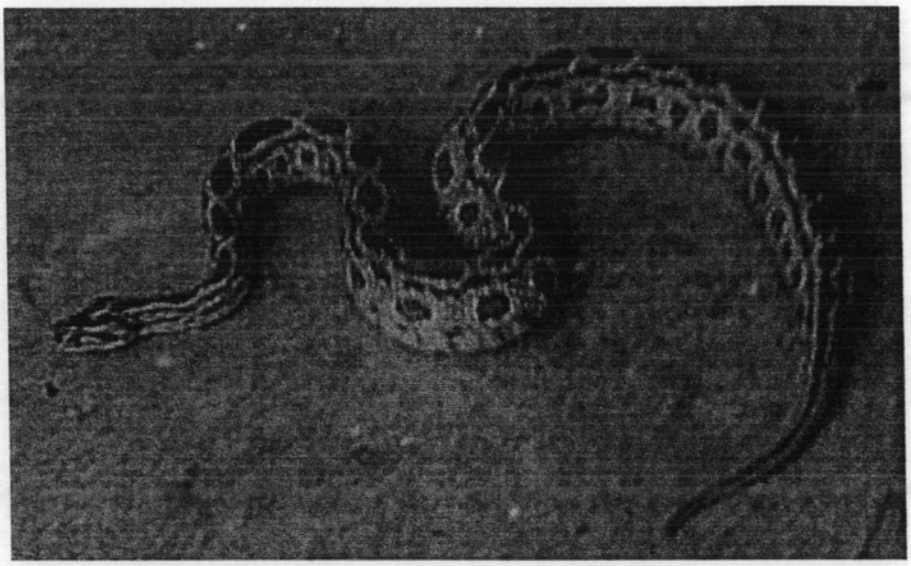
(Photo by lawan chanhome)

Fig. 2.6 Malayan krait

Habitat: Indian, Sri Lanka, eastern Pakistan and south East Asia

2.2.5 Siam Russell viper (*Vipera russeli siamensis*)

The average length is about 1.2 m grows to a maximum length of 1.7 m. The color pattern consists of a deep yellow, tan or brown ground color with three series of dark brown spots that run the length of its body. Each of these spots has a black ring around it. The outer border of which is intensified with a rim of white or yellow. The dorsal spots which usually number 23-30 may grow together while the side spots may break apart. The head has a pair of distinct dark patches, one on each temple, together with a pinkish, salmon or brownish V or X pattern that forms an apex towards the snout. Behind the eye, there is a dark streak, outlined in white, pink or buff. The venter is white, whitish, yellowish or pinkish often with an irregular scattering of dark spots.



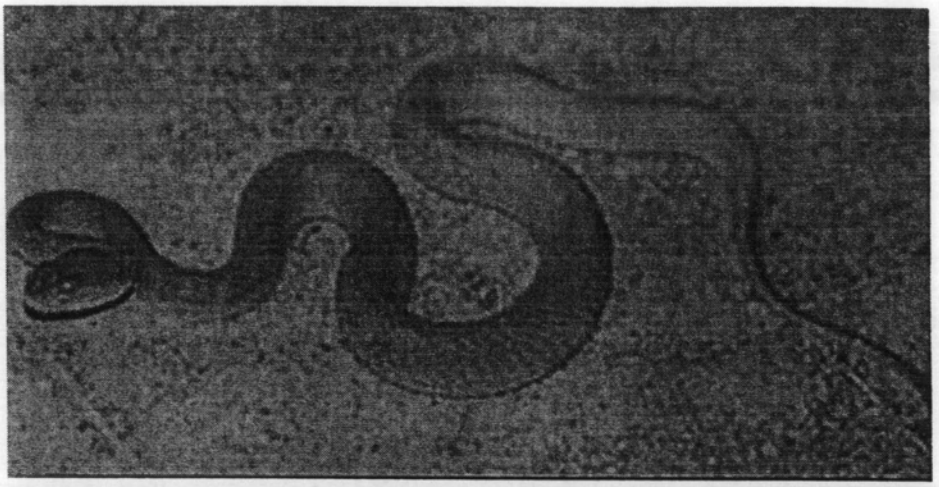
(http://www.kanchanapisek.or.th/kp7/science/Snake.html)

Fig. 2.7 Siam Russell viper

Habitat: Pakistan, India, Sri Lanka, Bangladesh, Nepal, Myanmar, Thailand, Cambodia, China (Guangxi, Guangdong), Taiwan and Indonesia

2.2.6 White-lipped tree viper, White-lipped pit viper (*Trimeresurus albolabris*)

Color pattern is green above the side of the head below the eyes is yellow, white or pale green, much lighter than rest of head. The belly is green, yellowish or white below. A light ventrolateral stripe present in all males but absent in females. The end of tail not mottled brown. Total length males 60 cm, females 80 cm tail length males 12 cm, females 13 cm.



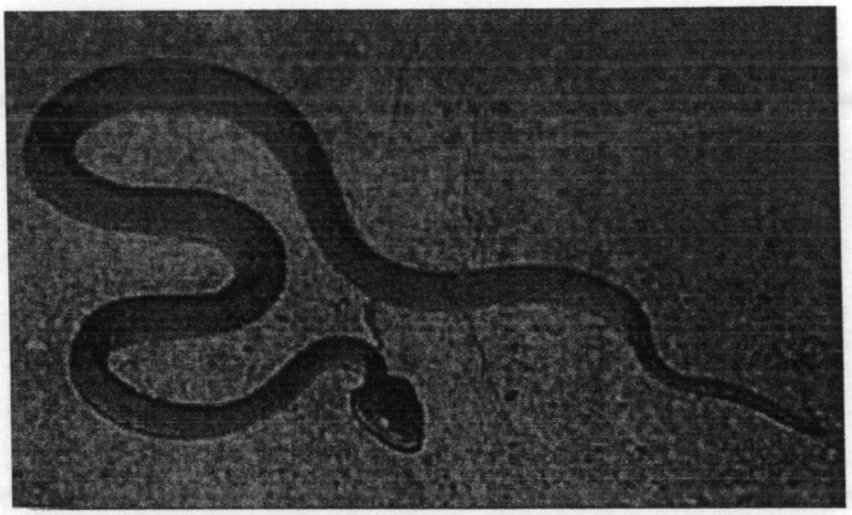
(http://www.kanchanapisek.or.th/kp7/science/Snake.html)

Fig. 2.8 White-lipped tree viper, White-lipped pit viper

Habitat: Northern India, Myanmar, Thailand, West Malaysia and Vietnam. In Indonesia, it occurs on the islands of Sumatra, Mentawai (Siberut, Sipora, North Pagai) and Borneo

2.2.7 Pope's tree viper, Pope's bamboo pit viper (*Trimeresurus popeorum*)

Color pattern is green above. A single supraocular above green below pale green to whitish. The two separated by a bright bicolored orange or brown (below) and white (above) (males) or white (females). Ventrolateral stripe which occupies the whole of the outermost scale row and a portion of the second row. Grows to a total length 70 cm and tail length 17 cm.



(http://www.kanchanapisek.or.th/kp7/science/Snake.html)

Fig. 2.9 Pope's tree viper, Pope's bamboo pit viper

Habitat: Northern India, Myanmar, Thailand, West Malaysia and Vietnam. In Indonesia, it occurs on the islands of Sumatra, Mentawai (Siberut, Sipora, North Pagai) and Borneo

2.2.8 Malayan pit viper (*Calloselasma rhodostoma*)

The Malayan pit viper, *Calloselasma rhodostoma* (formerly knows as *Agkistrodon rhodostoma*) is the terrestrial snake occurring in southern Vietnam, Laos, Cambodia, Thailand, northern Malaysia, Java and various offshore islands (Fig 2.10) (Daltry *et al.*, 1996). The snake has Reddish running into pink tinge toward the belly with triangular-shaped, brown markings bordered with light-colored scales. The base of the triangular-shaped markings end at the midline. It has dark brown, arrow-shaped markings on the top and each side of its head. The average size is 60 cm long, maximum 1 m (Fig 2.11). It lives at rubber plantations, farms, rural villages and rain forests.

In this research we study the toxicity and purify fibrinolytic protein from Malayan pit viper.

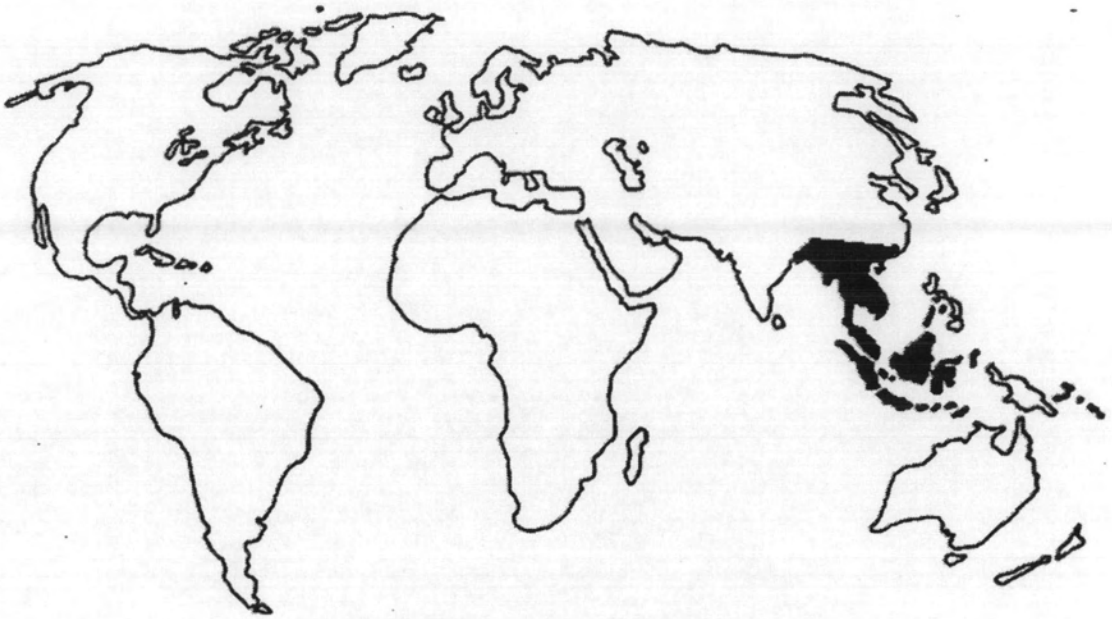


Fig. 2.10 Habitat of Malayan pit viper

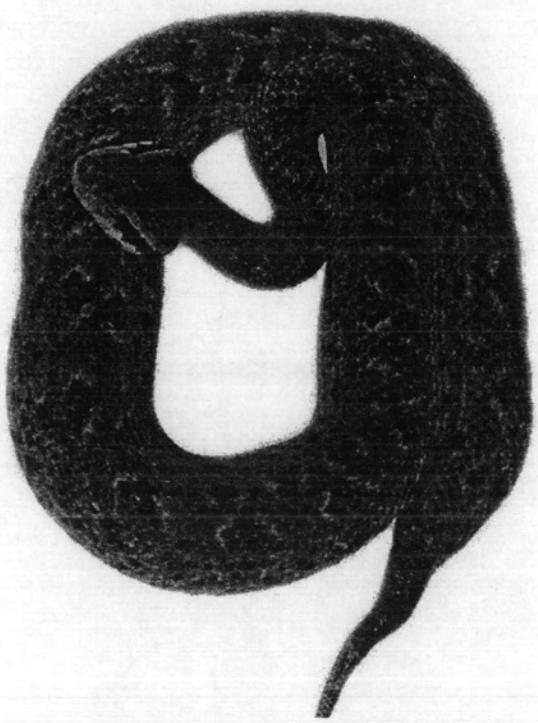


Fig. 2.11 Malayan pit viper

2.3 Snake bite in Thailand

Snake envenomation is a serious medical problem in Thailand and south Asia country since the economic activities of the country are mainly agricultural (Pithayanukul et al., 2004). A national survey of snake bites in Thailand shows that 70% are of venomous species. The most common species involved in envenomation are the Malayan pit viper (38%), White-lipped green pit viper (27%) and Russell's viper (14%) (Viravan et al., 1992).

Malayan pit viper envenomation is a major health problem relative to the expansion of civilization to the snake's natural habitat. During envenomation, the venom component mainly effects the hemostatic system. Malayan pit viper antivenom (IgG) was tested against the proteolytic effects of the venom. Though the antivenom does combat the protelytic effects of the venom, side effects can still arise due to the Fc region of the molecule. Thus, alternatives for a new antivenom will be explored.

2.4 Natural products use to against venom

2.4.1 Plant exact

Pithayanukul *et al.* (2004) found that the butanolic and purified butanolic extracts of False daisy (*Eclipta prostrate*) inhibited the lethality, hemorrhagic, proteolytic and phospholipase activity of Malayan pit viper venom.

Fig. 2.12 False daisy (*Eclipta prostrata*)

2.4.2 Virginia opossum serum

The Virginia opossum (*Didelphis virginiana*) is North America's only marsupial. A marsupial is an animal with a pouch like a kangaroo or a koala. Opossum has been around for at least 70 million years and is one of Earth's oldest surviving mammals.

The animal has a triangular head and a long pointed nose and grayish fur everywhere but on its ears, feet and tail. Its tail is prehensile. A prehensile tail is adapted for grasping and wrapping around things like tree limbs (Fig. 2.13). The Virginia opossum can be found in most of the United States east of the Rocky Mountains and on the West Coast. It is also found in Mexico, Central America, British Columbia and Canada (Fig. 2.14).

Virginia opossum is an animal that is naturally resistant to the proteolytic effects of Crotalid venoms. It has proteinase inhibitors in their sera that bind to and neutralize

the hemorrhagic and other proteolytic activity in many snake venoms (McKeller & Perez, 2002; Perez *et al.*, 1979; Sanchez *et al.*, 1998).

http://www.nhptv.org/natureworks/opossum.htm

Fig. 2.13 Virginia opossum

Fig. 2.14 Habitat of Virginia opossum (*D. virginiana*)

In this research, we study the antiproteolytic protein in opossum serum against to Malayan pit viper toxin. The information is hope to be useful in developing new anti-venom rug in the future.

2.5 Fibrinolytic protein from snake venom

2.5.1 History

Fibrinolytic protein from snake was found since 1700s (Swenson & Markland, 2005) Recently, it widely was investigated because it may be used as drug for thrombosis. The inventor of this agent was descried.

1700s Fontana observed that blood remained fluid in animals dying from viper envenomation. This effect most likely due to the combination action of fibrinogenolytic and defibrinogenation enzyme found in the venom.

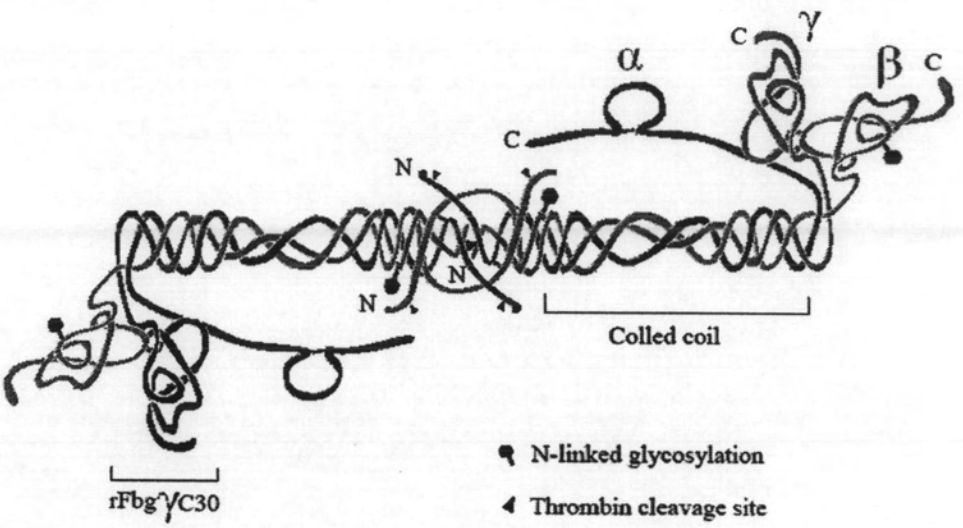
1800s Mitchell and Reichert studies and descried the effect of rattlesnake (*Crotalus* species) venom on a number of animal organs including blood. They made the observation that in animals dying soon after envenomation the blood was coagulable, but blood from animal whose death was delayed was incouagluble. They also reported that the blood looses its ability to coagulate following prolonged contact with venom .Additionally, clotted blood incubated at room temperature with venom for 24 hours showed complete clot dissolution. These findings suggested that component in the venom possessed the ability to break fibrin clot .

1937 Eagle observed fibrinolytic activity from the venom of a variety of viperid species including those from *Agkistrodon, Crotalus, Bitis* and *Vipera.*

1956 the new discoveries was reported, Didisheim and Lewis were among the first to suggest a clinical use for the observed fibrinolytic activity in snake venom. In this study, they examined *Akistrodon, Crotalus* and *Bothop* venoms. Interestingly, cobra venoms were observed to possess fibrinogenolytic activity but not fibrinolytic activity. Didisheim and Lewis suggested that purified venom proteinase should have an advantage in lysis of human clot due to a decrease susceptibility to human serum proteinase inhibitors.

2.6 Classification of fibrinolytic protein

These enzymes were classified into two groups according to the reaction with human fibrinogen protein (Fig. 2.15)



(http://www.bmsc.washington.edu/people/teller/fig1.gif)

Fig. 2.15 Human Fibrinogen, this agent composed with α, β and γ chain

2.6.1. α Fibrinogenase

α fibrinogenase is a fibrinogenolytic enzyme which mainly digest to α chain fibrinogen. Because the enzyme acts with metal it was classified to be metalloproteinase group. Fibrolase is α fibrinogenase which was isolated from *Akistrodon contortrix contortrix* which cleaved at the α chain of fibrinogen. It also cleaves at the β chain at a slower rate and no effect on the γ chain. This enzyme compose with single chain protein which containing one mol of zinc per mol of protein (Fig. 2.16) (Guan *et al.*, 1991) and cleave the α chain of fibrinogen preferentially at position Lys^{413} Leu^{414} . The metalloproteinase activity has been confirmed by the

observation that complete and rapid inhibit by the addition of EDTA, tetraethylenepentamine, or 1,10–phenanthroline (Markland, 1998).

The inhibition of enzymatic activity by agent such as zinc chelate supports the conclusion from atomic absorption spectroscopy which shows one mol of zinc per mol of enzyme that shows signature sequence of metzinzinc (Bode *et al.*, 1993; Bode *et al.*, 1996; Stocker & Bode, 1995). That confirmed, enzyme is zinc metalloproteinase and not a serine proteinase or cysteine proteinase.

Fibrinogenolysis metalloproteinases are stores in the venom gland as inactive zymogen and activated by a cysteine switch-like mechanism similar to that described for the closely related hemorrhagic metalloproteinase (Hite *et al.*, 1992).

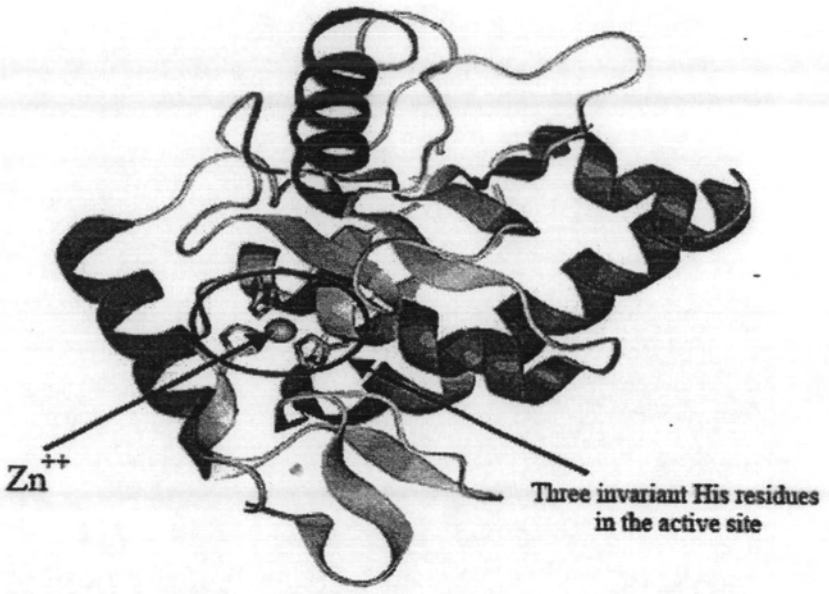


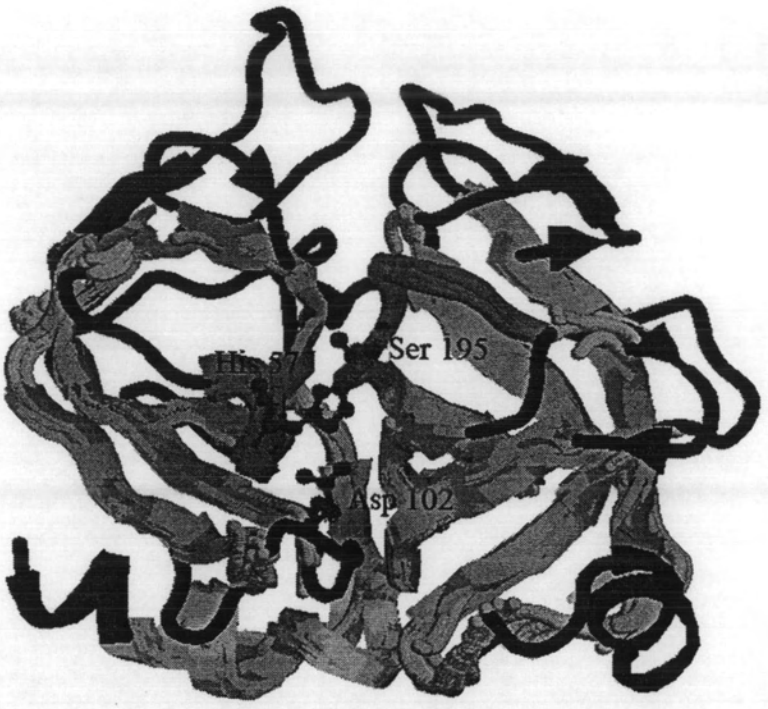
Fig. 2.16 Metalloproteinase model. Three dimension structure of fibrolase, the active site of enzyme span amino acid 139 -159 and contains a zinc atom (the position of which is indicate by the arrow), which is complexed by three histidine residue (circle). This three dimension representation is position to allow a direct view down into the active-site. In this three dimensional view, the amino terminus is on the opposite of the molecule distance from active-site and can be seen (Swenson & Markland, 2005)

2.6.2 β Fibrinogenase

β fibrinogenase is a fibrinogenolytic enzyme which mainly digest to β chain fibrinogen. The enzyme was classified into serine proteinase group because there have small change sequence and structure similar to serine proteinase (Fig. 2.17). Serine proteinase mechanism was start by the first step in the catalysis by the formation

of an acyl enzyme intermediate between the substrate and the essential serine. Formation of this covalent intermediate proceeds through a negatively charged tetrahedral transition state intermediate and then the peptide bond is cleaved. During the second step or deacylation, the acyl-enzyme intermediate is hydrolyzed by a water molecule to release the peptide and to restore the Ser-hydroxyl of the enzyme. The deacylation which also involves the formation of a tetrahedral transition state intermediate proceeds through the reverse reaction pathway of acylation. A water molecule is the attacking nucleophile instead of the Ser residue. The His residue provides a general base and accept the OH group of the reactive Ser.

The high homology sequence in venom serine proteinase, include ancrod (Hung *et al.*, 1994), barotoxin (Itoh *et al.*, 1987) and crotalid (Markland, 1976) make the enzyme not direct act on fibrin or fibrinogen. It opposite form fibrinogen with direct act on either the N- or C- terminal regions (Swenson & Markland, 2005).



(http://bioinfo3d.cs.tau.ac.il/MASS/figures/examples/serine-proteases/serine_proteinaseB.png)

Fig. 2.17 serine proteinase model. Three residues which form the catalytic triad are essential in the catalytic process His 57, Asp 102 and Ser 195.

2.7 Biochemical prosperities of fibrinolytic protein from snake venom

The information of fibrinolytic protein was reported and compared between difference sources.

Table 2.1 Representative properties of α chain and β chain fibrinogenase (Swenson & Markland, 2005)

Properties	α- chain fibrinogenase	β-chain fibrinogenase
Source	*Akistrodon contortrix contortrix*	*Vipera lebetina*
Class of enzyme	Metalloproteinase	Serine proteinase
Amino acid residue per molecule	203	232
Molecular weight	22,713 Da	~26,000 Da
PI	6.8	~3
Carbohydrate content	None	~30%
Enzymatic inhibitors	EDTA, DTT	PMSF, DFP
Inhibition by human plasma proteinase inhibitor	+	N.D.
Optimal Ph	7.1-7.4	8.5-9.5
Optimal temperature	37 °C, heat labile	Heat stable
Hemorrhagic activity	-	-
Fibrinogenolytic activity	α >β	α <<β
Fibrinolytic activity	+	-

N.D. : Not Determined

Table 2.2 Representative properties of fibrinogenase from difference source (Swenson & Markland, 2005)

Properties	α fibrinogenase	α fibrinogenase	α fibrinogenase	β fibrinogen
Source (genus species)	*Bothops neuwiedi*	*Agkistrodon halys brevicaudus*	*Lachesis stenophrys*	*Agkistrodon blomhoffi brevicadus*
Common name	Neuwiedase	Brevilysin L6	LSF	Brevinase
Amino acid residues per molecule	198	203	~200 partial sequence	233 Total AA (Heterodimer)
Molecular weight	22,524 Da	22,713 Da	24,000 Da	25,725 Da (2 chain total)
PI	5.9	4.8	N.D.	5.5 (theoretical)
Carbohydrate content	<1%	None	Glycosylated	N.D.
Enzymatic inhibitors	EDTA, 1,10-phenantroline	EDTA and o-phenanthroline	EDTA, DTT	PMSF, DFP, Pefabloc, and DTT
Inhibition by human plasma proteinase inhibitors	+	N.D.	N.D.	N.D.
Optimal pH	7.4–8.0	8.5–9.5	N.D.	5.5–8.5
Optimal temperature	37 °C, heat labile	Heat stable	N.D.	Heat stable
Hemorrhagic activity	–	–	–	N.D.
Fibrinogenolytic	Aα>Bβ	Bβ≫Aα	Aα>Bβ	Bβ>Aα
Fibrinolytic activity	+	–	+	+

N.D.: Not Determined

2.8 Recent report on fibrinolytic protein

In a recent inventory, 67 direct acting fibrinolytic enzymes were discovered which 46 are metalloproteinase and 21 is serine proteinase. The geological distribution of fibrinolytic enzyme is shown on fig. 2.18. The summary of fibrinolytic protein from various snake was reported on table 2.3.

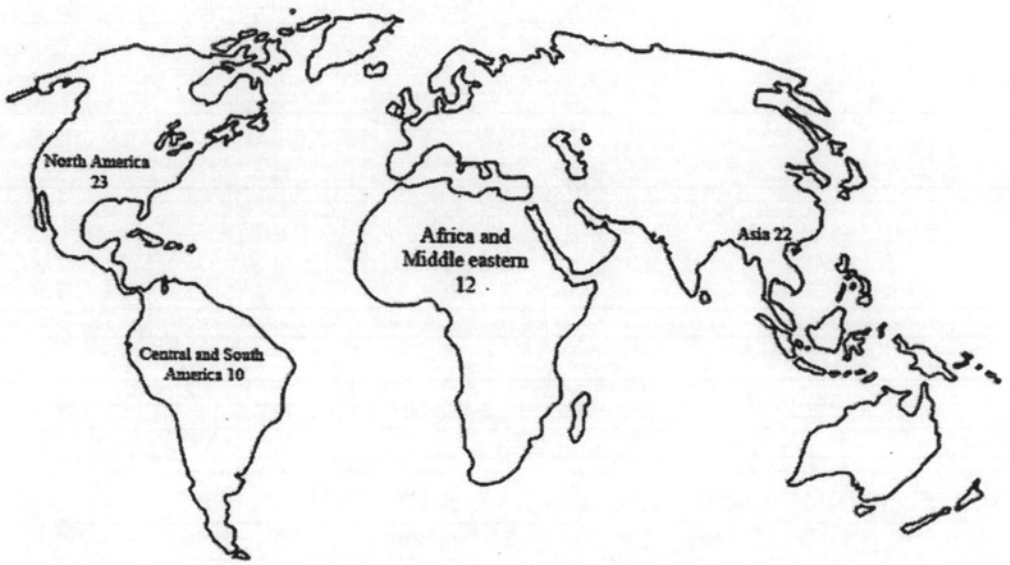


Fig. 2.18 Fibrinolytic enzyme reported by geological area. Asia 22, North America 23, Africa and Middle East 12 and Central and South Africa 10 (Swenson & Markland, 2005).

Table 2.3 Fibrinolytic enzyme from various snakes

Fibrinolytic enzyme	Snake species	Reference
Atrolase	*Crotalus atrox*	(Willis & Tu, 1988)
Fibrolase	*Agkistrodon contortrix contortrix*	(Pretzer *et al.*, 1993)
Lebetese I and II	*Vipera lebetina*	(Siigur *et al.*, 1998)
Neuwiedase	*Botrops neuwiedi*	(Rodrigues *et al.*, 2000)
M5	*Crotalus molossus molossus*	(Chen & Rael, 1997)
Piscivorase I and II	*Akistrodon piscovorus piscovorus*	(Hahn *et al.*, 1995)
F IIa	*Akistrodon acutus*	(Xiuxia *et al.*, 2001)
Basilase	*Crotalus basiliscus basiliscus*	(Datta *et al.*, 1995)
Brevinase	*Akistrodon blomhoffii brevicaudus*	(Lee *et al.*, 1999)
Leucurolysin-a (leuc-a)	*Bothrop leucurus*	(Bello *et al.*, 2006)
Brevilysin L6	*Akistrodon halys brevicaudus*	(Terada *et al.*, 1999)
LSF	*Lachesis stenophrys*	(Leonardi *et al.*, 1999)

Neuwiedase was isolated from *Botrops neuwiedi*, a metalloproteinase with active toward the α chain fibrinogen (Rodrigues *et al.*, 2000).This enzyme shares approximately the same level of homology (70%) with hemorrhagic metalloproteinase, but it does not show hemorrhagic activity. Fibrinolytic enzyme with out hemorrhagic activity has not been previously identified in the *Bothops* genus (Swenson & Markland, 2005)

Brevilysin L6 was isolated from *Akistrodon halys brevicaudus*. The enzyme is characterized as non-hemorrhagic metalloproteinase (Terada *et al.*, 1999) with molecular weigh 22,731 Da and composed of 203 residues (Bode *et al.*, 1996; Stocker *et al.*, 1993). It effectively cleaves the α chain of fibrinogen but shows little or no activity on the β and γ chain.

The *Lachesis* genera has been investigated previously for fibrinolytic enzyme, but those that have been identified are also hemorrhagic (Sanchez *et al.*, 1995; Sanchez *et al.*, 1991; Sanchez *et al.*, 2003b). Recently, LSF (*Lachesis stenophrys* fibrinogenase) was isolated from *Lachesis stenophrys* and the molecular weight is 24 kDa and act with out hemorrhagic activity (Leonardi *et al.*, 1999).

Brevinase was isolated from *Akistrodon blomhoffi brevicadus* (Lee & Park, 2000; Lee *et al.*, 1999) which compose with two chain heterodimers. It belongs to the serine proteinase family of β fibrinogenase (Lee *et al.*, 1999). The enzyme requires disulfide bonds to stabilize the heterodimer with 16.5 kDa and 17 kDa. The purified dimers displays direct fibrinolytic without fibrinogen clotting confirm that enzyme is not thrombin like enzyme. Sequence analysis of the two chains that comprise brevinase indicates significant difference between the two chains. This enzyme would represent a new class of snake venom fibrinolytic enzyme not pervious report (Lee *et al.*, 1999).

Aside from the possible release of plasminogen activator, a plasminogen activator serine proteinase has been isolated from *Trimeresurus stejnegeri* (Zhang *et al.*, 1997; Zhang *et al.*, 1995).This enzyme lacks kringle and finger domains that

provides fibrin-binding specificity to mammalian plasminogen activator, but it dose cleave the same bound in plasminogen as its mammalian counterparts.

Recent advance have been made in the use of snake venom, direct acting fibrinolytic metalloproteinase for clinical treatment of occlusive thrombi. Several of these enzymes have been tested with in vivo animal models and promising results were obtained.

2.9 Hemostatic system

2.9.1 Blood coagulation

The blood coagulation cascade and platelets collaborate to mediate thrombus formation and maintain homeostasis. This system must be functional to prevent excessive blood loss at sites of vascular injury. Linkages between the platelet and coagulation pathways are multifaceted and necessary to achieve efficient thrombus formation. Thrombin formed in the blood coagulation system, converts fibrinogen to fibrin, the major protein component of the hemostatic plug, and thrombin also is a major physiological and potent activator of platelets. Fibrinogen not only forms fibrin but also aggregates platelets by engaging its receptor, integrin $\alpha_{IIb}\beta_3$. Platelets influence thrombin generation by providing a surface for assembly of the prothrombinase complex, consisting of the zymogen, prothrombin, Factor Xa, Factor Va, and phospholipids (Byzova & Plow, 1997).The blood coagulation cascade shows on fig 2.19. Complete formation of fibrin is shown on fig. 2. 20.

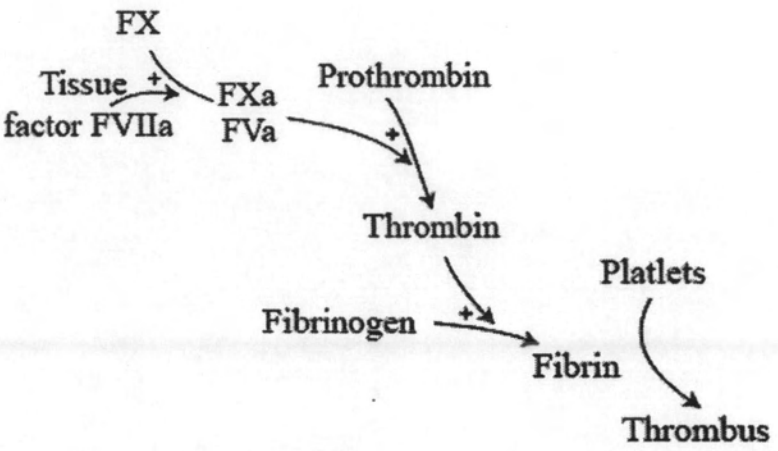
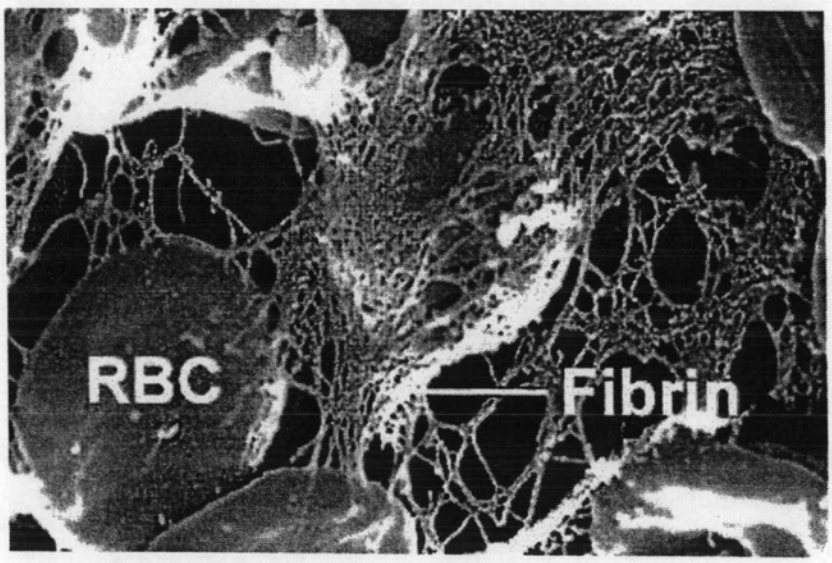


Fig. 2.19 Blood coagulation mechanism

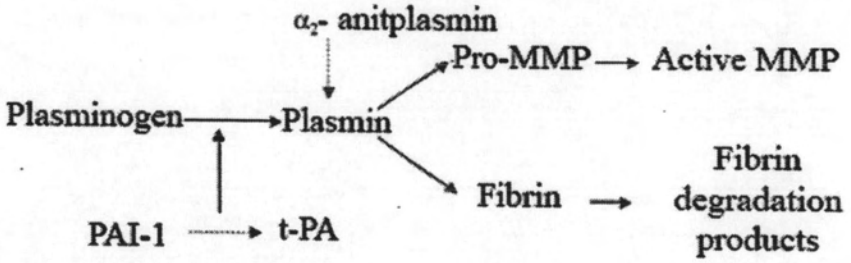


http://www.chemsoc.org/ExemplarChem/entries/2003/imperial_Bhono/bloodclot2.jpg

Fig. 2.20 Fibrin

2.9.2 Fibrinolytic

Thrombotic disorders are the major cause of morbidity and mortality in many countries sparked intense interest in the human fibrinolytic system, which normally provides a counterbalance to the blood coagulation cascade. The rate-limiting step in the fibrinolytic cascade, conversion of the circulating zymogen plasminogen into the active protease plasmin, is catalyzed by t-PA,[1] a member of the chymotrypsin family of serine proteases (Zhang *et al.*, 1999). P lasmin digests fibrin to fibrin degradation product (Fig. 2.21).



PAI-1: tissue-type plasminogen inhibitor

t-PA : plasminogen activator

MMP: matrix metalloproteinase.

Dotted arrow reflect inhibitoratory influent

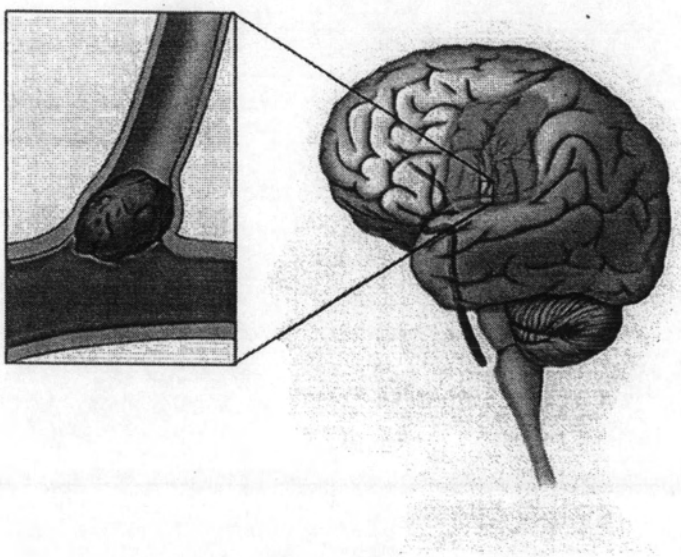
Fig. 2.21 Fibrinolytic mechanism (Marin *et al.*, 2003)

2.10 Thrombosis disease

Cerebrovascular disease, stroke and transient ischaemic attacks (TIAs) are some of the most common causes of morbidity and mortality in developed nations and are rapidly growing in importance in developing countries as they adopt the worst aspects of a western lifestyle (Gatenby, 2004). Stroke happens when blood flow to the brain stops. There have two type of stroke, ischemic and hemorrhagic stroke.

2.10.1 Ischemic stroke

The most common one is an ischemic stroke, occurs by blood clot that blocks a blood vessel in brain (Fig . 2.22).

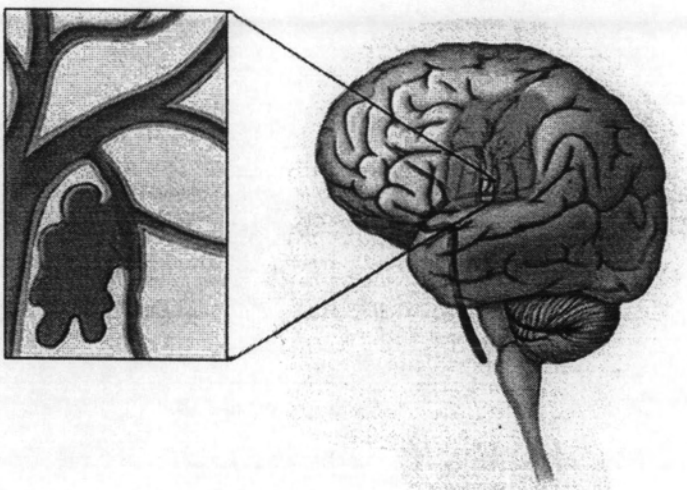


(http://www.hoinews.com/uploadedImages/whoi/News/Stories/stroke(2).jpg)

Fig. 2.22 Pathology of ischemic stroke

2.10.2 Hemorrhagic stroke

Hemorrhagic stroke, caused when blood vessel in the brain ruptures and spills blood into the surrounding tissue. Brain cells in the area begin to die, either because they stop getting the oxygen and nutrients needed for functions, or they are killed by the rupture of the vessel and sudden spill of blood (Fig. 2.23).



http://www.ahaf.org/hrtstrok/about/strokehemorrhagicBorder.jpg

Fig. 2.23 Pathology of hemorrhagic stroke

2.11 Thrombolytic agent

2.11.1 Streptokinase

Streptokinase is the first drug used as thrombolytic agent. It was invented in 1930s and used as thrombolytic drug in 1940s.This potent agent is derived from the beta hemolytic streptococci. The agent is consequently associated with the risk of anaphylaxis. Streptokinase treatment or streptococcal infections induce rapid, strong and persistent immune response. The new agent was investigated.

2.11.2 Tissue plasmin activator (TPA)

Tissue plasmin activator (TPA) is the most advances drug used to lysis clot (Cox et al., 1997; Moreno et al., 1997; White et al., 1997). TPA is indirect fibrinolytic, this agent dissolving clot with activate plasminogen. Plasminogen is converted to plasmin, which break down fibrin.

TPA was found in 1940s, but it does not success in drug industry until 1980s. The fist invention of recombinant DNA technique. Recently, TPA was produced by recombinant DNA technique, bacterial clones containing human tissue-type plasminogen activator (T-PA), cDNA sequences were identified in a cDNA library prepared using gel-fractionated mRNA from human melanoma cells (Pennica *et al.*, 1983). In 1996 the U.S. Food and Drug Administration (FDA) approved the use of TPA to treat ischemic stroke in the first three hours after the onset of symptoms.

The thrombolytic agent currently used causes excessive bleeding and reocclusion at the site of residual thrombosis (Markland et al., 1994). For that reason a better clot dissolving drug must be developed. Snake venom are new source, enzyme from snake venom that can target blood clot without casing trauma (Ramirez *et al.*, 1999; Retzios & Markland, 1994).

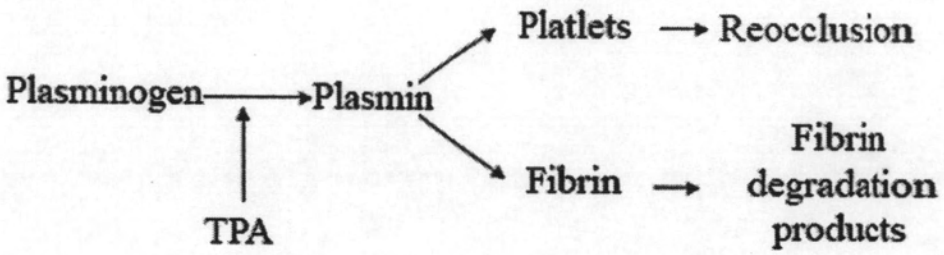


Fig. 2.24 Mechanism of Tissue plasmin activator (TPA)

2.11.3 Fibrinolytic protein from snake venom

Disintigrin, metalloproteinase and fibrinolytic enzyme are among the most important protein form snake venom that have potential application in cancer, cardiovascular and cerebrovascular disorder. In addition, venoms are abundant and stable source of enzymes and structurally similar to other enzymes found in mammals (Perez et al., 2001). Direct act fibrinogen metalloproteinase from snake venom was developed for clinical treatment of occlusive thrombi (Toombs, 2001a; Toombs, 2001b).

Fibrinolytic enzyme from snake are advance thrombosis agent. It has different mechanism from clinical used thrombolytic agent which are tissue plasminogen activator and streptokinase (Retzios & Markland, 1988). The α and β chain fibrinogenase can be defined as venom enzyme degradation preferentially either α or β chains of fibrinogen, respectively. These direct act end proteinases do not require any other factor for activity. They do not release fibrinopeptides A or B and do not induce fibrin formation.

The venom of a number of snakes of the Viperidae family contains components with anticoagulation activity. These can act either direct fibrinogenolysis or fibrinolysis or indirectly through activation of the host anticoagulant system by stimulate conversion of the plasminogen to plasmin (Burkhart *et al.*, 1992).Ancrod was isolated from Malayan pit viper, this specific proteinase instead of releasing fibrinopeptides A and B in thrombin like fashion from the α chain of fibrinogen, cleave fibrinopeptides A,

AP, and AY instead. Ancrod have sequence homology with thrombin-like venom serine proteinase.

Although, fibrinolytic enzyme from snake venom is an advance drug for thrombosis. But, many steps to purified and low yield production are the business problems. Such as, three HPLC steps were used to purify fibrolase enzyme with production yield of 15-30 mg/g from crude venom (Loayza *et al.*, 1994). The process is not reasonable for business. Recombinant DNA technique is the advance method using to develop this agent. The assay can produce more products with low cost. In the future, it can help this drug to be available on the market.

2.12 Recently report of fibrinolytic enzyme from snake venom

Alfimeprase is a recombinantly produced truncated from fibrolase has been produced and introduced into clinical trials. Alfimeprase and fibrolase contain a slight amino acid sequence difference. Recombinant alfimeprase is 201 amino acid in length encompassing residue 3-203 of native fibrolase, with only change in amino acid sequence begin the serine substitution at the position three (Toombs, 2001a; Toombs, 2001b).

Plasminogen activators are the standard therapeutic agent for clot lysis. A direct comparison of the speed of clot lysis between plasminogen activators, urokinase and alfimeprase, demonstrate that alfimeprase rapidly and effective clear occlusion thrombi. In order to evaluate the effect of alfimeprase therapy, blood shed from surgical sites

remote from the administration site was used to assess the hemorrhagic potential of alfimeprase as compared to plasminogen activators. In these studies the volume of blood lost at the site of surgical incision was determined following thrombolytic therapy. The amount of blood loss following alfimeprase treatment was not different from the negative control (saline treated) groups. The ability of α2M to rapidly bind and neutralize alfimeprase is directly related to this reduction in hemorrhagic potential.

Clinical evaluations of alfimeprase may lead to the approval of a new therapeutic for vascular occlusive condition.

2.13 The advance of fibrinolytic protein from snake venom

There are several specific advances to the venom enzymes over the plasminogen activator for clinical use. (Swenson & Markland, 2005).

(i) The venom enzymes, particular the metalloproteinase, are not inhibited by the blood serine proteinase inhibitor.

(ii) The venom enzyme do not activate plasmin, therefore secondary effect such as pletlet activator relate to plasmin formation are avoided.

(iii) The luck of pathologic alteration and the absent of demonstrable histologic change that can be significant therapeutic potential for the novel class of direct acting, venom fibrinolytic enzyme.