

Chapter 30

Thrombin-Like Enzymes in Snake Venoms

Stephen P. Mackessy

Abstract Snake venoms, particularly from vipers, are rich sources of serine proteinases, some of which contain thrombin-like activity. Following human envenomations, these toxins often produce rapid coagulopathies via the depletion of circulating fibrinogen, typically via specific proteolysis of the A α and B β subunits. Hypofibrinogenemia following bites may be prolonged, contributing to hemorrhagic effects of the venom and occasionally leading to life-threatening conditions such as disseminated intravascular coagulation. However, they have also been used as therapeutic drugs for treating a diversity of human disorders, including strokes, deep vein thromboses and cerebral and myocardial infarctions. Many snake venom thrombin-like enzymes (SV-TLEs) have been sequenced, and important structural elements (six disulfides, the catalytic triad) are highly conserved. SV-TLEs are commonly glycosylated, and this modification may confer a high level of stability; unlike trypsin, they are exceptionally stable in aqueous solution. Structurally, they are closely related to other serine proteinases such as trypsin and chymotrypsin, but as a result of gene duplication, accelerated point mutations and ASSET, venom TLEs have evolved a diversity of activities. The relationship between structure and function of the different venom serine proteinases is still unclear, and future studies of substrate specificity of this diverse family of toxins will help resolve this uncertainty.

Introduction

Thrombin is the key element of blood coagulation, and its activation is tightly regulated via a series of factor-dependent reactions of coagulation cascade. Disruption of thrombin's normal activity, via genetic defects, disease or severe injury, can result in serious health concerns, and so it is not surprising that there is an extensive literature

S.P. Mackessy (✉)
School of Biological Sciences, University of Northern Colorado, 501 20th St., CB 92, Greeley, CO
80639-0017, USA
e-mail: stephen.mackessy@unco.edu

on this critically important serine proteinase. For example, a search of the PubMed citation database (<http://www.ncbi.nlm.nih.gov/sites/entrez>) in January 2010 using the term “thrombin” returned over 38,500 hits. It is therefore well beyond the scope of this chapter to discuss the many roles of thrombin in hemostasis, but numerous recent reviews are available (e.g., di Cera, 2008), and this chapter will focus on select aspects of thrombin-like enzymes from snake venoms.

A notable result of human envenomations by many species of snakes is the rapid onset of coagulopathies, including the loss of blood coagulability. Though most pronounced following viperid bites, profound (consumptive) coagulopathies are not infrequently observed following elapid bites, particularly by Australian species (Isbister et al., 2009; White, 2005, 2009). Many of the coagulopathies induced by snake venoms result from the activities of snake venom serine proteinases (SVSPs), and among these, the thrombin-like enzymes (TLEs) are among the best characterized and have been the subject of many studies and recent reviews (e.g., Phillips et al., 2009; Serrano and Maroun, 2005; Swenson and Markland, 2005). SVSPs are broadly distributed among venomous reptiles, and like several other toxin families, the diversity of pharmacologies associated with these enzymes has resulted from gene duplication and accelerated evolution (Deshimaru et al., 1996; Kordis and Gubensek, 2000; Nakashima et al., 1993, 1995; Ohno et al., 1998), insertion of new exons (Pawlak and Kini, 2008), extension of intron-exon boundary (Tamiya et al., 1999) and exchange of exon segments (Doley et al., 2008, 2009). Although studies on the additive/synergistic effects of venom components are uncommon, it is likely that TLEs potentiate the effects *in vivo* of other components, such as the hemorrhagic metalloproteinases. Serine proteinases are most abundant among viperid venoms (e.g., Braud et al., 2000), and members of this protein family may comprise 20% or more of the venom proteome (e.g., Alape-Girón et al., 2008; Calvete et al., 2009; Sanz et al., 2006). Serine proteases which affect other components of hemostasis also have been characterized from a colubrid snake venom (Assakura et al., 1994) and from lizard venoms and saliva (Fry et al., 2006; Utaisincharoen et al., 1993), but at present, none appear to induce life-threatening coagulopathies. However, one colubrid, *Dispholidus typus* (Boomslang), does produce a potent procoagulant glycoprotein of ~55–67 kDa (Hiestand and Hiestand, 1979), but it remains incompletely characterized and may not be a serine protease.

Clotting disorders can occur at many levels in the blood clot cascade, but among venomous reptiles, targeting of fibrinogen patency is most common. Thrombin-like enzymes in venoms are serine proteinases and are typically responsible for the specific cleavage of fibrinogen A α or/and B β chains, and their actions *in vivo* lead to rapid defibrinogenation (Phillips et al., 2009; Serrano and Maroun, 2005; Swenson and Markland, 2005). Metalloproteinases, abundant in reptile venoms (Fox and Serrano, 2008, 2009), also typically hydrolyze fibrinogen *in vitro* (e.g., Mukherjee, 2008; Weldon and Mackessy, 2010), and some may also catalyze hydrolysis of the γ chain of fibrinogen and fibrin clots (Mackessy, 1993a, 1996). Many metalloproteinases can be considered to be less specific in their actions toward fibrinogen, as suggested by time course digests which result in production of numerous degradation fragments (Mackessy, 1993a) and their activities toward other structural

Table 30.1 Some properties of thrombin-like snake venom serine proteinases

Species	Name	Activity	UniProtKB accession #	Mass	Inhibitors	References
<i>Agkistrodon bilineatus</i>	Bilineobin	Fibrinogenolytic (A α and B β)	Q9PSN3	57 kDa 26,479 (deglyco-sylated)	Heparin + Dithiothreitol + TLCK + Antithrombin III + Leupeptin + Argatroban - Hirudin -	Komori et al. (1993); Nikai et al. (1995)
<i>Agkistrodon contortrix</i>	Contortrioxobin	Fibrinogenolytic (B β); Factor V activation; Factor XIII activation	P82981	26 kDa	Benzamide + DAPI + Antithrombin III -	Amiconi et al. (2000)
<i>Bothrops atrox</i>	Batroxobin	Fibrinogenolytic (A α)	P04971	41.5 kDa	Benzamide + α 2-macroglobulin + Antithrombin III - Heparin - Hirudin - Aprotinin - SBTI - ϵ -ACA - Tranexamic acid - Iodoacetamide -	Itoh et al. (1987); Stocker and Barlow (1976); Stocker et al. (1982); Sturzebecher et al. (1986)
<i>Bothrops atrox</i>	Thrombocytin	Fibrinogenolytic (A α); Factor VIII activation	none	36 kDa	Pro-Phe-ArgCH ₂ Cl + PRCK + SBTI + Antithrombin III + Heparin + FPRCK + FARCK +	Castro et al. (2004); Kirby et al. (1979); Serrano and Maroun (2005)

Table 30.1 (continued)

Species	Name	Activity	UniProtKB accession #	Mass	Inhibitors	References
<i>Bothrops jararaca</i>	KN-BJ	Fibrinogenolytic (A α); Kinin release	O13069	38 kDa	Benzamine derivatives +	Serrano et al. (1998)
<i>Bothrops jararaca</i>	Bothrobin	Fibrinogenolytic (A α); Platelet aggregation; Factor VIII activation	P81661	35 kDa	Platelet Aggregation: Anti-GP IIb/IIIa + Anti-GP Ib +	Nishida et al. (1994)
<i>Bothrops leucurus</i>	Leucurobin	Fibrinogenolytic (A α); Gyratory	none	35 kDa	Benzamine + β -mercaptoethanol + SBTI - EDTA -	Magalhães et al. (2007)
<i>Calloselasma (Agkistrodon) rhodostoma</i>	Ancrod	Fibrinogenolytic (A α)	P47797	35.4 kDa	NPGb + Agmatine + α 2-macroglobulin + Antithrombin III +	Au et al. (1993); Burkhart et al. (1992); Castro et al. (2004); Nolan et al. (1976)
<i>Cerastes vipera</i>	Cerastobin	Fibrinogenolytic (A α and B β); Platelet aggregation	P18692	38 kDa	Iodoacetamide + Trasylol - SBTI -	Farid et al. (1989, 1990)
<i>Cerastes cerastes</i>	Cerastocytin	Fibrinogenolytic (A α); Platelet aggregation; Factor X activation	Q7SYF1	38 kDa	SBTI + TLCK + TPCK + Antithrombin III - Hirudin -	Dekhil et al. (2003a); Marrakchi et al. (1995)
<i>Cerastes cerastes</i>	Cerastotin	Fibrinogenolytic (A α); Platelet aggregation	P81038	40 kDa	TPCK + TPLK + SBTI + Hirudin - Antithrombin III -	Marrakchi et al. (1997)

Table 30.1 (continued)

Species	Name	Activity	UniProtKB accession #	Mass	Inhibitors	References
<i>Crotalus adamanteus</i>	Crotalase	Fibrinogenolytic (A α); Kinin release	Q9P555	32.7 kDa	TLCK + Pro-Phe-ArgCH ₂ Cl + PFRCK + AFRCK + GVRCK + IPRCK + AFCKK + Tetranitromethane + 2-mercaptoethanol + Hirudin - TPCK -	Markland (1976, 1998); Markland et al. (1982); Henschen-Edman et al. (1999)
<i>Crotalus durissus terrificus</i>	Gyroxin-like B2.1	Fibrinogenolytic (A α); Gyrotory	Q58G94	32 kDa (26.6 no glyco)	Dithiothreitol +	Alexander et al. (1988)
<i>Deinagkistrodon acutus</i>	Acutin	Coagulant	Q9YGS1	38 kDa	x	Pan et al. (1999)
<i>Deinagkistrodon acutus</i>	Venom serine proteinase Dav-PA	Fibrinogenolytic; amidolytic	Q9I8X1	28,032	x	Zhu et al. (2005)
<i>Deinagkistrodon acutus</i>	Acutobin	Fibrinogenolytic (A α)	Q9I8X2	40 kDa (28.8 no glyco)	x	Wang et al. (2001)
<i>Gloydus (Agkistrodon) blomhoffii brevicaudus</i>	Brevinase	Fibrinogenolytic (A α and B β)	Q9PT51	2 Chains: 16.5 and 17 kDa	Pefabloc + Dithiothreitol +	Lee et al. (1999, 2000)

Table 30.1 (continued)

Species	Name	Activity	UniProtKB accession #	Mass	Inhibitors	References
<i>Gloydus</i> (<i>Agkistrodon</i>) <i>halys blomhoffi</i>	Halytase	Fibrinogenolytic (B β); bradykinin release	P81176	38 kDa	Leupeptin + Hirudin -	Matsui et al. (1998)
<i>Gloydus</i> <i>ussuriensis</i> (formerly <i>Agkistrodon</i> <i>caliginosus</i>)	CPI-enzyme-2	Fibrinogenolytic (B β)	O42207	44 kDa	x	Hahn et al. (1998); Shimokawa and Takahashi (1993a, b; 1997)
<i>Gloydus</i> <i>ussuriensis</i> (formerly <i>Agkistrodon</i> <i>caliginosus</i>) <i>Lachesis muta</i>	Calobin	Fibrinogenolytic (A α); coagulant	Q91053	34 kDa	Aprotinin + Hirudin +	Hahn et al. (1996)
	LM-TL	Fibrinogenolytic (A α); Gyratory	P33589	41–47 kDa	Agmatine + <i>p</i> -aminobenzamidine + BPTI - Ecotin - Hirugen - Bothrojaracin - Bothroaltermis - <i>p</i> -APMSF +	Castro et al. (2001); Magalhaes et al. (1993); Silveira et al. (1989)
<i>Trimeresurus</i> <i>elegans</i>	Elegaxobin II	Fibrinogenolytic (A α); Kinin release	P84787	35 kDa	x	Oyama and Takahashi (2003)
<i>Trimeresurus</i> <i>flavoviridis</i>	Flavoxobin	Fibrinogenolytic (A α)	P05620	23.5 kDa	x	Shieh et al. (1985, 1988); Yamamoto et al. (2002)

Table 30.1 (continued)

Species	Name	Activity	UniProtKB accession #	Mass	Inhibitors	References
<i>Trimeresurus mucrosquamatus</i>	Mucrosobin	Fibrinogenolytic (B β)	U31417	28 kDa	x	Guo et al. (2001)
<i>Viridovipera (formerly Agkistrodon stejnegeri)</i>	Stejnefibrase-1	Fibrinogenolytic (A α)	Q8AY80	28.3 kDa	NPGb +, PMSF +	Gao et al. (1998)
<i>Viridovipera (formerly Agkistrodon stejnegeri)</i>	Stejnobin	Fibrinogenolytic (A α)	Q8AY81	29.3 kDa	DFP +, PMSF +	Zhang et al. (1998)

+, Inhibition; -, No inhibition. All tested enzymes were also inhibited by DFP and PMSF. AFKCK, Ala-Phe-Lys chloromethyl ketone; AFRCK, Ala-Phe-Arg chloromethyl ketone; BPTI, bovine pancreatic trypsin inhibitor; DAPI, 4',6-diamidino-2-phenylindole; DFP, diisopropyl fluorophosphate; ϵ -ACA, epsilon-aminocaproic acid; FAKCK, Phe-Ala-Lys chloromethyl ketone; FARCK, Phe-Ala-Arg chloromethyl ketone; FPRCK, Phe-Pro-Arg chloromethyl ketone; GVRCK, Gly-Val-Arg chloromethyl ketone; glyco, carbohydrate moiety; IPRCK, Ile-Pro-Arg chloromethyl ketone; NPGb, *p*-nitro-phenyl-*p*-guanidino benzoate HCl; PFRCK, Pro-Phe-Arg chloromethyl ketone; p-APMSF, *p*-amidinophenylmethanesulfonyl fluoride; PMSF, phenylmethylsulfonyl fluoride; PRCK, Pro-Arg chloromethyl ketone; SBTI, Soybean trypsin inhibitor; TLCK *p*-Tosyl-L-lysine chloromethyl ketone hydrochloride; TPCK, *p*-Tosyl-L-Phenylalanine chloromethyl ketone hydrochloride; x, inhibitor not reported. Table largely from Serrano and Maroun (2005) and Phillips et al. (2009)

proteins (Escalante et al., 2006)), and their pro- or anticoagulant activities will not be considered further here. However, it is likely that functionally important interactions occur among these venom components, and hypofibrinogenemia (TLE-catalyzed) accompanied by structural degradation catalyzed by metalloproteinases may produce uncontrollable hemorrhage. For the snake, these actions are important for prey incapacitation and facilitation of digestion; in human envenomations by vipers, these proteinases produce some of the more debilitating and difficult to manage effects (Gutiérrez et al., 2009).

Defining which of the myriad serine proteases often found in a single venom is a TLE-SVSP can be challenging, as many have similar activities toward model substrates such as paranitroaniline-derived peptides as well as toward native protein substrates such as fibrinogen. Further, the term “thrombin-like” is also problematic, because unlike most TLEs, thrombin is a multifunctional enzyme with rather different roles depending on physiological environment (Kini, 2005; Phillips et al., 2009). Additionally, most TLEs have not been assayed with a wide series of substrates (some are defined by sequence homology only), so the true specificity of activity, or lack thereof, is not well defined. Some of the SVSPs labeled as thrombin-like enzymes in the databases are incorrectly assigned to this activity, and some (particularly sequences derived from cDNA libraries) are labeled as TLEs because of sequence homologies but without any activity data. A more limiting definition of venom TLEs is needed, but for the present review, SVSPs which have specific catalytic activity toward fibrinogen A α or/and B β chains will be considered as TLEs. A list of many well-defined venom TLEs is provided in Table 30.1, along with major activities and biochemical attributes.

Structural Features of Snake Venom TLEs

Primary and Secondary Structure

Snake venom thrombin-like enzymes are serine endopeptidases which are members of the trypsin-like serine protease superfamily, the S1 family of peptidases, clan PA and subclan S (Marchler-Bauer et al., 2009; Rawlings et al., 2004a, b). Well over 100 sequences of SVSPs are available in databases such as NCBI (<http://www.ncbi.nlm.nih.gov/>) and UniProtKB (<http://www.uniprot.org/>), with 77 entered as thrombin-like enzymes in UniProt (December 2009). These enzymes have a high level of sequence identity (>60%; Kini, 2005) and are structurally constrained by the presence of six highly conserved disulfide bridges (Fig. 30.1), five of which are common to all S1 serine proteinases. The catalytic triad typical of the chymotrypsin/trypsin superfamily (His57, Asp102, Ser195; chymotrypsinogen numbering) is also highly conserved, as are numerous flanking residues, and SVSPs are typically classified as serine proteinases by the common sensitivity to the serine active site inhibitors PMSF and DFP (Serrano and Maroun, 2005).

Fig. 30.1 Sequences of snake venom thrombin-like enzymes, TLEs and several additional SVSPs which show greater than 50% sequence identity with batroxobin are shown. Sequences are listed by UniProt Accession numbers, and names and species identities are provided in Appendix. *Shaded residues:* H, D and S – conserved histidine 57, aspartic acid 102 and serine 195 residues of the catalytic triad; residues 35–41, 59–66, 92–99 – segments i, ii and iii undergoing ASSET and likely important surface residues involved in substrate recognition/binding (Doley et al., 2009); C – represent highly conserved cysteine disulfide pairs (residues 22–157, 42–58, 91–245, 136–201, 168–182, 191–220). Numbering above sequences is based on chymotrypsin

Table with 3 columns: ID (e.g., P84788, Q9180), Name (e.g., --VIGDEENINHERPELVIAV), and Description (e.g., --YDDYGGOTLINNEWVLAAR). The table lists numerous entries, many of which are highlighted in red in the original image.

Fig. 30.1 (continued)

Biological solutions to structural needs are often frugal, and biological designs are typically conservative; this truism is notably observed among the SVSPs, which, in spite of a highly conserved primary structure and molecular fold, have evolved into a diversity of different pharmacologies (Serrano and Maroun, 2005), most of which cause dysregulation of the highly ordered coagulation cascade of vertebrates. Thrombin-like SVSPs typically target fibrinogen, but even within this target, TLEs from different sources may catalyze preferential hydrolysis of the $\text{A}\alpha$ and/or $\text{B}\beta$ chains. SVSPs are typically very stable molecules, in large part due to the six disulfides found in most. Purified thrombin-like and kallikrein-like SPs from *Crotalus* and *Sistrurus* venoms are unaffected by the relatively harsh conditions of RP-HPLC, and they retain most activity even after long storage (>1 year) in solution at 4°C (Mackessy, unpublished observations). As noted below, part of this exceptional stability may be due to the carbohydrate moiety typical of most SVSPs.

Glycosylation

Venom serine proteinases are commonly glycosylated, and the carbohydrate moiety is commonly Asn-linked (Tanaka et al., 1992). The extent of *N*- or *O*-glycosylation appears to be significant but quite variable, and the functional significance of this variation is incompletely understood (Serrano and Maroun, 2005). Carbohydrate content may account for 5–30% of the total mass, and in a few cases the mass of the carbohydrate moiety (62%) may exceed that of the protein component (Paes Leme et al., 2008). However, the presence of these carbohydrate moieties can have important functional consequences. Glycosylation appears to confer greater thermal stability to BJ-48, a TLE isolated from *B. jararacussu* venom, and deglycosylation also greatly increases susceptibility to trypsin inhibitors such as STI (Silva-Junior et al., 2007). Glycosylation also conferred to *Bothrops* protease A (*B. jararaca* venom) thermal protection and protection against protein inhibitors (soybean trypsin inhibitor {STI}, bovine pancreatic trypsin inhibitor {BPTI}, antithrombin III) but did not protect against inhibition by benzamidine, further indicating a steric protective effect against the larger molecules (Botos and Wlodawer, 2007; Paes Leme et al., 2008). The smaller inhibitor molecule appears to enter/bypass the “protective cage” of the carbohydrate moiety essentially unimpeded. Interestingly, a similar protective mechanism has been demonstrated for glycosylation of the nicotinic acetylcholine receptor of *Naja haje* snake muscle: the small neurotransmitter (acetylcholine) does not experience steric hindrance by the carbohydrate “cage”, but the much larger α -neurotoxins are excluded from nAChR α -subunit binding sites, conferring resistance to the snake (Takacs et al., 2001). When glycosylation is eliminated, the receptor showed sensitivity to the neurotoxin similar to that of mammalian preparations. Partial deglycosylation of BPA did not increase susceptibility of the enzyme to protein inhibitors, nor did it significantly increase inhibition by benzamidine, but activity toward both fibrinogen and D-Val-Leu-Arg-pNA was enhanced (Paes Leme et al., 2008). These results indicate a type of

functional trade-off between optimal enzyme activity and in vivo stability, suggesting that the protective effect of glycosylation is not without some cost to enzyme efficacy.

Two SVSPs with apparent thrombin-like activity were isolated from *Deinagkistrodon acutus* venom and shown to be *N*-glycosylated at Asn35, as observed from electron density maps of this region of the crystal structures (Zhu et al., 2005). Because this glycosylation site occurs close to the active site, it was interpreted to restrict access of larger molecules like STI and BPTI. Structural analyses using superimposition of the venom SVSPs and trypsin-STI complex demonstrated collision between side-chain residues of STI and the carbohydrate moieties of the SVSPs. Again, it appears that glycosylation of these serine proteases creates steric hindrance of inhibitor binding, thereby protecting the enzyme. Most TLEs also are glycosylated, and it is likely that glycosylation has been selected for as a protective mechanism against endogenous serine protease inhibitors of snake prey species. The effect of glycosylation may therefore be to increase effective half-life in prey tissues and to increase probability of fibrinogen depletion. In human envenomations by rattlesnakes (*Crotalus*), recurrent coagulopathies are commonly encountered following antivenom treatment (Boyer et al., 1999), and it would be of interest to determine if glycosylated TLEs are involved in these persistent and recurrent cases of hypofibrinogenemia and thrombocytopenia.

Crystal Structures and Structural Predictions

At present, no crystal structures of snake venom TLEs have been solved. The structure of a plasminogen activator from *Trimeresurus stejnegeri* venom was resolved at 2.5 Å (Parry et al., 1998), and this protein has a high degree of sequence identity with snake venom TLEs. Several glycosylated serine proteases from *Deinagkistrodon acutus* venom have also been crystallized and their structures solved at 2.1 Å (Zhu et al., 2005); because they have fibrinogenolytic activity, they were considered to be TLEs (Philipps et al., 2009), but activity toward pNA-derived peptide substrates showed that they lacked the specificity often observed for other TLEs, for example from *Crotalus* venoms (e.g., Mackessy, 1993b). A preliminary crystallographic analysis of RVV-V from *Daboia russelli* venom was just published (Nakayama et al., 2009), and the structure should be available soon. Although this enzyme has been considered a TLE, it appears to be a specific activator of Factor V only, although it also showed amidase activity toward the TLE substrate Phe-pipecolyl-Arg-pNA. A theoretical model of this protein has been constructed (Segers et al., 2006), based on the several related SVSP structures which are currently available.

Because of conserved primary structure and disulfide pattern of SVSPs generally, it is probable that venom TLEs have a structure similar to that of other S1

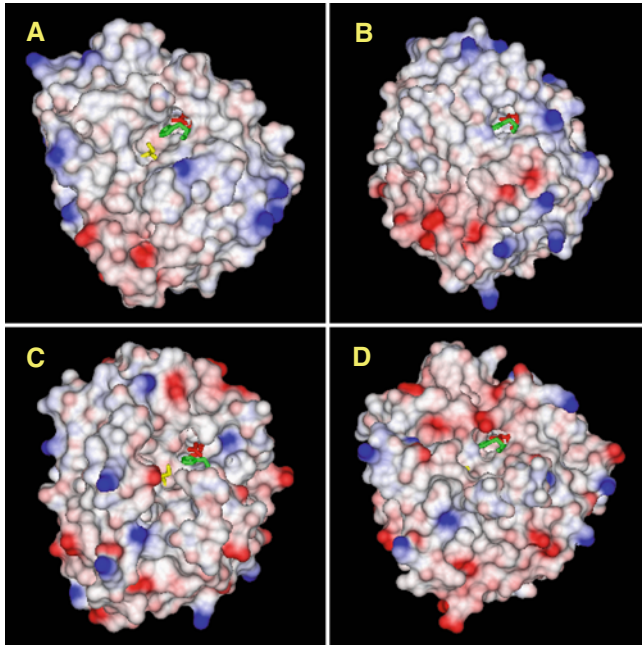


Fig. 30.2 Space-filling models of serine proteases. (a) Bovine trypsin (PDB ID: 1AQ7); (b) Bovine chymotrypsin B (PDB ID: 1DLK); (c) Venom serine proteinase Dav-PA (*Deinagkistrodon acutus* venom; PDB ID: 1OP0). (d) Venom plasminogen activator TSV-PA (*Viridovipera (Trimeresurus) stejnegeri* venom (PDB ID: 1BQY). Models were drawn with Discovery Studio ViewerPro showing van der Waals radii and partial charges (red, negative; blue, positive). Active site residues His57 (green), Asp102 (red) and Ser195 (yellow) are shown in stick models. In B and D, Ser195 is barely visible. Note that although overall topology is similar, surface charge density and apparent accessibility to the active site vary between the molecules

serine proteinases, as molecular modeling of LM-TL (a TLE from *Lachesis muta* venom) has indicated (Castro et al., 2001). The overall similarity in molecular fold and active site orientation can be seen from comparisons of structural models of bovine chymotrypsin (MacSweeney et al., 2000) and trypsin and the SVSPs Dav-PA and TSV-PA (Fig. 30.2). Differences in surface charge densities are likely important for conferring observed differences in substrate specificity.

Gene Structures

At present, the gene structure for batroxobin (from *Bothrops moojeni* venom) remains the only published genomic representative of a snake venom TLE (Itoh et al., 1988). The gene is approximately 8 kbp, and the generally shorter exons (exon 1 = 240 bp; exon 2 = 151 bp; exon 3 = 260 bp; exon 4 = 134 bp; exon 5 = 728 bp) are interrupted by introns varying in length from 2.5 kbp (intron 1) to 0.35 kbp

(intron 4). In this respect, batroxobin is similar to trypsin, which also has 5 exons and 4 introns (Craik et al., 1984); both differ in organization from chymotrypsin, which has 7 exons and 6 introns (Bell et al., 1984).

Relations to the Charge Relay Catalytic System of Model Serine Proteases: Chymotrypsin/Trypsin

Thrombin-like serine proteases from snake venoms possess the highly conserved functional residues of the catalytic triad (Ser195, His57, Asp102) characteristic of the well-known serine proteases (e.g., Polgár, 1971) such as chymotrypsin (EC 3.4.21.1). They are members of the S1 serine proteinase family, PA clan of endopeptidases and they share the same basic fold in the catalytic domain with other members of the family, such as trypsin, chymotrypsin and thrombin. Peptide bond hydrolysis occurs via a two step acylation–deacylation reaction, with serine acting as a nucleophile and histidine as a proton donor/acceptor within the catalytic cleft; a hydrogen on the histidine imidazole ring is transferred to the aspartate carboxylate, forming a charge relay system. The mechanism of catalysis has been known for a long time (Hartley and Kilby, 1954; Polgár, 1971; Polgár and Bender, 1969) and has become a classic example in biochemistry courses of enzyme-catalyzed hydrolysis. The importance of the serine residue to snake venom TLEs, as well as unequivocal demonstration of a SVSP, is now routinely confirmed via specific active site inhibitors such as DFP, PMSF and AEBSF, which irreversibly and covalently modify serine in the catalytic cleft.

Distribution and Evolution of Snake Venom TLEs

Occurrence and Relative Abundance in Snake Venoms

Thrombin-like enzymes are found most frequently and as major components in the venoms of viperid snakes (Mackessy, 2009). Uncommonly, TLEs are present in elapid venoms (e.g., Jin et al., 2007), but they appear not to be broadly distributed among species of this family. Although there are reports of serine proteases in venoms from “colubrid” rear-fanged snakes (Assakura et al., 1994; Mackessy, 2002) and several lizards (Fry et al., 2006), TLEs do not appear to occur in venoms and salivas from these species. Serine proteases make up ~2.6% of the venom proteome of *Philodryas olfersii* (Ching et al., 2006), a colubrid snake capable of producing serious human envenomations, but this venom also does not appear to contain TLEs. Venom from *Alsophis portoricensis* (Puerto Rican Racer, family Dipsadidae) was recently shown to contain low activity toward the thrombin substrate Bz-PheValArgpNA (Weldon and Mackessy, 2010), but the levels are an order of magnitude lower than seen in most rattlesnake venoms.

Mechanisms of Evolution of SV-TLEs

Gene Duplication

A common means by which diversity is generated in a specific protein family is via gene duplication. In a critically important component of a complex system, such as thrombin in the blood clot cascade, mutation of specific residues which change activity levels or specificity of substrate recognition could be lethal mutations. However, when the gene is duplicated and one member remains static, the functional product of the original gene remains intact. The other gene copy is freed from selective constraints favoring conservation of original structure/function, and mutations can then lead to production of a novel activity. Repeated gene duplication can result in multiple copies of closely related genes being present, and over evolutionary time, a diverse set of pharmacologies within a structurally conserved protein family may result (Ogawa et al., 1996; Nobuhisa et al., 1996; Kini and Chan, 1999). This appears to be of common occurrence among snake venoms, and venom gene duplication has resulted in a multigene family of SVSPs (Deshimaru et al., 1996), giving rise to venoms with numerous serine proteinases. For example, in the venom of the Desert Massasauga (*Sistrurus catenatus edwardsii*), 24% of the proteome (Sanz et al., 2006) and >37% of the transcriptome consists of serine proteinases (Pahari et al., 2007). The transcriptome analysis showed 12 distinct isoforms of SVSPs, and fractionation of the venom has revealed at least 8 distinct serine proteinases are translated (Mackessy, unpublished observations). At least three of these SVSPs show thrombin-like activity, as assessed by preferential hydrolysis of the substrate Bz-Phe-Val-Arg-pNA over other Arg-terminal substrates (Mackessy, 1993b).

Accelerated Point Mutation

Single nucleotide replacement within an exon can have ramifications on function ranging from neutral to lethal mutation. One of the best documented single point mutations resulting in extreme alteration of function involves the sickle mutant of human hemoglobin which has resulted from the selective pressures of malarial parasites on humans (Huisman, 1993). Among snake venom toxins, point mutations have also occurred commonly and contribute to the primary structure diversity seen among homologous toxins from different species. Many of these point mutations appear to be functionally inconsequential; for example, small variations in sequence of α -neurotoxins from closely related species still results in a three-finger toxin with very high affinity for nicotinic acetylcholine receptors of skeletal muscle (Nirthanan and Gwee, 2004). However, among many toxin families, including SVSPs (Deshimaru et al., 1996), point mutations within protein-coding regions have occurred at a rate greatly accelerated relative to other proteins and even to the highly conserved UTRs within the same protein. Analysis of substitution rates between untranslated and translated regions demonstrated that nucleotide substitutions occurred much more frequently in protein-coding regions, and this unusually

high rate of substitution has contributed in part to the diversification of functionality. The ratios of non-synonymous to synonymous substitutions within coding regions were generally greater than 1 (0.67–1.64), whereas these ratios in typical isozyme genes were typically less than 0.2 (Deshimaru et al., 1996), again highlighting the rapid evolution of these toxin genes relative to non-toxins. This accelerated evolution within the SVSP multiple gene family is likely driven by selective pressures favoring multiple toxin activities against snake prey and predators.

ASSET

Recently we have shown that in addition to accelerated point mutations, many snake venom toxins also evolve via accelerated segment switch in exons to alter targeting (ASSET: Doley et al., 2009). The occurrence of ASSET is particularly interesting, as it seems to occur primarily in specific surface segments, while accelerated

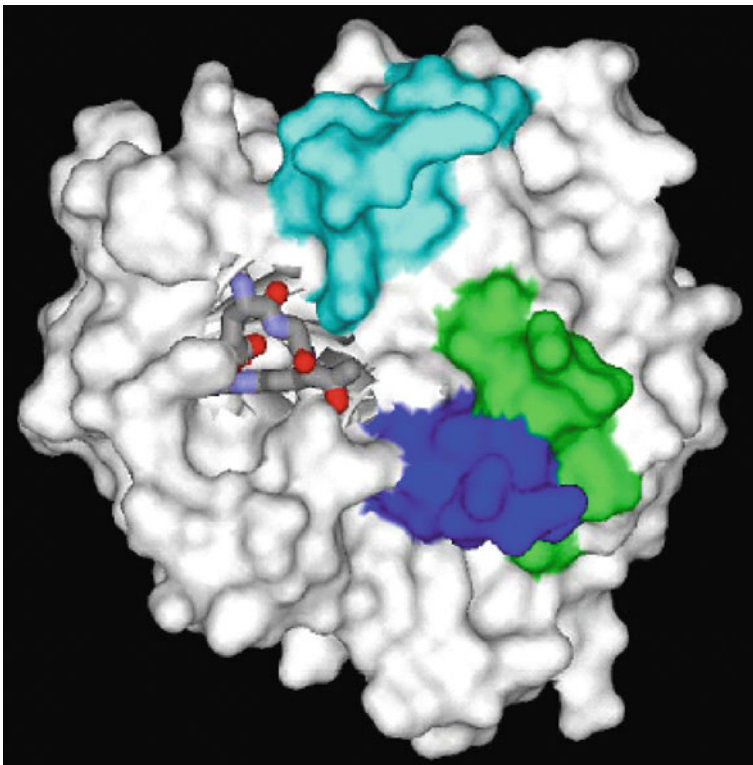


Fig. 30.3 Surface model of venom plasminogen activator (TSV-PA) from *Viridovipera (Trimeresurus) stejnegeri* venom (PDB ID: 1BQY). The segments that undergo exchange are shown in green, dark blue and turquoise color; note that the substrate binding area is the region shown in turquoise. The side chains of the active site residues are shown as ball and stick. From Doley et al. (2009)

point mutations occur in the rest of the molecule; among SVSPs, this includes those regions associated with substrate binding. ASSET therefore could produce rapid functional differentiation of gene products which share a highly conserved molecular fold and apparent surface topology (see Fig. 30.2). In viperid venoms in particular, numerous SVSP cDNAs have been sequenced, including many from *Viridovipera stejnegeri* (Tsai and Wang, 2001), *Deinagkistrodon acutus* (Zhang et al., 2006) and *Sistrurus catenatus edwardsii* (Pahari et al., 2007), demonstrating the high level of multiplicity of SVSPs in the venom and gland transcriptome of even a single individual. At least part of this diversification has occurred via ASSET.

ASSET has been hypothesized to be a mechanism of accelerated evolution of venom toxins which can confer new pharmacological functionalities on a conserved molecular fold, as is common among venom proteins. By switching functionally important segments of gene (protein) sequence, such as that important to substrate binding, rapid large scale changes in substrate specificity can occur. Such a mechanism appears to be important in the evolution of SVSPs (Doley et al., 2009), as the regions of exchange include those known to involve substrate binding (Fig. 30.3). ASSET can result in large-scale functional changes, with accelerated point mutations “fine-tuning” substrate fit. This hypothetical scenario may explain the variety of substrate specificities (thrombin-like, kallikrein-like, plasmin-like, arginine esterase, etc) seen among the SVSPs.

Structural and Phylogenetic Relationships

As mentioned above, SVSPs including TLEs show a high level of sequence identity, and the genes are obviously closely related. One might predict that functional classes of the SVSPs (thrombin-like, kallikrein-like, plasmin-like, etc) should cluster following structural cladistic analyses, and this prediction has been borne out by some studies (i.e., Wang et al., 2001). In this study, three functional subtypes clustered into discrete groups (thrombin-like {coagulating}, kallikrein-like {kininogenase} and plasminogen activators). However, a different analysis (Lee and Park, 2000) resulted in the clustering of functionally different SVSPs. A more recent analysis of sequence relationships among TLEs indicated a common ancestry among the SVSPs analyzed but did not demonstrate unequivocal clustering of functional subtypes (Castro et al., 2004). Because of these discrepancies, a phylogenetic analysis of TLEs and other SVSPs was undertaken using ClustalX and bootstrapped neighbor-joining method. One hundred and fifty-one snake venom serine protease sequences were retrieved from the UniProtKB database (<http://www.uniprot.org/>; January 2010) using the primary sequence of batroxobin in a BLAST search and the criterion of having >50% sequence identity with this target. Five SP sequences from lizards (sequence identity ~40%) were also included. Bovine and human trypsin sequences were used as outgroups, and bovine chymotrypsinogen and human thrombin were included in alignments and subsequent analyses (160 sequences

total). Sequences were aligned using ClustalX 1.81(Thompson et al., 1994) and sequence similarities were evaluated using the bootstrapped neighbor-joining algorithm (1,000 bootstrap trials). The resulting NJ tree was drawn using FigTree 1.2.1 (available at <http://tree.bio.ed.ac.uk/software/figtree/>) and nodes were coded as to

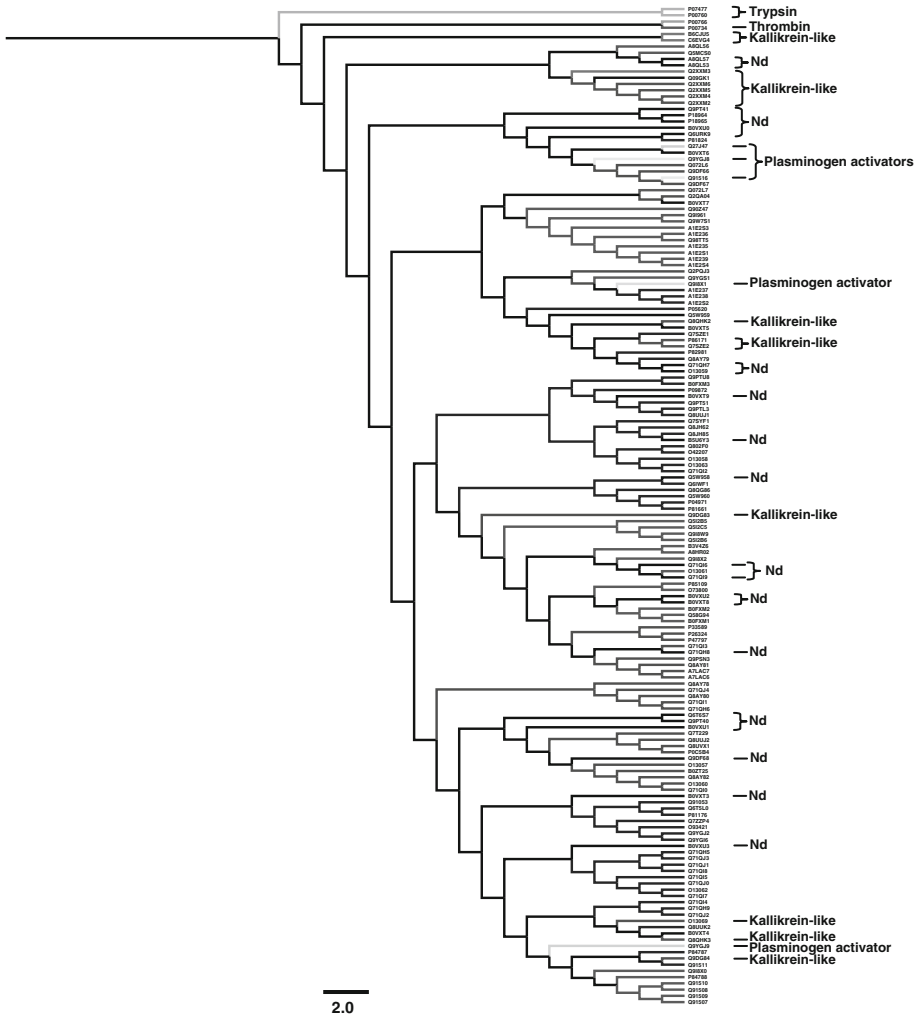


Fig. 30.4 Bootstrapped neighbor-joining tree of relationships among TLE and SVSP. The sequences are shown in Fig. 30.1. Although there is a trend toward clustering of similar activities (such as kallikrein-like activities), kallikrein-like and plasminogen-activating activities are distributed throughout many clades dominated by TLEs, suggesting that similar primary sequence motifs alone are not responsible for differences between activities. Kallikrein and plasminogen activating SVSPs are labeled; unlabeled nodes are all thrombin-like enzymes. Nd, activity not determined

functionality based on database-reported activity. The majority of sequences were identified as thrombin-like enzymes in the UniProt database, with a smaller number of kallikrein-like (14) and plasminogen activator (5) sequences identified; 28 were of unknown/undefined activity. Although there is a tendency for similar function sequences to cluster (Fig. 30.4), there are instances of all three activities occurring within many clades, suggesting that functionality is not dependent on sequence features alone. Effects of phylogenetic constraint (closer evolutionary relatedness of species) were not specifically analyzed but seem unlikely to play a major role. A more likely limitation of this analysis is that the absolute function of many SVSPs is equivocal or unknown. As others have noted, the relationship between sequence homology and biological activity remains paradoxical (Serrano and Maroun, 2005).

Biomedical Applications of SV-TLEs

Therapeutic Use and Drug Discovery

Snake venoms consist of a myriad of potent biological activities which have been recruited from various tissues and which mimic many natural regulatory components (Fry, 2005; Stocker and Meier, 1989). Because venom protein components profoundly affect homeostasis at numerous levels, there is a long-standing interest in venoms as a source of drug discovery and development (for a review, see Fox and Serrano, 2007). One of the earliest drugs related to hemostasis developed from a venom and approved for human use is Captopril, an inhibitor of angiotensin-converting enzyme which was based on bradykinin-potentiating peptides isolated from *Bothrops jararaca* venom (Ferreira, 1965; Smith and Vane, 2003). Several SVSPs have also been developed as actual or potential drugs for human use, and these have been discussed in several more recent reviews (Fox and Serrano, 2007; Marsh and Williams, 2005; Phillips et al., 2009; Serrano and Maroun, 2005; Swenson and Markland, 2005); only those with defined thrombin-like properties will be included here.

One of the most promising SV-TLEs for clinical use was Ancrod (Viprinex), a serine proteinase originally isolated from the venom of *Calloselasma* (formerly *Agkistrodon*) *rhodostoma* (Au et al., 1993). Its utility in treating stroke victims was evaluated some years ago (Sherman, 2002), and initial indications for acute ischemic stroke were very promising. However, in late 2008, Ancrod failed phase 3 clinical trials for acute ischemic stroke, and it is no longer being developed as a drug for human use in strokes (Neurobiological Technologies, <http://www.ntii.com>). In a recently published study involving 500 patients at several different facilities who received Ancrod within 6 h of symptom onset, Ancrod treatment of acute ischemic stroke victims was halted due to lack of efficacy (Levy et al., 2009). No difference between placebo and Ancrod treatment was seen in positive response of

patients or in ninety day mortality levels, and the incidence of symptomatic intracranial bleeding was approximately twice as great in patients receiving Ancrod. This lack of efficacy was particularly disappointing considering that desired changes in fibrinogen levels (rapid initial defibrinogenation and avoidance of prolonged hypofibrinogenemia) were seen in >90% of Ancrod-treated patients (Levy et al., 2009). Ancrod is still used outside of the United States for several coagulopathies, including deep vein thrombosis and coronary artery bypass surgery (Cole, 1998), but the outlook for more extensive clinical use is poor.

Batroxobin (Itoh et al., 1987) is another TLE (from *Bothrops moojeni* venom) which has been investigated extensively for use in a variety of disorders, including cerebral and myocardial infarction, ischemic stroke, angina and for prevention/treatment of surgical bleeding (Phillips et al., 2009; Xu et al., 2007). A subsidiary of the Chinese biopharmaceutical company Sinobiomed was granted a patent for recombinant batroxobin (rBAT, Defibrase) in 2007, and some recent reports indicate that this TLE may be more successful in clinical trials than Ancrod. In a study of a small group of patients with deep vein thrombosis (DVT), a serious and potential fatal disorder of the lower limbs, batroxobin was found to be effective in abolishing symptoms of DVT (pain, swelling/erythema of lower limbs) and achieving limb salvage (Zhang et al., 2009). Patient monitoring for 1–3 month post-treatment indicated no abnormalities or return of symptoms, and fibrinogen levels were lowered for at least 14 days. An interesting effect of batroxobin treatment was the increase in endothelial progenitor cells in peripheral blood, which were greatly elevated from normal levels (statistically significant) at 7 and 14 days post-administration. Because these cells (CD34+, CD31+, VE-cadherin+ cells) are important in neovessel formation in adults and may be an essential component of vasculogenesis following disease/injury (Allegra et al., 2009; Asahara et al., 1999), batroxobin-stimulated mobilization may be an important component of recovery (Zhang et al., 2009). The mechanism of this action is presently unknown, but the positive results obtained support extending clinical trials to include a larger patient group.

Batroxobin has also been recommended for treatment of patients with ischemic stroke and transient ischemic attack who also show hyperfibrinogenemia (Xu et al., 2007). Intravenous batroxobin was administered in clusters immediately following and at 3 and 6 months after ischemic events; fibrinogen levels decreased significantly after administration at 0 and 3 months, but the decrease was not significant at 6 months. One year survivorship was also significantly greater in the batroxobin-treated group, and incidence of intracranial hemorrhage was not different in the two treatment groups. These indications suggest that batroxobin may be a safer and more effective treatment for ischemia than Ancrod. However, a recent review of the effectiveness of batroxobin to control hemorrhage during thoracic surgery concluded that although some differential effects were observed, no clinically relevant benefit was observed following batroxobin use (Zeng et al., 2009). The authors conclude that there is insufficient evidence supporting any benefit of batroxobin for hemorrhage during thoracic surgery. These conflicting reports suggest that while

batroxobin may have efficacy for controlling some specific types of coagulopathies, it is not generally indicated for all such conditions.

Several additional TLEs are undergoing evaluation in animal models for antithrombotic use. Acutobin, a TLE derived from *Deinagkistrodon acutus* venom, was reported to be effective in reducing mortality and brain damage following ischemia and reperfusion of the cerebral artery in hyperglycemic rats (Wei et al., 2004). This model mimics a condition particularly at risk of brain tissue injury following ischemia, and acutobin treatment resulted in increased brain tissue perfusion and a reduction in the size of infarct. In a very different application of a snake venom TLE, a dental fibrin adhesive was produced from fibrinogen hydrolyzed by TLE from *Crotalus durissus terrificus* venom (Barbosa et al., 2008). Free gingival grafts were immobilized using either TLE or sutures, and at 7 days post-treatment, inflammatory cell density was lower in the TLE treatment group. By 14 and 45 days, no difference was observed between the two treatments. This study demonstrates that TLEs from snake venom can have utility in production of biological products, with medical applications which are only tangentially related to their in vivo direct actions as fibrinolytic serine proteases.

Use in Basic Research and Diagnostics

Thrombin-like enzymes have been developed for use as diagnostic reagents for clinical and research labs, and Reptilase[®], derived from *B. jararaca* venom, is commonly used in clinical lab diagnosis of bleeding and other coagulation disorders (Phillips et al., 2009; Stocker, 1998). Like several TLEs, Reptilase[®] shows high specificity for the A α chain of fibrinogen and is not affected by fibrin degradation products (FDPs). Clinically, a thrombin time/Reptilase[®] time (TT/RT) of >1, which is correlated with the presence of FDPs, is indicative of high potential for disseminated intravascular coagulation, a potentially fatal coagulopathy.

Several other TLEs have been used in a variety of other applications, such as in the production of dental adhesives (Barbosa et al., 2008; see above). Gyroxin, purified from *Crotalus durissus terrificus* venom, was labeled with ¹²⁵I and used to study biodistribution in a mouse model system (Alves da Silva et al., 2006). The authors suggested that distribution and exogenous compound metabolism could be evaluated with these methods, and it was hypothesized that the distribution kinetics/metabolism patterns observed may be correlated with tissue-specific distribution of different protease activated receptors (PARs) for this (and other) serine proteinases. Ancrod, which may be removed from therapeutic use, has been used as a reagent to study polymerized collagen-fibrin matrices (Rowe and Stegemann, 2009). Collagen-fibrin mixed scaffolds showed strength and tensile stiffness greater than collagen alone, and this mixed matrix may have applications in bioengineering of tissues and biomaterials design generally. Potential further applications were indicated for use in cell culture work, in vitro modeling of vascular flow dynamics and in vivo wound healing (Rowe and Stegemann, 2009). These are but a few examples

of the creative ways in which venom TLEs are being utilized in both applied clinical and basic research.

Future Potential for SV-TLEs

There is a continuing need for safe and effective drugs to treat coagulation disorders such as venous thromboembolic disease and stroke (Spyropoulos, 2008), and research into thrombin-like enzymes from snake venoms could provide novel lead compounds or enzymes which could be directly useful. There is an obvious diversity of SVSPs from front-fanged snakes which could provide a source of novel compounds. However, though variation in component number can exceed 100 protein/peptide compounds in a single venom, there is a relatively small diversity of protein families so far described from venoms (Juárez et al., 2004), and TLEs described thus far appear to possess conserved functional variation along a common theme. This scenario may change as newer methods allow a deeper probe of the venom proteome and reveal diversity of structure (and likely function) among the much less abundant minor venom components (Bandow, 2010; Calvete et al., 2009; Polaskova et al., 2010), but other sources, such as among venoms from rear-fanged snakes, may prove to contain novel TLEs.

Snake venom serine proteases have proven to be useful in various applications in biotechnology and basic research (Wisner et al., 2001), and their specificities could perhaps be exploited for use in mass spectrometry applications currently dominated by trypsin use, such as peptide fingerprinting, MS/MS sequencing, etc. It may be that the most useful applications of SVSPs like the TLEs may lie in research purposes rather than drug development.

Summary and Conclusion

Snake venom thrombin-like enzymes are important components of most viperid snake venoms and are less broadly occurring among other squamate reptile venoms. As part of the biological weaponry of venomous species, their actions *in vivo* can cause cataclysmic coagulopathies which may become life-threatening. Purified and characterized, TLEs have many applications in biomedicine as well as basic and applied research. Rapid advances in genomics and proteomics have provided sequences for many venom serine proteinases, including TLEs, and detailed structure/activity data is available for a smaller subset of these. There is a need for rigorous substrate specificity studies to be conducted with the naturally expressed venom serine proteinases, particularly for those species with extensive transcriptome and proteome datasets. Such functional data will help to answer the remaining questions related to the observed diversity of actions of these structurally conservative venom components. Further, there are many species of front-fanged and rear-fanged snakes whose venoms are poorly known, and it is likely that additional interesting variants of this family of proteinases remain to be described.

Appendix: Identification of Serine Proteinases Used in Sequence Alignments (Fig. 30.1) and Sequence Similarity Analysis (Fig. 30.4), Ordered by UniProt Accession Number

Accession	Protein names	Organism	Length
P00760	Trypsin – Bovine cationic	<i>Bos taurus</i>	246
P07477	Trypsin-1 (EC 3.4.21.4)	<i>Homo sapiens</i> (Human)	247
P00766	Chymotrypsin – bovine	<i>Bos taurus</i>	245
P00734	Thrombin – human	<i>Homo sapiens</i> (Human)	259
A1E235	Venom thrombin-like enzyme (Fragment)	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	235
A1E236	Venom thrombin-like enzyme (Fragment)	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	235
A1E237	Venom thrombin-like enzyme (Fragment)	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	235
A1E238	Venom thrombin-like enzyme (Fragment)	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	217
A1E239	Venom thrombin-like enzyme (Fragment)	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	235
A1E2S1	Venom thrombin-like enzyme	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	235
A1E2S2	Venom thrombin-like enzyme	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	235
A1E2S3	Venom thrombin-like enzyme	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	235
A1E2S4	Venom thrombin-like enzyme	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	235
A7LAC6	Thrombin-like serine protease 1	<i>Trimeresurus albolabris</i> (White-lipped pit viper)	260
A7LAC7	Thrombin-like serine protease 2	<i>Trimeresurus albolabris</i> (White-lipped pit viper)	260
A8HR02	Thrombin-like protein (Fragment)	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	236
A8QL53	Putative serine protease (Fragment)	<i>Naja atra</i> (Chinese cobra)	282
A8QL56	Thrombin-like serine protease	<i>Ophiophagus hannah</i> (King cobra)	260
A8QL57	Putative serine protease (Fragment)	<i>Bungarus multicinctus</i> (Many-banded krait)	282
B0FXM1	Gyroxin-like B1_3 serine protease	<i>Crotalus durissus terrificus</i> (South American rattlesnake)	262
B0FXM2	Gyroxin-like B1_4 serine protease	<i>Crotalus durissus terrificus</i> (South American rattlesnake)	262
B0FXM3	Gyroxin-like B1_7 serine protease	<i>Crotalus durissus terrificus</i> (South American rattlesnake)	259
B0VXT3	Serine proteinase isoform 1	<i>Sistrurus catenatus edwardsii</i> (Desert massasauga)	262
B0VXT4	Serine proteinase isoform 2	<i>Sistrurus catenatus edwardsii</i> (Desert massasauga)	259

Accession	Protein names	Organism	Length
B0VXT5	Serine proteinase isoform 3	<i>Sistrurus catenatus edwardsii</i> (Desert massasauga)	257
B0VXT6	Serine proteinase isoform 4	<i>Sistrurus catenatus edwardsii</i> (Desert massasauga)	258
B0VXT7	Serine proteinase isoform 5	<i>Sistrurus catenatus edwardsii</i> (Desert massasauga)	258
B0VXT8	Serine proteinase isoform 6 (Fragment)	<i>Sistrurus catenatus edwardsii</i> (Desert massasauga)	242
B0VXT9	Serine proteinase isoform 7	<i>Sistrurus catenatus edwardsii</i> (Desert massasauga)	273
B0VXU0	Serine proteinase isoform 8	<i>Sistrurus catenatus edwardsii</i> (Desert massasauga)	258
B0VXU1	Serine proteinase isoform 9	<i>Sistrurus catenatus edwardsii</i> (Desert massasauga)	260
B0VXU2	Serine proteinase isoform 10	<i>Sistrurus catenatus edwardsii</i> (Desert massasauga)	260
B0VXU3	Serine proteinase isoform 11	<i>Sistrurus catenatus edwardsii</i> (Desert massasauga)	257
B0ZT25	Snake venom serine protease homolog (TjsvSPH)	<i>Protobothrops</i> (<i>Trimeresurus</i>) <i>jerdonii</i> (Jerdon's pit-viper)	260
B3V4Z6	Thrombin-like protein DAV-WY	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	260
B5U6Y3	Serine proteinase	<i>Echis ocellatus</i> (Ocellated saw-scaled viper)	258
B6CJU5	Kallikrein toxin Var13	<i>Varanus komodoensis</i> (Komodo dragon)	258
C6EVG4	Kallikrein toxin 1	<i>Heloderma suspectum cinctum</i>	255
O13057	Venom serine proteinase 2 (EC 3.4.21)	<i>Protobothrops</i> (<i>Trimeresurus</i>) <i>flavoviridis</i> (Habu)	260
O13058	Venom serine proteinase 3 (EC 3.4.21)	<i>Protobothrops</i> (<i>Trimeresurus</i>) <i>flavoviridis</i> (Habu)	257
O13059	Venom serine proteinase 1 (EC 3.4.21)	<i>Trimeresurus gramineus</i> (Indian green tree viper)	258
O13060	Venom serine proteinase 2A (EC 3.4.21)	<i>Trimeresurus gramineus</i> (Indian green tree viper)	260
O13061	Venom serine proteinase 2B (EC 3.4.21)	<i>Trimeresurus gramineus</i> (Indian green tree viper)	260
O13062	Venom serine proteinase 2C (EC 3.4.21)	<i>Trimeresurus gramineus</i> (Indian green tree viper)	257
O13063	Venom serine proteinase 3 (EC 3.4.21)	<i>Trimeresurus gramineus</i> (Indian green tree viper)	258
O13069	Kinin-releasing/fibrinogen-clotting serine proteinase 2 (EC 3.4.21) (KN-BJ 2)	<i>Bothrops jararaca</i> (Jararaca)	257
O42207	Capillary permeability-increasing enzyme 2 (CPI-enzyme 2) (EC 3.4.21)	<i>Gloydius ussuriensis</i> (Ussuri mamushi) (<i>Agkistrodon caliginosus</i>)	258
O73800	Thrombin-like enzyme salmobin (EC 3.4.21)	<i>Gloydius</i> (<i>Agkistrodon</i>) <i>halys pallas</i> (Chinese water mocassin)	260

Accession	Protein names	Organism	Length
O93421	Thrombin-like enzyme pallas (EC 3.4.21)	<i>Gloydius</i> (Agkistrodon) <i>halys pallas</i> (Chinese water mocassin)	236
P04971	Batroxobin (BX) (EC 3.4.21.74) (Venombin-A) (Defibrase) (Reptilase)	<i>Bothrops atrox</i> (Barba amarilla) (Fer-de-lance)	255
P05620	Thrombin-like enzyme flavoxobin (EC 3.4.21) (Venom serine proteinase 1)	<i>Protobothrops</i> (Trimeresurus) <i>flavoviridis</i> (Habu)	260
P09872	Ancrod (EC 3.4.21.74) (Venombin-A) (Protein C activator) (ACC-C)	<i>Agkistrodon contortrix contortrix</i> (Southern copperhead)	231
P0C5B4	Thrombin-like enzyme gloshedobin (EC 3.4.21) (Defibrase)	<i>Gloydius shedaensis</i> (Shedao island pit viper)	260
P18964	Vipera russelli proteinase RVV-V alpha (EC 3.4.21.95) (Factor V-activating)	<i>Daboia russelli siamensis</i> (Eastern Russell's viper)	236
P18965	Vipera russelli proteinase RVV-V gamma (EC 3.4.21.95) (Factor V-activating)	<i>Daboia russelli siamensis</i> (Eastern Russell's viper)	236
P26324	Ancrod (EC 3.4.21.74) (Venombin-A) (Protein C activator) (ACC-C)	<i>Calloselasma</i> (Agkistrodon) <i>rhodostoma</i> (Malayan pit viper)	234
P33589	Gyroxin analog (EC 3.4.21.74) (Thrombin-like enzyme) (LM-TL) (Venombin-A)	<i>Lachesis muta muta</i> (Bushmaster)	228
P47797	Ancrod (EC 3.4.21.74) (Venombin-A) (Protein C activator) (ACC-C)	<i>Calloselasma</i> (Agkistrodon) <i>rhodostoma</i> (Malayan pit viper)	258
P81176	Halystase (EC 3.4.21)	<i>Agkistrodon halys blomhoffii</i> (Mamushi) (<i>Gloydius blomhoffii</i>)	238
P81661	Thrombin-like enzyme bothrombin (EC 3.4.21.74) (Reptilase)	<i>Bothrops jararaca</i> (Jararaca)	232
P81824	Platelet-aggregating proteinase PA-BJ (EC 3.4.21)	<i>Bothrops jararaca</i> (Jararaca)	232
P82981	Thrombin-like enzyme contortrixobin (EC 3.4.21)	<i>Agkistrodon contortrix contortrix</i> (Southern copperhead)	234
P84787	Thrombin-like enzyme elegaxobin-2 (EC 3.4.21) (Elegaxobin II)	<i>Protobothrops</i> (Trimeresurus) <i>elegans</i> (Sakishima habu)	233
P84788	Thrombin-like enzyme elegaxobin-1 (EC 3.4.21) (Elegaxobin I)	<i>Protobothrops</i> (Trimeresurus) <i>elegans</i> (Sakishima habu)	233

Accession	Protein names	Organism	Length
P85109	Thrombin-like enzyme kangshuanmei (EC 3.4.21)	<i>Gloydius</i> (Agkistrodon) <i>halys brevicaudus</i> (Korean slamosa snake)	236
P86171	Kinin-releasing enzyme KR-E-1 (EC 3.4.21)	<i>Gloydius ussuriensis</i> (Ussuri mamushi) (Agkistrodon caliginosus)	235
Q072L6	Venom serine proteinase-like	<i>Bothrops asper</i> (Terciopelo)	259
Q072L7	Thrombin-like enzyme (EC 3.4.21)	<i>Lachesis stenophrys</i> (Central American bushmaster)	258
Q09GK1	Venom serine protease (EC 3.4.21)	<i>Philodryas olfersii</i> (Green snake)	261
Q27J47	Plasminogen-activating proteinase (LV-PA) (EC 3.4.21) (LMUT0402S)	<i>Lachesis muta muta</i> (Bushmaster)	258
Q2PQJ3	Venom serine protease 1 (EC 3.4.21) (BjussuSP-I)	<i>Bothrops jararacussu</i> (Jararacussu)	232
Q2QA04	Serine proteinase (EC 3.4.21)	<i>Crotalus durissus durissus</i> (Central American rattlesnake)	262
Q2XXM2	Kallikrein-Phi5 (Fragment)	<i>Philodryas olfersii</i> (Green snake)	248
Q2XXM3	Kallikrein-Phi4 (Fragment)	<i>Philodryas olfersii</i> (Green snake)	244
Q2XXM4	Kallikrein-Phi3	<i>Philodryas olfersii</i> (Green snake)	229
Q2XXM5	Kallikrein-Phi2	<i>Philodryas olfersii</i> (Green snake)	229
Q2XXM6	Kallikrein-Phi1	<i>Philodryas olfersii</i> (Green snake)	229
Q58G94	Gyroxin-like B2.1 (EC 3.4.21)	<i>Crotalus durissus terrificus</i> (South American rattlesnake)	238
Q5I2B5	Thrombin-like protein 3	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	260
Q5I2B6	Thrombin-like protein 1	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	260
Q5I2C5	Thrombin-like enzyme 2	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	260
Q5MCS0	Serine protease	<i>Lapemis hardwickii</i> (Hardwick's sea snake)	265
Q5W958	Venom serine proteinase-like HS120	<i>Bothrops jararaca</i> (Jararaca)	253
Q5W959	Venom serine proteinase HS114 (EC 3.4.21)	<i>Bothrops jararaca</i> (Jararaca)	258
Q5W960	Venom serine proteinase HS112 (EC 3.4.21)	<i>Bothrops jararaca</i> (Jararaca)	255
Q6IWF1	Venom serine protease BthaTL (EC 3.4.21)	<i>Bothrops alternatus</i> (Urutu)	233
Q6T5L0	Thrombin-like enzyme shedaenase (EC 3.4.21)	<i>Gloydius</i> (Agkistrodon) <i>shedaoensis</i> (Shedao island pit viper)	238
Q6T6S7	Venom serine proteinase-like protein 1	<i>Bitis gabonica</i> (Gaboon viper)	260
Q6URK9	Platelet aggregating serine peptidase (Fragment)	<i>Bothrops jararaca</i> (Jararaca)	167
Q71QH5	Venom serine protease KN8 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	257
Q71QH6	Venom serine protease KN13 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	258

Accession	Protein names	Organism	Length
Q71QH7	Venom serine protease PA (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	258
Q71QH8	Serine protease CL4	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	257
Q71QH9	Venom serine protease KN14 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	260
Q71QI0	Venom serine protease KN7 homolog	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	260
Q71QI1	Venom serine protease KN12 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	258
Q71QI2	Venom serine protease CL2 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	258
Q71QI3	Venom serine protease CL5 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	257
Q71QI4	Venom serine protease KN5 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	260
Q71QI5	Venom serine protease KN3 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	257
Q71QI6	Serine protease CL3	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	257
Q71QI7	Venom serine protease KN11 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	257
Q71QI8	Venom serine protease KN10 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	257
Q71QI9	Serine protease CL1	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	260
Q71QJ0	Venom serine protease KN2 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	257
Q71QJ1	Venom serine protease KN9 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	257
Q71QJ2	Venom serine protease KN6 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	260
Q71QJ3	Venom serine protease KN1 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	257
Q71QJ4	Venom serine protease KN4 homolog	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	260
Q7SYF1	Cerastocytin (EC 3.4.21.74) (Proaggregant serine proteinase) (CC-PPP)	<i>Cerastes cerastes</i> (Horned desert viper)	256
Q7SZE1	Thrombin-like enzyme defibrase (EC 3.4.21)	<i>Gloydius</i> (Agkistrodon) <i>saxatilis</i> (Rock mamushi)	258
Q7SZE2	Bradykinin-releasing enzyme KR-E-1 (Thrombin-like defibrase) (EC 3.4.21)	<i>Gloydius ussuriensis</i> (Ussuri mamushi) (<i>Agkistrodon caliginosus</i>)	234
Q7T229	Venom serine protease homolog	<i>Bothrops jararacussu</i> (Jararacussu)	260
Q7ZZP4	Thrombin-like enzyme PTLE3	<i>Gloydius</i> (Agkistrodon) <i>halys pallas</i> (Chinese water mocassin)	190

Accession	Protein names	Organism	Length
Q802F0	Thrombin-like enzyme PTLE1 (EC 3.4.21)	<i>Gloydius</i> (Agkistrodon) <i>halys pallas</i> (Chinese water mocassin)	258
Q8AY78	Venom serine protease 5 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	258
Q8AY79	Thrombin-like enzyme stejnefibrase-2 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	258
Q8AY80	Thrombin-like enzyme stejnefibrase-1 (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	258
Q8AY81	Thrombin-like enzyme stejnobin (EC 3.4.21)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	260
Q8AY82	Venom serine protease 1 homolog	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	260
Q8JH62	Serine beta-fibrinogenase (EC 3.4.21) (VLBF)	<i>Vipera lebetina</i> (Elephant snake) (Leventine viper)	257
Q8JH85	Serine alpha-fibrinogenase (EC 3.4.21) (VLAf)	<i>Vipera lebetina</i> (Elephant snake) (Leventine viper)	258
Q8QG86	Serine proteinase BITS01A (EC 3.4.21)	<i>Bothrops insularis</i> (Island jararaca)	257
Q8QHK2	Catroxase-2 (EC 3.4.21) (Catroxase II) (EI)	<i>Crotalus atrox</i> (Western diamondback rattlesnake)	258
Q8QHK3	Catroxase-1 (EC 3.4.21) (Catroxase I)	<i>Crotalus atrox</i> (Western diamondback rattlesnake)	262
Q8UUJ1	Thrombin-like enzyme ussurase (EC 3.4.21)	<i>Gloydius ussuriensis</i> (Ussuri mamushi) (Agkistrodon caliginosus)	233
Q8UUJ2	Thrombin-like enzyme ussurin (EC 3.4.21)	<i>Gloydius ussuriensis</i> (Ussuri mamushi) (Agkistrodon caliginosus)	236
Q8UUK2	Venom serine proteinase Sp1 (EC 3.4.21)	<i>Crotalus adamanteus</i> (Eastern diamondback rattlesnake)	259
Q8UVX1	Thrombin-like enzyme gussurobin (EC 3.4.21)	<i>Gloydius ussuriensis</i> (Ussuri mamushi) (Agkistrodon caliginosus)	260
Q90Z47	Venom thrombin-like enzyme	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	235
Q91053	Thrombin-like enzyme calobin-1 (EC 3.4.21) (Calobin I)	<i>Gloydius ussuriensis</i> (Ussuri mamushi) (Agkistrodon caliginosus)	262
Q91507	Mucrofibrase-1 (EC 3.4.21)	<i>Protobothrops</i> (<i>Trimeresurus</i>) <i>mucrosquamatus</i> (Taiwan habu)	257
Q91508	Mucrofibrase-2 (EC 3.4.21) (Trimubin)	<i>Protobothrops</i> (<i>Trimeresurus</i>) <i>mucrosquamatus</i> (Taiwan habu)	257
Q91509	Mucrofibrase-3 (EC 3.4.21)	<i>Protobothrops</i> (<i>Trimeresurus</i>) <i>mucrosquamatus</i> (Taiwan habu)	257
Q91510	Mucrofibrase-4 (EC 3.4.21)	<i>Protobothrops</i> (<i>Trimeresurus</i>) <i>mucrosquamatus</i> (Taiwan habu)	257
Q91511	Mucrofibrase-5 (EC 3.4.21)	<i>Protobothrops</i> (<i>Trimeresurus</i>) <i>mucrosquamatus</i> (Taiwan habu)	257
Q91516	Venom plasminogen activator (EC 3.4.21) (TSV-PA)	<i>Trimeresurus stejnegeri</i> (Chinese green tree viper)	258
Q98TT5	Thrombin-like enzyme	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	258

Accession	Protein names	Organism	Length
Q9DF66	Venom serine proteinase 3 (SP3) (EC 3.4.21)	<i>Protobothrops</i> (Trimeresurus) <i>jerdonii</i> (Jerdon's pit-viper)	258
Q9DF67	Venom serine proteinase 2 (SP2) (EC 3.4.21)	<i>Protobothrops</i> (Trimeresurus) <i>jerdonii</i> (Jerdon's pit-viper)	258
Q9DF68	Venom serine proteinase-like protein (SP1)	<i>Protobothrops</i> (Trimeresurus) <i>jerdonii</i> (Jerdon's pit-viper)	260
Q9DG83	Serpentokallikrein-1 (EC 3.4.21)	<i>Protobothrops</i> (Trimeresurus) <i>microsquamatus</i> (Taiwan habu)	260
Q9DG84	Serpentokallikrein-2 (EC 3.4.21)	<i>Protobothrops</i> (Trimeresurus) <i>microsquamatus</i> (Taiwan habu)	257
Q9I8W9	Venom serine proteinase Dav-X (EC 3.4.21)	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	260
Q9I8X0	Venom serine proteinase Dav-KN (EC 3.4.21)	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	257
Q9I8X1	Venom serine proteinase Dav-PA (AaV-SP-I) (AaV-SP-II) (EC 3.4.21)	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	258
Q9I8X2	Thrombin-like enzyme acutobin (EC 3.4.21) (Acuthrombin) (Acutase)	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	260
Q9I961	Acubin2	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	258
Q9PSN3	Thrombin-like enzyme bilineobin (EC 3.4.21)	<i>Agkistrodon bilineatus</i> (Cantil) (Tropical moccasin)	235
Q9PT40	Venom serine proteinase-like protein 2	<i>Vipera lebetina</i> (Leventine viper)	260
Q9PT41	Factor V-activating enzyme (FVA) (EC 3.4.21)	<i>Vipera lebetina</i> (Leventine viper)	259
Q9PT51	Brevinase (EC 3.4.21) [Brevinase chain A & B]	<i>Gloydius</i> (Agkistrodon) <i>halys blomhoffi</i> (Mamushi)	233
Q9PTL3	Thrombin-like enzyme salmonase (EC 3.4.21)	<i>Gloydius</i> (Agkistrodon) <i>halys brevicaudus</i> (Korean slamosa snake)	257
Q9PTU8	Venom serine proteinase A (EC 3.4.21)	<i>Bothrops jararaca</i> (Jararaca)	258
Q9W7S1	Acubin	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	258
Q9YGI6	Thrombin-like enzyme pallabin-2 (EC 3.4.21)	<i>Gloydius</i> (Agkistrodon) <i>halys pallas</i> (Chinese water mocassin)	260
Q9YGJ2	Thrombin-like enzyme pallabin (EC 3.4.21)	<i>Gloydius</i> (Agkistrodon) <i>halys pallas</i> (Chinese water mocassin)	260
Q9YGJ8	Plasminogen activator Haly-PA (EC 3.4.21)	<i>Gloydius</i> (Agkistrodon) <i>halys pallas</i> (Chinese water mocassin)	258
Q9YGJ9	Serine protease Haly-2 (EC 3.4.21)	<i>Gloydius</i> (Agkistrodon) <i>halys pallas</i> (Chinese water mocassin)	257
Q9YGS1	Thrombin-like defibrase 1 (Fragment)	<i>Deinagkistrodon acutus</i> (Hundred-pace snake)	234

References

- Alape-Girón, A., Sanz, L., Escolano, J., Flores-Díaz, M., Madrigal, M., Sasa, M., Calvete, J.J., 2008. Snake venomomics of the lancehead pitviper *Bothrops asper*: geographic, individual, and ontogenetic variations. *J. Proteome Res.* 7, 3556–3571.
- Alexander, G., Grothusen, J., Zepeda, H., Schwartzman, R.J., 1988. Gyroxin, a toxin from the venom of *Crotalus durissus terrificus*, is a thrombin-like enzyme. *Toxicon* 26, 953–960.
- Allegra, A., Coppolino, G., Bolignano, D., Giacobbe, M.S., Alonci, A., D'Angelo, A., Bellomo, G., Teti, D., Loddo, S., Musolino, C., Buemi, M., 2009. Endothelial progenitor cells: pathogenetic role and therapeutic perspectives. *J. Nephrol.* 22, 463–475.
- Alves da Silva, J.A., Muramoto, E., Ribela, M.T.C., Rogero, J.R., Camillo, M.A.P., 2006. Biodistribution of gyroxin using ^{125}I as radiotracer. *J. Radioanal. Nucl. Chem.* 269, 579–583.
- Amiconi, G., Amoresano, A., Boumis, G., Brancaccio, A., De Cristofaro, R., De Pascalis, A., Di Girolamo, S., Maras, B., Scaloni, A., 2000. A novel venombin B from *Agkistrodon contortrix contortrix*: evidence for recognition properties in the surface around the primary specificity pocket different from thrombin. *Biochemistry* 39, 10294–10308.
- Asahara, T., Masuda, H., Takahashi, T., Kalka, C., Pastore, C., Silver, M., Kearne, M., Magner, M., Isner, J.M., 1999. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ. Res.* 85, 221–228.
- Assakura, M.T., Reichl, A.P., Mandelbaum, F.R., 1994. Isolation and characterization of five fibrin(ogen)olytic enzymes from the venom of *Philodryas olfersii* (green snake). *Toxicon* 32, 819–831.
- Au, L.C., Lin, S.B., Chou, J.S., Teh, G.W., Chang, K.J., Shih, C.M., 1993. Molecular cloning and sequence analysis of the cDNA for ancrod, a thrombin-like enzyme from the venom of *Calloselasma rhodostoma*. *Biochem. J.* 294, 387–390.
- Bandow, J.E., 2010. Comparison of protein enrichment strategies for proteome analysis of plasma. *Proteomics* 2010 Feb 1. [Epub ahead of print].
- Barbosa, M.D.S., Stipp, A.C., Passanezi, E., Greggi, S.L.A., 2008. Fibrin adhesive derived from snake venom in periodontal surgery. *Histological analysis. J. Appl. Oral Sci.* 16, 310–315.
- Bell, G.I., Quinto, C., Quiroga, M., Valenzuela, P., Craik, C.S., Rutter, W.J., 1984. Isolation and sequence of a rat chymotrypsin B gene. *J. Biol. Chem.* 259, 14265–14270.
- Botos, I., Wlodawer, A., 2007. The expanding diversity of serine hydrolases. *Curr. Opin. Struct. Biol.* 17, 683–690.
- Boyer, L.V., Seifert, S.A., Clark, R.F., McNally, J.T., Williams, S.R., Nordt, S.P., Walter, F.G., Dart, R.C., 1999. Recurrent and persistent coagulopathy following pit viper envenomation. *Arch Intern Med.* 159, 706–710.
- Braud, S., Bon, C., Wisner, A., 2000. Snake venom proteins acting on hemostasis. *Biochemie* 82, 851–859.
- Burkhart, W., Smith, G.F., Su, J.L., Parikh, I., LeVine, H., 3rd., 1992. Amino acid sequence determination of ancrod, the thrombin-like alpha-fibrinogenase from the venom of *Agkistrodon rhodostoma*. *FEBS Lett.* 297, 297–301.
- Calvete, J.J., Fasoli, E., Sanz, L., Boschetti, E., Righetti, P.G., 2009. Exploring the venom proteome of the western diamondback rattlesnake, *Crotalus atrox*, via snake venomomics and combinatorial peptide ligand library approaches. *J. Proteome Res.* 8, 3055–67.
- Castro, H.C., Silva, D.M., Craik, C., Zingali, R.B., 2001. Structural features of a snake venom thrombin-like enzyme: thrombin and trypsin on a single catalytic platform? *Biochim. Biophys. Acta* 1547, 183–195.
- Castro, H.C., Zingali, R.B., Albuquerque, M.G., Pujol-Luz, M., Rodrigues, C.R., 2004. Snake venom thrombin-like enzymes: from reptilase to now. *Cell. Mol. Life Sci.* 61, 843–856.
- Ching, A.T.C., Rocha, M.M.T., Paes Leme, A.F., Pimenta, D.C., Furtado, M.F.D., Serrano, S.M.T., Ho, P.L., Junqueira-de-Azevedo, I.L.M., 2006. Some aspects of the venom proteome of the

- Colubridae snake *Philodryas olfersii* revealed from a Duvernoy's (venom) gland transcriptome. *FEBS Lett.* 580, 4417–4422.
- Cole, C.W., 1998. Controlling acute elevation of plasma fibrinogen with anicrod. *Cerebrovasc. Dis.* 1, 29–34.
- Craik, C.S., Choo, Q.L., Swift, G.H., Quinto, C., MacDonald, R.J., Rutter, W.J., 1984. Structure of two related rat pancreatic trypsin genes. *J. Biol. Chem.* 259, 14255–14264.
- Dekhil, H., Wisner, A., Marrakchi, N., El Ayeb, M., Bon, C., Karoui, H., 2003. Molecular cloning and expression of a functional snake venom serine proteinase, with platelet aggregating activity, from the *Cerastes cerastes* viper. *Biochemistry* 42, 10609–10618.
- Deshimaru, M., Ogawa, T., Nakashima, K., Nobuhisa, I., Chijiwa, T., Shimohigashi, Y., Fukumaki, Y., Niwa, M., Yamashina, I., Hattori, S., Ohno, M., 1996. Accelerated evolution of crotalinae snake venom gland serine proteases. *FEBS Lett.* 397, 83–88.
- Di Cera, E., 2008. Thrombin. *Mol. Aspects Med.* 29, 203–254.
- Doley, R., Mackessy, S.P., Kini, R.M., 2009. Role of accelerated segment switch in exons to alter targeting (ASSET) in the molecular evolution of snake venom proteins. *BMC Evol. Biol.* 9, 146.
- Doley, R., Pahari, S., Mackessy, S.P., Kini, R.M., 2008. Accelerated exchange of exon segments in Viperid three-finger toxin genes (*Sistrurus catenatus edwardsii*; Desert Massasauga). *BMC Evol. Biol.* 8, 196.
- Escalante, T., Shannon, J., Moura-da-Silva, A.M., Gutiérrez, J.M., Fox, J.W., 2006. Novel insights into capillary vessel basement membrane damage by snake venom hemorrhagic metalloproteinases: a biochemical and immunohistochemical study. *Arch. Biochem. Biophys.* 455, 144–153.
- Farid, T.M., Tu, A.T., el-Asmar, M.F., 1989. Characterization of cerastobin, a thrombin-like enzyme from the venom of *Cerastes vipera* (Sahara sand viper). *Biochemistry* 28, 371–377.
- Farid, T.M., Tu, A.T., el-Asmar, M.F., 1990. Effect of cerastobin, a thrombinlike enzyme from *Cerastes vipera* (Egyptian sand snake) venom, on human platelets. *Haemostasis* 20, 296–304.
- Ferreira, S.H., 1965. A bradykinin-potentiating factor (bpf) present in the venom of *Bothrops jararaca*. *Br. J. Pharmacol. Chemother.* 24, 163–169.
- Fox, J.W., Serrano, S.M.T., 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. Pharm. Des.* 13, 2927–2934.
- Fox, J.W., Serrano, S.M.T., 2008. Insights into and speculations about snake venom metalloproteinases (SVMP) synthesis, folding and disulfide bond formation and their contribution to venom complexity. *FEBS J.* 275, 3016–3030.
- Fox, J.W., Serrano, S.M.T., 2009. Snake venom metalloproteinases, in: Mackessy, S.P. (Ed.), *Handbook of Venoms and Toxins of Reptiles*. CRC Press/Taylor & Francis Group, Boca Raton, FL, pp. 95–113.
- Fry, B.G., 2005. From genome to “venome”: molecular origin and evolution of the snake venom proteome inferred from the phylogenetic analysis of toxin sequences and related body proteins. *Genome Res.* 15, 403–420.
- Fry, B.G., Vidal, N., Norman, J.A., Vonk, F.J., Scheib, H., Ramjan, S.F., Kuruppu, S., Fung, K., Hedges, S.B., Richardson, M.K., Hodgson, W.C., Ignjatovic, V., Summerhayes, R., Kochva, E., 2006. Early evolution of the venom system in lizards and snakes. *Nature* 439, 584–588.
- Gao, R., Zhang, Y., Meng, Q.-X., Lee, W.-H., Li, D.-S., Xiong, Y.-L., Wang, W.-Y., 1998. Characterization of three fibrinogenolytic enzymes from Chinese green tree viper (*Trimeresurus stejnegeri*) venom. *Toxicon* 36, 457–467.
- Guo, Y.W., Chang, T.Y., Lin, K.T., Liu, H.W., Shih, K.C., Cheng, S.H., 2001. Cloning and functional expression of the mucrosobin protein, a beta-fibrinogenase of *Trimeresurus mucrosquamatus* (Taiwan Habu). *Protein Exp. Purif.* 23, 483–490.
- Gutiérrez, J.M., Rucavado, A., Escalante, T. 2009. Snake venom metalloproteinases. Biological roles and participation in the pathology of envenomation, in: Mackessy, S.P. (Ed.), *Handbook of Venoms and Toxins of Reptiles*. CRC Press/Taylor & Francis Group, Boca Raton, FL, pp. 115–138.

- Hahn, B.S., Yang, K.Y., Park, E.M., Chang, I.M., Kim, Y.S., 1996. Purification and molecular cloning of calobin, a thrombin-like enzyme from *Agkistrodon caliginosus* (Korean viper). *J. Biochem. (Tokyo)* 119, 835–843.
- Hahn, B.S., Baek, K., Kim, W.S., Lee, C.S., Chang, I.L., Kim, Y.S., 1998. Molecular cloning of capillary permeability-increasing enzyme-2 from *Agkistrodon caliginosus* (Korean viper). *Toxicon* 36, 1887–1893.
- Hartley, B.S., Kilby, B.A., 1954. The reaction of p-nitrophenyl esters with chymotrypsin and insulin. *Biochem. J.* 56, 288–297.
- Henschen-Edman, A.H., Theodor, I., Edwards, B.F., Pirkle, H., 1999. Crotalase, a fibrinogen-clotting snake venom enzyme: primary structure and evidence for a fibrinogen recognition exosite different from thrombin. *Thromb. Haemost.* 81, 81–86.
- Hiestand, P.C., Hiestand, R.R. 1979. *Dispholidus typus* (Boomslang) snake venom: purification and properties of the coagulant principle. *Toxicon* 17, 489–498.
- Huisman, T.H., 1993. The structure and function of normal and abnormal haemoglobins. *Baillieres Clin. Haematol.* 6, 1–30.
- Isbister, G.K., Duffull, S.B., Brown, S.G., ASP Investigators. 2009. Failure of antivenom to improve recovery in Australian snakebite coagulopathy. *QJM* 102, 563–568.
- Itoh, N., Tanaka, N., Mihashi, S., Yamashina, I., 1987. Molecular cloning and sequence analysis of cDNA for batroxobin, a thrombin-like snake venom enzyme. *J. Biol. Chem.* 262, 3132–3135.
- Itoh, N., Tanaka, N., Funakoshi, I., Kawasaki, T., Mihashi, S., Yamashina, I., 1988. Organization of the gene for batroxobin, a thrombin-like snake venom enzyme. Homology with the trypsin/kallikrein gene family. *J. Biol. Chem.* 263, 7628–7631.
- Jin, Y., Lee, W.H., Zhang, Y., 2007. Molecular cloning of serine proteases from elapid snake venoms. *Toxicon* 49, 1200–1207.
- Juárez, P., Sanz, L., Calvete, J.J., 2004. Snake venomics: characterization of protein families in *Sistrurus barbouri* venom by cysteine mapping, N-terminal sequencing, and tandem mass spectrometry analysis. *Proteomics* 4, 327–338.
- Kini, R.M., 2005. Serine proteases affecting blood coagulation and fibrinolysis from snake venoms. *Pathophysiol. Haemost. Thromb.* 34, 200–204.
- Kini, R.M., Chan, Y.M., 1999. Accelerated evolution and molecular surface of venom phospholipase A₂ enzymes. *J. Mol. Evol.* 48, 125–132.
- Kirby, E.P., Niewiarowski, S., Stocker, K., Kettner, C., Shaw, E., Brudzynski, T.M., 1979. Thrombocytin, a serine protease from *Bothrops atrox* venom 1. Purification and characterization of the enzyme. *Biochemistry* 18, 3564–3570.
- Komori, Y., Nikai, T., Ohara, A., Yagihashi, S., Sugihara, H., 1993. Effect of bilineobin, a thrombin-like proteinase from the venom of common cantil (*Agkistrodon bilineatus*). *Toxicon* 31, 257–270.
- Kordis, D., Gubensek, F., 2000. Adaptive evolution of animal toxin multigene families. *Gene* 261, 43–52.
- Lee, J.W., Park, W., 2000. cDNA cloning of brevinase, a heterogeneous two-chain fibrinolytic enzyme from *Agkistrodon blomhoffii brevicaudus* snake venom, by serial hybridization polymerase chain reaction. *Arch. Biochem. Biophys.* 377, 234–240.
- Lee, J.W., Seu, J.H., Rhee, I.K., Jin, I., Kawamura, Y., Park, W., 1999. Purification and characterization of brevinase, a heterogeneous two-chain fibrinolytic enzyme from the venom of Korean snake, *Agkistrodon blomhoffii brevicaudus*. *Biochem. Biophys. Res. Commun.* 260, 665–670.
- Levy, D.E., del Zoppo, G.J., Demaerschalk, B.M., Demchuk, A.M., Diener, H.C., Howard, G., Kaste, M., Pancioli, A.M., Ringelstein, E.B., Spatareanu, C., Wasiewski, W.W., 2009. Ancrod in acute ischemic stroke: results of 500 subjects beginning treatment within 6 hours of stroke onset in the ancrod stroke program. *Stroke* 40, 3796–3803.
- Mackessy, S.P., 1993a. Fibrinogenolytic proteases from the venoms of juvenile and adult northern Pacific rattlesnake (*Crotalus viridis oreganus*). *Comp. Biochem. Physiol.* 106B, 181–189.
- Mackessy, S.P., 1993b. Kallikrein-like and thrombin-like proteases from the venom of juvenile northern Pacific rattlesnakes (*Crotalus viridis oreganus*). *J. Nat. Toxins* 2, 223–239.

- Mackessy, S.P., 1996. Characterization of the major metalloprotease isolated from the venom of *Crotalus viridis oreganus*. *Toxicon* 34, 1277–1285.
- Mackessy, S.P. 2002. Biochemistry and pharmacology of colubrid snake venoms. *J. Toxicol.-Toxin Rev.* 21, 43–83.
- Mackessy, S.P., 2009. The field of reptile toxinology: snakes, lizards and their venoms, in: Mackessy, S.P. (Ed.), *Handbook of Venoms and Toxins of Reptiles*. CRC Press/Taylor & Francis Group, Boca Raton, FL, pp. 3–23.
- MacSweeney, A., Birrane, G., Walsh, M.A., O'Connell, T., Malthouse, J.P., Higgins, T.M. 2000. Crystal structure of delta-chymotrypsin bound to a peptidyl chloromethyl ketone inhibitor. *Acta Crystallogr., Sect. D* 56, 280–286.
- Magalhães, A., Da Fonseca, B.C., Diniz, C.R., Gilroy, J., Richardson, M., 1993. The complete amino acid sequence of a thrombin-like enzyme/gyroxin analogue from venom of the bushmaster snake (*Lachesis muta muta*). *FEBS Lett.* 329:116–20.
- Magalhães, A., Magalhães, H.P.B., Richardson, M., Gontijo, S., Ferreira, R.N., Almeida, A.P., Sanchez, E.F., 2007. Purification and properties of a coagulant thrombin-like enzyme from the venom of *Bothrops leucurus*. *Comp. Biochem. Physiol.* 146A, 565–575.
- Marchler-Bauer, A., Anderson, J.B., Chitsaz, F., Derbyshire, M.K., DeWeese-Scott, C., Fong, J.H., Geer, L.Y., Geer, R.C., Gonzales, N.R., Gwadz, M., He, S., Hurwitz, D.I., Jackson, J.D., Ke, Z., Lanczycki, C.J., Liebert, C.A., Liu, C., Lu, F., Lu, S., Marchler, G.H., Mullokandov, M., Song, J.S., Tasneem, A., Thanki, N., Yamashita, R.A., Zhang, D., Zhang, N., Bryant, S.H. 2009. CDD: specific functional annotation with the Conserved Domain Database. *Nucleic Acids Res.* 37, 205–210.
- Markland, F.S., Jr., 1976. Crotalase. *Methods Enzymol.* 45, 223–236.
- Markland, F.S., Jr., 1998. Snake venoms and the hemostatic system. *Toxicon* 36, 1749–1800.
- Markland, F.S., Jr., Kettner, C., Schiffman, S., Shaw, E., Bajwa, S.S., Reddy, K.N., Kirakossian, H., Patkos, G.B., Theodor, I., Pirkle, H., 1982. Kallikrein-like activity of crotalase, a snake venom enzyme that clots fibrinogen. *Proc. Natl Acad. Sci. U.S.A.* 79, 1688–1692.
- Marrakchi, N., Zingali, R.B., Karoui, H., Bon, C., el Ayeb, M., 1995. Cerastocytin, a new thrombin-like platelet activator from the venom of the Tunisian viper *Cerastes cerastes*. *Biochim. Biophys. Acta* 1244, 147–156.
- Marrakchi, N., Barbouche, R., Guermazi, S., Karoui, H., Bon, C., El Ayeb, M., 1997. Cerastotin, a serine protease from *Cerastes cerastes* venom, with platelet-aggregating and agglutinating properties. *Eur. J. Biochem.* 247, 121–128.
- Marsh, N., Williams, V., 2005. Practical applications of snake venom toxins in haemostasis. *Toxicon* 45, 1171–1181.
- Matsui, T., Sakurai, Y., Fujimura, Y., Hayashi, I., Oh-Ishi, S., Suzuki, M., Hamako, J., Yamamoto, Y., Yamazaki, J., Kinoshita, M., Titani, K., 1998. Purification and amino acid sequence of halystase from snake venom of *Agkistrodon halys blomhoffii*, a serine protease that cleaves specifically fibrinogen and kininogen. *Eur. J. Biochem.* 252, 569–575.
- Mukherjee, A.K., 2008. Characterization of a novel pro-coagulant metalloprotease (RVBCMP) possessing α -fibrinogenase and tissue hemorrhagic activity from the venom of *Daboia russelli russelli* (Russell's viper): evidence of distinct coagulant and hemorrhagic sites in RVBCMP. *Toxicon* 51, 923–933.
- Nakashima, K., Nobuhisa, I., Deshimaru, M., Nakai, M., Ogawa, T., Shimohigashi, Y., Fukumaki, Y., Hattori, M., Sakaki, Y., Hattori, S., 1995. Accelerated evolution in the protein-coding regions is universal in crotalinae snake venom gland phospholipase A₂ isozyme genes. *Proc. Natl. Acad. Sci. U.S.A.* 92, 5605–5609.
- Nakashima, K., Ogawa, T., Oda, N., Hattori, M., Sakaki, Y., Kihara, H., Ohno, M., 1993. Accelerated evolution of *Trimeresurus flavoviridis* venom gland phospholipase A₂ isozymes. *Proc. Natl. Acad. Sci. U.S.A.* 90, 5964–5968.
- Nakayama, D., Ben Ammar, Y., Takeda, S., 2009. Crystallization and preliminary X-ray crystallographic analysis of blood coagulation factor V-activating proteinase (RVV-V) from Russell's viper venom. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* 65, 1306–1308.

- Nikai, T., Ohara, A., Komori, Y., Fox, J.W., Sugihara, H., 1995. Primary structure of a coagulant enzyme, bilineobin, from *Agkistrodon bilineatus* venom. Arch. Biochem. Biophys. 318, 89–96.
- Nishida, S., Fujimura, Y., Miura, S., Ozaki, Y., Usami, Y., Suzuki, M., Titań, K., Yoshida, E., Sugimoto, M., Yoshioka, A., Fukui, H., 1994. Purification and characterization of bothrombin, a fibrinogen-clotting serine protease from the venom of *Bothrops jararaca*. Biochemistry 33, 1843–1849.
- Nirthanan, S., Gwee, M.C.E., 2004. Three-finger α -neurotoxins and the nicotinic acetylcholine receptor, forty years on. J. Pharmacol. Sci. 94, 1–17.
- Nobuhisa, I., Nakashima, K., Deshimaru, M., Ogawa, T., Shimohigashi, Y., Fukumaki, Y., Sakaki, Y., Hattori, S., Kihara, H., Ohno, M., 1996. Accelerated evolution of *Trimeresurus okinavensis* venom gland phospholipase A₂ isozyme-encoding genes. Gene 172, 267–272.
- Nolan, C., Hall, L.S., Barlow, G.H., 1976. Ancrod, the coagulating enzyme from Malayan pit viper (*Agkistrodon rhodostoma*) venom. Methods Enzymol. 45, 205–213.
- Ogawa, T., Nakashima, K., Nobuhisa, I., Deshimaru, M., Shimohigashi, Y., Fukumaki, Y., Sakaki, Y., Hattori, S., Ohno, M., 1996. Accelerated evolution of snake venom phospholipase A₂ isozymes for acquisition of diverse physiological functions. Toxicon 34, 1229–1236.
- Ohno, M., Menez, R., Ogawa, T., Danse, J.M., Shimohigashi, Y., Fromen, C., Ducancel, F., Zinn-Justin, S., Le Du, M.H., Boulain, J.C., Tamiya, T., Menez, A., 1998. Molecular evolution of snake toxins: is the functional diversity of snake toxins associated with a mechanism of accelerated evolution? Prog. Nucleic Acid Res. Mol. Biol. 59, 307–364.
- Oyama, E., Takahashi, H., 2003. Purification and characterization of a thrombin like enzyme, ele-gaxobin II, with lys-bradykinin releasing activity from the venom of *Trimeresurus elegans* (Sakishima-Habu). Toxicon 41, 559–568.
- Paes Leme, A.F., Prezoto, B.C., Yamashiro, E.T., Bertholim, L., Tashima, A.K., Klitzke, C.F., Camargo, A.C., Serrano, S.M., 2008. *Bothrops* protease A, a unique highly glycosylated serine proteinase, is a potent, specific fibrinogenolytic agent. J. Thromb. Haemost. 6, 1363–1372.
- Pahari, S., Mackessy, S.P., Kini, M.R., 2007. The venom gland transcriptome of the Desert Massasauga Rattlesnake (*Sistrurus catenatus edwardsii*): towards an understanding of venom composition among advanced snakes (Superfamily Colubroidea). BMC Mol. Biol. 8, 115.
- Pan, H., Du, X., Yang, G., Zhou, Y., Wu, X., 1999. cDNA cloning and expression of acutin. Biochem. Biophys. Res. Commun. 255, 412–415.
- Parry MA, Jacob U, Huber R, Wisner A, Bon C, Bode, W. 1998. The crystal structure of the novel snake venom plasminogen activator TSV-PA: a prototype structure for snake venom serine proteinases. Structure 6, 1195–1206.
- Pawlak, J., Kini, R.M., 2008. Unique gene organization of colubrid three-finger toxins: complete cDNA and gene sequences of denmotoxin, a bird-specific toxin from colubrid snake *Boiga dendrophila* (Mangrove Catsnake). Biochimie 90, 868–877.
- Phillips, D.J., Swenson, S.D., Markland, F.S., Jr. 2009. Thrombin-like snake venom serine proteinases, in: Mackessy, S.P. (Ed.), Handbook of Venoms and Toxins of Reptiles. CRC Press/Taylor & Francis Group, Boca Raton, FL, pp. 139–154.
- Philipps, J., Thomalla, G., Glahn, J., Schwarze, M., Rother, J., 2009. Treatment of progressive stroke with tirofiban – experience in 35 patients. Cerebrovasc. Dis. 28, 435–438.
- Polaskova, V., Kapur, A., Khan, A., Molloy, M.P., Baker, M.S., 2010. High-abundance protein depletion: comparison of methods for human plasma biomarker discovery. Electrophoresis 31, 471–482.
- Polgár, L., 1971. On the mechanism of proton transfer in the catalysis by serine proteases. J. Theor. Biol. 31, 165–169.
- Polgár, L., Bender, M.L., 1969. The nature of general base-general acid catalysis in serine proteases. Proc. Natl. Acad. Sci. USA 64, 1335–1342.
- Rawlings, N.D., Tolle, D.P., Barrett A.J., 2004a. MEROPS: the peptidase database. Nucleic Acids Res. 32, D160–164.
- Rawlings, N.D., Tolle, D.P., Barrett A.J., 2004b. Evolutionary families of peptidase inhibitors. Biochem. J. 378, 705–716.

- Rowe, S.L., Stegemann, J.P., 2009. Microstructure and mechanics of collagen-fibrin matrices polymerized using ancrod snake venom enzyme. *J. Biomech. Eng.* 131, 061012. doi: 10.1115/1.3128673.
- Sanz, L., Gibbs, H.L., Mackessy, S.P., Calvete, J.J., 2006. Venom proteomes of closely related *Sistrurus* rattlesnakes with divergent diets. *J. Proteome Res.* 5, 2098–2112.
- Segers, K., Rosing, J., Nicolaes, G.A., 2006. Structural models of the snake venom factor V activators from *Daboia russelli* and *Daboia lebetina*. *Proteins* 64, 968–984.
- Serrano, S.M., Hagiwara, Y., Murayama, N., Higuchi, S., Mentele, R., Sampaio, C.A., Camargo, A.C., Fink, E., 1998. Purification and characterization of a kinin-releasing and fibrinogen-clotting serine proteinase (KN-BJ) from the venom of *Bothrops jararaca*, and molecular cloning and sequence analysis of its cDNA. *Eur. J. Biochem.* 251, 845–853.
- Serrano, S.M.T., Maroun, R.C. 2005. Snake venom serine proteinases: sequence homology vs. substrate specificity, a paradox to be solved. *Toxicon* 45, 1115–1132.
- Sherman, D.G., 2002. Ancrod. *Curr. Med. Res. Opin.* 18, s48–s52.
- Shieh, T.-C., Kawabata, S., Kihara, H., Ohno, M., Iwanaga, S., 1988. Amino acid sequence of a coagulant enzyme, flavoxobin, from *Trimeresurus flavoviridis* venom. *J. Biochem.* 103, 596–605.
- Shieh, T.C., Tanaka, S., Kihara, H., Ohno, M., Makisumi, S., 1985. Purification and characterization of a coagulant enzyme from *Trimeresurus flavoviridis* venom. *J. Biochem. (Tokyo)* 98, 713–721.
- Shimokawa, K., Takahashi, H., 1993a. Purification of a capillary permeability-increasing enzyme-2 from the venom of *Agkistrodon caliginosus* (Kankoku-Mamushi). *Toxicon* 31, 1213–1219.
- Shimokawa, K., Takahashi, H., 1993b. Some properties of a capillary permeability-increasing enzyme-2 from the venom of *Agkistrodon caliginosus* (Kankoku-Mamushi). *Toxicon* 31, 1221–1227.
- Silva-Junior, F.P., Guedes, H.L., Garvey, L.C., Aguiar, A.S., Bourguignon, S.C., Di Cera, E., Giovanni-De-Simone, S., 2007. BJ-48, a novel thrombin-like enzyme from the *Bothrops jararacussu* venom with high selectivity for Arg over Lys in P1: role of N-glycosylation in thermostability and active site accessibility. *Toxicon* 50, 18–31.
- Silveira, A.M.V., Magalhães, A., Diniz, C.R., Oliveira, E.B., 1989. Purification and properties of the thrombin-like enzyme from the venom of *Lachesis muta muta*. *Int. J. Biochem.* 21, 863–868.
- Smith, C.G., Vane, J.R., 2003. The discovery of captopril. *FASEB J.* 17, 788–789.
- Spyropoulos, A.C., 2008. Brave new worlds: the current and future use of novel anticoagulants. *Thromb. Res.* 123, S29-S35.
- Stocker, K.F., 1998. Research, diagnostic and medicinal uses of snake venom enzymes, in: Bailey, G.S. (Ed.), *Enzymes From Snake Venom*. Alaken Press, Fort Collins, CO, pp. 705–736.
- Stocker, K., Meier, J., 1989. Snake venom proteins in hemostasis: new results. *Folia Haematol. Int. Mag. Klin. Morphol. Blutforsch* 116, 935–953.
- Stocker, K., Barlow, G.H., 1976. The coagulant enzyme from *Bothrops atrox* venom (batroxobin). *Methods Enzymol.* 45, 214–223.
- Stocker, K., Fischer, H., Meier, J., 1982. Thrombin-like snake venom proteinases. *Toxicon* 20, 265–273.
- Sturzebecher, J., Sturzebecher, U., Markwardt, F., 1986. Inhibition of batroxobin, a serine proteinase from *Bothrops* snake venom, by derivatives of benzamidine. *Toxicon* 24, 585–595.
- Swenson, S., Markland, F.S., Jr., 2005. Snake venom fibrin(ogen)olytic enzymes. *Toxicon* 45, 1021–1039.
- Takacs, Z., Wilhelmsen, K.C., Sorota, S., 2001. Snake α -neurotoxin binding site on the Egyptian Cobra (*Naja haje*) nicotinic acetylcholine receptor is conserved. *Mol. Biol. Evol.* 18, 1800–1809.
- Tamiya, T., Ohno, S., Nishimura, E., Fujimi, T.J., Tsuchiya, T., 1999. Complete nucleotide sequences of cDNAs encoding long chain alpha-neurotoxins from sea krait, *Laticauda semifasciata*. *Toxicon* 37, 181–185.

- Tanaka, N., Nakada, H., Itoh, N., Mizuno, Y., Takanishi, M., Kawasaki, T., Tate, S., Inagaki, F., Yamashina, I., 1992. Novel structure of the N-acetylgalactosamine containing N-glycosidic carbohydrate chain of batroxobin, a thrombin-like snake venom enzyme. *J. Biochem.* 112, 68–74.
- Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22, 4673–4680.
- Tsai, I.-H., Wang, Y.-M., 2001. Identification of geographic variations and cloning of venom proteins of *Trimeresurus stejnegeri*: serine proteases and phospholipases. EMBL/GenBank/DDBJ databases.
- Utaisinchareon, P., Mackessy, S.P., Miller, R.A., Tu, A.T. 1993. Complete primary structure and biochemical properties of gilatoxin, a serine protease with kallikrein-like and angiotensin-degrading activities. *J. Biol. Chem.* 268, 21975–21983.
- Wang, Y.M., Wang, S.R., Tsai, I.H., 2001. Serine protease isoforms of *Deinagkistrodon acutus* venom: cloning, sequencing and phylogenetic analysis. *Biochem. J.* 354, 161–168.
- Wei, J.N., Wang, Q.C., Liu, G.F., Ezell, E.L., Quast, M.J., 2004. Reduction of brain injury by antithrombotic agent acutobin after middle cerebral artery ischemia/reperfusion in the hyperglycemic rat. *Brain Res.* 1022, 234–243.
- Weldon, C.L., Mackessy, S.P., 2010. Biological and proteomic analysis of venom from the Puerto Rican Racer (*Alsophis portoricensis*: Dipsadidae). *Toxicon* 55, 558–569.
- White, J., 2005. Snake venoms and coagulopathy. *Toxicon* 45, 951–967.
- White, J., 2009. Envenomation: prevention and treatment in Australia, in: Mackessy, S.P. (Ed.), *Handbook of Venoms and Toxins of Reptiles*. CRC Press/Taylor & Francis Group, Boca Raton, FL, pp. 423–451.
- Wisner, A., Braud, S., Bon, C., 2001. Snake venom proteinases as tools in hemostasis studies: structure-function relationship of a plasminogen activator purified from *Trimeresurus stejnegeri* venom. *Haemostasis* 31, 133–140.
- Yamamoto, C., Tsuru, D., Oda-Ueda, N., Ohno, M., Hattori, S., Kim, S.T., 2002. Flavoxobin, a serine protease from *Trimeresurus flavoviridis* (habu snake) venom, independently cleaves Arg726–Ser727 of human C3 and acts as a novel, heterologous C3 convertase. *Immunology* 107, 111–117.
- Xu, G., Liu, X., Zhu, W., Yin, Q., Zhang, R., Fan, X., 2007. Feasibility of treating hyperfibrinogenemia with intermittently administered batroxobin in patients with ischemic stroke/transient ischemic attack for secondary prevention. *Blood Coagul. Fibrinolysis* 18, 193–197.
- Zeng, Z., Xiao, P., Chen, J., Wei, Y., 2009. Are batroxobin agents effective for perioperative hemorrhage in thoracic surgery? A systematic review of randomized controlled trials. *Blood Coagul. Fibrinolysis* 20, 101–107.
- Zhang, B., Liu, Q., Yin, W., Zhang, X., Huang, Y., Luo, Y., Qiu, P., Su, X., Yu, J., Hu, S., Yan, G., 2006. Transcriptome analysis of *Deinagkistrodon acutus* venomous gland focusing on cellular structure and functional aspects using expressed sequence tags. *BMC Genomics*, 7, 152.
- Zhang, L., Lu, S.H., Li, L., Tao, Y.G., Wan, Y.L., Senga, H., Yang, R., Han, Z.C., 2009. Batroxobin mobilizes circulating endothelial progenitor cells in patients with deep vein thrombosis. *Clin. Appl. Thromb. Hemost.* 2009 Oct 13. [Epub ahead of print]
- Zhang, Y., Gao, R., Lee, W.-H., Zhu, S.-W., Xiong, Y.-L., Wang, W.-Y., 1998. Characterization of a fibrinogen-clotting enzyme from *Trimeresurus stejnegeri* venom, and comparative study with other venom proteases. *Toxicon* 36, 131–142.
- Zhu, Z., Liang, Z., Zhang, T., Zhu, Z., Xu, W., Teng, M., Niu, L., 2005. Crystal structures and amidolytic activities of two glycosylated snake venom serine proteinases. *J. Biol. Chem.* 280:10524–10529.