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

Species	Name	Activity	UniProtKB accession #	Mass	Inhibitors	References
Agkistrodon bilineatus	Bilineobin	Fibrinogenolytic (Aα and Bβ)	Q9PSN3	57 kDa 26,479 (deglyco-sylated	Heparin + Dithiothreitol + TLCK + Antithrombin III + Leupeptin + Argatroban - Hirudin -	Komori et al. (1993); Nikai et al. (1995)
Agkistrodon contortrix contortrix	Contortrixobin	Fibrinogenolytic (Bβ); Factor V activation; Factor XIII activation	P82981	26 kDa	Benzamidine + DAPI + Antithrombin III -	Amiconi et al. (2000)
Bothrops atrox	Batroxobin	Fibrinogenolytic (Aα)	P04971	41.5 kDa	Benzamidine + α2-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)
Bothrops atrox	Thrombocytin	Fibrinogenolytic (Aα); Factor VIII activation	none	36 kDa	Pro-Phe-ArgCH_2Cl + PRCK + SBTI + Antithrombin III + Heparin + FPRCK + FARCK +	Castro et al. (2004); Kirby et al. (1979); Serrano and Maroun (2005)

Table 30.1 (continued)

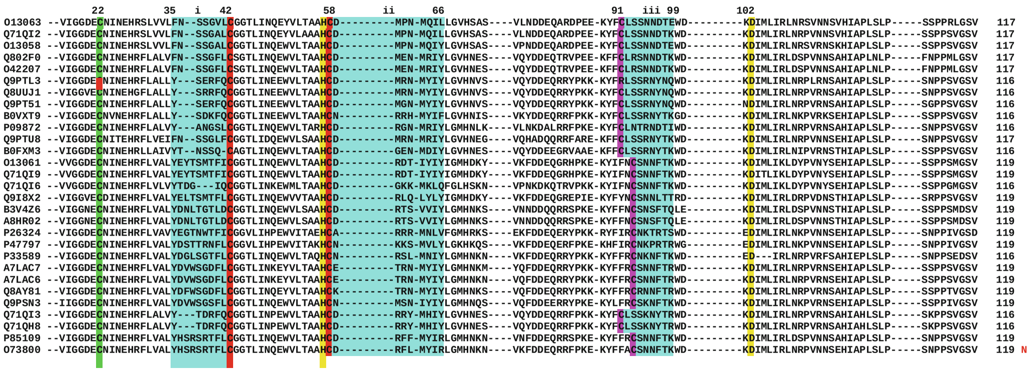
Species	Name	Activity	UniProtKB accession #	Mass	Inhibitors	References
Bothrops jararaca *Bothrops jararaca*	KN-BJ	Fibrinogenolytic (Aα); Kinin release	O13069	38 kDa	Benzamine derivatives +	Serrano et al. (1998)

Table 30.1 (continued)

Species	Name	Activity	UniProtKB accession #	Mass	Inhibitors	References
Crotalus adamanteus	Crotalase	Fibrinogenolytic (Aα); Kinin release	Q9PS55	32.7 kDa	TLCK + Pro-Phe-ArgCH_2Cl + PFRCK + AFRCK + GVRCK + IPRCK + AFKCK + Tetranitromethane + 2-mercaptoethanol + Hirudin - TPCK -	Markland (1976, 1998); Markland et al. (1982); Henschen-Edman et al. (1999)
Crotalus durissus terrificus	Gyroxin-like B2.1	Fibrinogenolytic (Aα); Gyratory	Q58G94	32 kDa (26.6 no glyco)	Dithiothreitol +	Alexander et al. (1988)
Deinagkistrodon acutus	Acutin	Coagulant	Q9YGS1	38 kDa	x	Pan et al. (1999)
Deinagkistrodon acutus	Venom serine proteinase Dav-PA	Fibrinogenolytic; amidolytic	Q9I8X1	28,032	x	Zhu et al. (2005)
Deinagkistrodon acutus	Acutobin	Fibrinogenolytic (Aα)	Q9I8X2	40 kDa (28.8 no glyco)	x	Wang et al. (2001)Acutob/84(g).4(a8s)o0

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 produced 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 Aα or/and Bβ chains will be considered as TLEs. A



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              22           35  i   42              58            ii  66                           91  iii 99           102
O13063 --VIGGDECNINEHRSLVVLFN--SSGVLCGGTLINQEYVLTAAHCD---------MPN-MQILLGVHSAS----VLNDDEQARDPEE-KYFCLSSNNDTEWD---------KDIMLIRLNRSVNNSVHIAPLSLP-----SSPPRLGSV   117
Q71QI2 --VIGGDECNINEHRSLVVLFN--SSGALCGGTLINQEYVLAAAHCD---------MPN-MQILLGVHSAS----VLNDDEQARDPEE-KYFCLSSNNDTKWD---------KDIMLIRLNRPVNNSVHIAPLSLP-----SSPPSVGSV   117
O13058 --VIGGDECNINEHRSLVVLFN--SSGALCGGTLINQEWVLTAAHCD---------MPN-MQIYLGVHSAS----VPNDDEQARDPEE-KYFCLSSNNDTEWD---------KDIMLIRLNRSVRNSKHIAPLSLP-----SSPPSVGSV   117
Q802F0 --VIGGDECNINEHRFLALVFN--SSGFLCSGTLINQEWVLTAAHCD---------MEN-MRIYLGVHNES----VQYDDEQTRVPEE-KFFCLRSNNDTKWD---------KDIMLIRLDSPVNNSAHIAPLNLP-----FNPPMLGSV   117
O42207 --VIGGDECNINEHRFLALVFN--SSGFLCSGTLINQEWVLTAAHCD---------MEN-MRIYLGVHNES----VQYDDEQTRVPEE-KFFCLRSNNDTKWD---------KDIMLIRLDSPVNNSAHIAPLNLP-----FNPPMLGSV   117
Q9PTL3 --VIGGDE NINEHRFLALLY---SERFQCGGTLINEEWVLTAAHCD---------MRN-MYIYLGVHNVS----VQYDDEQRRYPKK-KYFRLSSRNYNQWD---------KDIMLIRLNRPLRNSAHIAPLSLP-----SNPPSVGSV   116
Q8UUJ1 --VIGGVECNINEHGFLALLY---SRRFQCGGTLINEEWVLTAAHCD---------MRN-MYIYLGVHNVS----VQYDDEQRRYPKK-KYFCLSSRNYNQWD---------KDIMLIRLNRPVRNSAHIAPLSLP-----SNPPSVGSV   116
Q9PT51 --VIGGDECNINEHRFLALLY---SERFQCGGTLINEEWVLTAAHCD---------MGN-MYIYLGVHNVS----VQYDDEQRRYPKK-KYFCLSSRNYNQWD---------NDIMLIRLNRPVRNSAHIAPLSLP-----SGPPSVGSV   116
B0VXT9 --VIGGDECNVNEHRFLALLY---SDKFQCGGTLINEEWVLTAAHCN---------RRH-MYIFLGVHNIS----VKYDDEQRRFPKK-KYFCLSSRNYTKGD---------KDIMLIRLNKPVRKSEHIAPLSLP-----SSPPSVGSV   116
P09872 --VIGGDECNINEHRFLALVY---ANGSLCGGTLINQEWVLTARHCD---------RGN-MRIYLGMHNLK----VLNKDALRRFPKE-KYFCLNTRNDTIWD---------KDIMLIRLNRPVRNSAHIAPLSLP-----SNPPSVGSV   116
Q9PTU8 --VIGGDECNITEHRFLVEIFN--SSGLFCGGTLIDQEWVLSAAHCD---------MRN-MRIYLGVHNEG----VQHADQQRRFARE-KFFCLSSRNYTKWD---------KDIMLIRLNRPVNNSEHIAPLSLP-----SNPPSVGSV   117
B0FXM3 --VIGGDECNINEHRLLAIVYT--NSSQ-CAGTLINQEWVLTAAHCD---------GEN-MDIYLGVHNES----VQYDDEEGRVAAE-KFFCLSSRNYTKWD---------KDIMLIRLNIPVRNSTHIAPLSLP-----SSPPSVGSV   116
O13061 --VVGGDECNINEHRFLVALYEYTSMTFICGGTLINQEWVLTAAHCD---------RDT-IYIYIGMHDKY----VKFDDEQGRHPKE-KYIFNCSNNFTKWD---------KDIMLIKLDYPVNYSEHIAPLSLP-----SSPPSMGSV   119
Q71QI9 --VVGGDECNINEHRFLVALYEYTSMTFICGGTLINQEWVLTAAHCD---------RDT-IYIYIGMHDKY----VKFDDEQGRHPKE-KYIFNCSNNFTKWD---------KDITLIKLDYPVNYSEHIAPLSLP-----SSPPSMGSV   119
Q71QI6 --VVGGDECNINEHRFLVLVYTDG---IQCGGTLINKEWMLTAAHCD---------GKK-MKLQFGLHSKN----VPNKDKQTRVPKK-KYIFNCSNNFTKWD---------KDIMLIKLDYPVNYSEHIAPLSLP-----SSPPGMGSV   116
Q9I8X2 --VIGGVECDINEHRFLVALYELTSMTFLCGGTLINQEWVVTAAHCD---------RLQ-LYLYIGMHDKY----VKFDDEQGREPIE-KYFYNCSNNLTTRD---------KDIMLIRLDRPVDNSTHIAPLSLP-----SRPPSVGSV   119
B3V4Z6 --VIGGNECNINEHRFLVALYDNLTGTLDCGGTLINQEWVLSAAHCD---------RTS-VVIYLGMHNKS----VNNDDQQRRSPKE-KYFFNCSNSFTQLE---------KDIMLIRLDSPVNNSTHIAPLSLP-----SSPPSMDSV   119
A8HR02 --VIGGNECNINEHRFLVALYDNLTGTLDCGGTLINQEWVLSAAHCD---------RTS-VVIYLGMHNKS----VNNDDQQRRSPKE-KYFFNCSNSFTQLE---------KDIMLIRLDSPVNNSTHIAPLSLP-----SSPPSMDSV   119
P26324 --VIGGDECNINEHRFLVAVYEGTNWTFICGGVLIHPEWVITAEHCA---------RRR-MNLVFGMHRKS----EKFDDEQERYPKK-RYFIRCNKTRTSWD---------EDIMLIRLNKPVNNSEHIAPLSLP-----SNPPIVGSD   119
P47797 --VIGGDECNINEHRFLVALYDSTTRNFLCGGVLIHPEWVITAKHCN---------KKS-MVLYLGKHKQS----VKFDDEQERFPKE-KHFIRCNKPRTRWG---------EDIMLIRLNKPVNNSEHIAPLSLP-----SNPPIVGSV   119
P33589 --VIGGDECNINEHRFLVALYDGLSGTFLCGGTLINQEWVLTAQHCN---------RSL-MNIYLGMHNKN----VKFDDEQRRYPKK-KYFFRCNKNFTKWD---------ED---IRLNRPVRFSAHIEPLSLP-----SNPPSEDSV   116
A7LAC7 --VIGGDECNINEHRFLVALYDVWSGDFLCGGTLINKEYVLTAAHCE---------TRN-MYIYLGMHNKM----YQFDDEQRRYPKK-KYFFRCSNNFTRWD---------KDIMLIRLNRPVRNSEHIAPLSLP-----SSPPSVGSV   119
A7LAC6 --VIGGDECNINEHRFLVALYDVWSGDFLCGGTLINKEYVLTAAHCE---------TRN-MYIYLGMHNKN----VQFDDEQRRYPKK-KYFFRCSNNFTRWD---------KDIMLIRLNRPVRNSEHIAPLSLP-----SSPPSVGSV   119
Q8AY81 --VIGGDECNINEHRFLVALYDFWSGDFLCGGTLINQEYVLTAAHCK---------TRN-MYIYLGMHNKS----VQFDDEQRRYPKK-KYFFRCRNNFTRWD---------KDIMLIRLNRPVRNSAHIAPLSLP-----SSPPTVGSV   119
Q9PSN3 --IIGGDECNINEHRFLVALYDVWSGSFLCGGTLINQEWVLTAAHCN---------MSN-IYIYLGMHNQS----VQFDDEERRYPKE-KYLFRCSKNFTKWD---------KDIMLIRLNKPVRNSEHIAPLSLP-----SSPPIVGSV   119
Q71QI3 --VIGGDECNINEHRFLALVY---TDRFQCGGTLINPEWVLTAAHCD---------RRY-MHIYLGVHNES----VQYDDEQRRFPKK-KYFCLSSKNYTRWD---------KDIMLIRLNRPVRNSAHIAHLSLP-----SKPPSVGSV   116
Q71QH8 --VIGGDECNINEHRFLALVY---TDRFQCGGTLINPEWVLTAAHCD---------RRY-MHIYLGVHNES----VQYDDEQRRFPKK-KYFCLSSKNYTRWD---------KDIMLIRLNRPVRNSAHIAHLSLP-----SKPPSVGSV   116
P85109 --VIGGDECNINEHRFLVALYHSRSRTFLCGGTLINQEWVLTAAHCD---------RFF-MYIRLGMHNKN----VNFDDEQRRSPKE-KYFFRCSNNFTKWD---------KDIMLIRLDSPVNNSAHIAPLSLP-----SNPPSVGSV   119
O73800 --VIGGDECNINEHRFLVALYHSRSRTFLCGGTLINQEWVLTAAHCD---------RFL-MYIRLGMHNKN----VKFDDEQRRFPKE-KYFFACSNNFTKWD---------KDIMLIRLNRPVNNSEHIAPLSLP-----SNPPSVGSV   119 N 6776538 4385 2324 67451 19 14118 055 87451 273333 290
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P81824 --VVGGRPCKINVHRSLVLLYNS--SSLLCSGTLINQEWVLTAAHCD---------SKN-FKMKLGVHSIK----IRNKNERTRHPKE-KFICPNRKKDDVLD---------KDIMLIRLNRPVSNSEHIAPLSLP-----SSPPSVGSV   117
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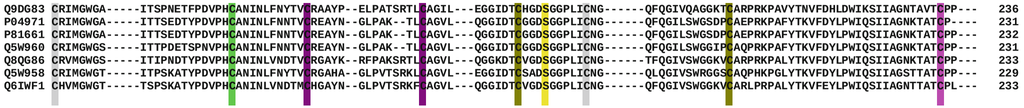

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P04971 CRIMGWGA-----ITTSEDTYPDVPHCANINLFNNTVCREAYN--GLPAK--TLCAGVL---QGGIDTCGGDSGGPLICNG------QFQGILSWGSDPCAEPRKPAFYTKVFDYLPWIQSIIAGNKTATCP----   231
P81661 CRIMGWGA-----ITTSEDTYPDVPHCANINLFNNTVCREAYN--GLPAK--TLCAGVL---QGGIDTCGGDSGGPLICNG------QFQGILSWGSDPCAEPRKPAFYTKVFDYLPWIQSIIAGNKTATCPP---   232
Q5W960 CRIMGWGS-----ITTPDETSPNVPHCANINLFNNTVCREAYN--GLPAK--TLCAGVL---QGGIDTCGGDSGGPLICNG------QFQGILSWGGIPCAQPRKPAFYTKVFDYLPWIQSIIAGNKTATCPP---   231
Q8QG86 CRVMGWGS-----ITIPNDTYPDVPHCANINLVNDTVCRGAYK--RFPAKSRTLCAGVL---QGGKDTCVGDSGGPLICNG------TFQGIVSWGGKVCARPRKPALYTKVFDYLPWIQSIIAGNKTATCPP---   233
Q5W958 CRIMGWGT-----ITPSKATYPDVPHCANINLFNYTVCRGAHA--GLPVTSRKLCAGVL---EGGIDTCSADSGGPLICNG------QLQGIVSWRGGSCAQPHKPGLYTKVFDYLPWIQSIIAGSTTATCPP---   229
Q6IWF1 CHVMGWGT-----TSPSKATYPDVPHCANINLVNDTMCHGAYN--GLPVTSRKFCAGVL---QGGIDTCVGDSGGPLICNG------QFQGIVSWGGKVCARLPRPALYTKVFEYLPWIQSIIAGNTTATCPL---   233
```

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 Structure and Structural Prediction

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*

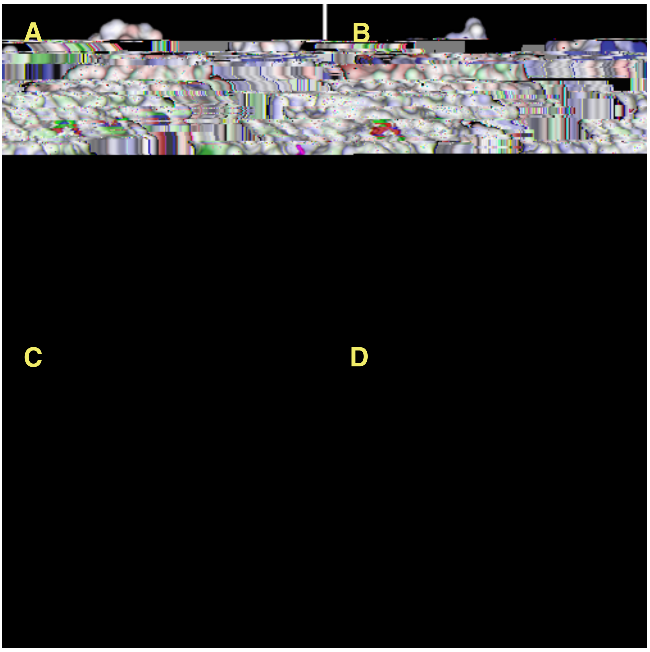

Fig. 30.2 Space-filling models of serine proteases. (**a**) Bovine trypsin (PDB ID: 1AQ7); (**b**) Bovine chymotrypsin B (PDB ID: 1DLK); (**c**) Venom serine proteinase Dav-PA (*Deinagkistrodon*

Mechanisms of Evolution of SV-TLE

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 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 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 Relationship

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

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 feapatients or in ninety day mortality levels, and the incidence of symptomatic intracrabatroxobin 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, of the creative ways in which venom TLEs are being utilized in both applied clinical and basic research.

Future Potential for SV-TLE

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.

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

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

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

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

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