Serine Proteases Affecting Blood Coagulation and Fibrinolysis from Snake Venoms

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Key Words

Procoagulant · Anticoagulant · Thrombin-like enzyme · Protein C activator · Factor V activator · Prothrombin activator · Plasminogen activator

Abstract

Snake venom proteases, in addition to their contribution to the digestion of the prey, affect various physiological functions. They affect platelet aggregation, blood coagulation, fibrinolysis, complement system, blood pressure and nervous system. This review provides a ready reference for serine proteases that interfere in blood coagulation and fibrinolysis. They exhibit their activity by activation of specific zymogens of coagulation factors. These serine proteases serve as tools to study molecular details in the activation of specific factors involved in coagulation and fibrinolytic cascades and are useful in treating various thrombotic and hemostatic conditions.

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Introduction

Snake venoms are cocktails of pharmacologically active proteins and peptides. They are used as offensive weapons in immobilizing, killing and digesting the preys

[1, 2]. Some of these toxins exhibit various enzymatic activities, whereas others are nonenzymatic proteins. Most enzymes found in snake venoms are hydrolases that breakdown biological molecules including proteins, nucleic acids and phospholipids. In addition to their contribution to the digestion of the prey, a number of these hydrolases exhibit specific pharmacological effects. Snake venoms, particularly crotalid and viperid venoms, are rich sources of serine proteases and metalloproteases. These proteases affect various physiological functions. They affect platelet aggregation, blood coagulation, fibrinolysis, complement system, blood pressure and nervous system. This short review describes a number of serine proteases that interfere in blood coagulation and fibrinolysis.

Procoagulant Proteases

All the procoagulants from snake venoms characterized to date are proteases; they activate a zymogen of specific coagulation factor in the coagulation cascade and hasten clot formation. Some venom proteases also activate the protein cofactor, factor V. For example, RVV-V (*Daboia russelli*) and thrombocytin (*Bothrops atrox*) activate factor V, the cofactor in the prothrombinase complex (reviewed in [3, 4]).

Factor VII Activators

So far, snake venom proteins that specifically activate only factor VII are not known. However, oscutarin, a group C prothrombin activator (see below) from *Oxyuranus scutellatus* venom activates factor VII [5]. Gel electrophoresis analysis of the cleavage products indicated that activation occurred at a site similar to the natural cleavage site. The activation of factor VII, similar to that of prothrombin, was greatly potentiated by Ca^{2+} ions and phospholipids, and was not dependent upon the presence of its factor Va-like subunit [6]. The structural details of oscutarin and related proteins are given below.

Factor X Activators

Venoms from Viperidae, Crotalidae and Elapidae contain a variety of proteases capable of activating factor X (for reviews, see [6, 7]). They are either metalloproteases or serine proteases. The factor X activator from Russell's viper (*Daboia russelli*) venom (RVV-X) is a metalloprotease that has been well characterized (for details, see [6]). So far, a few serine proteases that activate factor X have also been isolated from the venoms of *Ophiophagus hannah*, *Bungarus fasciatus*, *Cerastes cerastes* and *Cerastes vipera* (for references, see the review [6]). These serine proteases vary in their size. However, currently no structural details are available on any of these proteins.

Prothrombin Activators

A large number of snake species contain prothrombin activators in their venoms (for an inventory, see [8] and for reviews, see [9–14]). Based on their structural properties, functional characteristics and cofactor requirements, they have been categorized into four groups [8, 15–16]. Groups A and B prothrombin activators are metalloproteases and they convert thrombin to meizothrombin. In contrast, groups C and D prothrombin activators are serine proteases and they convert prothrombin to mature thrombin. Here, I will discuss some of the salient features of only these two latter groups. They have been found so far only in the venoms of Australian elapids. For more details, readers are advised to read recent reviews on prothrombin activators [12–14].

Group C Prothrombin Activators

This group of serine proteases requires only Ca^{2+} ions and negatively charged phospholipids, but not factor Va, for maximal activity [15]. They have been purified and characterized from *Oxyuranus scutellatus* [17–19] and *Pseudonaja textilis* venoms [20–23]. The native activators have a molecular mass of ~ 300 kDa and consist of a

factor Xa-like catalytic subunit (60 kDa) and a factor Va-like cofactor subunit (~ 200 kDa). The two chains of the factor Xa-like catalytic subunit are disulfide-linked, whereas the two chains of the factor Va-like cofactor are held together by non covalent interactions. The catalytic subunit, similar to factor Xa, has weak catalytic activity in isolation, which is greatly stimulated by the presence of the factor Va-like subunit [19, 21]. These activators cleave at both $\text{Arg}_{271}\text{-Thr}_{272}$ and $\text{Arg}_{320}\text{-Ile}_{321}$ bonds of prothrombin [19], converting it to mature thrombin, in contrast to groups A and B activators, which only convert prothrombin to meizothrombin. We have shown that the group C prothrombin activators structurally and functionally resemble the mammalian factor Xa-Va complex [21–23].

Group D Prothrombin Activators

The activities of these serine proteases are strongly stimulated by Ca^{2+} ions, factor Va and negatively charged phospholipid vesicles [24–27]. They are serine proteases with molecular mass ranging from 45 to 47 kDa [28, 29]. They have two chains held together by disulfide bonds. The serine protease active site is located in the heavy chain [27, 28].

They exhibit potent procoagulant effects, comparable to mammalian factor Xa, through activation of prothrombin [27, 28]. Similar to factor Xa, they cleave prothrombin at two sites, $\text{Arg}_{271}\text{-Thr}_{272}$ and $\text{Arg}_{320}\text{-Ile}_{321}$. Their poor activator activity is stimulated by more than a million-fold by negatively charged phospholipids, factor Va and Ca^{2+} ions. They also hydrolyze factor Xa-specific chromogenic substrates [27, 28]. Recently, we determined the complete amino acid sequences of trocarin D [28] and hopsarin D [29]. They share high identity (53–60%) and similarity (62–70%), and hence similar domain architecture, with factor Xa [28, 29]. Their light chains consist of an N-terminal Gla domain (residues 1–39), followed by two epidermal growth factor-like domains, EGF-I (residues 50–81) and EGF-II (residues 89–124). Thus, group D venom prothrombin activators are true structural and functional homologues of blood coagulation factors [28, 29].

Thrombin-Like Enzymes

The thrombin-like enzymes (TLEs) are widely distributed within several pit viper genera (*Agkistrodon*, *Bothrops*, *Crotalus*, *Lachesis* and *Trimeresurus*), as well as some true vipers (*Bitis* and *Cerastes*) and the colubrid, *Dispholidus typus* (for an inventory and reviews, see [30–32]). They are single-chain serine proteases (for example,

see [33]), except for the enzyme from *Cerastes cerastes*, which is reported to consist of two identical disulfide-linked chains [34]. These serine proteases have a molecular mass of 26–33 kDa. Some of them are glycoproteins. They share a high degree of sequence identity among themselves (~67%). However, they show less than 40% similarity with human thrombin. The presence of Asp 189 in all the TLEs explains their cleavage of arginyl bonds in fibrinogen, as this is the specificity pocket residue common to all serine proteases that hydrolyze peptide bonds after basic residues [32]. They preferentially release either fibrinopeptide A or B, rarely both with equal efficiency, unlike thrombin [31, 35]. Secondary cleavages are also often seen. They also show a fair degree of species specificity in the efficiency of fibrinogen conversion. Classical low-molecular-weight serine protease inhibitors inhibit TLEs, but most are not inhibited by thrombin inhibitors like antithrombin III and hirudin [31, 35, 36]. They act on blood plasma and induce friable and translucent clots presumably due to lack of crosslinking of fibrin by factor XIIIa. TLEs often also act on coagulation factor XIII, but appear to degrade rather than activate it [36]. Unlike thrombin, TLEs do not activate other coagulation factors [35]. Thus, although TLEs ‘resemble’ thrombin to an extent, they are structurally and functionally dissimilar to the coagulation factor [12, 31, 36]. These unique properties enable their clinical use as defibrinogenating agents; for example, ancrod (Arvin®; *Calloselasma rhodostoma*) and batroxobin (Defibrase®; *Bothrops atrox moojeni* [37]) (reviewed in [37, 38]). Since the fibrin formed is not cross-linked, it is readily degraded by the fibrinolytic system. TLEs are clinically well tolerated with no or only minimal side effects [31].

Anticoagulant Proteases

Anticoagulants from snake venoms prolong clot formation; they are enzymes, such as serine and metalloproteases, or nonenzymatic proteins, such as C-type lectin-related proteins and three-finger toxins. The anticoagulant effects of phospholipase A₂ enzymes, metalloproteases and nonenzymatic proteins are beyond the scope of this review and interested readers should consult recent reviews on these topics [39–43].

Protein C Activators

Protein C is an anticoagulant protein circulating in the blood as zymogen. Upon activation by thrombin, activated protein C degrades factors V/Va and VIII/VIIIa

and releases a tissue-type plasminogen activator. It also stimulates fibrinolysis through its interaction with plasminogen activator inhibitor [44–46]. A number of snake venoms specifically activate protein C [47]. Venoms from snake species belonging *Agkistrodon* genus (*A. contortrix contortrix*, *A. c. mokasen*, *A. c. pictigaster*, *A. piscivorus*, *A. p. leucostoma*, *A. halys halys*, *A. blomhoffi ussuriensis* and *A. bilineatus*) contain protein C activators. They have been purified from *A. c. contortrix* [47–52], *A. bilineatus* [53], *A. h. halys* [54], and *A. blomhoffi ussuriensis* [55]. They are glycoproteins with a molecular mass of ~36–40 kDa. These serine proteases activate protein C at low salt concentration in the absence of Ca²⁺ ions. High salt and Ca²⁺ ions inhibit their ability to activate protein C [49, 51, 54]. So far, amino acid sequence of only the protein C activator from *A. c. contortrix* venom has been determined [56]. Protein C activators are used as diagnostic agents in the estimation of protein C [57, 58]. They prolong clotting times [47, 48] and thrombus formation in the arterio-venous shunt [55] in vivo.

Fibrinolytic Proteases

Physiologically, plasmin plays a pivotal role in fibrinolysis. Some venom proteases contribute to fibrinolysis directly by the activation of plasminogen to plasmin (see below). Metalloproteases that digest and degrade fibrinogen and fibrin are beyond the scope of this review.

Plasminogen Activators

Plasminogen activators have been purified from the venoms of *Trimeresurus stejnegeri* (TSV-PA) [59], *Agkistrodon halys* (Halys-PA) [60] and *Lachesis muta muta* (LV-PA) [61]. These serine proteases are acidic glycoproteins with a molecular mass of 33 kDa. TSV-PA activates plasminogen by a single cleavage at the peptide bond Arg₅₆₁-Val₅₆₂ [59]. Based on the molecular model and crystal structure [62], Bon and coworkers [63–65] have studied the structure-function relationships of TSV-PA. They have shown that Phe193 plays an important role in binding to the substrate plasminogen and the inhibitors [64, 65]. Replacement of Phe193 by Gly results in 8–9-fold increased activity on plasminogen [64]. The mutant is 100-fold more susceptible to plasminogen activator inhibitor 1 (PAI-1) and α₂-antiplasmin [65]. In addition, 37-loop of TSV-PA plays a crucial role in its resistance to inhibition by protease inhibitors [65] (for details, see the review [66]).

Summary and Future Prospects

Snake venom serine proteases and their complexes have evolved by gene duplication and accelerated evolution [67, 68] to activate 'critical' proteins and interfere in

the coagulation cascade. They contribute to venom toxicity. However, a number of these proteases are being used in diagnostics as well as clinical treatment. Further, their studies contribute to our understanding of structure-function relationships of blood coagulation factors.

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