ISSN 1996-3351

Asian Journal of Biological Sciences

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OPEN ACCESS **Asian Journal of Biological Sciences**

ISSN 1996-3351
DOI: 10.3923/ajbs.2019.396.403

Review Article
Major Enzymes from Snake Venoms: Mechanisms of Action and Pharmacological Applications

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Abstract
Biochemically, snake venoms are complex mixtures of pharmacologically active proteins and polypeptides. Some of these proteins exhibit enzymatic activities, whereas others are non-enzymatic. The most common enzymes in snake venoms are phospholipase A_2s, serine proteinases, metalloproteinases, acetylcholinesterases, L-amino acid oxidases and hyaluronidases. This review was aimed at presenting the salient features of these enzymes, their structures, mechanisms of action and some pharmacological applications. The activities of snake venom enzymes are considered to be target specific and mimic the whole venom poisoning by exhibiting a wide variety of toxic effects. These enzymes are now target interest to researchers as they can be employed in the treatment of certain clinical conditions. Phospholipases A2 and L-amino acid oxidases are used in the treatment of certain cancers. Serine proteinases are employed in the treatment of thrombocytopenia, thrombosis and acute ischemic stroke. They also serve as important tools in the study of hemostasis and are clinically used for clotting assays, diagnosis, study of platelet function, as defibrinogenating agents to investigate dysfibrinogenemias. Metalloproteinases can be used for treating neuro-degenerative disorders such as Parkinson's and Alzheimer's. Hyaluronidases have been used as diffusion promoters for active substances like drugs, the treatment of hyaluronan-induced diseases like cancers and in the aesthetic medicine. Some of these enzymes exhibit multiple pharmacological functions, hence, they are of significant importance in developing therapeutic prototypes and lead agents for various human diseases and ailments due to their higher catalytic efficiency, heat stability and resistance to proteolysis as well as their abundance compared with non-venom homologs.

Key words: Snakes, venoms, enzymes, toxicity, pharmacological applications

Received: December 10, 2018 Accepted: February 01, 2019 Published: June 15, 2019

Citation: Ibrahim Sani, Rabi'u Aliyu Umar, Sanusi Wara Hassan, Umar Zaki Faruq, Abubakar Abdulhamid, Fatima Bello and Isah Musa Fakai, 2019. Major enzymes from snake venoms: Mechanisms of action and pharmacological applications. Asian J. Biol. Sci., 12: 396-403.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Snake venoms have evolved into complex mixtures of pharmacologically active proteins and peptides that exhibit potent, lethal and debilitating effects to assist in prey capture[1]. Snakes use their venoms as offensive weapons in incapacitating and immobilizing their prey (the primary function), as defensive tools against their predators (the secondary function) and to aid in digestion[1,2]. Biochemically, snake venoms are complex mixtures of pharmacologically active proteins and polypeptides. All of them in concert help in immobilizing the prey. A large number of protein toxins have been purified and characterized from snake venoms[2] and snake venoms typically contain from 30 to over 100 protein toxins. Some of these proteins exhibit enzymatic activities, whereas, several others are non-enzymatic proteins and polypeptides[3].

The most common enzymes in snake venoms are phospholipase A_2s (PLA2s), serine proteinases, metalloproteinases, acetylcholinesterases (AChEs), L-amino acid oxidases and hyaluronidases[1,4]. Snake venom enzymes are catalytically more active than their counterparts. In general they are more heat stable and more resistant to proteolysis due to the presence of additional disulfide bridges[5]. Some of these enzymes exhibited exquisite substrate specificity, while others are more promiscuous[6]. In this review, we present the salient features of the major classes of snake venom enzymes, their structures, mechanisms of action and pharmacological applications. The inhibition of the enzymes by synthetic and natural inhibitors is also highlighted.

SNAKE VENOM ENZYMES

Phospholipases A2 (PLA2s): The PLA2s (phosphatide 2-acylhydrolase, EC 3.1.14) represent a superfamily of lipolytic enzymes which specifically catalyze the hydrolysis of the ester bond at the sn-2 position of glycerophospholipids resulting in the generation of fatty acid (arachidonate) and lysophospholipids[4]. The PLA2 superfamily consists of about 15 groups which are further subdivided into several subgroups, all of which display differences in terms of their structural and functional specificities. However, the 4 main types or classes of PLA2s are the secreted (sPLA2s), the cytosolic (cPLA2s), the Ca^{2+}-independent (iPLA2s) and the lipoprotein-associated (LpPLA2s) phospholipases[5] A2.

Venom PLA2s belong to class I (terrestrial elapids and sea snake venoms), II (viperid snake venoms) and III (bee and lizard venoms), among the growing family of secreted PLA2s. According to the recent phylogenetic study, snake venom PLA2s can be classified into two groups based on their evolutionary derivation, (1) The calcium-dependent catalytically active enzymes (Asp49-, Asn49- and Gln49-PLA2s) and (2) The catalytically inactive PLA2s that exert their effects through a calcium-independent mechanism (Lys49-, Arg49- and some Asp49-PLA2s)[5].

Snake venom PLA2s are considered to be target specific and mimic the whole venom poisoning by exhibiting a wide variety of toxic effects. Type II are the chief toxins responsible for the expression of inflammatory, vasodilating and vasoconstriction mediators including prostaglandins, histamine, kinins, eicosanoids, platelet activating factor, catecholamines, dopamine, nitric oxide and endothelins during envenomation[1,6] leading to systemic toxicity. In addition, type I PLA2s are also involved in exerting several additional effects including cardiotoxicity, myotoxicity, pre or postsynaptic neurotoxicity, edema, hemolysis, hypotension, convulsion and can also lead to platelet aggregation inhibition and anti-coagulation[7].

Inhibition of PLA2: The binding affinities of all known ligands of PLA2 are in the range 10^{-8} to 10^{-4} M, which makes them poor to moderate candidates as drugs. Examination of the structures, PLA2 complexed with the known ligands showed that the poor potency can be attributed to the fact that these compounds are able to occupy only a few of the subsites within the overall substrate binding space, hence, generating only a limited number of interactions with the protein. Thus, keeping the stereochemical features of the subsites in the substrate binding site in mind, there is an immense possibility to design highly potent inhibitors[8].

Inhibition of PLA2 by natural compounds: There have been numerous reports on natural compounds inhibiting PLA2, these compounds include aristolochic acid, vitamin E and atropine. All the natural compounds studied so far have been shown to fit in the active site with the classical 'head to tail' hydrogen bonded interactions between the hydroxyl groups or oxygen atoms of the ligand with the active site residues of PLA2 molecule, in which His48 and Asp49 form hydrogen bonds either directly or through the conserved water molecule that bridges His48 and Asp49. They bind to PLA2 in a similar manner at the substrate binding site but occupy the subsites according to the size of their hydrophobic moiety. As a result, these compounds are similarly placed in the hydrophobic channel. While subsites near the active site residues are similarly saturated, subsites distant from the active sites are dissimilarly occupied[9].

The hydroxyl groups of both aristolochic acid and vitamin E form two hydrogen bonds with the side chains of His48 and Asp49. The conserved water molecule in both these cases has been replaced by the hydroxyl moieties of these compounds and generates direct hydrogen bonding interactions. In the case of atropine, while the oxygen atom of the atropine makes a direct hydrogen bond with His48, it also makes indirect interactions with the active site residues His48 and Asp49 through the conserved water molecule. Additionally, the hydroxyl group of atropine forms a hydrogen bond with the carbonyl group of Asp49. Unlike that of vitamin E and aristolochic acid, the conserved water molecule in the active site of the PLA2 is not displaced by atropine[7-10].

L-AMINO ACID OXIDASES

The L-amino acid oxidases (LAAOs, EC 1.4.3.2) are flavoenzymes found in such diverse organisms as bacteria, fungi, algae, fish, snails as well as venoms of snakes from the families Viperidae, Crotalidae and Elapidae[11].

Almost all LAAOs described to date are flavoproteins of dimeric structure, with each subunit presenting a non-covalent bond with flavin mono-nucleotide (FMN) or flavin adenine dinucleotide (FAD). The latter cofactor is commonly found in snake venom L-amino acid oxidases (SV-LAAOs). Flavins present in LAAOs are responsible for the characteristic yellow color of many snake venoms and contribute to their toxicity because of the oxidative stress that results from the production[12] of H_2O_2. This feature allows the classification of LAAOs as FAD-dependent oxidoreductases. They are capable of catalyzing the stereospecific oxidative deamination of L-amino acid substrates to α-keto acids.

The catalytic cycle starts with a reduction half-reaction involving the conversion of FAD to $FADH_2$ and the concomitant oxidation of the amino acid into an imino acid, which subsequently undergoes a non-enzymatic hydrolysis releasing α-keto acid and ammonia. Another half-reaction completes the cycle with the oxidation of $FADH_2$ by molecular oxygen, producing hydrogen peroxide[13].

Mechanism of catalysis: The oxidation of L-amino acid by the enzyme proceeds in two steps, forming α-imino acid as the intermediate product as detected by the borohydride trapping experiments[11,13]. During the reductive half-reaction, the α-hydrogen atom of the amino group is abstracted by FAD, producing the α-imino acid intermediate, which then reacts with water to form the α-keto acid. Two alternative mechanisms have been proposed for the reductive half reaction: (1) The carbanion mechanism, in which the proton is transferred, leaving a negative charge on the alpha-carbon atom and (2) A hydride-transfer mechanism, in which the hydrogen atom and the two electrons are transferred simultaneously.

Toxicity of LAAO: A number of studies have indicated that LAAO contributes a role to the toxicity of the venom. However, there is not a clear consensus on the mechanism of this role. Although some reports suggested that the enzyme causes induction of apoptosis, cytotoxicity, inhibition or induction of platelet aggregation, hemorrhage and hemolysis[14]. In the early 1990s, studies by several groups showed that snake venom induced apoptotic activity in vascular endothelial cells. The apoptotic activity is most likely related to an increase in the concentration of H_2O_2. Torii *et al.*[15] reported complete inhibition of apoptosis upon incubation of cells with catalase, a scavenger of H_2O_2. However, a number of other studies showed that cell viability was not completely recoverable in the presence of catalase, suggesting that the apoptotic effect of LAAO is not solely due to the production of H_2O_2. Ande *et al.*[16] showed that apoptotic activity may be partially due to the depletion of essential amino acids from the cell.

Role of glycosylation in the toxicity of LAAO: Another factor thought to play a role in the cell death process is the presence of the glycan moiety on the enzyme, which may interact with structures at the cell surface. Fluorescence microscopy using LAAO conjugated with a fluorescence label revealed a direct attachment of the protein to the cell surface of mouse lymphocytic leukemia cells, human umbilical vein endothelial cells, human promyelocytic leukemia cells, human ovarian carcinoma cells and mouse endothelial cells but not to human epithelioid carcinoma cells[15]. The differing levels of cytotoxic effects of the enzyme on the different cell lines suggested varying extents of cell-surface interaction between the cells and the enzyme[17].

The localization of the enzyme at the cell surface has been implicated in producing high concentrations of H_2O_2 localized at the membrane and attributed to apoptotic activity[16]. The structure of LAAO from snake venom revealed electron density consistent with a carbohydrate moiety attached to the side chains of Asn172 and Asn361. The glycan moiety at Asn172 lies near to the proposed O_2 entry and H_2O_2 exit channel. The co-localization of the enzyme's host-interacting glycan moiety with the H_2O_2 release site on the enzyme has been suggested as a possible mechanism for facilitating apoptosis activity. These apoptotic processes and cell damage are some of the action mechanisms proposed for many SV-LAAOs suggesting that these enzymes could be used as models for the development of more effective chemotherapeutic and other antitumor agents[18].

Antitumor potential of SV-LAAOs: Numerous studies of snake venoms show that SVLAAOs are capable of promoting cytotoxicity in different cell lines, such as S180 (murine sarcoma 180 tumor), SKBR-3 (breast adenocarcinoma), Jurkat (human acute T cell leukemia), EAT (Ehrlich ascites tumor), B16F10 (murine melanoma), PC12 (rat adrenal gland pheochromocytoma), as well as in non-tumor cells (lymphocytes and macrophages)[13-16]. It is noteworthy that the damage in normal cells is usually negligible when compared to the damage caused in tumor cells. Although the cytotoxicity mechanisms of SV-LAAOs have not been fully clarified, it is known that lipids present in cell membranes can be damaged by reactive oxygen species (ROS)[15]. Considering that membranes of tumor cells present higher concentrations of lipids than normal cells, it is speculated that the H_2O_2 produced by LAAOs exerts direct action on the membrane of tumor cells with lower toxicity on normal cells. The apoptosis-inducing activity was abolished by catalase and other H_2O_2 scavengers, proving that the H_2O_2 generated by LAAO action plays an important role in the apoptosis.

SNAKE VENOM SERINE PROTEINASES (SVSPS)

Serine proteinases (EC 3.4.21) catalyze the cleavage of covalent peptide bonds in proteins and play key roles in diverse biological processes ranging from digestion to the control and regulation of blood coagulation, the immune system and inflammation[19]. The SVSPs interfere with the regulation and control of key biological reactions in the blood coagulation cascade, fibrinolysis and blood platelet activation. Based on their biological roles, they have been classified as activators of the fibrinolytic system, procoagulant, anticoagulant and platelet aggregating enzymes[20].

The procoagulant SVSPs activate FVII, FX and prothrombin and shorten the coagulation times. Some SVSPs also possess fibrinogen-clotting activity[21] and are often referred to as thrombin-like enzymes. Thrombin-like enzymes have been extensively investigated for potential therapeutic uses. For example, ancrod, batroxobin and reptilase are available commercially for the treatment of cardiovascular diseases[22]. Ancrod is used clinically for the treatment of heparin-induced thrombocytopenia and thrombosis and acute ischemic stroke. Batroxobin is used for the treatment of thrombotic diseases. Batroxobin and ancrod are under clinical trials for the treatment of deep vein thrombosis. Additionally, reptilase is used as a diagnostic tool for disfibrinogenemia[23].

The anticoagulant SVSPs activate protein C via a thrombomodulin-independent mechanism. The most studied SVSP enzyme is from *Agkistrodon contortrix contortrix* venom, commercially referred to as Protac, which specifically converts protein C to activated protein C by hydrolyzing the Arg169-Leu170 bond, functioning independently of plasmatic factors. This is in contrast to the physiological activation of protein C by thrombin, which is dependent on thrombomodulin[22]. Protac is used clinically in functional assays of protein C determination, total protein S content and other protein S assays in plasma[24].

Fibrinolytic SVSPs have been isolated from the venoms of *Trimeresurus stejnegeri*, *Agkistrodon blomhoffii* and *Lachesis muta muta*[25]. These enzymes convert plasminogen to plasmin that rapidly degrades preexisting clots. The most studied fibrinolytic SVSP is the *T. stejnegeri* venom plasminogen activator (TSV-PA), which cleaves the Arg561-Val562 bond in plasminogen with high specificity and is resistant to inhibition[26].

From the above-mentioned clinical applications of SVSPs, it is clear that, in addition to their importance in snake envenomation, these venom enzymes also serve as important tools in the study of hemostasis and are clinically used for clotting assays, diagnosis, determination of protein C, protein S, plasma fibrinogen, study of platelet function as defibrinogenating agents to investigate dysfibrinogenemias, test the contractile system of platelets and for defibrinogenation of plasma[27].

Mechanism of catalysis: The first step to the highly efficient acid–base catalytic mechanism of SVSP involves Ser195, which initiates the attack on the carboxyl group of the peptide. The reaction is assisted by His57 which acts as a general base to form the tetrahedral intermediate, stabilized by interactions with the main-chain NHs of the oxyanion hole[24]. Following the collapse of the tetrahedral intermediate and the expulsion of the leaving group, His57-H^+ plays the role of a general acid and the acyl-enzyme intermediate is formed. In the second step of the reaction, His57 deprotonates a water molecule which then interacts with the acyl-enzyme complex to yield a second tetrahedral intermediate, the collapse of which results in the liberation of the carboxylic acid product[24,25].

Prothrombin activators: Serine proteinases which activate prothrombin are found exclusively in Australian snake venoms. The two groups differ in their co-factor requirements and structure: Prothrombin activators consist of enzymes (e.g., trocarin D from *Tropidechis carinatus* venom) that require Ca^{2+}, FVa and negatively charged phospholipids for their optimal activities[28], whereas, other enzymes (e.g., pseutarin C from *Pseudonaja textilis* venom) require Ca^{2+} and negatively charged phospholipids but not FVa for optimal

activity. Trocarin D is structurally and functionally similar to FXa, it has a light chain consisting of one Gla domain and two epidermal growth factor domains, linked by a single inter-chain disulfide bond to a heavy chain consisting of a serine proteinase domain[29]. In contrast, pseutarin C consists of two subunits, a catalytic subunit and a non-enzymatic subunit, which are structurally and functionally similar to FXa and FVa, respectively[30]. The catalytic subunit has similar light and heavy chains to trocarin D. The non-enzymatic subunit has a heavy chain (consisting of A1 and A2 domains) and a light chain (consisting of A3, C1 and C2 domains) that are held together by non-covalent interactions. Similar to FVa, the non-enzymatic subunit significantly increases the catalytic efficiency of the enzymatic subunit. Both these groups of prothrombin activators activate prothrombin by targeting the same cleavage sites as endogenous FXa and its complex with FVa. Thus these prothrombin activators are similar to blood coagulant factors and are probably evolved from blood coagulant factors by gene duplication[31].

SNAKE VENOM METALLOPROTEINASES (SVMPs)

It is estimated that SVMPs (EC 3.4.17) comprise at least 30% of the total protein of most viperid venoms[32]. The SVMPs are primarily responsible for the hemorrhagic activity and the induction of local and systemic bleeding. The SVMPs also possess diverse functions such as the disruption of hemostasis mediated by procoagulant or anti-coagulant effects, platelet aggregation and apoptotic or pro-inflammatory activities. Recent crystallographic studies of high-molecular-weight SVMPs and phylogenetically related ADAM (a disintegrin and metalloproteinase) and ADAMTS (ADAM with thrombospondin type-1 motif) family proteins have shed new light on the structure-function properties of this class of metalloproteinases[33].

Classification of SVMPs: The SVMPs range in size from 20-100 kDa and are classified into three groups (P-I to P-III) according to their domain organization[32]. The P-I SVMPs are the simplest ones and they contain only a metalloproteinase (M) domain in their mature form. The P-II SVMPs contain an M domain followed by a disintegrin (D) domain. In most cases, P-II SVMPs further undergo proteolysis to produce non-enzymatic disintegrins that have strong platelet aggregation inhibitory activity. The P-III SVMPs contain M, disintegrin-like (D) and cysteine-rich (C) domains. The P-III SVMPs are further divided into subclasses based on their distinct post-translational modifications, such as dimerization (P-IIIc) or proteolytic processing (P-IIIb). The heterotrimeric subclass of SVMPs formerly called P-IV[33] is now included in the P-III group as a subclass (P-IIId) representing another post-translational modification of the canonical P-IIIa SVMPs[32]. All the classes have a signal (pre) and a pro domain sequence before the M domain in their gene structures, but none of the SVMPs with the pro domain has been isolated from the venom.

Pharmacological application of SVMPs: Snake venom contains panoply of metalloproteinases with diverse biochemical activities and it is possible to identify biomedically relevant enzymes for treating neurodegenerative diseases. Certain oligomeric forms of highly phosphorylated α-synuclein are highly resistant to chymotrypsin or to proteinase K treatment[27]. As Parkinson's disease patients are characterized by the accumulation of phosphorylated extracellular protein aggregates termed Lewy bodies[25] or senile plaques in Alzheimer's disease patients, it is conceivable that these highly phosphorylated protein aggregates may be proteolytically cleaved by basic non-hemorrhagic metalloproteinases to help slow the progression of the disease. Hence, by applying sophisticated molecular modeling techniques, affinity docking simulations in combination with biochemical and *in vivo* testing, it is possible to identify basic non-hemorrhagic (i.e., Adamalysin II) but highly proteolytic SVMPs that can be further developed as novel anti-neurodegenerative agents for dissolving extracellular protein aggregates[34].

ACETYLCHOLINESTERASE (AChE)

The AChE (EC 3.1.1.7) is a member of the cholinesterase family[35] and plays a vital role in acetylcholine (ACh) transmission in the nervous system by ensuring the hydrolysis of ACh to choline and an acetate group, thereby terminating the chemical impulse. The transmission of a chemical impulse takes place within 1 msec and demands precise integration of the structural and functional components at the synapse[36]. Incidentally, AChE may also be one of the fastest enzymes known, hydrolyzing ACh at a rate that is close to the diffusion-controlled rate[7].

Significant amounts of AChE are found in the venom of snakes, particularly in species belonging to the family Elapidae, with the exception of Dendroaspis species. In contrast, AChE is not found in venoms of snakes belonging to the Viperidae and Crotalidae families. Incidentally, snake venom AChEs are also more active than Torpedo and mammalian AChEs in hydrolyzing Ach[37].

Mechanism of catalysis: The structure of AChE is remarkably similar to serine hydrolases and lipases. It belongs to the α/β hydrolase family, one of the largest groups of structurally related enzymes with diverse catalytic functions. It has a β-sheet platform that bears the catalytic machinery and in its overall features, is rather similar in all members of the family. Ser200, Glu327 and His440 residues form the catalytic triad[38]. As in lipases and serine proteinases, glutamate residue replaces aspartate. The triad displays opposite handedness to that of serine proteinases, such as chymotrypsin, but they are in the same relative orientation in the polypeptide chain in all α/β hydrolase enzymes. The most interesting feature of AChE is the presence of a deep and narrow cleft (20 A°) which penetrates halfway into the enzyme and widens close to its base. This cleft is lined by 14 aromatic residues and it contains the catalytic triad. Two acidic residues, Asp285 and Glu273 are at the top and one, Glu199, at the bottom of the cleft. In addition, there is also a hydrogen-bonded Asp72 residue in the cleft. Rings of aromatic residues represent major elements of the anionic site of AChE, Trp84 and Phe330 contributing to the so-called catalytic anionic site (CAS) and Tyr70, Tyr121 and Trp279 to the peripheral anionic site (PAS) located on the opposite side of the gorge entrance[39]. The aromatic surface of the gorge might serve as a kind of weak affinity column down which the substrate could hop or slide towards the active site via successive π-cation interactions. The AChE possesses a very large dipole moment and the axis of the dipole moment is oriented approximately along the axis of the active site gorge. This dipole moment might serve to attract the positively charged substrate of AChE into and down the active site gorge, this being a means of overcoming the penalty of the buried active site. A potential gradient exists along the whole length of the active site gorge, which can serve to pull the substrate down the gorge once it has entered its mouth[40]. The weak hydration of ACh is thought to favor its π-cation interaction with the aromatic residues, principally Trp279 and Tyr70, at the top of the gorge, as well as subsequent interactions along the gorge towards the active site, including the two residues at the bottleneck, Tyr121 and Phe330. The strong hydration of alkali metal cations should preclude their entering the gorge due to their large diameters in their hydrated forms. It was showed that the PAS traps the substrate, ACh, thus increasing the probability that it will proceed on its way to the CAS and provided evidence for an allosteric effect of substrate bound at the PAS on the acylation step[38-40]. Torpedo AChE is a classical serine hydrolase that bears a catalytic triad consisting of serine, histidine and a glutamate. Consistent with the mechanism of other serine proteases, the serine residue of the catalytic triad acts as a nucleophile, while the histidine residue acts as the acid/base catalyst for the hydrolysis of the substrate.

SNAKE VENOM HYALURONIDASES (SVHYS)

The SVHYs are rightly referred to as a 'Spreading factor' as they facilitate easy diffusion of systemic toxins from the site of bite into general circulation. Rapid hydrolysis of mega structure hyaluronic acid (HA) into fragments of varied molecular size resulting in the decreased viscosity of the envenomed milieu aiding rapid diffusion of toxins into circulation, which would otherwise, diffused much slowly[41]. Further, the degraded end products of HA with high molecular mass are reported to be anti-angiogenic, anti-inflammatory and immunosuppressive, while low molecular mass fragments are pro-inflammatory, immune-stimulatory and angiogenic leading to complications[42]. In addition, during snakebite the bite site becomes the depot of venom and rapid delivery of venom into the circulating blood is critical. The activity of SVHYs not only damages the tissue at the bite site, but also facilitates easy diffusion of systemic toxins into the circulation resulting in systemic toxicity[43].

The hyaluronidases are a class of endo-β-glycosidases distributed throughout the animal kingdom including various human organs and body fluids, external secretions of microorganisms, leeches and in the venoms of snakes, scorpions, lizards, bees and other insects[41]. The enzymatic degradation of HA is mediated through the coordinated activity of three different enzymes. The initial endoglycosidase activity on intact HA generates oligosaccharides of different chain length which become substrate for the two exoglycosidase namely the β-glucuronidase and β-N-acetyl hexosaminidase enzymes[44].

The biopolymer hyaluronic acid (HA) is a linear, megadalton, non-sulfated, glycosaminoglycan (GAG) present in ECM of soft connective tissues. Being a major component of ECM, HA interacts with the collagen fibers, protein filaments, growth factors and hold water as well as metal ions[45].

Hyaluronidases from different sources have been used in different applications in medicine. Examples include the use as a diffusion promoter for active substances (drugs, for instance), the treatment of hyaluronan-induced diseases (such as some types of cancers) and in the aesthetic medicine[46]. Despite their great therapeutic potential, these enzymes are found in small proportions in snake venoms and they have an extremely unstable catalytic activity. These issues hamper their isolation from venoms as well as their in-depth functional and structural characterization[47]. This is reinforced by the few hyaluronidase amino acids sequences deposited in databases, which are currently 14 for Uniprot and seventeen for NCBI.

CONCLUSION

The primary purpose of having a lethal concoction of toxins in snake venoms is for prey capture and defense and venom proteins have certainly evolved to exhibit a plethora of novel pharmacological functions with impressive specificity and functions. Due to the higher catalytic efficiency, heat stability and resistance to proteolysis as well as abundance of snake venom enzymes compared with non-venom homologs, they are currently attractive models for biochemists and structural biologists. Despite sharing similar structural scaffolds, some of these enzymes exhibit multiple pharmacological functions. Thus, structure-function relationships of such enzymes pose intriguing and exciting challenges to scientists. Structural studies of the enzymes would not only contribute to the understanding of the mechanism of catalysis but also to that of their inhibition. The inhibitors have significant importance in developing therapeutic prototypes and lead compounds for various human diseases and ailments.

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