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REVIEW

An Overview on Nucleases (DNase, RNase, and Phosphodiesterase) in Snake Venoms

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Abstract—In this review, we have compiled the data on pharmacological activities associated with endogenous purine release related enzymes—nucleases (DNases, RNases, and phosphodiesterases). The results of studies on toxic effects of these enzymes, emphasizing the future directions in this field, are summarized. One of the major problems facing toxicologists is the identification and characterization of specific venom nucleases since they share similar substrate specificities and biochemical properties. In this review, we have attempted to clarify some of the discrepancies about these enzymes. Further, we have tried to correlate the existence of nuclease enzymes in relation to endogenous release of purines, a multitoxin, during snake envenomation, and we also discuss the possible actions of purines. We hope that this review will stimulate renewed interest among toxicologists to biologically characterize these enzymes and elucidate their role in envenomation.

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Snake venom is a complex mixture of biologically active components comprising hydrolytic enzymes, non-enzymatic proteins/peptides, and small amounts of organic and inorganic molecules [1-3]. The venom components, known to vary with geographic location, season, and age of the snake [4-8], is not primarily for self-defense, but has a more important role in prey immobilization and its subsequent digestion [2, 9-11]. Hence, several digestive enzymes in venoms, in addition to their hydrolytic activity, have evolved to interfere with diverse physiological processes that help in the immobilization of prey/victim [2, 12, 13]. For example, hydrolytic enzymes such as proteases and phospholipase A_2 (PLA_2) of snake venoms are known to induce both systemic and local effects. Several PLA_2 enzymes are known to exhibit neurotoxicity (post-/presynaptic) and cardiotoxicity and are pro-/anticoagulant, thereby interfering with hemostasis. PLA_2 and proteases are responsible for local tissue damage and cause hemorrhage, necrosis, and edema [14, 15]. Though hydrolytic enzymes (DNase, RNase, and phosphodiesterase) are ubiquitously present in almost all snake venoms, they are less characterized for their pharmacological activities [2, 16, 17]. The lack of interest among toxicologists in these enzymes seems to be because of the assumption that they are only involved in digestion and are nontoxic. However, recently there is renewed interest among toxicologists in these enzymes as they are known to endogenously liberate purines, which act as multitoxins [2, 3].

The identification of free purines as endogenous constituents of venoms has further supported the role of purinergic signaling in envenomation [3, 18]. Purines are known to potentiate venom-induced hypotension and paralysis [2] *via* purine receptors, which are ubiquitously distributed among various organisms envenomed by snakes [3, 19-21].

The distribution of these enzymes in snake venoms, their catalytic mechanisms, and assay systems to determine their activities have been described in detail in earlier reviews [16, 17]. However, only a few reviews are available suggesting the possible pharmacological actions of these enzymes [2, 3].

NUCLEASES

Nucleases are enzymes that act on nucleic acids (DNA/RNA) and their derivatives. Snake venom nucleases are classified as endonucleases and exonucleases.

Abbreviations: EST, expressed sequence tag; PDE, phosphodiesterase; PLA_2, phospholipase A_2.

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Endonucleases include DNases, which specifically hydrolyze DNA, and RNases, which specifically hydrolyze RNA. Exonucleases include phosphodiesterases (PDE), which hydrolyze both DNA and RNA. PDE is also known to exhibit endonuclease activity [22, 23]. An endonuclease activity in snake venom was first reported by Delezenne and Morel [24] in 1919. Differentiating between specific venom endonuclease activity and PDE activity is difficult since endonucleolytic activity is an inherent property of venom PDE [22, 23]. Therefore, most of the reported nuclease activities might actually be due to PDE action [25, 26].

In order to differentiate PDE from endonucleases, biochemical parameters have to be considered in addition to substrate specificities. Even though both endonucleases and PDE hydrolyze DNA and RNA, they exhibit distinct pH optima and metal ion requirement. A unique venom protein with an acidic pH optimum that does not require divalent cations for the hydrolysis of DNA or RNA has been considered as an endonuclease [17, 27], whereas all PDEs are active at basic pH and require divalent metal ion for activity [16, 17]. The DNase activity reported by Sittenfeld et al. [25] might be due to the action of phosphodiesterase, since the activity was measured at pH 7.0 using calf thymus DNA. A more recent study by de Roodt et al. [26] showing DNase activity using plasmid and calf thymus DNA in a zymogram is likely to be PDE rather than DNase since EDTA was shown to inhibit the activity. Specific endonuclease activity with a pH optimum of 5.0 in addition to phosphodiesterase activity at basic pH optimum of 8.9 in the same venoms has been reported [27-31]. These data clearly indicate that the PDEs are distinctly different from endonucleases. However, PDE and exonuclease activities are still difficult to differentiate since there are no reports describing exclusive exonuclease activity in snake venoms. Thus, venom exonuclease activity is attributed to PDE.

DNases (EC 3.1.21.1). Relatively few studies have been carried with regard to specific DNases; as a result, it is difficult to say how widely they are distributed among snake venoms. A DNase activity with pH optimum of 5.0 was purified from *Bothrops atrox* venom [27]. However, it is interesting to note that this preparation also showed activity towards RNA and poly(AU) in addition to DNA. During the course of preparation of PDE from *Crotalus adamantus* venom, an endonuclease activity was separated from exonuclease activity [32]. Since the main aim of the author was to eliminate contaminating nuclease activity, very little is known about this isolated enzyme. This study is important as it indicates the presence of distinct DNase activity in venoms apart from PDE. To date, no biological activity has been assigned to venom DNases apart from their role in digestion. The properties of specific endonucleases purified from various snake venoms are given in Table 1.

RNases (EC 3.1.21.-). Like DNases, RNases are also not well characterized. A specific ribonuclease was isolated from the venom of *Naja oxiana* hydrolyzing double stranded RNA (now called as RNase V_1). The enzyme was shown to hydrolyze RNA without showing any base preference and produced oligonucleotides of 2-4 bases terminated in a 5′-phosphate [28, 29]. More recently, an RNase with specificity for polycytidine was purified from *Naja naja* venom [30, 31]. Both these enzymes had an apparent molecular mass of ~14 to 16 kDa. Although the authors claim that the RNase preparation from *N. naja* did not show phospholipase and phosphodiesterase activity, its N-terminal sequence was identical to that of PLA_2. None of the endonucleases are reported to exhibit any pharmacological activities.

Phosphodiesterases (EC 3.1.4.1). PDEs are known to catalyze the hydrolysis of phosphodiester bonds in a progressive fashion beginning at the 3′-end of polynucleotides liberating 5′-mononucleotides at basic pH. Uzawa [33] was the first to describe PDE activity in venoms. Since then, PDE activity has been surveyed against a wide variety of snake taxa and found ubiquitously distributed in venoms [1, 16, 17, 34]. Crotalid and viperid venoms are known to contain higher PDE activity than elapid venoms [2, 17]. PDEs act on several native substrates like DNA, rRNA, and tRNA without showing any preference for purine or pyrimidine bases. It is shown that native DNA is a better substrate than denatured DNA [16]. They also hydrolyze oligonucleotides including polyadenylic acid [35] and cyclic nucleotides. In addition, PDE also hydrolyze adenosine 5′-tetraphosphate, TDP-rhamnose, UDP-glucose, GDP-mannose, poly(ADP)-ribose, NAD^+, $NADP^+$, and other nucleic acid derivatives [16]. They are also known to hydrolyze ATP and ADP, liberating adenosine [36, 37].

Table 1. Properties of purified endonucleases from snake venoms

Snake	Enzyme	Substrate	Molecular mass, Da	p*I*	Reference
B. atrox	DNase	DNA, RNA, poly(AU)		5.0	[27]
N. naja oxiana	RNase	RNA	~15 900		[30, 31]
N. naja	RNase	poly(ribocytidine), rRNA	~14 000		

Table 2. Properties of purified phosphodiesterases from snake venoms

Snake	Substrate	Molecular mass, kDa	p*I*	Carbohydrate	Isoforms	Inhibitors	Reference
B. atrox	bis-pNPP, poly(A)	130	9.2		yes (2)	EDTA	[35]
B. alternatus	bis-pNPP	105	8-9.8	no		–"–	[42]
Cerastes cerastes	–"–	110	9.0	no		EDTA, cysteine, AMP, ADP	[49]
Crotalus adamentus	–"–	115, 140	9.0	yes			[23, 50]
Cr. mitchilli pyrrhus	cAMP, ATP, ADP	110	8.5			EDTA	[36]
Cr. rubber rubber	native DNA/RNA, cAMP	98	8.5		yes	EDTA, PCMB	[22]
Cr. viridis oreganus	–"–	114				EDTA	[37]
Trimeresures flavoviridis	bis-pNPP			yes	yes (4)	–"–	[38]
T. mucrosquamatus	DNA/RNA	140		no		EDTA, PCMB	[41]

Note: Bis-pNPP, bis-*p*-nitrophenyl phosphate; PCMB, *p*-chloromercuribenzoate.

Venom PDEs have been isolated and characterized from numerous species of snakes. The properties of several isolated/purified venom PDEs are summarized in Table 2. In general, unlike RNases, PDEs are high-molecular-mass (>90 kDa) single polypeptide chain proteins. However, some exist as homodimers [22, 36, 37]. They can be present in multimolecular forms or in only one form [22, 35, 38]. All PDEs are metalloenzymes, and metal chelators are generally known to inhibit PDE activity [16, 17, 39, 40]. Mori et al. [22] showed that *Crotalus ruber ruber* PDE contained 1.04 mol zinc per mol of enzyme. Further, zinc is also shown to be inhibitory at higher concentrations [22, 41, 42]. It is suggested that zinc is necessary for catalysis, whereas calcium and magnesium are involved in substrate binding [43]. Isoforms of PDEs are known to exist in *Vipera palastinae* and *Trimeresures flavoviridius* venoms [36, 38]. Though PDE has been isolated from several venoms, there is no information on amino acid or full-length cDNA sequence. However, the expressed sequence tag (EST) sequences generated from cDNA library of *Deinagkistrodon acutus*, *Lachesis muta*, and *Sistrurus catenatus edwardsi* species are shown to have representatives for the PDE gene [44-46]. Accession number of phosphodiesterase ESTs from various snake species are given in Table 3.

Table 3. Accession number of phosphodiesterase ESTs from various snakes

Snake	Accession number	Reference
D. acutus	DV561486, DV563305	[46]
L. muta	DY403207, DY403416	[45]
S. catenatus edwardsii	DY587965.1	[47]

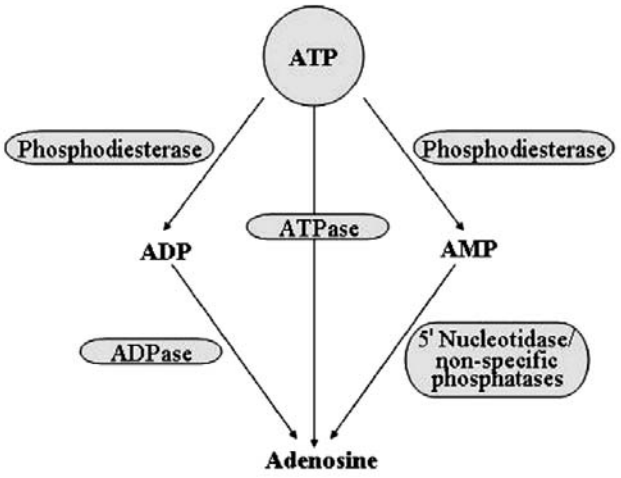
Although venom PDEs are widely distributed among several snake taxa, only few laboratories have investigated the biological activity of this near ubiquitous venom component. An earlier study by Russell et al. [48] showed a reduction in mean arterial pressure and locomotor depression with partially purified PDE preparation from several snake venoms. These effects can be due to the reduction of cAMP levels. Although this preparation had contaminating proteins, the study is significant because it indicated that even in the absence of cellular disruption there is adequate substrate available for the enzyme PDE in the circulation to cause profound hypotension. Though PDEs are known to hydrolyze a wide variety of biologically important nucleotides such as ATP, NAD^+, $NADP^+$, and GDP, this enzyme has not been investigated for other potential pharmacological activities.

ADENOSINE GENERATION BY NUCLEASES

The synergistic action of nucleases, i.e. DNase, RNase, and phosphodiesterase, along with other enzymes

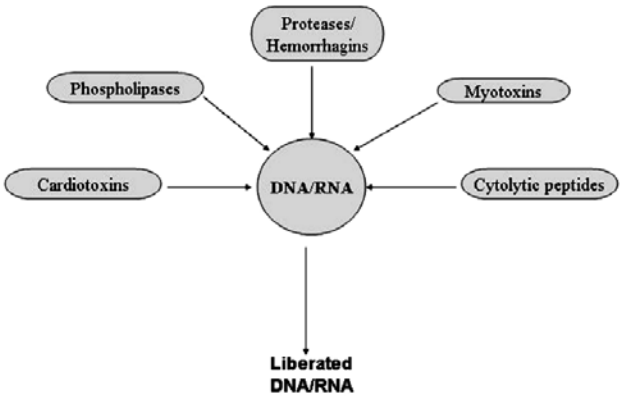
in venoms results in the generation of purine and pyrimidine nucleotides [2, 3]. Among these nucleotides, adenosine generation is pharmacologically important as it exhibits several snake envenomation related symptoms [2, 19-21].

Generation of adenosine by synergistic actions of venom enzymes can take place by different pathways. Enzymes like nucleotidase and PDE act immediately upon envenomation on available ATP molecules to release adenosine as shown in Scheme 1. DNases, RNases, and PDEs liberate purine and pyrimidine nucleotides from the cell genome. The action of these enzymes requires cell necrosis brought about by proteases/hemorrhagins, phospholipases, myotoxins, cardiotoxins, and cytolytic peptides as shown in Scheme 2 [51-54].



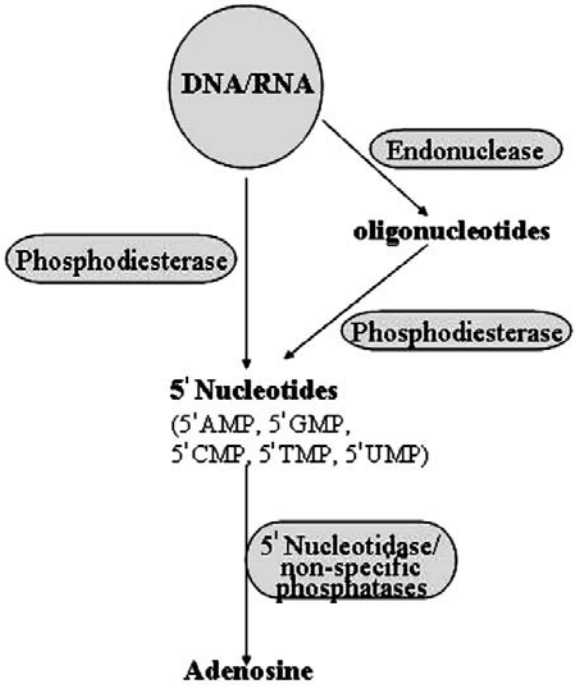
Adenosine generation by venom enzymes from ATP hydrolysis. Venom constituents are outlined with ovals and bold letters indicate end products released upon enzyme action

Scheme 1



Necrotic cell death brought about by venom enzymes. Venom constituents are outlined with ovals and bold letters indicate end products released upon enzyme action

Scheme 2



Schematic representation of adenosine generation by venom enzymes from DNA/RNA hydrolysis. Venom constituents are outlined with ovals and bold letters indicate end products released upon enzyme action

Scheme 3

Once the cell is ruptured by the venom, PDEs and DNases/RNases act on prey DNA/RNA and release 5′-nucleotide monophosphates (NMPs). 5′-Nucleotidase specifically or phosphatases nonspecifically acting on these 5′-NMPs liberate adenosine (Scheme 3 shows the schematic representation of adenosine generation by venom enzymes from DNA/RNA hydrolysis). There is always a possibility that the released adenosine *in vivo* due to the action of adenosine deaminase of the prey/victim will be converted into inosine, which is also an important purine metabolite since it is responsible for inducing many pharmacological actions. In brief, some of the pharmacological actions in relation to snake envenomation are described here (for more details see [2]).

Liberated adenosine could help in the diffusion of toxins into prey tissues by inducing increased vascular permeability through vasodilation [55, 56] and/or inhibition of platelet aggregation [57]. Thus, adenosine could act as potent spreading agent. Along with vascular permeability effects, adenosine induced edema [58] helps in potentiating venom-induced hypertension [2]. In addition, adenosine is also known to cause paralysis by inhibiting neurotransmitter release at both central and peripheral nerve termini [19, 59], thus potentiating venom-induced paralysis [2]. Further, along with hemolytic, myolytic, and cardiolytic toxins of snake venom, adenosine can also be involved in venom-induced

renal failure and cardiac arrest [2, 60, 61]. Other common behavioral disturbances, such as nociceptive, locomotor alterations, and pain observed upon envenomation, can also be brought about by adenosine [2, 62-69]. Therefore, it seems that adenosine plays a central role in envenomation strategies of prey immobilization [2, 3].

Although experimental data in deciphering these actions by purified enzymes are lacking, there is enough evidence for direct involvement of adenosine and adenosine signaling in snake envenomation [3, 18]. Apart from endogenous liberation of adenosine that can bring about various pharmacological actions, it is also possible that these enzymes can interfere in many physiological process of an organism directly. Since they are hydrolytic enzymes, their pharmacological actions might not only be based on their catalytic activity, but also could perform additional pharmacological activities. Venom enzymes have evolved to interfere with diverse physiological process [12, 13]. Hence it is likely that nucleases also posses distinct pharmacological activities like those of venom PLA_2 and proteases [14, 15]. Our recent work on anticoagulant effect of *N. naja* 5′-nucleotidase confirms that the observed pharmacological effect is independent of catalytic activity [70].

Although nucleases—DNase, RNase, and phosphodiesterase—are near ubiquitous in distribution, little progress has been made towards understanding these enzymes with a toxicological perspective. As discussed, characterization of individual nucleases has not been clearly established as they hydrolyze similar substrates and share similar biochemical properties. Future research on complete biophysical characterization of the purified enzymes could clearly reveal the existence of unique venom proteins or proteins having multiple domains to perform different catalytic functions. Determination of complete cDNA and/or amino acid sequence will also enable evaluation of the degree of homology of these enzymes from various species and families of snakes. The renewed interest in these nuclease enzymes is based on the involvement in generation of adenosine, a multitoxic component, but the direct involvement of these enzymes in the generation of adenosine *in vivo* has to be established. Apart from these, nucleases could also possess distinct pharmacological properties that are independent of catalytic activity, which has to be verified by carrying out pharmacological studies with purified proteins. Further research is needed to isolate and biologically characterize these enzymes in snake venoms such that their role in venom is clearly established.

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