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A Comparative Quantitative Study of Kinin-Releasing and Kinin-Degrading Enzymatic Activities in Snake Venoms

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ABSTRACT: Kinin-releasing (kininogenase) and kinin-degrading (kininase) activities of a number of crotalid viperid and elapid venoms were measured with the aid of a kinin radioimmunoassay. A titrimetric assay using an arginine ester or a tyrosine ester was utilised to measure trypsin-like and chymotrypsin-like activities respectively. The crotalid and viperid venoms contained a wide range of these enzymatic activities but none of the elapid venoms tested showed such activities.

INTRODUCTION

Snake bite is an important medical problem in rural areas of West African savanna region especially in Ghana, Nigeria, Cameroon and Benin (1). Snake bite poisoning is a complex and often a life-threatening condition, that involves tissues such as blood, cardiovascular, respiratory and nervous system. Snake venom is composed of several constituents (both proteins and non proteins) such as neurotoxins, cardiotoxins, haemolysins, coagulating factors and many enzymes like kallikrein, which acts mainly through production of biologically active kinin to cause hypotensive shock and severe localized pain characteristics of the viperid and crotalid snake species bite.

Certain crotalid venoms are known to posses both kinin-releasing (Kininogenase, kallikrein-like) and kinin-degrading (kininase) activities (2). Other snake venoms have been assayed for the presence of kinin-releasing enzymes but not directly for kinin-degrading enzymes (3,4). Chymotrypsin is a potent kininase (5) but chymotrypsin-like enzymes have been reported to be absent from snake venoms (6). This short report describes a comparative study of kininreleasing, arginine esterolytic (trypsin-like), kinin-degrading and tyrosine esterolytic (chmotrypsin-like) activities in various crotalid, viperid and elapid venoms.

MATERIALS AND METHODS

Lyophilized samples of the venoms of *Crotalus varids helliri*, *Agkistrodon halys ussuriensis*, *Vipera xanthina xanthina*, *V. aspis aspis*, *V.lebetina schweizeri*, *V. lebetina obtusa*, *V. lebetina deserti*, *V. berus*, *V. ammodytes*, *Naja nigricollis*, *N. naja kaouthia*, *N. naja*, *Bungarus candidus* and *Micrurus fulvius* were purchased from Laxtoxan, Rosans, France. Lyophilized venom samples from Crotalus atrox, *Crotalus adamanteus*, *Crotalus scutulatus* and *Agkistrodon piscivorus* were donated by Prof. F.E. Russell, Tucson, Arizona U.S.A A freeze-dried sample of the venom of Echis carinatus ocellatus was donated by Dr. R.D.G. Theakson, Liverpool, U.K. Solutions of each venom were made in Tris-HCl buffer pH 7.4 immediately prior to assay. Kinin-releasing activity were determined by the procedure of Bailey *et al* (7). Kinin-degrading activities were measured in terms of the amount of kinin remaining after

incubation at 25°C of bradykinin (60 ng in 0.3 ml 0.9% NaCl) with suitable dilution of venom (0.1 ml) to ensure saturation of the enzyme with sybstrate over the period of incubation of 10 minutes. In both cases kinin was measured by a specific radioimmunoassay (7). The trypsin-like activity of each venom was assayed at 25°C and pH 8.0 using 10 mM N-α-p-tosyl-L-arginine methyl ester (Tos-Arg-OEt) as substrate in a titrimetric assay (8). Chymotrypsin-like activity was measured by a titrimetric assay at 25°C and at pH 8.0 using 10mM N-acetyl-L-tryosine ethyl ester (Ac-Tyr-OEt) as substrate (9).

RESULTS AND DISCUSSION

All of the crotalid and viperid venoms tested, with the exception of that of E.c. ocellatus, showed kininogenase and trypsin-like activities (Table 1). The venom of A.h. ussuriensis possessed high levels of kinin-releasing and arginine-eseterolytic activities. However, hydrolytic activity towards Tos-Arg-OMe should not be used as a measure of the kininogenase activity of a venom as can be seen from the lack of correlation between those two activities for the venoms in the present study.There is good agreement between the levels of kininogenase activity in the venoms of C.atrox, C.adamanteus, A.P. piscivorous and V.a. ammodytes found in the present study and those recorded by Mebs (4), especially considering the variability of the enzyme content of venoms seen even at the subspecies level (11). Similarly there are reasonable agreements between some venom hydrolytic activities towards Tos-Arg-OMe found in the present study and those recorded by Oshima *et al* (3).

Table 1 Enzymatic activities of snake venoms.

Venom	Kinin-releasing SPA	Trypsin-like SPA	Kininase SPA	Chymotrypsin-like SPA
C.atrox	4.6 ± 0.4*	2.7 ± 0.2	0.16 ± 0.04	0.19 ± 0.09
C.adamanteus	1.7 ± 0.2*	1.9 ± 0.1	0.17 ± 0.03	0.16 ± 0.02
C.v. helleri	1.8 ± 0.2	3.8 ± 0.2	N.D.	N.D.
C.s. scutulatus	17.0 ± 1.3*	8.3 ± 0.2	0.23 ± 0.04	0.1 ± 0.01
A.p. piscivorus	5.1 ± 0.6*	4.1 ± 0.2	0.17 ± 0.05	0.27 ± 0.04
A.h. ussuriensis	17.0 ± 0.9	12.0 ± 0.5	0.09 ± 0.01	0.46 ± 0.03
E.c. ocellatus	0.1	0.2 ± 0.1	0.42 ± 0.01	1.40 ± 0.10
V.x. xanthina	0.8 ± 0.1**	1.3 ± 0.1	0.08 ± 0.01	0.3 ± 0.03
V.a. aspis	4.7 ± 0.5**	0.7 ± 0.1	0.23 ± 0.03	1.40 ± 0.10
V.l. schweizeri	2.2 ± 0.2**	1.7 ± 0.1	0.12 ± 0.02	1.00 ± 0.10
V.l. Obtusa	2.3 ± 0.2**	3.7 ± 0.1	0.12 ± 0.04	1.20 ± 0.10
V.l. deserti	1.0 ± 0.1	0.7 ± 0.1	0.09 ± 0.01	0.60 ± 0.02
V.b. berus	11.0 ± 1.4**	0.8 ± 0.1	0.16 ± 0.03	0.80 ± 0.10
V.a. ammodytes	5.4 ± 0.7**	1.0 ± 0.2	0.06 ± 0.02	1.10 ± 0.20

SPA = Specific activity, is expressed as units per mg venom together with the standard deviation (n = 6). For kinin-releasing activity the unit is ug kinin released per minute, and for trypsin-like activity the unit is umole Tos-Arg-OMe hydrolysed per minute. For kininase activity the unit is ug kinin degraded per minute and for chymotrypsin-like activity it is umole Ac-Tyr-OEt hydrolysed per minute.

*taken from Bailey (7)
**taken from Al-Joufi *et al* (10).
N.D. means none detected.

All of the crotalid and viperid venoms tested, with exception of that of C.V. Helleri, showed kininase and chymotrypsin-like activities (Table 1). The venom of *E. c. ocellatus* possessed high levels of both kinin-degrading and chymotrypsin-like activities. It is possible that a chymotrypsin-like enzyme is responsible for the kininase activity of that venom. On the other hand, the venoms of V.a. aspis, V.l. obtusa showed the same or similar levels of chymotrypsin-like activity as that of E.c. ocellatus but were much less potent in degrading kinin. Thus hydrolytic activity towards Ac-Try-OEt should not in general be used as a measure of the chymotrypsin-like kininase activities of venoms. In the main, the viperid venoms contained higher amounts of chymotrypsin-like activity than crotalid venoms.

It has been previously reported that snake venoms lack chymotrypsin-like enzymes on the basis that they do not hydrolyze tyrosine ester (6). Earlier measurements of venom chymotryptic activity utilized spectrophotometric assays which employed much lower concentrations of tyrosine ester than in the titrimetric assay (12, 13). When the titrimetric assay was repeated for some of the venoms using 1 mM Ac-Tyr-OEt as substrate much lower levels of activity were recorded (Bailey and Al-Joufi, unpublished data). The need to use 10 mM Ac-Tyr-OEt in the normal titrimetric assay in order to detect venom chymotrypsin-like enzymes may be due to their relatively high values of Km, or due to the presence of competitive inhibitors of those enzymes in the venoms. Full analysis must await the isolation and characterization of the enzymes. The elapid venoms which were assayed in this study, those of *N. nigricollis, N.n. kaouthia, N.n. naja, B. candidus* and *M. fulvius* did not show any of the enzymatic activities tested for, in agreement with the lack of proteolytic enzymes previously reported for elapid venoms (13).

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