

Classification and Nomenclature of Disintegrins Isolated from Snake Venoms

On behalf of the Registry of Exogenous Hemostatic Factors of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis

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Viper venoms from the Elapidae, Hydrophiidae, Atractaspididae, Viperidae and Colubridae families contain at least 25 separate classes of biologically active compounds [1]. Nomenclature standardization of these molecules by structure and function have been reported for prothrombin activators [2; 3]. No such agreement has been made for the nomenclature and classification of disintegrins which, for this report, are defined as those small molecular weight (4 – 16 kDa), non-enzymatic soluble monomeric or dimeric molecules from viper venom possessing an RGD-like motif and a cysteine arrangement significantly homologous to others in this protein family [4]. At the 51st Annual SSC Meeting in Sydney Australia (August 2005), we reported the variety of names given to the 78 disintegrins then known: 36% called “-tin” (and half of those being “-statin”), and 43% placing “-in” after some combination of genus, species or both. Seventeen percent were named according to the eluted fraction they had during HPLC purification, and 3% used an acronym based on the genus and species plus a number to indicate isomers of the same protein. Only 1% of all disintegrins used the suffix “-or” in the name (Table 5 in [4]). For all of these disintegrins, the naming was done concurrently with the biochemical characterization of the protein in its initial purification from crude venom. Since that time, 15 additional disintegrins have been described (Table 1). The technology of proteomics [5; 6], deduced sequences from cloned cDNA [5; 7], as well as actual recombinant expression of disintegrins even before the protein has been isolated from the crude venom [8; 9], underscore the need for a standardized method of nomenclature for these molecules. The most confusing naming pattern happens when cDNA clones, used to deduce a disintegrin amino acid sequence and named with a letter abbreviation followed by a molecular clone number, are linked with an actual venom-derived protein isolated by HPLC, but the protein is not called the same name as the “gene”. An example is the cDNA clone ML2/8/15 shown to be the same sequence as HPLC fractions ML11 and ML12 [5]. It becomes very challenging for future investigators to know which “name” to associate with their own experiments with that particular viper’s venom. In addition, naming a disintegrin by its HPLC elution fraction number also becomes confusing if the venom is purified under a different gradient or purification method than originally used. Multiple disintegrins, both monomers and dimers, are being isolated each year from the snakes in the Family Viperidae, and we can anticipate this to continue as the venoms of heretofore untested species are being characterized, especially by molecular cloning. Use of a standard nomenclature system will lessen confusion as well as assist in valid comparisons of purified disintegrin activities.

To that end, we suggest the following nomenclature standard be used. We strongly recommend no longer naming isolated venom proteins only by their HPLC fraction elution number. DNA, mRNA and cDNA clones should be named according to the accepted mode described below, and

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written in italics to distinguish each from the protein derived from that gene. Both gene products and protein should have the same name. This is a standard practice in the molecular technology literature [10]. Therefore, *mojavestatin* will clearly be recognized as a gene sequence for the protein mojavestatin. For those instances where the disintegrin has only been isolated by recombinant DNA techniques, the letter “r-“ should be placed before the name, as in “r-jerdostatin”.

Disintegrins named prior to 2006 will retain their well-established trivial names. Each disintegrin will receive a number corresponding to its discovery date in order to establish what will be the next number to be used for future disintegrins named from that species (Table 2). For future discoveries, it will be critical to know the full genus-species-subspecies of the snake, especially in those instances when the crude venom is obtained from a commercial distributor rather than directly from a herpetologist. The investigators will also need to look up the NCBI protein database (<http://www.ncbi.nlm.nih.gov/entrez/>) for any disintegrins named as a result of cDNA analysis but which have not actually been isolated or characterized. It would be helpful to include within the disintegrin name some indication of whether it is a monomer or dimer. A new disintegrin’s name could consist of a combination of genus-subspecies or species-subspecies Latin names followed by the suffix “-min” (for a monomer) or “-din” (for a dimer), plus the next sequential number. For example, four disintegrins from *Echis carinatus sochurecki* have been isolated thus far: echistatin [11], EC3 [12], EC6 [12] and schistatin [13]. The next disintegrins from this subspecies to be discovered would be named ecarisomin 5 (a monomer), ecarisodin 6 (a dimer), and so on. If it is not known whether the disintegrin is a monomer or dimer when first described (as with cDNA analysis), “-tin” could be used as the suffix, followed by the next sequential number, such as “ecarisotin 5”. Further characterization of that disintegrin as a monomer would allow that investigator to call the disintegrin “ecarisomin 5”. The number therefore becomes the constant, allowing future investigators to know that the protein is actually the same. This will also apply when isoforms of the same disintegrin are discovered. As another example, five disintegrins have been isolated from *Gloydus* species: adinbitor from *Gloydus blomhoffi brevicaudus*, brevicaudin 1a, 1b and 2b from *Gloydus halys brevicaudus* and saxatilin from *Gloydus saxatilis*. The next disintegrins to be isolated from each species could be blobrevidin 2 (a dimer), halybrevimin 4 (a monomer) and gloysaxatin 2 (unknown structure), respectively. The heterodimer described by Bilgrami et al. [9], with subunits originally called 1TEJ_A and _B, could now be called “ecaridin 2” since it is the second disintegrin from *Echis carinatus* named without indication of a subspecies (echistatin $\alpha 2$ being the first).

This nomenclature protocol may also be used in those instances where hybrids of disintegrin DNA, made from sequences from two different venom subfamilies, are named using word roots from BOTH subfamilies.

Unfortunately, this will not eliminate the situation where the same disintegrin, described by two different investigators in the same year, is named uniquely, and correctly by this system, since the genus is now named differently in the herpetologist literature. Such a situation occurred with naming kistrin from *Agkistrodon rhodostoma* and rhodostomin from *Calloselasma rhodostoma*.

One caution also needs to be noted for using the suffix “-tin”. Snake proteins which target GPIIb α , GPVI and/or α 2 β 1, known as C-type lectin-like proteins, oftentimes are named with the suffix “-cetin” [14].

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Table 1. Disintegrins named 2004 – 2006, arranged alphabetically by species name, and then by the year in which the disintegrin was discovered in that species. Note that in some instances, the disintegrin is only deduced from cDNA and has not yet been isolated from the crude venom

<i>Species</i>	<i>Disintegrin</i>	<i>Reference</i>
<i>Bothrops jararaca</i>	r-bothrostatin ^a	[15]
<i>Cerastes vipera</i>	CV3	[5]
<i>Crotalus horridus</i>	horrdistatin 1	Galan, in press
<i>Crotalus horridus</i>	horrdistatin 2	Galan, in press
<i>Crotalus scutulatus scutulatus</i>	mojavestatin ^b	[16]
<i>Crotalus scutulatus scutulatus</i>	scutustatin ^b	[16]
<i>Crotalus scutulatus scutulatus</i>	mojastin 1	[17]
<i>Crotalus scutulatus scutulatus</i>	mojastin 2	[17]
<i>Echis carinatus (unknown subspecies)</i>	1TEJ_A and _B (heterodimer)	[9]
<i>Echis ocellatus</i>	EO4-KGD	[6]
<i>Macrovipera lebetina transmediterranea</i>	lebestatin	[18]
<i>Macrovipera lebetina transmediterranea</i>	ML4 ^c	[5]
<i>Macrovipera lebetina transmediterranea</i>	ML2/8/15 ^b	[19]
<i>Macrovipera lebetina transmediterranea</i>	ML11, ML12 ^d	[19]
<i>Trimeresurus jerdonii</i>	r-jerdostatin ^a	[8]

^aderived from cDNA analysis and created as recombinant protein; never actually isolated from crude venom

^bderived from cDNA analysis; never actually isolated from crude venom

^cML4 is most likely lebestatin as described by Olfa et al.[18]

^dML11, ML12 (from HPLC purification) is most likely the same as ML2/8/15 deduced from cDNA

Table 2. Disintegrins named 1988 – 2006, arranged alphabetically by species name, and then numbered sequentially.

<i>Species</i>	<i>Disintegrin</i>	#	<i>Reference</i>
<i>Agkistrodon (Gloydus) halys</i>	halystatin	1	(Fujisawa et al., 1994)
<i>Agkistrodon acutus</i>	accutin	1	(Yeh et al., 1998)
<i>Agkistrodon contortrix contortrix</i>	contortrostatin	1	(Trikha et al., 1994b)
<i>Agkistrodon contortrix contortrix</i>	acostatin	2	(Okuda et al., 2002)
<i>Agkistrodon halys</i>	halysin	1	(Huang et al., 1991a)
<i>Agkistrodon halys</i>	halysetin	2	(Liu et al., 2000)
<i>Agkistrodon halys brevicaudus</i>	salmosin	1	(Kang et al., 1998)
<i>Agkistrodon piscivorus piscivorus</i>	applaggin	1	(Chao et al., 1989)
<i>Agkistrodon piscivorus piscivorus</i>	piscivostatin	2	(Okuda and Morita 2001)
<i>Agkistrodon rhodostoma</i>	kistrin	1	(Dennis et al., 1990)
<i>Agkistrodon ussuriensis</i>	ussuristatin 1	1	(Oshikawa and Terada, 1999)
<i>Agkistrodon ussuriensis</i>	ussuristatin 2	2	(Oshikawa and Terada, 1999)
<i>Bitis arietans</i>	bitistatin	1	(Shebuski et al., 1989)
<i>Bitis arietans</i>	arietin	2	(Huang et al., 1991c)
<i>Bitis gabonica</i>	gabonin	1	(Huang et al., 1992)
<i>Bitis gabonica</i>	gabonin 1	2	(Francischetti et al., 2004)
<i>Bitis gabonica</i>	gabonin 2	3	(Francischetti et al., 2004)
<i>Bothrops asper</i>	bothrasperin	1	(Pinto et al., 2003)
<i>Bothrops atrox</i>	batroxostatin	1	(Rucinski et al., 1990)
<i>Bothrops cotiara</i>	cotiarin	1	(Scarborough et al., 1993)
<i>Bothrops jararaca</i>	jararacin	1	(Scarborough et al., 1993)
<i>Bothrops jararaca</i>	jarastatin	2	(Coelho et al., 1999)
<i>Bothrops jararaca</i>	bothrostatin	3	(Fernandez et al., 2005)
<i>Calloselasma rhodostoma</i>	rhodostomin	1	(Huang et al., 1987)
<i>Cerastes cerastes</i>	cerastatin	1	(Marrakchi et al., 1997)
<i>Cerastes cerastes</i>	CC5	2	(Calvete et al., 2002)
<i>Cerastes cerastes</i>	CC8	3	(Calvete et al., 2002)
<i>Cerastes cerastes cerastes</i>	cerastin	1	(Scarborough et al., 1993)
<i>Cerastes vipera</i>	CV3 ^a	1	(Bazaa et al., 2005)
<i>Crotalus atrox</i>	crotatroxin	1	(Scarborough et al., 1993)
<i>Crotalus basilicus</i>	basilicin	1	(Scarborough et al., 1993)
<i>Crotalus durissus durissus</i>	durissin	1	(Scarborough et al., 1993)
<i>Crotalus molossus molossus</i>	molossin	1	(Scarborough et al., 1993)
<i>Crotalus scutulatus scutulatus</i>	mojastin 1	1	(Sánchez et al., 2006)
<i>Crotalus scutulatus scutulatus</i>	mojastin 2	2	(Sánchez et al., 2006)
<i>Crotalus scutulatus scutulatus</i>	mojavestatin ^a	3	(Sánchez et al., 2005)
<i>Crotalus scutulatus scutulatus</i>	scutustatin ^a	4	(Sánchez et al., 2005)
<i>Crotalus viridis</i>	crotavirin	1	(Liu et al., 1995)
<i>Crotalus viridis cereberus</i>	cereberin	1	(Scarborough et al., 1993)
<i>Crotalus viridis lutosus</i>	lutosin	1	(Scarborough et al., 1993)
<i>Crotalus viridis viridis</i>	viridin	1	(Scarborough et al., 1993)
<i>Echis carinatus (unknown subspecies)</i>	echistatin α 2	1	(Dennis et al., 1990)
<i>Echis carinatus (unknown subspecies)</i>	1TEJ_A, -B	2	2005
<i>Echis carinatus leakyi</i>	echistatin β	1	(Chen et al., 1995)
<i>Echis carinatus leakyi</i>	echistatin γ	2	(Chen et al., 1995)
<i>Echis carinatus leukogaster</i>	leukogastin A	1	(Okuda et al., 2001)
<i>Echis carinatus leukogaster</i>	leukogastin B	2	(Okuda et al., 2001)

<i>Echis carinatus multisquamatus</i>	multisquamatin	1	(Trikha et al., 1994b)
<i>Echis carinatus multisquamatus</i>	EMS 11	2	(Calvete et al., 2003)
<i>Echis carinatus sochurecki</i>	echistatin	1	(Gan et al., 1988)
<i>Echis carinatus sochurecki</i>	EC3	2	(Marcinkiewicz et al., 1999a)
<i>Echis carinatus sochurecki</i>	EC6	3	(Marcinkiewicz et al., 2000)
<i>Echis carinatus sochurecki</i>	schistatin	4	(Tomar et al., 2001)
<i>Echis ocellatus</i>	ocellatin	1	(Okuda et al., 2001)
<i>Echis ocellatus</i>	ocellatusin	2	(Smith et al., 2002)
<i>Echis ocellatus</i>	EO4	3	(Calvete et al., 2003)
<i>Echis ocellatus</i>	EO4-KGD	4	
<i>Echis ocellatus</i>	EO5	5	(Calvete et al., 2003)
<i>Echis pyramidum</i>	pyramidin	1	(Okuda et al., 2001)
<i>Eristicophis macmahoni</i>	eristostatin	1	(Gould et al., 1990)
<i>Eristicophis macmahoni</i>	eristocophin	2	(Scarborough et al., 1991)
<i>Eristicophis macmahoni</i>	EMF10	3	(Marcinkiewicz et al., 1999b)
<i>Gloydus blomhoffi brevicaudus</i>	adinbitor*	1	(Wang et al., 2004)
<i>Gloydus halys brevicaudus</i>	brevicaudin 1a, 1b, 2b	1-3	(Terada, 2000)
<i>Gloydus saxatilis</i>	saxatilin	1	(Hong et al., 2002)
<i>Lachesis mutus</i>	lachesin	1	(Scarborough et al., 1993)
<i>Macrovipera lebetina transmediterranea</i>	lebestatin	1	(Olfa et al., 2005)
<i>Macrovipera lebetina transmediterranea</i>	ML4	2	
<i>Macrovipera lebetina transmediterranea</i>	ML2/8/15	3	
<i>Macrovipera lebetina transmediterranea</i>	ML11, ML12	3*	
<i>Sistrurus catenatus tergeminus</i>	tergeminin	1	(Scarborough et al., 1991)
<i>Sistrurus miliarius barbouri</i>	barbourin	1	(Scarborough et al., 1991)
<i>Trimeresurus albolabris</i>	albolabrin	1	(Williams et al., 1990)
<i>Trimeresurus elegans</i>	elegantin	1	(Williams et al., 1990)
<i>Trimeresurus flavoridis</i>	flavoridin	1	(Musial et al., 1990)
<i>Trimeresurus flavoridis</i>	triflavin	2	(Huang et al., 1991b)
<i>Trimeresurus flavoridis</i>	CTF-I,II	3, 4	(Yamakawa et al., 1991)
<i>Trimeresurus flavoridis</i>	flavostatin	5	(Kawasaki et al., 1996)
<i>Trimeresurus flavoridis</i>	trimestatin	6	(Okuda and Morita, 2001)
<i>Trimeresurus gramineus</i>	trigramin	1	(Huang et al., 1987)
<i>Trimeresurus jerdonii</i>	jerdonatin	1	(Zhou et al., 2004)
<i>Trimeresurus jerdonii</i>	jerdonin	2	(Zhou et al., 2004)
<i>Vipera ammodytes</i>	VA6	1	(Calvete et al., 2003)
<i>Vipera berus</i>	VB7	1	(Calvete et al., 2003)
<i>Vipera lebetina obtusa</i>	VLO4	1	(Calvete et al., 2003)
<i>Vipera lebetina obtusa</i>	VLO5	2	(Calvete et al., 2003)
<i>Vipera lebetina obtusa</i>	obtustatin	3	(Moreno-Murciano et al., 2003)
<i>Vipera palestinae</i>	viperostatin	1	(Kisiel et al., 2004)

*ML11 and ML12 are proposed to be the same protein, derived from the gene ML2/8/15, according to Sanz et al., 2005 [19]