

Article

Genomic Confirmation of the P-IIIe Subclass of Snake Venom Metalloproteinases and Characterisation of Its First Member, a Disintegrin-Like/Cysteine-Rich Protein

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		VaaMPIII-2 CDS		VaaMPIII-3 CDS			
		Exon (bp)	Intron (bp)	Exon (bp)	Intron (bp)		
Exon 1	>	52		52		Exon 1	
	Intron 1 (1)		2084		2295		Intron 1 (1)
Exon 2		107		107		Exon 2	
	Intron 2 (0)		975		883		Intron 2 (0)
Exon 3		71		71		Exon 3	
	Intron 3 (2)		1835		2309		Intron 3 (2)
Exon 4		79		79		Exon 4	
	Intron 4 (0)		1111		837		Intron 4 (0)
Exon 5		77		77		Exon 5	
	Intron 5 (2)		660		4391		Intron 5 (2)
Exon 6		196		127		Exon 6	
	Intron 6 (0)		2281		1916		Intron 6 (0)
Exon 7		57					
	Intron 7 (0)		655				
Exon 8		72					
	Intron 8 (0)		2195				
Exon 9		170					
	Intron 9 (2)		1408				
Exon 10		85					
	Intron 10 (0)		306				
Exon 11		128					
	Intron 11 (2)		2755				
Exon 12		178					
	Intron 12 (0)		830				
Exon 13		90					
	Intron 13 (0)		1057				
Exon 14		196		196		Exon 7	
	Intron 14 (1)		2307		2619		Intron 7 (1)
Exon 15		103		103		Exon 8	
	Intron 15 (2)		7931		5870		Intron 8 (2)
Exon 16		133		118		Exon 9	
	Intron 16 (0)		545		1038		Intron 9 (0)
Exon 17	>	48		45		Exon 10	
Σ exons		1842		975			
Σ introns			28935		22158		

Figure S1. Exon and intron lengths, and intron phases in the VaaMPIII-2 and VaaMPIII-3 genes. Intron phase is shown in parentheses (0, 1 or 2). Intron phases in VaaMPIII-2: 1,0,2,0,2,0,0,0,2,0,2,0,0,1,2,0; and in VaaMPIII-3: 1,0,2,0,2,-,-,-,-,-,-,-,0,1,2,0 (dashes denote missing introns according to VaaMPIII-2).

N-terminal sequencing	LC-ESI-MS/MS	NCBI accession number
RAGTECRPARSECDVAEYC	(R)KENDVPIPCAPEDIK(C), (R)KENDVPIPCAPEDIKCGR(L), (K)ENDVPIPCAPEDIKCGR(L), (R)LFCELIKNTCK(Y), (K)YDYSEDPDYGMVDHGK(C), (K)NTCKYDYSEDPDYGMVDHGK(C), (R)HCVDVTTAY	MG958499

RAGTECRPARSECDVAEYCTGQSAECPDVFHSNGKPLNNGYCYNGNCPIMYHQCIALFG
 PNATVGQDGCFEWNKKGESYFYCRKENDVPIPCAPEDIKCGRLFCELIKNTCKYDYSEDPDY
 GMVDHGKCGDGKVCINRHCVDVTTAY

Figure S2. Structural identification of the isolated protein as VaaMPIII-3. After reduction and alkylation of Cys residues, one part of the isolated protein was N-terminally sequenced, and the other fragmented with trypsin and subjected to tandem mass spectrometry analysis (LC-ESI-MS/MS). Sequences of seven tryptic peptides were determined. All sequenced parts of the isolated protein are displayed in the table and correspond to VaaMPIII-3 (NCBI accession number: MG958499). On the primary structure of VaaMPIII-3, the identified parts of the sequence (49%) are typed either on black (Edman analysis) or gray background (MS analysis).

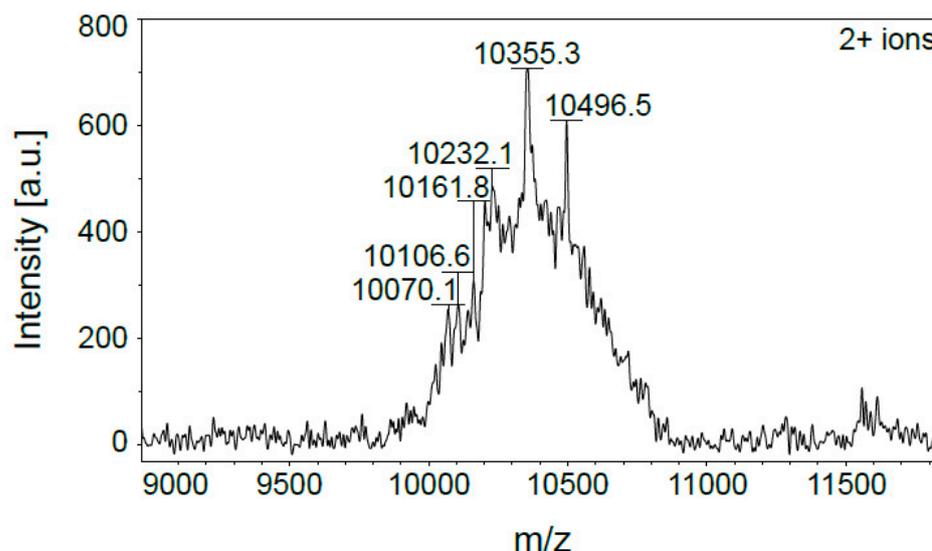


Figure S3. Mass spectroscopy of VaaMPIII-3. The VaaMPIII-3 sample was analysed using UltrafleX-treme III MALDI-TOF/TOF mass spectrometer (Bruker, Billerica, MA, USA). Only doubly charged VaaMPIII-3 ions are shown, where the existence of multiple glycoforms of this protein is nicely visible.