Characterization and Preliminary Crystallographic Studies of EMS16, an Antagonist of Collagen Receptor (GPIa/IIa) from the Venom of *Echis multisquamatus*

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Received March 13, 2003; accepted April 21, 2003

EMS16 is a member of the snake venom-derived C-type lectin family of proteins (CLPs) found in the venom of *Echis multisquamatus*. It binds to glycoprotein Ia/IIa (integrin $\alpha 2\beta 1$), a major collagen receptor of platelets, acting as a potent antagonist of platelet aggregation and cell migration. Amino acid sequencing and cDNA cloning of EMS16 have revealed that it is composed of an A chain of 134 amino acid residues and a B chain of 128 residues. Crystals of EMS16 belong to space group $P2_12_12_1$, with unit-cell parameters a = 46.57, b = 59.93, and c = 115.74 Å, and diffract to a resolution of 1.9 Å. Phase determination is underway by means of molecular replacement with the structure of blood coagulation factor IX-binding protein (IX-bp) from habu snake venom (PDB code 1bj3) as the search model.

Key words: collagen receptor, C-type lectin-like protein, *Echis*, Glycoprotein Ia/IIa, snake venom.

Snake venom contains many protein components that affect haemostasis and the nervous system in various ways. A great many of these venom proteins are classified into superfamilies, such as metalloproteases, phospholipases and ion channel antagonists. The C-type lectin family proteins (CLPs) are some of the major components of snake venom. Even though these proteins have regions of amino acid sequence homology, the protein targets differ: some are directed at the haemostatic system, in which blood coagulation factors play an important role, while others are directed at plasma proteins and platelet glycoproteins. Recently, we reported the Xray crystal structures of several CLPs, blood coagulation factors IX/X binding protein (IX/X-bp) (1), factor X binding protein (X-bp) (2), factor IX binding protein (IX-bp) (3), flavocetin-A (4), and bitiscetin (5). They all have a heterodimeric structure $(\alpha\beta)$ or form heterodimeric tetramers $(\alpha\beta)_{4}$ and also have a unique 3D domain-swapping loop in their molecules.

The glycoprotein Ia/IIa complex (also commonly referred to as "integrin $\alpha 2\beta 1$ ") is a major collagen receptor found on platelet membranes and is responsible for platelet activation with another collagen receptor, GPVI. Many researchers have studied the mechanisms of interaction between GPIa/IIa and GPVI during platelet aggregation, but because there are numerous complexes produced during signal transduction, as well as other factors, the true mechanism is not clearly understood. Currently, snake venom CLPs such as convulxin (6, 7), a GPVI-agonist, and aggretin (rhodocytin) (8, 9), a GPIa/IIa-agonist, are used as tools for studying the mechanism of collagen-induced platelet aggregation.

Recently, a new CLP, EMS16 from the venom of *Echis* multisquamatus, was reported by Marcinkiewicz *et al.* (10) as the first selective GPIa/IIa antagonist from snake venom. It binds to the integrin $\alpha 2$ -I domain, which is the collagen-binding site of integrin $\alpha 2$, causing inhibition of collagen-induced platelet aggregation and cell migration.

The X-ray crystal structure of the integrin α 2-I domain was previously reported by Emsley *et al.* (11). As the first step to clarify the binding mechanism in a complex of integrin α 2-I domain and EMS16, the amino acid sequence of EMS16 was deduced by cDNA cloning, and its crystal structure analysis is underway.

MATERIALS AND METHODS

Materials—Pre-packed columns, Superdex 75 pg and S-Sepharose High Performance, were purchased from Amersham Pharmacia Biotech (UK), the COSMOSIL 5C18 AR-300 HPLC column was from Nacalai Tesque (Kyoto), endoprotease Asp-N was from Boehringer Mannheim, and N-glycosidase F was from Pierce. The DNA polymerase Advantage 2 polymerase mix and Pyrobest were purchased from CLONTECH (CA) and TaKaRa (Shiga), respectively. Chemicals of reagent grade were obtained from Amersham Bioscience (UK), Sigma (MO), or Wako Pure Chemicals (Osaka).

Protein Purification—The crude venom of *E. multisq-uamatus* was dissolved in 50 mM Tris-HCl buffer, pH 8.0, and insoluble materials were removed by centrifugation. The supernatant was fractionated by gel filtration with Superdex 75pg and collected fractions were assayed by determining the cross-reactivity with anti-IX/X-bp antibody. The cross-reacting fractions were pooled and purified by successive chromatographies on a Q-Sepharose HP column and then a reversed-phase HPLC column (COSMOSIL 5C18 AR-300). EMS16 was identified by N-

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20

-79															ACG	CGG	GGGG	AAA	GCC	TGGA	-61
-60	GT	TGC	CTC	TGA	.GCA	GAG	TTG	CTA	ССТ	GGG	GAG	GCI	GAA	GGA	CAG	GGF	AGG	SAAC	GAA	GACC	-1
1	AT	GGG	GCG	ATT	CAT	CTC	CGI	CAG	CTT	CGG	CTT	GCI	GGI	CGT	GTI	CCI	сто	CCI	GAG	TGGG	60
-23	Μ	G	R	F	I	S	V	S	F	G	L	L	V	V	F	L	S	L	S	G	-4
61	AC	TGG	AGC	TGA	CTT	CGA	TTG	TCC	ATC	TGA	TTG	GAC	cgc	СТА	TGA	TCA	AGCA	TTG	СТА	CCTG	120
-3	T	G	А	D	F	D	С	P	S	D	W	Т	A	Y	D	Q	Н	С	Y	L	17
121	GC	CAT	TGG	TGA	ACC	CCA	GAA	сто	GTA	TGA	AGC	CGA	GAG	GTT	CTG	CAC	GGA	GCA	GGC	GAAA	180
18	A	I	G	Е	Р	Q	N	W	Y	Е	A	Е	R	F	С	т	Е	Q	A	ĸ	37
181	GA	ccc	GCA	тст	የርርጥ	ירייר	аат		AAG	CAG	AGA	AGA	AGG		CTT	ייזיפיו	າດດດ	'CCA	GCT	GGTC	240
38	D.	соо с	ч	T.	17	g	т	0	c c	D	F	F	C	N	F	101		0	T.	W	57
30	<u> </u>	9	11	<u> </u>	v	5	<u> </u>	<u>v</u>	5	K	Ľ	E	_ ^G	IN	r	v	л	Ŷ	ш	v	57
241	TC	TGG	CTT	CAT	GCA	TAG	ATC	CGA	AAT	CTA	TGT	CTG	GAT	TGG	GCI	GAG	GGGA	TCG	ACG	CGAA	300
58	s	G	F	М	Н	R	s	Е	I	Y	v	W	I	G	L	R	D	R	R	Е	77
301	GA	GCA	GCA	ATG	CAA	ccc	AGA	ATG	GAA	TGA	CGG	CTC	CAA	GAT	'CA'I	TTA	ACGT	מאמי	CTG	GAAA	360
78	Е	Q	Q	с	N	P	Е	W	N	D	G	s	ĸ	I	I	Y	v	N	W	K	97
	_																				
361	GA	AGG	AGA	ATC	CAA	AAT	GTG	TCA	AGG	GTT	GAC	TAA	ATG	GAC	AAA	TTT	TCA	CGA	CTG	GAAC	420
98	E	G	Е	S	K	М	С	Q	G	_L	Т	K	W	Т	N	F	H	D	W	N	117
421	AA	TAT	TAA	CTG	TGA	AGA	тст	TTA	TCC	TTT	CGI	CTG	CAA	ATT	стс	AGC	AGI	GTG	AAG	TCTG	480
118	N	I	N	С	Е	D	L	Y	Ρ	F	v	С	K	F	s	А	v	*			134
	_																	_			
481	GA	GAA	TCA	AGG	AAG	CCC	CCC	ACC	TAC	TCC	CCG	CCA	CCA	GCC	GCC	ATC	тст	GCI	CTG	CCCC	540
541	СТ	TCG	CTC	AGT	GGA	TGC	TCI	CTO	TGG	CCG	GGA	TCI	GGI	TTT	GCI	GCI	CCI	GAT	GGG	CCAG	600
601	GA	GGT	CCA	ATA	AAT	TCT	GCC	TAG	CAC	C-p	oly	(A)									627

terminal amino acid sequence analysis of the intact and S-pyridylethylated proteins. Deglycosylation of EMS16 was carried out by treatment with N-glycosidase F at 37°C for 48 h.

Determination of the cDNA Sequence-cDNA cloning of EMS16 was carried out by the RT-PCR method using total RNA isolated from the venom gland of E. multisquamatus. The template for RACE-PCR was prepared by RT-PCR using MMLV reverse transcriptase. 5'- and 3'-RACE were carried out to determine the nucleotide sequences of both the 5'- and 3'- cDNA ends using Pyrobest (TaKaRa)

-42							ΤG	CTA	CCT	GTG	GAG	GCI	GAA	GGA	CAG	IGG <i>P</i>	AGG	AAG	GAA	GACC	-1
1	AT	GGG	GCG	ACT	CAT	CTC	CGT	CAG	GTT	CAG	CTI	GCI	GGT	CGI	GTI	CCI	стс	сст	GAG	TGGA	60
-26	М	G	R	L	I	s	V	R	F	s	L	L	V	V	F	L	S	L	s	G	-7
61	AT	TGG	AGC	TGG	TTT	GTG	TTG	TCC	CTT	GGG	TTG	GTC	стс	стт	TGA	TCA	GCA	TTG	СТА	TAAG	120
-6	I	G	А	G	L	С	c	P	L	G	W	S	S	F	D	Q	Н	С	¥	ĸ	14
121	GT	TTT	CGA	ACC	AGT	CAA	ААА	CTG	GAC	CGA	GGC	CGA	GGA	AAT	CTG	CAI	GCA	ACA	.GCA	CAAA	180
15	v	F	Е	Ρ	V	K	_N	W	Т	Е	A	E	Е	I	C	М	Q	Q	H	ĸ	34
181	GG	CAG	CCG	TCT	GGC	СТС	CAT	CCA	CGG	CAG	TGA	AGA	AGA	AGC	TTT	TGI	GTC		GCI	GGCC	240
35	G	s	R	L	A	s	I	н	G	s	Е	Е	Е	A	F	v	s	ĸ	\mathbf{L}	A	54
241	тс	CAA	AGC	TTT	GAA	ATT	TAC	TTC	CAT	GTG	GAI	CGG	ACT	GAA	TAA	TCC	ATG	GAA	AGA	CTGC	300
55	s	ĸ	A	L	K	F	т	s	М	W	I	G	L	N	N	Р	W	к	D	с	74
301	АА	ATG	GGA	GTG	GAG	TGA	таа	TGC	CAG	АТТ	CGA	CTA	CAA	AGC	ATG	GAA	GCG	AAG	ACC	CTAC	360
75	ĸ	W	Е	W	s	D	N	A	R	F	_D	Y	ĸ	A	W	K	R	R	Р	Y	94
361	тC	ሞልሮ	ACT	יייגע	сст	CCT	0 2 2	ccc		ጥልር	CAT	CTTT	የጥጥር	CTT	יריאר	ሞልር	ACC	ምምር	CCA	AAAG	420
95	c	Т	v	м	v	v	ĸ	Р	<u>D</u>	R	I	F	W	F	Т	R	G	с	_E	K	114
421	тс	GGT	ATC	TTT	TGT	CTG	CAA	GT1	сст	GAC	AGA	TCC	AGC	TGT	GTG	AAG	TCI	GGA	GAA	GCAA	480
115	s	v	s	F	v	С	K	F	L	т	D	Ρ	A	v	*						128
481	GG	AAG	ccc	ccc	ACC	CCA	CTT	GGC	ccc	CAC	CTG	cco	GCCA	TCT	CTG	сто	TGC	ccc	CTI	CACT	540
541	CA	GTG	GAT	GCT	стс	TGT	AGC	CGG	GAT	CTG	GCI	TTG	CTG	CTC	CTG	ATG	GGC	ccc	GAG	GTCC	600
601	AA	TAA	ATT	CTG	сст	AGC	ACG	-pc	ly(A)											620

Fig. 1. cDNA and deduced amino acid sequence of the EMS16 A chain. The numbering of nucleotides and amino acids (bold) are indicated on both sides of the sequence. 1 indicates the position of the start codon and the N-terminus of the EMS16 A chain, respectively. The putative signal peptide, mature EMS16 A chain, and polyadenylation signal are indicated by italics. underlining and bold underlining, respectively. The cDNA sequence of the EMS16 A chain has been deposited in GenBank under accession number AB098253.

with degenerate primers based on partial amino acid sequences of EMS16, and single primers identified by the nucleotide sequence. The nucleotide sequence was determined with a DSQ2000L DNA sequencer (Shimadzu, Crystallization of EMS16-Initial crystallization experiments on EMS16 were performed using the microbatch technique (12) with Crystal Screen I (Hampton Research). Crystals were grown at 293 K under a thin layer of Al's

> Fig. 2. cDNA and deduced amino acid sequence of the EMS16 B chain. The numbering of nucleotides and amino acids (bold) are indicated on both sides of the sequence. 1 indicates the position of the start codon and the N-terminus of the EMS16 B chain, respectively. The putative signal peptide, mature EMS16 B chain and polyadenylation signal are indicated by italics, underlining and bold underlining, respectively. The cDNA sequence of the EMS16 B chain has been deposited in GenBank under accession number AB098254.

oil (Hampton Research) on a 96-well plate (Nunc), 0.6 µl

of the purified protein solution (5 mg/ml in 50 mM Tris

Kvoto)

A/α chain	1	20 40	60
EMS16 A (GPIa/IIa) Aggretin A (GPIa/IIa) Convulxin A (GPVI) Flavocetin-A α (GPIb)	GLEDCPSDWTAYDQHCYL GLEDCDFGWSPYDQHCYQ GLHCPSDWYYYDQGCYR DFDCIPGWSAYDRYCYQ	AIGEPONWYEAERFCTEOAKDGH AFNEOKTWDEAEKFCRAOENGAH IFNEEMNWEDAEWFCTKOAKGAH AFSKPKNWEDAESFCEEGVKTSH	JVSIQSREEGNFVAQLVSGFMHR-SEIYVWI LASIESNGEADFVSWLISQKDELADEDYVWI LVSIESAKEADFVAWWVTQNIEE-SFSHVSI JVSIESSGEGDFVAQLVAEKIKTFQYVWI
EMS16 A Aggretin A Convulxin A Flavocetin-Aα	80 GLRDRREEQOCNPEWND GLRAQNKEOQCSSEWSD GLRVQNKEKQCSTKWSD GLRIQNKEQOCRSEWSD	100 GSKIIYVNWKEGESKMCOGLTKW GSSVSYENLIDLHTKKCGALEKL GSSVSYENLVKQFSKKCYALKKG	120 134 identity I I (%) INFHDWNNINCEDLYPFVCKFSAV 100.0 rGFRKWVNYYCEQMHAFVCKLLPY 46.9 rGFRKWFVASCIGKIPFVCKFPPQC 45.1 rELRTWFNVYCGTENPEVCKYTPEC 48.1
B /β chain EMS16 B (GPIa/IIa) Aggretin B (GPIa/IIa) Convulxin B (GPVI) Flavocetin-A β (GPIb) RVV-X LC B (F.X)	1 I CPLGWSSFDQHCYKVFE DCPSGWSSYEGHCYKPFN DFCCPSHWSSYDFYCYKVFK DFCCPLGWSSYDEHCYQVFQ VLDCPSGWLSYEQHCYKGFN	20 40 I PVKWTEAEEICMQQHKGSRLAS EPKIWADAERFCKLQPKHSHLVS QEMTWADAEKFCTQQHTGSHLVS QKMNWEDAEKFCTQQHKGSHLVS DLKKWTDAEKFCTEQKKGSHLVS	60 HGSEEEAFVSKLASKALKFTSMWIGLNNP FQSAEEADFV-KLTRPRLKANLVWMGLSNI FHSTEEVDFVVKMTHQSLKS-FFWIGLNNI FHSSEEVDFVTSKTFPILKYDFVWIGLSNV LHSREEEEFVVNLISENLEYPATWIGLGNM
EMS16 B Aggretin B Convulxin B Flavocetin-A β RVV-X LC B	80 - WKDCKWEWSDNARFDY VWHGCNWQWSDGARLNY - WNKCNWQWSDGTKDY - WNECTKEWSDGTKLDY - WKDCRMEWSDRGNVKY	100 KAWKRRPYCTVMVVKPDRIFWFT KDWQEQSECLAFRGVHTEWLN KEWHEEFECLISRTFDNQWLS KAWSGGSDCIVSKTTDNQWLS KALAEESYCLIMITHEKEWKS	120 128 identity I I (%) RGCEKSVSFVCKFLTDPAV 100.0 MDCSSTCSFVCKFKA 44.3 APCSDTYSFVCKFEA 43.4 MDCSSKYVVVCKFQA 48.4 MTCNFIAPVVCKF 47.5

Fig. 3. Alignment of the amino acid residues in the primary structures of snake venom C-type lectin proteins. The numbering at the top refers to EMS16. The ligand of each CLP is shown in parentheses. The conserved amino acid residues are shaded and the positions of N-glycosylated Asn residues have a black background. The dots indicate the location of the consensus sequence of the N-gly-

HCl buffer at pH 7.5) being mixed with 1.2 μ l of the precipitant solution. Initial investigation revealed protein microcrystals to be present in solutions containing the precipitant PEG8000 (Crystal Screen I: No. 42). The crystallization conditions were thereafter optimized by adjusting the volume ratio of protein solution to reservoir solution using the hanging-drop vapor-diffusion method. After mixing 1 μ l of protein solution and 3 μ l of precipitant solution (0.04 M potassium dihydrogen phosphate, 16% PEG8000, 20% glycerol suspended over 0.5 ml of reservoir solution), good diamond-shaped crystals appeared in about a week and grew to dimensions of $0.4 \times 0.4 \times 0.1$ mm (Fig. 5).

RESULTS AND DISCUSSION

EMS16 was purified by three-step column chromatography (yield: 1.1 mg/g venom). The N-terminal sequences of the S-pyridylethylated (Pe-) subunits were NH_2 -DFD-CPSDWTAYDQHCYLAIGE for the Pe-A chain and NH_2 -CPLGWSSFDQHCYKVFPVKXWTEAE for the Pe-B chain. These sequences are identical to those of EMS16, which were previously reported by Marcinkiewicz *et al.* (10). The S-pyridylethylated subunits of EMS16 were digested with endoprotease Asp-N and the peptide fragments were analyzed as to their amino acid sequences. The PTH-amino acid of residue 21 in the B chain could not be detected on sequencing of either the Pe-B chain or the Asp-N digested fragment.

The cDNAs encoding the EMS16 subunits were obtained by the RT-PCR method from the total RNA of the venom gland of *E. multisquamatus*. The nucleotide cosylation site in EMS16 and the RVV-X light chain B (13). The unconserved cysteine residues, including the C-terminus of the A chain and the N-terminal portion of the B chain of convulxin and flavocetin-A, and Cys115 in the B chain of aggretin, bridge the heterodimeric units ($\alpha\beta$) to give multimeric molecules ($\alpha\beta$)_n. RVV-X LC, Russell's viper venom coagulation factor X activator light chain.

sequence of the EMS16 A chain comprised 706-bp, including a 79 bp 5'-untranslated region (UTR), a 479 bp open reading frame (ORF), and a 156 bp 3'-UTR (Fig. 1), while the B chain comprised 662-bp, including a 42 bp 5'-UTR, a 462 bp of ORF, and a 158 bp 3'-UTR (Fig. 2). Figure 3 shows the deduced amino acid sequence of mature EMS16 compared with those of other snake venomderived CLPs that bind to platelet glycoproteins. The amino acid sequence of EMS16 was approximately 50% homologous with those of other snake CLPs, indicating that EMS16 belongs to the snake venom class of CLPs. The deduced amino acid sequence indicated that the 21st residue of the B chain is Asn. Alignment of the 21st to 23rd residues (Asn-Trp-Thr) suggested the possibility of N-glycosylation of the Asn at position 21 in the B chain,

(kDa)

67

43

30

was performed under non-reducing conditions.

20.1 -

14.4 ->

(-) (+) Fig. 4. N-Glycosidase treatment of EMS16. EMS16 (2 μg) was treated with N-glycosidase F at 37°C for 48 h. (–) without N-glycosidase F treatment; (+) after N-glycosidase F treatment. SDS-PAGE



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22

Fig. 5. Crystals of EMS16. Photograph of EMS16 crystals grown from PEG8000. The maximum dimension of the crystal was approximately 0.4 mm.

which also agrees with the result of amino acid sequencing of the B chain, in which the 21st residue could not be identified. As shown in Fig. 3, RVV-X (Daboia russelli viper venom coagulation factor-X activator) has an N-glycoside attached to Asn24 of light chain-B [previously called "light chain-1(LC1)" (13)]. The apparent mass of EMS16, as determined by SDS-PAGE, was decreased (31-kDa to 28-kDa) on treatment with N-glycosidase F (Fig. 4), resulting in an approximately 2.4 kDa difference between the 33,292 Da reported for EMS16 by Marcinkiewicz et al. (10) and the value of 30,942 Da calculated from the deduced amino acid sequence. This indicates that EMS16 very likely has an N-glycoside attached to the Asn at position 21 of the B chain.

Diffraction data for EMS16 were collected in-house using an R-AXIS IV⁺⁺ imaging-plate detector mounted on a $CuK\alpha$ rotating-anode generator equipped with an Osmic mirror system (Rigaku). A crystal was flash-cooled to 120 K in a stream of cold nitrogen-gas (Rigaku Cryogenic System) and then rotated through 90°, with 0.5° oscillation per frame. The raw data were processed and scaled using the program CRYSTAL CLEAR ver. 1.3 (Rigaku).

The crystals of EMS16 belong to orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 46.57, b =59.93, and c = 115.74 Å, and diffract to a resolution of 1.9 Å. Analysis of the packing density showed that one molecule of EMS16 per asymmetric unit would yield a reasonable solvent content $[V_{\rm M} = 2.6 \text{ Å}^3\text{Da}^{-1} \text{ and estimated solvent content } V_{\rm solv} = 53\%; (14)]$. The statistics for data collection are listed in Table 1.

Structural analysis of EMS16 was carried out by the molecular-replacement method using program AMoRe in the CCP4 suite [Collaborative Computational Project, 1994; (15)]. A search model was constructed by homology modeling using Insight II 2000 (Accelrys) based on the atomic coordinates of factor IX-binding protein (IX-bp) from the venom of the habu snake [PDB code 1bj3; (3)]. EMS16 and IX-bp show amino acid sequence homology of 54.7 and 43.9% for the A and B chains, respectively. After rotation and translation calculations, a clear peak was found with a correlation coefficient of 0.624 and an R factor of 39.0% (10–4 Å). Structural refinement is currently in progress using program CNS (16) and XtalView (17).

Table 1. Data collection statistics. Values in parentheses refer to the highest resolution shell.

0	
Wavelength (Å)	1.5418
Space group	$P2_{1}2_{1}2_{1}$
Cell dimensions (Å)	$a = 46.57 \ b = 59.93 \ c = 115.74$
Resolution range (Å)	30-1.9 (1.97-1.9)
Measured reflections	91,401
Unique reflections	26,199
Ι/σ (Ι)	16.1 (5.6)
Completeness (%)	99.7 (99.7)
Mosaicity (°)	0.6
$R_{ m merge}^{ m a}$ (%)	3.1 (11.6)
Unique reflections I/σ (I) Completeness (%) Mosaicity (°) R_{merge}^{a} (%)	26,199 16.1 (5.6) 99.7 (99.7) 0.6 3.1 (11.6)

 ${}^{a}R_{\text{merge}} = \Sigma_{h} \Sigma_{i} | I(h, i) - \langle I(h) \rangle | / \Sigma_{h} \Sigma_{i} I(h, i)$, where I(h, i) is the intensity value of the *i*th measurement of h and $\langle I(h) \rangle$ is the corresponding mean value of I(h) for all *i* measurements.

Platelet collagen receptors such as integrin $\alpha 2\beta 1$ (GPIa/IIa) and GPVI play an important role in platelet activation. Many researchers are currently investigating the mechanism of platelet function, including signal transduction, whereby binding of collagen to integrin $\alpha 2\beta 1$ and GPVI leads to platelet activation. The snake venom proteins that are specific for platelet glycoproteins are therefore useful tools for studying the process of platelet activation. For example, convulxin, which is a potent platelet agonist for GPVI, is a major tool being used in the study of the collagen-platelet interaction. However, there is no specific antagonist against collagen receptors, except for monoclonal antibodies to integrin $\alpha 2\beta 1$ and GPVI. EMS16, a snake venom CLP, is the first specific antagonist of integrin $\alpha 2\beta 1$, especially of the integrin α2-I domain, where collagen interacts to initiate platelet activation.

We have previously reported the crystal structures of several CLPs: coagulation factor IX/X-binding protein (bp) (1), factor X-bp (2), factor IX-bp (3), flavocetin-A (4), and bitiscetin (5). EMS16 shows significant sequence similarity to other CLPs, suggesting that the overall 3D structure of EMS16 should be similar, with unique 3D domain swapping. Determination of the structure of EMS16 and its complex with the integrin α 2-I domain should provide valuable information regarding the collagen-integrin $\alpha 2\beta 1$ interaction and permit the design of an anti-haemostasis drug.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (T.M.). We would like to thank Dr. Yuri Utkin (Russian Academy of Science, Moscow, Russian Federation) for helping to catch the *Echis multisquamatus*, and Drs Michihisa Toriba and Atsushi Sakai (Japan Snake Institute, Gunma) for extracting the venom glands. We thank Tomoko Yokomizo and Yuko Tokunaga for their help with the peptide sequencing of EMS16.

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