Molecular Cloning and Sequence Analysis of Aggretin, a Collagen-like Platelet Aggregation Inducer

Ching-Hu Chung,* Lo-Chun Au,†,‡ and Tur-Fu Huang‡,1

*Department of Pharmacology, College of Medicine, National Taiwan University, Taiwan; †Department of Medical Research, Veterans General Hospital-Taipei, Taiwan; and ‡Graduate Institute of Medical Biotechnology, National Yang-Ming University, Taipei, Taiwan

A cDNA library derived from the Malayan-pit-viper (*Calloselasma rhodostoma*) venom gland was constructed in the phagemid vector. Using the information of the N-terminal amino acid sequences of two subunits of aggretin, synthetic mixed-base oligonucleotides were employed as a screening probe for colony hybridization. Separate cDNA clones encoding for the α and β chains of aggretin were isolated and sequenced. The results revealed that mature α and β chains contain 136 and 123 amino acid residues, respectively. Aggretin subunits show high degrees of identity with respective subunits (50–60% for α, 49–58% for β) of C-type lectin-like snake venoms. The identity to rattlesnake lectin is relatively lower (i.e., 39 and 30%). All cysteine residues in each chain of aggretin are well conserved and located at the positions corresponding to those of C-type lectins. Thus, three intracatenary disulfide bridges and an interchain disulfide bond between Cys83(α) and Cys75(β) may be allocated. This is the first report regarding the entire sequence of venom GPIa/IIa agonist. According to the alignment of amino acid sequences, hypervariable regions among these C-type lectin-like proteins were revealed. These hypervariable regions are proposed to be the counterparts directly interacting with different receptors or different domains of a receptor on the surface of platelet. © 1999 Academic Press

Snake venoms affect blood coagulation and platelet function in a complex manner (1). The most pronounced anti-platelet constituents are Arg-Gly-Asp containing trigramin-like peptides, which have been identified as specific fibrinogen receptor antagonists and named as disintegrins (2–4). Several non-coagulant, non-enzymatic proteins that cause platelet aggregation have been purified from different snake venoms (5–7). Aggretin, a potent platelet-aggregating protein purified from *Calloselasma rhodostoma* venom, is a 29 kDa heterodimeric protein consisting of two subunits with molecular masses of 18 kDa and 15 kDa, linked by disulfide bridge (5). It elicited platelet aggregation in both human platelet-rich plasma and washed platelet suspension. It activates platelet through a Ca^{2+}-dependent mechanism and is devoid of enzyme activity. It may activate platelets through the binding of a collagen-like receptor (i.e. glycoprotein Ia/IIa) on the platelet membrane (5).

Rattlesnake lectin (RSL) from *Crotalus atrox* venom was determined as a C-type lectin and it consists of a carbohydrate-recognition domain (CRD) (8). RSL is composed of covalently linked and formed as a homodimer molecule. Many distinct proteins isolated from snake venoms consist of two heterogeneous CRD-like domain linked by an interchain S-S bond. For example, the sequences of glycoprotein Ib agonist or antagonist revealed high homology with those of C-type lectins (9), including botrocetin isolated from venom of *Bothrops jararaca* (10), bitiscetin from *Bitis arietans* (11), habu IX/X-bp from *Trimeresurus flavovoridis* (12), and echicetin from *Echis carinatus* (13, 14). A collagen-like platelet aggregating protein, convulxin, from venom of *Crotalus durissus terrificus* binds to a specific platelet receptor GPVI. Convulxin consists of two subunits, α and β, and the two chains were homologous to those of the CRD of C-type lectins (15). From the available data concerning the N-terminal sequences of α and β-chain of aggretin, we found that they share a high degree of homology as compared with the respective chain of these GPIb-binding proteins, even though aggretin acts on a distinct target, GPIa/IIa rather than GPIb (5).

In this study, we isolated cDNA clones of aggretin α and β subunits. The amino-acid sequences were deduced after the determination of nucleotide sequence of each clone. The polypeptide sequences were found to align well with those of CRD containing GPIb-binding proteins, although they may act in different way to induce platelet activation or to inhibit the interaction between von Willebrand factor and GPIb (16, 17).

MATERIALS AND METHODS

Determination of N-terminal amino acid sequence of aggretin. Aggretin was purified from crude venom of *Calloselasma rhodostoma* according to the method described previously (5). Aggretin (10 μg) was dissociated by boiling in protein denaturing buffer containing β-mercaptoethanol and then electrophoresed into 12% SDS-PAGE. Two bands (subunits), α, 18 kDa and β 15 kDa were revealed after electro-blotted to PVDF membrane according to the method of Matsudarin *et al.* (18), and stained with Coomassie blue. The protein of each band was subjected to determination of N-terminal amino acid sequencing by using an Applied Biosystems model 477A pulsed liquid-phase sequencer equipped with an on-line 120 PTH amino acid analyzer. The N-terminal sequence of 24 residues for the α subunit and N-terminal sequence of 18 residues for the β subunit were determined (Fig. 1).

Construction of venom gland cDNA library. The cDNA library of the *Calloselasma rhodostoma* venom gland was constructed previously (19). A set of cDNAs derived from a poly(A)$^+$ RNA preparation of the venom glands of *Calloselasma rhodostoma* were cloned into the Uni-ZAP XR vector (20) by inserting the cDNAs into the EcoRI and XhoI sites of the Uni-ZAP XR phage DNA. For convenience, the Uni-ZAP XR vectors was excised *in vivo* to generate a pBluescript SK-phagemid cDNA library by co-transfecting the Uni-ZAP XR phage and an R408 helper phage (21) into *Escherichia coli* XL1-Blue host cells according to the manufacturer's instructions (Stratagene, La Jolla, CA, USA).

Primer design. A 20meric mixed-base oligonucleotide probe, based on the N-terminal amino-acid sequence of α subunit was synthesized (Fig. 1). For the screening of β subunit, a 20meric oligonucleotide probe was synthesized according to the conserved sequence, mamushigin's coding region (22), of GPIb-binding protein (Fig. 1).

Isolating of cDNA clones of aggretin. E. coli XL1-Blue was infected with the rescued phagemid. The infected bacteria were plated out on to Luris broth/ampicillin plates. Ampicillin-resistant colonies were transferred on to nitrocellulose membrane and prepared for colony hybridization (23). The 20meric oligonucleotide probe (see Fig. 1) was ^{32}P-end-labeled with T4 polynucleotide kinase and (γ-^{32}P)ATP (24). The membrane replicas were pre-hybridized at 65°C for 3 h in hybridization buffer (0.45 M sodium chloride, 0.045 M sodium citrate, 0.02 M potassium phosphate, pH 6.8, 0.1% (w/v), 0.1% (w/v) ficoll and 0.02% (w/v) polyvinylpyrrolidone) plus 100 μg/ml sheared and denatured herring-sperm DNA. The membrane replicas were then hybridized at 49°C for 8 h in 30 ml of hybridization buffer containing 5 pmol of the labeled oligonucleotide probe. Subsequent washes of the hybridized membrane filters were carried out in 6 × SSC (1 × SSC is 0.15 M NaCl/0.015 M sodium citrate) and 0.1% (w/v) SDS as follows: four times at 4°C for 5 min each, once at room temperature (5 min) and once at 47°C (2 min). The positive clones were detected by autoradiography, and were further purified by the isolation of single cell colonies.

DNA sequencing and nucleotide analysis. DNA sequencing was performed by the PCR/dideoxyl method using a ABI PRISM BigDye

Aggretin α chain N-terminal

^{1}G	L	E	D	C	D	F	G	W	S	P
Y	D	Q	H	C	Y	Q	A	F	N	E
TAPy	GAPy	CAPu	CAPy	TGPy	TAPy	CA				

20meric mixed-base oligonucleotide probe

Q	K	T	W	D	E	A	-------

Aggretin β chain N-terminal

^{1}D	C	P	S	G	W	S	S	Y	E	G	H
					TGG	TCC	TCC	TAT	GAA	GGG	CA

20meric oligonucleotide probe

C	Y	K	P	F	N	-------

FIG. 1. The N-terminal amino acid sequences of aggretin α/β chains (bold letters) and the sequences of the oligonucleotides used as hybridization probes. Py, pyrimidines (T or C); Pu, purines (A or G).

Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, USA) and an automatic sequencer (ABI PRISM 377-96 DNA Sequencer, Perkin-Elmer, CA, USA). The two primers used, T3 and T7, are located in the flanking regions of cDNA.

T3 primer: 5′ AATTAACCCTCACTAAAGGG 3′

T7 primer: 3′ CGGGATATCACTCAGCATAATG 5′

RESULTS AND DISCUSSION

Screening of a cDNA Library

In order to design the oligonucleotide probes for screening the cDNA library, N-terminal sequences of α and β polypeptide chain were first determined (see Fig. 1). A 20meric mixed-based oligonucleotide probe with 64 combinations was synthesized for the isolation of α-subunit. On the other hand, we used defined-sequence probe for β subunit, which was referred to C-type lectin conserved mamushigin's cDNA codons (22), since too many combinations existed in mixed-base probe. Six positive clones of each subunit were isolated separately after colony hybridization. All clones had been sequenced. The nucleotide sequences for clones of the same group shared an identical sequence although they were different in length (different clones).

Analysis of cDNA Clones

The longest cDNA sequences and the open reading frames deduced from them are shown in Fig. 2. The results indicate that these cDNA do encode α and β subunits, since N-terminal sequences of α and β subunit shown in Fig. 1 appear in open reading frames of these two clones. Mature α and β subunits are composed of 136 and 123 amino-acid residues, respectively.

(A) aggretin α chain

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 -24  attcggcacgagggaactagagctggtttggaggattgtgactttggttggtctccctat  36
       I  R  H  E  G  T  R  A  G  L  E  D  C  D  F  G  W  S  P  Y
                                    +1
  37  gatcagcattgctaccaggcattcaatgaacaaaaaacctgggatgaggcagagaagttc  96
       D  Q  H  C  Y  Q  A  F  N  E  Q  K  T  W  D  E  A  E  K  F
  97  tgcagggcgcaggagaatggtgcgcatctggcctctatcgaaagcaatggagaagcagac  156
       C  R  A  Q  E  N  G  A  H  L  A  S  I  E  S  N  G  E  A  D
 157  tttgtttcctggctgatttctcagaaagacgaactggcagacgaagactacgtctggatc  216
       F  V  S  W  L  I  S  Q  K  D  E  L  A  D  E  D  Y  V  W  I
 217  ggactgagggctcaaaacaaagaacagcaatgcagctcggagtggagcgatggctccagt  276
       G  L  R  A  Q  N  K  E  Q  Q  C  S  S  E  W  S  D  G  S  S
 277  gtcagttatgagaacttgattgatctacatacgaaaaagtgtggtgcgctggaaaaactg  336
       V  S  Y  E  N  L  I  D  L  H  T  K  K  C  G  A  L  E  K  L
 337  acagggtttcgtaagtgggtcaattattactgtgaacaaatgcatgctttcgtctgcaag  396
       T  G  F  R  K  W  V  N  Y  Y  C  E  Q  M  H  A  F  V  C  K
 397  ctcctgccatattaaagatccagttgtgtgaagtctggagaaggaagcccccacccaca   456
       L  L  P  Y  *
                  +136
 457  tcccccaccccccccacctgccgcaatctctgtagctggatctggttttgctgctcctga  516
 517  tgggccagaaggtccaatataatttctgcctagcatgaaaaa..........................
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(B) aggretin β chain

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-102  ttgtggccgagggacagggacggaaggaagaccatggggcgattcatcttcgtgagcttc  -43
                                       M  G  R  F  I  F  V  S  F
 -42  ggcttgctggtcgtgttcctctccctgagtggtactggagctgattgtccctctggttgg  18
       G  L  L  V  V  F  L  S  L  S  G  T  G  A  D  C  P  S  G  W
  19  tcctcctatgaagggcattgctacaagccctttcaatgaaccgaaaaactgggccgatgca  78
       S  S  Y  E  G  H  C  Y  K  P  F  N  E  P  K  N  W  A  D  A
  79  gagagattctgcaaactgcagccgaagcacagccatctggtctcctttcagacgcgctgaa  138
       E  R  F  C  K  L  Q  P  K  H  S  H  L  V  S  F  Q  S  A  E
 139  gaagcagattttgtggtcaagttgaccagaccaaggttgaaagccaatttagtctggatg  198
       E  A  D  F  V  V  K  L  T  R  P  R  L  K  A  N  L  V  W  M
 199  ggactgagcaatatctggcacggatgcaactggcagtggagtgatggcgccaggctcaac  258
       G  L  S  N  I  W  H  G  C  N  W  Q  W  S  D  G  A  R  L  N
 256  tacaaagactggcaggaacaatctgaatgtctcgcattcaggggagttcatacagaatgg  318
       Y  K  D  W  Q  E  Q  S  E  C  L  A  F  R  G  V  H  T  E  W
 319  ttaaatatggactgcagcagtacttgctctttcgtctgcaagttcaaggcatagtctgaa  378
       L  N  M  D  C  S  S  T  C  S  F  V  C  K  F  K  A  *
                                                      +123
 379  gatccagctgagtggagaagcaaggaaaccccccacccccacccctgttgctgaatctggt  438
 439  tttgctgctcctgatgggccagaaggtccaatataatttctgcctacatgaaaaaaaa......
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FIG. 2. The nucleotide sequences (top line) and the deduced amino acid sequences (bottom line) of the α- and β-subunits of aggretin. A +1 indicates the first amino acid residues of the mature subunits. The polyadenylation signal sequence AATAAA is underlined.

The peptide leading each N-terminal sequence is supposed to be the signal peptide or pre-peptide. We could not find a clone containing both α and β subunit. Besides, the α and β cDNAs shown in Fig. 2 are equipped with their own stop codons and polyadenylation-signal sequences. The results indicated that α/β heterodimer, although linked by a disulfide bond, are encoded by two genes. It is unlike the case of insulin which are originally synthesized by a single-chain precursor followed by folding, disulfide-bridge formation and cleavage to generate heterodimer (25). However, α and β subunit genes share 71% similarity in nucleotide sequence. They may originally duplicate from an ancestral gene and then evolved in diversely.

Amino Acid Sequence Homology

On comparison of the Swiss-Prot database, two groups of proteins, C-type lectins and C-type lectin-like proteins from snake venoms are found to show significant similarities with α and β subunits of aggretin (Fig. 3). The percentages of sequence similarity of these proteins are summarized in Table 1. The mature subunits of aggretin showed a high degree of similarity (38.5–59.7% identity for α subunit and 30.1–57.7% identity for β subunit) with those of C-type lectin-like proteins. Aggretin α chain contains seven cysteine residues, and β chain contains eight cysteine residues. All cysteine residues in each subunits of aggretin are well conserved and located at the positions corresponding to C-type lectins (9). According to the structural model of the CRD proposed by Spiess (26), six of these cysteines are involved in the intracatenary disulfide bridges (i.e. Cys 5-16, Cys 33-131, Cys 106-123 of the α subunit; and Cys 2-13, Cys 30-119, Cys 96-111 of the β subunit). According to the information of RSL and botrocetin, besides these six intracatenary disulfide bridges, a disulfide bridges between the two subunits has been shown to associate cysteine residues around position 80 (botrocetin αCys80-βCys75 and RSL 86-86) (8, 14). Therefore, it is likely that the interchain disulfide bond of aggretin may similarly exist around position 80 (Cys-83 of the α chain and Cys-75 of the β chain). It is noted that aggretin possesses an additional cysteine residue (Cys 115) in the β chain which is probably a candidate for disulfide linking. It is not known what a role the extra cysteine residue plays.

From alignment of the C-type lectin sequences, there was no specific fragment could be detected in the polypeptide sequence involved in the interaction between the C-type lectin and its receptor. Members of GPIb binding proteins show a high degree of similarity with C-type lectin, and most snake venom GPIb binding proteins do not induce direct platelet aggregation. However, one GPIb binding protein, AL-B from *Trimeresurus albolabris* does induce direct platelet aggregation (27). This is an open question because both categories of snake venom GPIb binding proteins having similar structural features exhibit different biological activities. From the result of the full sequences of α and β subunits of aggretin, we found that they share a high degree of homologous sequence as compared with the respective chain of these GPIb binding proteins. Therefore, the detailed study of their structure-activity in eliciting platelet responses for aggretin and these GPIb binding proteins will allow us to reveal which polypeptide sequences are likely to interact with receptor(s) on the platelet membrane.

A potent platelet-aggregating protein, convulxin, from the venom of *Crotalus durissus terrificus* has been recently cloned and fully sequenced (15). It

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                      *                    20                    *              40                                    60
aggretin α      1   G L E D C D F G W S P Y D Q H C Y Q A F N E Q K T W D E A E K F C R A Q E N G A H L A S I E S - N G E A D F V S W L I S Q K D E L A D E D Y - V   70
convulxin α     1   G L H - C P S D W Y Y Y D Q H C Y R I F N E E M N W E D A E W F C T K Q A K G A H L V S I K S - A K E A D F V A W M V T Q N I E E S F S H - - V   68
AL-B α          1   D C P S D W S S F K Q Y C Y Q I F K Q L K T W E D A E R F C M D Q V K G A H L V S I E S - Y R E A V F V A Q Q L S E N V K T T K Y D - - V   66
botrocetin α    1   D C P S G W S S Y E G N C Y K F F Q Q K M N W A D A E R F C S E Q A K G G H L V S I K I Y S K E K D F V G D I V T K N I Q S S D L Y - - A   67
mamushigin α    1   E D D S D C P S D W S S N G R F C Y K L F Q Q K M K W A D A E R F C T E Q R T G A H L V S I E S - N T E A A F V N Q M I S E N I K K T D Y - - - V   69
echicetin β     1   D Q D C L S G W S F Y E G H C Y Q L F R - L K T W D E A E K Y C N - Q W D G G H L V S I E S - N A K A E F V A Q L I S R K L P K S A I E D R V   68
hahu IX/X-bp α  1   D C L S G W S S Y E G H C Y K A F E K Y K T W E D A E R V C T E Q A K G A H L V S I E S - S G E A D F V A Q L V T Q N M K R L D F Y - - I   66
RSL             1   N N C P L D W L P M N G L C Y K I F N Q L K T W E D A E M F C R K Y K P G C H L A S F H R - Y G E S L E I A E Y I S D Y H K G Q E N - - - V   66

                      *                    80                    *             100                                   120                    *
aggretin α     71   W I G L R A Q N K E Q Q C S S E W S D G S S V S Y - - - - E N L I D L H T K K - - C G A L E K L T G F R K W V N Y Y C E Q M H A F V C K L L P Y   136
convulxin α    69   S I G L R V Q N K E K Q C S T K W S D G S S V S Y - - - - D N L L D L Y I T K - - C S L L K K E T G F R K W F V A S C I G K I P F V C K F P P Q C   135
AL-B α         67   W I G L S V V N K G Q Q C S S E W S D G S S V S Y - - - - E N L V K P L S K K - - C F V L K K G T E F R K W F N V A C E Q K H L F M C K F L R P R   133
botrocetin α   68   W I G L R V E N K E K Q C S S E W S D G S S V S Y - - - - E N V V E R T V K K - - C F A L E K D L G F V L W I N L Y C A Q K N P F V C K S P P P   132
mamushigin α   70   W I G L T V Q N E E Q Q C K S R W S D R S S V S Y - - - - E N L V K P N S K K - - C F V L K E Y E G S R K W F N V Y C G Q K Y A F M C K F L R P R   136
echicetin β    69   W I G L R D R S K R E Q C G H L W T D N S F V H Y - - - - E H V V P P - - T K - - C F V L E R Q T E F R K W I A V N C E F K P F V C K A K I P R   133
hahu IX/X-bp α 67   W I G L R V Q G K V K Q C N S E W S D G S S V S Y - - - - E N W I E A E S K T - - C L G L E K E T D F R K W V N I Y C G Q Q N P F V C E A   129
RSL            67   W I G L R D - - K K K D F S W E W T D R S C T D Y L T W D K N Q P D H Y Q N K E F C V E L V S L T G Y R L W N D Q V C E S K D A F L C Q C K F   135


                      *                    20                    *              40                                    60
aggretin β      1   D C P S G W S S Y E G H C Y K P F N E P K N W A D A E R F C K L Q P K H S H L V S F Q S A - E E A D F V V K L T R P R L K A N L V W M G L S N   70
convulxin β     1   G F C C P S H W S S Y D R Y C Y K V F K Q E M T W A D A E K F C T Q Q H T G S H L V S F H S T - E E V D F V V K M T H Q S L K S T F F W I G A N N   72
AL-B β          1   D C P S D W S S Y D L Y C Y R V F Q E K K N W E D A E K F C T Q Q T D S H L V S F D S S - E E V D F V A S K T E P V L K H D L V W I G L G S   70
botrocetin β    1   D C P P D W S S Y E G H C Y K F F K E W M H W D D A E E F C T E Q Q T G A H L V S F Q S K - E E A D F V R S L T S E M L K G D V V W I G L S D   70
mamushigin β    1   D C P S D W S S Y E G H C Y R V F Q K E M T W E D A E K F C T Q Q R K E S H L V S F H S S - E E V D F V V S M T W P I L K Y D F V W I G L N N   70
echicetin β     1   N C L P D W S V Y E G Y C Y K V F K E R M N W A D A E F C M K Q V K D G H L V S F R N S - K E V D F M I S L A F P M L K M E L V W I G L S D   70
hahu IX/X-bp β  1   D C P S D W S S Y E G H C Y K P F S E P K N W A D A E N F C T Q Q H A G G H L V S F Q S S - E E A D F V V K L A F Q T F G H S I F W M G L S N   70
RSL             1   N N C P L D W L P M N G L C Y K I F N Q L K T W E D A E M F C R K Y K P G C H L A S F H R Y G E S L E I A E Y I S D Y H K G Q E N V W I G L R D   72

                      *                    80                    *             100                                   *   120
aggretin β     71   I W H G C N W Q W S D G A R L N Y K D W Q E - - - - - - - - Q S E C L A F R G V H T E - - W L N M D C S S T C S F V C K F K A   123
convulxin β    73   I W N K C N W Q W S D G T K P E Y K E W H E - - - - - - - - E F E C L I S R T F D N Q - - W L S A P C S D T Y S F V C K F E A   125
AL-B β         71   V M N A C K L Q W S D G T E L K Y N A W S A - - - - - - - - E S E C I T S K S T D N E - - W L T R S C S R T Y P F V C K F Q A   123
botrocetin β   71   V W N K C R F E W T D G M E F D Y D D Y Y L I A - - - - - - E Y E C V A S K P T N N K - - W W I I P C T R F K N F V C E F Q A   125
mamushigin β   71   I W N E C M V E W T D G T R L S H N A W I T - - - - - - - E S E C I A A K T T Q N Q - - W L S R P C S R T Y N V V C K F Q E   123
echicetin β    71   Y W R D C Y W E W S D G A Q L D Y K A W D N - - - - - - - - E R H C F A A K T T D N Q - - W M R R K C S G E F Y F V C K C P A   123
hahu IX/X-bp β 71   V W N Q C N W Q W S N A A M L R Y K A W A E - - - - - - - - E S Y C V V F K S T N N K - - W R S R A C R M M A Q F V C E F Q A   123
RSL            73   K K K D F S W E W T D R S C T D Y L T W D K N Q P D H Y Q N K E F C V E L V S L T G Y R L W N D Q V C E S K D A F L C Q C K F   135
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FIG. 3. Comparison of the amino acid sequences deduced from aggretin in cDNAs with those of snake venom C-type lectin and group VII C-type lectins. The two aggretin chains are aligned corresponding with the two chains of the following snake venom protein: convulxin from *Crotalus durissus terrificus* venom (15), mamushigin from *Agkistrodon halys blomhoffii venom* (22), alboagreggin-B (AL-B) from *Trimeresurus albolabris* venom (17), botrocetin from *Bothrops jararaca* venom (9), echicetin from *Echis carinatus* venom (13, 14), habu IX/X-bp from *Trimeresurus flavoviridis* venom (12), and Rattlesnake lectin (RSL) from *Crotalus atrox* venom (8). Asterisks indicate the position of well conserved cysteine residues. Gaps (−) are inserted to obtain the maximum similarity.

consists of two subunits, α and β, and encodes sequences of 135 amino acids for the α chain and 125 amino acids for the β chain, existing as hexameric structure (28). The convulxin α/β subunits were homologous to each other and to those of the CRD of C-type lectins. Convulxin has been found to bind to a specific platelet receptor, GPVI, which is also one of the collagen receptors (29, 30). The mature subunits of aggretin showed a high degree of similarity (56.4% identity for α chain and 56.1% identity for β chain) with the mature subunits of convulxin (15). Aggretin is a collagen-like platelet-aggregation protein and it may bind to GPIa/IIa (5). Now, we are trying to define the biological function of aggretin α/β chains

TABLE 1

Sequence Identity among Aggretin and Other Venom Peptides

Class	Category	Venom peptide	% of sequence identity with aggretin	
			α chain	β chain
C-type lectin-like peptides	Collagen-like peptide	Aggretin	—	—
		Convulxin	56.4	56.1
	GPIb antagonists	Habu IX/X-bp	59.7	57.7
		Mamushigin	50.7	53.7
		Echicetin	49.6	49.6
	GPIb agonists	AL-B	56.4	53.7
		Botrocetin	52.3	48.8
C-type lectin	Platelet-aggregation inducer[a]	RSL	38.5	30.1

[a] The mechanism in molecular level is unclear.

and to re-examine the possibility that aggretin may bind to other receptor besides GPIa/IIa. The elucidation of the essential binding motif of this molecule in eliciting platelet aggregation will highlight the identity of collagen-receptor and the signal transduction involved at molecular level.

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