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Ussuristatin 2, a Novel KGD-Bearing Disintegrin from *Agkistrodon ussuriensis* Venom

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Two platelet aggregation inhibitors, ussuristatin 1 (US-1) and 2 (US-2), were newly isolated from the venom of Chinese viper (*Agkistrodon ussuriensis*) by SP-Toyopearl 650M column chromatography and reverse-phase HPLC. The M_rs of these polypeptides were estimated to be about 8,000 by SDS-PAGE. Analytical gel filtration revealed that US-2 exists as a dimer. Both polypeptides comprised 71 amino acids, whose sequences showed high similarities to those of other disintegrins. US-1 had a typical Arg-Gly-Asp (RGD) sequence, which is responsible for blocking the binding of fibrinogen to the receptor. In US-2, the corresponding sequence was Lys-Gly-Asp (KGD). US-1 strongly suppressed platelet aggregation induced by ADP, collagen, thrombin, and epinephrine with IC_{50}=17-33 nM. US-2 also inhibited the platelet aggregation, but the IC_{50}s were about ten times higher. US-1 also dose-dependently inhibited the adhesion of human melanoma cells to fibrinogen and fibronectin, while US-2 did not inhibit the cell adhesion to fibronectin. This indicates that the KGD-bearing disintegrin is a specific inhibitor for the fibrinogen receptor.

Key words: amino acid sequences, disintegrins, platelet aggregation, protein purification, snake venom.

Platelet aggregation, which is essential for effective primary hemostasis, involves the interaction of plasma fibrinogen with a specific receptor, glycoprotein (GP) II_b/III_a, on the platelet membrane (*1, 2*). Two binding domains for the fibrinogen receptor are present in the fibrinogen molecule: an Arg-Gly-Asp (RGD) sequence of the Aα-chain, and a C-terminal dodecapeptide of the γ-chain (*3*). Since the first report on the purification of trigramin, an anti-platelet aggregation peptide, from *Trimeresurus gramineus* venom (*4*), several platelet aggregation inhibitors have been isolated from various snake venoms (*5, 6*). Termed disintegrins, these peptides commonly possess the RGD sequence and compete with fibrinogen for GP II_b/III_a. Barbourin is the only exception (*7*). It was discovered as a KGD-containing disintegrin from the venom of the southeastern pigmy rattlesnake *Sistrurus m. barbouri*, and it selectively inhibits the binding of fibrinogen to GP II_b/III_a without affecting the binding of fibronectin or vitronectin to the receptor. Disintegrins contain many highly conservative Cys residues, which form the intramolecular disulfide bonds necessary for the development of full inhibitory activity to platelet aggregation as well as for the construction of a tight tertiary structure (*4, 8*).

Three kinds of *Agkistrodon* snakes inhabit China: *A. halys brevicaudus*, *A. halys intermedius*, and *A. ussuriensis*. Neither the evolutionary relationship between these snakes nor the protein components in their venoms have yet been analyzed. As a first step, we chose to study the disintegrins, since these proteins in general are well characterized and many homologues are known. In this paper, we report the isolation, characterization, and complete amino acid sequences of two disintegrins, ussuristatin (US) 1 and 2, from *A. ussuriensis* venom. Both proteins inhibited the aggregation of human platelets. US-1 was a monomeric protein containing an RGD sequence. In contract, US-2 was a unique disintegrin that exists as a homodimer and contains a KGD sequence in place of the RGD sequence. US-1 inhibited the attachment of C32 human melanoma cells to fibrinogen and fibronectin, while US-2 inhibited the attachment to fibrinogen but not to fibronectin.

MATERIALS AND METHODS

Materials—Crude *A. ussuriensis* venom which was collected in the northeast provinces of China was provided by Dr. W.-X. Hao (China Medical University) in lyophilized form. ADP was purchased from Niko Bioscience (Tokyo). *Achromobacter* protease I (Lys-C), arginylendopeptidase (Arg-C), and endoproteinase Asp-N were purchased from Takara (Kyoto). Human melanoma cell line C32 (ATCC CRL 1585) was purchased from American Type Culture Collection. Carboxypeptidase Y and all other reagents were purchased from Wako Pure Chemical (Osaka).

Purification of Ussuristatins—Crude *A. ussuriensis* venom (1.0 g) was dissolved in 20 ml of 0.5 M acetic acid. After 2 h at room temperature, the insoluble material was removed by centrifugation at 3,000 rpm for 10 min. The

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Abbreviations: Arg-C, arginylendopeptidase; GP, glycoprotein; Lys-C, *Achromobacter* protease I; PE, *S*-pyridylethylated; TFA, trifluoroacetic acid; US, ussuristatin.

supernatant was lyophilized, dissolved in 10 mM acetate buffer (pH 4.5), and loaded onto a SP-Toyopearl 650M column (1.6×35 cm, Tosoh) equilibrated with the same buffer. Elution was performed at 4°C with a linear gradient of NaCl from 0 to 0.2 M in the same buffer, and 5-ml fractions were collected. Proteins were detected by the absorption at 280 nm on a Ubest-30 spectrophotometer (Jasco). Active fractions that suppress ADP-induced platelet aggregation were further purified by reverse-phase HPLC using a TSK-gel ODS-120T column (4.6×250 mm, Tosoh) with a linear gradient of 0 to 80% acetonitrile in 0.1% TFA.

Platelet Aggregation Assay—Human blood was obtained from healthy adult donors in tubes containing 0.1 volume of 3.8% sodium citrate and used within 3 h. Platelet-rich plasma was separated from whole blood by centrifugation at 1,200 rpm for 10 min. Platelet aggregation was measured using an aggregometer (NBS Hema Tracer 601, Niko Bioscience). ADP (10 μM), thrombin (5 U/ml), collagen (2 μg/ml), epinephrine (2 μg/ml), and ristocetin (1 mg/ml) were used to induce the aggregation.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)—SDS-PAGE was carried out using a 16.5% polyacrylamide gel under the reduced and non-reduced conditions as described by Schagger and von Jagow (*9*). Protein bands were detected by staining gels with 0.1% Coomassie Brilliant Blue R-250.

Analytical Gel Filtration—Sample was dissolved in 10 mM phosphate buffer (pH 6.0) and applied to a TSK-gel G3000 SWXL column (7.8×300 mm, Tosoh). Elution was done with the same buffer at a flow rate of 1 ml/min and monitored at 280 nm. The column was calibrated with ovalbumin (45 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.5 kDa), α-lactalbumin (14.2 kDa), and aprotinin (6.5 kDa).

Proteolytic Digestions—*S*-Pyridylethylated proteins were prepared according to Friedman *et al.* (*10*). PE-proteins were digested at 37°C with Lys-C (E/S=1:100) for 6 h in 20 mM Tris-HCl (pH 9.0), Arg-C (E/S=1:50) for 6 h in 50 mM Tris-HCl (pH 8.0), or Asp-N (E/S=1:100) for 16 h in 50 mM Tris-HCl (pH 8.0). The digests were lyophilized, dissolved in 0.1% TFA and fractionated by reverse-phase HPLC on a TSK-gel ODS-120T column (0.46×25 cm) in 0.1% TFA with an appropriate gradient of acetonitrile.

Sequence Analysis—The amino acid sequences of peptides were determined by manual Edman degradation using the 4-*N*,*N*-dimethylaminoazobenzene-4′-isothiocyanate/phenylisothiocyanate double coupling method (*11*). Amino acid derivatives were identified as described previously (*12*). The C-terminal residues were identified by carboxypeptidase Y digestion as follows. PE-protein was dissolved in 20 mM acetate buffer (pH 5.5), and the enzyme (E/S=3:100) was added. The mixture was incubated at 37°C for 15-120 min. Aliquots were subjected to amino acid analysis.

Cell Attachment Assay—The human melanoma cell line C32 was cultured in Dulbecco's Modified Eagle medium containing 10% fetal calf serum and harvested at subconfluency with 0.2% trypsin and 1 mM EDTA. Wells (2 cm^2) of four-well dishes were treated with 50 μl of fibrinogen (50 μg/ml) or fibronectin (50 μg/ml) in PBS for 2 h at room temperature or overnight at 4°C. A 300-μl aliquot of cells (1.25×10^5 cells/ml in Hanks' balanced salt solution with glucose and 0.5% BSA) was added to each well. The cells were incubated for 1 h at 37°C, then nonadherent cells were removed by aspiration, and adherent cells were washed with PBS. Adherent cells were fixed with 2% glutaraldehyde for 10 min and stained with 2% Giemsa solution for 20 min. The total number of cells in each well was counted microscopically.

RESULTS

Purification of Ussuristatins—Crude ussuristatins were purified by cation-exchange chromatography on a SP-Toyopearl 650M (Fig. 1A). ADP-induced platelet aggregation inhibition activity was found in two peaks (fractions I and II). Each fraction was further purified by reverse-phase HPLC. Fraction I gave a peak of US-2 with platelet aggregation inhibitory activity at a retention time of 31 min (Fig. 1B). Fraction II yielded an active peak of US-1 at a retention time of 27 min (Fig. 1C). We obtained 1.7 mg of US-1 and 1.1 mg of US-2 from 1.0 g of *A. ussuriensis* venom.

The apparent molecular masses of purified US-1 and US-2 were estimated to be 24 and 30 kDa under non-reduced conditions, respectively (Fig. 2A). These values were

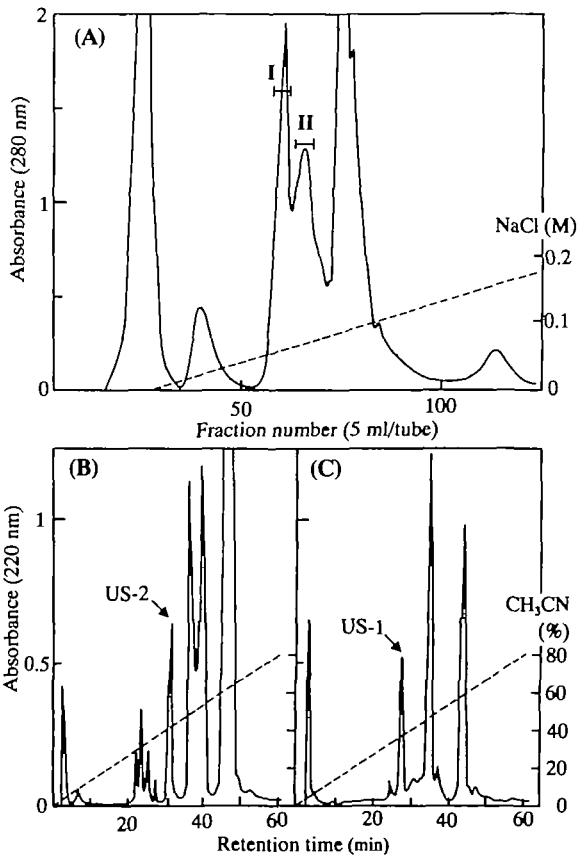

Fig. 1. **Purification of ussuristatins.** (A) Cation-exchange chromatography of acid-treated *A. ussuriensis* venom on an SP-Toyopearl 650M column. Broken line indicates concentration of NaCl in 10 mM acetate buffer (pH 4.5). Reverse-phase HPLC of fraction I (B) and fraction II (C) on a TSK-gel ODS-120T column. Broken lines indicate concentrations of acetonitrile in 0.1% TFA.

not changed in the presence of 8 M urea under non-reduced conditions (data not shown). On the other hand, both peptides gave a single band at about 8 kDa under reduced conditions. The molecular masses were estimated to be 8 kDa for US-1 and 16 kDa for US-2 by an analytical gel filtration (Fig. 2B). These results showed that US-1 exists as a monomeric polypeptide, and US-2 as a homodimer, probably linked by disulfide bridges.

Amino Acid Sequences of Ussuristatins—PE-US-1 was digested with Lys-C and Asp-N, and the fragment peptides were separated by reverse-phase HPLC (data not shown) to give K1-K4 and D1-D6, respectively. The peptide D6 was further digested with V8 protease to give three subfragments, D6V1-D6V3. Figure 3 shows the results of sequencing and the complete primary structure of US-1. The amino acid sequence of US-2 was determined similarly as shown

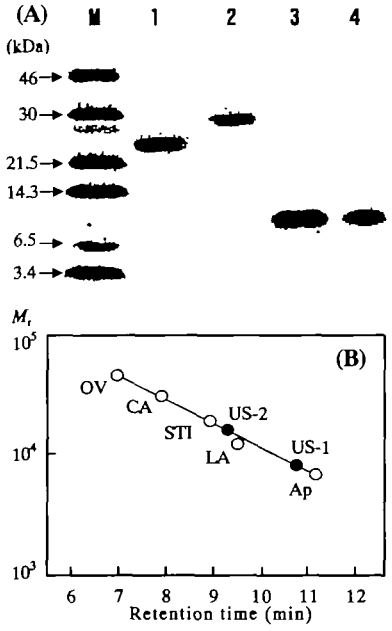

Fig. 2. **SDS-PAGE and analytical gel filtration of purified ussuristatins.** (A) SDS-PAGE was carried out under non-reduced (lanes 1 and 2) or reduced conditions (lanes 3 and 4). M, marker proteins; lanes 1 and 3, US-1; lanes 2 and 4, US-2. (B) Analytical gel filtration on a TSK-gel G3000 SWXL column in 10 mM phosphate buffer (pH 6.0) at a flow rate of 1 ml/min. M_r marker proteins are ovalbumin (OV), carbonic anhydrase (CA), soybean trypsin inhibitor (STI), α-lactalbumin (LA), and aprotinin (Ap).

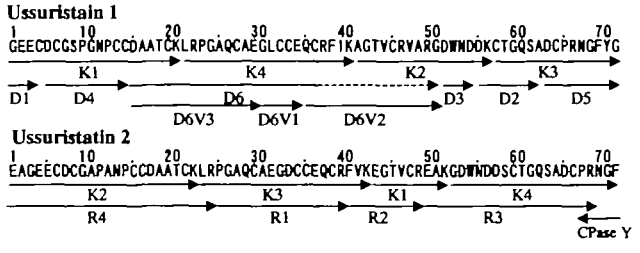

Fig. 3. **Amino acid sequences of ussuristatins.** Peptides derived from PE-US-1 and -2 by digestion with Arg-C (R), *S. aureus* V8 protease (V), or Lys-C (K) are shown below the sequence. CPase Y, the estimated sequence by carboxypeptidase Y digestion. Broken line indicates the estimated sequence from the amino acid composition.

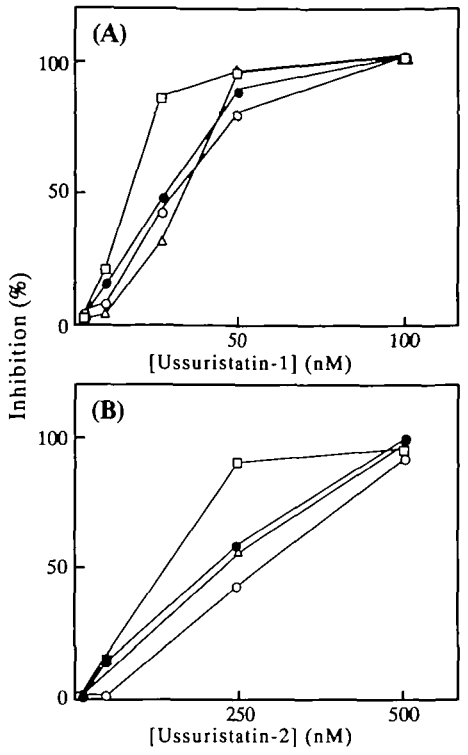

Fig. 4. **Platelet aggregation inhibition by ussuristatins.** Aggregation was initiated by the addition of 10 μM ADP (○), 5 U/ml of thrombin (●), 2 mg/ml of collagen (△), or 2 mg/ml of epinephrine (□). (A) US-1 and (B) US-2. Concentration of US-2 was calculated as the monomer.

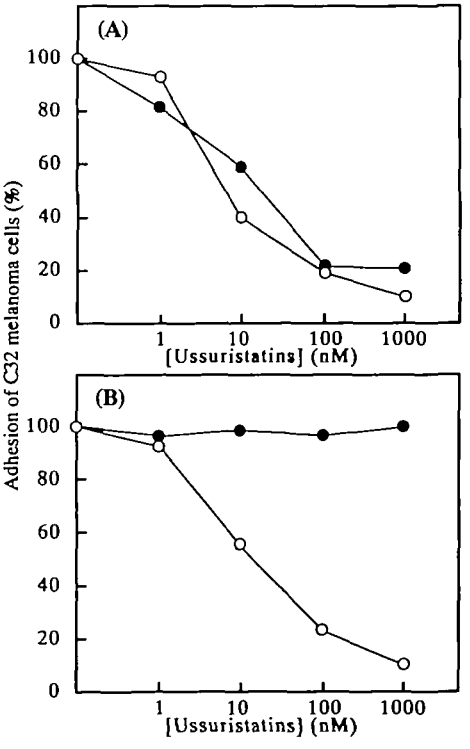

Fig. 5. **Effects of ussuristatins on adhesion of C32 melanoma cell to (A) fibrinogen and (B) fibronectin.** ○, US-1; ●, US-2.

in Fig. 3. PE-US-2 was digested with Lys-C and Arg-C to give K1-K4 and R1-R4, respectively (data not shown). The C-terminal five residues of US-2 were confirmed by carboxypeptidase Y digestion.

Both disintegrins are 71 amino acid residues long. The calculated molecular weight is 7,458 for US-1 and 7,385 for the monomer of US-2. An RGD sequence, which is known to act as a cell-adhesion signal (*13*), is present at residues 49-51 in US-1, but not in US-2. Instead, a KGD sequence is found at residues 51-53 in US-2.

Inhibition of Platelet Aggregation by Ussuristatins—The inhibitory effects of ussuristatins on human platelet aggregation were examined. US-1 strongly inhibited the fibrinogen-dependent platelet aggregation by ADP, thrombin, collagen, and epinephrine with IC_{50} of 32, 28, 33, and 17 nM, respectively (Fig. 4). These values were almost identical to those for other potent disintegrins such as echistatin (*14*) and applaggin (*15*). US-2 was about ten times less effective as an inhibitor (Fig. 4). The concentration of US-2 was calculated on the basis of the monomer, which behaves as a divalent ligand. The IC_{50}s of US-2 were 290, 230, 220, and 140 nM for ADP, thrombin, collagen, and epinephrine-induced platelet aggregation, respectively. Neither ussuristatin inhibited platelet aggregation induced by ristocetin (data not shown), which activates the receptor (GP I_b) for von Willebrand factor on human platelets (*16*).

Inhibition of C32 Cell Adhesion by Ussuristatins—We next examined whether ussuristatins block the adhesion of C32 human melanoma cells (C32 cells) to fibrinogen and fibronectin. C32 cells in the absence of serum adhere to and spread on fibrinogen or fibronectin-coated dishes through their cell surface integrins (*17*). Both ussuristatins dose-dependently inhibited the attachment of C32 cells to fibrinogen-coated dishes (Fig. 5A). A similar result was reported for trigramin (*18*). US-1 also blocked the C32 cell attachment to fibronectin-coated dishes, while US-2 did not (Fig. 5B). This is explained by the fact that an RGD sequence is indispensable for the interaction of cellular fibronectin receptor, integrin $\alpha_5\beta_1$, with fibronectin (*18, 19*) and the conservative Lys for Arg substitution in US-2 appeared not to be recognized by the receptor.

DISCUSSION

We isolated two new disintegrins, termed ussuristatins, from the venom of *A. ussuriensis*. The acid treatment of crude venom inactivates and precipitates most of the contaminating proteins in the venom and facilitates the purification of ussuristatins without affecting their anti-platelet aggregation activity. Purification by reverse phase HPLC on C18 column gave pure disintegrins.

On SDS-PAGE analysis, ussuristatins gave significantly higher M_rs than those calculated from the sequence in the absence of reductant. A similar phenomenon was observed with other disintegrins from *A. halys brevicaudus* (unpublished data). The M_r of US-1 was confirmed to be 8,000 by SDS-PAGE under reduced condition and by analytical gel filtration HPLC. The M_r of US-2 was determined to be 16,000 by analytical gel filtration, while SDS-PAGE under reduced condition gave a different value (8,000), which is close to the calculated value (7,385) from the sequence. This suggests that US-2 forms a homodimer linked by intermolecular disulfide bonds.

The deduced sequences of ussuristatins were compared with those of disintegrins from other snake venoms (Fig. 6). Twelve Cys residues are completely conserved in both ussuristatins. US-2 showed the highest similarity to US-1 (88% identity) among several disintegrins. Similarity between US-2 and barbourin (*7*), a KGD-bearing disintegrin from *Sistrurus m. barbouri*, was 86%. On the other hand, US-1 showed much higher similarities to lutosin (*20*) from *Crotalus viridis lutosus* (92% identity) and durissin (*20*) from *C. durissus durissus* (90% identity) than to US-2.

Both US-1 and 2 dose-dependently inhibited human platelet aggregation induced by ADP, thrombin, collagen, and epinephrine (Fig. 4). US-2 was less effective than US-1 with respect to the inhibition of ADP-, collagen-, and epinephrine-induced aggregations. This may be attributable to the substitution of the usual RGD sequence by a KGD sequence. Barbourin, another KGD-bearing disintegrin, also showed a little weaker inhibitory activity to platelet aggregation than other RGD-disintegrins from closely related species (*7*). US-1 inhibited adhesion of C32 cells to both fibrinogen and fibronectin, while US-2 did not inhibit the adhesion to fibronectin (Fig. 5B). Barbourin also does not bind to receptors for fibronectin and vitronectin (*7*). Only the RGD sequence is recognized by the fibronectin receptor.

Recently, two series of platelet aggregation inhibitors, lebetins 1 and lebetins 2, were isolated from *Vipera lebetina* venom (*25*). Lebetins lack both RGD/KGD-related sequences and other sequences with known anti-aggregating activity. They have a tetrapeptide PPKK sequence that is identical to residues 109-112 of a cow κ-casein (*26*), which inhibits ADP-induced platelet aggregation. According to Jia *et al.* (*27*), the platelet inhibitory activity of atrolysin A, a hemorrhagic metalloprotease obtained from *C. atrox* venom, is attributable to the RSECD sequence, in lieu of the RGD sequence, and two acidic residues as well as the S-S bridged cysteinyl residue are essential for inhibition of platelet aggregation. These results imply that the binding of ligand to fibrinogen receptor is not restricted to the RGD/KGD sequence.

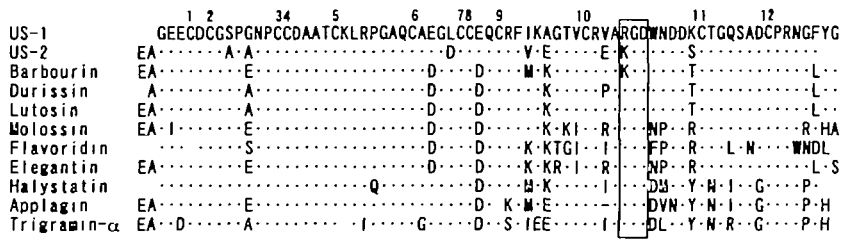

Fig. 6. **Sequence alignment of disintegrins from *Crotalinae* venoms.** Sequences of US-1 and 2 are compared with those of barbourin (*7*), durissin (*20*), lutosin (*20*), molossin (*20*), flavoridin (*21*), elegantin (*22*), halystatin (*23*), applagin (*15*), and trigramin-α (*24*). Dots indicate the identical residues to those of US-1. Cys residues are numbered above the sequences. The RGD or KGD sequence is boxed.

Applaggin (*15*) and contortrostatin (*28*) are disintegrins that form a disulfide-linked homodimer. Unlike monomeric disintegrins, contortrostatin behaves as a divalent ligand and differently regulates the intracellular signaling pathway leading to tyrosine phosphorylation of platelet proteins on activation (*29*). Since US-2 is also dimeric but a KGD-containing disintegrin, it will be interesting to examine how it affects the platelet tyrosine phosphorylation.

In this study, we could not determine the location of intermolecular disulfide bonds in US-2. The conformation of kistrin in solution (*8*) involves four disulfide bonds linking C-3 to C-9, C-6 to C-8, C-7 to C-11, and C-10 to C-12 (Fig. 6) that are partially or completely buried inside the molecule, and two disulfide bonds linking C-1 to C-5 and C-2 to C-4 that are exposed to the same direction and located apart from the RGD loop. If US-2 has a similar conformation to kistrin, these Cys residues (residue 5, 7, 14, and 19 in US-2) would be involved in the intermolecular linking. Finally, US-2 may provide an important model for designing potent and selective GP II_b/III_a antagonists that can be utilized as anti-platelet agents.

REFERENCES

1. Phillips, D.R., Charo, I.F., Parise, L.V., and Fitzgerald, L.A. (1988) The platelet membrane glycoprotein IIb-IIIa complex. *Blood* **71**, 831-843
2. Plow, E.F. and Ginsburg, M.H. (1989) Cellular adhesion: GPIIb-IIIa as a prototypic adhesion receptor. *Prog. Hemost. Thromb.* **9**, 117-156
3. Plow, E.F., Pierschbacher, M.D., Ruoslahti, E., Marguerie, G.A., and Ginsberg, M.H. (1985) The effect of Arg-Gly-Asp-containing peptides on fibrinogen and von Willebrand factor binding to platelets. *Proc. Natl. Acad. Sci. USA* **82**, 8057- 8061
4. Huang, T.F., Holt, J.C., Lukasiewicz, H., and Niewiarowski, S. (1987) Trigramin. A low molecular weight peptide inhibiting fibrinogen interaction with platelet receptors expressed on glycoprotein IIb-IIIa complex. *J. Biol. Chem.* **262**, 16157-16163
5. Huang, T.F., Wu, Y.J., and Ouyang, C. (1987) Characterization of a potent platelet aggregation inhibitor from *Agkistrodon rhodostoma* snake venom. *Biochim. Biophys. Acta* **925**, 248-257
6. Gan, Z.R., Gound, R.J., Jacobs, J.W., Friedman, P.A., and Polokoff, M.A. (1988) Echistatin. A potent platelet aggregation inhibitor from the venom of the viper, *Echis carinatus*. *J. Biol. Chem.* **263**, 19827-19832
7. Scarborough, R.M., Rose, J.W., Hsu, M.A., Phillips, D.R., Fried, V.A., Campbell, A.M., Nannizzi, L., and Charo, I.F. (1991) Barbourin. A GPIIb-IIIa-specific integrin antagonist from the venom of *Sistrurus m. barbouri*. *J. Biol. Chem.* **266**, 9359-9362
8. Adler, M., Lazarus, A.R., Dennis, S.M., and Wargner, G. (1991) Solution structure of kistrin, a potent platelet aggregation inhibitor and GPIIb-IIIa antagonist. *Science* **253**, 445-448
9. Schagger, H. and von Jagow, G. (1987) Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* **166**, 368-379
10. Friedman, M., Krull, L.H., and Cavins, J.F. (1970) The chromatographic determination of cystine and cysteine residues in proteins as *S*-β-(4-pyridylethyl)cysteine. *J. Biol. Chem.* **245**, 3868-3871
11. Chang, T.-Y. (1979) Manual solid phase sequence analysis of polypeptides using 4-*N,N'*-dimethylaminoazobenzene 4'-isothiocyanate. *Biochim. Biophys. Acta* **578**, 188-195
12. Terada, S., Fujimura, S., Noda, K., and Kimoto, E. (1992) Separation of dimethylaminoazobenzenethiohydantoin derivatives by a reversed-phase high-performance liquid chromatography. *Fukuoka Univ. Sci. Rep.* **22**, 35-39
13. Ruoslahti, E. and Pierschbacher, M.D. (1987) New perspectives in cell adhesion: RGD and integrins. *Science* **238**, 491-497
14. Gan, Z.-R., Gould, R.J., Jacobs, J.W., Friedman, P.A., and Polokoff, M.A. (1988) Echistatin. A potent platelet aggregation inhibitor from the venom of the viper, *Echis carinatus*. *J. Biol. Chem.* **263**, 19827-19832
15. Chao, B.H., Jakubouski, J.A., Savage, B., Chow, E.P., Marzec, U.M., Harker, L.A., and Maraganore, J.M. (1989) *Agkistrodon piscivorus piscivorus* platelet aggregation inhibitor: A potent inhibitor of platelet activation. *Proc. Natl. Acad. Sci. USA* **86**, 8050-8054
16. Scott, J.P., Montgomery, R.R., and Retzinger, G.S. (1991) Dimeric ristocetin flocculates proteins, binds to platelets, and mediates von Willebrand factor-dependent agglutination of platelets. *J. Biol. Chem.* **266**, 8149-8155
17. Dennis, M.S., Henzel, W.J., Pitti, R.M., Lipari, M.T., Napier, M.A., Deisher, T.A., Bunting, S., and Lazarus, R.A. (1990) Platelet glycoprotein IIb-IIIa protein antagonists from snake venoms: evidence for a family of platelet-aggregation inhibitors. *Proc. Natl. Acad. Sci. USA* **87**, 2471-2475
18. Knudsen, K., Tuszynski, G.P., Huang, T.F., and Niewiarowski, S. (1988) Trigramin, an RGD-containing peptide from snake venom, inhibits cell-substratum adhesion of human melanoma cells. *Exp. Cell. Res.* **179**, 42-49
19. Savage, B., Marzec, U.M., Chao, B.H., Harker, L.A., Maraganore, J.M., and Ruggeri, Z.M. (1990) Binding of the snake venom-derived proteins applaggin and echistatin to the arginine-glycine-aspartic acid recognition site(s) on platelet glycoprotein II_b-III_a complex inhibits receptor function. *J. Biol. Chem.* **265**, 11766-11772
20. Scarborough, R.M., Rose, J.W., Naughton, M.A., Phillips, D.R., Nannizzi, L., Arfsten, A., Campbell, A.M., and Charo, I.F. (1993) Characterization of the integrin specificities of disintegrins isolated from American pit viper venoms. *J. Biol. Chem.* **268**, 1058-1065
21. Musial, J., Niewiarowski, S., Rucinski, B., Stewart, G.J., Cook, J.J., Williams, J.A., and Edmunds, L.H., Jr. (1990) Inhibition of platelet adhesion to surfaces of extracorporeal circuits by disintegrins: RGD-containing peptides from viper venoms. *Circulation* **82**, 261-273
22. Williams, J., Rucinski, B., Holt, J., and Niewiarowski, S. (1990) Elegantin and albolabrin purified peptides from viper venoms: Homologies with the RGDS domain of fibrinogen and von Willebrand factor. *Biochim. Biophys. Acta* **1039**, 81-89
23. Fujisawa, Y., Kuroda, S., Notaya, K., Konishi, H., and Terashita, Z. (1994) Halystatin, a novel disintegrin from *Agkistrodon halys*, is a potent inhibitor of bone resorption and platelet aggregation. *J. Takeda Res. Lab.* **53**, 39-56
24. Huang, T.-F., Holt, J.C., Kirby, E.P., and Niewiarowski, S. (1989) Trigramin: Primary structure and its inhibition of von Willebrand factor binding to glycoprotein II_b/III_a complex on human platelets. *Biochemistry* **28**, 661-666
25. Barbouche, R., Marrakchi, N., Mansuelle, P., Krifi, M., Fenouillet, E., Rochat, H., and Ayeb, M.E. (1996) Novel anti-platelet aggregation polypeptides from *Vipera lebetina* venom: isolation and characterization. *FEBS Lett.* **392**, 6-10
26. Jolles, P., Levy-Toledano, S., Fiat, A.M., Soria, C., Gillessen, D., Thomaidis, A., Dunn, F.W., and Caen, J.P. (1986) Analogy between fibrinogen and casein. Effect of an undecapeptide isolated from kappa-casein on platelet function. *Eur. J. Biochem.* **158**, 379-382
27. Jia, L.-G., Wang, X.-M., Shannon, J.D., Bjarnason, J.B., and Fox, J.W. (1997) Function of disintegrin-like/cysteine-rich domains of atrolysin A. Inhibition of platelet aggregation by recombinant protein and peptide antagonists. *J. Biol. Chem.* **272**, 13094-13102
28. Trikha, M., Rote, W.E., Manely, P.J., Lucchesi, B.R., and Markland, F.S. (1994) Purification and characterization of platelet aggregation inhibitors from snake venom. *Thromb. Res.* **73**, 39-52
29. Clark, E.A., Trikha, M., Markland, F.S., and Brugge, J.S. (1994) Structurally distinct disintegrins contortrostatin and multisquamatin differentially regulate platelet tyrosine phosphorylation. *J. Biol. Chem.* **269**, 21940-21943