Purification, Cloning, and Molecular Characterization of a High Molecular Weight Hemorrhagic Metalloprotease, Jararhagin, from Bothrops jararaca Venom

INSIGHTS INTO THE DISINTEGRIN GENE FAMILY

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A large hemorrhagin, jararhagin, has been cloned from a Bothrops jararaca venom gland cDNA expression library. The cDNA sequence predicts a 421-amino acid residue molecule with strong amino acid sequence homology and similar domain structure to HR1B, a high molecular weight hemorrhagic metalloprotease isolated from Trimeresurus flavoviridis (Habu) venom. Like HR1B, jararhagin contains enzyme, disintegrin, and cysteine-rich carboxyl-terminal regions. In the disintegrin region, the Arg-Gly-Asp sequence is replaced by Glu-Cys-Asp, as found in non-Arg-Gly-Asp disintegrin regions of HR1B and a guinea pig sperm fusion protein PH-302. The cDNA sequence of jararhagin predicts a precursor protein (proprotein) with striking similarity to cryptic regions in precursors of the disintegrin peptides trigramin and rhodostomin. Comparison of jararhagin with disintegrin precursors highlights the modular arrangement of proprotein, metalloprotease, and disintegrin domains in the metalloprotease/disintegrin family and provides an insight into their biosynthesis and evolution.

The pit viper, Bothrops jararaca (Jararaca), is a snake of great medical importance in Brazil, where it is responsible for 90% of snake bite cases in the southeastern area (Cardoso and Brando, 1982). Its venom contains several enzymes which activate the blood-clotting cascade, causing either severe or complete consumption coagulopathy (Rosenfeld et al., 1989; Takeya et al., 1989, 1990a; Shannon et al., 1989; Sanchez et al., 1991). Large hemorrhagins generally range in size from 50 to 90 kDa (Takeya et al., 1990b). Only one, HR1B from T. flavoviridis, has been sequenced. Primary structural analysis of this large hemorrhagin has revealed a complex, multidomain molecule containing a metalloprotease domain, similar to small venom metalloproteases, a disintegrin-like domain, and a third region with no homology to known sequences (Takeya et al., 1990b).

Disintegrins are a family of Arg-Gly-Asp (RGD)-containing venom peptides, with high affinity for platelet glycoprotein IIb/IIIa integrin receptors (Gould et al., 1990). They prevent platelet aggregation through inhibition of fibrinogen and von Willebrand factor binding to platelets (Huang et al., 1989) and therefore have potential antithrombotic value. The presence of a disintegrin-like domain in HR1B is evidence of a close relationship with the disintegrins. The recent cloning of the gene for the disintegrin rhodostomin from Calloselasma rhodostoma (Malayan Pit Viper) venom has shown the presence of a metalloprotease precursor sequence in the disintegrin transcript (Au et al., 1991).

Here, we report the purification and molecular cloning and characterization of a large hemorrhagin from B. jararaca venom. A multidomain structure exhibiting a high level of amino acid sequence homology with the high molecular weight hemorrhagin HR1B from T. flavoviridis was found. In addition, a precursor protein sequence was present with striking sequence similarities to disintegrin proprotein, providing further evidence that viper hemorrhagins and disintegrins belong to the same gene family.

This is, to our knowledge, the first report of the cloning of a high molecular weight snake venom hemorrhagin and, in keeping with current disintegrin nomenclature (Gould et al., 1990), we propose the name jararhagin which combines a reference to the species origin (Jarar-) and the hemorrhagic action (-hagin) of the metalloprotease.

MATERIALS AND METHODS

Venom—Pooled lyophilized venom of B. jararaca from about 100 snakes was donated by the Instituto Butantan, Sao Paulo, Brazil.

Assay for Hemorrhagic Activity—Male CPW strain mice (18-20 g) were anesthetized with halothane, a portion of their back was shaved, and different amounts of venom fractions in a volume of 100 μl were injected intradermally. The fraction was considered hemorrhagic on the visualization of a hemorrhagic reaction 24 h after the injection.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) X62222.

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Control saline injections were included. Purification of the Hemorrhagin—B. jararaca venom (5–10 mg amounts) was dissolved in 0.5 ml of distilled water and centrifuged. The supernatant was mixed with 2 volumes of 2 M ammonium sulfate containing 20 mM Tris-Cl, pH 7.4, and applied directly to a phenyl-Sephrose hydrophobic interaction column (HR 5/5, 1 ml). Phenam- cia) equilibrated with starting buffer (1.2 M ammonium sulfate, 20 mM Tris-HCl, pH 7.4). The column was washed with 10 ml of starting buffer and eluted with a linear gradient to 100% eluting buffer (20 mM Tris-HCl, pH 7.4) over 20 ml at a flow rate of 0.5 ml/min on a Pharmacia FPLC system. The eluate was monitored continuously at 280 nm with a Cecil CE1220 variable wavelength spectrophotometer. Fractions containing hemorrhaginic activity were either pooled and used immediately for biochemical studies or were chromatographed further on anion-exchange FPLC. Anion-exchange chromatography was performed on a Pharmacia Mono Q column on an FPLC system as above. The column was eluted with a gradient of sodium chloride in 20 mM Tris-HCl, pH 6.8, from 0 to 350 mM over 25 min at a flow rate of 1 ml/min. For HPLC, samples were diluted 1:2 in starting buffer (0.1% aqueous trifluoroacetic acid) and applied to an 8x100 mm, Applied Biosystems, Warrington, Upland, California) on an ACN 500/94 HPLC system comprising a quaternary solvent pump, a Rheodyne injection valve, and a variable wavelength UV detector. Elution was with a linear gradient of ace- tonitrile to 50%.

Electrophoresis—Samples were run on SDS-containing polyacry- lamide slab gels on a Bio-Rad Mini Protean system. Separating gel containing 6.5% acrylamide and 4% bisacrylamide was used; the stacking gel concentration was 3%. Gels were stained with Coomassie Brilliant Blue R-250 (0.5% in methanol/water/acetic acid, 9:2:9) and destained with methanol/acetic acid. Molecular weight protein standards were obtained from Bio-Rad.

Hemorrhagin Antiserum Preparation—Rabbits were immunized with 10–50 μg of purified hemorrhagin emulsified in Freund's complete adjuvant, for the first injection, and boosted using Freund's incomplete adjuvant after 2 and 4 weeks. Blood was collected at regular intervals, and the serum was separated after clotting. The concentration was 3%. Gels were stained with Coomassie Brilliant Blue. The column was eluted with a gradient of sodium chloride, 20 mM, 50%.

Northern Blot Analysis—Total RNA (5 μg) from venom glands was electrophoresed on 1.5% formaldehyde-agarose gels and transferred onto nitrocellulose using a Bio-Rad Trans-Blot system according to the manufacturer's instructions. Antibody detection of polypeptides was as described for immunoscreening.

RESULTS AND DISCUSSION

Purification of the Hemorrhagin—Following initial fractionation of venom on phenyl-Superose, hemorrhagic activity was resolved into two components with approximately equal total hemorrhagic activity (Fig. 1). The early eluting fraction coeluted with phospholipase and procoagulant activity and was not studied further. The late eluting material was free of procoagulant, phospholipase, and platelet aggregation-inducing activity and had a minimum hemorrhagic dose of 20 μg. This fraction made up 5% by weight of the pooled venom and on cloned DNA digested with appropriate restriction enzymes and subcloned into M13 mp18/19. Oligonucleotide primers to internal sequence were used to obtain overlapping sequence information.

Affinity Purification of Antibodies Recognizing Recombinant Antigens—A modification of the method described by Snyder et al. (1987) was used. A nicked-circular plasmid (9 cm2) containing recombinant proteins induced from confluent BJ4 phage was dried and blocked with PBS, 2% low fat milk powder, 0.05% Tween 20. The filter was incubated with hemorrhagin antiserum at 4°C for 1 h and washed in PBS, 0.05% Tween 20. Filter-bound antibodies were eluted by incubation in 3.5 ml of 0.2 M glycine, pH 2.5, and neutralized with 1.75 ml of 1 M K2HPO4, pH 9.0. Antibodies were diluted in PBS, 2% bovine serum albumin. Reduced venom proteins were separated through 7.5% separating gel using Laemmli's discontinuous system (Laemmli, 1970), and stained with Coomassie Blue. Proteins were transferred from SDS-polyacrylamide gels onto nitrocellulose using a Bio-Rad Trans-Blot system according to the manufacturer's instructions. Antibody detection of polypeptides was as described for immunoscreening.

...continued with further details and analysis of the results.
used. Subsequently, other preparations of venom were found to contain higher concentrations of this hemorrhagin (up to 10% by weight). It was greater than 90% pure on anion-exchange FPLC and reverse phase HPLC (Fig. 2), with a molecular mass of 52 kDa (Fig. 2) and an isoelectric point of approximately 4.5. The recovery of biological activity exceeded 60%, and the mass recovery, determined by repeated chromatography on phenyl-Superose, was greater than 80%.

**DNA Cloning**—Complementary DNA clone banks were constructed in the expression vector, λZAPII, using mRNA purified from the venom glands of a specimen of *B. jararaca* as template. Antibodies raised against the hemorrhagin (see “Materials and Methods”) recognized a major band of 52 kDa in Western blot analysis of the whole venom (data not shown). This antibody preparation was used to screen the cDNA expression library in order to identify clones containing putative hemorrhagin coding sequences. A large proportion of the library, approximately 7% of all clones, were immunoreactive with the hemorrhagin antibodies, which correlates with a high abundance of immunologically related hemorhagic components present in *B. jararaca* venom (Assakura et al., 1986).

Several strongly immunoreactive clones were isolated from the clone bank with insert sizes ranging from 0.6 to 2.2 kb. The clone with the largest insert (approximately 2.2 kb), BjD4, was selected for further study. This clone hybridized to a major band of 2.2 kb in a Northern blot of the total venom gland RNA from *B. jararaca* (Fig. 3A), suggesting that the clone represented the full-sized transcript. However, later sequence analysis (see below) revealed that approximately 100 base pairs of cDNA were missing from the 5′ end. In order to provide further confirmation of its identity, antibodies recognizing the fusion protein produced by clone BjD4 were affinity-purified from antisera raised to the native hemorrhagin and used in a Western blot analysis of *B. jararaca* venom. These BjD4-specific antibodies were found to react to a single major protein with the same apparent molecular mass as the purified hemorrhagin (Fig. 3B).

**DNA Sequence Analysis**—The cDNA sequence of BjD4, excluding the poly(A) tail, is 2118 bases in length, and has an open reading frame of 1663 bases predicting a protein of 521 amino acids (Fig. 4). A search of the GenBank and EMBL Data Banks revealed strong sequence homology with snake venom metalloproteases and disintegrin peptides (Fig. 5).

The NH₂-terminal sequence of the mature protein was

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**Fig. 2.** *a*, fractionation of the hemorrhagin obtained from separation on phenyl-Superose on reverse phase HPLC on a C8 wide pore (4.6 × 100 mm, 300 Å) cartridge (the dotted line indicates the concentration of acetonitrile); *b*, on Mono Q anion-exchange FPLC eluted with a gradient of NaCl in 20 mM Tris-Cl, pH 6.8 (100% Buffer B contains 1 M NaCl); and *c*, electrophoretic separation of the fraction indicated by an arrow in Fig. 1 on a 10% SDS-polyacrylamide gel.

**Fig. 3.** *A*, Northern blot analysis of *B. jararaca* venom gland RNA with BjD4 insert. RNA size markers are shown on the right, with sizes given in kilobases (kb). *B*, Western blot analysis of *B. jararaca* venom with BjD4 affinity-purified antibodies. *Lanes 1* and *2* contain whole venom fractionated by SDS-polyacrylamide gel electrophoresis. *Lane 1* shows a Western blot of this *B. jararaca* whole venom reacted with BjD4 affinity-purified antibodies and *lane 2* the stained gel. Molecular sizes are indicated on the right in kDa.
predicted to be pyroglutamic acid. This is based on sequence homology to other venom metalloproteases (Takeya et al., 1990b), and in common with these proteases a pure preparation of the native hemorrhagin was found to be NH2-terminally blocked. In this reading frame there is a stop codon 1264 bases downstream predicting a mature protein, which we have called jararhagin, of 421 amino acids and a molecular mass of 47 kDa. The difference between this value and that estimated by polyacrylamide gel electrophoresis for the 52-kDa native component may be due to post-translational modification. A potential glycosylation site linked to asparginine is located at residue 183 (Fig. 5).

As shown in Fig. 5, jararhagin closely resembles HR1B, a large hemorrhagin from T. flavoviridis (Takeya et al., 1990). They share 60% identical and 80% similar amino acid residues, taking into account conservative amino acid substitutions, and have the same domain structure. Both contain an enzyme region (amino acids 1–205), disintegrin region (amino acids 206–297), and a carboxy-terminal region (amino acids 298–421), showing homology to other low molecular mass snake venom metalloproteases and disintegrin peptides. This indicates that jararhagin is a member of the same family as HR1B. Two large hemorrhagins, HR2 (46 kDa) and HR3 (64 kDa), have been described in B. jararaca venom (Assakura et al., 1986), as well as a 48-kDa hemorrhagic metalloprotease (bothropasin) (Mandelbaum et al., 1982). However, in the absence of primary structure data for these components, their relationship with jararhagin is unclear.

Structural Features—The predicted amino-terminal residue of jararhagin, pyroglutamate, is preceded by 150 amino acids of a putative proprotein sequence with striking homology to proprotein sequences found in the coding genes of the disintegrins trigramin (Neeper and Jacobson, 1990) and rhodostomin (Au et al., 1991) (Fig. 6). Neither projararhagin nor prorhodostomin extend to the signal sequence found in pro-trigramin and are, therefore, incomplete cDNA sequences. However, where sequence data are available, the jararhagin proprotein shares 85% identity with protrigramin (residues -150 to -1) and 80% identity with prorhodostomin (residues -115 to -1). The function of the proprotein is unknown, and a search of the GenBank and EMBL protein Data Banks with the jararhagin propeptide sequence failed to find any significant similarities to other proteins. The presence of adjacent metalloprotease domains in jararhagin, protrigramin, and rhodostomin suggests that the propeptide may maintain the protease in an inactive state prior to secretion into the lumen of the venom gland.

In common with other snake venom metalloproteases (Takeya et al., 1990a) a characteristic zinc-chelating sequence His-Glu-X-X-His (residues 145–149), similar to the zinc binding region of thermolysin (Matthews et al., 1974), is found in the enzyme domain of jararhagin (Fig. 5). Secondary structure prediction of jararhagin by the method of Garnier et al. (1978) (data not shown) also indicates the zinc chelating region to be situated in a helical stretch (residues 139–151) and presumably represents the catalytic region of the molecule. Three residues are present, His-94, Gln-99, and Ile-104 (Fig. 3), which are conserved in hemorrhagins (Takeya et al., 1990a); these may be involved in hemorrhagic activity.

Jararhagin contains a region (amino acids 206–297, Fig. 5) with sequence homology to disintegrins, a family of RGD containing snake venom peptides which inhibit platelet aggregation. Members of the disintegrin family include bitistatin (Shebushki et al., 1990), trigramin (Huang et al., 1989; Neep er and Jacobson, 1990), and rhodostomin (Gould et al., 1990; Au et al., 1991). In jararhagin the RGD sequence is replaced by SECD and, as shown in Fig. 6, the disintegrin region forms part of a family of “non-RGD” disintegrins along with HR1B.
A. Pro-peptide region

Jar:  AHPGCAVPVPKEDAMESYFQNVGEPVPVHHEKNKLFLSKYBEHNYSPSCG
pro-Trig:  NQVLLTVLCLAVFPPQGSSILIESGHLNOYEVYYPEKVATYKGAVDCEYDAMOEYDFVFQHVLPNH
pro-Rhod:  FAKNYSETYHSPDG
signal peptide

B. Enzyme region

Jar:  GTETTYPVPVEDCXYCQGDIADDIASIAACGKGLYFKLQGREYTFIEPKLDPSASEAANVFYNEYVEKEDAEAKPSCGT-NGWKSYEKPKKAGAFT
pro-Trig:  GTETTYPVPVEDCXYCQGDADDIASIAACGKGLYFKLQGREYTFIEPKLDPSASEAANVFYNEYVEKEDAEAKPSCGT
pro-Rhod:  GYRTTYPVPVEDCXYCQGDADDIASIAACGKGLYFKLQGREYTFIEPKLDPSASEAANVFYNEYVEKEDAEAKPSCGT

C. Disintegrin region

Jar:  LGTDLSPPCVGNELEQEGCDDGTPPSCCONEOECAATCKLKSQGSGUGHGDCCEKFXSSLGARMSKECDDPACPENGGSGSSPAPWVFK
pro-Trig:  SKTDIVSPPPCVGNELEQEGCDDGTPPSCCONEOECAATCKLKSQGSGUGHGDCCEKFXSSLGARMSKECDDPACPENGGSGSSPAPWVFK
pro-Rhod:  CRDTDIVSPPPCVGNELEQEGCDDGTPPSCCONEOECAATCKLKSQGSGUGHGDCCEKFXSSLGARMSKECDDPACPENGGSGSSPAPWVFK
Bitistatin:  LRT-TSPPCVGNELEQEGCDDGTPPSCCONEOECAATCKLKSQGSGUGHGDCCEKFXSSLGARMSKECDDPACPENGGSGSSPAPWVFK

D. Carboxyl-terminal region

Jar:  NGQPCGLNYGCGNYCNPIMNYHQCYALFG-AVDYEAEEDSCFQWNGKGYGYCCKENKXHIPACPVEDVCCGLRYCKDMSPGQNPCMKFNSDDEHGMWLG
pro-Trig:  NGQPCGLNYGCGNYCNPIMNYHQCYALFG-AVDYEAEEDSCFQWNGKGYGYCCKENKXHIPACPVEDVCCGLRYCKDMSPGQNPCMKFNSDDEHGMWLP
pro-Rhod:  TKCADGKVCNKSVDVDAYT

**Fig. 5. Comparison of the predicted amino acid sequence of jararhagin, with members of the snake venom zinc metalloprotease and disintegrin families.** Protrigramin and prorhodostomin are inferred from cDNA sequences of the disintegrins trigramin (Neeper and Jacobson, 1990) and rhodostomin (Au et al., 1991), respectively; HR1B is a high molecular mass hemorrhagin from T. flavoviridis (Takeya et al., 1990b); HR2A is a low molecular weight hemorrhagin from T. flavoviridis (Takeya et al., 1990b), and bitistatin is a long-chain disintegrin peptide from Bitis arietans (Shebuski et al., 1990). Alignments are divided into four functional regions based on sequence homology: A, proprotein (-150 to -1); B, enzyme ((1-205); C, disintegrin (206-297); and D, carboxyl-terminal (298-421) regions. Amino acids are numbered with respect to the putative amino-terminal residue E in jararhagin. Identical residues are shaded, conserved cysteine residues are indicated (*), and the zinc chelating motif (HEXXH) is underlined. Protrigramin was translated from the published cDNA sequence using the TRANSLATE program (DNASTAR).
FIG. 6. Similarity of non-RGD disintegrin regions in snake venom metalloproteases and guinea pig sperm fusion peptides. The disintegrin and carboxyl-terminal regions of jararhagin and HR1B are aligned with the amino-terminal disintegrin domains of mature PH-30α and PH-30β (Blobel et al., 1992). Alignment of these with the longest representative of the disintegrin peptide family, bitistatin, and with the partial amino-terminal sequence of one-chain botrocetin (Fujimara et al., 1991) is also shown. The RGD sequence in bitistatin is marked with an asterisk, and all aligning cysteines are shaded. Alignments were done by eye and differ slightly from those generated by computer.

FIG. 7. A, schematic representation of the zinc metalloprotease and disintegrin families showing percentage homology to the amino acid sequence deduced from the trigramin cDNA. The cDNA inferred trigramin protein sequence was used to scan Swiss-prot or PIR databases, using the FASTA program (Pearson and Lipman, 1988). A comparison of the amino acid sequences within the shaded region is shown in B. Echistatin (Gan et al., 1988); Kistrin (Gould et al., 1990); Flavoviridin (Gould et al., 1990); Triflavin (Huang et al., 1991); Bitistatin (Dennis et al., 1990); Elegantin (Williams et al., 1990); Batroxostatin (Rucinski et al., 1990); Applagin (Chao et al., 1989); Halyssin (Huang et al., 1991); Triгamin α (Huang et al., 1989); Triгamin β and γ (Dennis et al., 1990); HR1B (Takeya et al., 1990b); HR2A (Miyata et al., 1989); H2 (Takeya et al., 1989); HRTD (Shannon et al., 1989); HR2T (Takeya et al., 1990a); proTriгamin (Neep and Jacobson, 1990); proRhodostomin (Au et al., 1991).

and the guinea pig sperm fusion proteins PH-30α and PH-30β (Blobel et al., 1992). It has been suggested by Takeya et al. (1990b) that this region may play a role in adhesion of venom metalloproteases to target molecules, possibly to integrins located in the vascular endothelium. Similarly, the non-RGD disintegrin domain in sperm fusion proteins may bind sperm cells to integrins on the egg surface during fertilization (Blobel et al., 1992). Although it is clear that disintegrin peptides bind to platelet GP IIb-IIIa integrin targets via the RGD sequence (Savage et al., 1990), the mode of action of the “non-RGD disintegrins” is not yet understood. A conservative ECD replacement, containing an odd-numbered cysteine residue, is found in all these non-RGD disintegrin molecules (Fig. 6). This may be of functional significance.
Both jararhagin and HR1B contain a cysteine-rich carboxy-terminal region (amino acids 298–421) of unknown function (Fig. 5). However, there is a strong resemblance between the snake venom enzymes jararhagin and HR1B with the guinea pig sperm adhesive proteins PH-30α and PH-30β in this region (Fig. 6). Furthermore, amino acid homology and cysteine alignment extend beyond the disintegrin region (defined by hitistatin) to the end of the carboxy-terminal domain, indicating that the disintegrin/carboxy-terminal region may function as a single contiguous unit with an adhesive role.

Relationship with Disintegrins and Botrocetin—A schematic representation of the zinc metalloprotease and disintegrin families is shown in Fig. 7A. Homologies are shown in relation to protrigramin in order to highlight the modular arrangement of proteases and disintegrins present in the metalloprotease group of proteins. The structure and organization of the gene coding for jararhagin, containing proprotein, enzyme, and disintegrin domains, is very similar to that of trigramin and rhodostomin. This suggests that snake venom metalloproteases, hemorrhagins, and disintegrins have all evolved from the same ancestral gene, presumably via gene duplication and exon shuffling.

It is evident from the cDNA cloning of rhodostomin (Au et al., 1991) and trigramin (Neiper and Jacobson, 1990) that RGD-containing disintegrin peptides are derived from a metalloprotease precursor, presumably through cleavage at a site in the metalloprotease/disintegrin domain boundary. Sequences of rhodostomin and trigramin precursors are similar to jararhagin and HR1B in this region (Fig. 7B) and susceptibility to cleavage may be dependent on factors such as nearby disulfide bridges or glycosylation, as suggested by Kini and Evans (1992) in their recent review of the metalloprotease/disintegrin family. There is a possibility therefore that a class of non-RGD-containing disintegrin proteins might exist as products of metalloprotease/disintegrin precursors such as jararhagin and HR1B.

Interestingly, strong homology was found at the start of the disintegrin region in jararhagin (Fig. 6) to one-chain botrocetin, a 28–30-kDa platelet agonist from B. jararaca (Fujimura et al., 1991). Although only the partial NH2-terminal sequence of single chain botrocetin is known, only one mismatch (Val-222 and Glu) is found in the 24 amino residues identified (Fig. 6), indicating a structural relationship between one-chain botrocetin disintegrins and hemorrhagins. The biological properties of one-chain botrocetin show marked differences to RGD-containing disintegrin peptides. Disintegrin peptides are small and range in size from 5 kDa, in the case of echiatin (Gan et al., 1988), to 9 kDa, in the case of hitistatin (Goedl et al., 1990), and they inhibit the binding of fibrinogen and von Willebrand factor to GP IIb-IIIa on activated platelets through the RGD sequence (Savage et al., 1990). In contrast, the 28-kDa one-chain botrocetin is much larger in size and promotes platelet aggregation in a two-step process involving complex formation with von Willebrand factor. This complex then agglutinates platelets by interacting with GP Ib rather than to GP IIb-IIIa platelet receptors (Fujimura et al., 1991).

Although the predicted size of the protein derived from the disintegrin/carboxy-terminal region (amino acids 209–421) is 23 kDa and different to the one-chain botrocetin (28 kDa), a comparison of the amino acid composition of the one-chain botrocetin to the disintegrin/carboxy-terminal region (Table I) showed only slight variation. We are currently investigating the possibility that one-chain botrocetin is a product of jararhagin. Further work on the non-RGD disintegrins may reveal alternative ligand-receptor interactions of potential therapeutic interest, as in the case of RGD disintegrin peptides.

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REFERENCES


Molecular Cloning of Jararhagin