

## MONOMERIC AND DIMERIC DISINTEGRINS: PLATELET ACTIVE AGENTS FROM VIPER VENOM

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*When the term “disintegrin” was first coined in 1990, it described a family of naturally occurring proteins with low molecular weights and highly conserved sequences, both in their cysteine arrangements and adhesive Arg-Gly-Asp (RGD) motifs. Another common characteristic was the inhibitory potential these proteins demonstrated in interacting with cell-surface integrin receptors. Measurement of the effect by disintegrins on the interaction between the platelet receptor  $\alpha$ IIB $\beta$ 3 and its ligand, fibrinogen, has become a hallmark assay for comparing the activities of members of this increasingly diverse family discovered in the past two decades. This review focuses on the inhibitory profiles, based on platelet function, of the monomeric and heterodimeric disintegrins described to date, as well as the informative contributions of disintegrin mutations in our understanding of the structure–function relationships between ligand and  $\alpha$ IIB $\beta$ 3. The challenge of naming future examples of these proteins is also addressed.*

**Keywords:** Disintegrin, Inhibitory profiles, Platelet aggregation.

### Introduction

The discovery of disintegrins in the early 1980s resulted from the observation that something fundamentally different happened when prey were bitten by sea snakes versus vipers. The animals bitten by the former experienced neurological symptoms, while those envenomed by the latter would hemorrhage. The bleeding phenomenon suggested that the viper venom possessed either an enzymatic component capable of digesting extracellular matrix or blood vessel endothelial cells, or an anticoagulant thwarting

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the coagulation cascade, or an antiplatelet agent to prevent that cell's contribution to thrombus formation. It turned out that all viper venoms studied thus far possess examples of all three types of molecules. Some nonenzymatic proteins in the venom were found to be low-molecular-weight, cysteine-rich, and very potent inhibitors of platelet aggregation (Markland, 1998). Simultaneous to the study of these natural antiplatelet proteins in the 1980s came research on the receptors present on the platelet surface that mediate that aggregation: integrins. The  $\alpha\text{IIb}\beta 3$  receptor's interaction with fibrinogen was found to be the final common pathway of platelet aggregation, regardless of agonist used (Calvete, 2004; Huang and Hong, 2004; Shattil and Newman, 2004). The term "disintegrin" was coined in 1990 (Gould *et al.*, 1990) to indicate that these viper proteins could bind to, and interfere with the function of, integrin receptors on cell surfaces. In those early days of disintegrin research, platelets became a primary target of experimentation because of their clinical relevance and ease of procurement. Since then, disintegrins have been used to study integrin function in many diverse types of cells (McLane *et al.*, 2004). In addition, the definition of "disintegrin" has expanded to include molecules found in many non-viper species (McLane *et al.*, 2004). Platelets remain, however, a significant functional model for describing the activities of disintegrins, which, for this review, will be defined as those low-molecular-weight, nonenzymatic soluble monomeric or dimeric molecules from viper venom possessing an RGD-like motif and a cysteine arrangement significantly homologous to others in this protein family.

### Variability in Potency

The  $\text{IC}_{50}$  is the concentration of ligand that inhibits a defined cellular activity by 50%. A comparison of disintegrins by their antiplatelet function is made complex by the variety of agonist types and concentrations used. In addition, one must note that investigators have utilized platelets from species as diverse as human, mouse, and buffalo, and may have processed such platelets by filtering, washing, or diluting prior to analysis. The most common agonist/platelet combination used is adenosine diphosphate (ADP) and platelet-rich plasma (PRP). Some authors standardized the platelet concentration used (Huang *et al.*, 1991d; Marrakchi *et al.*,

**TABLE 1** Disintegrin Inhibition of Platelet Aggregation Induced by Collagen (1–10 ug/mL)

| 50% Inhibitory concentrations (IC <sub>50</sub> ) within the range shown |                                   |                                      |
|--------------------------------------------------------------------------|-----------------------------------|--------------------------------------|
| <400 nM                                                                  | 400–1000 nM                       | >1000 nM                             |
| Accutin (h) (Yeh et al., 1998)                                           | Bitistatin (h)                    | Jarastatin (r) (Coelho et al., 1999) |
| Applaggin (h) (Chao et al., 1989)                                        | (Huang et al., 1991d)             |                                      |
| Bitistatin (h) (Huang et al., 1991d)                                     | Halyssetin (h) (Liu et al., 2000) |                                      |
| Cerastatin (h, r) (Marrakchi et al., 1997)                               | Halysin (h) (Huang et al., 1991a) |                                      |
| Contortrostatin (h) (Kamiguti et al., 1997)                              |                                   |                                      |
| Crotavirin (h) (Liu et al., 1995)                                        |                                   |                                      |
| Echistatin (h) TW                                                        |                                   |                                      |
| Eristostatin (h) TW                                                      |                                   |                                      |
| Flavostatin (h) (Kawasaki et al., 1996; Maruyama et al., 1997)           |                                   |                                      |
| Gabonin (h) (Huang et al., 1992)                                         |                                   |                                      |
| Jerdonatin (h) (Zhou et al., 2004)                                       |                                   |                                      |
| Kistrin (h) (Dennis et al., 1990)                                        |                                   |                                      |
| Triflavin (h) (Huang et al., 1991b)                                      |                                   |                                      |
| Ussuristatin 1 (h) (Oshikawa and Terada, 1999)                           |                                   |                                      |
| Ussuristatin 2 (h) (Oshikawa and Terada, 1999)                           |                                   |                                      |

Testing reported from human (h) or rabbit (r) platelets. Disintegrins in more than one column showed differences based on the species or preparation of platelet used (washed, gel-filtered, or platelet-rich plasma). References in parentheses. TW = this work (present study).

1997; McQuade et al., 2004), while most make no mention of this. Tables 1 through 4 summarize disintegrin IC<sub>50</sub> values of platelet aggregation induced by collagen, ADP, and other agonists, respectively.

### *Collagen*

Collagen from the extracellular matrix is one of the first agonists to which platelets are exposed during an episode of blood vessel damage (Farndale et al., 2004). Platelets bind collagen through

their  $\alpha 2\beta 1$  integrin receptor and glycoprotein VI (Hynes, 2002) and undergo a series of intracellular signaling events that lead to the release of intracellular calcium, reorganization of the cytoskeleton, release of alpha and dense granule contents, and conformational activation of the fibrinogen integrin receptor  $\alpha \text{IIb}\beta 3$  (Andrews and Berndt, 2004). The source of collagen used in the study of disintegrin inhibition of collagen-induced platelet aggregation (Table 1) is usually described as commercially available Type I isolated from horse tendon. It has been noted, however, that all collagens exhibit the ability to induce platelet aggregation *in vitro* (Farndale et al., 2004). When experiments used 10  $\mu\text{g}/\text{mL}$  collagen, there was a significant difference in inhibitory potential based on the animal species and platelet preparation used. Washed human platelets showed an  $\text{IC}_{50}$  of 23 nM with crotoxin (*Crotalus viridis*) (Liu et al., 1995), 176 nM with accutin (*Agkistrodon acutus*) (Yeh et al., 1998), and 360 nM with halysin (*Agkistrodon halys*) (Huang et al., 1991a), while flavoridin (also called triflavin from *Trimeresurus flavoridis*), applagin (*Agkistrodon piscivorus piscivorus*), and gabonin (*Bitis gabonica*) gave values of 64, 89, and 342, respectively, using human PRP (Chao et al., 1989; Huang et al., 1991b, 1992). Huang et al. (1991c) compared the inhibition of collagen-induced platelet aggregation by arietin from *Bitis arietans* (also known as bitan [Dennis et al., 1990] and bitistatin [Shebuski et al., 1989]) using human washed platelets versus PRP and found  $\text{IC}_{50}$  values of 300 and 800 nM, respectively. Cerastin (*Cerastes cerastes*) was more potent in inhibiting collagen-induced aggregation using rabbit PRP (2.3 nM) than with human washed platelets (40 nM) (Marrakchi et al., 1997). Interestingly, Oshikawa and Terada (1999) used 2  $\mu\text{g}/\text{mL}$  collagen for their studies using human PRP and obtained values ranging from 33 nM to 220 nM, for two disintegrins both found in *Agkistrodon ussuriensis* venom, ussuristatin 1 (a monomer) and ussuristatin 2 (a dimeric disintegrin). Coelho et al. (1999), using rabbit washed platelets, found jarastatin from *Bothrops jararaca* to be minimally active in inhibiting collagen-induced platelet aggregation, with an  $\text{IC}_{50}$  of 1000 nM. Our laboratory compared the ability of native eristostatin (*Eristicophis macmahoni*) and recombinant echistatin (Wierzbicka-Patynowski et al., 1999) to inhibit collagen-induced platelet aggregation and found both to have comparable potencies, at 380 nM and 229 nM, respectively.

### ADP

Adenosine diphosphate (ADP) is the most common agonist used to assess disintegrin biological activity, as shown in Table 2. ADP is an important physiological activator of platelets (Andrews and Berndt, 2004; Murugappan et al., 2004), although it is not the initiator of such activation during a thrombotic event. After exposure of resting platelets to agonists, such as thrombin or collagen, the resulting activation signaling events will culminate, among other things, in the secretion of ADP from the platelets' dense granules. This ADP will bind to platelet G protein-coupled receptors P2Y<sub>1</sub> and P2Y<sub>12</sub>, causing localized platelet activation and stable aggregation (Andrews and Berndt, 2004). It has become a hallmark of disintegrin characterization to determine their inhibitory potency in ADP-induced platelet aggregation. As was seen with such studies using the agonist collagen, investigators have used varying amounts of ADP as well as different species and preparations of platelets, so IC<sub>50</sub> comparisons may not be straightforward. Overall, the data in Table 2 show that monomeric and homodimeric disintegrins have significant inhibitory potency (less than 400 nM), while most heterodimeric disintegrins show IC<sub>50</sub> values greater than this, suggesting that monomeric disintegrins are more potent than dimeric disintegrins in preventing fibrinogen from interacting with its  $\alpha$ IIb $\beta$ 3 receptor. It has long been established that a tripeptide motif, found at the tip of a flexible loop at the C-terminus, is responsible for the adhesive properties of disintegrins (McLane et al., 2004). For 50 of the 58 monomeric disintegrins listed by McLane et al. (2004), that tripeptide is arginine-glycine-aspartic acid (RGD). For three of the remaining disintegrins, arginine is replaced by lysine, forming a KGD motif demonstrated to impart not only potency but also selectivity for the  $\alpha$ IIb $\beta$ 3 receptor (Kang et al., 1999; Scarborough et al., 1991). An exception to this pattern is the RGD-containing monomeric adinbitor from *Gloydius blomhoffi brevicaudus*, with an IC<sub>50</sub> of 6,000 nM in ADP-induced platelet aggregation (Wang et al., 2004). This 89-residue disintegrin is comparable in size to the largest disintegrins, such as bitistatin (McLane et al., 2004), but in contrast to these, adinbitor has only 12 cysteines rather than 14. This suggests that its disulfide pattern, which is critical to disintegrin function, must be significantly different, and nonoptimal, to render this disintegrin protein virtually inactive.

**TABLE 2** Disintegrin Inhibition of Platelet Aggregation Induced by ADP (1–20  $\mu$ M)

| 50% inhibitory concentrations ( $IC_{50}$ ) within the range shown  |                                                                                                                            |                                                                                                                                                             |                                                 |                                                     |
|---------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|-----------------------------------------------------|
| <100 nM                                                             | 100–200 nM                                                                                                                 | 200–400 nM                                                                                                                                                  | 400–1000 nM                                     | >1000 nM                                            |
| Acostatin 1 (h) (Okuda et al., 2002)                                | Albolabrin (h, m) (Beviglia et al., 1995; McLane et al., 1994; Williams et al., 1990)                                      | Barbourin (h) (Scarborough et al., 1991)                                                                                                                    | Barbourin (d) (Knight et al., 1996)             | Adinbitor (h) (Wang et al., 2004)                   |
| Acostatin 2 (h) (Okuda et al., 2002)                                | Batroxostatin (h) (Rucinski et al., 1990)                                                                                  | Basilicin (h) (Scarborough et al., 1993)                                                                                                                    | EC3 $\delta$ (h) (Marcinkiewicz et al., 1999a)  | Contortrostatin $\delta$ (r) (Trikha et al., 1994b) |
| Accutin (h) (Yeh et al., 1998)                                      | Bitistatin (h,d) (Dennis et al., 1990; Huan et al., 1991d; Knight et al., 1996; McLane et al., 1994; McQuade et al., 2004) | Bitistatin (h) (Huang et al., 1991d; Shebuski et al., 1989)                                                                                                 | Echistatin $\alpha$ 2 (h) (Dennis et al., 1990) | Echistatin (rt) (Chen et al., 1995)                 |
| Applaggin (h) (Chao et al., 1989; Savage et al., 1990)              |                                                                                                                            |                                                                                                                                                             |                                                 | Echistatin $\gamma$ (rt) (Chen et al., 1995)        |
| Barbourin (h,m) (Beviglia et al., 1995; Knight et al., 1996)        |                                                                                                                            |                                                                                                                                                             |                                                 | EMF10 $\delta$ (h) (Marcinkiewicz et al., 1999b)    |
| Bitistatin (also called arietin, bitan) (d) (Shebuski et al., 1989) | Contortrostatin (d) (Trikha et al., 1994b)                                                                                 | Crotatroxin (h) (Scarborough et al., 1993)                                                                                                                  | Echistatin $\gamma$ (r) (Chen et al., 1995)     | Halyssetin (h) (Liu et al., 2000)                   |
| Bothrasperin (h) (Pinto et al., 2003)                               | Crotavirin (h) (Liu et al., 1995)                                                                                          | Echistatin $\alpha$ 1 (d) (Lombardi et al., 1999)                                                                                                           | Echistatin $\gamma$ (h,g) (Dennis et al., 1990) | Jarastatin (r) (Coelho et al., 1999)                |
| CC5 $\delta$ (h) (Calvete et al., 2002)                             | Durissin (h) (Scarborough et al., 1993)                                                                                    | Echistatin $\alpha$ (h,b,d) (Chen et al., 1995; Clark et al., 1994; Knight et al., 1996; McLane et al., 1994; Pfaff et al., 1994; Scarborough et al., 1991) | Echistatin $\gamma$ (h) (Dennis et al., 1990)   |                                                     |
| CC8 $\delta$ (h) (Calvete et al., 2002)                             |                                                                                                                            |                                                                                                                                                             |                                                 |                                                     |
| Cerastin (h) (Scarborough et al., 1993)                             |                                                                                                                            |                                                                                                                                                             |                                                 |                                                     |
| Cerastatin (h,r) (Marrakchi et al., 1997)                           |                                                                                                                            |                                                                                                                                                             |                                                 |                                                     |
| Cereberin (h) (Scarborough et al., 1993)                            |                                                                                                                            |                                                                                                                                                             |                                                 |                                                     |



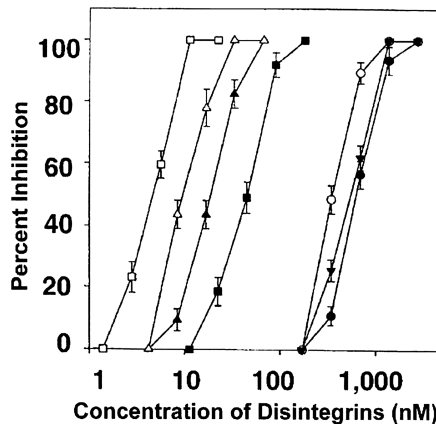
**TABLE 2** Disintegrin Inhibition of Platelet Aggregation Induced by ADP (1–20  $\mu$ M) (*Continued*)

| 50% inhibitory concentrations ( $IC_{50}$ ) within the range shown                    |                                                             |            |             |          |
|---------------------------------------------------------------------------------------|-------------------------------------------------------------|------------|-------------|----------|
| <100 nM                                                                               | 100–200 nM                                                  | 200–400 nM | 400–1000 nM | >1000 nM |
| Kistrin (h,d,ho,b) (Knight et al., 1996; Lombardi et al., 1999; Scaloni et al., 1996) | Ocellatusin (h) (Smith et al., 2002)                        |            |             |          |
| Multisquamatin (h) (Okuda et al., 2001; Trikha et al., 1994b)                         | Piscivostatin <sup>§</sup> (h) (Okuda and Morita, 2001)     |            |             |          |
|                                                                                       | Pyramidin A (h) (Okuda et al., 2001)                        |            |             |          |
| Trigramin a (h) (Huang et al., 1987; Rucinski et al., 1990)                           | Salmosin (h) (Kang et al., 1998)                            |            |             |          |
| Trimestatatin (h) (Fujii et al., 2003)                                                | Saxatilin (h) (Hong et al., 2002)                           |            |             |          |
|                                                                                       | Tergeminin (h) (Scarborough et al., 1991)                   |            |             |          |
| Ussuristatin 1 (h) (Oshikawa and Terada, 1999)                                        | Trigramin a (h) (Huang et al., 1987; Rucinski et al., 1990) |            |             |          |
|                                                                                       | Trigramin $\beta$ 2 (h) (Dennis et al., 1990)               |            |             |          |
|                                                                                       | Viridan (h) (Scarborough et al., 1993)                      |            |             |          |

Testing reported from human (h), rabbit (r), rat (rt), buffalo (b), guinea pig (g), horse (ho), dog (d), and mouse (m) platelets. Disintegrins in more than one column showed differences based on the species or preparation of platelet used (washed, gel-filtered, or platelet rich plasma).  
References in parentheses.  $\S$  = dimeric disintegrin



Dimeric disintegrins were first described in 1994 with the characterization of contortrostatin (Trikha et al., 1994b). This disintegrin was found to be potent in inhibiting ADP-induced platelet aggregation (Trikha et al., 1994a), with an  $IC_{50}$  of 49 nM. Marcinkiewicz et al. isolated additional dimers, including EC3 (Marcinkiewicz et al., 1999a) and EMF10 (Marcinkiewicz et al., 1999b), but these did not show significant potency with  $\alpha IIb\beta 3$ , making platelet aggregation a less sensitive method for analytical comparison (Calvete et al., 2003). These investigators developed a microplate cell adhesion inhibition assay, using Chinese hamster ovary (CHO) cells stably transfected with  $\alpha IIb\beta 3$ , called A5 cells (O'Toole et al., 1990). Figure 1 graphically emphasizes the difference in inhibitory potency of the first dimers tested in this system compared with monomers. Eristostatin ( $IC_{50} = 5$  nM) was most potent, and echistatin ( $IC_{50} = 50$  nM) was the least potent of



**FIGURE 1** Effects of various concentrations of monomeric and heterodimeric disintegrins (x-axis, in nM) on adhesion of A5 CHO cells stably transfected with  $\alpha IIb\beta 3$  to immobilized fibrinogen (y-axis, in percent inhibition). Fluorescently labeled cells ( $10^5$  cells/well) were mixed with disintegrins, added to 96-well plates coated overnight with the relevant ligand, incubated at  $37^\circ\text{C}$  for 30 min, and washed. Bound cells were lysed in 0.5% Triton X-100, fluorescence was measured, and percent inhibition of adhesion was calculated in comparison to fluorescence of adherent cells in the absence of disintegrins. Data are the mean  $\pm$  S.E. of at least three experiments. EC6 ○; EC3 ●; eristostatin □; kistrin ▲; flaviridin △; EMF10 ▼; echistatin ■. (Reprinted from *J. Biol. Chem.* 2000 Oct 13; 275(41):31930–31937 with permission.)

**TABLE 3** Comparison of Adhesive Tripeptide Sequences in Monomeric and Dimeric Disintegrins and the Inhibitory Effect on  $\alpha$  IIb $\beta$ 3 Activity

| Disintegrins    | Tripeptide Sequence* | IC <sub>50</sub> <sup>+</sup> |       | Reference                                       |
|-----------------|----------------------|-------------------------------|-------|-------------------------------------------------|
| CC8             | RGD/RGD              | 11                            | 2     | Calvete et al., 2002                            |
| Contortrostatin | RGD/RGD              | 49                            |       | Trikha et al., 1994b                            |
| Eristostatin    | RGD                  | 59                            | 5     | Marcinkiewicz et al., 1999b                     |
| Piscivostatin   | RGD/KGD              | 102                           |       | Okuda and Morita, 2001                          |
| Echistatin      | RGD                  | 136                           | 48    | Marcinkiewicz et al., 1999b; Smith et al., 2002 |
| Ocellatusin     | RGD                  | 168                           | 77    | Smith et al., 2002                              |
| VLO4            | RGD/RGD              |                               | 115   | Calvete et al., 2003                            |
| EO4             | RGD/RGD              |                               | 118   | Calvete et al., 2003                            |
| VA6             | RGD/RGD              |                               | 144   | Calvete et al., 2003                            |
| Lebein 1        | RGD/RGD              | 160                           |       | Gasmi et al., 2001                              |
| Ussuristatin 2  | KGD/KGD              | 290                           |       | Oshikawa and Terada, 1999                       |
| EMS11           | MLD/unknown          |                               | 350   | Calvete et al., 2003                            |
| EC6             | MLD/RGD              |                               | 420   | Marcinkiewicz, 2004                             |
| VB7             | RGD/KGD              |                               | 420   | Calvete et al., 2003                            |
| EC3             | VGD/MLD              | 1000                          | 500   | Marcinkiewicz et al., 2000                      |
| EMF10           | RGD/MGD              | 1600                          | 500   | Marcinkiewicz et al., 1999b                     |
| VLO5            | MLD/VGD              | 1600                          | 760   | Calvete et al., 2003                            |
| EO5             | MLD/VGD              | 1800                          | 980   | Calvete et al., 2003                            |
| Obtustatin      | KTS                  |                               | 10000 | Kisiel et al., 2004                             |
| Viperistatin    | KTS                  |                               | 10000 | Kisiel et al., 2004                             |

\*Dimeric sequences are those found in the alpha and beta chains, respectively, summarized in Calvete et al. (2003).

<sup>+</sup>50% inhibitory concentration is mean reported in literature cited. Note that the IC<sub>50</sub> values measured are ADP-induced aggregation of platelets (left column) or A5 cell adhesion (right column).

the monomerics, similar to what is seen in ADP-induced platelet aggregation. EC3, EC6, and EMF10 had comparable potencies at approximately 450 nM. Of the dimerics recently tested in this multiwell plate system (Table 3), EO4 from *Echis ocellatus* (Calvete et al., 2003) and VLO4 from *Vipera lebetina obtusa* (Calvete et al., 2003) showed the greatest inhibitory potential of  $\alpha$ IIb $\beta$ 3/fibrinogen interaction, while EO5, also from *Echis ocellatus* (Calvete et al., 2003), was least potent. Reviewing the motif present in the "RGD" location within the dimeric disintegrins reveals that these structures possess a greater variety of tripeptide motifs in place of RGD in their sequences. It is interesting to note that those dimeric disintegrins

that contain R/KGD within each of their chains show  $\alpha$ IIB $\beta$ 3 inhibitory activity comparable to monomeric disintegrins (Table 3), while there is decreasing potency if the disintegrin possesses sequences other than R/KGD. This has also been suggested as an explanation for their greater potency with other integrins like  $\alpha$ v $\beta$ 3,  $\alpha$ 1 $\beta$ 1,  $\alpha$ 4 $\beta$ 1,  $\alpha$ 5 $\beta$ 1, and  $\alpha$ 9 $\beta$ 1 (Calvete et al., 2003; Marcinkiewicz et al., 1999a, 2000). Interestingly, obtustatin and viperistatin, both monomers (Kisiel et al., 2004), are even less potent in this system than are the dimeric disintegrins (Table 3). The authors propose that the KTS tripeptide, replacing RGD in their adhesive loop, is responsible for these disintegrins' lack of interaction with  $\alpha$ IIB $\beta$ 3 and selectivity for the  $\alpha$ 1 $\beta$ 1 integrin.

#### *Platelet Agonists Other than Collagen and ADP*

Platelet aggregation inducers other than ADP and collagen have also, to a lesser extent, been used to test disintegrin inhibition of platelet aggregation. Thrombin is a very strong activator of platelets (Hirsh and Weitz, 1999), and disintegrins inhibit thrombin-induced platelet aggregation with potency similar to that when platelets are activated with collagen or ADP (Table 4). Noteworthy among those disintegrins tested are the two proteins isolated from *Agkistrodon ussuriensis* venom, ussuristatin 1 and ussuristatin 2. As with ADP and collagen-induced platelet aggregation described above, the monomer is about 10-fold more potent than the dimer in its inhibitory potency, with thrombin as the agonist. Using the thrombin-receptor-activating peptide (TRAP, also known as protease-activating peptide-1, PAR-1 [Selnick et al., 2003]), Maruyama et al. (1997) and Kawasaki et al. (1996) showed the disintegrin flavostatin (*Trimeresurus flavoridis*) to have an  $IC_{50}$  of 59 nM, which is similar to its potency in ADP-induced aggregation.

When platelets have become activated, the resulting intracellular signaling may cause production and release of a number of chemicals that will further activate additional platelets. Thromboxane A<sub>2</sub> is formed from arachidonic acid and released from platelets activated by collagen and thrombin, but not by low levels of ADP (FitzGerald, 1991). It has a short half-life (Roth and Calverley, 1994) and is partly responsible for the second wave of aggregation observed in aggregation tracings (Larson, 2004). U46619 (9,11-dideoxy-11 $\alpha$ ,9 $\alpha$ -epoxymethanoprostaglandin F<sub>2 $\alpha$</sub> ) is a stable

**TABLE 4** Disintegrin Inhibition of Platelet Aggregation Induced By Other Agonists

| Disintegrin    | Agonist used<br>Thrombin | 50% Inhibitory Concentration (nM)<br>with Platelet Type Indicated |               |               |                | Ref.                      |
|----------------|--------------------------|-------------------------------------------------------------------|---------------|---------------|----------------|---------------------------|
|                |                          | Human<br>WP*                                                      | Human<br>PRP* | Animal<br>WP* | Animal<br>PRP* |                           |
| Accutin        | 0.1 U/mL                 | 267                                                               |               |               |                | Yeh et al., 1998          |
| Applaggin      | 20 ug/mL                 | 128                                                               |               |               |                | Chao et al., 1989         |
| Bitistatin     | 0.1 U/mL                 | 160                                                               |               |               |                | Huang et al., 1991c,d     |
| Cerastatin     | 0.04 U/mL                | 40                                                                |               | Rabbit 2.3    |                | Marrakchi et al., 1997    |
| Crotavirin     | 0.2 U/mL                 | 114                                                               |               |               |                | Liu et al., 1995          |
| Halysin        | 0.1 U/mL                 | 310                                                               |               |               |                | Huang et al., 1991a       |
| Jarastatin     | Not specified            |                                                                   |               | Rabbit 800    |                | Coelho et al., 1999       |
| Triflavin      | 0.1 U/mL                 | 38                                                                |               |               |                | Huang et al., 1991b       |
| Ussuristatin 1 | 5 U/mL                   | 28                                                                |               |               |                | Oshikawa and Terada, 1999 |
| Ussuristatin 2 | 5 U/mL                   | 230                                                               |               |               |                | Oshikawa and Terada, 1999 |
|                | U46619                   |                                                                   |               |               |                |                           |
| Accutin        | 1 uM                     | 102                                                               |               |               |                | Yeh et al., 1998          |
| Bitistatin     | 1-5 uM                   | 150                                                               | 500           |               |                | Huang et al., 1991c,d     |
| Crotavirin     | 1 uM                     | 112                                                               |               |               |                | Liu et al., 1995          |
| Gabonin        | Not specified            | 382                                                               |               |               |                | Huang et al., 1992        |
| Halysin        | 4 uM                     |                                                                   | 1200          |               |                | Huang et al., 1991a       |
| Kistrin        | 100 ng/mL                |                                                                   | 135           |               |                | Dennis et al., 1990       |
| Triflavin      | 4uM                      | 84                                                                |               |               |                | Huang et al., 1991b       |
|                | Tumor cells              |                                                                   |               |               |                |                           |
| Albolabrin     | B16F10 melanoma          |                                                                   |               |               | Mouse 165      | Beviglia et al., 1995     |
| Barbourin      | B16F10 melanoma          |                                                                   |               |               | Mouse 46       | Beviglia et al., 1995     |
| Echistatin     | B16F10 melanoma          |                                                                   |               |               | Mouse 74       | Beviglia et al., 1995     |
| Eristostatin   | B16F10 melanoma          |                                                                   |               |               | Mouse 7        | Beviglia et al., 1995     |
| Kistrin        | Saos-2                   |                                                                   | 30            |               |                | Chiang et al., 1995b      |
|                | osteosarcoma             |                                                                   |               |               |                |                           |
| Kistrin        | MCF-7 breast             |                                                                   | 30            |               |                | Chiang et al., 1995a      |
|                | carcinoma                |                                                                   |               |               |                |                           |
| Trigramin      | MCF-7 breast             |                                                                   | 100           |               |                | Chiang et al., 1995a      |
|                | carcinoma                |                                                                   |               |               |                |                           |

\* Abbreviations used: WP = washed platelets; PRP = platelet-rich plasma. Values are the means provided in each literature source.

thromboxane A2 analog that leads to platelet activation and exposure of the fibrinogen binding site of  $\alpha\text{IIb}\beta 3$  (Mazurov et al., 1984). Of the disintegrins tested using these agonist conditions, accutin (Yeh et al., 1998) and halysin (Huang et al., 1991a) were the most and least potent, respectively, in inhibiting platelet aggregation (Table 4).

Platelet-activating factor (PAF-acether) is released from activated polymorphonuclear neutrophils (PMN) during inflammatory episodes and has the ability to activate platelets, thus inducing aggregation (Benveniste and Chignard, 1985). Lee et al. (1999) investigated the ability of triflavin from *Trimeresurus flavoviridis* to inhibit rabbit platelet aggregation in this system. When used alone, triflavin was minimally able to inhibit PMN-induced platelet aggregation ( $\text{IC}_{50} = 1,000 \text{ nM}$ ). In contrast, when 260 nM triflavin was used with varying concentrations of BN52521, an antagonist to the PAF receptor, platelet aggregation was completely inhibited. The authors hypothesized that the use of inhibitors to  $\alpha\text{IIb}\beta 3$  and the PAF receptor may be of benefit in the treatment of ischemic disorders. In contrast, Marrakchi et al. (1997) tested the medium-length disintegrin cerastatin, from *Cerastes cerastes*, with both human and rabbit washed platelets, and found significant potency with both species (100 and 2.3 nM, respectively) in inhibiting PAF-acether-induced aggregation.

Perhaps the most unique agonists used to induce platelet aggregation are tumor cells (Table 4). Beviglia et al. (1995) tested four disintegrins on murine platelets and found eristostatin to be the most potent and albolabrin the least potent ( $\text{IC}_{50} = 7$  and 165 nM, respectively) in inhibiting B16F10 mouse melanoma cell-induced aggregation. Chiang et al. (1995a,b) assessed kistrin's antiplatelet activity using Saos-2 osteocarcinoma or MCF-7 breast carcinoma cell-induced human platelet aggregation and found comparable  $\text{IC}_{50}$ s at 30 nM. In the latter system, trigramin was threefold less potent than kistrin (Chiang et al., 1995a). Sheu et al. (1994) described the effect of triflavin on J-5 hepatoma cell-induced platelet aggregation, with a potent inhibition of 20 nM.

There are a number of disintegrins for which amino acid sequences are available online but for which no platelet aggregation information is yet available (Figure 2). Based on the disintegrin structure-function studies done thus far, it can be predicted that all of the RGD-containing monomeric and dimeric disintegrins,

|                         |                                                                                      |
|-------------------------|--------------------------------------------------------------------------------------|
| Brevicaudin 1a (A59411) | GEEDCGSPGPCDAATCKLRQAQCAEGLCCDQCRFMKGTVCRIARGGDDDDYCNGISAGCPRNPFHA                   |
| Brevicaudin 1b (A59410) | EAGEEDCGSPGPCDAATCKLRQAQCAEGLCCDQCRFMKGTVCRIARGGDDDDYCNGISAGCPRNPFHA                 |
| Brevicaudin 2b (A59409) | GEEDCGSPGPCDAATCKLRQAQCAEGLCCDQCRFMKEGTICRRARGDDLDYCNGISAGCPRNPFHA                   |
| CTF 1/II (P23323)       | ELLEGEDCYCHIPPNPCDPAATCKLTPGSAEGLCCDQCRFKKKGTICRIARGGFPDDRCTGLSDDCPRMNDL             |
| Gabonin-1 (AAR24528)    | ..TGAMNSAHPCCDPVTCCKPRGEHCISGPCRNCKFLNAGTICKRGRGDSLHDYCTGTPDCPRNPKGESDELEWSAAATGSVLM |
| Gabonin-2 (AAR24529)    | ..TGAMNSAHPCCDPVTCCKPRGEHCISGPCRNCKFLNAGTICKKTMLDGLNDYCTGTPDCPRNPKGESDELEWSAAATGSVLM |
| Halystatin-2 (BAA06027) | EAGEDCDGAPANPCDDAATCKLRPGAQCAEGLCCDQCRFMKEGTICRMARGGDDDDYCNGISAGCPRNPFHA             |
| Schistatin (1RMB)       | NSVHPCCDPVICEPREGEHCISGPCNCYFLNSGTICKRARGDGNQDYCTGITTPDCPRNRYNV                      |

**FIGURE 2** Amino acid sequences of disintegrins for which platelet aggregation information is not yet available. The one-letter code for amino acids is used. The stars indicate the motif commonly referred to as the “RGD loop,” and the RGD or RGD-like motif is underlined. Data bank accession numbers, given in parentheses after the name of the disintegrin, are given from [www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed). Note that the sequences of gabonin 1 and 2 shown are the last 73 amino acids of the actual 128 residue sequence provided.

brevicaudins 1a, 1b, 2b . (Terada, 2000), CTF-I and II (Yamakawa et al., 1991), gabonin 1 (Francischetti et al., 2004), schistatin (Bilgrami et al., 2004), and halystatin-2 (Fujisawa et al., 1994), will have significant inhibitory potencies, while gabonin-2 (Francischetti et al., 2004), containing MLD, will instead be selective for  $\alpha 4\beta 1$  and  $\alpha 9\beta 1$ , similar to EC3 (Coelho et al., 2004).

### Disintegrin Binding Sites on $\alpha \text{IIb}\beta 3$

When disintegrins were first characterized as being antiplatelet molecules, it became obvious that they were not specific for  $\alpha \text{IIb}\beta 3$ , and they were still antigenic when infused into animals. Significant effort in the past two decades has been spent toward the development of disintegrin analogs that possess both  $\alpha \text{IIb}\beta 3$  specificity and low antigenicity. A successful drug thus created is eptifibatide (Integrelin<sup>®</sup>) (Harrington, 1997), a cyclic heptapeptide based on the sequence of the KGD-containing disintegrin barbourin (Scarborough et al., 1991). Finding the molecular mechanism for this specificity, however, has awaited the crystallization of the ligand-bound integrin. Great strides toward this happened when Xiong et al. (2001, 2002) described the crystal structure of the extracellular segment of  $\alpha \text{v}\beta 3$  in complex with a pentapeptide RGD ligand, Arg-Gly-Asp-[D-Phe]-[N-methylVal-]. The authors stated that the main chain conformation of the RGD motif in the pentapeptide was almost identical to that of the RGD tripeptide in the natural ligand echistatin, suggesting that the crystal structure presented from their research could serve as a basis for understanding the interaction of integrins with other RGD-containing ligands. The ligand-binding area was proposed to consist of a seven-bladed  $\beta$ -propeller from  $\alpha \text{v}$  and a  $\beta \text{A}$  domain from  $\beta 3$ , forming a narrow groove (Xiong et al., 2001). The ligand Arg and Asp side chains contact the integrin propeller and  $\beta \text{A}$  domains, respectively. Integrin residues most involved in the ligand binding include Asp150 and Asp 218 (for Arg), Arg216 (for Gly), while the side chain of the ligand Asp interacts with a divalent cation in the MIDAS (metal ion-dependent adhesion site) in  $\beta \text{A}$  as well as with  $\beta 3$  residues Tyr122, Arg214, and Asn215.

Xiao et al. (2004) have taken integrin structural studies one step further in their description of crystals of  $\alpha \text{IIb}\beta 3$  and in a

proposed hypothesis for ligand selectivity for this integrin versus  $\alpha v\beta 3$ . Recall that Lys-Gly-Asp (KGD) disintegrins are highly selective for  $\alpha IIb\beta 3$ . The hydrophobic component of Lys is one methylene longer than that of Arg in RGD. A comparison of the  $\beta$ -propeller of  $\alpha IIb$  and  $\alpha v$  reveals Phe231 in place of Arg218. This change favors the hydrophobic contacts provided by Lys but not the shorter side chain in Arg. In addition, Asp224 (available for hydrogen bonding) in  $\alpha IIb$  is more deeply buried than Asp150 and Asp218 in  $\alpha v$ , requiring a longer side chain to reach it. These studies have tremendously helped in our understanding of why disintegrins containing KGD are selective for  $\alpha IIb\beta 3$ . Future studies need to address how the KGD motif functions when it is present in one or both chains of a dimeric disintegrin.

### Functional Information from Disintegrin Mutations

Mutations of disintegrins have been informative on ligand structural requirements for interaction with  $\alpha IIb\beta 3$  (reviewed in McLane et al., 1998, 2004). In general, these studies have suggested that (1) increased constraints within the disintegrin RGD loop enhance their inhibitory activity (Chang et al., 2001; Lee et al., 1993; Yamada and Kidera, 1996); (2) the arginine of the disintegrin RGD motif is a critical amino acid for disintegrin interaction with  $\alpha IIb\beta 3$  (Dennis et al., 1993; Garsky et al., 1989; Tselepis et al., 1997; Wierzbicka-Patynowski et al., 1999); (3) the C-terminus of disintegrins plays a crucial role in determining potency of inhibition (Marcinkiewicz et al., 1997; Wright et al., 1993; Xiao et al., 2004); (4) the binding for disintegrin RGD sequences to  $\alpha IIb\beta 3$  is influenced by the residues flanking the RGD motif (Rahman et al., 1995, 1998, 2000; Wierzbicka-Patynowski et al., 1999); (5) a disintegrin's C-terminus binds  $\alpha IIb\beta 3$  at a location distinct from where the RGD sequence binds (Marcinkiewicz et al., 1997). Eristostatin is a potent small monomeric disintegrin, with an  $IC_{50}$  of 79 nM in its inhibition of ADP-induced platelet aggregation. The author has performed alanine mutagenesis on residues within eristostatin in order to compare the molecular mechanism this disintegrin is using for this inhibition compared with its ability to inhibit cancer cell metastasis in a hematogenous mouse model. Functional characterization using the recombinant alanine mutants shows that residues Q1, P4, V25, and G49 are not critical for eristostatin's inhibition



of ADP-induced platelet aggregation, Because all possess similar  $IC_{50}$  of about 100 nM. In contrast, alanine mutation of residues R24, N31, and N48 resulted in significantly less potency, with  $IC_{50}$  of about 500 nM. The glycine within the RGD motif is even more critical, because G28A showed no activity.

Most recently, Hantgan et al. (2004) used two forms of recombinant echistatin to study the paradox that small RGD ligands can bind to resting  $\alpha IIb\beta 3$ , while larger ligands cannot. Full-length echistatin with a single mutation within its RGD loop [Ech(1-49)M28L], and a truncated form [Ech(1-40)M28L] were used in a series of experiments involving sedimentation velocity/equilibrium and dynamic light scattering to examine the disintegrin's effect on an  $\alpha IIb\beta 3$  structure examined by transmission electron microscopy. Both full-length and truncated echistatin perturbed  $\alpha IIb\beta 3$ 's solution conformation, but only Ech(1-49)M28L inhibited  $\alpha IIb\beta 3$  function. This suggested that the C-terminus of echistatin is needed for binding to the receptor, but it is not involved in stabilizing the receptor conformation in solution.

One unique type of disintegrin mutation created and characterized has been functional hybrids, where the disintegrin is fused to the C-terminus of another protein. Zhou et al. expressed conortrostatin with an IgG heavy chain, and the resulting fused recombinant molecule inhibited ADP-induced platelet aggregation with an  $IC_{50}$  of 250 nM, which was four-fold less potent than the native molecule (Zhou et al., 2000). Butera et al. (2003) created an alkaline phosphatase (ALP)-fused eristostatin that showed selectivity for the  $\alpha IIb\beta 3$  integrin that matched the native disintegrin, while still possessing ALP enzymatic function. These same investigators have developed a fusion between eristostatin and enhanced green fluorescent protein (Butera et al., 2005) that shows promise for maintaining inhibitory potential with  $\alpha IIb\beta 3$  and ease of visualizing the disintegrin's binding on platelets. Yang et al. (2001) fused the 13-residue RGD loop of eristostatin to an inactive proinsulin moiety, producing a chimera that inhibited ADP-induced platelet aggregation ( $IC_{50} = 200$  nM), which is less potent than the native molecule. Chang et al. (1999) expressed rhodostomin (kistrin) as a fusion with glutathione-S-transferase (GST) and used this to investigate platelet intracellular signaling accompanying shape change. Not only did platelet shape change result in pp125FAK phosphorylation, but platelets spread on immobilized

GST-kistrin at a rate double that observed when using immobilized fibrinogen.

### The Challenge of Nomenclature

Viper venoms have been shown to contain a pharmacopeia of biologically active compounds, with at least 25 separate classes described in Markland (1998). Efforts to standardize the naming of these molecules by structure and function have been reported for prothrombin activators (Kini et al., 2000; Pirkle and Stocker, 1991). For these, the recommended naming criteria is to attach “-arin” or “-activase” to prefixes derived from the Latin names of species, plus a designation of A, B, C, or D based on cofactor requirements, followed by a number for any isoforms. No such agreement has been made for the nomenclature and classification of disintegrins. Of the 73 disintegrins named since 1987 (Table 5), 80% have the suffix “-in”, 36% called “-tin” (and half of those being “-statin”), and the remaining 43% placing the “-in” after some combination of genus, species, or both. Twenty percent of all disintegrins do not have “-in” for their name, with 17% being named according to the eluted fraction number they had during high-performance liquid chromatography (HPLC) purification and 3% using an acronym based on genus and species, plus a number to indicate isomers of the same protein. Only 1% of all disintegrins use the suffix “-or” in the name. Some of the challenges faced when recommending a universally accepted nomenclature method for disintegrins include the following realities. “Statin” as a suffix is now commonly used for naming a class of HMG-CoA reductase inhibitors for controlling cholesterol blood levels (Ovbiagele et al., 2005). Discovery of subspecies has caused taxonomic renaming of some snakes, while disintegrins already isolated from the snake were named based on the previous genus and species designation. Probably the best example of this is the disintegrin echistatin, originally described as being isolated from *Echis carinatus* (Gan et al., 1988). In subsequent years, however, multiple isoforms of this disintegrin have been discovered in this species, and the species expressing the classical echistatin is now called *Echis carinatus sochurecki* (Okuda et al., 2001). The naming systems for these isoforms over the years have employed Greek letters (for example, echistatin  $\gamma$ ) or variations based on the genus and species

**TABLE 5** Disintegrins Discovered 1988–2004, Arranged Alphabetically By Species name

| Species                                     | Disintegrin           | Ref.                       |
|---------------------------------------------|-----------------------|----------------------------|
| <i>Agkistrodon (Gloydius) halys</i>         | Halystatin            | Fujisawa et al., 1994      |
| <i>Agkistrodon acutus</i>                   | Accutin               | Yeh et al., 1998           |
| <i>Agkistrodon contortrix</i>               | Contortrostatin       | Trikha et al., 1994b       |
| <i>Agkistrodon contortrix</i>               | Acostatin             | Okuda et al., 2002         |
| <i>Agkistrodon halys</i>                    | Halysin               | Huang et al., 1991a        |
| <i>Agkistrodon halys</i>                    | Halysetin             | Liu et al., 2000           |
| <i>Agkistrodon halys brevicaudus</i>        | Salmosin              | Kang et al., 1998          |
| <i>Agkistrodon piscivorus</i>               | Applaggin             | Chao et al., 1989          |
| <i>Agkistrodon piscivorus</i>               | Piscivostatin         | Okuda and Morita 2001      |
| <i>Agkistrodon rhodostoma</i>               | Kistrin               | Dennis et al., 1990        |
| <i>Agkistrodon ussuriensis</i>              | Ussuristatin 1        | Oshikawa and Terada, 1999  |
| <i>Agkistrodon ussuriensis</i>              | Ussuristatin 2        | Oshikawa and Terada, 1999  |
| <i>Bitis arietans</i>                       | Bitistatin            | Shebuski et al., 1989      |
| <i>Bitis arietans</i>                       | Arietin               | Huang et al., 1991c        |
| <i>Bitis gabonica</i>                       | Gabonin               | Huang et al., 1992         |
| <i>Bitis gabonica</i>                       | Gabonin 1             | Francischetti et al., 2004 |
| <i>Bitis gabonica</i>                       | gabonin 2             | Francischetti et al., 2004 |
| <i>Bothrops asper</i>                       | Bothrasperin          | Pinto et al., 2003         |
| <i>Bothrops atrox</i>                       | Batroxostatin         | Rucinski et al., 1990      |
| <i>Bothrops cotiara</i>                     | Cotiarin              | Scarborough et al., 1993   |
| <i>Bothrops jararaca</i>                    | Jararacin             | Scarborough et al., 1993   |
| <i>Bothrops jararaca</i>                    | Jarastatin            | Coelho et al., 1999        |
| <i>Calloselasma rhodostoma</i>              | Rhodostomin           | Huang et al., 1987         |
| <i>Cerastes cerastes</i>                    | Cerastatin            | Marrakchi et al., 1997     |
| <i>Cerastes cerastes</i>                    | CC5                   | Calvete et al., 2002       |
| <i>Cerastes cerastes</i>                    | CC8                   | Calvete et al., 2002       |
| <i>Cerastes cerastes cerastes</i>           | Cerastin              | Scarborough et al., 1993   |
| <i>Crotalus atrox</i>                       | Crotatroxin           | Scarborough et al., 1993   |
| <i>Crotalus basilicus</i>                   | Basilicin             | Scarborough et al., 1993   |
| <i>Crotalus durissus durissus</i>           | Durissin              | Scarborough et al., 1993   |
| <i>Crotalus molossus molossus</i>           | Molossin              | Scarborough et al., 1993   |
| <i>Crotalus viridis</i>                     | Crotavirin            | Liu et al., 1995           |
| <i>Crotalus viridis cereberus</i>           | Cereberin             | Scarborough et al., 1993   |
| <i>Crotalus viridis lutosus</i>             | Lutosin               | Scarborough et al., 1993   |
| <i>Crotalus viridis viridis</i>             | Viridin               | Scarborough et al., 1993   |
| <i>Echis carinatus</i>                      | Schistatin            | Tomar et al., 2001         |
| <i>Echis carinatus</i> (unknown subspecies) | Echistatin $\alpha$ 2 | Dennis et al., 1990        |
| <i>Echis carinatus leakyi</i>               | Echistatin $\beta$    | Chen et al., 1995          |

(Continued on next page)

**TABLE 5** Disintegrins Discovered 1988–2004, Arranged Alphabetically By Species Name (*Continued*)

| Species                               | Disintegrin            | Ref.                         |
|---------------------------------------|------------------------|------------------------------|
| <i>Echis carinatus leakeyi</i>        | Echistatin $\gamma$    | Chen et al., 1995            |
| <i>Echis carinatus leukogaster</i>    | Leukogastin A          | Okuda et al., 2001           |
| <i>Echis carinatus leukogaster</i>    | Leukogastin B          | Okuda et al., 2001           |
| <i>Echis carinatus multisquamatus</i> | Multisquamatin         | Trikha et al., 1994b         |
| <i>Echis carinatus multisquamatus</i> | EMS 11                 | Calvete et al., 2003         |
| <i>Echis carinatus sochurecki</i>     | Echistatin             | Gan et al., 1988             |
| <i>Echis carinatus sochurecki</i>     | EC3                    | Marcinkiewicz et al., 1999a  |
| <i>Echis carinatus sochurecki</i>     | EC6                    | Marcinkiewicz et al., 2000   |
| <i>Echis ocellatus</i>                | Ocellatin              | Okuda et al., 2001           |
| <i>Echis ocellatus</i>                | Ocellatusin            | Smith et al., 2002           |
| <i>Echis ocellatus</i>                | EO4                    | Calvete et al., 2003         |
| <i>Echis ocellatus</i>                | EO5                    | Calvete et al., 2003         |
| <i>Echis pyramidum</i>                | Pyramidin              | Okuda et al., 2001           |
| <i>Eristicophis macmahoni</i>         | Eristostatin           | Gould et al., 1990           |
| <i>Eristicophis macmahoni</i>         | Eristocophin           | Scarborough et al., 1991     |
| <i>Eristicophis macmahoni</i>         | EMF10                  | Marcinkiewicz et al., 1999b  |
| <i>Gloydus blomhoffi brevicaudus</i>  | Adinbitor*             | Wang et al., 2004            |
| <i>Gloydus halys brevicaudus</i>      | Brevicaudin 1a, 1b, 2b | Terada, 2000                 |
| <i>Gloydus saxatilis</i>              | Saxatilin              | Hong et al., 2002            |
| <i>Lachesis mutus</i>                 | Lachesin               | Scarborough et al., 1993     |
| <i>Sistrurus catenatus tergeminus</i> | Tergeminin             | Scarborough et al., 1991     |
| <i>Sistrurus miliarius barbouri</i>   | Barbourin              | Scarborough et al., 1991     |
| <i>Trimeresurus albolabris</i>        | Albolabrin             | Williams et al., 1990        |
| <i>Trimeresurus elegans</i>           | Elegantin              | Williams et al., 1990        |
| <i>Trimeresurus flavoridis</i>        | Flavoridin             | Musial et al., 1990          |
| <i>Trimeresurus flavoridis</i>        | Triflavin              | Huang et al., 1991b          |
| <i>Trimeresurus flavoridis</i>        | CTF-I,II               | Yamakawa et al., 1991        |
| <i>Trimeresurus flavoridis</i>        | Flavostatin            | Kawasaki et al., 1996        |
| <i>Trimeresurus flavoridis</i>        | Trimestatin            | Okuda and Morita, 2001       |
| <i>Trimeresurus gramineus</i>         | Trigramin              | Huang et al., 1987           |
| <i>Trimeresurus jerdonii</i>          | Jerdonatin             | Zhou et al., 2004            |
| <i>Vipera ammodytes</i>               | VA6                    | Calvete et al., 2003         |
| <i>Vipera berus</i>                   | VB7                    | Calvete et al., 2003         |
| <i>Vipera lebetina obtusa</i>         | VLO4                   | Calvete et al., 2003         |
| <i>Vipera lebetina obtusa</i>         | VLO5                   | Calvete et al., 2003         |
| <i>Vipera lebetina obtusa</i>         | Obtustatin             | Moreno-Murciano et al., 2003 |
| <i>Vipera palestinae</i>              | Viperostatin           | Kisiel et al., 2004          |

\*Adinbitor is noted in PubMed Protein ([www.ncbi.nlm.nih.gov/entrez](http://www.ncbi.nlm.nih.gov/entrez)) as coming from *Gloydus blomhoffi brevicaudus* rather than *Agkistrodon halys brevicaudus stejneger* as described in Wang et al., (2004).

(schistatin, carinatin, EC3) with no hint of an ordered scheme for naming future discoveries from the same snakes.

Multiple disintegrins, both monomers and dimers, are being isolated each year from the snakes in the family Viperidae, and we can anticipate this to continue as the venoms of heretofore untested species are being characterized. Use of a standard nomenclature system will lessen confusion as well as assist in valid comparisons of purified disintegrin activities.

### Conclusions

For two decades, naturally occurring proteins from vipers and pit vipers have provided structural and functional information about the platelet fibrinogen integrin,  $\alpha\text{IIb}\beta 3$ . With fewer than 40% of all Viperidae subfamily members having been characterized for the presence of disintegrins, much work remains to be done. Because disintegrins have the proven ability to be selective for this integrin, and long-term success with antiplatelet agents such as eptifibatide has been disappointing (Bennett, 2001; Collier, 2001), it remains possible that a disintegrin may yet be found that can be a molecular model for an effective drug with  $\alpha\text{IIb}\beta 3$ .

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### References

- Andrews, R. K., Berndt, M. C. (2004). Platelet physiology and thrombosis. *Thromb. Res.* 114(5-6):447-453.
- Bennett, J. S. (2001). Novel platelet inhibitors. *Annu. Rev. Med.* 52(1):161-184.
- Benveniste, J., Chignard, M. (1985). A role for PAF-acether (platelet-activating factor) in platelet-dependent vascular diseases? *Circulation* 72(4):713-717.
- Beviglia, L., Stewart, G. J., Niewiarowski, S. (1995). Effect of four disintegrins on the adhesive and metastatic properties of B16F10 melanoma cells in a murine model. *Oncol. Res.* 7(1):7-20.

- Bilgrami, S., Tomar, S., Yadav, S., Kaur, P., Kumar, J., Jabeen, T., Sharma, S., Singh, T. P. (2004). Crystal structure of schistatin, a disintegrin homodimer from saw-scaled viper (*Echis carinatus*) at Å resolution. *J. Mol. Biol.* 341 (3):829–837.
- Butera, D., Fontes Piazza, R. M., McLane, M. A., Chammass, R., Moura da Silva, A. M. (2005). Molecular Engineering of an EGFP/disintegrin-based integrin marker. *Toxicon* 46:178–184.
- Butera, D., Skielka, K., McLane, M., Paquette-Straub, C., Ducancel, F., Moura da Silva, A. (2003). Cloning, expression, and characterization of a bi-functional disintegrin/alkaline phosphatase hybrid protein. *Prot. Expr. Purif.* 31 (2):286–291.
- Calvete, J. J. (2004). Structures of integrin domains and concerted conformational changes in the bidirectional signaling mechanism of  $\alpha$ II(b) $\beta$ 3. *Exp. Biol. Med.* 229 (8):732–744.
- Calvete, J. J., Fox, J. W., Agelan, A., Niewiarowski, S., Marcinkiewicz, C. (2002). The presence of the WGD motif in CC8 heterodimeric disintegrin increases its inhibitory effect on  $\alpha$ II(b) $\beta$ 3,  $\alpha$ (v) $\beta$ 3, and  $\alpha$ 5 $\beta$ 1 integrins. *Biochemistry* 41 (6):2014–2021.
- Calvete, J. J., Moreno, M., Theakston, R. D., Kisiel, D. G., Marcinkiewicz, C. (2003). Snake venom disintegrins: novel dimeric disintegrins and structural diversification by disulphide bond engineering. *Biochem. J.* 372 (Pt 3):725–734.
- Chang, C. P., Chang, J. C., Chang, H. H., Tsai, W. J., Lo, S. J. (2001). Positional importance of Pro53 adjacent to the Arg49-Gly50-Asp51 sequence of rhodostomin in binding to integrin  $\alpha$ II(b) $\beta$ 3. *Biochem. J.* 357 (Pt1):57–64.
- Chang, H. H., Lin, C. H., Lo, S. J. (1999). Recombinant rhodostomin substrates induce transformation and active calcium oscillation in human platelets. *Exp. Cell Res.* 250 (2):387–400.
- Chang, M. C., Wang, B. R., Huang, T. F. (1995). Characterization of endothelial cell differential attachment to fibrin and fibrinogen and its inhibition by Arg-Gly-Asp- containing peptides. *Thromb. Haemost.* 74 (2):764–769.
- Chao, B. H., Jakubowski, J. A., Savage, B., Chow, E. P., Marzec, U. M., Harker, L. A., Maraganore, J. M. (1989). *Agkistrodon piscivorus piscivorus* platelet aggregation inhibitor: a potent inhibitor of platelet activation. *Proc. Natl. Acad. Sci. USA* 86:8050–8054.
- Chen, Y. L., Huang, T. F., Chen, S. W., Tsai, I. H. (1995). Determination of the structure of two novel echistatin variants and comparison of the ability of echistatin variants to inhibit aggregation of platelets from different species. *Biochem. J.* 305 (Pt 2):513–520.
- Chiang, H. S., Swaim, M. W., Huang, T. F. (1995a). Characterization of platelet aggregation induced by human breast carcinoma and its inhibition by snake venom peptides, trigramin and rhodostomin. *Breast Canc. Res. Treat.* 33 (3):225–235.
- Chiang, H. S., Yang, R. S., Huang, T. F. (1995b). The Arg-Gly-Asp-containing peptide, rhodostomin, inhibits *in vitro* cell adhesion to extracellular matrices and platelet aggregation caused by saos-2 human osteosarcoma cells. *Br. J. Canc.* 71 (2):265–270.

- Clark, E. A., Trikha, M., Markland, F. S., Brugge, J. S. (1994). Structurally distinct disintegrins contortrostatin and multisquamatin differentially regulate platelet tyrosine phosphorylation. *J. Biol. Chem.* 269(35):21940–21943.
- Coelho, A. L., de-Freitas, M. S., Oliveira-Carvalho, A., Moura-Neto, V., Zingali, R. B., Barja-Fidalgo, C. (1999). Effects of jarastatin, a novel snake venom disintegrin, on neutrophil migration and actin cytoskeleton dynamics. *Exp. Cell Res.* 251(2):379–387.
- Coelho, A. L., De Freitas, M. S., Mariano-Oliveira, A., Rapozo, D. C., Pinto, L. F., Niewiarowski, S., Zingali, R. B., Marcinkiewicz, C., Barja-Fidalgo, C. (2004). RGD- and MLD-disintegrins, jarastatin and EC3, activate integrin-mediated signaling modulating the human neutrophils chemotaxis, apoptosis and IL-8 gene expression. *Exp. Cell Res.* 292(2):371–384.
- Coller, B. S. (2001). Anti-GPIIb/IIIa drugs: current strategies and future directions. *Thromb. Haemost.* 86(1):427–443.
- Dennis, M. S., Carter, P., Lazarus, R. A. (1993). Binding interactions of kistrin with platelet glycoprotein IIb-IIIa: analysis by site-directed mutagenesis. *Proteins* 15(3):312–321.
- Dennis, M. S., Henzel, W. J., Pitti, R. M., Lipari, M. T., Napier, M. A., Deisher, T. A., Bunting, S., 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(7):2471–2475.
- Farndale, R. W., Sixma, J. J., Barnes, M. J., De Groot, P. G. (2004). The role of collagen in thrombosis and hemostasis. *J. Thromb. Haemost.* 2(4):561–573.
- FitzGerald, G. A. (1991). Mechanisms of platelet activation: thromboxane A2 as an amplifying signal for other agonists. *Am. J. Cardiol.* 68(7):11B–15B.
- Franchichetti, I. M. B., My-Pham, V., Harrison, J., Garfield, M. K., Ribeiro, J. M. C. (2004). *Bitis gabonica* (Gaboon viper) snake venom gland: toward a catalog for the full-length transcripts (cDNA) and proteins. *Gene* 337:55–69.
- Fujii, Y., Okuda, D., Fujimoto, Z., Horii, K., Morita, T., Mizuno, H. (2003). Crystal structure of trimestatin, a disintegrin containing a cell adhesion recognition motif RGD. *J. Mol. Biol.* 332(5):1115–1122.
- Fujisawa, Y., Kuroda, S., Notoya, K., Konishi, H., 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.
- Gan, Z. R., Gould, R. J., Jacobs, J. W., Friedman, P. A., Polokoff, M. A. (1988). Echistatin. A potent platelet aggregation inhibitor from the venom of the viper, *Echis carinatus*. *J. Biol. Chem.* 263(36):19827–19832.
- Garsky, V. M., Lumma, P. K., Freidinger, R. M., Pitzenberger, S. M., Randall, W. C., Veber, D. F., Gould, R. J., Friedman, P. A. (1989). Chemical synthesis of echistatin, a potent inhibitor of platelet aggregation from *Echis carinatus*: synthesis and biological activity of selected analogs. *Proc. Natl. Acad. Sci. U.S.A.* 86(11):4022–4026.
- Gasmi, A., Srairi, N., Guermazi, S., Dkhil, H., Karoui, H., El-Ayeb, M. (2001). Amino acid structure and characterization of a heterodimeric disintegrin from *Vipera lebetina* venom. *Biochim. Biophys. Acta* 1547(1):51–56.

- Gould, R. J., Polokoff, M. A., Friedman, P. A., Huang, T. F., Holt, J. C., Cook, J. J., Niewiarowski, S. (1990). Disintegrins: a family of integrin inhibitory proteins from viper venoms. *Proc. Soc. Exp. Biol. Med.* 195(2):168–171.
- Hantgan, R. R., Stahle, M. C., Connor, J. H., Lyles, D. S., Horita, D. A., Rocco, M., Nagaswami, C., Weisel, J. W., McLane, M. A. (2004). The disintegrin echistatin stabilizes integrin  $\alpha$ II(b) $\beta$ 3's open conformation and promotes its oligomerization. *J. Mol. Biol.* 342(5):1625–1636.
- Harrington, R. A. (1997). Design and methodology of the PURSUIT trial: evaluating eptifibatide for acute ischemic coronary syndromes. Platelet Glycoprotein IIb-IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy. *Am. J. Cardiol.* 80(4A):34B–38B.
- Hirsh, J., Weitz, J. I. (1999). New antithrombotic agents. *Lancet* 353(9162):1431–1436.
- Hong, S. Y., Koh, Y. S., Chung, K. H., Kim, D. S. (2002). Snake venom disintegrin, saxatilin, inhibits platelet aggregation, human umbilical vein endothelial cell proliferation, and smooth muscle cell migration. *Thromb. Res.* 105(1):79–86.
- Huang, F., Hong, E. (2004). Platelet glycoprotein IIb/IIIa inhibition and its clinical use. *Curr. Med. Chem. Cardiovasc. Hematol. Agents* 2(3):187–196.
- Huang, T. F., Holt, J. C., Lukasiewicz, H., 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(33):16157–16163.
- Huang, T. F., Liu, C. Z., Ouyang, C. H., Teng, C. M. (1991a). Halysin, an antiplatelet Arg-Gly-Asp-containing snake venom peptide, as fibrinogen receptor antagonist. *Biochem. Pharmacol.* 42(6):1209–1219.
- Huang, T. F., Sheu, J. R., Teng, C. M. (1991b). A potent antiplatelet peptide, triflavin, from *Trimeresurus flavoviridis* snake venom. *Biochem. J.* 277(Pt 2):351–357.
- Huang, T. F., Wang, W. J., Teng, C. M., Liu, C. S., Ouyang, C. (1991c). Purification and characterization of an antiplatelet peptide, arietin, from *Bitis arietans* venom. *Biochim. Biophys. Acta* 1074(1):136–143.
- Huang, T. F., Wang, W. J., Teng, C. M., Ouyang, C. (1991d). Mechanism of action of the antiplatelet peptide, arietin, from *Bitis arietans* venom. *Biochim. Biophys. Acta* 1074(1):144–150.
- Huang, T. F., Peng, H. C., Teng, C. M., Ouyang, C. (1992). An antiplatelet peptide, gabonin, from *Bitis gabonica* snake venom. *Arch. Biochem. Biophys.* 298(1):13–20.
- Hynes, R. O. (2002). Integrins: Bidirectional, Allosteric Signaling Machines. *Cell* 110(6):673–687.
- Kamiguti, A. S., Markland, F. S., Zhou, Q., Laing, G. D., Theakston, R. D., Zuzel, M. (1997). Proteolytic cleavage of the  $\beta$ 1 subunit of platelet  $\alpha$ 2 $\beta$ 1 integrin by the metalloproteinase jararhagin compromises collagen-stimulated phosphorylation of pp72. *J. Biol. Chem.* 272(51):32599–32605.
- Kang, I. C., Chung, K. H., Lee, S. J., Yun, Y., Moon, H. M., Kim, D. S. (1998). Purification and molecular cloning of a platelet aggregation inhibitor from the snake (*Agkistrodon halys brevicaudus*) venom. *Thromb. Res.* 91(2):65–73.



- Kang, I. C., Lee, Y. D., Kim, D. S. (1999). A novel disintegrin salmosin inhibits tumor angiogenesis. *Cancer Res.* 59(15):3754–3760.
- Kawasaki, T., Sakai, Y., Taniuchi, Y., Sato, K., Maruyama, K., Shimizu, M., Kaku, S., Yano, S., Inagaki, O., Tomioka, K., Yanagisawa, I., Takenaka, T. (1996). Biochemical characterization of a new disintegrin, flavostatin, isolated from *Trimeresurus flavoviridis* venom. *Biochimie* 78(4):245–252.
- Kini, R. M., Morita, T., Rosing, J. (2000). Classification and nomenclature of prothrombin activators isolated from snake venoms. Registry of Exogenous Hemostatic Factors of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis 2000, www.med.unc.edu/isth/SSC/communications/exogenous/kinisnake.pdf
- Kisiel, D. G., Calvete, J. J., Katzhendler, J., Fertala, A., Lazarovici, P., Marcinkiewicz, C. (2004). Structural determinants of the selectivity of KTS-disintegrins for the  $\alpha 1\beta 1$  integrin. *FEBS Lett.* 577(3):478–482.
- Knight, L. C., Maurer, A. H., Romano, J. E. (1996). Comparison of iodine-123-disintegrins for imaging thrombi and emboli in a canine model. *J. Nucl. Med.* 37(3):476–482.
- Larson, L. (2004). Primary Hemostasis, in: McKenzie, S. (ed.), *Clinical Laboratory Hematology*, Upper Saddle River, NJ: Pearson Education.
- Lee, G., Chan, W., Hurle, M. R., Desjarlais, R. L., Watson, F., Sathe, G. M., Wetzel, R. (1993). Strong inhibition of fibrinogen binding to platelet receptor  $\alpha$  IIb  $\beta$  3 by RGD sequences installed into a presentation scaffold. *Protein Eng.* 6(7):745–754.
- Lee, L. W., Peng, H. C., Ko, W. C., Hung, W. C., Su, C. H., Lin, C. H., Huang, T. F., Yen, M. H., Sheu, J. R. (1999). Triflavin potentiates the antiplatelet activity of platelet activating factor receptor antagonist on activated neutrophil-induced platelet aggregation. *Eur. J. Pharmacol.* 364(2–3):239–246.
- Liu, C. Z., Peng, H. C., Huang, T. F. (1995). Crotavirin, a potent platelet aggregation inhibitor purified from the venom of the snake *Crotalus viridis*. *Toxicol.* 33(10):1289–1298.
- Liu, J. W., Du, X. Y., Liu, P., Chen, X., Xu, J. M., Wu, X. F., Zhou, Y. C. (2000). Purification, characterization, and cDNA sequence of halysetin, a disintegrin-like/cysteine-rich protein from the venom of *Agkistrodon halys* Pallas. *Biochem. Biophys. Res. Comm.* 278(1):112–118.
- Lombardi, P., Pelagalli, A., Avallone, L., Angelo, D., Belisario, M. A., Angelo, A., Staiano, N. (1999). Species-dependent specificity of platelet aggregation inhibitors from snake venom. *J. Comp. Pathol.* 121(2):185–190.
- Marcinkiewicz, C. (2005). Functional characteristics of snake venom disintegrins: potential therapeutic implication. *Curr. Pharm. Design* 11(7):815–827.
- Marcinkiewicz, C., Vijay Kumar, S., McLane, M. A., Niewiarowski, S. (1997). Significance of RGD loop and C-terminal domain of echistatin for recognition of  $\alpha$  IIb  $\beta$  3 and  $\alpha$  (v)  $\beta$  3 integrins and expression of ligand-induced binding site. *Blood* 90(4):1565–1575.
- Marcinkiewicz, C., Calvete, J. J., Marcinkiewicz, M. M., Raida, M., Vijay, K. S., Huang, Z., Lobb, R. R., Niewiarowski, S. (1999a). EC3, a novel heterodimeric disintegrin from *Echis carinatus* venom, inhibits  $\alpha 4$  and  $\alpha 5$

- integrins in an RGD-independent manner. *J. Biol. Chem.* 274(18):12468–12473.
- Marcinkiewicz, C., Calvete, J. J., Vijay-Kumar, S., Marcinkiewicz, M. M., Raida, M., Schick, P., Lobb, R. R., Niewiarowski, S. (1999b). Structural and functional characterization of EMF10, a heterodimeric disintegrin from *Eristocophis macmahoni* venom that selectively inhibits  $\alpha 5\beta 1$  integrin. *Biochemistry* 38(40):13302–13309.
- Marcinkiewicz, C., Taooka, Y., Yokosaki, Y., Calvete, J. J., Marcinkiewicz, M. M., Lobb, R. R., Niewiarowski, S., Sheppard, D. (2000). Inhibitory effects of MLDG-containing heterodimeric disintegrins reveal distinct structural requirements for interaction of the integrin  $\alpha 9\beta 1$  with VCAM-1, tenascin-C, and osteopontin. *J. Biol. Chem.* 275(41):31930–31937.
- Markland, F. S. (1998). Snake venoms and the hemostatic system. *Toxicon* 36(12):1749–1800.
- Marrakchi, N., Barbouche, R., Bon, C., Ayeb, M. E. (1997). Cerastatin, a new potent inhibitor of platelet aggregation from the venom of the Tunisian viper, *Cerastes cerastes*. *Toxicon* 35(1):125–135.
- Maruyama, K., Kawasaki, T., Sakai, Y., Taniuchi, Y., Shimizu, M., Kawashima, H., Takenaka, T. (1997). Isolation and amino acid sequence of flavostatin, a novel disintegrin from the venom of *Trimeresurus flavoviridis*. *Peptides* 18(1):73–78.
- Mazur, P., Henzel, W. J., Seymour, J. L., Lazarus, R. A. (1991). Ornatin: potent glycoprotein IIb-IIIa antagonists and platelet aggregation inhibitors from the leech *Placobdella ornata*. *Eur. J. Biochem.* 202(3):1073–1082.
- Mazurov, A. V., Leytin, V. L., Repin, V. S., Forster, W. (1984). Platelet prostanoids in interaction of platelets with collagen substrates. II. Effects of exogenous arachidonic acid, stable PG endoperoxides analogue, and aspirin. *Biomed. Biochim. Acta* 43: S373–376.
- McLane, M. A., Kowalska, M. A., Silver, L., Shattil, S. J., Niewiarowski, S. (1994). Interaction of disintegrins with the  $\alpha$  IIb  $\beta 3$  receptor on resting and activated human platelets. *Biochem. J.* 301(Pt 2):429–436.
- McLane, M. A., Marcinkiewicz, C., Vijay-Kumar, S., Wierzbicka-Patynowski, I., Niewiarowski, S. (1998). Viper venom disintegrins and related molecules. *Proc. Soc. Exp. Biol. Med.* 219(2):109–119.
- McLane, M. A., Sanchez, E. E., Wong, A., Paquette-Straub, C., Perez, J. C. (2004). Disintegrins. *Curr. Drug Targets Cardiovasc. Haematol. Disord* 4(4):327–355.
- McQuade, P., Knight, L. C., Welch, M. J. (2004). Evaluation of  $^{64}\text{Cu}$ - and  $^{125}\text{I}$ -radiolabeled bitistatin as potential agents for targeting  $\alpha \nu \beta 3$  integrins in tumor angiogenesis. *Bioconjug. Chem.* 15(5):988–996.
- Moreno-Murciano, M., Paz, Monleon, D., Calvete, J. J., Celda, B., Marcinkiewicz, C. (2003). Amino acid sequence and homology modeling of obtustatin, a novel non-RGD-containing short disintegrin isolated from the venom of *Vipera lebetina obtusa*. *Prot. Science* 12, 366–371.
- Murugappan, S., Shankar, G., Kunapuli, S. P. (2004). Platelet receptors for adenosine nucleotides and thromboxane A<sub>2</sub>. *Semin. Thromb. Hemost.* 30:411–418.
- Musial, J., Niewiarowski, S., Rucinski, B., Stewart, G. J., Cook, J. J., Williams, J. A., Edmunds, L. H., Jr. (1990). Inhibition of platelet adhesion to surfaces of

- extracorporeal circuits by disintegrins. RGD-containing peptides from viper venoms. *Circulation* 82(1):261–273.
- O'Toole, T. E., Loftus, J. C., Du, X. P., Glass, A. A., Ruggeri, Z. M., Shattil, S. J., Plow, E. F., Ginsberg, M. H. (1990). Affinity modulation of the  $\alpha$ IIb  $\beta$ 3 integrin (platelet GPIIb-IIIa) is an intrinsic property of the receptor. *Cell Regul.* 1(12):883–893.
- Okuda, D., Morita, T. (2001). Purification and characterization of a new RGD/KGD-containing dimeric disintegrin, piscivostatin, from the venom of *Agkistrodon piscivorus piscivorus*: the unique effect of piscivostatin on platelet aggregation. *J. Biochem.* 130(3):407–415.
- Okuda, D., Nozaki, C., Sekiya, F., Morita, T. (2001). Comparative biochemistry of disintegrins isolated from snake venom: consideration of the taxonomy and geographical distribution of snakes in the genus *Echis*. *J. Biochem.* 129(4):615–620.
- Okuda, D., Koike, H., Morita, T. (2002). A new gene structure of the disintegrin family: a subunit of dimeric disintegrin has a short coding region. *Biochemistry* 41(48):14248–14254.
- Oshikawa, K., Terada, S. (1999). Ussuristatin 2, a novel KGD-bearing disintegrin from *Agkistrodon ussuriensis* venom. *J. Biochem.* 125(1):31–35.
- Ovbiagele, B., Kidwell, C. S., Saver, J. L. (2005). Expanding indications for statins in cerebral ischemia: a quantitative study. *Arch. Neurol.* 62(1):67–72.
- Pfaff, M., McLane, M. A., Beviglia, L., Niewiarowski, S., Timpl, R. (1994). Comparison of disintegrins with limited variation in the RGD loop in their binding to purified integrins  $\alpha$ IIb  $\beta$ 3,  $\alpha$ V  $\beta$ 3 and  $\alpha$ 5  $\beta$ 1 and in cell adhesion inhibition. *Cell Adhes. Commun.* 2(6):491–501.
- Pinto, A., Angulo, Y., Jiminez, R., Lomonte, B. (2003). Isolation of bothrasperin, a disintegrin with potent platelet aggregation inhibitory activity, from the venom of the snake *Bothrops asper*. *Rev. Biol. Trop.* 51(1):253–259.
- Pirkle, H., Stocker, K. (1991). Thrombin-like enzymes from snake venoms: an inventory. For the Subcommittee on Nomenclature of Exogenous Hemostatic Factors of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. *Thromb. Haemost.* 65(4):444–450.
- Rahman, S., Aitken, A., Flynn, G., Formstone, C., Savidge, G. F. (1998). Modulation of RGD sequence motifs regulates disintegrin recognition of  $\alpha$ IIb  $\beta$ 3 and  $\alpha$ 5  $\beta$ 1 integrin complexes. Replacement of elegantin alanine-50 with proline, N-terminal to the RGD sequence, diminishes recognition of the  $\alpha$ 5  $\beta$ 1 complex with restoration induced by  $Mn^{2+}$  cation. *Biochem. J.* 335( Pt 2):247–257.
- Rahman, S., Flynn, G., Aitken, A., Patel, Y., Hussain, F., Lu, X., Loftus, J. C., French, D., Wijelath, E., Strand, K., Savidge, G. F. (2000). Differential recognition of snake venom proteins expressing specific Arg-Gly-Asp (RGD) sequence motifs by wild-type and variant integrin  $\alpha$ IIb $\beta$ 3: further evidence for distinct sites of RGD ligand recognition exhibiting negative allostery. *Biochem. J.* 345Pt 3:701–709.
- Rahman, S., Lu, X., Kakkar, V. V., Authi, K. S. (1995). The integrin  $\alpha$ IIb  $\beta$ 3 contains distinct and interacting binding sites for snake-venom RGD

- (Arg-Gly-Asp) proteins. Evidence that the receptor-binding characteristics of snake-venom RGD proteins are related to the amino acid environment flanking the sequence RGD. *Biochem. J.* 312(Pt 1):223–232.
- Roth, G. J., Calverley, D. C. (1994). Aspirin, platelets, and thrombosis: theory and practice. *Blood* 83(4):885–898.
- Rucinski, B., Niewiarowski, S., Holt, J. C., Soszka, T., Knudsen, K. A. (1990). Batroxostatin, an Arg-Gly-Asp-containing peptide from *Bothrops atrox*, is a potent inhibitor of platelet aggregation and cell interaction with fibronectin. *Biochim. Biophys. Acta.* 1054(3):257–262.
- Savage, B., Marzec, U. M., Chao, B. H., Harker, L. A., Maraganore, J. M., 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 IIb/IIIa complex inhibits receptor function. *J. Biol. Chem.* 265(20):11766–11772.
- Scaloni, A., Di Martino, E., Miraglia, N., Pelagalli, A., Della Morte, R., Staiano, N., Pucci, P. (1996). Amino acid sequence and molecular modelling of glycoprotein IIb/IIIa and fibronectin receptor iso-antagonists from *Trimeresurus elegans* venom. *Biochem. J.* 319(Pt 3):775–782.
- Scarborough, R. M., Rose, J. W., Hsu, M. A., Phillips, D. R., Fried, V. A., Campbell, A. M., Nannizzi, L., Charo, I. F. (1991). Barbourin. A GPIIb-IIIa-specific integrin antagonist from the venom of *Sistrurus m. barbouri*. *J. Biol. Chem.* 266(15):9359–9362.
- Scarborough, R. M., Rose, J. W., Naughton, M. A., Phillips, D. R., Nannizzi, L., Arfsten, A., Campbell, A. M., Charo, I. F. (1993). Characterization of the integrin specificities of disintegrins isolated from American pit viper venoms. *J. Biol. Chem.* 268(2):1058–1065.
- Selnick, H. G., Barrow, J. C., Nantermet, M. G., Connolly, T. M. (2003). Non-peptidic small-molecule antagonists of the human platelet thrombin receptor PAR-1. *Curr. Med. Chem. Cardiovasc. Hematol. Agents* 1(1):47–59.
- Shattil, S. J., Newman, P. J. (2004). Integrins: dynamic scaffolds for adhesion and signaling in platelets. *Blood* 104(6):1606–1615.
- Shebuski, R. J., Ramjit, D. R., Bencen, G. H., Polokoff, M. A. (1989). Characterization and platelet inhibitory activity of bitistatin, a potent arginine-glycine-aspartic acid-containing peptide from the venom of the viper *Bitis arietans*. *J. Biol. Chem.* 264(36):21550–21556.
- Sheu, J. R., Lin, C. H., Huang, T. F. (1994). Triflavin, an antiplatelet peptide, inhibits tumor cell-extracellular matrix adhesion through an arginine-glycine-aspartic acid-dependent mechanism. *J. Lab. Clin. Med.* 123(2):256–263.
- Smith, J. B., Theakston, R. D., Coelho, A. L., Barja, F., Calvete, J. J., Marcinkiewicz, C. (2002). Characterization of a monomeric disintegrin, ocellatusin, present in the venom of the Nigerian carpet viper, *Echis ocellatus*. *FEBS Lett.* 512(1–3):111–115.
- Terada, S. (2000). Isolation and primary structures of platelet aggregation inhibitors from *Gloydius halys brevicaudus* venom. *Fukuoka Univ. Sci. Reports* 30:71–78.

- Tomar, S., Yadav, S., Chandra, V., Kumar, P., Singh, T. P. (2001). Purification, crystallization and preliminary X-ray diffraction studies of disintegrin (schistatin) from saw-scaled viper (*Echis carinatus*). *Acta Crystallogr. D. Biol. Crystallogr.* 57(Pt 11):1669–1670.
- Trikha, M., De Clerck, Y. A., Markland, F. S. (1994a). Contortrostatin, a snake venom disintegrin, inhibits  $\beta 1$  integrin-mediated human metastatic melanoma cell adhesion and blocks experimental metastasis. *Cancer Res.* 54(18):4993–4998.
- Trikha, M., Rote, W. E., Manley, P. J., Lucchesi, B. R., Markland, F. S. (1994b). Purification and characterization of platelet aggregation inhibitors from snake venoms. *Thromb. Res.* 73(1):39–52.
- Tselepis, V. H., Green, L. J., Humphries, M. J. (1997). An RGD to LDV motif conversion within the disintegrin kistrin generates an integrin antagonist that retains potency but exhibits altered receptor specificity. Evidence for a functional equivalence of acidic integrin-binding motifs. *J. Biol. Chem.* 272(34):21341–21348.
- Wang, J. H., Wu, Y., Ren, F., Lu, L., Zhao, B. C. (2004). Cloning and characterization of Adinbitor, a novel disintegrin from the snake venom of *Agkistrodon halys brevicaudus stejneger*. *Acta Biochim. Biophys. Sin.* 36(6):425–429.
- Wierzbicka-Patynowski, I., Niewiarowski, S., Marcinkiewicz, C., Calvete, J. J., Marcinkiewicz, M. M., McLane, M. A. (1999). Structural requirements of echistatin for the recognition of  $\alpha v \beta 3$  and  $\alpha 5 \beta 1$  integrins. *J. Biol. Chem.* 274(53):37809–37814.
- Williams, J., Rucinski, B., Holt, J., 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(1):81–89.
- Wright, P. S., Saudek, V., Owen, T. J., Harbeson, S. L., Bitonti, A. J. (1993). An echistatin C-terminal peptide activates GPIIb/IIIa binding to fibrinogen, fibronectin, vitronectin and collagen type I and type IV. *Biochem. J.* 293(Pt 1):263–267.
- Xiao, T., Takagi, J., Collier, B. S., Wang, J. H., Springer, T. A. (2004). Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. *Nature* 432(7013):59–67.
- Xiong, J. P., Stehle, T., Diefenbach, B., Zhang, R., Dunker, R., Scott, D. L., Joachimiak, A., Goodman, S. L., Arnaout, M. A. (2001). Crystal structure of the extracellular segment of integrin  $\alpha v \beta 3$ . *Science* 294(5541):339–345.
- Xiong, J. P., Stehle, T., Zhang, R., Joachimiak, A., Frech, M., Goodman, S. L., Arnaout, M. A. (2002). Crystal structure of the extracellular segment of integrin  $\alpha V \beta 3$  in complex with an Arg-Gly-Asp ligand. *Science* 296(5565):151–155.
- Yamada, T., Kidera, A. (1996). Tailoring echistatin to possess higher affinity for integrin  $\alpha (IIb) \beta 3$ . *FEBS Lett.* 387(1):11–15.
- Yamakawa, Y., Omori, S., Maeyama, J. (1991). Primary structures of cytotoxic factors isolated from habu (*Trimeresurus flavoviridis*) venom. *J. Biochem.* 109(5):667–669.

- Yang, Z. H., Jing, J., Tang, J. G. (2001). Inhibition of platelet aggregation of a mutant proinsulin molecule engineered by introduction of a native Arg-Gly-Asp sequence. *Appl. Biochem. Biotechnol.* 90(1):1–10.
- Yeh, C. H., Peng, H. C., Yih, J. B., Huang, T. F. (1998). A new short chain RGD-containing disintegrin, accutin, inhibits the common pathway of human platelet aggregation. *Biochim. Biophys. Acta* 1425(3):493–504.
- Zhou, Q., Hu, P., Ritter, M. R., Swenson, S. D., Argounova, S., Epstein, A. L., Markland, F. S. (2000). Molecular cloning and functional expression of con-tortrostatin, a homodimeric disintegrin from southern copperhead snake venom. *Arch. Biochem. Biophys.* 375(2):278–288.
- Zhou, X. D., Dinga, C. H., Taia, H., Jina, Y., Chena, R. Q., Lua, Q. M., Wanga, W. Y., Xiong, U. L. (2004). A novel disintegrin, jerdonatin, inhibits platelet aggregation and sperm-egg binding. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 139(1):117–122.