Harel, M., Su, C.-T., Frolow, F., Ashani, Y., Silman, I., & Sussman, J. L. (1990) Abstracts of the 40th Meeting, American Crystallography Association, New Orleans, April 1990, p 61.
Henderson, R. (1970) *J. Mol. Biol. 54*, 341–354.
Hendrickson, W. A. (1985) *Methods Enzymol. 115*, 252–270.
Hope, H. (1988) *Acta Crystallogr. B44*, 22–26.
Huber, R., & Bode, W. (1978) *Acc. Chem. Res. 11*, 114–121.
James, M. N. G., Sielecki, A. R., Brayer, G. D., & Delbaere, L. T. J. (1980) *J. Mol. Biol. 144*, 43–88.
Jones, T. A. (1978) *J. Appl. Crystallogr. 11*, 268–272.
Kunitz, M. (1938) *J. Gen. Physiol. 22*, 207–237.
Matthews, B. W., Sigler, P. B., Henderson, R., & Blow, D. M. (1967) *Nature 214*, 652–656.
Pflugrath, J. W., Saper, M. A., & Quiocho, F. A. (1984) in *Methods and Applications in Crystallographic Computing* (Hall, S., & Ashida, T., Eds.) pp 404–407, Clarendon Press, Oxford.
Ringe, D., Seaton, B. A., Gelb, M. H., & Abeles, R. H. (1985) *Biochemistry 24*, 64–68.
Ringe, D., Mottonen, J. M., Gelb, M. H., & Abeles, R. H. (1986) *Biochemistry 25*, 5633–5638.
Segal, D. M., Powers, J. C., Cohen, G. H., & Davies D. R. (1971) *Biochemistry 10*, 3728–3738.
Sheriff, S. (1987) *J. Appl. Crystallogr. 20*, 55–57.
Sielecki, A. R., Hendrickson, W. A., Broughton, C. G., Delbaere, L. T. J., Bayer, G. D., & James, M. N. G. (1979) *J. Mol. Biol. 134*, 781–804.
Tsukada, H., & Blow, D. M. (1985) *J. Mol. Biol. 184*, 703–711.
Yapel, A., Han, M., Lumry, R., Rosenberg, A., & Shiao, D. F. (1966) *J. Am. Chem. Soc. 88*, 2573–2584.

Identification of the Disulfide Bond Pattern in Albolabrin, an RGD-Containing Peptide from the Venom of *Trimeresurus albolabris*: Significance for the Expression of Platelet Aggregation Inhibitory Activity†

Juan J. Calvete,‡ Wolfram Schäfer,‡ Tomasz Soszka,§ Weiqi Lu,§ Jacquelynn J. Cook,§ Bradford A. Jameson,∥ and Stefan Niewiarowski*,§

Max Planck Institut für Biochemie, D-8033 Martinsried/München, Federal Republic of Germany, and Department of Physiology, Thrombosis Research Center, School of Medicine, and Fels Research Institute, Temple University, Philadelphia, Pennsylvania 19140

Received November 28, 1990; Revised Manuscript Received February 14, 1991

ABSTRACT: Albolabrin is a 73 amino acid peptide isolated from the venom of *Trimeresurus albolabris*. It contains an RGD sequence and 12 cysteines and is a potent inhibitor of both platelet aggregation and fibrinogen binding to the GPIIb/IIIa complex. This protein shows a high degree of analogy (primarily due to the alignment of all cysteines and the RGD) with a number of other viper venom proteins which inhibit cell adhesion and platelet aggregation and are referred to as disintegrins: rhodostomin, trigramin, flavoridin, applagin, elegantin, and batroxostatin. In this study, we found that the reduction and vinylpyridylethylation of albolabrin and flavoridin decreased their platelet aggregation inhibitory activity approximately 40–50 times. It can be postulated that the higher potency of native and reduced flavoridin as compared to albolabrin depends on the substitution of the Asp of albolabrin with a Phe at the C-terminal end of the RGD in flavoridin. The activity of a synthetic C-terminal peptide derived from flavoridin (residues 35–65) containing four cysteines was about 75 times lower than that of the original flavoridin. The substitution of a pair of cysteine residues with alanines in this peptide resulted in further loss of activity. In order to identify the disulfide bonds in albolabrin, the molecule was digested consecutively by trypsin and porcine pancreatic elastase. Peptides resulting from this digestion were isolated by reverse-phase HPLC and identified by amino acid composition and mass spectrometry. Direct evidence for the existence of a linkage between cysteine-10 and cysteine-12 and between cysteine-6 and cysteine-11 was obtained, and indirect evidence suggested links between cysteines-1–3, -2–4, -5–8, and -7–9. On the basis of this information, we propose a model for albolabrin which may be appropriate for the structure of the entire disintegrin family.

It is well established that short RGDX peptides, representing the cell recognition site of a number of adhesive proteins such as fibronectin, fibrinogen, vitronectin, von Willebrand factor, and thrombospondin, prevent cell adhesion and platelet aggregation (Ruoslahti & Pierschbacher, 1987). The concentrations of these peptides required to inhibit platelet aggregation range from 10 to 200 μM. Huang et al. (1987a, 1989) isolated a potent platelet aggregation inhibitor from the snake venom of the *Trimeresurus gramineus* snake. This peptide, referred to as trigramin, is composed of a single polypeptide chain of 72 amino acid residues; it contains the RGD sequence and 6 disulfide bridges. Trigramin blocks platelet aggregation and binding of ^{125}I-fibrinogen to ADP-stimulated platelets ($K_i = 2 \times 10^{-8}$ M). It binds to the glycoprotein IIb/IIIa complex on the platelet surface (Huang et al. 1987a), and it blocks the adhesion of cultured human melanoma cells and fibroblasts

† This work was supported by NIH Grants HL15226 and HL36579 (to S.N.) and BMFT (Bonn) Grant PTB 038824 (to W.S.). The provision of a Max-Planck-Gesellschaft fellowship to Juan J. Calvete is gratefully acknowledged. B.A.J. is a scholar of the American Foundation for Aids Research.

* Correspondence should be addressed to this author at the Department of Physiology, Temple University School of Medicine, Philadelphia, PA 19140.
‡ Max Planck Institut für Biochemie.
§ Department of Physiology, Temple University.
∥ Fels Research Institute, Temple University.

0006-2960/91/0430-5225$02.50/0

to fibronectin (Knudsen et al., 1988). A number of trigramin analogues have been isolated from the venoms of various vipers including echistatin (Gan et al., 1988), bitistatin or bitan (Shebuski et al., 1989; Dennis et al., 1990), applagin (Chao et al., 1989), albolabrin and elegantin (Williams et al., 1990), batroxostatin (Rucinski et al., 1990), rhodostomin or kistrin (Huang et al., 1987b; Gould et al., 1990; Dennis et al., 1990), and flavoridin, eristostatin, and agkistrostatin (Gould et al., 1990). These peptides represent a new class of proteins that we call disintegrins because they interfere with the interaction of adhesive ligands with their integrin receptors (Niewiarowski et al., 1989; Musial et al., 1990; Gould et al., 1990). All disintegrins were isolated from the Viperidae family of snakes, and the names of these inhibitor proteins were modified from the genera of the snakes from which the venoms were obtained. Disintegrins fall into 3 subfamilies: a short group containing 48–49 amino acids and 4 disulfide bridges (echistatin and eristostatin); a medium group which contains 70–73 amino acids and 6 disulfide bridges (trigramin, albolabrin, elegantin, agkistrostatin, applagin, batroxostatin, flavoridin, and rhodostomin); and a long group with 83–84 residues and 7 disulfide bridges (bitistatin and its variants) (Gould et al., 1990). On a molar basis, the concentration of disintegrins required to cause 50% inhibition of ADP-induced platelet aggregation in platelet-rich plasma was 1000–30 000 times lower than the required concentrations of RGDS (Musial et al., 1990; Dennis et al., 1990). Similarly, trigramin was several hundred times more potent as compared to RGDS in blocking the formation of in vivo hemostatic plugs in hamster mesenteric arteries (Cook et al., 1989). In addition, trigramin and batroxostatin were about 400–500 times more potent than GRGDS in inhibiting melanoma cell adhesion to fibronectin (Knudsen et al., 1988; Rucinski et al., 1990).

It has been proposed that the RGD sequence represents a cell recognition site of disintegrins (Gould et al., 1990; Dennis et al., 1990; Savage et al., 1990). RGD is conserved in all of these molecules, and the alteration of these amino acids changes the activity of the peptide. For instance, Garsky et al. (1989) demonstrated that the replacement of arginine with ornithine or alanine results in greatly diminished activity of echistatin. Ruoslahti and Pierschbacher (1987) and Reed et al. (1988) proposed that the appropriate conformation of the RGD sequence is essential for the attachment of adhesive proteins to integrins. It has been shown that the reduction of the disulfide bridges in trigramin and echistatin and subsequent alkylation of the sulfhydryl groups result in a significant decrease of their biological activity (Huang et al., 1987a, 1989; Gan et al. 1988; Knudsen et al., 1988). Therefore, it appears that the RGD conformation in disintegrins may be determined by the appropriate pairing of cysteine residues.

The purpose of the present study was to determine the location of the disulfide bridges in albolabrin, a medium-length disintegrin, and to identify the cysteines which are critical for the expression of the biological activity of this molecule. For this purpose, we used synthetic peptides derived from flavoridin, which has slightly higher antiplatelet aggregation activity as compared to albolabrin. There is a high degree of sequence analogy between albolabrin and flavoridin including identical alignment of the RGD sequence and the number and position of the 12 cysteines (Gould et al., 1990). It is likely that the location of the disulfide bridges in both molecules is identical.

Materials and Methods

RGDX Tetrapeptides. RGDS and RGDF were purchased from Peninsula Laboratories, Belmont, CA. The tetrapeptide RGDD was kindly provided by Dr. S. Kahn, Wistar Institute, Philadelphia, PA. The purity of these peptides was abount 95% as determined by amino acid analysis and HPLC.

Purification of Albolabrin and Flavoridin. Albolabrin was purified from the snake venom of *Trimeresurus albolabris* supplied by Latoxan (Rosans, France) using reverse-phase HPLC as described previously (Williams et al., 1990). An additional purification step included chromatography on a Lichrospher 100 RP-18 (5 μm, Merck, Darmstadt) column using a gradient solution of (A) 0.1% (v/v) trifluoroacetic acid (TFA) in water and (B) 0.1% (v/v) TFA in acetonitrile. The column was eluted at 1 mL/min under isocratic conditions (10% B) for the first 5 min followed by a 15-min linear gradient up to 25% B and then up to 60% B for 70 min. The albolabrin peak eluted at 28–29% acetonitrile. The purification of flavoridin from the crude venom of *Trimeresurus flavoviridis* (Sigma Chemical Co., St Louis, MO) was accomplished by two-step reverse-phase chromatography on a wide-pore (300 Å) C-18 silica matrix column (4.6 mm × 25 cm, Vydac; Separation Group, Hesperia, CA). The conditions were similar to those described previously for the purification of batroxostatin (Rucinski et al., 1990). Active flavoridin was eluted at 35% acetonitrile. The purity of both proteins was tested by SDS–polyacrylamide gel electrophoresis (Phast Gel, Pharmacia) and by N-terminal amino acid sequencing. Figures 1 and 2 show the amino acid sequences of albolabrin and flavoridin, respectively, with special reference to the position of the cysteine residues. Albolabrin and flavoridin are composed of 73 and 70 amino acids, respectively, with all cysteines and the RGD sequence aligned (66% amino acid sequence identity). Reduction and vinylpyridylethylation of both peptides were accomplished as previously described (Huang et al., 1987a).

Proteolytic Degradation of Albolabrin. Albolabrin (2 mg/mL in 100 mM NH_4HCO_3, pH 8.0) was digested with TPCK-trypsin (Sigma Chemical Co.) at a protein:enzyme ratio of 50:1 (w/w) for 18 h at 37 °C. The tryptic peptides were separated by reverse-phase HPLC using a Lichrospher 100 RP-18 (5 μm, Merck, Darmstadt) column equilibrated in a mixture of 0.1% (v/v) TFA in water (A) and 0.1% (v/v) TFA in acetonitrile (B) (90% A/10% B) and eluted at 1.0 mL/min first isocratically for 5 min followed by a linear gradient up to 25% B for 10 min and then up to 40% B for 30 min. A large substructure containing the six disulfide bridges of albolabrin cross-linking four different tryptic-derived fragments was identified by a combination of amino acid analysis (after hydrolysis with 6 N HCl at 110 °C for 24 h using a Biotronik amino acid analyzer) and amino-terminal sequence analysis (using a Beckman 890 C sequencer following the manufacturer's instructions). This substructure (Figure 1) was further degraded with porcine pancreatic elastase (Sigma Chemical Co.) in 100 mM NH_4HCO_3/1 mM $CaCl_2$, pH 8.0, at an enzyme:peptide ratio of 1:25 (w/w) overnight at 37 °C. The resulting peptides were separated by using a similar chromatographic system. The column was eluted isocratically with 100% A for 5 min followed by a linear gradient up to 10% B for 10 min, up to 30% B for 60 min, and finally up to 40% B for 15 min. The isolated peptides were analyzed by amino acid analysis as described. Mass spectra were recorded with a mass spectrometer MAT 900 (von Finnigan MAT, Bremen) equipped with liquid SIMS ionization and with an atom gun (Ion Tech. Ltd., Teddington, U.K.).

Synthesis of Flavoridin-Derived Peptides. These peptides were synthesized by solid-phase methods (Jameson et al., 1988) using a fully automated 430 Applied Biosystems peptide

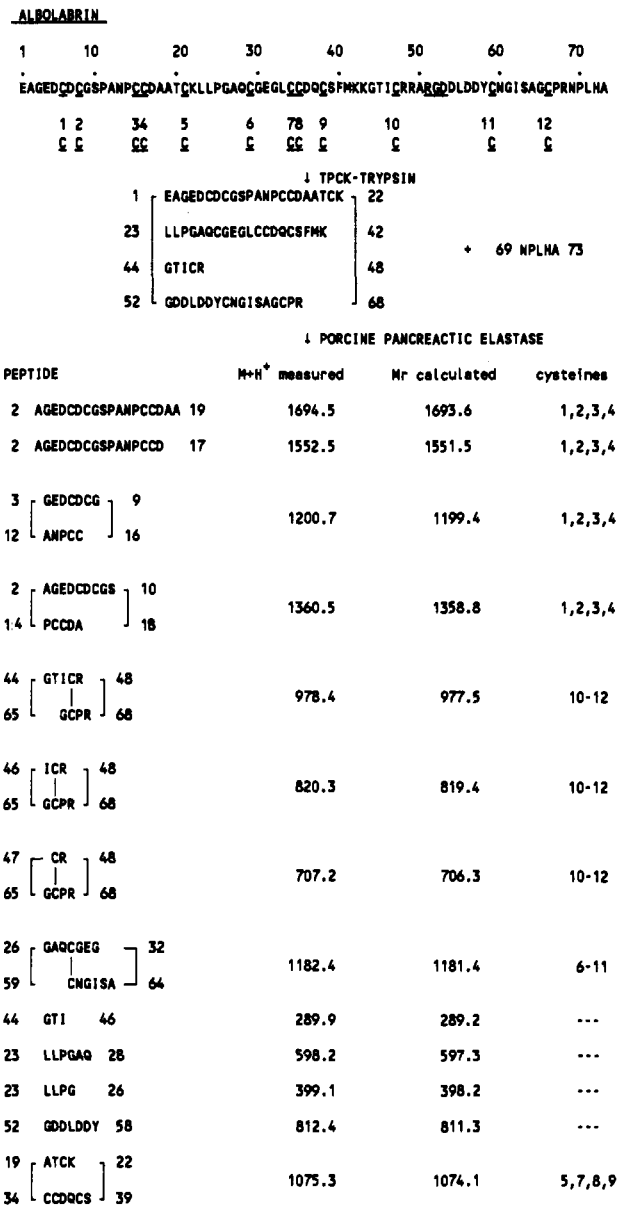

FIGURE 1: Amino acid sequence of albolabrin and of peptides obtained by its digestion with TPCK-trypsin and porcine pancreatic elastase. The numbers of amino acid residues are marked on the top of albolabrin, and the numbers of the cysteine residues are marked below the sequence of the protein. The numbers at the left and right side of each peptide indicate its N- and C-terminal residues, respectively, and its localization within the albolabrin molecule. Peptides within brackets represent disulfide-bonded polypeptides. $M+H^+$ indicates the molecular mass of the protonated molecular ion determined by mass spectrometry. Mr corresponds to the molecular mass of the peptide calculated from its amino acid composition. In the right column, the cysteines contained in each substructure are indicated. Established disulfide bridges are represented by a dash.

synthesizer, and they were purified by two-step reverse-phase HPLC using a C-18 silica Vydac Column (Hesperia, CA). The predicted sequences of the peptides were confirmed by means of amino acid composition and sequence analyses. The amino acid sequences of flavoridin and of flavoridin-derived peptides are summarized in Figure 2. Each of the 4 peptides was composed of 31 amino acids corresponding to amino acid residues 35–66 of flavoridin. Peptide 1 contained four cysteine residues in identical positions with cysteines-9, -10, -11, and -12 of native flavoridin. In peptide 2, cysteine-10 and cysteine-11 were substituted with alanines. In peptide 3, alanines replaced cysteines-10 and -12, and in peptide 4, alanines replaced cysteines-9 and -11. The reverse-phase HPLC profile

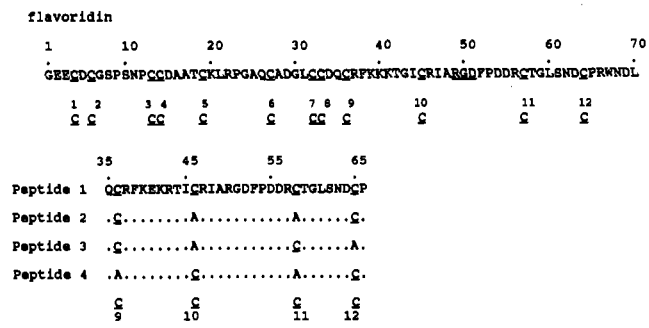

FIGURE 2: Amino acid sequence of flavoridin and of synthetic peptides derived from flavoridin. The numbering is as in Figure 1. Dots below the sequence of peptide 1 indicate identical amino acid residues as in peptide 1.

Table I: Inhibition of Platelet Aggregation by RGD-Containing Peptides

peptide	IC_{50}[a]
RGDF	25 μM
RGDS	105 μM
RGDD	1250 μM
flavoridin	40 nM
albolabrin	220 nM
reduced and vinylpyridylethylated flavoridin	1530 nM
reduced and vinylpyridylethylated albolabrin	8030 nM

[a] Mean values from three to six experiments.

showed that these peptides did not dimerize, and amino acid analysis after treatment with 4-vinylpyridine confirmed that the peptides had the predicted composition and did not contain any free sulfhydryl groups. The existence of intermolecular disulfide bonds within peptides 1, 2, 3, and 4 was ruled by measuring the molecular weight by mass spectrometry; $(M + H)^+$ for peptide 1 was 3598 while that for peptides 2, 3, and 4 was 3535. In fact, peptide 1 showed two peaks of $(M + H)^+$ 3582.4 and 3598.0, respectively. The peak at 3582.4 corresponds to dehydrated peptide. This is not unexpected since the peptide was dissolved in HCl.

Protein concentration was estimated with the Pierce reagent (Sorenson & Brodbeck, 1986) according to the manufacturer's instructions.

Inhibition of ADP-Induced Platelet Aggregation. Blood was collected in sodium citrate (pH 7.35) (final concentration 0.01 M) and centrifuged at room temperature to obtain platelet-rich plasma. Platelet aggregation was recorded at 37 °C in a Sienco aggregometer (Morrison, CO) following addition of 5 μL of ADP (final concentration 10 μM) to 400-μL samples of platelet-rich plasma. The tested peptides dissolved in water were added in a small volume (5 μL) to platelet-rich plasma 2 min prior to the addition of ADP. In each experiment, the inhibition of platelet aggregation by various concentrations of peptide was tested, and the IC_{50} (molar concentration of peptide required to cause 50% inhibition of platelet aggregation) was calculated by linear regression.

Results and Discussion

Table I compares the inhibition of ADP-induced platelet aggregation by RGDF, RGDS, and RGDD and by native and reduced albolabrin and flavoridin. As has been shown previously (Musial et al., 1990), flavoridin was 5 times more potent than albolabrin. It is conceivable that the higher activity of flavoridin as compared to albolabrin is related to the substitution of the aspartic acid adjacent to the RGD sequence with phenylalanine. It is established that the activity of RGDX peptides in blocking platelet aggregation depends on the hy-

Table II: Inhibition of ADP-Induced Platelet Aggregation by Flavoridin-Derived Peptides[a]

peptide	IC_{50} (μM)
peptide 1 (C_9, C_{10}, C_{11}, C_{12})	3.01 ± 0.47
peptide 2 (C_9, A_{10}, A_{11}, C_{12})	11.58 ± 4.50
peptide 3 (C_9, A_{10}, C_{11}, A_{12})	3.50 ± 2.40
peptide 4 (A_9, C_{10}, A_{11}, C_{12})	7.30 ± 0.80

[a] Mean ± SD from three to six experiments; the IC_{50} for peptide 1 is statistically different from the IC_{50} for peptides 2 and 4; the IC_{50} for peptide 3 is statistically different from the IC_{50} from peptide 4 ($p \leq 0.05$).

drophobicity of the amino acid residue adjacent to the RGD sequence (Tranqui et al., 1989) and that RGDF is one of the most potent RGDX inhibitory peptides (Plow et al., 1987).

Reduction and vinylpyridylethylation of albolabrin and flavoridin decreased their platelet aggregation inhibitory activities about 40–50-fold (Table I). Thus, not only the sequence around the RGD tripeptide but also its conformation maintained by the appropriate cysteine pairings is necessary for full expression of the inhibitory activity of disintegrins. In order to evaluate the biological significance of the cysteine pairings surrounding the RGD sequence, we have synthesized four peptides derived from the sequence of flavoridin (Figure 2). Peptide 1 corresponded to the polypeptide representing amino acid residues 35–66 of flavoridin and contained cysteines-9, -10, -11, and -12 of this molecule. In platelet aggregation assay, its activity was about 75 times lower as compared to flavoridin (Table II). In cell adhesion assay (inhibition of adhesion of B16 melanoma cells to fibronectin), flavoridin was at least 500 times more active than flavoridin-derived peptide 1 (data not shown). We assume that peptide 1 may contain three species with three possible cysteine pairings: (a) Cys-1 linked to Cys-3 and Cys-2 linked to Cys-4; (b) Cys-1 linked to Cys-2 and Cys-3 linked to Cys-4; (c) Cys-1 linked to Cys-4 and Cys-2 linked to Cys-3. The final IC_{50} of the mixture would be influenced by these three species. It is possible that different pairings of cysteines in peptide 1 may result in different biological activities, however, the occurrence of a very active species of peptide 1 at a very low concentration is not likely. In view of a low activity of peptide 1, these data suggest that formation of two disulfide bridges around the RGDF sequence is not sufficient for the expression of the biological activity of flavoridin. Peptides 2, 3, and 4 were variants of peptide 1 in which a pair of cysteines in each peptide was replaced with alanine residues and, thus, contained single intrachain disulfides. It is noteworthy that peptides 2 and 4, as compared to peptides 1 and 3, showed statistically significant greater IC_{50} values when tested for the ability to inhibit platelet aggregation (Table II). This may indicate that different disulfide bridges around the RGD sequence contribute nonequivalently to the maintenance of its biologically active conformation.

To gain information about the structure of disintegrins, we have investigated the disulfide bridge pattern of albolabrin, and elaborated a model for this protein which may be relevant for the whole disintegrin family. Figure 1 shows the amino acid sequences of the peptides isolated after tryptic digestion of albolabrin. The short peptide corresponds to the C-terminal region of the molecule and confirms the previous assignment of its C-terminus by carboxypeptidase Y digestion analysis (Williams et al., 1990). The large substructure, composed of four fragments, corresponds to the amino acid stretches 1–22, 23–42, 44–48, and 52–68 cross-linked by the six disulfide bonds of albolabrin, which remain intact in this tryptic product. The observation that peptides 44–48 and 52–68, each containing

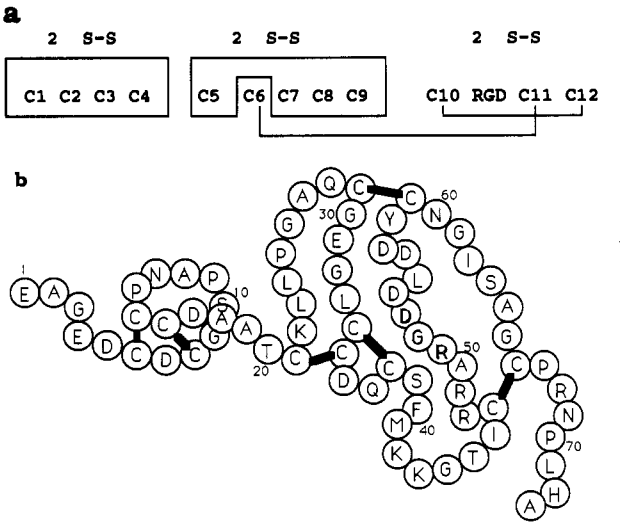

FIGURE 3: (a) Localization of disulfide bonds in albolabrin. The exact arrangement of the two disulfides of the cysteine residues in the boxes is unknown (see Figure 1). (b) Two-dimensional model of albolabrin elaborated according to the information in (A) and assuming disulfide linkage between cysteines-1–3, -2–4, -5–8, and -7–9.

a single cysteine residue, remain disulfide-bonded within this albolabrin substructure suggests that the last two cysteines in albolabrin do not form the loop maintaining the active RGD conformation. To work out the disulfide arrangement in albolabrin, the tryptic substructure containing the six disulfide bonds was further degraded with porcine pancreatic elastase, an enzyme of broad cleavage specificity. The resulting degradation products were isolated by HPLC as described under Materials and Methods. Among the peptides identified by amino acid analysis and mass spectrometry (Figure 1), five different cleavage products contained two peptides linked by a single disulfide bond. Three of these products suggest a linkage between cysteines-10 and -12, while the other two contained a disulfide bridge between cysteines-6 and -11 of the parent albolabrin. Other peptides analyzed (Figure 1) contained either the first four cysteines of albolabrin or cysteines-5, -7, -8, and -9, respectively. Each group of four cysteines involved the formation of two disulfide bridges between themselves. Since this pairing pattern was consistently found in different experiments working with different batches of purified albolabrin, we conclude that disulfide rearrangement did not take place. Although the exact pairing in the substructures containing two disulfides was not further investigated, a bridge between cysteines-1 and -2, -3 and -4, and -7 and -8 would be sterically hindered as the formation of a disulfide bond requires at least two amino acid residues between cysteine residues (Richardson, 1981).

Figure 3 shows the experimentally determined disulfide bonds and a model for albolabrin elaborated according to this information. In this model, we have favored the cross-pairing of the nonassigned disulfide bonds because this arrangement is very commonly found in nature. The alternative pairing possibilities (1–4, 2–3, 5–7, and 8–9) would, however, not significantly alter the overall folding pattern of the molecule, which may be supported mainly by the long-range disulfide bonds between cysteines-6–11 and -10–12.

Due to the high sequence identity existing between all the known snake venom disintegrins (Gould et al., 1990; Dennis et al., 1990), we hypothesized that the disulfide bridge pattern outlined here for albolabrin may be the same in the other medium-range disintegrins. If this is the case, the extra pair of cysteines found in the long disintegrins (Shebuski et al.,

1990; Dennis et al., 1990) may form a disulfide bridge between themselves. Interestingly, in the small disintegrins, the N-terminal polypeptide stretch, containing the first five cysteines in the medium disintegrins, is missing, and an additional cysteine residue is located between the penultimate and the last cysteine residues (Gan et al., 1988). It is tempting to speculate that in the short disintegrins this extra cysteine residue will be paired to the third cysteine in the molecule which corresponds to the eight cysteine (residue 35) in the medium-range disintegrins. Moreover, if the overall three-dimensional folding pattern has been evolutionary conserved among the members of the disintegrin family, then in albolabrin cysteine-8 and alanine-64 (replaced by the nonanalogous cysteine in the short disintegrins) may be close to each other. Initial evidence for this hypothesis was obtained by computer modeling of the structure of albolabrin using an energy minimization program starting with a random configuration with the only constraints imposed by disulfide bonds between cysteines-6–11 and -10–12 (data not shown). In the short disintegrins, a disulfide bridge in this position (residues 7–37 or 8–37) may restrain the conformational freedom of the RGD-containing loop and may, therefore, be responsible for the increased potency in inhibiting platelet aggregation.

In conclusion, we propose a model for albolabrin which may be appropriate for the structure of the entire disintegrin family. Comparison of the structure of albolabrin with echistatin (a 49 amino acid disintegrin) suggests that cross-links between the first four N-terminal cysteines are less important for the expression of biological activity than cross-links between the C-terminal cysteines. The cross-links between cysteine-6 and cysteine-11 and between cysteine-10 and cysteine-12 appear to be very critical for the structure of albolabrin, flavoridin, and other medium disintegrins and for the expression of their biological activity.

References

Chao, B. H., Jakubowski, J. M., Savage, B., Ping, Chow, E., Marzec, U. M., Harker, L. A., & Maraganore, J. M. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 8050–8054.

Cook, J. J., Huang, T.-F., Rucinski, B., Strzyzewski, M., Tuma, R. F., Williams, J. A., & Niewiarowski, S. (1989) *Am. J. Physiol. 266*, H1038–H1043.

Dennis, M. S., Henzel, W. J., Pitti, R. M., Lipari, M. T., Napier, M. A., Deisher, T. A., Bunting, S., & Lazarus, R. A. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 2471–2475.

Gan, Z.-R., Gould, R. J., Jocbs, J. W., Friedman, P. A., & Polokoff, M. A. (1988) *J. Biol. Chem. 263*, 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) *Proc. Natl. Acad. Sci. U.S.A. 86*, 4022–4926.

Gould, R. J., Polokoff, M. A., Friedman, P. A., Huang, T.-F., Holt, J. C., Cook, J. J., & Niewiarowski, S. (1990) *Proc. Soc. Exp. Biol. Med. 195*, 168–171.

Huang, T.-F., Holt, J. C., Lukasiewicz, H., & Niewiarowski, S. (1987a) *J. Biol. Chem. 262*, 16157–16163.

Huang, T.-F., Wu, Y.-J., & Ouyang, C. (1987b) *Biochim. Biophys. Acta 925*, 248–257.

Huang, T.-F., Holt, J. C., Kirby, E. P., & Niewiarowski, S. (1989) *Biochemistry 28*, 661–666.

Jameson, B. A., Rao, P. E., Kong, L. I., Hahn, B. H., Shaw, G. M., Hood, L. E., & Kent, S. B. H. (1988) *Science 240*, 1335–1339.

Knudsen, K. A., Tuszynski, G. P., Huang, T.-F., & Niewiarowski, S. (1988) *Exp. Cell Res. 179*, 42–49.

Musial, J., Niewiarowski, S., Rucinski, B., Stewart, G. J., Cook, J. J., Williams, J. A., & Edmunds, L. H., Jr. (1990) *Circulation 82*, 261–273.

Niewiarowski, S., Huang, T.-F., Rucinski, B., Cook, J. J., Williams, J. J., Musial, J., Edmunds, L. H., Jr., Bush, L., Shebuski, R., & Friedman, P. A. (1989) *Thromb. Haemostasis 62*, 319.

Plow, E. F., Pierschbacher, M. D., Rouslahti, E., Marguerie, G., & Ginsberg, M. H. (1987) *Blood 70*, 110–115.

Reed, J., Hull, W. E., vonder Lieth, C.-W., Kubler, D., Suhaj, S., & Kinzel, W. (1988) *Eur. J. Biochem. 178*, 141–154.

Richardson, J. S. (1981) *Adv. Protein Chem. 34*, 167–339.

Rucinski, B., Niewiarowski, S., Holt, J. C., Soszka, T. & Knudsen, K. A. (1990) *Biochim. Biophys. Acta 1054*, 257–262.

Ruoslahti, E., & Pierschbacher, M. D. (1987) *Science 238*, 491–497.

Savage, B., Marzec, U. M., Chao, B. H., Harker, L. A., Maraganore, J. M., & Ruggeri, Z. M. (1990) *J. Biol. Chem. 265*, 11766–11772.

Shebuski, R. J., Ramjit, D. R., Bencern, G. H., & Polokoff, M. A. (1989) *J. Biol. Chem. 264*, 21550–21556.

Sorensen, K., & Brodbeck, U. (1986) *J. Immunol. Methods 95*, 291–293.

Tranqui, L., Andrieux, A., Hudry-Clergeon, G., Ryckewaert, J.-J., Soyez, S., Chapel, A., Ginsberg, M. H., Plow, E. F., & Marguerie, G. (1989) *J. Cell Biol. 108*, 2519–2527.

Williams, J., Rucinski, B., Holt, J. C., & Niewiarowski, S. (1990) *Biochim. Biophys. Acta 1039*, 81–89.