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# FIBRONECTIN AND FIBRINOGEN DEGRADATION PRODUCTS STIMULATE PMN-LEUKOCYTE AND MAST CELL DEGRANULATION

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The ability of various peptides cleaved by plasmin from human fibrinogen and fibronectin or fibrinogen- and fibronectin- related synthetic peptides to induce histamine release from mast cells and collagenase and elastase from PMN-leukocytes was examined.

Low molecular weight fibrinogen degradation products showed dose dependent secretion of collagenase. These peptides (mol. wt. 1.4 kD) at the concentration of $10^{-5}$ M released about 47% of collagenase and 13% of elastase. Synthetic fibrinopeptides A and B had a similar strong collagenase releasing potency and also released histamine from mast cells.

Peptides from plasmin digestion of fibronectin containing cell attachment site with sequence Arg-Gly-Asp-Ser and also synthetic peptide reproducing this amino-acid sequence at the concentration of 1000 $\mu$g/ml released about 50% of collagenase and 55% of elastase from PMN-leukocytes. Moreover peptides containing cell attachment and gelatin binding site induced histamine release from mast cells.

The association of fibrinogen and fibronectin degradation with activation of mast cells may motivate the treatment with antihistaminic drugs of all pathological conditions where the intensive protein degradation takes place.

Key words: *Collagenase, histamine, mast cells, PMN — leukocytes, fibronectin degradation products, fibrinogen degradation products.*

## INTRODUCTION

Proteins destruction in inflammatory diseases involves proteinases released from activated cells or generated upon activation of the clotting and complement system. Proteolytic degradation products could lose certain biological function of the parent molecules or could acquire new biological activities (1). For instance fibronectin fragments generated by endogenous proteases are potent chemoattractants for human peripheral blood monocytes (2), fibrob-

lasts (3) and endothelial cells (4) and stimulate elastase and lactoferin release from PMN-leukocytes (5). Low molecular weight fibrinogen degradation products inhibit platelets aggregation (6), are chemotactic to human monocytes (7), suppres immunoreactivity (8), damage kidney (9) and enhance the permeability of capillaries and the blood-brain barrier (10).

In the present study we examined the activities of various peptides cleaved by plasmin from human fibrinogen and fibronectin or corresponding synthetic peptides as inducers of histamine release from mast cells and PMN-collagenase and elastase release.

## MATERIALS AND METHODS

Fibronectin was isolated from heparinized human plasma freshly drawn into protease inhibitors (25 mM EDTA, 1 mM phenylmethylsulphonyl fluoride, 10 mM benzamidine -HCl and 10 mM E-aminocaproic acid) and purified by two-step affinity chromatography procedure using gelatin-Sepharose and heparin-Sepharose (11) Fibronectin degradation products were obtained by digestion of fibronectin by plasmin. Fibronectin was incubated at a 50:1 molar ratio (substrate/enzyme) with plasmin at 37°C for 5 h and proteolysis was stopped by addition of soybean inhibitor. Fibronectin degradation products were fractionated by gelatin-Sepharose and heparin-Sepharose affinity chromatography (11).

Digestion of human fibrinogen with plasmin was performed at 37°C at the enzyme/substrate ratio of 1:100 for 18 h. Reaction was stopped by the addition of soybean trypsin inhibitor. Peptide were fractionated by the gel filtration on Sephadex G-25 column (2 × 75 cm) and eluted with 0.14 M NaCl. The column was calibrated using bradykinin (mol. wt 1240), trasylol (mol. wt 6500) and cytochrome c (mol. wt 12600) as standards.

Peritoneal fluids were collected from male Wistar rats and cell suspension containing about 5% mast cells was purified by centrifugation in Percoll (12). The purity of the final mast cell preparation was greater than 95% and viability averaged 98% as assessed by trypan blue exclusion. The mast cell suspension contained $2 \times 10^5$ cells/0.2 ml of 145 mM NaCl, 0.9 mM $CaCl_2$, 2.4 mM KCl, 0.1% glucose and 0.1% serum albumin adjusted to pH 7.4 with Sörensen phosphate buffer. The suspension was exposed to various concentration of peptides at 37°C for 30 min. Cells were separated from the medium by centrifugation (400 xg, 5 min) at 4°C and histamine was determined fluorimetrically in both the supernatant and cells using the o-phthalaldehyde method of Shore (13). Histamine released into the supernatant was expreased as a percentage of the total cellular histamine content. All values were corrected for the spontaneous histamine release (5—8%) which occured in the absence of any inducer.

Rat PMN-leukocytes were isolated from carrageenin induced pleural exudate by centrifugation at 100 xg for 15 min and hypotonic lysis of remaining red cells. The purity of the final cell suspension averaged 90—95%, viability of PMN-leukocytes was always greater than 90% as assessed by trypan blue exclusion. The PMN-leukocytes suspension ($20 \times 10^6$ cells) in Hanks solution was incubated with various concentration of peptides for 60 min. at 37°C. Incubation was terminated by centrifugation (200 xg, 10 min.) at 4°C and the supernatant solution was assayed for extracellular collagenase and elastase level. The total concentration of enzyme in the cell sediment was also determined following cells lysis with 0.2% Triton X-100 in 0.05 M Tris-HCl buffer, pH 7.5. Enzyme release was expressed as the percentage of the total enzyme activity and all values were corrected for the spontaneous release in the absence of any stimulus.

Collagenase activity was determined at 37°C by measuring hydroxyproline released from reconstituted collagen fibrils (14) and elastase activity on synthetic substrate N-t butyloxycarbonyl-L-alanine-p-nitrophenyl ester (Boc-Ala-ONp) (15). The following reagents were used: fibrinogen from human plasma (Sigma) containing 65% protein (91% of protein clottable), plasmin from human plasma (Sigma) with activity 3,3 units/mg protein, Tyr-fibrinopeptide A and fibrinopeptide B — human sequence (Sigma).

## RESULTS

Analysis of plasmin digested fibrinogen samples on Sephadex G-25 indicated that peptides of various sizes were generated. The effect of these fractions on the release of collagenase and elastase from PMN-leukocytes was investigated.

The activity of the fibrinogen degradation products was directly related to the degree of fragmentation of the molecule. Intact fibrinogen and high molecular weight peptides were inactive, whereas low molecular weight fibrinogen degradation products (average mol. wt 1.4 kD) showed dose dependent secretion of collagenase and elastase. These peptides at the concentration of $10^{-5}$ M released about 47% of collagenase and 13% of elastase (Fig. 1). Synthetic fibrinopeptides A and B had a similar strong collagenase releasing

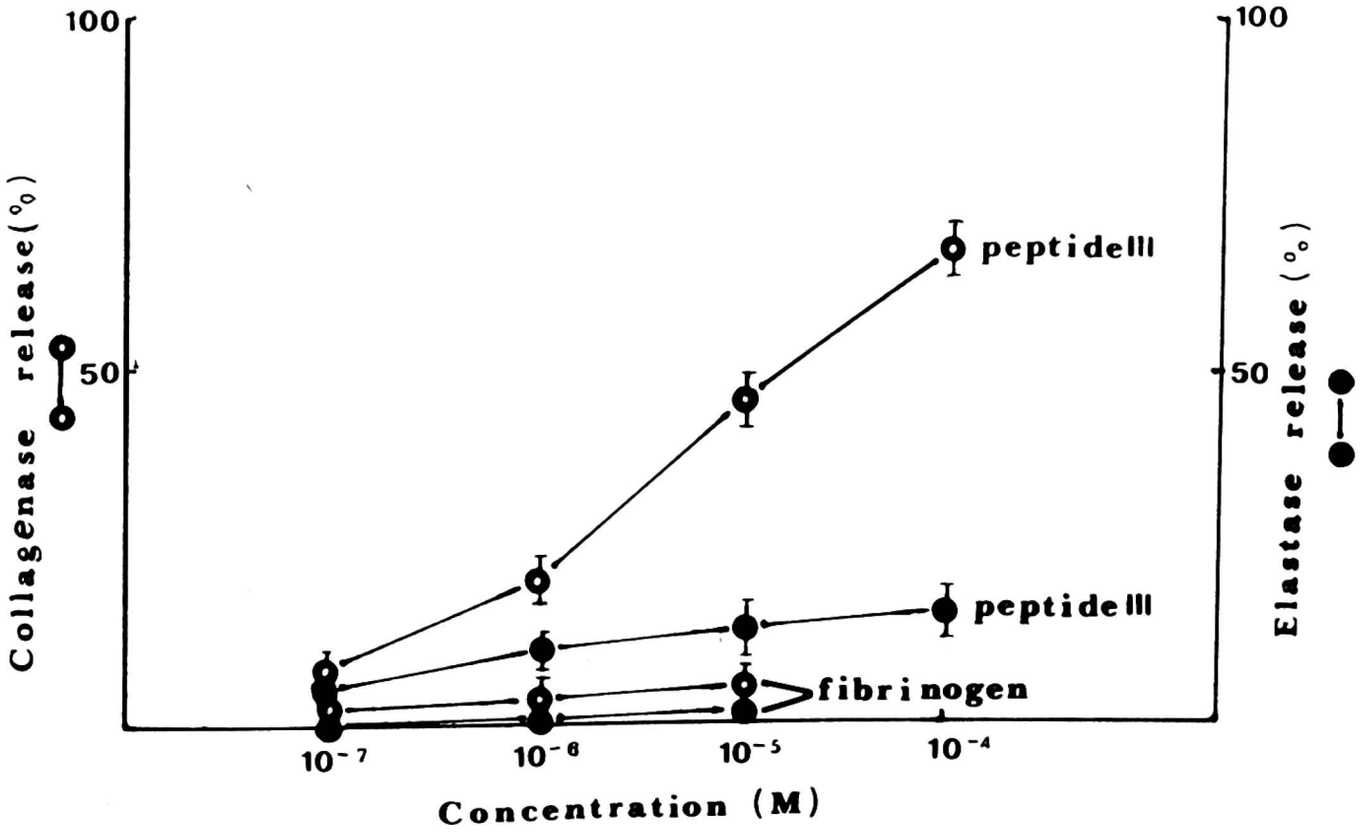


*Fig. 1.* Concentration dependent effect of fibrinogen degradation products (peptides III) on collagenase and elastase release from PMN-leukocytes. Each value represents the mean ± SEM of 8 experiments.

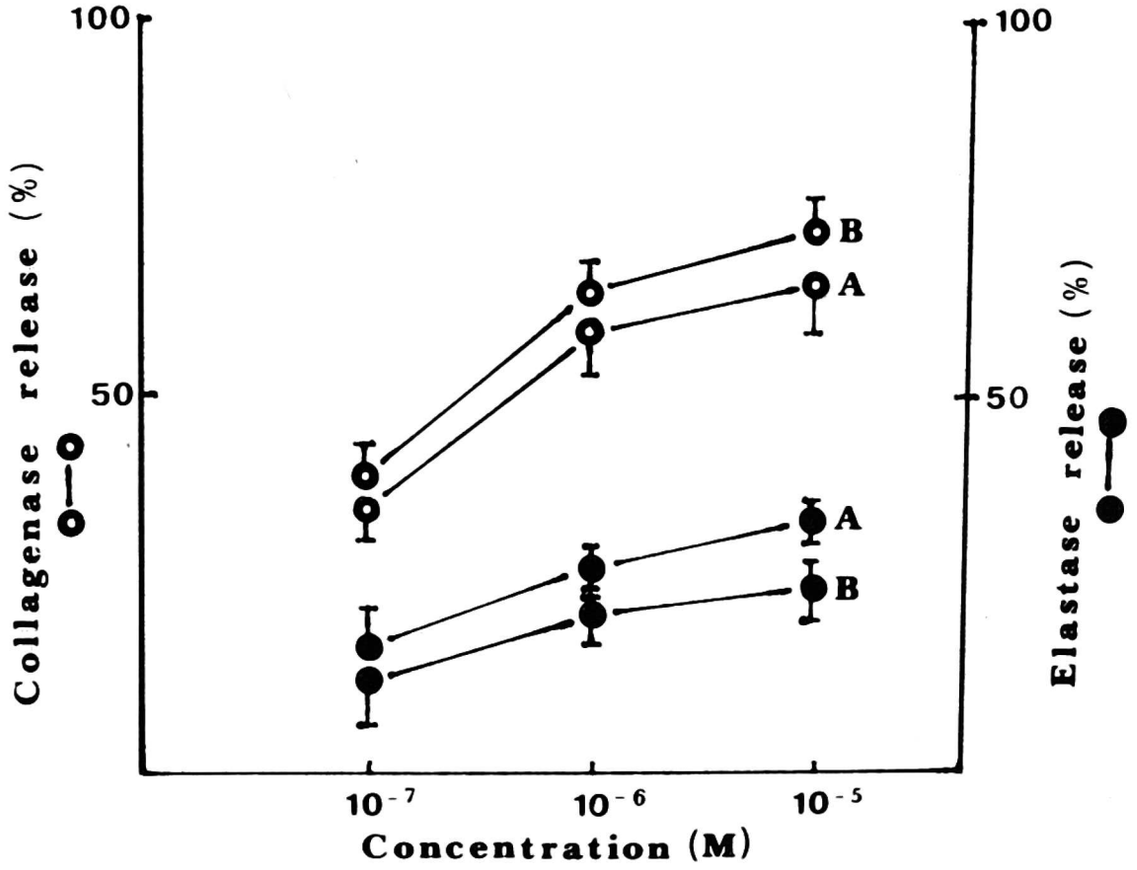


*Fig. 2.* The effect of fibrinopeptides (A) and (B) on collagenase and elastase release from PMN-leukocytes. Each value represents the mean ± SEM of 10 experiments.

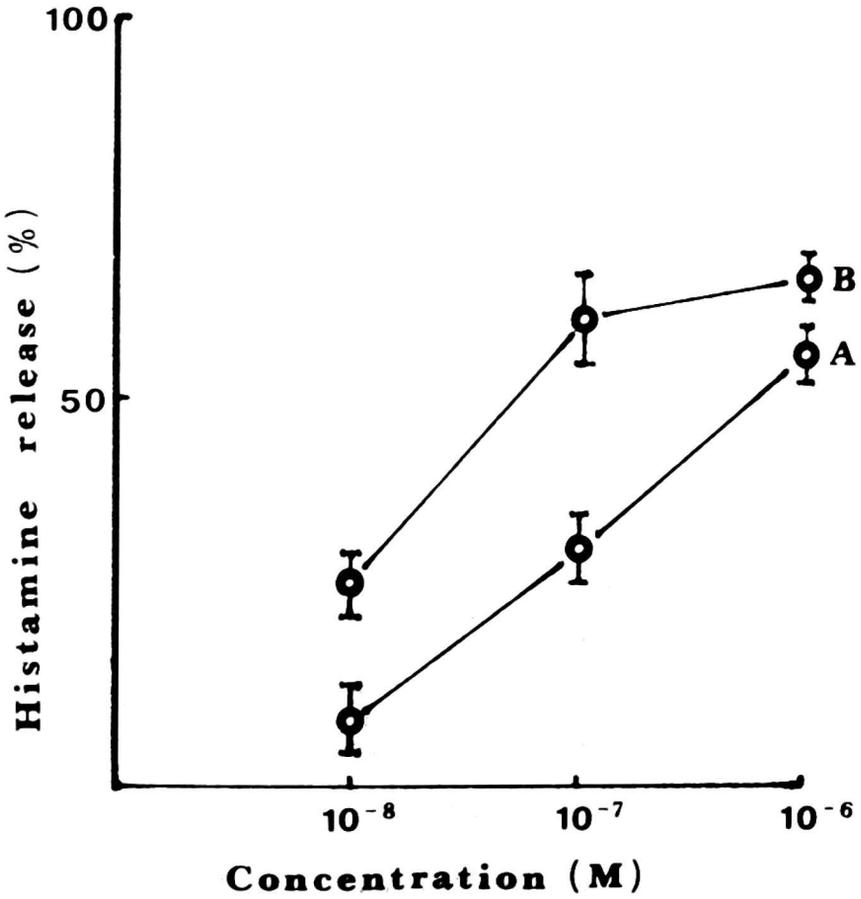


*Fig. 3.* Histamine release from mast cells induced by fibrinopeptides (A) and (B). Each value represents the mean ± SEM of 7 experiments.

potency *Fig. 2)*. Exposure of mast cells to different concentration of fibrinopeptides A and B caused secretion of histamine *(Fig. 3)*. The time course of the peptides mediated histamine release was apparently completed within 20 minutes *(Fig. 4)*.

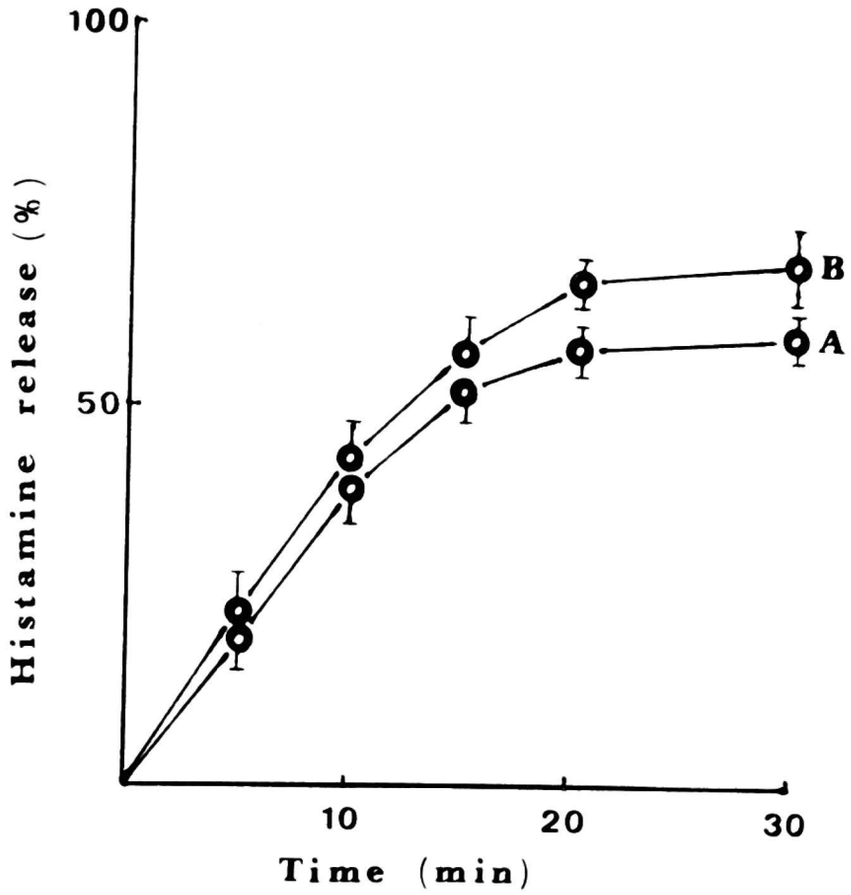


*Fig. 4.* Time course of histamine release from mast cells by fibrinopeptides (A) and (B). Each value represents the mean ± SEM of 4 experiments. Concentration of peptides $10^{-6}$ M.

Digestion of fibronectin by plasmin generated many peptides retaining the biological activities of the parent molecule such as binging to many substrates. Peptides with a strong affinity for heparin and gelatin were separated from the whole digest by the binding to heparin and gelantin-Sepharose. The effect of these separated peptides on release of collagenase and elastase from PMN-leukocytes and histamine from mast cells is shown in *Table 1*. PMN-leukocytes were stimulated to secretion by whole digest and by peptides without affinity to gelatin and heparin. These peptides contained probably the cell attachment site, because synthetic tetrapeptide (Arg-Gly-Asp-Ser) reproducing the amino-acid sequences of cell binding domain of fibronectin, was active in collagenase and elastase secretion.

Mast cells were also stimulated by peptides containing cell attachment site, synthetic tetrapeptide, dipeptide Gly-Asp and additionally by peptides possesing collagen-binding domains *(Table 1)*.

*Table 1* The ability of fibronectin degradation products and related synthetic peptides to release histamine from mast sells and collagenase and elastase from PMN

| | Stimulating agent Concentrations μg/ml | Release (% of total) from: | | |
|---|---|---|---|---|
| | | PMN Collagenase | PMN Elastase | Mat cells Histamine |
| Whole digest | 100 | 10 | 18 | 20 |
| | 500 | 42 | 51 | 45 |
| | 1000 | 58 | 68 | 60 |
| Peptides bound to Heparin-Sepharose | 100 | 3 | 4 | 2 |
| | 1000 | 11 | 7 | 11 |
| Peptides bound to Gelatin-Sepharose | 100 | 7 | 5 | 21 |
| | 1000 | 12 | 8 | 64 |
| Unbound peptides | 100 | 8 | 15 | 18 |
| | 500 | 30 | 45 | 38 |
| | 1000 | 50 | 55 | 52 |
| Arg-Gly-Asp-Ser | 100 | 11 | 16 | 16 |
| | 500 | 25 | 40 | 38 |
| | 1000 | 45 | 56 | 58 |
| Gly-Asp | 100 | 16 | 20 | 11 |
| | 1000 | 41 | 50 | 44 |
| Fibronectin | 100 | 4 | 6 | 3 |
| | 1000 | 7 | 9 | 11 |

## DISCUSSION

The proteolytic peptides of human plasma fibrinogen and fibronectin but not intact molecules were found to induce the release of collagenase and elastase from PMN-leukocytes and histamine from mast cells.

Low molecular weight fibrinogen degradation products (mol. wt. 1.4 kD) and synthetic fibrinopeptides A and B showed the highest dose dependent collagenase releasing activity at concentrations from $10^{-4}$ to $10^{-5}$ M and were less effective in elastase secretion over the same concentration range. Collagenase and elastase are located within separate compartments of PMN-leukocytes, in specific and azurophilic granules respectively, and various effect of peptides on enzyme release is probably connected with a different susceptibility of two types of granules to stimulation.

Mast cells were stimulated by lower concentrations of fibrinopeptides A and B than those used in the experiments with PMN-leukocytes.

Proteolytic fragments of fibronectin containing cell attachment promoting activity as well as synthetic peptides reproducing the amino-acid sequences

(Arg-Gly-Asp-Ser) of such fragments stimulated neutrophil degranulation. The sequence Arg-Gly-Asp-Ser is the minimal sequence necessary to mediate cell attachment (16) but not indispensable for activation of PMN-leukocytes and mast cells as the dipeptide Gly-Asp located in the central sequence of cytoadhesive tetrapeptide, had a similar activity.

Intact fibrinogen and fibronectin are the substrates for multiple plasma proteinases such as trombin and plasmin, for leukocyte neutral proteinases and likely for tissue proteinases, most of which are probably present at inflammatory sites (17). Fibronectin degradation products have been demonstrated in synovial fluids obtained from patients with an active rheumatoid arthritis (18).

Limited proteolysis of proteins by enzymes generates peptides with biological properties not necessarily expressed by the parent molecule. Proteolytic digestion of fibronectin leads for example, to increased inhibitory activity toward endothelial cells growth (19), tumor growth-enhancing activity (20), chemotaxis of monocytes (2) and inhibition of fibronectin function (21). Fibrinogen degradation products inhibit platelets aggregation (6) and diminish edema formation (22) and also enhance the microvascular permeability mediated in part by the release of histamine from mast cells (23).

Our experiments indicate that fibrinogen and fibronectin degradation products had some pro-inflammatory activities. The activation of PMN-leukocytes and mast cells and subsequent release of collagenase, elastase and histamine may produce deleterious effects. Histamine released from mast cells, as we have previously shown (24), is involved in the process of activation of PMN-leukocyte collagenase intensifying the proteolytic processes in the course of inflammation.

The association of fibrinogen and fibronectin degradation with activation of mast cells may motivate the treatment with antihistaminic drugs of all pathological conditions where the intensive protein degradation takes place. To some extent the process of the release of histamine from mast cells may by modulated by some anti-inflammatory drugs (25).

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