Vol. 85, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 14, 1978 Pages 1113-1118

STIMULATION OF HUMAN GRANULOCYTE ELASTASE BY PLATELET FACTOR 4 AND HEPARIN

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Received October 27, 1978

SUMMARY

Highly purified platelet factor 4 (PF_4) was found to be a potent stimulator of human granulocyte elastase activity against native elastin and solubilized α elastin. Heparin neutralized this stimulation of elastolysis by PF_4, but independently stimulated granulocyte elastase activity. Chondroitin sulfate, a constituent of the PF_4 carrier molecule, also stimulated granulocyte elastase activity. The stimulation of granulocyte elastase by PF_4 occurs at known serum concentrations of PF_4.

INTRODUCTION

Human granulocytes contain an elastase (EC 3.4.21.11) which is an anionic glycoprotein with activity at neutral pH against elastin isolated from various tissues (1). This granulocyte elastase is also active against elastic tissue in experimental animals treated with the enzyme (2), and granulocyte elastase has been implicated in the pathogenesis of human emphysema (3). The granulocyte enzyme is inhibited by serum antiproteases (4), and by synthetic elastase active-site directed low molecular weight compounds (5). No information is currently available concerning the effects of small anionic or cationic molecules on the activity of granulocyte elastase. Platelet factor 4 (PF_4) is a low molecular weight cationic protein which is released from platelets during clotting (6) and after mechanical damage (7). PF_4 is capable of neutralizing the anticoagulant effect of heparin (8), and has recently been shown to

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0006-291X/78/0853-1113$01.00/0

inhibit human skin and granulocyte collagenase (8). Since both collagenase and elastase are serine proteases which may play a role in the pathogenesis of human disease, we examined the effects of highly purified PF_4 on granulocyte elastase. We have found PF_4 to be a potent stimulator of granulocyte elastase activity.

MATERIALS AND METHODS

Platelet factor 4 was isolated from human platelets by affinity chromatography as previously described (10). No phenylalanine or methionine was found by amino acid analysis, and the protein displayed a single band on soidum dodecyl sulfate polyacrylamide gel electrophoresis. PF_4 was dialyzed against .01M phosphate (pH 7.4) with 0.30 M NaCl prior to experiments with granulocyte elastase.

Granulocytes were collected from freshly drawn human blood by dextran sedimentation of the white-cell rich fraction (11). Following granulocyte lysis, the lysozomal granules were collected by centrifugation. These granules were lysed by repeated freeze-thawing, and purified granulocyte elastase was isolated from the granular extract by affinity chromatography using an elastin-sepharose column (12). Esterase activity of the column effluent was measured using N-t-BOC-L-alanine-p-nitrophenyl ester (NBA) as described by Visser and Blout (13). The peak containing the NBA esterase activity displayed a typical granulocyte elastase isoenzyme pattern on polyacrylamide gel electrophoresis, and a single band on sodium dodecyl sulfate polyacrylamide gel electrophoresis. Human α-1 antitrypsin was prepared from freshly drawn blood as previously described (14). The purity of this preparation was confirmed by immuno-electrophoresis and by polyacrylomide gel electrophoresis.

Elastolytic activity was measured using elastin-agar plates (15) containing 7.75 μg of bovine ligamentum nuchae elastin per mm^2. Reactants were mixed in a test tube and allowed to incubate for 30 minutes before being added to the plate. Plates were incubated at 37°c for 24 hours. The areas of clearing were read by 2 observers, using a comparator (Bausch and Lomb), and the results averaged. Elastolytic activity was also measured using oxalic acid treated bovine ligament elastin (16) as a substrate according to the method of Keller and Mandl (17).

RESULTS AND DISCUSSION

We found platelet factor 4 to be a potent stimulator of elastolytic activity (figure 1). Concentrations of PF_4 as low as 10 μg/ml stimulated granulocyte elastase activity against both insoluble elastin and soluble α elastin. This stimulation was linear up to PF_4 concentrations of 200 μg/ml. Using solubilized α elastin as the substrate, the addition of as little as 20 μg/ml PF_4 to 5 μg of granulocyte elastase resulted in an increase of elastolysis of 38%, and the addition of 100 μg/ml

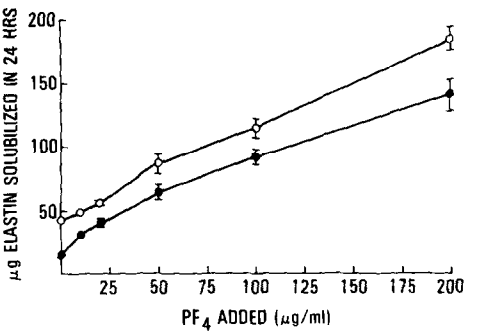

Figure 1: µg of elastin solubilized by human granulocyte elastase in 24 hours as PF_4 is added to reaction tube. Open circles represent data for 5 µg elastase plus PF_4; closed circles represent data for 5 µg elastase, 6.6 µg α-1 antitrypsin, and PF_4. Each point is the mean ± S.E. of 3 separate determinations on an elastin-agar plate.

PF_4 to 5 µg of granulocyte elastase resulted in a doubling of elastase activity (not shown). Figure 1 also demonstrates that this effect of PF_4 on granulocyte elastase occurs in the presence of alpha-1-antitrypsin. The addition of 6.6 µg of α-1-antitrypsin to 5 µg of granulocyte elastase lowered the elastolytic activity of the enzyme to 35% of control levels, but the addition of PF_4 resulted in the stimulation of the non-inhibited enzyme in a linear fashion (figure 1). When the elastolytic activity of granulocyte elastase was completely abolished by α-1-antitrypsin, no activity was restored by the addition of up to 500 µg/ml PF_4. The reported concentration of PF_4 in human serum is 8-15 µg/ml (18). Therefore, the reported effect of PF_4 on granulocyte elastase occurs at serum levels, and higher.

In view of the known binding of PF_4 to heparin resulting in a neutralization of heparin's anticoagulant effect, the effect of heparin on the granulocyte elastase - PF_4 system was examined. We found that the addition of more than 0.5 units of heparin alone to granulocyte elastase resulted in the stimulation of enzyme activity against elastin (figure 2, open circles). This stimulation of granulocyte elastase by heparin has not been previously reported, and the possible physiologic significance

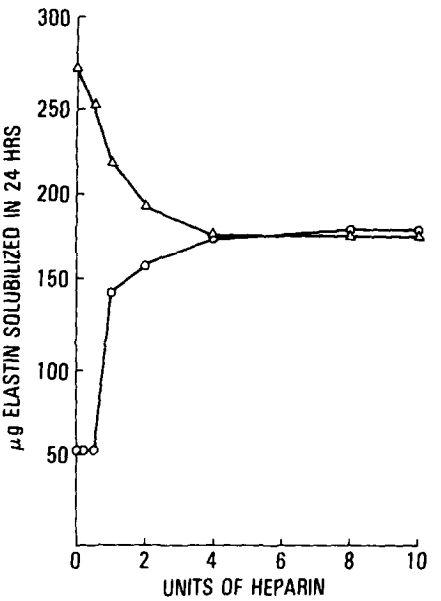

Figure 2: Experiment showing effect of added heparin on 5 μg of granulocyte elastase alone (O) or 5 μg of granulocyte elastase plus 20 μg/ml PF_4 (Δ) as measured by elastin plate assay. Each point is the average μg elastin solubilized per 24 hours of 2 separate determinations.

of this stimulation is unknown. Furthermore, it can be seen from figure 2 that the stepwise addition of heparin to a mixture of granulocyte elastase and PF_4 results in a stepwise neutralization of the PF_4 effect until the PF_4 stimulation of granulocyte elastase has been abolished and only the heparin effect remains (figure 2, open triangles). Heparin shows no such neutralization of the inhibitory effect of PF_4 on granulocyte collagenase (8). When PF_4 was added to a mixture of granulocyte elastase and 1 unit of heparin, no further stimulation of elastolysis was seen until the PF_4 concentration reached 100 μg/ml (not shown).

Since PF_4 has been shown to exist in both a free and a bound form in human serum, and since the carrier protein is complexed with 4 molecules of chondroitin sulfate (19), we also studied the effect of chondroitin sulfate on granulocyte elastase, and on the PF_4 stimulation

TABLE 1

STIMULATION OF GRANULOCYTE ELASTASE BY PF_4:

EFFECTS OF CHONDROITIN SULFATE

Reaction Mixture	Enzyme activity[+]	% Stimulation
Granulocyte elastase 5 µg	110.4	
Granulocyte elastase 5 µg + 30 µg/ml PF_4	164.4	49%
Granulocyte elastase 5 µg + 30 µg/ml PF_4 + 0.5 mg/ml chondroitin SO_4	255.7	132%
Granulocyte elastase 5 µm + 0.5mg/ml chondroitin SO_4	173.9	58%

[+]Enzyme activity expressed as µg elastin solubilized in 24 hours. Reaction mixtures of 0.1 ml were incubated 30 minutes at 22°c before being added to elastin-agar plates. Condroitin sulfate was made up in 0.01M phosphate (pH 7.4) with 0.30M NaCl.

of elastolysis. As is shown in table 1, the addition of chondroitin sulfate (0.5 mg/ml) to granulocyte elastase alone results in a stimulation of elastolysis (58%), and the addition of the same amount of chondroitin sulfate to a mixutre of GE and PF_4 results in further stimulation of elastolysis. It appears that the PF_4 and chondroitin sulfate effects are additive.

Kagan, et al have reported that the elastolytic activity of pancreatic elastase is increased when small anionic molecules such as sodium dodecyl sulfate or bile acids are added to reaction mixtures of pancreatic elastase and elastin (20). These anions attach to elastin, which is amphiphilic, and increase the attraction of cationic enzyme. Low molecular weight cationic proteins, like PF_4, have not previously been tested for their effect on either the pancreatic or granulocyte enzyme. Because platelets are normally activated during clotting and tissue injury, and since such injury induces inflammation with the attraction of granulocytes, it is possible that PF_4-granulocyte elastase interactions play an important physiologic role in tissue injury.

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