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PRESENCE OF A GELATIN-SPECIFIC PROTEINASE AND ITS LATENT FORM IN HUMAN LEUCOCYTES

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SUMMARY

A gelatin-specific proteinase has been isolated from the human leucocyte homogenate using the column chromatography technique on DEAE-Sephadex A-50 and Sephadex G-200 Gelatin-specific proteinase is active toward temperature-denatured collagen (gelatin) or collagen fragments resulting from the action of collagenase on collagen No activity toward native collagen, casein, hemoglobin, elastin, histones or *p*-phenylazobenzyloxycarbonyl-peptide has been detected Azocoll was susceptible to the action of gelatin-specific proteinase far less than denatured collagen The optimum activity of gelatin-specific proteinase is at pH 7 8 Below pH 5 0 the enzyme is inactive The activity is inhibited by EDTA, dithiothreitol, cysteine or human serum (at 1 5 or 1 10 dilution) and enhanced by 60–80% by *p*-chloromercuribenzoate or iodoacetamide Soybean trypsin inhibitor or DFP have been ineffective

The final preparation of gelatin-specific proteinase contains changeable amounts of its latent form which can be activated by the action of trypsin, a specific agent present in the rheumatoid synovial fluid or, to a lesser extent by chymotrypsin

The activator → zymogens → enzymes sequence, as a part of a possible mechanism acting in a situation of a substantial increase in collagen degradation has been proposed

INTRODUCTION

Evidence is accumulating that tissue collagenase is not the only enzyme specifically involved in collagen degradation in vivo The main, if not the sole, role of collagenase seems to be the cleavage of the native collagen molecule into two polypeptide fragments [1] The activity of a partly purified collagenase toward denatured collagen or its polypeptide fragments is low [2] and can be ascribed to the activity of contaminating proteinase rather than to collagenase itself [3]

Recently, McCroskery et al [4] have shown that highly purified mammalian tissue collagenase does not attack gelatin In vitro, gelatin can be degraded by com-

Abbreviation Pz-, *p*-phenylazobenzyloxycarbonyl-

mon proteinases, as for example pronase, trypsin, etc However, in vivo, degradation of denatured collagen or, more likely, of its polypeptide fragments resulting from collagenase action is catalyzed probably by a specific proteinase(s) active at physiologic pH Such a proteinase accompanying collagenase has been demonstrated to be released into the culture medium by tadpole tissue [5], rheumatoid synovial tissue [2] or rheumatoid subcutaneous nodule tissue [3] Indirect evidence has been presented that neutral proteinase is also associated with human granulocyte collagenase [6] The authors have not established the identity of this enzyme pointing only to its striking similarity to the granulocyte elastase

The results presented in this paper indicate that human leucocytes contain a neutral proteinase specific toward temperature-denatured collagen (gelatin) The enzyme is accompanied by its latent form, which can be activated by the action of trypsin, chymotrypsin or an activator isolated from the rheumatoid synovial fluid

METHODS

Acid-soluble collagen was prepared from calf skin and was purified according to Kang et al [7] Collagen solutions were made from the stored, lyophilized material by dissolving it in 0 05% acetic acid, dialysing against 0 05 M Tris–HCl buffer (pH 7 6), containing 0 005 M $CaCl_2$ and 0 2 M NaCl and centrifuging (1 h at 10 000 $\times$ g) off the non-dissolved material The gelatin was prepared from this collagen solution by its thermal denaturation at 45 °C for 15 min

Isolation of gelatin-specific proteinase

Leucocytes were separated from the human whole blood obtained from donors and homogenized as described by Kruze and Wojtecka [8] The homogenate was brought to 65% satn by the gradual addition of solid $(NH_4)_2SO_4$ The resulting precipitate was separated by centrifugation (30 min at 10 000 $\times$ g), and dissolved in 0 05 M Tris–HCl buffer (pH 7 6), containing 0 005 M $CaCl_2$ and 0 2 M NaCl The solution was dialysed against 0 02 M Tris–HCl buffer (pH 8 5), containing 0 002 M $CaCl_2$ A dialysed sample was applied to a DEAE-Sephadex A-50 column (1.4 cm $\times$ 19 cm) equilibrated previously with the same buffer Elution was started with the same buffer and was followed by adding a NaCl gradient up to a 0 4-M concentration Effluents were assayed for collagenase and gelatin-specific proteinase activity The active fractions were pooled separately for each enzyme, concentrated (using Aquacid) and applied to a column of Sephadex G-200 (2 cm $\times$ 75 cm) equilibrated with 0 01 M Tris–HCl buffer (pH 7 6), containing 0 005 M $CaCl_2$ and 0 2 M NaCl The effluents were again assayed for collagenase and gelatin-specific proteinase activity

Detection of the latent form of gelatin-specific proteinase

The presence of the latent form of gelatin-specific proteinase was detected by assaying the activity of the enzyme pretreated with trypsin, chymotrypsin or activator isolated from the rheumatoid synovial fluid The amount of the activator used is indicated in Results

Enzyme assays

The activity of gelatin-specific proteinase was assayed in a system containing

in a total volume of 2 ml 0 5 ml of 0 15% thermally denatured collagen (gelatin), the enzyme preparation as indicated and 0 05 M Tris–HCl buffer (pH 7 6), containing 0 005 M $CaCl_2$ and 0 2 M NaCl, up to 2 ml The system was incubated at 37 °C for periods up to 15 h and the incubation was terminated by adding 1 ml of 45% trichloroacetic acid, mixing and cooling in an ice bath for 10 min The amount of gelatin dissolved in 15% trichloroacetic acid solution was taken as a measure of the proteinase activity This amount was determined by analyzing the hydroxyproline content in the 0 5-ml aliquot portions of the supernatant separated from the precipitate by centrifugation at 3500 × *g* for 15 min Collagenase activity was estimated similarly by determining the amount of soluble, hydroxyproline-containing peptides released from thermally reconstituted calf skin collagen fibrils [8] Collagenolytic activity was also estimated from changes in the viscosity of the reaction mixtures containing 0 1% collagen solution and collagenase or gelatin-specific proteinase (150 μg) in 0 05 M Tris–HCl buffer (pH 7 6), containing 0 005 M $CaCl_2$ and 0 3 M NaCl, measured at 26 °C

Viscosity measurements

These were carried out using an Ostwald-type viscometer The water flow time in this apparatus was 120–130 s at 26 °C

Polyacrylamide-gel electrophoresis

Electrophoresis of the purified gelatin-specific proteinase was performed as described by Davis [9]

Assays for the specificity of gelatin-specific proteinase

To ascertain the specificity of the enzyme, its activity toward various substrates was tested Assay with *p*-phenylazobenzyloxycarbonyl-(Pz)-L-Pro–L-Leu–Gly–L-Pro–D-Arg as a substrate was performed as described by Wunsch and Heidrich [10] except that the buffer used was 0 05 M Tris–HCl buffer (pH 7 6), containing 0 005 M $CaCl_2$ and 0 2 M NaCl Incubation at 37 °C lasted 15 h and thereafter the mixture was acidified and extracted with ethyl acetate [2] Assay with azocoll as a substrate was performed in a system containing 15 mg of azocoll suspended in 2 8 ml of 0 01 M Tris–HCl buffer (pH 7 6), containing 0 005 M $CaCl_2$ and 0 2 M NaCl, and 0 2 ml of the enzyme solution The system was incubated at 37 °C and the release of the dye was monitored at 520 nm

The caseinolytic activity of gelatin-specific proteinase was tested using a 0 6% casein solution according to Kunitz [11] and a 5% casein solution as described by Koj et al [12] The activity of the gelatin-specific proteinase toward the urea-denatured hemoglobin was determined according to the procedure of Anson [13] and the activity toward histones by the method of Davies et al [14]

The elastinolytic activity of gelatin-specific proteinase was measured by its ability to degrade resorcin–fuchsin–elastin, using the method of Banga and Ardelt [15], except that the buffer used was 0 2 M Tris–HCl (pH 8 8) and the incubation time was 15 h

Protein

Protein was determined by the method of Lowry et al [16] using bovine albumin as a standard

Hydroxyproline

The hydroxyproline concentration was determined according to the procedure of Stegemann and Stalder [17]

Materials

Special reagents were obtained from the following sources DFP, Tris, trypsin, iodoacetamide (Koch-Light), hydroxy-L-proline (Nutritional Biochem), bovine serum albumin (Fluka), methyl cellosolve (Bio-Rad), soybean trypsin inhibitor (Mann Res Lab), casein (BDH), Clostridial collagenase (Worthington), DEAE-Sephadex A-50, Sephadex G-75 and G-200 (Pharmacia), Azocoll (Calbiochem), *p*-chloromercuribenzoate, sodium salt (Chemapol) Histones (prepared from calf thymus) were kindly offered by Dr I Szumiel, Institute of Nuclear Research, Warszawa Human leucocyte collagenase was provided by Dr E Wojtecka from this laboratory The enzyme was isolated and purified according to the method [27] involving the following steps isolation and homogenization of leucocytes, mixing the homogenate with dioxan (1 1, by vol), exhaustive dialysis of the resulting supernatant, chromatography on DEAE-Sephadex A-50 and subsequently molecular sieve chromatography on Sephadex G-200 and G-100 columns

All other reagents were Polish commercial products of analytical grade

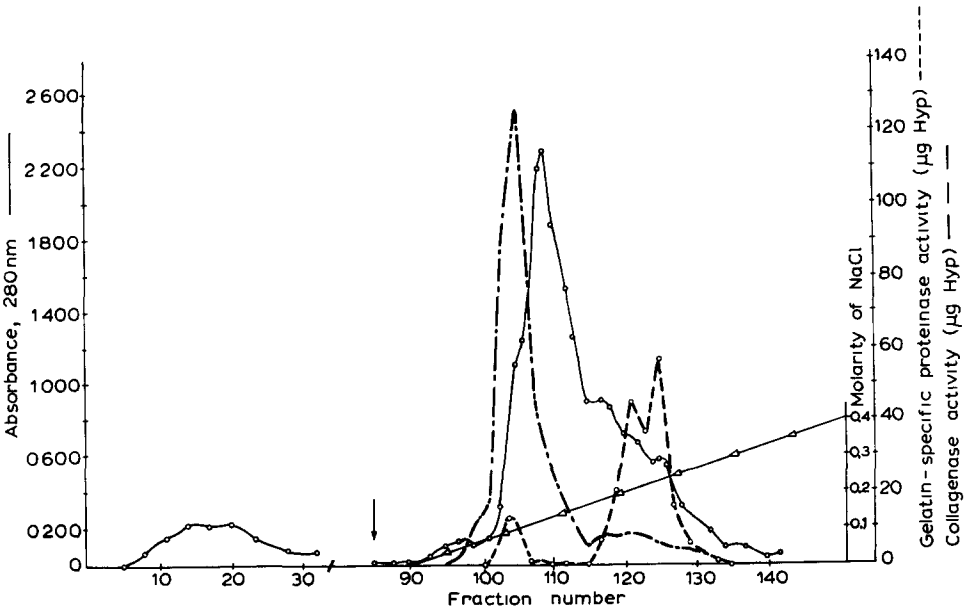


Fig 1 Ion-exchange chromatography of the crude leucocyte gelatin-specific proteinase on DEAE-Sephadex A-50 100 mg protein (redissolved $(NH_4)_2SO_4$ ppt) was applied to the column (1 4 cm × 19 cm) and eluted with 0 02 M Tris–HCl buffer (pH 8 5), containing 0 002 M $CaCl_2$ at a flow rate of 12 ml/h The arrow indicates the start of a linear gradient of NaCl from 0 0 to 0 4 M Effluent fractions of 2 5 ml were collected and assayed for collagenase and gelatin-specific proteinase activities as described under Methods (Hyp, hydroxyproline)

RESULTS

Gelatin-specific proteinase was isolated from the homogenate of human leucocytes by a procedure involving 65 % satn with $(NH_4)_2SO_4$ and separation by chromatography on a column of DEAE-Sephadex A-50, using NaCl linear-gradient elution The effluents were assayed using native collagen and thermally denatured collagen (gelatin) as substrates for differentiating the activity of collagenase and gelatin-specific proteinase The elution pattern obtained, shown in Fig 1 demonstrates a good separation of both activities

Pooled fractions from the peak activity of each enzyme were purified further by passing them separately through a Sephadex G-200 column As illustrated in Fig 2 the activity of gelatin-specific proteinase eluted closer to the void volume of the column This step also brought a further purification of the enzyme (Table I)

Table I shows that the specific activity of the final preparation of gelatin-specific proteinase was 50–200 times higher than the activity of the homogenate

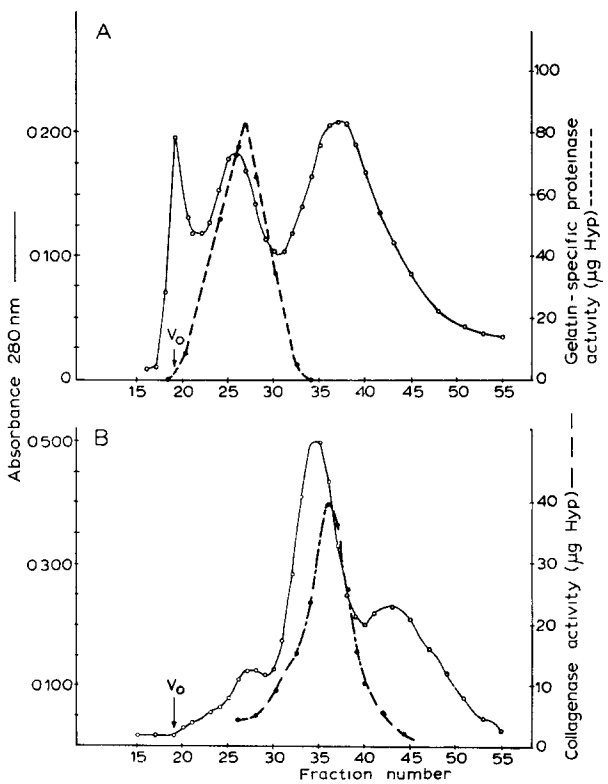


Fig 2 Gel filtration of the human leucocyte gelatin-specific proteinase (A) and collagenase (B) on Sephadex G-200 A partially purified gelatin-specific proteinase (13 mg of protein, Fractions 119–127 from DEAE-Sephadex A-50) and collagenase (16 mg of protein, Fractions 102–109 from DEAE-Sephadex A-50) were applied separately to a 2 cm × 75 cm column and eluted with 0 01 M Tris–HCl buffer (pH 7 6), containing 0 005 M $CaCl_2$ and 0 2 M NaCl at a flow rate of 12 ml/h Effluent fractions of 3 5 ml were collected and gelatin-specific proteinase and collagenase activities assayed as described under Methods

TABLE I

PURIFICATION OF HUMAN LEUCOCYTE GELATIN-SPECIFIC PROTEINASE

Specific activity refers to the μg of hydroxyproline solubilized per 1 mg of enzyme protein in the cold 15% trichloroacetic acid after a 15-h incubation at 37 °C

Purification step	Total protein (mg)	Spec act (μg hydroxyproline)	Total activity
Expt I			
Leucocyte homogenate	250 0	40 0	10 000
$(NH_4)_2SO_4$ ppt	106 0	88 0	9 328
DEAE-Sephadex A-50	16 0	200 0	3 200
Sephadex G-200	2 7	4000 0	10 000
Expt II			
Leucocyte homogenate	250 0	46 3	11 575
$(NH_4)_2SO_4$ ppt	143 0	41 0	5 863
DEAE-Sephadex A-50	20 0	473 0	9 460
Sephadex G-200	3 9	2200 0	8 580
Expt III			
Leucocyte homogenate	160 0	6 5	1 040
DEAE-Sephadex A-50	20 0	29 1	582
Sephadex G-200	1 7	1400 0	2 380

During the last step of purification the total activity was increased indicating the probable presence of a latent form of the enzyme in the preparation The latent form of the enzyme can be considered as an inactive complex of the enzyme or its zymogen which can autoactivate itself [18] Our preliminary results (unpublished) indicate that separation on Sephadex G-200 did not result in splitting off any inhibitor of gelatin-

TABLE II

ACTIVATION OF LATENT GELATIN-SPECIFIC PROTEINASE BY TRYPSIN, CHYMOTRYPSIN AND AN ACTIVATOR FROM THE RHEUMATOID SYNOVIAL FLUID

150 μg of partially purified gelatin-specific proteinase (a preparation rich in the latent form of the enzyme) was preincubated with activators for 1 h at 37 °C Thereafter trypsin and chymotrypsin were inhibited with 200 μg/ml of soybean trypsin inhibitor and the system was supplemented with 0 75 mg (100 μg of hydroxyproline) of thermally denatured collagen and incubated for an additional 15 h After incubation the hydroxyproline in the material which was soluble in 15% trichloroacetic acid was determined

Addition	Activity of gelatin-specific proteinase (μg hydroxyproline)
None	3 0
Trypsin (5 μg/ml)	85 0
Chymotrypsin (5 μg/ml)	15 6
Activator from rheumatoid synovial fluid (5 μg/ml)	85 0
Activator from rheumatoid synovial fluid, alone	0 0

specific proteinase This leaves us with a supposition that autoactivation of zymogen is likely to account for the increase in activity during the purification procedure

Pretreatment of the crude preparation or purified enzyme with trypsin, chymotrypsin or activator from rheumatoid synovial fluid also resulted in an increase of the activity of gelatin-specific proteinase In this respect trypsin was equally effective as an activator from rheumatoid synovial fluid, and chymotrypsin was less effective (Table II) It should be added that the extent of activation of gelatin-specific proteinase varies depending on the proportion of the latent form present in the preparation of the enzyme and on the purity and activity of the activator from rheumatoid synovial fluid

Properties of gelatin-specific proteinase

The gelatinolytic specificity of the enzyme was demonstrated by its action toward thermally denatured collagen and to a lesser extent toward commercial gelatin (azocoll) Other substrates, specific for elastase, trypsin or bacterial collagenase were practically not susceptible to the action of gelatin-specific proteinase (Table III) Ad-

TABLE III

ACTIVITY OF PARTIALLY PURIFIED HUMAN LEUCOCYTE GELATIN-SPECIFIC PROTEINASE TOWARD DIFFERENT SUBSTRATES

Gelatin-specific proteinase, trypsin or bacterial collagenase was incubated with the indicated amount of appropriate substrate in 0 01 M Tris–HCl buffer (pH 7 6)–0 005 M $CaCl_2$–0 2 M NaCl for 15 h at 37 °C In experiment with elastin 0 2 M Tris–HCl buffer (pH 8 8) was used The activity of the enzymes was measured as indicated and described in Methods — not measured

Substrate	Amount added (mg)	Enzyme activity measured as	Gelatin-specific proteinase		Trypsin 5 μg	Bacterial collagenase 5 μg
			10 μg	100 μg		
Gelatin (thermally collagen)	0 75	μg hydroxyproline	50 0	—	82 0	—
Pz-peptide	0 20	$\Delta A_{320\ nm}$	0 02	0 03	—	0 60
0 6% casein	6 0	$\Delta A_{280\ nm}$	0 00	0 10	0 500	—
5 0% casein	100 0	$\Delta A_{280\ nm}$	0 00	—	0 570	—
Hemoglobin	100 0	$\Delta A_{650\ nm}$	0 02	0 03	0 800	—
Histones	20 0	$\Delta A_{700\ nm}$	0 03	0 11	0 800	—
Azocoll	15 0	$\Delta A_{520\ nm}$	0 02	0 65	2 400	—
Elastin	50 0	$\Delta A_{560\ nm}$	0 00	—	—	—

ditional evidence for the gelatinolytic action of the enzyme was obtained by demonstrating that the reaction products are composed of small peptides migrating near the solvent front in disc-gel electrophoresis (Fig 3, 15-h incubation) and well separated from the substrate and the enzyme itself by molecular-sieve chromatography on a Sephadex G-75 column (Fig 4)

The homogeneity of the final preparation of gelatin-specific proteinase was checked by disc gel electrophoresis Fig 5 indicates that the enzyme preparation was quite homogenous showing only a single protein band

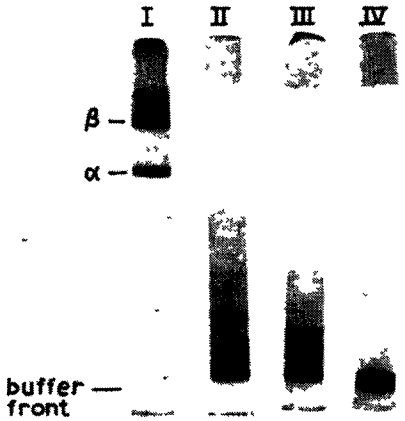


Fig 3 Polyacrylamide-gel electrophoresis of thermally denatured collagen before (I) and after 6 h (II), 9 h (III) and 15 h (IV) of incubation with gelatin-specific proteinase 0 75 mg of thermally denatured collagen was mixed with 30 μg protein of the enzyme in 1 ml of 0 01 M Tris–HCl buffer (pH 7 6), containing 0 005 M $CaCl_2$ and 0 2 M NaCl and incubated at 37 °C After incubation the mixture was acidified with acetic acid to pH 4 0 and applied to the disc-gel electrophoresis The electrophoresis was carried out as described by Nagai et al [26]

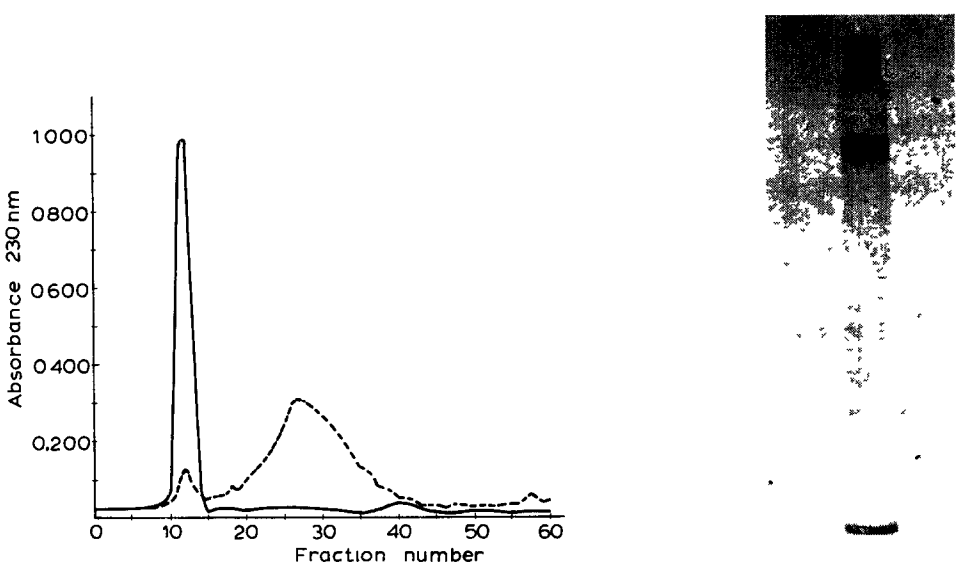


Fig 4 Molecular sieve chromatography on Sephadex G-75 of thermally denatured collagen before and after a 15-h incubation with gelatin-specific proteinase 5 mg of thermally denatured collagen was mixed with 100 μg of enzyme protein in 4 ml of 0 01 M Tris–HCl buffer (pH 7 6) containing 0 005 M $CaCl_2$ and 0 2 M NaCl and incubated at 37 °C After incubation the mixture was applied to the 1 4 cm × 75 cm column of Sephadex G-75 and eluted with the same buffer Effluents were monitored for protein ($A_{230\ nm}$) ——— substrate (thermally-denatured collagen), — — — products

Fig 5 Polyacrylamide-gel electrophoresis of purified human leucocyte gelatin specific proteinase 12 5% gels were used at 5 mA/tube A 50-μg protein of purified gelatin-specific proteinase sample was applied

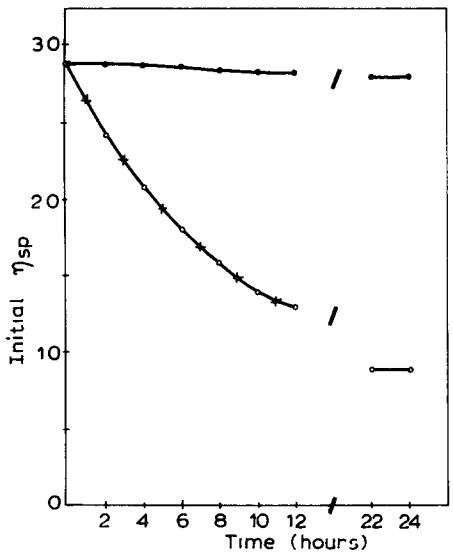


Fig 6 Changes in the viscosity of the collagen solution (0 1% in 0 05 M Tris–HCl buffer (pH 7 6), containing 0 005 M $CaCl_2$ and 0 3 M NaCl) due to the activity of (○) collagenase (150 μg), (●) gelatin-specific proteinase (150 μg), or (×) collagenase and gelatin-specific proteinase Measurements were performed at 26 °C in an Ostwald viscometer

The specificity of this preparation toward native collagen and collagen fragments resulting from the action of collagenase on collagen was estimated by measuring the viscosity of a system incubated with a stepwise increased temperature Fig 6 shows that gelatin-specific proteinase was ineffective toward native collagen at 26 °C Viscosity changes brought about by collagenase at this temperature were not affected by the addition of gelatin-specific proteinase At higher temperatures the viscosity curve changed its shape (Fig 7) when the system contained gelatin-specific proteinase

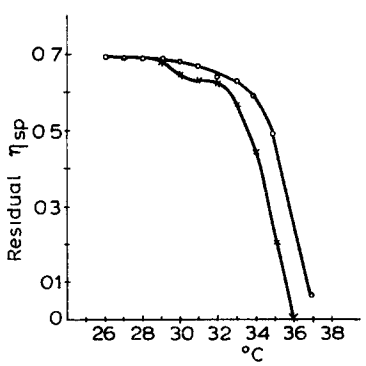


Fig 7 Temperature-dependent effect of human leucocyte collagenase and leucocyte gelatin-specific proteinase on the residual specific viscosity (η sp) following the reaction of collagen in solution at 26 °C with leucocyte collagenase A 0 1% collagen solution in 0 05 M Tris–HCl buffer (pH 7 6), containing 0 005 M $CaCl_2$ and 0 3 M NaCl was incubated with 150 μg collagenase for 24 h at 26 °C Thereafter the incubated sample was divided into two parts One was supplemented with 50 μg of gelatin-specific proteinase (×) while the second with a new portion of collagenase (○) The temperature of the samples was increased stepwise by 1 °C every 15 min and the viscosity measured

TABLE IV

PROPERTIES OF THE HUMAN LEUCOCYTE GELATIN-SPECIFIC PROTEINASE

0 9 mg of thermally denatured collagen (120 μg Hyp) was incubated with 20 μg of enzyme protein and the indicated final concentration of inhibitors or activators in a Tris–HCl buffer (pH 7 6), containing 0 005 M $CaCl_2$ and 0 2 M NaCl The activity was measured as μg Hyp released after a 15-h incubation at 37 °C

Additions	Concn	Enzyme activity (μg hydroxyproline)	Enzyme activity (%)
None		61 0	100 0
EDTA	10 mM	0 0	0 0
Dithiothreitol	10 mM	2 2	3 6
Human serum	diluted 1 5	4 4	7 2
Human serum	diluted 1 10	11 1	18 2
Cysteine	5 mM	30 5	50 0
DFP	1 mM	46 2	75 7
DFP	2 mM	46 2	75 7
Soybean trypsin inhibitor	0 1 mg/ml	51 0	83 6
Soybean trypsin inhibitor	0 2 mg/ml	47 3	77 5
p-Chloromercuribenzoate	0 025 mg/ml	100 0	164 0
Iodoacetamide	0 025 mg/ml	112 5	184 0

Under this condition the macromolecular products of collagenase action on collagen were denatured and degraded further by gelatin-specific proteinase causing a faster decrease in the viscosity of the system

The effect of inhibitors and activators on the activity of gelatin-specific proteinase was estimated using compounds known to affect proteinases The results obtained are presented in Table IV Complete inhibition of the activity was attained at a 10 mM concentration of EDTA A similar effect was observed when dithiothreitol was used Inhibition by cysteine was distinctly lower The stimulation of gelatin-specific proteinase was observed when *p*-chloromercuribenzoate or iodoacetamide were used A quite effective inhibition of gelatin-specific proteinase activity was obtained sup-

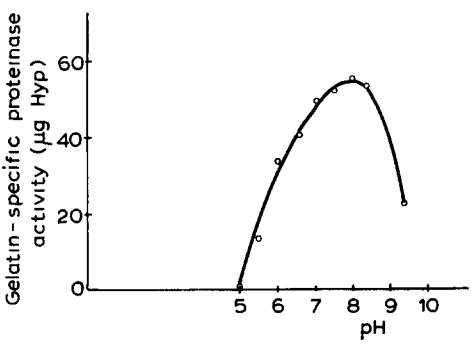


Fig 8 The pH dependence of the activity of gelatin-specific proteinase Buffers used to obtain the appropriate pH values in the range of 5 0–9 3 were Tris–maleate buffer (pH 5 0–7 0), Tris–HCl buffer (pH 7 5–8 35) at a final concentration of 0 2 M and glycine–NaOH (pH 9 3) at 0 1 M The reaction mixtures consisted of 15 μg of enzyme protein and 0 75 mg of thermally denatured collagen (100 μg Hyp)

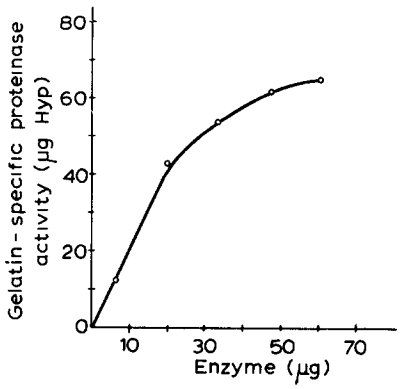


Fig 9 Proteolytic activity as a function of gelatin-specific proteinase concentration The reaction mixtures consisted of 0 75 mg of thermally denatured collagen (100 μg Hyp) and varying concentrations of gelatin-specific proteinase (from 6 5 to 60 μg of protein)

plementing the incubation system with human blood serum (1 5 or 1 10 dilution) DFP and soybean trypsin inhibitor were shown to have a small effect on the activity of gelatin-specific proteinase

The pH optimum for the activity of gelatin-specific proteinase was at 7 8 (Fig 8) The enzyme was not active at pH lower than pH 5 0 The activity was proportional to the enzyme concentration (Fig 9) The linear portion of the curve was utilized in most of the other determinations of the enzyme activity

The activity of gelatin-specific proteinase at 37 °C is stable No decline was observed up to 15 h of incubation (Fig 10)

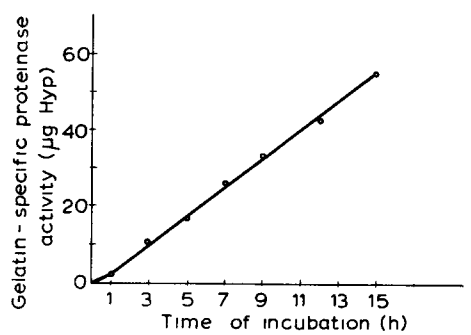


Fig 10 Time dependence curve of the activity of gelatin-specific proteinase The reaction mixture containing 0 75 mg of thermally denatured collagen (100 μg Hyp) and 15 μg of enzyme protein was incubated at 37 °C for different time periods After incubation the material soluble in cold 15% trichloroacetic acid was determined as described under Methods

DISCUSSION

In 1962 Gross and Lapiere [19] demonstrated, for the first time, the existence of a specific tissue collagenase catalyzing the degradation of collagen in vivo Shortly thereafter, tissue collagenase has been purified and shown to be active toward native collagen and less specifically toward denatured collagen or its fragments It was then hypothesized that another enzyme must accompany collagenase in order to accom-

plish the degradation process started by collagenase This hypothesis has been confirmed in the case of tadpole explant tissue [5], rheumatoid synovial tissue [2] and rheumatoid subcutaneous nodule tissue [3] All these tissues release into culture medium neutral proteinase which can be separated from collagenase and which is active toward denatured collagen or Pz-peptide

In this work a similar proteinase has been demonstrated to be present in human leucocytes Gelatin-specific proteinase from human leucocytes has been isolated and purified In many respects the enzyme is similar to neutral gelatin-specific proteinases isolated from other sources It is active over a pH range from 6 to 9 with an optimum at 7 8 and no activity below pH 5 It is inhibited by EDTA, thiol reagents and human blood serum and slightly susceptible to inhibition by soybean trypsin inhibitor or DFP

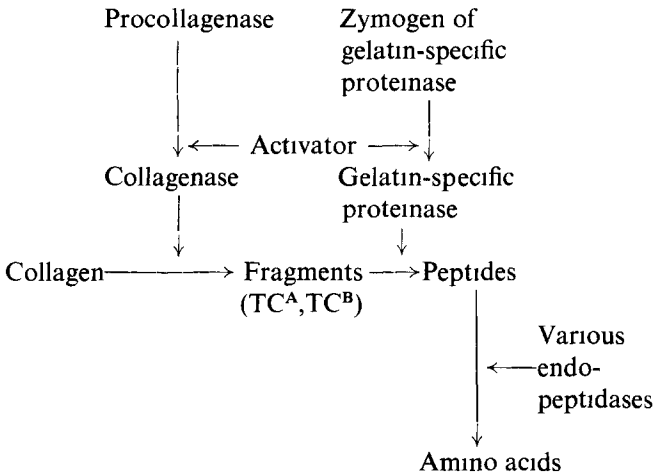
Gelatin-specific proteinase has no activity toward native collagen or its undenatured fragments produced by the action of collagenase at 26 °C Thermal denaturation of these fragments or collagen renders them susceptible to the action of gelatin-specific proteinase A commercial gelatin (azocoll) is much less susceptible to gelatin-specific proteinase and Pz-peptide is not attacked by the enzyme The latter observation is at variance with other neutral proteinases which have been shown to attack not only gelatin but also Pz-peptide The fact that our gelatin-specific proteinase does not attack elastin indicates that the enzyme is not identical with granulocyte elastase as has been suspected by Lazarus et al [6] for neutral proteinase present in granulocytes Comparison of the properties of granulocytic elastases ascertained recently by Ohlsson and Olsson [20] with the properties of gelatin-specific proteinase demonstrates that both enzymes cannot be identical

In spite of the difference in substrate specificity it seems justified to consider the human leucocyte gelatin-specific proteinase as belonging to the same group of neutral proteinase accompanying collagenase and accomplishing collagenolysis started by splitting collagen into two fragments by collagenase

The question which still remains unanswered is how the activity of gelatin-specific proteinase is controlled in vivo The fact that human blood serum inhibits collagenase and gelatin-specific proteinase activities leads to the suggestion that the reversible complexing of these enzymes with α_2-macroglobulin and/or α_1-antitrypsin can play a role in the mechanism of regulating and controlling collagenolysis [21, 22]

Another mechanism which should be considered involves the conversion of zymogens of the enzymes into active forms by the action of a specific agent released or produced during demanding situations Such a situation seems to exist in rheumatoid arthritis where the synovial fluid contains the agent acting both on the zymogen of leucocyte collagenase [8] and the zymogen of gelatin-specific proteinase The production of an activator and procollagenase by tadpole tissue explants in culture has been demonstrated by Harper and Gross [23] Bone collagenase is also produced from procollagenase and for this process an additional agent, probably some kind of proteinase is needed [24] The proteolytic nature of this agent has been inferred from the fact that procollagenase has been activated by trypsin and by a proteolytically active preparation from lysosomes Conversion of leucocyte procollagenase to collagenase by trypsin has also been demonstrated [25] while in this paper evidence for the conversion of zymogen of gelatin-specific proteinase to its active form by trypsin, chymotrypsin and activator from rheumatoid synovial fluid has been presented

Evidence is thus accumulating which points out that the sequence



seems to be a mechanism acting in a situation of increased collagen degradation due to physiologic or pathologic demand It remains to be demonstrated that this supposition is true It poses also another, quite important question concerning the origin and nature of the activator The activator isolated from rheumatoid synovial fluid has been studied by our group and the results will be published elsewhere

ACKNOWLEDGEMENTS

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