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SOME PROPERTIES OF LATENT COLLAGENASE FROM HUMAN SYNOVIAL FLUID *

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Latent collagenase has been isolated in pure form from the rheumatoid synovial fluid. The final preparation, activated by trypsin, yielded a collagenase of specific activity 2 227 units/mg. Electrophoresis in sodium dodecyl sulfate polyacrylamide gels revealed a protein doublet of 54 and 50 kDa. Trypsin or $HgCl_2$ activation resulted in disappearance of the doublet and emergence of a new doublet of 47 and 43 kDa. The latent collagenase could also be activated by leucocyte cathepsin G or plasmin. Neither the latent nor the active collagenase from synovial fluid showed any cross-reactivity with the antibodies against leucocyte collagenase. The trypsin activated collagenase degraded collagen type I, II, III giving typical cleavage products but did not degrade type IV and V collagen.

Collagen degradation in rheumatoid arthritis has been attributed to collagenase (E.C. 3.4.24.7) which may arise from both proliferative lesion or inflammatory cells in the joint cavity [1].

There is general agreement that collagenase are synthesized and released in the latent form [2]. Activation can be achieved in cell-free systems with proteinases, mercurial compounds or chaotropic agents [3, 4, 5].

We have found that rheumatoid synovial fluid contains inactive, trypsin activable collagenase [6]. Since previous attemps at studying the properties of the enzyme have been made using partially purified preparations, we report here on some biochemical characteristics pure synovial fluid collagenase, and present further evidence for the enzyme not being granulocytic origin.

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MATERIALS AND METHODS

Enzyme preparation. Latent synovial fluid collagenase was isolated from cell-free rheumatoid synovial fluid as described previously [6] except that Heparin-Sepharose chromatography was included for further enzyme purification.

Collagen substrate. Acid soluble calf skin collagen was isolated and purified by the method of Kang *et al.* [7].

Enzyme assay. Collagenase assay, activation of latent collagenase and protein determination were carried out as described in a previous paper [6]. One unit of latent collagenase activity was the amount of the latent enzyme which after full trypsin activation hydrolysed 1 μg collagen gel per minute at 37°C.

Electrophoretic techniques. SDS-polyacrylamide gel electrophoresis was performed by the method of Weber & Osborn [8] or Laemmli [9] using 7% gels. Protein samples were denatured in 1% SDS and reduced in 5% 2-mercaptoethanol at 100°C. Phosphorylase b (94000 Da), bovine serum albumin (68000 Da), ovalbumin (43000 Da), carbonic anhydrase (30000 Da) and soybean trypsin inhibitor (20100 Da) were used as standards for determination of molecular mass of proteins.

Western immunoblots. Immunologic blot analysis was performed after SDS-polyacrylamide gel electrophoresis and electrophoretic transfer [10] of proteins to PVDF blotting membrane (Millipore) for 1 h at 150 mA using the Biometra Fast Blot System. The blotting membrane was incubated with antibodies against human leucocyte collagenase. Another antibody used was goat anti rabbit IgG-alkaline phosphatase conjugate (Sigma); the blot was developed and stained with 5-bromo-4-chloro-3-indoyl phosphate and nitroblue tetrazolinum.

RESULTS AND DISCUSSION

Collagenase was purified from synovial fluid mainly in the latent form. The final preparation activated with trypsin had a specific activity of 2227 units/mg representing about 9000 fold purification with a 20% recovery. The purification procedure is shown in Fig. 1.

Electrophoresis of the latent preparation in SDS-polyacrylamide gel revealed a protein doublet at 54 and 50 kDa. Trypsin treatment resulted in full activation of latent collagenase and emergence of a new doublet of 47 and 43 kDa (Fig. 2). Incubation of latent enzyme with $HgCl_2$ brought about incomplete collagenase activation. The upper doublet became converted to the two lower molecular mass species which corresponded to those produced by trypsin (Fig. 2); however, conversion of the 54 kDa form to lower molecular mass species was slower. Thus, trypsin or $HgCl_2$ treatment resulted in the loss

of an about 10000 Da unit from each component of the upper doublet leading to the formation of the lower doublet.

From these results it appears that the upper doublet represents precursor forms, while the lower one consists of enzyme that had been activated by trypsin or $HgCl_2$. Electrophoresis of purified collagenase preparation stored at

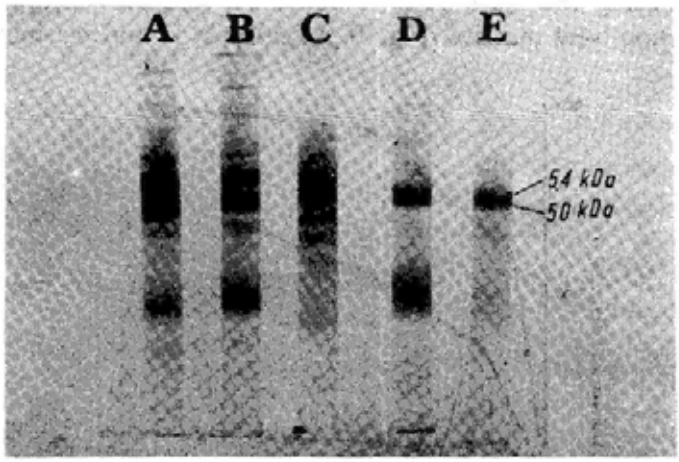

Fig. 1. SDS-polyacrylamide disc gel electrophoresis of samples from each stage of the collagenase purification. A, synovial fluid (120 μg); B, $(NH_4)_2SO_4$ ppt, 50% saturation (100 μg); C, Sephadex G-150 (40 μg); D, DEAE Sephadex A-50 (30 μg); E, Heparin-Sepharose (10 μg). The gels were stained with Coomassie brilliant blue R-250

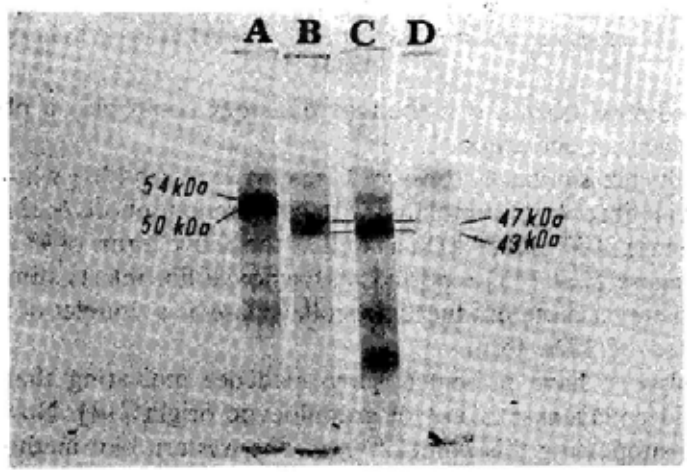

Fig. 2. SDS-polyacrylamide disc gel electrophoresis of latent and active forms of collagenase. A, latent collagenase (20 μg); B, latent collagenase (20 μg) treated with trypsin (1 μg) for 5 min at 20°C then trypsin inhibited by diisopropylfluorophosphate (DFP) (2 mM); C, latent collagenase (20 μg) treated with $HgCl_2$ (0.2 mM) for 1 h at 20°C; D, trypsin (1 μg)

process can take place. This is in accordance with the mechanism proposed by Grant *et al.* [11] assuming that both trypsin and mercurial compounds activate procollagenase by proteolytic cleavage (trypsin) or conformational rearrangement (mercurial salts) initiating an intramolecular autoproteolytic reaction resulting in the formation of lower molecular mass enzyme.

Latent collagenase was also activated by plasmin and leucocyte cathepsin G, the latter being the most effective activator. In contrast, thrombin and leucocyte elastase were inactive (Fig. 3). As catepsin G and plasmin are often

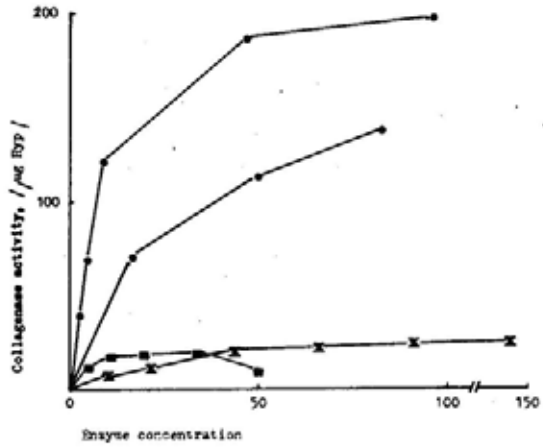

Fig. 3. Activation of latent synovial fluid collagenase with proteinases. Latent collagenase (2 μg) was treated with cathepsin G, ● ; plasmin, ○; or elastase, x; for 20 min at 20°C at concentrations shown (μg/ml), or with thrombin, ■; (units/ml). The reaction was stopped by adding DFP to 2 mM concentration and collagenase was then assayed and expressed as μg of hydroxyproline (Hyp) released

present in inflamed tissues, it is thought that they could play a physiological role in collagenase activation.

Our results are similar to those of Unemori & Werb [12] who found that rabbit synovial fibroblasts stimulated with calcium ionophore A 23187 secreted a procollagenase of 57 and 53 kDa as well as the active forms of 47 and 43 kDa. Likewise, Nagase *et al.* [13] reported that synovial fibroblasts stimulated with phorbol miristate acetate produced procollagenase as a doublet of 53 kDa and a glycosylated 57 kDa form.

Previously, we have presented some evidence indicating that the latent synovial fluid collagenase is not of granulocytic origin [14]. Now we report further data supporting this point of view. The western blot method was used to study the immunologic cross-reactivity of synovial fluid collagenase with specific antibodies against human leucocyte collagenase. Neither the latent nor the active collagenase from synovial fluid showed any cross-reactivity with the antibody against the leucocyte enzyme (Fig. 4). Considering the molecular

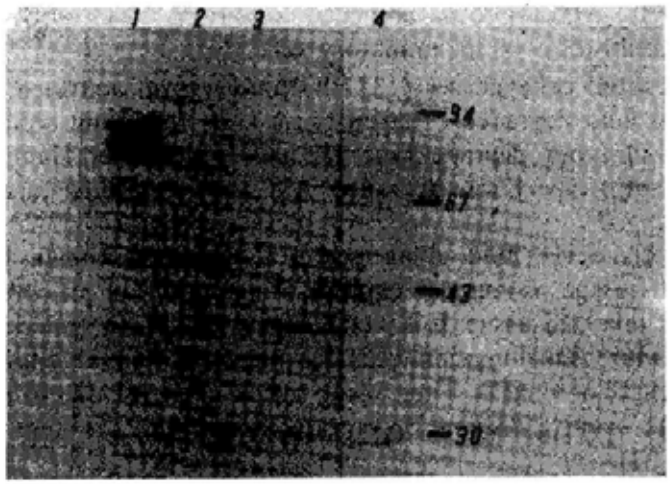

Fig. 4. Western blot analysis of immunologic cross-reactivity of synovial fluid collagenase with antibodies against leucocyte collagenase. Human leucocyte collagenase, 1; synovial fluid collagenase, latent, 2; and active, 3; molecular mass markers ($\times 10^{-3}$), 4. For details see Materials and Methods

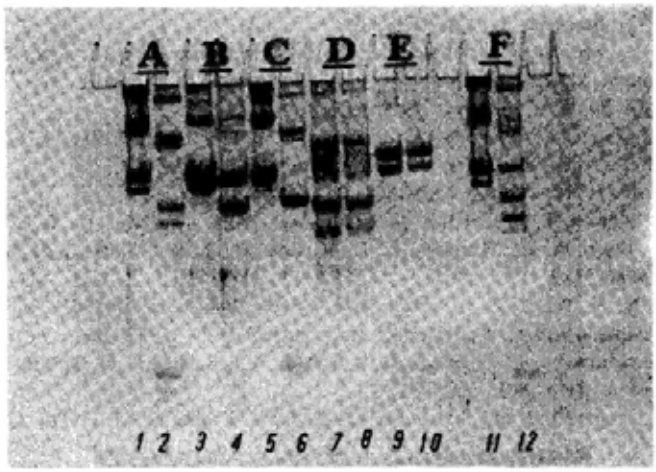

Fig. 5. Degradation of type I-V collagens and gelatin type I by synovial fluid collagenase. Collagens (40 μg) type I, A; type II, B; type III, C; type IV, D; type V, E; and gelatin (40 μg) type I, F; were incubated without (1, 3, 5, 7, 9, 11) or with trypsin-activated collagenase (2, 4, 6, 8, 10, 12) at 27°C for 30 min. Portions (5 μl) of reduced samples were analysed by SDS-polyacrylamide slab gel electrophoresis

properties of enzyme and the immunological data we conclude that the latent collagenase present in synovial fluid is not of leucocytic origin but probably derives from synovial cells. Recent findings show that these cells in culture are stimulated to secrete latent collagenase by soluble factors released from monocyte-macrophages [15], granulocytes [16] and mast cells [17]. These

observations are relevant to pathological conditions which are characterized by massive infiltration of inflammatory cells.

Like interstitial collagenases [18], the pure trypsin activated collagenase from synovial fluid degraded collagen types I, II, III giving typical cleavage products but did not degrade type IV and V collagen. Under the same conditions gelatin type I was degraded in a way resembling type I collagen (Fig. 5).

The fate of synovial fluid collagenase is at present unknown. It is evident that numerous tissue proteinases capable of activation of procollagenase are present in the inflamed tissue. It is worth noting that when synovial fluids had been frozen before centrifugation (cells disrupted) we were able to isolate only active collagenase of 42 kDa. Thus, it can not be excluded that in pathological conditions the inactive enzyme becomes activated and the joint destruction may follow.

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