DIFFERENCES IN THE PHYSICAL PROPERTIES OF COLLAGENASES ISOLATED FROM RHEUMATOID SYNOVIUM AND HUMAN SKIN

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SUMMARY

During purification of human collagenase from normal skin and rheumatoid synovium differences have been observed with regard to the size and charge properties of the two enzymes. Gel filtration experiments in Sephadex G-100 superfine have allowed molecular weights of approximately 63,000 and 32,000 daltons to be calculated for the skin and rheumatoid synovial enzyme respectively. Ion exchange chromatography using Sephadex QAE, A-50 has shown the rheumatoid synovial enzyme to possess charge properties more basic than that of the skin enzyme. Elution of collagenase activity from $7\frac{1}{2}\%$ polyacrylamide gels has produced no evidence for a 'fast-moving' component of either enzyme.

INTRODUCTION

Enzymes capable of degrading undenatured collagen at neutral pH and physiological ionic strength have been detected in the medium from organ cultures of both rheumatoid synovium (1) and human skin (2). Bauer et al, have purified collagenase from these cultures using gel filtration and affinity chromatography techniques (3,4), and have reported that each tissue produces two collagenases described as 'fast' or 'slow'-moving according to their electrophoretic properties in polyacrylamide gels (3,5). We also have developed purification procedures for the rheumatoid and human skin collagenases (6). Originally it was thought that development of a purification procedure for the rheumatoid collagenase would be facilitated by using the more readily available skin enzyme, especially in view of the reported immunological identity of the two enzymes (3). However, although the enzymic properties of these collagenases are similar (1,2), we have found that they have quite different size and charge characteristics and consequently the purification procedures had to be modified for each enzyme. We report here the differences in physical properties observed during the purification of these two enzymes.

METHODS

Source of Collagenases. The human skin and rheumatoid synovial enzymes

were obtained using tissue culture techniques previously described (1,2). Initial concentration of each collagenase was achieved by ultrafiltration of enzymatically active culture medium.

Purification Procedure. The techniques employed in the purification of each collagenase have comprised gel filtration on Sephadex G-200, ion exchange chromatography using Sephadex QAE, A-50, further gel filtration on Sephadex G-100 superfine, and polyacrylamide gel electrophoresis with subsequent enzyme elution (6,7). All procedures were performed at 0-3°C and ultrafiltration with Amicon PM-10 membranes was used for the concentration of collagenase activity when required.

Ion exchange chromatography using the strongly basic anion exchanger QAE, A-50 revealed differences in the charge properties of the two enzymes. Partially purified enzyme was applied to a resin column equilibrated with 20mM Tris-HCl - 5mM $CaCl_2$ buffer, pH 8.1. Elution with this buffer was followed by a salt gradient elution up to 0.3M NaCl.

Gel filtration using Sephadex G-100 superfine demonstrated a size difference between the two enzymes. Partially purified enzyme was applied to a column measuring 35 x 1.6 cm and eluted with 0.17M NaCl - 20mM Tris-HCl - 5mM $CaCl_2$ buffer, pH 7.6. The elution positions of the protein standards phophorylase a, transferrin, ovalbumin, carbonic anhydrase and trypsin provided a size calibration of the column.

Polyacrylamide Disc Gel Electrophoresis. Samples of each of the partially purified enzymes were applied to polyacrylamide gels that had been polymerized with riboflavin (8,9). These were subjected to electrophoresis at 2° in a Tris-glycine buffer of pH 8.3 using a current of 0.6mA per tube for 12 hours. After electrophoresis one gel of each enzyme was stained with Coomassie Brilliant Blue and another cut into transverse discs using a 'Canalco' gel slicer. Each disc was eluted with 300 µl of 0.17M NaCl - 50mM Tris-HCl - 5mM $CaCl_2$, pH 7.4 by gentle shaking overnight at 2° and the eluate assayed for collagenase activity.

Assays. Collagenase activity was measured by the release of soluble ^{14}C-glycine-containing peptides from thermally reconstituted guinea pig skin collagen (10). Protein was determined by the method of Lowry et al, (11).

RESULTS AND DISCUSSION

Figure 1 demonstrates that the synovial collagenase activity does not bind to the QAE, A-50 resin under the experimental conditions employed. The appearance of the enzyme in the first few eluant fractions provided a good purification step in as much as 95% of the

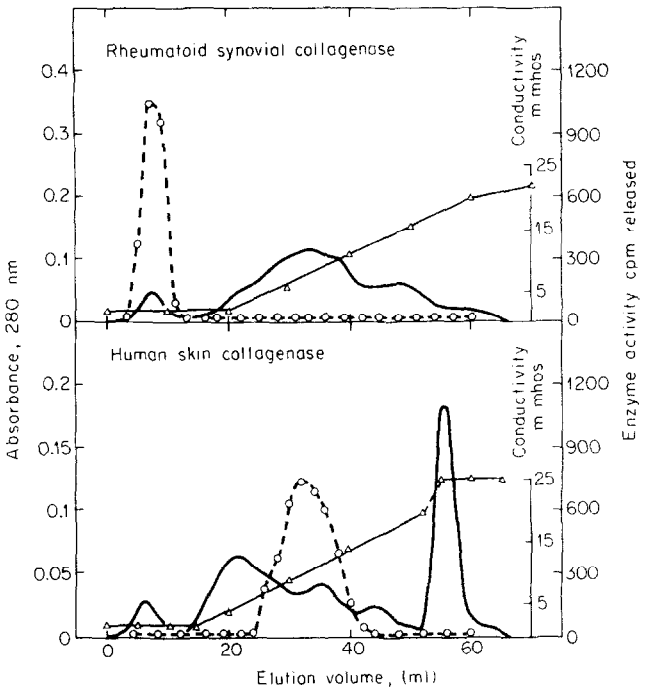

Figure 1. Ion exchange chromatography of the rheumatoid synovial and human skin collagenase on Sephadex QAE, A-50. Each partially purified enzyme, 70 mg skin and 40 mg rheumatoid synovial enzyme, was applied to the resin column measuring 30 x 1.6 and eluted with 20 mM Tris-HCl - 5mM $CaCl_2$, pH 8.1, followed by a linear salt gradient at a flow rate of 20 ml/hr. Eluant fractions of 2 ml were collected and assayed for collagenase activity by incubating 50 µl of eluant on ^{14}C-collagen fibrils for 6 hours at 37°(10). O—O, enzyme activity; ———, absorbance at 280 nm; Δ—Δ, conductivity.

protein sample remained bound to the resin. In contrast, the skin collagenase was bound to the resin under identical conditions and was eluted in a position along the salt gradient between 0.07M and 0.13M NaCl. One of the purification advantages of this resin step is that serum albumin, which we have found to be a major contaminant of skin collagenase preparations, remains bound to the column until eluted with 0.3M NaCl. The behaviour of the two enzymes on Sephadex QAE, A-50 at pH 8.1 suggests that protein charge differences exist, with the rheumatoid synovial enzyme appearing to be more basic than the skin enzyme.

Figure 2 demonstrates the different collagenase activity profiles obtained when each enzyme, partially purified by Sephadex G-200 and QAE, A-50, was subjected to gel filtration on the same column of

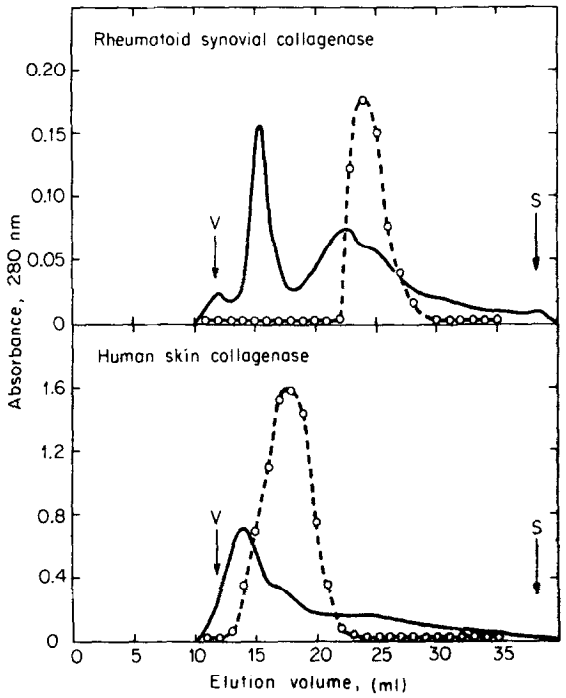

Figure 2. Gel filtration of the rheumatoid synovial and human skin collagenase on Sephadex G-100 superfine. A partially purified collagenase preparation from skin (10 mg) and rheumatoid synovium (2 mg) was applied to a column measuring 32 x 1.6 cm and eluted with buffer containing 0.17M NaCl - 20mM Tris-HCl - 5mM $CaCl_2$, pH 7.6, at a flow rate of 4 ml/hr. Eluant fractions of 1 ml were collected and collagenase activity determined. O- -O, enzyme activity; ———, absorbance at 280 nm; V, void volume; S, salt exclusion volume.

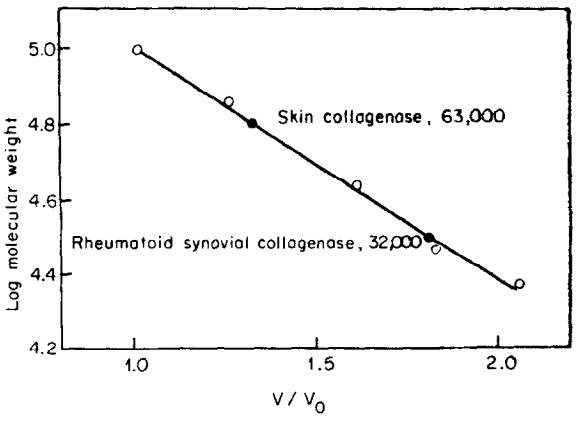

Figure 3. Plot of elution volume/void volume versus log molecular weight for proteins eluted from a Sephadex G-100 superfine column measuring 32 x 1.6 cm. Conditions as for Figure 2. Standard proteins (see Methods) were determined by measuring the absorbance at 280 nm and the collagenases were plotted from their peak of enzyme activity.

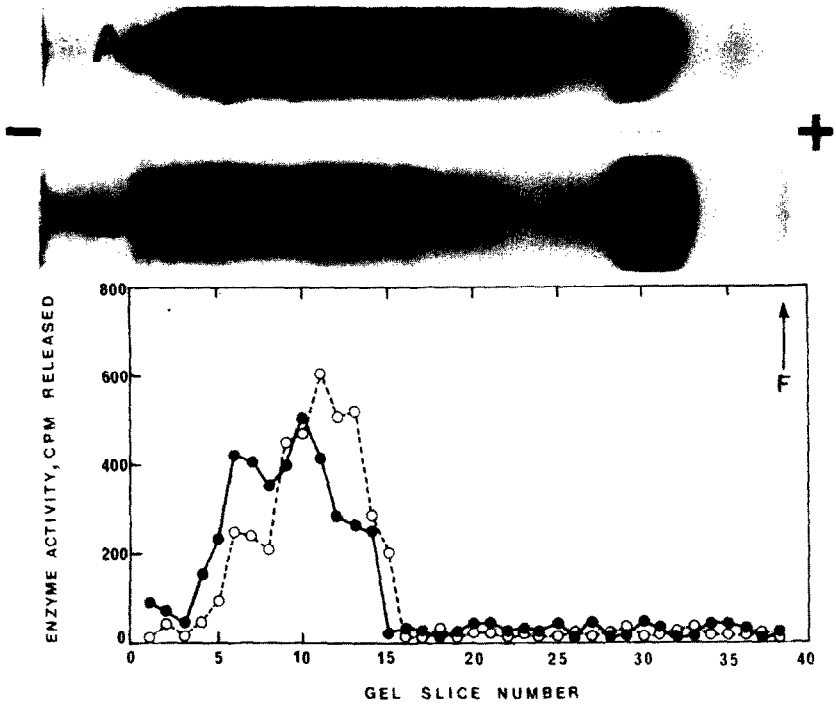

Figure 4. Positions of collagenase activity eluted from $7\frac{1}{2}$% polyacrylamide gels which contained preparations of the rheumatoid synovial (**A**, o- -o) and skin enzymes (**B**, ●—●). 100 µg of each enzyme, partially purified by a Sephadex G-200 gel filtration, was subjected to electrophoresis as described in Methods. F, position of tracking dye.

Sephadex G-100 superfine. Protein standards of known molecular weights were used to calibrate the same column and assuming both enzymes to be globular proteins, the elution positions correspond to molecular weights of 63,000 for the skin enzyme and 32,000 for the rheumatoid enzyme (Figure 3). These values are also supported by molecular weight determinations of the purified enzyme preparations (7) using polyacrylamide gels containing sodium dodecyl sulphate (12). Possible explanations to account for the higher molecular weight of the skin enzyme were that it represented either a polymer of the smaller rheumatoid enzyme or an enzyme-substrate or enzyme-protein complex. However, gel filtration experiments in the presence of dissociating agents such as 0.1% Triton, 6M urea and 1M NaCl failed to alter the molecular size of the skin enzyme (7).

Enzyme preparations partially purified by Sephadex G-200 gel filtration were subjected to disc gel electrophoresis in either $7\frac{1}{2}$ or $10\frac{1}{2}$% polyacrylamide gels in the cold. Subsequent elution experiments showed that both enzyme activities were localised in the upper region

of the gels. No enzyme activity was eluted from the lower regions of the gels which might correspond to the 'fast-moving' skin collagenase purified by Bauer et al (3). Even when culture media concentrates before purification were subjected to electrophoresis no activity was eluted from the lower region.

The two enzymes described here were purified from the large single peaks of collagenase activity obtained from gel filtration on Sephadex G-200 of concentrated tissue culture medium. Further purification using ion exchange chromatography and gel filtration has produced no evidence for more than one skin or rheumatoid synovial collagenase. These findings are thus at variance with those of Bauer et al (3,5), but as yet the reason for the differences has not been elucidated. Further studies are in progress.

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