THE PRESENCE OF COLLAGENASE IN KUPFFER CELLS OF THE RAT LIVER

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Summary: Collagenase was first demonstrated as an active form in the Kupffer cells of the rat liver. The cells were isolated by the method of protease digestion and bovine serum albumin floatation. The activity was approximately three to five times higher than that of the rat granulocytes.

Since Gross and Lapiere (1) succeeded in detecting collagenase activity from the culture medium of the tadpole tailfin, the enzyme has been found also in the culture media of the several mammalian tissues; skin (2), bone (3), uterus (4), cornea (5), gingiva (6) and synovial membrane (7).

In 1968, Lazarus and his co-workers (8) reported the presence of collagenase in human granulocytic leucocytes, which is the only report that has presented an active form of collagenase from the cell in situ.

This paper describes the presence of an active collagenase in the Kupffer cells of the liver. The enzyme activity was also estimated in comparison with that of the granulocytes.

Materials and Methods

Isolation of Kupffer cells: Kupffer cells were isolated according to the Roser's method (9) partially modified as follows: male Wistar rats, each weighing 200±20g, were anaesthetized by an intraperitoneal injection of sodium pentobarbital, and 200 I.U. of heparin was given through the portal vein. The liver then was perfused via the portal vein with 40 ml of Eisen's solution (10), and was quickly excised. About 45g of the livers were cut into 1-2mm^3 pieces and incubated in 300 ml of Eisen's solution containing 0.04% Pronase E (a protease, Kaken Chemical Co., Japan) at 37°C for 20 minutes with

constant shaking. The cell suspension yielded was removed and further 300 ml of the Pronase solution were added. The same procedures were repeated five times. Each of the cell suspensions collected was filtered through 3 sheets of gauze piled on and centrifuged at 300xg for 8 minutes. The cell pellet obtained was washed with Eisen's solution (sample 1) and resuspended in 5 ml of 20% bovine serum albumin solution. The same volume of Eisen's solution was overlaid and centrifuged at 1000xg for 15 minutes. The cell layer at the bovine serum albumin solution-Eisen's solution interface (sample 2) and the residue at the bottom of albumin layer (sample 3) were collected. Each sample was washed with 40 ml of Eisen's solution three times. All the procedures except for incubation were carried out at 4°C.

Separation of granulocytes: The blood was obtained by puncturing the heart of male Wistar rats with a heparinized syringe and the granulocytes were separated by Böyum's method (11) (sample 4).

Method for collagenase assay: The collagenase activity was measured by liberating soluble collagen labeled with ^{14}C-glycine, which was extracted and purified from the guinea pig skin, as a substrate (12). The above labeled collagen was dissolved in the ratio of 0.1% (g/v) in 0.05M Tris-HCl buffer pH 7.4 containing 0.4M NaCl and 5mM $CaCl_2$ and was divided to 0.4ml each in 2.5 ml centrifuge tubes (approximately 2000cpm/tube) and was incubated at 37°C for 12 hours to form collagen gel. The above enzyme samples (1-4) were all homogenized in 1 ml volumes of Eisen's solution with 0.1% Triton X-100 and 0.4 ml of each was added to the tubes of the substrate collagen gel. After incubating at 37°C for 20 hours the contents of the tubes were centrifuged for 15 minutes at 3000 rev./min. Aliquots of the supernatant were transfered in 10 ml of Bray's solution for determining radioactivity on a liquid scintillation counter.

Identification of collagenase: The enzyme sample (sample 2) was purified by affinity chromatography according to the Nagai's method (13), then incubated with acid soluble rat skin collagen at 25°C for 12 hours.

Table 1 Collagenase Activity in Kupffer Cells of the Rat Liver

	Collagenase activity (cpm/mg Prot.)		
	Exp. 1	Exp. 2	Exp. 3
Liver homogenate	0.3	0.7	trace
Cells after protease digestion (sample 1)	55.3	97.5	51.0
Cells at the albumin-Eisen's solution interface (sample 2)	169.2	154.5	168.0
Cells at the bottom of albumin layer (sample 3)	15.8	22.6	27.0

After adding EDTA and denaturing at 60°C for 3 minutes, the reaction products were separated by disc electrophoresis.

Protein determination of the samples: Protein contents in the samples were determined by the Lowry's method (14).

Results

Kupffer cell preparations: The cell pellet obtained following the Pronase digestion mostly consisted of numerous cells not stainable with Trypan blue and some of parenchymal cells and cell debris stainable with Trypan blue. However, after applying the bovine serum albumin floatation method more than 95% of the cells were found to be viable, not stainable with Trypan blue. Although these cells were different in size, they were almost uniform in appearance and containing some granules other than a round nucleus in their cytoplasm. Besides, as the number of granules seemed to be rather higher in proportion to the size of the cytoplasm, these cells were morphologically identified as the Kupffer cells.

Table 2 Collagenase Activity in Kupffer Cells and Granulocytes of Rats

	Collagenase activity (cpm/10^6 cells)	
	Exp. 1	Exp. 2
Kupffer cells	180.3	169.0
Granulocytes	47.4	28.5

Collagenase activity of the samples: As shown in Table 1, collagenase activity was negligible in the homogenate of the liver as a whole, but markedly increased in the sample of the cell pellet obtained after Pronase digestion (sample 1). This activity was more evidently high in the cells at the albumin solution-Eisen's solution interface (sample 2). The collagenase activity was also found in the cells at the bottom of albumin layer (sample 3), but the activity in which was much less than that in the sample 2. These date indicate that the enzyme activity was localized in the Kupffer cells. The collagenase activity per cell in the purified Kupffer cells was approximately three to five times higher than that of the granulocytes (Table 2).

Disc electrophoresis of the reaction products: Disc electrophoretic patterns of the denatured products by incubating collagen with the enzyme (sample 2) are illustrated in Fig. 1. The control pattern consisted of monomers, dimers and specimens of a higher molecular weight, while the pattern treated with the enzyme sample showed α^A, β^A, and some smaller fragments.

Discussion

Resorption of collagen in the experimental fibrosis of the liver has

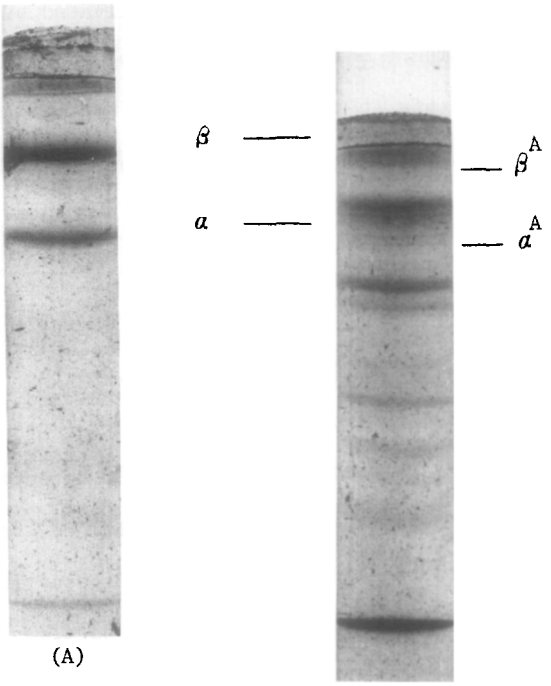

Fig. 1. Disc electrophoretic patterns of the denatured products by incubating collagen with the Kupffer cell collagenase. (A) control collagen (B) the enzyme treated collagen

been widely observed after interupting the administration of the etiologic agent (15). However, the exact mechanism of the regression has not been clarified yet.

Collagenase, which acts on the native fiber under physiological condition, may be reasonably considered to be the first enzyme to demolish collagen (16) in the liver, although there has been no evidence for the presence of collagenase in the liver.

The present data indicate that the collagenase activity is present in the viable cells isolated and purified by the combined protease digestion and bovine serum albumin floatation method. Roser (9) identified the cells obtained by this method as Kupffer cells autoradiographically with radioactive colloidal gold. Our observations also supported his work and views.

Recently the presence of an inactive precursor of bone collagenase was

reported in the tissue culture medium which was activated by trypsin (17). In the concurrent experiments it is unlikely that Pronase activated an inactive form of collagenase in the Kupffer cells, because the Pronase activity was not detectable at all after washing the cell pellet samples with Eisen's solution.

The fact that the enzyme activity found in the Kupffer cells was higher than that of the granulocytes suggests that the collagenase activity is related to phagocytosis and that cells which belong to the reticuloendothelial system have collagenase.

The reason why the collagenase activity was not detected in the homogenate of the liver tissue is not properly explained, but we already observed a masked form of collagenase in the liver tissue which was activated by SCN treatment following the Abe and Nagai's method (18) (unpublished data). Studies concerning a relationship between this form of collagenase in the liver tissue and the collagenase in the Kupffer cells are now in progress.

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