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## Abstract

Type V collagen-degrading enzyme activity was detected as a metalloprotease acting at neutral pH in the human liver. Type V collagen extracted from human placenta and labeled with [1-14C] acetic anhydride was used as the substrate in the assay. Four major degradation products with relatively high molecular weights were observed upon polyacrylamide gel electrophoresis of the incubation mixture of type V collagen and liver homogenate. The significance of the measurement of this enzyme activity was discussed in relation to the clarification of the mechanism of liver fibrosis.

**KEYWORDS:** type V collagen-degrading enzyme activity, human liver, liver fibrosis, collagen degradation products

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— Brief Note —

# Detection of Type V Collagen-Degrading Enzyme Activity in Human Liver

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Type V collagen-degrading enzyme activity was detected as a metalloprotease acting at neutral pH in the human liver. Type V collagen extracted from human placenta and labeled with [1-$^{14}$C] acetic anhydride was used as the substrate in the assay. Four major degradation products with relatively high molecular weights were observed upon polyacrylamide gel electrophoresis of the incubation mixture of type V collagen and liver homogenate. The significance of the measurement of this enzyme activity was discussed in relation to the clarification of the mechanism of liver fibrosis.

*Key words :* type V collagen-degrading enzyme activity, human liver, liver fibrosis, collagen degradation products

Liver fibrosis is a pathological state of collagen deposition in the liver. Recent studies (1, 2) have demonstrated the presence of type IV and V collagens (approximately one-third of the total collagen content) as well as type I and III collagens (two-thirds) in normal liver. In the fibrotic liver, the levels of these types of collagen are high (1). However, type IV and V collagens may play a role different from that of interstitial (type I and III) collagen. Collagen accumulation results from increased production and/or decreased degradation of collagen. However, the mechanism of type IV and V collagen degradation in the fibrotic liver is not known.

Vertebrate collagenase (EC 3. 4. 24. 7) degrades type I and III collagens at a specific site, but does not act on type IV and V collagens. The presence of type V collagen-degrading enzyme has been reported in media from cultured murine reticulum cell sarcoma (3), human leiomyosarcoma (3) and rabbit pulmonary alveolar macrophages (4), and in sputum from patients with chronic obstructive pulmonary disease (5). However, this enzyme has not been detected in the liver. The present paper describes the detection of type V collagen-degrading enzyme activity in the human liver.

Type V collagen was extracted from the placenta using pepsin at an enzyme/substrate ratio of 1 : 400 by weight for 24 h (6). NaCl precipitation at acidic and neutral pHs (pH 2.8 and 7.5, respectively) was performed on the supernatant of the digest. Type V collagen thus purified was labeled with [1-$^{14}$C] acetic anhydride (New England Nuclear) (7). The labeled collagen was stored at −70℃ until use. All procedures were done at 4℃, unless otherwise indicated.

The human liver, obtained by autopsy of

patients within 9 h of death, was homogenized with 4 volumes of 50 mM Tris-HCl buffer (pH 7.5) containing 5 mM $CaCl_2$, 200 mM NaCl and 0.1% Triton X-100 using a Polytron homogenizer at a speed of 18,000 rpm for 2 min. Type V collagen-degrading activity was measured by the method of Liotta *et al.* (3) with some modifications: The liver homogenate was preincubated with 1 mM 4-aminophenylmercuric acetate (APMA) at 37℃ for 1 h to activate the enzyme. Then, 160 $\mu$g of [1-$^{14}$C] type V collagen (124 dpm/$\mu$g collagen) was incubated at 37℃ for 1 h with 8 $\mu$l of the liver homogenate in a final volume of 650 $\mu$l of 50 mM Tris-HCl buffer (pH 7.5) containing 5 mM $CaCl_2$, 200 mM NaCl and 1 mM diisopropyl fluorophosphate (DFP, Sigma). Control tubes contained 10 mM ethylenediamine tetraacetic acid (EDTA) which inhibits the enzyme. The reaction was terminated by adding trichloroacetic acid (final 2%) and tannic acid (0.1%) with bovine serum albumin (20 $\mu$g) as a precipitation carrier. After standing at 4℃ for 30 min, each tube was centrifuged at 3,000 rpm for 30 min and radioactivity of the supernatant was determined with a liquid scintillation counter (Aloka LSC-700). Each assay was run in duplicate.

Type V collagen and its degradation products were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (8) and PAGE (9).

Purified type V collagen showed 2 bands

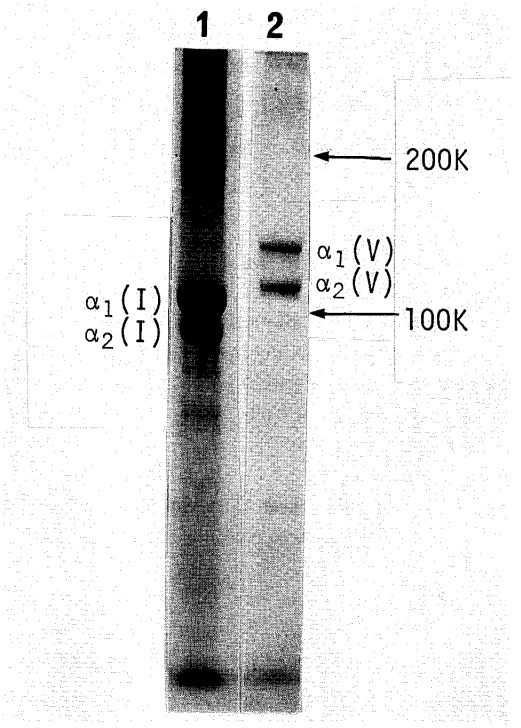
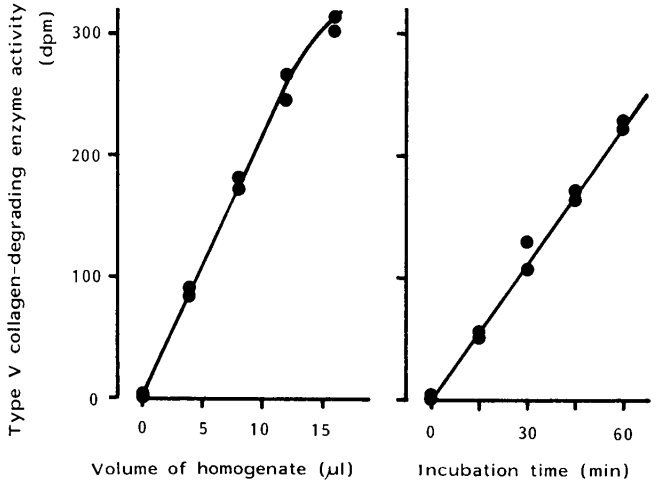



**Fig. 1** SDS-PAGE (7.5% gel) patterns of type I and type V collagens. Untreated collagens (20 $\mu$g each) purified from human placenta were applied: Lane 1, type I collagen; lane 2, type V collagen.

**Fig. 2** Effect of the amount of liver homogenate and the reaction time on type V collagen-degrading enzyme activity.



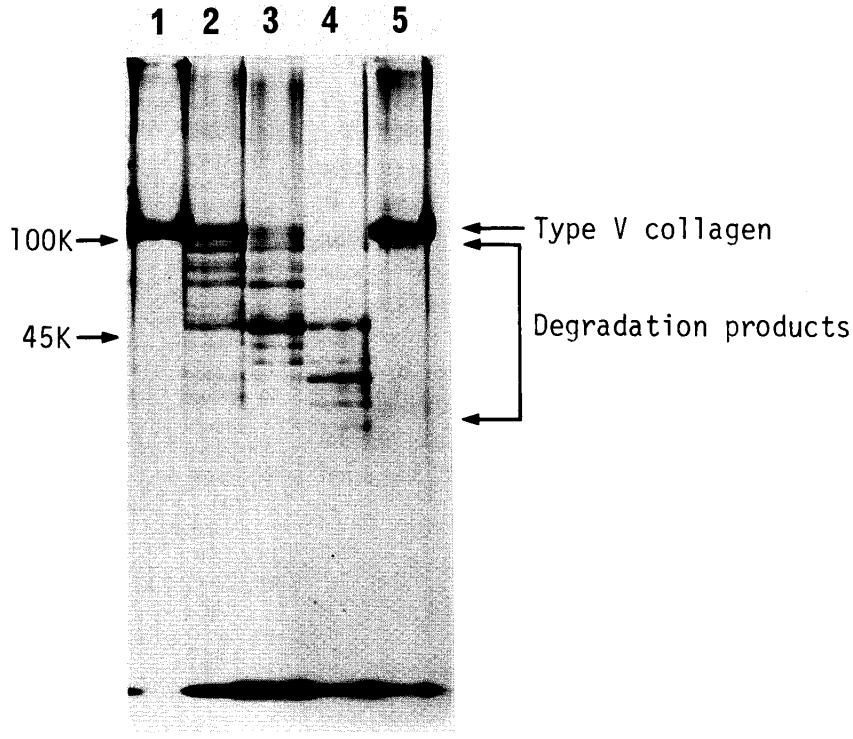



**Fig. 3** PAGE (7.5% gel) patterns of type V collagen treated with human liver homogenate. Purified type V collagen (40 $\mu$g) was incubated with 10 $\mu$l of liver homogenate in the presence of 1 mM diisopropyl fluorophosphate at 37°C. Lane 1, type V collagen alone; lane 2, type V collagen+homogenate for 2 h; lane 3, type V collagen+homogenate for 6 h; lane 4, type V collagen+homogenate for 24 h; lane 5, type V collagen+homogenate in the presence of EDTA for 24 h.

on SDS-PAGE, which corresponded to $\alpha_1$ (V) and $\alpha_2$ (V) (Fig. 1). Type V collagen-degrading enzyme activity was maximally activated when the liver homogenate was preincubated with 1 mM APMA for 1 h. Trypsin also activated the enzyme activity, but not as much as APMA. The velocity of the reaction was proportional to the amount of the homogenate up to 12 $\mu$l and to the reaction time up to 60 min (Fig. 2). The degradation products were examined by PAGE (Fig. 3). Four major degradation products with relatively large molecular weights were seen after 2 h of the incubation of type V collagen with liver homogenate. Two smaller degradation products appeared 6 h after the start of the incubation; further degradation was observed after 24 h. The degradation was completely inhibited by the addition of EDTA as shown in lane 5 of Fig. 3.

Type V collagen-degrading enzyme activity was detected in the human liver. The enzyme was active at neutral pH. The enzyme activity was inhibited by EDTA, indicating that this enzyme is a metalloprotease. Prior activation of this enzyme with APMA was necessary for the measurement of the activity in the liver homogenate, suggesting that the enzyme is present as an enzyme-inhibitor complex *in vivo* (10). Degradation products with relatively large molecular weights, observed after 2 h of the incubation, were similar to those produced by the enzyme in cultured rabbit macrophages (4) or human sputum (5).

Type V collagen was increased in the

sinusoidal area and portal tract in the cirrhotic liver (2). Furthermore, remarkable accumulation of type V collagen started at the early stage of liver cirrhosis (11). However, no study of the degradation of type V collagen in the liver has been reported. In the present study, type V collagen-degrading enzyme activity was detected in the human liver. Measurement of this enzyme activity in various stages of fibrosis may contribute to the clarification of the mechanism of liver fibrosis. The assay method described herein will be useful because the activity can be determined with a small amount of biopsied liver.

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