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DENTAL PULP COLLAGENASE: INITIAL DEMONSTRATION AND CHARACTERIZATION

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SUMMARY

A collagenase, active against native helical collagen, was initially found in the explant medium of bovine dental pulp. In contrast to the collagenases from other oral tissues, all the pulp enzyme released was in a latent form which was activated by trypsin treatment, 4-aminophenylmercuric acetate, and some chaotropic agents. The activated enzyme was inhibited by low concentrations of EDTA and calf serum. The molecular weight of activated enzyme was tentatively estimated at 45,000 daltons by gel filtration. The enzyme attacked undenatured collagen in solution at 20°C producing characteristic products $\alpha^{A}(3/4)$ and $\alpha^{B}(1/4)$.

Since Gross and Lapiere reported a specific collagenase in tadpole tails (1), many mammalian collagenases (E.C. 3.4.24.3) have been identified in a wide variety of animal tissues (2) including skin, bone, uterus, rheumatoid synovium, cornea and gingiva. Recently, we have demonstrated a collagenase in bovine dental sac (3). Mammalian collagenases are capable of degrading native collagen under physiological conditions to produce characteristic fragments: an amino-terminal 75 % fragment and a carboxy-terminal 25 % fragment.

The dental pulp, which is responsible for the formation and maintenance of dentine, contains a relatively small amount (16.8 % wt) of collagen (4). However, it shows fairly high rate of collagen synthesis (5). These results suggest the presence of collagenolytic activity in dental pulp. This paper describes the initial demonstration and some properties of dental pulp collagenase.

Abbreviations used are 4-APMA, 4-aminophenylmercuric acetate; SDS, sodium dodecyl sulfate.

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MATERIALS AND METHODS

[U-^{14}C]glycine (10 mCi/mmole) was obtained from The Radiochemical Centre, Amersham, Bucks, England, 4-Aminophenylmercuric acetate (4-APMA) from Tokyo Kasei Kogyo Co. Ltd., Tokyo, and calf serum from GIBCO, Grand Island, N. Y.

Dental pulps (50 g wet wt) were obtained from unerupted premolars and wisdom teeth in bovine mandibules and explanted in Tyrode's solution (80 ml) containing antibiotics as reported previously (3). The explant media were harvested and replenished every 2 days for 23 days and stored at -20°C.

Collagenase activity was measured according to Terato et al. (6) by the release of ^{14}C-labelled peptide from [^{14}C]glycine labelled collagen in solution (780 cpm/0.4 mg/tube) during 20 hours at 35°C. One unit of collagenase was defined as the activity which hydrolyzed 1 µg of native soluble collagen per min. To demonstrate collagenase digestion products, 7 µg of partially purified pulp collagenase were incubated at 20°C with 500 µg of collagen in 50 mM Tris-HCl buffer, pH 7.8, containing 200 mM NaCl and 5 mM $CaCl_2$ for 63 hours and electrophoresed on sodium dodecyl sulfate (SDS) polyacrylamide gels (7).

Protein was determined by the method of Hartree (8) by using bovine serum albumin as a standard.

RESULTS

The release of protein and collagenase activity into pulp explant medium is shown in Figure 1. In contrast to other oral tissues (3,9), all the pulp collagenase released into the medium was in a latent form which was most effectively activated by 4-APMA (10), a thiol-blocking reagent, at a final concentration of 1 mM. Alternatively, the latent collagenase was activated by trypsin treatment and chaotropic agents such as sodium thiocyanate and sodium iodide (11). Ammonium sulfate fractionation also activated partly the latent enzyme.

The activated pulp collagenase was inhibited by EDTA and calf serum at final concentrations of 10 mM and 10 % (v/v), respectively.

The pooled explant media from day 5 through 21 were combined and partially purified by ammonium sulfate fractionation. The precipitate formed in 60 % saturated ammonium sulfate was dissolved in a minimal amount of 30 mM Tris-HCl, pH 7.8, containing 200 mM NaCl and 5 mM $CaCl_2$, and dialyzed against the same buffer. The molecular weight of the activated collagenase was estimated at 45,000 daltons on Sephadex G-150 column using bovine serum albumin (68,000), ovalbumin (45,000), and myoglobin (17,800) as calibration proteins.

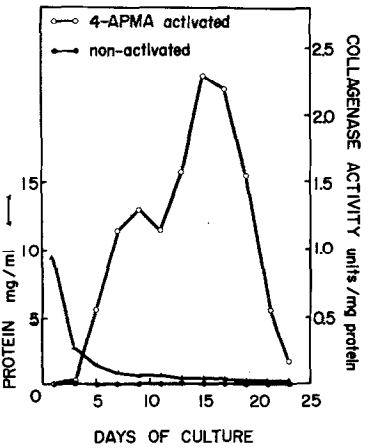

Fig. 1. Protein and collagenase activity released into explant medium from bovine dental pulp (50 g wet wt). For the activation of latent enzyme, 10 mM 4-APMA solution was added to the collagenase assay to give a final concentration of 1 mM.

Two major groups of collagen degradation products corresponding in size to $\alpha^{A}(3/4)$ and $\alpha^{B}(1/4)$ fragments were observed on SDS polyacrylamide-gel electrophoresis (Fig. 2). Electron micrographs of segment-long-spacing aggregates (12) prepared from the enzyme-collagen reaction mixture revealed the presence of an amino-terminal fragment (α^{A}), 75 % the length of the collagen molecule, and a carboxy-terminal fragment (α^{B}) representing the remaining one-quarter.

DISCUSSION

Unexpectedly high collagenolytic activity, which specifically cleaves native helical collagen under physiological conditions, was demonstrated in the explant medium of bovine dental pulp. The activated collagenase is inhibited by EDTA and calf serum which have been regarded as common inhibitors of mammalian collagenases (2). Furthermore, the cleavage pattern revealed by both SDS polyacrylamide-gel electrophoresis and electron micrographs of segment-long-spacing aggregates confirmed that the pulp enzyme was a typical mammalian collagenase.

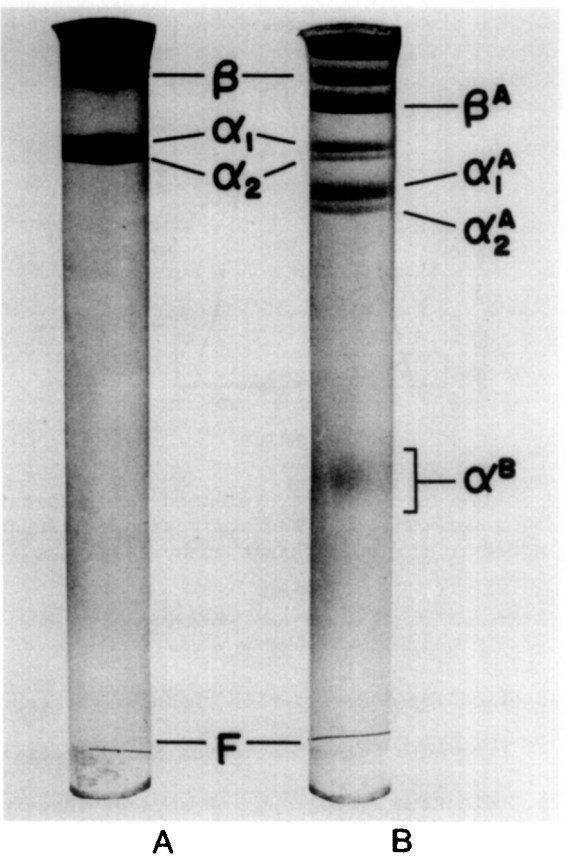

Fig. 2. Gel electrophoresis patterns of collagen degradation products. The control samples without enzyme (A) and the reaction products after exposure to dental pulp collagenase at 20°C (B) were subjected to 7.5 % SDS polyacrylamide-gel electrophoresis. Each sample was equivalent to approximately 80 µg of collagen.

Most of the animal tissues including oral tissues like gingiva (9) and dental sac (3) generally release the active form of collagenase in the late stages of their explant, while all the collagenase found in the explant medium of dental pulp is in a latent form all through the stages of explant.

Histologically, the ground substance of dental pulp contains relatively low amounts of collagen fibril which is much more fine than the ordinary collagen fiber (13). Orlowski (4) confirmed by hydroxyproline analysis that the bovine dental pulp contains low amounts (16.8 % wt) of collagen. However, there have been some reports which suggest the fairly high metabolic activity in the collagen synthesis in rat (5) and bovine pulps (14). The presence of

high collagenase activity in bovine dental pulp seems to explain these two rather conflicting evidences.

In conclusion, there is a typical mammalian collagenase in bovine dental pulp, all of which excreted in explant medium is in a latent form. Further purification and characterization of dental pulp collagenase and inhibitor are in progress.

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