BASAL LAMINA DEGRADATION: THE IDENTIFICATION OF MAMMALIAN-LIKE COLLAGENASE ACTIVITY IN MESENCHYMAL-DERIVED MATRIX VESICLES

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Summary: Isolated matrix vesicles from 26-day embryonic New Zealand White rabbit incisor tooth organs were incubated on ^{14}C labeled reconstituted Type I collagen at 25°C for 18 hours. Under these conditions the matrix vesicles demonstrated the ability to degrade collagen as demonstrated by radioactivity solubilized. Analysis of the released radioactivity using SDS-PAGE demonstrated the presence of two molecular species with molecular weights of 67,000 and 32,000 daltons. These results are in accordance with the presence of a mammalian-like collagenase associated with the matrix vesicles, whose function may be the degradation of the basal lamina.

INTRODUCTION

Developing epidermal organs require a sequence of differentiative events which are mediated by reciprocal interactions between epithelium and mesenchyme. The architecture of various epidermal organs during embryonic development indicates specific spatial restrictions on the types of epithelial-mesenchymal interactions which can occur. Since the epithelia and mesenchymal tissues are separated by a basal lamina, intercellular communication between these heterotypic tissues requires either that a diffusible substance migrate across the basal lamina, or that the basal lamina is selectively degraded to permit direct heterotypic cell-cell contacts.

In many epithelial-mesenchymal systems (1, 2) the basal lamina is degraded *in situ* prior to overt histogenesis and morphogenesis. Banerjee and Bernfield (3) observed that mouse submandibular gland morphogenesis is accompanied by active remodeling of the basal lamina. Recently, Slavkin and Bringas (4) described

basal lamina degradation *in situ* prior to direct heterotypic cell-cell contact and epithelial cell differentiation during embryonic and neonatal incisor and molar tooth organ development.

Using the developing rabbit tooth as a model, we have investigated the mechanism of basal lamina degradation. The extracellular matrix interface of the developing tooth, which separates mesenchyme and epithelium, contains mesenchymal-derived (5) matrix vesicles along the undersurface of the basal lamina. These matrix vesicles have been implicated in epithelial-mesenchymal interactions (5) and as a loci for incipient crystalline calcium hydroxyapatite formation prior to dentine mineralization (6).

One function of matrix vesicles may be the compartmentalization of enzymatic activities which would serve to degrade the basal lamina in a specific topographical location and at a precise time during tooth morphogenesis. Since the basal lamina in the developing tooth is composed of collagenase-labile materials (allegedly Type IV collagen) and proteoglycans, we postulated that if matrix vesicles functioned to degrade the basal lamina, they would possess protease activities. Matrix vesicles located in proximity to the undersurface of the basal lamina, prior to degradaton, contain ATPase and acid phosphatase activity (6, 7). In this preliminary report we demonstrate mammalian-like collagenase activity associated with matrix vesicles isolated from the interface between inner enamel epithelium and adjacent preodontoblasts.

MATERIALS AND METHODS

Preparation of Matrix Vesicles. The matrix vesicle preparations (a matrix vesicle enriched, membrane particulate fraction) were physically isolated from 26-day old New Zealand White rabbit embryos as previously described (7). Briefly, for each experiment 80 to 160 incisor tooth organs were extracted, the dental papillae were removed, the mineralized incisal tip was excised, the resulting matrix specimens were sonicated to remove adhering cells, digested with bacterial collagenase (Worthington CLS-AP, 1 mg/ml) in 0.05 M Tris-0.005 M $CaCl_2$ (pH 7.6), and the resulting supernatant was chromatographed on a Bio-Gel A-50 m column (20 x 0.6 cm) using Tris-$CaCl_2$ as the eluting buffer. The void volume contained matrix vesicles *per se* and membrane particulate structures. The void volume of the column was determined using Blue Dextran 2000 (Pharmacia Fine Chemicals Inc.). Bacterial collagenase alone eluted in the included volume of the column.

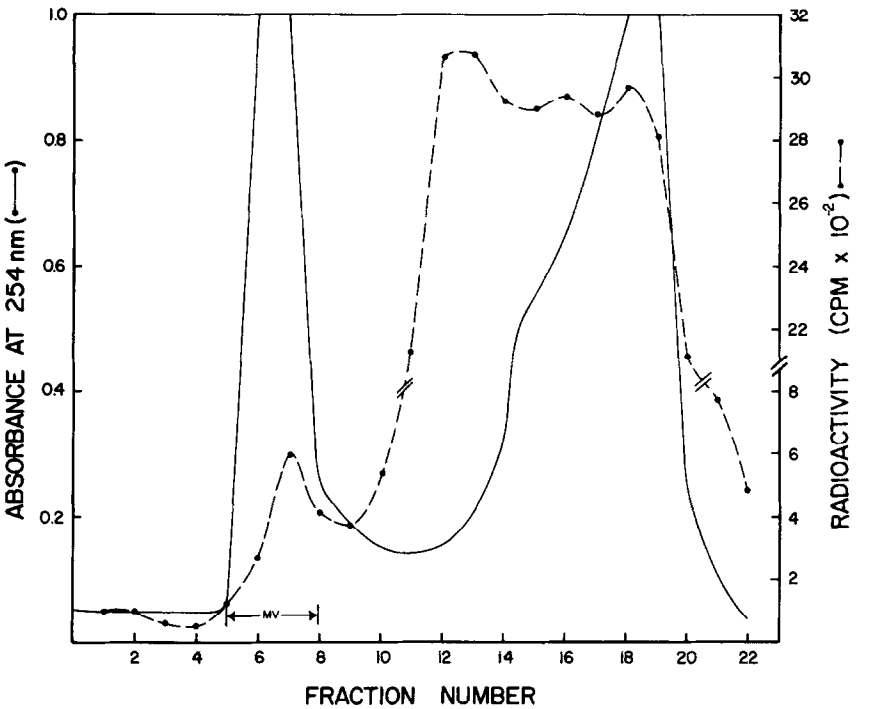

Figure 1 Elution pattern and collagenolytic activity of matrix vesicles of embryonic tooth organ digest eluted from Bio-Gel A-50 m; 0.7 ml fractions collected. ——— Absorbance at 254 nm; o---o Collagenolytic activity as cmp of ^{14}C-collagen/200 µl reaction mixture solubilized after 18 hr incubation at 25°C.

Collagenase Assays. Collagenase activity was assayed by a modification of the radiofibril method of Nagai et al. (8). Collagenase activity was assayed using ^{14}C-proline labeled collagen substrate prepared from the skin of β-aminoproprionitrile-treated five-week old rabbits which had been injected with 200 µCi of ^{14}C-proline (Schwarz-Mann, 260 mCi/mM) on days 11, 13 and 15 after initiation of β-aminopropionitrile treatment (9). The reaction mixture consisted of 0.5 ml reconstituted ^{14}C-labeled collagen in 0.05 M Tris-0.005 M CaCl (pH 7.6), 0.4 ml of each fraction eluted from the column used to isolate the matrix vesicles, and 0.1 ml of a protease inhibitor mixture containing 39 µg of ε-aminocaproic acid, 37 µg of p-tosyllysylchloromethylketone, and 340 µg of p-methylsulphonyl-fluoride.

Each experiment included appropriate buffer blanks and trypsin controls to assess that the collagen used in the assays was not denatured. The reaction mixture was incubated at 25°C for 18 hrs. At the termination of the incubation, the reaction mixture was centrifuged at 5400 x g and 200 µl aliquots of the supernatant were counted for radioactivity in a Beckman LS230 liquid scintillation counter. The remaining supernatant from selected assay samples was dialyzed, lyophylized and analyzed using 5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the method of Furthmayr and Timpl (10). Molecular weights were estimated by comparison to the relative electrophoretic migration distances of the γ(MS 385,000), β_{11}(MW 190,000) and α_1(MW 95,000) bands of Type I collagen used in these studies.

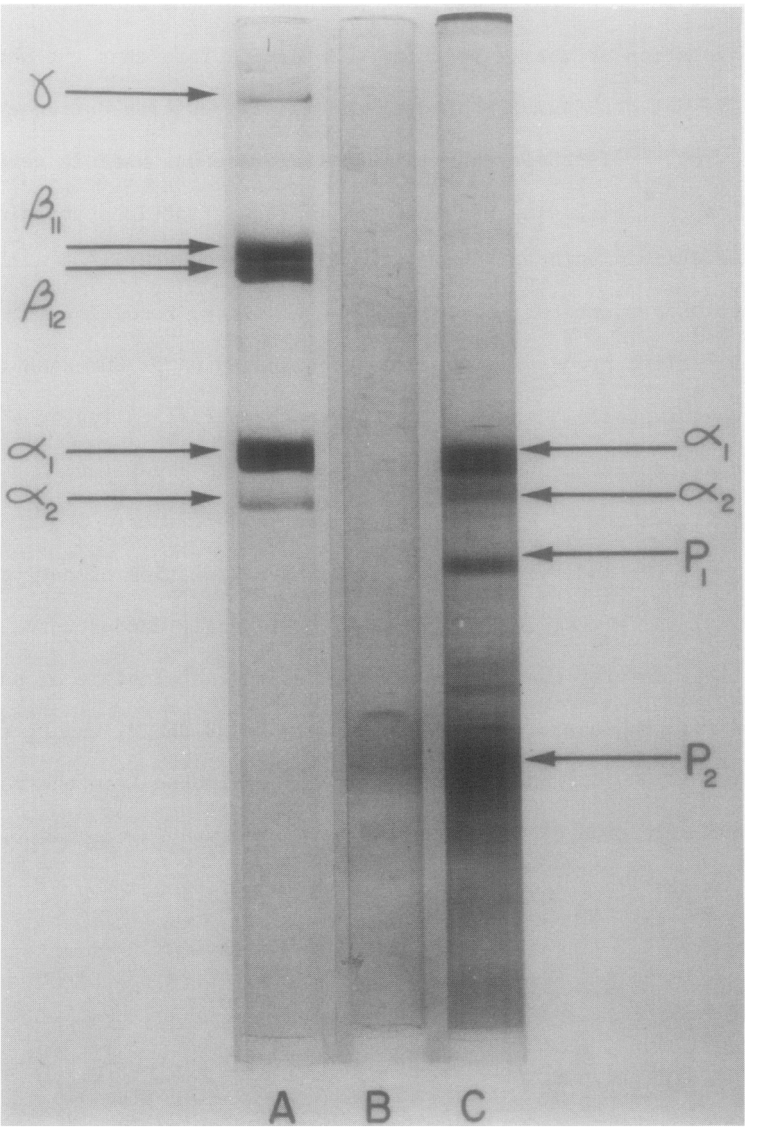

Figure 2 5% SDS-polyacrylamide gel electrophoresis of (A) native collagen used as the substrate for the collagenase assays; (B) reaction products of bacterial collagenase; and (C) reaction products of matrix vesicle preparation.

RESULTS

The collagenase activity of the various fractions collected from the Bio-Gel A-50 m column is illustrated in Figure 1. Collagenase activity was associated with those fractions containing matrix vesicles and also with a second

set of fractions which corresponded to the elution of the bacterial collagenase used in the isolation of matrix vesicles. To demonstrate that the bacterial collagenase did not artificially bind to the matrix vesicles during the isolation procedures, ^{125}I-labeled bacterial collagenase was used in several experiments. No radioactivity was detected in those fractions containing matrix vesicles as determined using a Packard 520 Autogamma counter.

The reaction products of the collagenase activity, resulting from assays of the matrix vesicle preparations, were fractionated on 5% SDS-PAGE (Fig. 2). Our results unequivocally demonstrated that the bacterial collagenase reaction products were not detected on 5% SDS-PAGE (Fig. 2B) as predicted, and these observations further served as a control for our experiments. The two major reaction products (P_1 and P_2) resulting from the degradation of native Type I collagen by the matrix vesicle preparation unequivocally demonstrated a mammalian collagenase-like activity characterized by an enzymatic cleavage of native collagen into two fragments. The molecular weights of the P_1 and P_2 fragments were 67,000 and 32,000 daltons, respectively, as estimated from the relative electrophoretic migration distances of the standard Type I collagen γ, β_{11} and α_1 bands.

DISCUSSION

The results of this study have demonstrated that mesenchymal-derived matrix vesicles contained a collagenase which cleaves native Type I collagen at one locus giving rise to two products with molecular weights of 67,000 and 32,000, respectively. Preliminary studies indicate that matrix vesicles also contain neutral protease activity.

During tooth morphogenesis, mesenchymally-derived matrix vesicles accumulate along the undersurface of the inner enamel epithelia adjacent to and prior to basal lamina degradation (4). The demonstration of collagenase and protease (6, 7) activities within matrix vesicles strongly suggests that these enzymes are responsible for the degradation of the basal lamina. The developmental function *in situ* of basal lamina degradation may be to "unmask" the outer

cell surfaces of the epithelia, and, thereby, permit direct mesenchymal-epithelial heterotypic cell-cell contact (11). Membrane-limited particulate structures, termed dense bodies, interstitial bodies, extracellular vesicles, and matrix vesicles have been observed with transmission electron microscopy at the interface of a number of interacting tissues and cells (12, 13, 14). Perhaps one common function of all these trilaminar membrane-limited structures is the transport of proteolytic activities involved in basal lamina or basement membrane degradation. If such an assumption is correct, matrix vesicle formation by mesenchymal cells associated with various epidermal organ systems (e.g. mammary gland, tooth, hair and feather formation, limbs) would diagnostically precede basal lamina degradation.

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