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ARTICLES

Prolidase Activity in Fibroblasts Is Regulated by Interaction of Extracellular Matrix With Cell Surface Integrin Receptors

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Abstract Prolidase (EC 3.4.13.9) is a ubiquitously distributed imidodipeptidase that catalyzes the hydrolysis of C-terminal proline or hydroxyproline containing dipeptides. The enzyme plays an important role in the recycling of proline for collagen synthesis and cell growth. An increase in enzyme activity is correlated with increased rates of collagen turnover indicative of extracellular matrix (ECM) remodeling, but the mechanism linking prolidase activity and ECM is poorly understood. Thus, the effect of ECM-cell interaction on intracellular prolidase activity is of special interest. In cultured human skin fibroblasts, the interaction with ECM and, more specifically, type I collagen mediated by the β_1 integrin receptor regulates cellular prolidase activity. Supporting evidence comes from the following observations: 1) in sparse cells with a low amount of ECM collagen or in confluent cells in which ECM collagen was removed by collagenase (but not by trypsin or elastase) treatment, prolidase activity was decreased; 2) this effect was reversed by the addition of type I collagen or β_1 integrin antibody (agonist for β_1 integrin receptor); 3) sparse cells (with typically low prolidase activity) showed increased prolidase activity when grown on plates coated with type I collagen or on type IV collagen and laminin, constituents of basement membrane; 4) the relative differences in prolidase activity due to collagenase treatment and subsequent recovery of the activity by β_1 integrin antibody or type I collagen treatment were accompanied by parallel differences in the amount of the enzyme protein recovered from these cells, as shown by Western immunoblot analysis. Thus, we conclude that prolidase activity responded to ECM metabolism (tissue remodeling) through signals mediated by the integrin receptor. J. Cell. Biochem. 67:166–175, 1997.

Key words: prolidase; fibroblasts; collagen; integrins; extracellular matrix–cell interaction

Collagen, which accounts for about one-third of total body proteins, is essential for the maintenance of connective tissue. It is specifically digested by tissue and bacterial collagenases [Birkedal-Hansen, 1987]. Although extracellular collagenases initiate the breakdown of collagen, the final step of collagen degradation is catalyzed by intracellular prolidase (EC 3.4.13.9). This cytosolic enzyme is necessary for specifically splitting imidodipeptides with C-terminal proline or hydroxyproline [Myara et al., 1984; Endo et al., 1989] because the tertiary amide bond is not recognized by other peptidases [Mock et al., 1990]. Similarly, the final step in digestion of ingested proteins is catalyzed by prolidase because intestinal hydrolases and peptidases are also ineffective against imidodipeptides [Phang and Scriver, 1989]. Thus, this enzyme potentially links protein nutrition and the metabolism of ECM proteins.

A model of this linkage is found in the inherited disorder with prolidase deficiency [Royce and Steinmann, 1993]. This is a rare autosomal recessive disorder characterized by elevated plasma imidodipeptides, massive imidodipeptiduria, skin lesions, recurrent infections, and mental retardation [Freij et al., 1984]. In cultured fibroblasts from patients with prolidase

Abbreviations: ECM, extracellular matrix; ECMR, extracellular matrix receptor; FBS, fetal bovine serum; Gly-Pro, glycyl-proline; kDa, kilodalton; MEM, minimal essential medium; MEM-0, MEM without serum; MEM-10, MEM with 10% FBS; PBS, phosphate buffered saline; SDS-PAGE, sodium dodecylsulfate–polyacrylamide gel electrophoresis; TBS-T, Tris-buffered saline–Tween 20; Tris, tris-(hydroxymethyl)aminomethane.

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deficiency, an increase in the rapidly degraded collagen pool and a decrease in the free proline pool has been found [Chamson et al., 1989]. In normal fibroblasts, the addition of exogenous glycyl-L-proline caused an increase in rapidly degraded collagen and a decrease in the proline pool [Chamson et al., 1989]. Although these findings suggest that prolidase activity may be related to collagen metabolism, the mechanism and end points by which this enzyme is regulated remain largely unknown.

Recent studies of the regulation of normal cell growth and tissue organization have focused on the interaction between cell surface and extracellular matrix (ECM) proteins. Mediated by the integrin family of cell surface receptors, this interaction can regulate gene expression, cellular differentiation, and proliferation [Akiyama et al., 1990; Albelda and Buck, 1990; Bissell, 1981; Carey, 1991; Donjacour and Cunha, 1991]. Of special interest, the metastatic potential of tumor cells may depend on the loss or derangement of cellular interaction with ECM proteins [Ruoslahti, 1992]. The major component of ECM proteins is collagen, and it may play a central role in the interaction with integrin receptors on cell surfaces [Akiyama et al., 1990]. In this receptor family, there are two receptors which recognize collagen. The first, designated ECMR I, is a heterodimer containing α (147 kDa) and β (125 kDa) subunits, which bind to collagen, fibronectin, and laminin. A second heterodimer, termed ECMR II, has a 145 kDa α subunit associated with a 125 kDa β subunit, the same as in ECMR I, but it binds only to collagen. ECMR I and ECMR II have been identified as $\alpha_3\beta_1$ and $\alpha_2\beta_1$, respectively [Akiyama et al., 1990].

The observation that prolidase activity in cultured cells increased with cell growth and density [Myara et al., 1985] led us to consider whether the activity of the enzyme mediating the final step in collagen degradation might in turn be regulated by the interaction between cell surface and ECM proteins (e.g., collagen). We now report that this indeed is the case and that β_1 integrin receptor is involved. Ligation of this receptor, either by anti-β_1 integrin antibody or by collagen, increases intracellular prolidase activity and the cellular content of prolidase protein.

MATERIALS AND METHODS

Materials

L-proline, L-glycyl-L-proline, collagenase (from *C. histolyticum*, type VII, chromatographically purified), elastase, and trypsin were purchased from Sigma Chemical (St. Louis, MO), as were most other chemical and buffers used. Eagle's medium and fetal bovine serum used in cell culture were products of Gibco (Gaithersburg, MD). Plates coated with collagen type I, collagen type IV, laminin, and fibronectin were obtained from Collaborative Biomedical Products (Bedford, MA). Monoclonal anti-β_1-integrin antibody, anti-αIIb and anti-β_3 integrin antibodies were obtained from ICN Biomedicals Inc. (Costa Mesa, CA). Soluble calf skin type I collagen was purchased from ICN Biochemicals (Cleveland, OH). Polyclonal anti-human prolidase antibody was the gift of Dr. C.R. Scriver (Montreal Children's Hospital, Montreal, Quebec, Canada) [Boright et al., 1989]. Nitrocellulose membrane (0.2 µm) and SDS-PAGE molecular weight standards were received from Bio-Rad Laboratories (Hercules, CA). Horseradish peroxidase–labeled antirabbit immunoglobuin G antibody was purchased from Promega Corp. (Madison, WI). L-5[^{3}H] proline, 28 Ci/mmol, was from Amersham (Arlington Heights, IL).

Tissue Culture

Normal human skin fibroblasts (KIM cells obtained by punch biopsy from an 11-year-old male donor) were maintained in 162 cm^2 Costar tissue culture flasks in Eagle's medium supplemented with 10% fetal bovine serum (FBS), 2 mM/L glutamine, nonessential aminoacids (Quality Biologicals Inc., Gaithersburg, MD), 50 U/ml penicillin, and 50 µg/ml streptomycin at 37°C in a 5% CO_2 incubator. For growth experiments and prolidase studies, cells between the eleventh and sixteenth passages were cultured in Costar six-well plates at a density of 10^5 cells/well in 2 ml growth medium.

Determination of Prolidase Activity

The activity of prolidase was determined by the method of Myara et al. [1982] which colorimetrically quantitates proline with Chinard's reagent [Chinard, 1952]. Briefly, the monolayer was washed three times with 0.15 mol/l of NaCl. Cells were detached by scraping, suspended in

0.15 mol/l NaCl, and harvested by low speed (200*g*) centrifugation. The cell pellet (from six wells) was suspended in 0.3 ml of 0.05 mol/l Tris-HCl, pH 7.8, and sonicated three times for 10 s at 0°C. Samples were then ultracentrifuged (100,000*g*, 15 min) at 4°C. Supernatant was used for protein determination and assay of prolidase activity. Activation of prolidase required preincubation with manganese: 100 µl of supernatant incubated with 100 µl of 0.05 mol/l Tris-HCl, pH 7.8 containing 2 mmol/l $MnCl_2$ for 24 h at 37°C. After preincubation, the prolidase reaction was initiated by adding 100 µl of the preincubated mixture to 100 µl of 94 mmol/l Gly-Pro (final concentration, 47 mmol/l). After additional incubation for 1 h at 37°C, the reaction was terminated with 1 ml of 0.45 mol/l trichloroacetic acid. In parallel tubes, the reaction was terminated at time zero (without incubation). The released proline was determined by adding 0.5 ml of the trichloroacetic acid supernatant to 2 ml of a 1:1 mixture of glacial acetic acid:Chinard's reagent (25 g of ninhydrin dissolved at 70°C in 600 ml of glacial acetic acid and 400 ml of 6 mol/l orthophosphoric acid) and incubated for 10 min at 90°C. The amount of proline released was determined colorimetrically by absorbance at 515 nm and calculated by using proline standards. Protein concentration was measured by the method of Lowry et al. [1951]. Enzyme activity was reported as nanomoles per minute per milligram of protein.

Preparation of ECM

Preparation of extracellular matrix (ECM) was performed by a modification of the method described by Jones and De Clerck [1980]. Cells were seeded at 1×10^5 per well in 6 well plates in 2 ml of growth medium. For radiolabeling of ECM, 1 mCi/ml of L-5[^{3}H] proline (28 Ci/mmol) was added to wells 3 days after seeding. After an additional 3 days of incubation, the monolayer was washed with PBS, and cells were removed by incubation for 10 min with 1 ml of 0.5% Triton X-100 in PBS. Thereafter, the cultures were treated with 1 ml 0.25 M NH_4OH for 30 min at room temperature to remove cytoskeletal elements and then washed three times with PBS. As examined by phase microscopy, no cells remained attached, whereas the ECM remained firmly attached to the plating surface.

Analysis of ECM

The ECM labeled with 5-[^{3}H] proline was analyzed for glycoprotein, elastin, and collagen content by sequential enzyme treatment with trypsin, elastase, and collagenase. Because commercial trypsin was contaminated with elastase, the preparation (10 ml of a 0.05% solution) was incubated with 5 mg bovine elastin (type IV; Sigma) for 30 min at 37°C to adsorb elastase. The suspension of "trypsin" and insoluble elastin was centrifuged, and the supernatant containing trypsin was used in the experiments. The respective enzymes were used at a final concentration of 10 µg/ml in 0.1 mol/l Tris-HCl, pH 7.6, containing 10 mmol/l $CaCl_2$. After ECM was incubated in 1 ml of the specified enzyme for 3 h, the enzyme solution containing released proteins was transferred to a scintillation vial. The monolayer then was washed with 1 ml of PBS, and the wash was added to the enzyme solution in the scintillation vial. The amount of radioactivity solubilized by each treatment (corrected for nonspecific release of radioactivity during the time of incubation without enzyme added) was determined by liquid scintillation spectrometry. The sequential treatment with trypsin, elastase, and collagenase resulted in the release of all of the radiolabeled ECM.

SDS-PAGE

Slab SDS-PAGE was used according to the method of Laemmli [1970]. Supernatants from cell homogenates (10–20 µg of protein) were incubated at 100°C for 5 min in 62.5 mmol/l Tris-HCl, pH 6.8, containing 2.0% (w/v) SDS, 5% (v/v) β-mercaptoethanol, 10% (v/v) glycerol, and 0.001% Bromophenol blue. Samples were applied to a polyacrylamide slab gel (4% stacking and 7.5% separating in 0.1% SDS) and were electrophoresed at 50 V per gel for 1.5 h at room temperature in a pH 8.3 running buffer containing 25 mmol/l Tris, 192 mmol/l glycine, and 0.1% SDS. The following unstained high molecular weight standards (Bio-Rad) were used: myosin (200 kDa), galactosidase (116.2 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), and ovalbumin (45 kDa).

Western Blot Analysis

After SDS-PAGE, the gels were allowed to equilibrate for 5 min in 25 mmol/l Tris, 0.2 mol/l glycine in 20% (v/v) methanol. The protein was

transferred to 0.2 μm pore-sized nitrocellulose at 100 mA for 1 h by using a LKB 2117 Multiphor II electrophoresis unit. Nitrocellulose was blocked by soaking with gentle shaking in 5% dried milk in TBS-T solution (20 mmol/l Tris-HCl buffer, pH 7.4, containing 150 mmol/l NaCl and 0.05% Tween 20) for 1 h at room temperature. Prolidase was identified by a polyclonal antibody against human prolidase [Boright et al., 1989] at a concentration 1:3,000 in 5% dried milk in TBS-T. After 1 h incubation with the antiprolidase antibody, the membrane was washed with TBS-T (1×15 min and 2×10 min) followed by incubation with horseradish peroxidase–labeled antibody against rabbit Fc IgG (1:5,000 in TBS-T for 30 min). The nitrocellulose was extensively washed with TBS-T (5×10 min) and submitted to the ECL Western blotting detection system (Amersham) for 1 min. The nitrocellulose membrane was wrapped with Saran Wrap and exposed to film for 30 s to 1 min.

Statistical Analysis

In all experiments, the mean values for three independent experiments done in triplicates ± standard deviation (SD) were calculated. The results were submitted to statistical analysis using the Student's *t*-test, accepting $P < 0.05$ as significant.

RESULTS

In proliferating, normal human skin fibroblasts, the level of prolidase activity increased with time. At first, this increase appeared related to cell density (Fig. 1A). In sparsely plated cells (about 1×10^5/well), prolidase activity was low on day 1 but increased with growth and reached a level double to that of day 1 when cells reached confluency (about 3×10^5/well on day 5). One possible explanation for this phenomenon is that prolidase activity in fibroblasts is affected by cell-cell interaction. However, when cells were seeded at different densities and grown for 48 h, prolidase activity showed no density-dependent difference (Fig. 1B). Alternatively, growth-related changes could be due to time-dependent cellular interactions with extracellular matrix (ECM). If such were the case, a disturbance of specific interactions between ECM molecules and the cell surface may affect intracellular prolidase activity. Among ECM molecules, proteins (i.e., glycoproteins, elastins, and collagens) are likely candidates. We measured prolidase activity in confluent cells for which ECM protein composition has been altered by specific enzyme treatment (e.g., removal of glycoproteins (trypsin treatment), elastins (elastase treatment), or collagens [collagenase treatment]. After 6 h of collagenase treatment, the activity decreased to 30% of control (Fig. 2). Similar treatment with trypsin or elastase did not result in decreased prolidase activity. In fact, with trypsin treatment, the activity showed significant increases in activity after 2 h of treatment. These results suggested that collagen (or some other collagenase digestible protein) was the critical component in the ECM which played a key role in influencing the observed changes in intracellular prolidase activity.

Additional evidence supporting the importance of ECM collagens in regulating prolidase

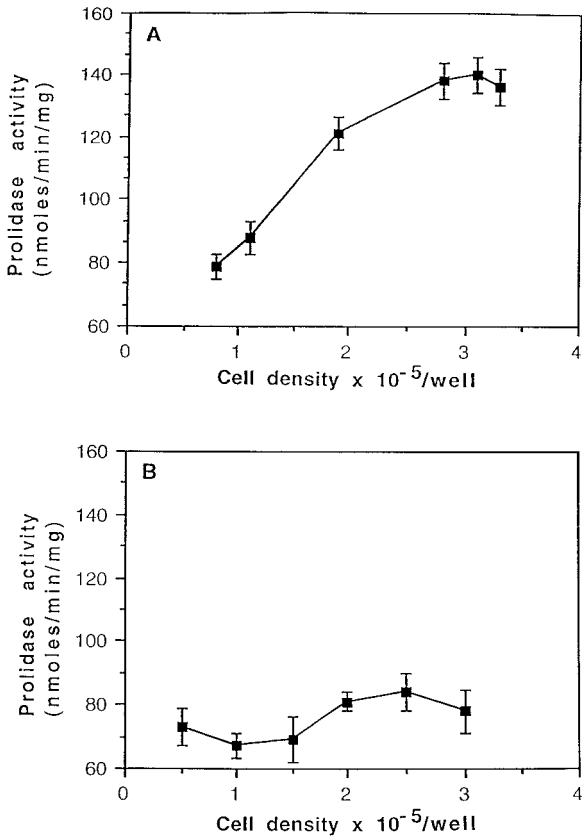

Fig. 1. Cell density–dependent prolidase activity in normal human skin fibroblasts. **A:** Cells were replicate-plated in six-well plates at 10^5 per well in 1 ml of MEM with 10% FBS. Every 24 h cells were counted and prolidase activity determined in the cell extract. **B:** Cells were replicate-plated at different densities in six-well plates in 1 ml of 10% FBS per well. After 48 h of incubation, cells were counted and prolidase activity measured in the cell extract.

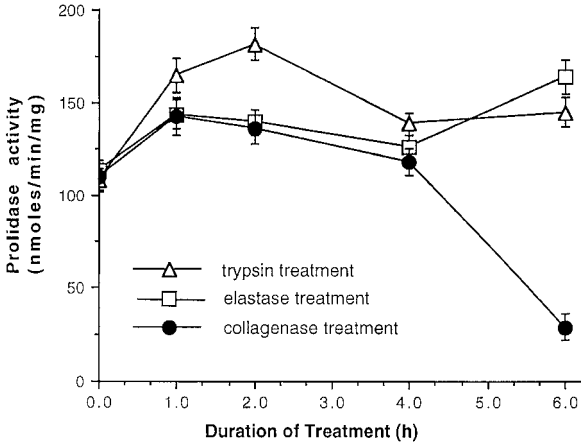

Fig. 2. Prolidase activity in confluent human skin fibroblasts treated with proteolytic enzymes each at a concentration of 10 µg/ml. Cells were replicate-plated in six-well plate at 10^5 per well in 1 ml of MEM with 10% FBS. When cells reached confluency at day 5, the monolayer was washed three times with PBS and incubated in 1 ml of 0.1 M Tris-HCl, pH 7.6, containing 10 mM $CaCl_2$ and the indicated protease at 37°C.

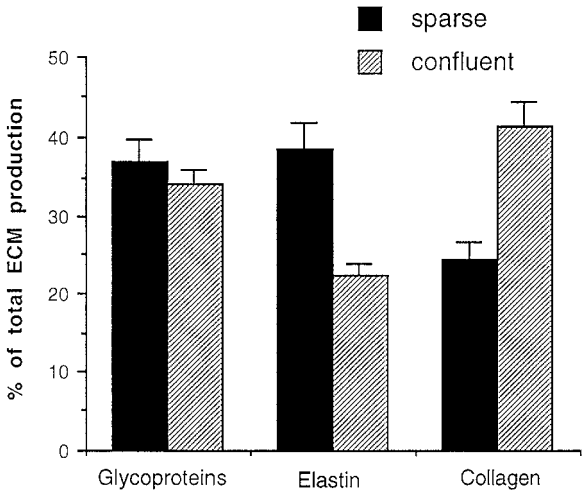

Fig. 3. Trypsin-, elastase-, and collagenase-digestable proteins of ECM produced by confluent (3×10^5/well) and subconfluent (1.4×10^5/well) human skin fibroblasts. The amount of each ECM protein solubilized by the respective protease is calculated as a percentage of total radioactivity. Details are described in Materials and Methods.

came from examining the content of ECM proteins accompanying sparse and confluent cells (Fig. 3). The content of elastins and collagens is markedly different between sparse and confluent fibroblasts. Elastin content is lower in confluent cells than in sparse cells. By contrast, collagen content is higher in confluent cells than in sparse cells. Although total ECM in sparse cells is much lower than in confluent cells, the percentage of glycoproteins in ECM did not differ between sparse and confluent cells. Under these conditions, the specific activity of prolidase was correlated with the collagen content. Accompanying the 75% increase in collagen content as sparse cells became confluent, the level of prolidase activity increased 120% (data not shown), suggesting that ECM collagen-cell interaction affects intracellular prolidase activity.

We considered that the β_1 subunit of the integrin receptor is involved in regulating prolidase activity in fibroblasts. To test this hypothesis, we employed a monoclonal anti-β_1 integrin antibody, which is known to be an agonist for β_1 integrin receptors [Schwartz et al., 1991a,b]. The rationale for the experiment was that the agonist activity of anti-β_1 antibodies might substitute for collagen in collagenase-treated cells in regulating prolidase activity. As shown in Table I, prolidase activity in confluent, collagenase-treated cells decreased to 50% of control values. Refeeding these cells with serum-free medium containing anti-β_1 integrin antibodies or type I collagen restored prolidase activity to control values. In contrast, anti-β_3 or anti-αIIb antibodies did not produce such an effect (Table I).

Similar results were observed when fibroblasts were sparsely seeded on plates coated with other ECM proteins (Table II). Sparse cells, with typically low prolidase activity, showed increased activity when grown on plates coated with type I collagen, type IV collagen, laminin, or in the presence of soluble anti-β_1 integrin antibody. All these treatments produced an increase of prolidase activity, with the notable exception of cells grown on fibronectin. We conclude from these results that ligation of the β_1 integrin receptor is an early step in the sequence of events increasing intracellular prolidase activity.

It is likely that type I collagen is the natural ligand for β_1 integrin receptor-mediated regulation of prolidase in fibroblasts. In experiments monitoring prolidase activity in fibroblasts grown in 10% serum, prolidase increased linearly over 24 h (Fig. 4). Presumably, the observed increase results from ligation of β_1 integrin receptors by ECM proteins produced by these cells. Importantly, the rate of increase was augmented by the addition of anti-β_1 integrin antibodies or type I collagen. To show that anti-β_1 integrin antibodies can replace type I collagen as an agonist for stimulating prolidase

TABLE I. Effect of Collagen or Anti-β_1 Integrin Antibody on Prolidase Activity in Collagenase-Treated Fibroblasts*

	Treatment	Postcollagenase incubation	Prolidase activity (nmoles/min/mg protein)
A	Control	MEM-10% FBS	168 ± 12
B	Collagenase	None	88 ± 14
C	Collagenase	MEM-0% FBS	78 ± 8
D	Collagenase	MEM-0% FBS + type I collagen	155 ± 11
E	Collagenase	MEM-0% FBS + anti-β_1 integrin antibody	160 ± 12
F	Collagenase	MEM-0% FBS + anti-β_3 integrin antibody	70 ± 15
G	Collagenase	MEM-0% FBS + anti-αIIb integrin antibody	78 ± 8
H	Collagenase	MEM-10% FBS	122 ± 12

*Cells were cultured as described in the legend to Fig. 2. Confluent cells were incubated with bacterial collagenase (10 μg/ml) for 6 h (B). After treatment with collagenase, the monolayer was incubated for 24 h with MEM with (H) or without FBS (C), with MEM-0% FBS and type I collagen at final concentration of 2 μg/ml (D), or with MEM-0% FBS and anti-β_1 (E), anti-β_3 (F), or anti-αIIb (G) integrin antibodies at final concentrations of 10 ng/ml. Cells without collagenase treatment incubated in MEM with 10% FBS (A) served as control. Prolidase activity is expressed as nanomoles/minute/milligram of protein.

TABLE II. Effect of Protein-Coated Plates on Prolidase Activity in Sparse Fibroblasts*

	Extracellular matrix protein	Integrin receptor subtype	Prolidase activity (nmoles/min/mg)
A	Control	—	32 ± 4
B	Collagen, type I	$\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$	67 ± 7
C	Collagen, type IV	$\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$	107 ± 7
D	Laminin	$\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_6\beta_1$	77 ± 10
E	Fibronectin	$\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$ $\alpha_v\beta_1$, $\alpha_v\beta_6$	22 ± 5
F	Anti-β_1 integrin	β_1	69 ± 4

*Cells were cultured as described in the legend to Fig. 2. After cells were trypsinized, they were suspended in MEM without serum (MEM-0), spun down, and plated at low density in six-well plates (10^5 cells/well). Plates were coated with extracellular matrix proteins. Anti-β_1 integrin antibody was added to the medium at a final concentration of 10 ng/ml. After 24 h of incubation in medium without FBS (MEM-0), cells from three wells were harvested and combined for prolidase activity determination. Subtypes of integrin receptor which recognize the specific ECM protein are indicated [Dedhar, 1995].

activity and to rule out the involvement of other collagenase-digestible proteins, we performed the following experiment. Confluent fibroblasts were treated with collagenase for 6 h, the monolayer was washed three times with PBS, and the cells were refed with serum-free medium or serum-free medium containing collagenase or both collagenase and β_1 integrin antibody. This design prevented the accumulation of collagen (i.e., collagenase-digestible proteins) in the ECM. Prolidase activity was measured after collagenase treatment and after 12 and 24 h of refeeding (Fig. 5). The results clearly showed that anti-β_1 integrin antibodies can restore prolidase activity after collagenase treatment even when exposure to collagenase continued to prevent the accumulation of any collagenase-digestible protein at the cell surface, thereby ruling out their involvement in the increase in prolidase activity. More prolonged incubation (>24 h) with collagenase and anti-β1 integrin antibody resulted generally in a decrease in prolidase activity (data not shown), but these levels were not consistent, presumably due to sporadic attrition of the antibody-receptor complex.

It was likely that the measured differences in prolidase activity under the aforementioned perturbations were due to differences in intracellular enzyme content. Direct measurement established that this was the case. The relative differences in prolidase activity due to collagenase treatment and subsequent recovery of the activity by β_1 integrin antibody or type I collagen treatment were accompanied by parallel differences in the amount of the enzyme protein recovered from these cells, as shown by Western immunoblot analysis (Fig. 6).

DISCUSSION

Our studies suggest that ECM proteins and ECM reorganization (tissue remodeling) may affect intracellular prolidase activity. First we confirmed the previous observation [Myara et al., 1985] that in cultured human skin fibroblasts prolidase activity was strongly dependent on cell density and rose when cell density increased. It is well known that increased fibroblasts density is accompanied by increased col-

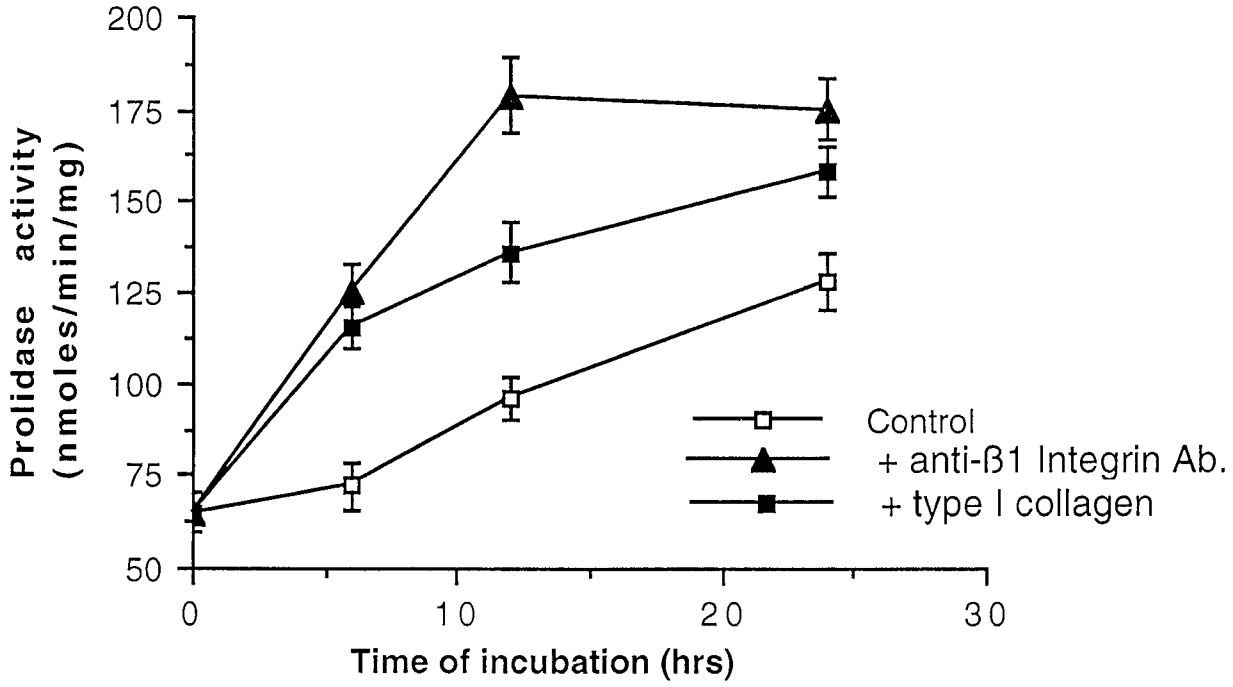

Fig. 4. Prolidase activity in sparse cells (1×10^5/well) growing for 24 h in 10% FBS alone or containing collagen type I (2 μg/ml) or β_1 integrin monoclonal antibody (10 ng/ml). The experiment was done in a six-well plate. Cells were seeded at 0.5×10^5/well in MEM-10% FBS. After 24 h (0.62×10^5/well), the medium was changed into MEM-10% FBS with or without additives and cultured for an additional 24 h. At the end of the experiment, the cell number per well was $1.0 \pm 0.1 \times 10^5$/well.

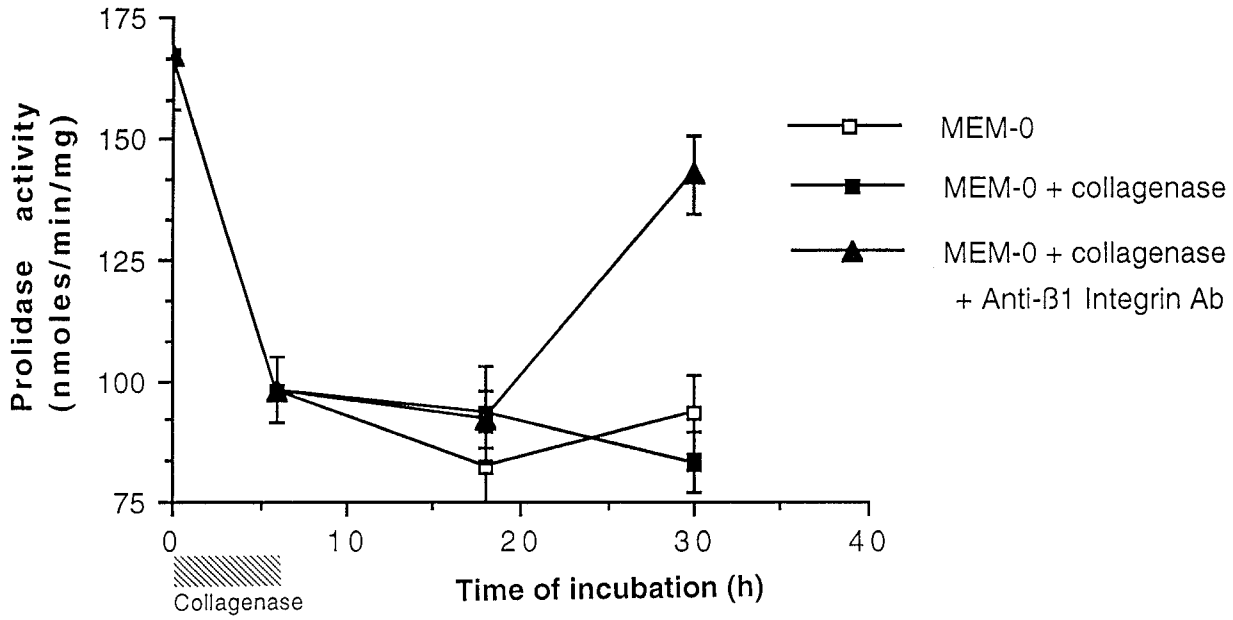

Fig. 5. The effect of collagenase on anti-β_1 integrin antibody-stimulated prolidase activity in collagenase-treated confluent fibroblasts. Confluent fibroblasts were treated with 10 μg/ml of bacterial collagenase for 6 h, and then the cells were refed MEM-0 with or without collagenase or collagenase + anti-β_1 integrin antibody (10 ng/ml).

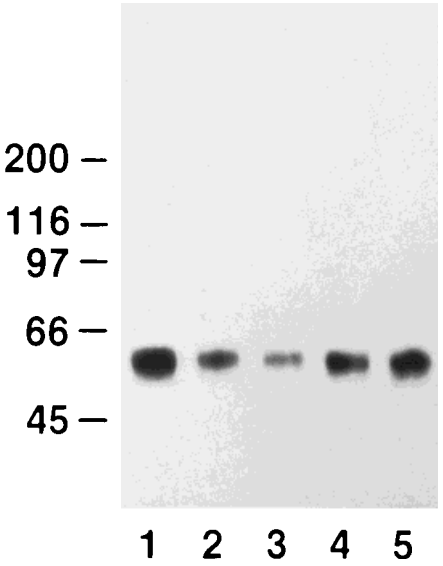

Fig. 6. Western blot analysis of prolidase from cytosol of control fibroblasts (**lane 1**), collagenase-treated for 6 h (**lane 2**), collagenase-treated/refed MEM-0 for 24 h without (**lane 3**) and with collagen type I, 2 μg/ml (**lane 4**), or with β_1 integrin monoclonal antibody, 10 ng/ml (**lane 5**). The same amount of supernatant protein (20 μg) was run in each lane.

lagen production by these cells. We then provided evidence that the stimulation of prolidase activity is mediated by interaction between type I collagen and cell surface β_1 integrin receptors. Our experiments showed that type I collagen might be the ECM component responsible for maintaining activity of intracellular prolidase. In sparse cells (demonstrating low prolidase activity), 24 h incubation with type I collagen increased prolidase activity to the values observed in confluent cells. The same effect was achieved by incubation of the cells on type I collagen–coated plates. Similar results were obtained when cells were grown on type IV collagen– or laminin-coated plates. Since type IV collagen and laminin are not produced by cultured fibroblasts, it is reasonable to infer that type I collagen is the ECM component in cultured fibroblasts responsible for activation of prolidase activity. Moreover, treatment of confluent fibroblasts (demonstrating high prolidase activity) with bacterial collagenase decreased intracellular prolidase activity. This effect can be reversed by exposing cells to type I collagen or β_1 integrin antibody. Importantly, the differences in prolidase activity were corroborated by the intracellular enzyme content, as shown by Western immunoblot analysis. Whether these differences were due to the regulation of new protein synthesis or modulation of enzyme turnover rate are being intensely studied.

The reaction catalyzed by prolidase serves two important physiological functions in mammals. First, it catalyzes the final step in collagen degradation which completes the recycling of proline [Jackson and Heininger, 1973; Yaron and Naider, 1993]. The efficiency for proline recycling is about 90% [Jackson et al., 1975]. The best and most abundant substrate for prolidase is Gly-Pro, which comes from intracellular degradation of procollagen (intracellular form of collagen) and extracellular degradation of collagen. A second important physiological function for prolidase is in the digestion of dietary proteins [Emmerson and Phang, 1993]. The imido bond of proline- and hydroxyproline-containing oligopeptides cannot be cleaved by intestinal hydrolases. Instead, these as well as other oligopeptides circulate in the plasma and enter cells through specific dipeptide transport systems for imidodipeptides and are substrates for specific hydrolases (e.g., prolidase and prolinase [the latter hydrolyzes dipeptides with proline or hydroxyproline in the amino terminus]). Thus, prolidase participates in the final step of protein digestion. In view of the above, prolidase catalyzes reactions which may be a metabolic interface between degradation of ECM proteins on the one hand and the digestion of dietary proteins on the other. This linkage may help explain the findings in the inherited disorder with prolidase deficiency, which is accompanied by skin lesions (ulcerative dermatitis), recurrent infections, mental retardation, and elevated proline-containing dipeptides in plasma [Freij et al., 1984; Phang and Scriver, 1989]. Collagen, which accounts for about one-third of total body proteins, is a polypeptide containing the highest amount of imido bonds compared to all known proteins. In α_1 chains of type I collagen (containing 235 amino acid residues), Gly-Pro occurs 25 times [Jackson et al., 1975]. It is evident that the deficiency of prolidase in humans with the inherited disorder will severely impede the recycling of collagen proline as well as the release of dietary proline. Based on the occurence of skin lesions and the observed collagen defect resembling lathyrism, Goodman et

al. [1968] suggested that impaired recycling of proline may be an underlying mechanism.

Other than collagen, only the heavy chain of immunoglobulin and the C1q component of complement contains Gly-Pro approaching the abundance in collagen [Jackson et al., 1975]. It is likely that immunodeficiency is a result of disturbances in the biosynthesis of immunoglobulin and C1q. In view of the collagen-like amino acid sequence in both substances, one may speculate that the immunologic deficit is related to the prolidase deficiency and the unavailability of proline. In most cases of prolidase deficiency, mental retardation is present [Phang and Scriver, 1989]. It may result from a decrease in proline concentration in the central nervous system. Proline may play an important role in modulation of glutamatergic neurons [Fremeau et al., 1992], and prolidase activity may serve as a regulator of proline concentration in central nervous system.

The linkage of collagen metabolism to prolidase activity may be also important in wound healing [Senboshi et al., 1996]. Although the linkage between collagen and cellular prolidase activity was shown in fibroblasts, its full significance may be in the interaction between cells of various types. The complex events occurring in wound healing may serve as an useful model. During wound healing, platelets, immune cells (e.g., macrophages), lymphocytes, and neutrophils are exposed to collagen through β_1 integrin receptors [Romanic et al., 1997; Hauzenberger et al., 1997; Keely and Parise, 1996]. A presumed upregulation of prolidase in these cells concomitant to the breakdown of collagen by collagenase released by some of these cells would recycle proline in the area of the wound. The proline would be used for fibroblast proliferation and the production of collagen in granulation tissue. The local production of immunoglobulins may be critical for defense against bacterial organisms. It is tempting to speculate that the constellation of abnormalities found in prolidase deficiency can be understood based on a defect in this tissue microcosm with diverse cells. Nevertheless, the interaction between β_1 integrins on cell surfaces and ECM collagen may serve as a universal mechanism for regulation of prolidase both in physiologic and pathophysical tissue responses.

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