Cell-layer-associated proteolytic cleavage of the telopeptides of type I collagen in fibroblast culture

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In human skin fibroblast cultures a fraction of the procollagen that was processed to collagen and remained in the cell layer was further proteolytically modified by removal of both *N*- and *C*-terminal telopeptides. The proteolytic activity was associated with the cell layer, since secreted collagens were found always to contain intact telopeptides. The inclusion of neutral polymers, which caused the accumulation of the collagen in the cell layer [Bateman, Cole, Pillow & Ramshaw (1986) *J. Biol. Chem.* **261**, 4198–4203], made the telopeptide cleavage more apparent in those cells which expressed the proteolytic activity. The extent of this cleavage was variable from cell culture to cell culture and between experiments with the same fibroblast line. The proteolytic activity was pH-dependent; cleavage was greatest at a culture-medium pH of 7.5 and 8.0 and was completely inhibited at a culture-medium pH of 7.0 and 6.5. The activity was significantly inhibited by soybean trypsin inhibitor, an elastase-specific inhibitor (*N*-acetylalanylalanylprolylvalylchloromethane) and the thrombin inhibitor hirudin. This cell-associated proteolytic activity may play a role in collagen degradation by removing the telopeptides, which are the primary sites of collagen cross-linking, thus destabilizing the collagen matrix sufficiently to render it susceptible to further proteolytic breakdown.

INTRODUCTION

The major protein component of the fibroblast extracellular matrix is type I collagen, which consists of two $\alpha 1(I)$ chains and one $\alpha 2(I)$ chain in a triple-helical configuration. This protein is synthesized as a precursor, procollagen, which is converted into collagen by the action of specific procollagen peptidases (Prockop & Tuderman, 1982). After this processing, each chain has short non-helical telopeptide extensions at both the *N*- and the *C*-termini, and the involvement of these regions in covalent cross-links stabilizes the mature collagen fibrillar structure (Kühn, 1982; Eyre *et al.*, 1984).

The formation and maintenance of a functional extracellular matrix involves a tightly controlled balance of degradation, as well as synthesis, of the matrix macromolecules. The mechanism and control of degradation is complex, involving many specific proteinases, enzyme inhibitors and activators (Werb, 1982). Fibroblasts are known to produce several proteolytic enzymes which have activity against collagens and other extracellular matrix components. In addition to the well-characterized mammalian collagenases (Werb, 1982) and other secreted proteinases, fibroblasts also produce proteinases, such as elastases (Godeau *et al.*, 1982; Schwartz *et al.*, 1986) and serine proteinases (Harper *et al.*, 1984), which are cell-surface-associated. However, less is known about these cell-associated enzymes, and their physiological roles are generally not known. It has been suggested that mammalian collagenase may be less active towards insoluble fibrillar collagen than towards soluble collagen (Leibovich & Weiss, 1971; Vater *et al.*,

1979) and that a proteolytic activity that can remove the cross-linking telopeptides may be necessary before collagenase action in matrix degradation (Werb, 1982; Weiss, 1984). In the present paper we describe a proteolytic activity associated with the fibroblast cell layer which can remove the collagen telopeptides and may thus have a role in collagen turnover.

EXPERIMENTAL

Materials

SBTI, hirudin and pepsin were obtained from Sigma Biochemical Co., St. Louis, MO, U.S.A. The elastase inhibitor Ac-Ala-Ala-Pro-Val-CH₂Cl was a gift from Professor D. Lowther, Department of Biochemistry, Monash University, Melbourne, Australia, and Professor J. Powers, School of Chemistry, Georgia Institute of Technology, Atlanta, GA, U.S.A. Phenylmethanesulphonyl fluoride was obtained from Merck, Darmstadt, Germany, and *N*-ethylmaleimide was from BDH Biochemicals, Poole, Dorset, U.K. Purified fibroblast collagenase was kindly given by Dr. J. Jeffrey, Division of Dermatology, Department of Medicine, Washington University, St. Louis, MO, U.S.A. PEG was obtained from BDH and purified from acetone by precipitation with diethyl ether (Albertsson, 1960). Dextran T-40 was obtained from Pharmacia, Uppsala, Sweden, and PVP (*M_r* 30000–40000) was obtained from May and Baker Ltd., Dagenham, Essex, U.K. L-[5-³H]Proline (30 Ci/mmol) was purchased from Amersham Australia Pty., Sydney, Australia. All other chemicals were commercially available analytical-grade reagents. Cell-

Abbreviations used: SBTI, soybean trypsin inhibitor; Ac-Ala-Ala-Pro-Val-CH₂Cl, *N*-acetylalanylalanylprolylvalylchloromethane; PEG, poly(ethylene glycol)4000; PVP, polyvinylpyrrolidone; pNa1 and pNa2, processing intermediates in the conversion of procollagen to collagen that contain the *N*- but not the *C*-propeptides of the pro $\alpha 1$ and pro $\alpha 2$ chains.

culture media, including those containing polymer, were filter-sterilized before use.

Fibroblast culture and procollagen labelling

Human dermal fibroblast cultures were established and cultured as previously described (Bateman *et al.*, 1984, 1986). For biosynthetic labelling of procollagen, 2–4-day post-confluent cultures were labelled with L-[5-³H]proline for 18 h in Dulbecco's modification of Eagle's medium containing 10% (v/v) foetal-calf serum and 0.15 mM-sodium ascorbate. The cell layer and medium fractions were then prepared and analysed separately (Bateman *et al.*, 1984, 1986). Briefly, the cell culture medium was pooled with a phosphate-buffered saline (0.1 M-NaH₂PO₄/0.15 M-NaCl, pH 7.4) wash of the cell layer and the proteinase inhibitors phenylmethanesulphonyl fluoride (0.1 mM), *N*-ethylmaleimide (1 mM) and EDTA (10 mM) were added. The cell layers were scraped from each dish in 0.05 M-Tris/HCl, pH 7.5, containing 0.15 M-NaCl and the proteinase inhibitors, and then sonicated. Procollagens were precipitated from both the cell-layer extract and medium fractions with 25% -satd. (NH₄)₂SO₄ at 4 °C and then dissolved in 0.05 M-Tris/HCl, pH 7.5, containing 0.15 M-NaCl, and portions were taken for further analysis.

Medium was routinely buffered at pH 7.5 with 20 mM-Hepes/22 mM-NaHCO₃ (Bateman *et al.*, 1984), but this was modified in some experiments by the use of different buffering systems. These were 10 mM-Pipes/10 mM-Mops/22 mM-NaHCO₃, pH 6.5; 10 mM-Mops/10 mM-Hepes/22 mM-NaHCO₃, pH 7.0, and 15 mM-Hepes/10 mM-Tricine/22 mM-NaHCO₃, pH 8.0 (Eagle, 1971). In certain experiments the culture medium was also modified by the addition of 5% (w/v) PEG, dextran or PVP; in one experiment the concentration of PVP was varied from 0 to 4% (w/v) to assess the effects of different polymer concentrations. In some experiments the specific proteinase inhibitors SBTI, hirudin or Ac-Ala-Ala-Pro-Val-CH₂Cl were included at the final concentrations noted in the Figure legend.

Pepsin cleavage of the procollagens

Portions of labelled procollagen were subjected to limited pepsin digestion at 4 °C for 6 h at a final pepsin concentration of 100 µg/ml (Bateman *et al.*, 1984). Samples were then freeze-dried.

Mammalian-collagenase digestion

Portions of the labelled procollagen or pepsin-digested collagens in 0.05 M-Tris/HCl, pH 7.5, containing 0.15 M-NaCl and 0.01 M-CaCl₂ were mixed with the trypsin-activated mammalian collagenase (Wilhelm *et al.*, 1984) at an enzyme/substrate ratio of approx. 1:70 and digestion was performed at 22 °C for 24 h. Protein was then precipitated by addition of cold ethanol to 75% (v/v) and the precipitate at 4 °C was collected by centrifugation and freeze-dried.

CNBr cleavage of collagen

CNBr cleavage was performed in 70% (v/v) formic acid containing 50 mg of CNBr/ml for 4 h at room temperature as described by Scott & Veis (1976). After cleavage samples were diluted 10-fold with water and then freeze-dried.

SDS/polyacrylamide-gel electrophoresis

The preparation of samples and electrophoresis conditions were described previously (Laemmli, 1970; Bateman & Peterkofsky, 1981). Electrophoretic analysis of the collagen chains was performed on 5% (w/v) separating gels with a 3.5% (w/v) stacking gel, both gel solutions containing 2.0 M-urea. Collagen fragments released by digestion with mammalian collagenase were resolved on 7.5% (w/v) separating gels, and collagen CNBr peptides were separated on 12.5% (w/v) separating gels in the absence of urea. The labelled collagen chains and peptides were detected and quantified by fluorography (Bonner & Laskey, 1974; Bateman *et al.*, 1986).

RESULTS AND DISCUSSION

In the present study, using human dermal fibroblasts, most of the type I collagen produced during the labelling period was secreted from the cell layer into the culture medium, where most remained as procollagen and its partially processed intermediates (Fig. 1, lanes 1 and 2). These findings are in accord with many other studies on collagen production by fibroblasts in culture (Goldberg

Fig. 1. SDS/polyacrylamide-gel electrophoresis of skin fibroblast cell-layer and secreted collagens

Human skin fibroblasts were labelled with [³H]proline (see the Experimental section for details) and the collagens retained in the cell layer (C, lane 1) and secreted into the medium (M, lane 2) were examined on 5% (w/v) polyacrylamide separating gels. All samples were electrophoresed unreduced so that the disulphide-bonded procollagen species would remain near the top of the gel and the processed α -chains could be more readily identified. Duplicate samples were treated with pepsin before electrophoretic analysis (lanes 3 and 4). The migrations of the unreduced procollagen species, (1), and the type I collagen $\alpha 1$, $\alpha 2$, pN $\alpha 1$ and pN $\alpha 2$ chains are shown. $\alpha 1^*$ and $\alpha 2^*$ designate the α -chains that, having lost their telopeptide regions, show a faster electrophoretic migration.

& Sherr, 1973; Lichtenstein *et al.*, 1975; Goldberg, 1977). The small amount of fully processed type I collagen in the medium (Fig. 1, lane 2) had α -chains with slower electrophoretic mobilities when compared with those from pepsin-digested collagen (Fig. 1, lane 4). This indicated that the processed collagen in the medium fraction had proteinase-sensitive regions. Since removal of these regions by pepsin did not fragment the collagen α -chains, these data are consistent with them being the proteinase-sensitive telopeptides at the *N*- and *C*-termini of the molecule, which are left intact after procollagen processing (Fessler & Fessler, 1978).

A small proportion of the type I collagen produced during the labelling period remained associated with the cell layer (Fig. 1, lane 1). The processed collagen $\alpha 1(I)$ and $\alpha 2(I)$ chains that remained in the cell layer had faster electrophoretic mobilities than the $\alpha 1(I)$ and $\alpha 2(I)$ chains of the processed collagen in the medium fraction. The migration of these chains, which were associated with the cell layer, was not increased by treatment with pepsin (Fig. 1, lane 3), which indicated that these chains did not have intact telopeptides. These data suggested that there was proteolytic activity associated with the cells which removed the telopeptides from collagen that remained within the cell layer.

In cell culture it is possible that trypsin may be internalized during cell passaging and later released slowly into the culture medium. This possible cause for the observed activity was assessed by comparing recently trypsin-treated cells with those that had been grown for long periods with many medium changes since trypsin treatment, and by examining other cultures which had been passaged by Pronase digestion. The pattern of telopeptide cleavage was not altered in either of these experiments (results not shown). Other workers studying cell-surface associated proteinase activity have also demonstrated that the trypsin activity was not carried over from cell passaging (Bosmann *et al.*, 1976; Hatcher *et al.*, 1977; Wiseman & Hammond, 1978).

The telopeptide cleavage was only apparent in the collagen fraction that remained associated with the cell layer (Fig. 1, lane 1). Since this represents less than 10% of the total type I collagen synthesized in fibroblast culture, the telopeptide cleavage is not readily seen unless the gels are over-exposed. This need for extended exposures and the fact that most workers studying collagen synthesis have concentrated on examination of the fraction of collagen secreted into the medium, may explain why this collagen cleavage has not been demonstrated previously.

Since the cleavage of collagen telopeptides appeared to be related to its retention near the cell layer, experimental conditions were employed which promoted the accumulation of collagen in the cell layer. In previous studies we have shown that when neutral polymers were included in the medium during procollagen labelling, the procollagen was efficiently processed to collagen which was retained in the cell layer (Bateman *et al.*, 1986). The inclusion of polymer was examined by using a cell culture that had shown some telopeptide cleavage in the absence of polymer (Fig. 2, lane 1). Various concentrations of PVP were included in the cell-labelling medium (Fig. 2). When the concentration of polymer was increased, more of the collagen was retained in the cell layer, and more of the collagen was cleaved to the smaller form without the telopeptides (Fig. 2, lanes 3–6). Similar results were

Fig. 2. Electrophoresis of skin fibroblast collagens in the presence of neutral polymer (PVP)

Fibroblasts were labelled in [3 H]proline in various concentrations of PVP and cell layer (C) and medium (M) fractions analysed on 5% gels (see the Experimental section for details). Lanes 1 and 2, incubated without addition of polymer; lanes 3 and 4, with 1.75% (w/v) PVP; lanes 5 and 6, with 4.0% (w/v) PVP. All samples were reduced with dithiothreitol before electrophoresis. The migrations of type-I-procollagen pro $\alpha 1$, pro $\alpha 2$, $\alpha 1$ and $\alpha 2$ chains are shown. $\alpha 1^*$ and $\alpha 2^*$ designate the α -chains which have proteolytically lost their telopeptides. FN indicates the electrophoretic migration of fibronectin.

also obtained when other polymers such as PEG or dextran were used (results not shown). The increased telopeptide removal in the presence of neutral polymers was probably because the polymer promoted the accumulation of collagen in the cell layer where the proteolytic activity was present. In addition to altering the effective concentration of the substrate, the volume-exclusion properties of the polymer could also cause effects which lead to changes in the reaction kinetics (Laurent, 1971; Minton, 1983; Medina *et al.*, 1985).

The cleavage of collagen telopeptides was a variable finding. In some fibroblast cultures the cell-layer collagen was present only as the form lacking the telopeptides, whereas in others the collagen telopeptides remained intact. In some cultures an intermediate state with some normal and some cleaved chains existed (Fig. 2, lane 1). The extent of telopeptide cleavage did not apparently correlate with cell passage number nor with the state of confluency, and, even for a single cell line, it varied from experiment to experiment (results not shown). Although the presence of neutral polymers often increased telopeptide cleavage, in some fibroblast cultures no proteolysis occurred even when polymers were included in the culture medium. A similar variability of proteinase activity has been described for other cell-layer-associated proteinases produced by fibroblast cultures (Schwartz *et al.*, 1986). These proteinase activities, which were not assessed for collagen telopep-

Fig. 3. Electrophoresis of fibroblast cell-layer collagen labelled at different culture-medium pH values

Fibroblast cultures were labelled with [^3H]proline in culture media buffered at different pH values and containing 5% (w/v) PEG (see the Experimental section for details). Collagens in the cell layer were analysed without reduction on 5% gels. Lane 1, medium buffered at pH 6.5; lane 2, buffered at pH 7.0; lane 3, buffered at pH 7.5; lane 4, buffered at pH 8.0. The migration of type I collagen cross-linked α -chain dimers (β -components), $\alpha 1$ and $\alpha 2$ chains, and the α -chains without telopeptides, $\alpha 1^*$ and $\alpha 2^*$, are shown.

tide degradation, not only varied independently of the passage number or the site of biopsy, but also varied during the cell cycle, leading to both high and low levels of activity (Schwartz *et al.*, 1986). The variability is probably due, in part, to the artificial nature of cell-culture systems.

To maximize the proteolytic cleavage of the telopeptides and to allow a more detailed study of this process, a cell line which exhibited high levels of this activity was chosen and was labelled in the presence of 5% (w/v) PEG in subsequent experiments.

The previous experiments (Figs. 1 and 2) were performed under normal culture conditions where the medium was buffered at pH 7.5. In order to determine whether the telopeptide removal was pH-dependent, labelling was performed in media buffered and maintained at pH 6.5, 7.0, 7.5 and 8.0 (Fig. 3). At pH 6.5 (Fig. 3, lane 1) and at pH 7.0 (Fig. 3, lane 2), the $\alpha 1$ and $\alpha 2$ chains had a significantly slower migration than those chains produced at pH 7.5 (Fig. 3, lane 3) and at pH 8.0 (Fig. 3, lane 4). It was concluded that the enzyme activity which removed the telopeptides was greater at pH 7.5 and 8.0. This pH optimum for the proteolytic cleavage of the telopeptides argued against a role for lysosomal

Fig. 4. Electrophoresis of the mammalian-collagenase digests of fibroblast cell-layer collagen labelled at different culture-medium pH values

Fibroblast collagen labelled at different pH values in the presence of 5% (w/v) PEG from the experiment shown in Fig. 3 was digested with mammalian collagenase and analysed on 7.5% (w/v) polyacrylamide gels (see the Experimental section for details). Samples were not reduced before electrophoresis. Lane 1, collagen labelled in medium buffered at pH 6.5; lane 2, buffered at pH 7.0; lane 3, buffered at pH 7.5; lane 4, buffered at pH 8.0; $\alpha 1^A$, $\alpha 2^A$ and $\alpha 1^A^*$, $\alpha 2^A^*$ designate the mammalian-collagenase cleavage fragments from the *N*-terminal portion of the $\alpha 1$ and $\alpha 2$ chains and the $\alpha 1$ and $\alpha 2$ chains with degraded telopeptides respectively. The *C*-terminal collagenase fragments are designated $\alpha 1^B$ and $\alpha 1^B^*$.

enzymes, which have acidic pH optima (Burleigh, 1977).

At pH 6.5 and pH 7.0 the collagen formed cross-linked β -components (Fig. 3, lanes 1 and 2). However, these β -components were not present at the higher pH (Fig. 3, lanes 3 and 4), where the cleavage of telopeptides had occurred. Since specific lysine residues in the telopeptides are involved in forming cross-links (Kühn, 1982; Eyre *et al.*, 1984), the telopeptide cleavage must be at sites closer to the triple-helical domain than are these lysine residues.

Studies on intact α -chains did not show whether one or both telopeptides were subject to cleavage. The telopeptide cleavage was therefore examined by analysis of the fragments of collagen produced by mammalian collagenase digestion (Fig. 4). Mammalian collagenase digestion cleaves the helix at a single site to give two fragments of the $\alpha 1$ and $\alpha 2$ chains (Werb, 1982). The $\alpha 1^A$ and $\alpha 2^A$ fragments represent the *N*-terminal-3/4' fragments of these chains. The $\alpha 1^A$ and $\alpha 2^A$ fragments

Fig. 5. Electrophoresis of the CNBr cleavage products of fibroblast cell-layer collagen labelled at different culture medium pH values

Collagen labelled at different culture-medium pH values in the presence of 5% (w/v) PEG from the experiment shown in Fig. 3 was digested with CNBr and the products analysed on 12.5% (w/v) polyacrylamide gels (see the Experimental section for details). Lane 1, medium buffered at pH 6.5; lane 2, buffered at pH 7.0; lane 3, buffered at pH 7.5; lane 4, buffered at pH 8.0; lane 5, medium buffered at pH 6.5 and sample subjected to limited digestion with pepsin before CNBr cleavage. The major type I collagen CNBr peptides are indicated. $\alpha 1CB6^*$ and $\alpha 2CB3.5^*$ designate the smaller form of these C-terminal peptides with degraded telopeptides.

produced at pH 7.5 and pH 8.0 (Fig. 4, lanes 3 and 4), were smaller than these fragments produced at pH 6.5 and pH 7.0 (Fig. 4, lanes 1 and 2), where proteolytic activity was negligible (Fig. 3). These data demonstrated that part of the N-terminal telopeptides of both the $\alpha 1$ and the $\alpha 2$ chains were removed by the cell-associated proteolytic activity.

Examination of the mobilities of the C-terminal- $\alpha 1^B$ fragment suggested the proteolytic removal of a portion of the C-terminal telopeptide. This was confirmed by examination of CNBr-cleavage fragments (Fig. 5). As anticipated, the proteolytic activity which was present at pH 7.5 and pH 8.0 (Fig. 3, lanes 3 and 4) did not alter the migration of any of the CNBr peptides within the central regions of the $\alpha 1$ -chains (Fig. 5, lanes 3 and 4). However, the $\alpha 1CB6$ and $\alpha 2CB3.5$ peptides, which contain the C-terminal telopeptides of both the $\alpha 1$ and $\alpha 2$ chains, were reduced in size by the proteolytic activity (Fig. 5). The size of the degraded $\alpha 1CB6$ peptide was smaller than the $\alpha 1CB6$ peptide produced by pepsin digestion (Fig. 5, lanes 4 and 5), further showing that the cell-associated proteinase activity acted at a different site to the pepsin. The CNBr peptides from the N-terminal

Fig. 6. Electrophoresis of collagens from the fibroblast cell layer labelled in the presence of specific proteinase inhibitors

Fibroblast cultures were labelled in medium at pH 7.5 containing 5% (w/v) PEG and analysed on 5% (w/v) gels (see the Experimental section for details). Samples in lanes 2, 4, 6 and 8 were pepsin-digested before analysis. All samples were unreduced. Lanes 1 and 2, no proteinase inhibitors added (control); lanes 3 and 4, labelling medium contained 100 μ g of SBTI/ml; lanes 5 and 6, medium contained 2 units of hirudin/ml; lanes 7 and 8, medium contained 0.01 mM-Ac-Ala-Ala-Pro-Val-CH₂Cl (AcA₂PVC). The migrations of the type I collagen chains containing telopeptides ($\alpha 1$ and $\alpha 2$) and those without telopeptides ($\alpha 1^*$ and $\alpha 2^*$) are shown.

telopeptides were too small to allow analysis by polyacrylamide-gel electrophoresis.

To gain some knowledge on the nature of the cell-associated proteolytic activity, inhibitors were included during the biosynthetic labelling (Fig. 6). The choice of inhibitors was limited to those which were not toxic to cell culture; thus many inhibitors such as phenylmethanesulphonyl fluoride, N-ethylmaleimide and EDTA were not tested, since they have generalized effects on cell metabolism. SBTI was the most effective inhibitor (Fig. 6, lane 3) of those examined, and prevented most of the cleavage seen with the control, which contained no inhibitors (Fig. 6, lane 1). The extent of SBTI inhibition was variable, ranging from 50 to 80%, but 100% inhibition was never achieved. Hirudin, a potent thrombin inhibitor (Markwardt, 1970), and the elastase inhibitor Ac-Ala-Ala-Pro-Val-CH₂Cl (Powers *et al.*, 1977) also significantly inhibited the proteolytic activity (Fig. 6, lanes 5 and 7), although the effectiveness was again variable, ranging from 40 to 70%. $\alpha 1$ -Antitrypsin also had a similar inhibitory effect, whereas a comparison between cells labelled in the presence or absence of serum indicated that there was no serum inhibitor which lead to major inhibition (results not shown). This limited assessment of inhibitor specificity of the proteolytic activity, along with the pH-optima data, suggested that the enzyme (or enzymes) involved are serine proteinases and may be similar to the elastase-type proteinases previously reported in fibroblast culture

(Godeau *et al.*, 1982; Schwartz *et al.*, 1986). It was not determined whether these proteases could specifically cleave the collagen telopeptides.

The failure of the specific inhibitors to completely prevent telopeptide proteolysis may be due to several effects. For example, there could be a number of proteinases, not all of which could be inhibited, which can act on the telopeptides; fibroblast cultures have previously been shown to contain a number of cell-associated proteolytic activities (Godeau *et al.*, 1982; Harper *et al.*, 1984; Schwartz *et al.*, 1986). Alternatively, the incomplete inhibition may result from steric hindrance at the cell surface, preventing adequate access for the macromolecular inhibitors (Steven *et al.*, 1982). Also, it may be impossible in cell culture to supply the active-site inhibitors in sufficient concentration to overcome competition from the continuing synthesis of the substrate, collagen.

Insoluble fibrillar collagen is not as readily degraded by mammalian collagenase as soluble collagen (Leibovich & Weiss, 1971; Vater *et al.*, 1979), and it has been suggested, therefore, that a preliminary depolymerase activity may be required to allow effective degradation (Burleigh, 1977; Werb, 1982; Weiss, 1984). This postulated depolymerase could remove important cross-linking regions and destabilize the collagen matrix sufficiently to render it susceptible to further proteolytic breakdown. Such a depolymerase role has been proposed for granulocyte elastase (Burleigh, 1977). The fibroblast enzyme activity described here may be analogous to the granulocyte elastase and fulfill this role in the initiation of collagen breakdown by cleavage of both C- and N-terminal telopeptides. This activity appears to be different to the telopeptide-degrading neutral proteinase detected in gingival fibroblast cultures (Scott *et al.*, 1983), since this enzyme only removed the C-terminal telopeptides and was secreted into the culture medium.

The association of the skin fibroblast proteinase with the cell may be of importance physiologically, allowing the cells to direct localized collagen degradation during cell migration, tissue remodelling and growth. The increasing number of reports of cell-associated proteolytic activities, and their roles in various processes, including cell growth, cell proliferation, senescence and matrix degradation (Bosmann *et al.*, 1976; Hatcher *et al.*, 1977; Ku *et al.*, 1983; Pitts & Scott, 1983; Schwartz *et al.*, 1986), stress the need for more comprehensive studies of these activities.

In most cases the inclusion of neutral polymers in the culture medium made the cell-associated telopeptide-cleaving activity more apparent. The inclusion of neutral polymers may therefore be a useful tool in further studies of this and other cell proteolytic activities. In other studies using neutral polymers, for example those on genetic defects which affect collagen biosynthesis, the presence of this proteinase activity could be a disadvantage. In such cases the proteolytic activity may be overcome by a decrease in the pH of the culture medium to pH 7.0 or lower or by the inclusion of inhibitors such as SBTI in the medium.

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