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Stratum Corneum Chymotryptic Enzyme: A Proteinase Which May Be Generally Present in the Stratum Corneum and With a Possible Involvement in Desquamation

ANITA LUNDSTRÖM and TORBJÖRN EGELRUD

Department of Dermatology, University Hospital, Umeå, Sweden

A chymotrypsin-like proteinase that may be involved in the desquamation process in plantar stratum corneum has recently been partially characterized. The aim of the present study was to elucidate whether a similar proteinase is also present in non-palmo-plantar stratum corneum. Stratum corneum was obtained by tape stripping of volar forearm skin after the skin surface had been painted with colourless nail varnish. The adherent tissue was released from the tape strips by acetone treatment, then extracted with diethyl ether and dried. Extracts of this acetone-ether powder were analyzed with respect to proteolytic activity by means of electrophoresis under non-reducing conditions in polyacrylamide gels containing sodium dodecyl sulphate and casein. The extracts were found to contain one major chymotrypsin-like proteinase with an apparent molecular weight of around 25 kDa, and several minor proteinases with trypsin-like activity. The 25 kDa proteinase was active at pH 5.5–8, and could be inhibited by aprotinin, chymostatin and zinc ion, but not by leupeptin. No difference could be found between the 25 kDa enzyme in forearm stratum corneum and the recently described chymotrypsin-like enzyme in dissociated plantar stratum corneum cells as regards electrophoretic mobility, pH dependency, and inhibitor profile. The fact that the enzyme could degrade casein at pH 5.5 and that it appears to be present in stratum corneum in general suggests that it may play a role in the desquamation process under in vivo conditions. The tentative name »stratum corneum chymotryptic enzyme« is proposed for this newly discovered proteinase. ***Key word: Chymotrypsin.***

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Anita Lundström, Department of Dermatology, University Hospital, S-901 85 Umeå, Sweden.

In an epidermis in steady state the continuous production of new stratum corneum is balanced by a well-regulated shedding of corneocytes from the skin surface. Little is known about this desquamation process on the molecular level.

We have recently described a simple in vitro system in which cell shedding from isolated slices of plantar stratum corneum can be studied (1). When a piece of plantar stratum corneum is incubated in a simple buffer there is cell dissociation at the surface that had faced outwards in vivo. This unipolar cell shedding, that mimicks desquamation, is dependent on the activity of an endogenous chymotrypsin-like proteinase (1, 2). A preliminary characterization of a previously undescribed proteinase that fulfils the criteria for being the enzyme responsible for the in vitro cell shedding in plantar stratum corneum has recently been presented (3).

Since palmo-plantar stratum corneum in several aspects differs from stratum corneum at other body sites, it can not be taken for granted that results obtained from plantar stratum corneum are applicable to stratum corneum in general. Some disorders of cornification affect palms and soles exclusively. A unipolar, proteinase dependent cell shedding in vitro takes place also in non-palmo-plantar stratum corneum, but only if the medium is supplemented with certain detergents (4). Taken together these observations may suggest significant differences in mechanisms of cell cohesion and/or desquamation between palmo-plantar and non-palmo-plantar stratum corneum. Nevertheless it appears that proteolytic degradation of intercellular cohesive structures may be of crucial importance in desquamation irrespective of body site (1, 2, 4). It should be of interest to know if this degradation is mediated by the same enzyme in the two major types of stratum corneum. The aim of the present work was to elucidate whether an enzyme similar to the recently described chymotrypsin-like-enzyme in plantar stratum corneum with possible involvement in the desquamation process (3), is also present in non-palmo-plantar stratum corneum.

MATERIALS AND METHODS

Aprotinin, chymostatin and leupeptin were obtained from Boehringer Mannheim, Germany.

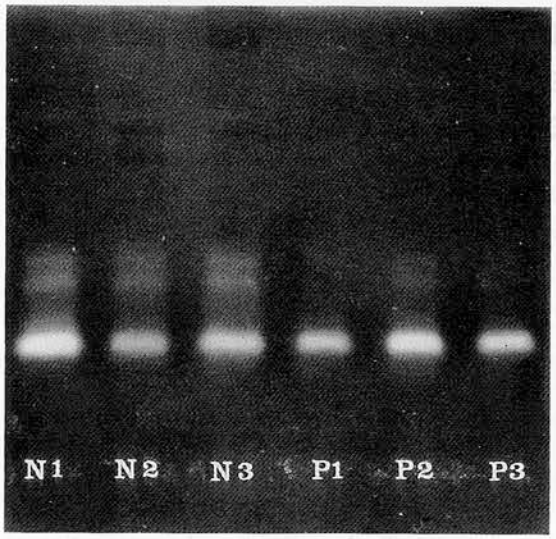

Fig. 1. Casein hydrolysing activity in extracts of stratum corneum from non-palmo-plantar stratum corneum and from plantar stratum corneum cells. Buffer for pretreatment with Triton X-100 and incubation: 0.1 M Tris-HCl, pH 8. **Lane N1–3**: Extracts from volar forearm stratum corneum from three different individuals. **Lane P1–3**: Extracts from plantar stratum corneum cells from three different individuals.

Forearm stratum corneum cells

A part of the skin surface of the volar aspect of the forearm of volunteers with normal skin was painted with acetone soluble colourless nail varnish and allowed to dry for a few min. The painted area was then stripped with pieces of Scotch tape. In one experiment 18 consecutive strips were taken from the same area with nail varnish being applied before each of the 18 tape strippings. In all the other experiments only the most superficial tape strip was used. After the strippings tape strips with a surface area of about 15 cm^2 were briefly treated with 5 ml of acetone in order to dissolve the nail varnish and release the tissue that had adhered to the tape. The tissue was recovered by centrifugation at 500 *g* for one min. The pellets obtained were treated once with 1.5 ml of acetone followed by 1.5 ml of diethyl ether and then air dried. In this way 0.3–2 mg of white powderish material was obtained from each strip, the yields being highest from the most superficial strips and decreasing with tissue depth.

Dissociated plantar stratum corneum cells

Slices of plantar stratum corneum were obtained with a skin transplantation knife from under the heels of volunteers with normal skin. The slices were incubated in 0.1 M Tris-HCl, pH 8, 0.1% sodium azide for 24 h at 37°C. This resulted in a partial dissociation of cells from the surface that had faced outwards in vivo (1, 2). These cells were scraped off and collected by centrifugation at 6,000 g for 5 min and washed once in 0.1 M Tris-HCl, pH 8. The pelleted material was treated twice with 10 volumes of acetone, once with 10 volumes of diethyl ether, and then air dried.

Analysis of caseinolytic activity

The acetone-ether treated stratum corneum preparations were extracted for 1.5 h at room temperature with Laemmli's sample buffer (5) with no reducing agent (0.625 M Tris-HCl pH 6.8, 2% sodium dodecyl sulphate (SDS), 10% glycerol); 30 µl buffer per mg powder. The clear supernatants obtained after centrifugation at 10,000 *g* for 10 min were immediately transferred to new test tubes. The extracts were analysed by electrophoresis in 12.5% polyacrylamide gels with SDS (SDS-PAGE) and co-polymerized casein. This procedure was adopted from Horie et al. (6) and carried out as described earlier (3). In brief, gels were prepared according to Laemmli (5) but 1% heat denatured bovine casein was included in the polymerisation mixture; 10 µl of the extracts were applied to each lane. After electrophoresis the gels were soaked in a buffer (0.1 M Tris-HCl pH 7–8 or 0.1 M sodium acetate pH 4–5.5 with 0.1% sodium azide) containing 2.5% Triton X-100 for one hour at room temperature. The gels were then incubated in the same buffer but without Triton X-100 at 37° C for 15 h. In experiments with inhibitors these were included in the buffers during soaking with detergent as well as during the incubation. For further details see the legends to the figures. Caseinolytic activity appeared as clear bands when the gels were stained with Coomassie blue after incubation. Electrophoretic mobility was related to the mobility of fully denatured and reduced molecular weight markers.

RESULTS

SDS-extracts of non-palmo-plantar stratum corneum and of dissociated stratum corneum cells from three different individuals were subjected to SDS-PAGE under nonreducing conditions in gels containing casein. The results of the analysis are shown in Fig. 1. The caseinolytic activity in extracts of preparations of stratum corneum from volar forearm skin could be separated into one major component with an apparent molecular weight of around 25 kDa and at least two minor components with apparent molecular weights of around 31 and 29 kDa respectively. The interindividual variations in this pattern were very small. The 25 kDa component had the same electrophoretic mobility as the major caseinolytic enzyme in extracts of plantar stratum corneum cells (3). The 29 and 31 kDa components were also present in these extracts, but in smaller amounts than in extracts of non-palmo-plantar stratum corneum.

In order to study the vertical distribution of the caseinolytic activity in non-palmo-plantar stratum corneum, 18 consecutive tape strips were taken from the same area of volar forearm skin. After the last stripping the skin surface was red with glistening patches. The material obtained was extracted at a fixed volume per weight ratio and analysed for caseinolytic activity. The results are shown in Fig. 2.

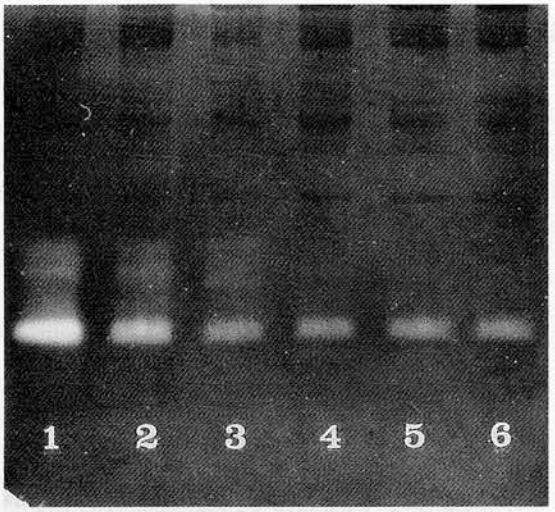

Fig. 2. Vertical distribution of the casein hydrolyzing activity in non-palmo-plantar stratum corneum (see Materials and Methods). Acetone-ether powder from three consecutive tape strips was pooled before extraction. Buffers used for pretreatment with Triton X-100 and for incubation: 0.1% Tris-HCl, pH 8. **Lane 1**: strips no 1–3; **lane 2**: strips no 4–6; **lane 3**: strips no 7–9; **lane 4**: strips no 10–12; **lane 5:** strips no 13–15; **lane 6:** strips no 16–18.

The major 25 kDa caseinolytic component could be detected at all levels examined, although the activity seemed to be greater in the superficial layers. Significant amounts of the minor 29 and 31 kDa components were detected only in material from strips no. 1–9.

The 25 kDa caseinolytic enzyme in extracts of non-palmo-plantar stratum corneum was active at pH 5.5–8, but not at pH 4 (Fig. 3). Its activity at pH 7–8 was greater than at pH 5.5, at which pH-value it was the only caseinolytic enzyme detected.

The effects of some inhibitors on the various components of the caseinolytic activity in extracts of non-palmo-plantar and plantar stratum corneum are shown in Fig. 4. Aprotinin and zinc ion inhibited all caseinolytic activity. Chymostatin inhibited the major 25 kDa component but not the minor 29 and 31 kDa components. Leupeptin, on the other hand, inhibited only the minor components.

DISCUSSION

We have recently presented evidence that cohesion between cells in the stratum corneum, at palmoplantar as well as at non-palmo-plantar sites, is dependent on protein structures (1, 2, 4, 7–9). These structures must be degraded before cell dissociation

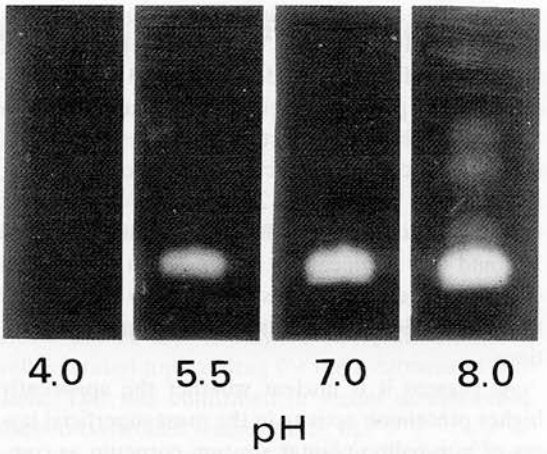

Fig. 3. The pH dependency of the caseinolytic activity in extracts of non-palmo-plantar stratum corneum. Buffers for pretreatment with Triton X-100 and incubation: 0.1 M sodium acetate (pH 4 and pH 5.5) and 0.1 M Tris-HCl (pH 7 and pH 8).

can occur. This implies that proteolytic degradation of intercellular cohesive structures may play a central role in the events that lead to a decreased cohesion in the superficial layers of the stratum corneum and eventually to desquamation. The identification of the proteolytic enzymes involved and the elucidation of their origin and regulation should be important goals in efforts to understand the final steps of epidermal turnover.

No difference could be found between the 25 kDa caseinolytic enzyme extracted from volar forearm stratum corneum described in this report and the recently described chymotrypsin-like enzyme that

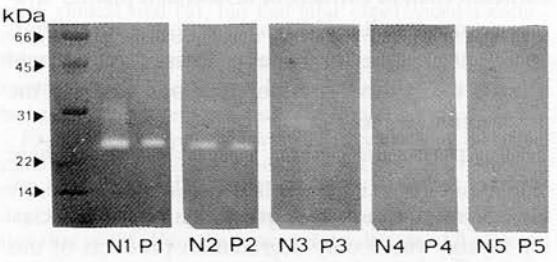

Fig. 4. The effects of inhibitors on casein hydrolysing activity in extracts of non-palmo-plantar stratum corneum and of plantar stratum corneum cells. Buffer used for pretreatment with Triton X-100 and incubations: 0.1 M Tris-HCl, pH 8, 1% dimethyl sulphoxide with the following additions: **1**: No inhibitor added, **2**: leupeptin 160 μM, **3**: chymostatin 160 μM, **4**: zinc sulphate 100 μM, **5**: aprotinin 15 μM .(Leupeptin and chymostatin were added as solutions in dimethyl sulphoxide.) **N**: non-palmo-plantar stratum corneum (volar forearm). **P**: plantar stratum corneum. Molecular weight markers to the left.

can be extracted from dissociated plantar stratum corneum cells as regards electrophoretic mobility, inhibitor profile, and pH dependency (3). The properties of the plantar proteinase are compatible with this enzyme having a function in the degradation of intercellular cohesive structures during cell shedding in vitro. The fact that the 25 kDa chymotrypsin-like enzyme can degrade casein at pH-values as low as 5.5, and that it appears to be present in the stratum corneum in general, suggests that it may also play an important role in desquamation under in vivo conditions.

At present it is unclear whether the apparently higher proteinase activity in the most superficial layers of non-palmo-plantar stratum corneum as compared to deeper layers reflects a physiologically relevant difference. One reasonable explanation could be that the partial dissociation of cells in the superficial layers facilitates the extraction of enzymes present in the extracellular space.

All caseinolytic activity that could be detected with the procedures used in this study could be inhibited by aprotinin, i.e. all detected enzymes appeared to be serine proteinases (10). In addition to the 25 kDa enzyme that could be inhibited by chymostatin (an inhibitor of chymotrypsin-like proteinases (11)) extracts from superficial parts of the stratum corneum contained proteinases with apparent molecular weights around 29 and 31 kDa. These enzymes could be inhibited by leupeptin, an inhibitor of trypsin-like enzymes (11), but not by chymostatin. The activity of the leupeptin-sensitive enzymes was greater in extracts of non-palmo-plantar stratum corneum than in extracts of dissociated plantar stratum corneum cells. This could reflect a true difference between the two types of tissue, but it could also be due to the different methods used for the preparation of the tissues.

In hypertrophic plantar stratum corneum there appears to be a rather sharp boundary between cohesive tissue with intact desmoglein I and dissociating layers, where only degradation products of this desmosomal protein can be found (8, 9). If the 25 kDa caseinolytic enzyme is responsible for the degradation of intercellular cohesive structures preceding desquamation, the fact that it could be detected at all levels of non-palmo-plantar stratum corneum could imply either that it is inhibited in deeper layers, or that its physiological substrates are not susceptible to proteolysis except in the superficial, dissociating cell layers.

In conclusion, palmo-plantar as well as non-palmo-plantar stratum corneum contains a chymotrypsin-like enzyme which may be involved in desquamation. We want to propose the tentative name »stratum corneum chymotryptic enzyme« for this newly discovered proteinase.

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