Proteolytic Degradation of Desmosomes in Plantar Stratum Corneum Leads to Cell Dissociation in vitro

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Pieces of plantar stratum corneum were incubated with trypsin. This resulted in cell dissociation. The only observable ultrastructural change caused by trypsin was a degradation of desmosomal plates between dissociating cells. This suggests that desmosomes are of primary importance in plantar stratum corneum cell adhesion. (Received September 21, 1987.)

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Epidermal steady state requires that desquamation at the skin surface balances *de novo* production of stratum corneum. The mechanisms involved in desquamation have not yet been elucidated. Before these mechanisms can be understood, the structures primarily responsible for cell adhesion in stratum corneum must be known.

When considering structures in cornified skin with possible adhesive functions, there appear to be two possible candidates: the desmosomes and the lipid-rich intercellular substance.

In living epithelia the adhesive function of desmosomes is well established (1). In the transition between the granular and the cornified layers of epidermis, the ultrastructure of desmosomes appears to undergo some changes (2). It is not known whether these changes have functional implications. The role of desmosomes in stratum corneum cell adhesion has been questioned (3).

Since the discovery of steroid sulphatase deficiency in X-linked ichthyosis (4), possible roles of lipids in stratum corneum cell adhesion have been considered (3, 5, 6, 7). A model that entirely excludes desmosomes has been presented, where stratum corneum is compared to a brick wall with the corneocytes embedded in and held together by intercellular lipids, like bricks and mortar (8). In this model it must be assumed that desmosomes, which are also found at high levels of the stratum corneum (9), lose their adhesive capacity soon after the transition between the granular and cornified layers, well before the deterioration of the stratum corneum as a physico-chemical barrier. Thus, according to the model, the elimination of desmosome-mediated cell adhesion ought not to be directly related to desquamation.

In this paper we report that cell dissociation can be induced in plantar stratum corneum by trypsinization and that the ultrastructural changes that occur in this process are compatible with an important role for desmosomes in stratum corneum cell adhesion.

MATERIALS AND METHODS

Bovine trypsin, 2×crystallized (cat. no. T 8253) was obtained from Sigma Chemical Co., St. Louis, Mo., USA. Incubations of tissue pieces were carried out in 0.01 M sodium phosphate, pH 7.2, containing 0.14 M sodium chloride and 0.1% (w/v) sodium azide (PBS).

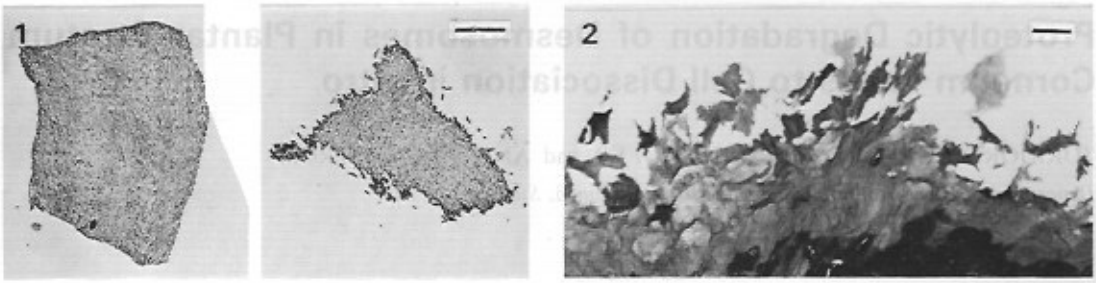

Fig. 1. Cryostat sections of pieces of plantar stratum corneum incubated for 15 h at 37°C without (*left*) or with trypsin 1 mg/ml (*right*). Phase contrast. Bar = 200 μm. Note release of cells from all surfaces of the trypsinized tissue piece and no release from the control.

Fig. 2. Semithin section, toluidine blue stain, of plantar stratum corneum incubated with trypsin. Bar = 20 μm. Note dissociation of cells only at the surface of the tissue piece.

Tissues

Plantar stratum corneum was obtained by means of horizontal cuts with a scalpel from under the heels of persons with normal skin. Samples from 5 individuals with normal skin were used in this study. The flakes were soaked in PBS for 2 h at room temperature. Cells loosely attached to the part that had faced the skin surface were then removed by firm scraping with a scalpel. The specimens were then cleaved horizontally into two halves under a dissection microscope. Cubic pieces measuring 0.3–0.5 mm were cut from the lower half and incubated in PBS containing trypsin 1 mg/ml on an agitated water bath at 37°C for 15 h. Controls were incubated in PBS without trypsin. Released cells were examined directly by phase-contrast microscopy or sedimented and prepared for electron microscopy. Incubated tissue pieces were frozen on dry ice for freeze sectioning or prepared for electron microscopy.

Processing for electron microscopy

Incubated tissue pieces and sedimented cells were fixed in 2.5% glutaraldehyde in 0.2 M sodium phosphate, pH 7.3, for at least 15 h at 4°C and postfixed in 1% osmium tetroxide for 1 h at 4°C. After dehydration in ethanol (total time 1 h) the preparations were embedded in epoxy resin and sectioned. Semi-thin sections were stained with toluidine blue. Thin sections were contrasted with lead citrate and uranyl acetate following routine procedures and viewed in a Philips EM 300 at 80 kV.

RESULTS

Light microscopy

Small pieces of plantar stratum corneum, cut from layers well below a zone above which surface cells could be removed by scraping with a scalpel, were incubated at 37°C in PBS with and without trypsin. A macroscopically observable release of cells from trypsinized tissue started after a lag period of 2–4 h. There was no observable release of cells from controls. After 15 h the incubations were stopped and the remaining tissue pieces as well as released cells were processed for microscopic examination. Freeze-cut sections showed that cells had been released from all trypsin-treated surfaces, whereas all surfaces in controls remained intact (Fig. 1).

Semi-thin sections of trypsinized tissue (Fig. 2) showed many partially dissociated cells on the surfaces. Phase-contrast microscopy of released cells (not shown) showed these to have polygonal shapes and sharp contrast, suggesting retained intracellular keratin. On semi-thin sections (not shown) the majority of released cells were intensively stained. Only occasionally were 'cell ghosts' found. The impression was that trypsin was effective only on the outermost cell layer, leaving deeper layers unaffected until overlying cells had been removed. This could be verified by electron microscopy (see below).

Fig. 3. Low-power electron micrograph from the surface of a trypsinized piece of plantar stratum corneum, showing partially dissociated cells and widened intercellular spaces. Bar = 1900 nm.

Electron microscopy

The only observable ultrastructural differences between trypsinized tissue and controls were found in the intercellular parts of desmosomes close to the surfaces of the tissue pieces where there was partial cell dissociation (Figs. 3, 6, 7). In controls (Fig. 4) and in deeper parts of trypsinized pieces (Fig. 5) the intercellular plates of desmosomes had an appearance typically found in stratum corneum with a homogenous density and an apparent lack of the midline structure found in desmosomes in living epithelium (1). The earliest change induced by trypsin was found in the lateral parts of the desmosomal plates where there was a decrease in electron density (Figs. 6, 7). This revealed a central part with an apparent resistance to trypsin as well as a structure probably equivalent to the outer leaflet of the plasma membrane. In the next following steps the central part became fragmented and was detached from one or both of the surfaces of dissociating cells (Fig. 6). No desmosomal remnants were found on the surfaces of fully dissociated cells. There were no observable effects of trypsin on keratin filaments or on the cell envelopes.

DISCUSSION

Plantar stratum corneum is a tissue with exceptional mechanical resistance, which must be due to strong intercellular adhesive structures. In our in vitro experiment, trypsin was found to cause cell dissociation in plantar stratum corneum. This suggests that structures built up by proteins are of primary importance in plantar stratum corneum cell adhesion.

A significant fraction of the intercellular space of plantar stratum corneum is occupied by intercellular desmosomal plates (10, 11), structures that are believed to be the result of an interaction between proteins anchored in opposing cells (12). The only effect that could be ascribed to trypsin in the experiment presented in this paper was an apparent degradation of desmosomal plates, suggesting that these structures are mainly responsible for cell adhesion in plantar stratum corneum. Whether the same is true also for stratum corneum of non-palmo-plantar skin is still an open question, but evidence that desmosomes or protein structures are important in stratum corneum cell adhesion also at these sites has been presented (13, 14, 15).

If lipids were mainly responsible for cell adhesion in plantar stratum corneum, it should be possible to obtain cell dissociation by extraction with lipid solvents (3, 5). This we have failed to do (unpublished). Similar results have been reported for stratum corneum from

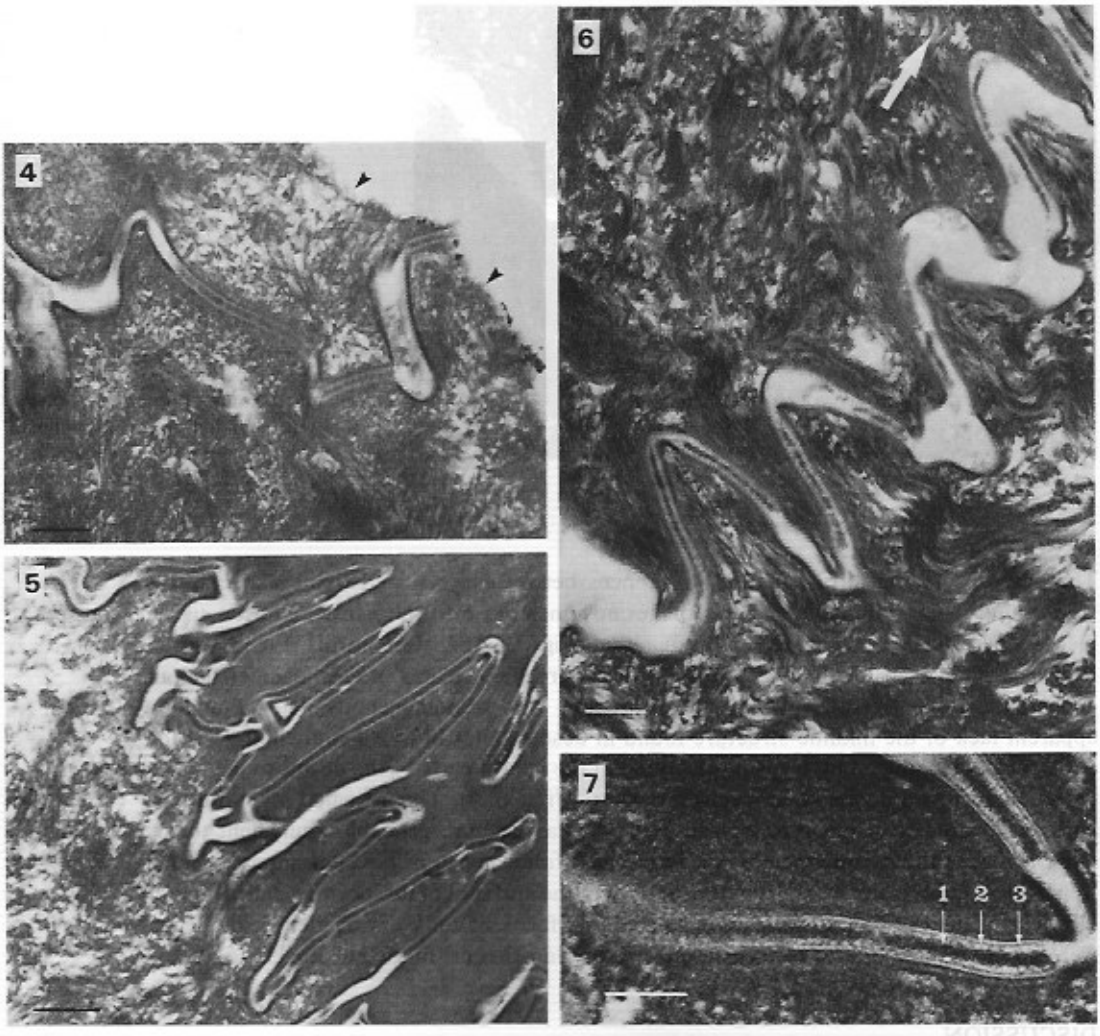

Fig. 4. Electron micrograph from the surface of a piece of plantar stratum corneum incubated in PBS without trypsin for 15 h at 37°C (control experiment). Note intact desmosomal plates. *Arrowheads*: the tissue surface that had faced the medium during the incubation. Bar = 200 nm.

Fig. 5. Electron micrograph of trypsinized plantar stratum corneum. The area shown is situated in deeper parts of the tissue piece where cell dissociation has not yet started. Note numerous apparently intact desmosomal plates in the intercellular space. Bar = 200 nm.

Fig. 6. Electron micrograph from close to the surface of a trypsinized piece of plantar stratum corneum, showing the intercellular contact zone between two partially dissociated cells. Note partially degraded desmosomal plates (*lower left*) and fragmented desmosomal plates that have been detached from one of the cells as the intercellular space has widened (*upper right*). *Arrow*: directed towards the surface of the tissue sample. Bar = 200 nm.

Fig. 7. Electron micrograph of desmosomal plates, partially degraded by trypsinization, in plantar stratum corneum. *1*: central part apparently resistant to trypsin; *2*: lateral part with decreased electron density; *3*: electron-dense structure equivalent to the outer leaflet of the trilaminar plasma membrane. Bar = 100 nm.

other body sites (14, 16). In spite of this we find it quite easy to envisage how a disorder of lipid metabolism could have an impact on desquamation. Since desmosomes in stratum corneum are embedded in the lipid-rich intercellular substance (3), changes in the composition of this substance could have (e.g. regulatory) effects on desmosome-degrading enzymes. Another possibility is that inter-cellular lipids provide weak adhesive forces of importance in the outermost layers of stratum corneum, the cells of which can be removed by mild mechanical trauma (14, 17). In this context it should be noted though, that in the so-called retention ichthyoses (among them X-linked ichthyosis) desmosomes are abundant in the thickened stratum corneum, suggestive of a delayed desmosomal degradation in these disorders (18).

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