

## Expression of Stratum Corneum Chymotryptic Enzyme in Human Sebaceous Follicles

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**Stratum corneum chymotryptic enzyme (SCCE) may be involved in desquamation, a process necessary for maintaining a normal anatomy at all sites where there is continuous turnover of cornified epithelia. Using immunohistochemistry and *in situ* hybridization, we have, in this work, analysed SCCE expression in the sebaceous follicle. We found expression of SCCE in luminal parts of the pilary canal, common sebaceous ducts and proximal sebaceous ducts. In addition, SCCE was seen in cells apparently situated within the distal parts of the glandular lobules. Co-expression of SCCE and keratin 10 was seen only in the pilary canal and the common sebaceous ducts. The results give further support for SCCE being involved in desquamation-like processes. The association with cornification seems to be more general for SCCE than for keratin 10. The possible role of SCCE in diseases involving disturbances in the turnover of cornified cells in the sebaceous follicle, such as acne vulgaris, is a question for future studies. Key words: desquamation; immunohistochemistry; *in situ* hybridization.**

(Accepted March 26, 1998.)

Acta Derm Venereol (Stockh) 1998; 78: 343–347.

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The serine protease stratum corneum chymotryptic enzyme (SCCE) may have a function in catalysing the degradation of intercellular cohesive structures in the stratum corneum of the interfollicular epidermis, i.e. one of the events involved in desquamation. Evidence supporting this function of SCCE has been obtained in studies of its enzymatic properties (1–4) and ultrastructural localization (5), and of an *in vitro* model of desquamation (6–8). So far, expression of SCCE has been demonstrated only in squamous epithelia undergoing terminal differentiation and cornification (9, 10).

The need for a desquamation-like process in order to maintain a normal anatomy is not unique for the interfollicular epidermis, but must be present at all sites where there is continuous production of a cornified cell layer with a regulated thickness, including various parts of the pilosebaceous unit. In the sebaceous follicle, disturbances of the turnover of the cornified surface epithelium of the pilosebaceous duct may be one of the earliest events in the development of the acne lesion (11, 12).

In addition to its proposed function in desquamation, SCCE has the potential to be involved in inflammatory processes, e.g. in acne. It has recently been shown that SCCE can function as an interleukin-1 $\beta$  (IL-1 $\beta$ ) activating enzyme *in vitro* (13). This should be seen in relation to the fact that whereas keratinocytes can produce the IL-1 $\beta$  precursor, they do not produce the active IL-1 $\beta$  converting enzyme (14, 15), i.e. the enzyme usually associated with IL-1 $\beta$  activation (16, 17). The possible importance of alternative activation mechanisms for IL-1 $\beta$  in the epi-

dermis has also been supported by results from biochemical studies of biologically active IL-1 $\beta$  in epidermal extracts (18, 19).

As part of our efforts to further elucidate the possible functions of SCCE in the skin under normal and pathological conditions, we have studied its expression in the sebaceous follicle with immunohistochemical techniques and by means of *in situ* hybridization.

### MATERIALS AND METHODS

#### *Skin biopsies*

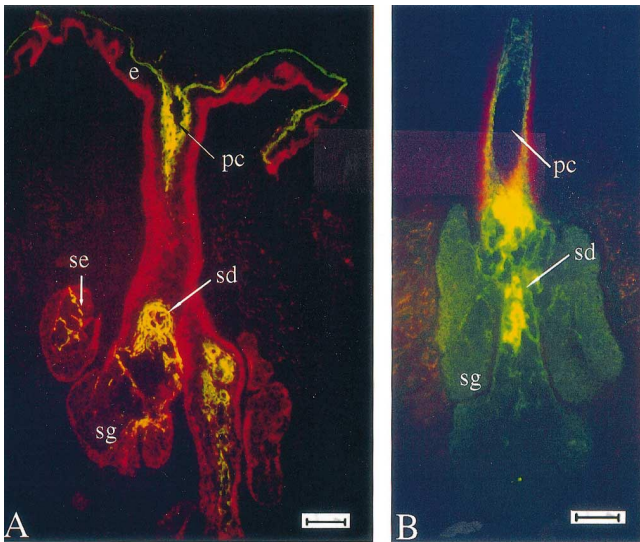
Four-mm punch biopsies were taken under local anaesthesia from the chests of 12 volunteers with healthy skin. Biopsies were also taken from the scalps of 4 volunteers with no skin diseases. For immunofluorescence microscopy, the biopsies were fixed for 1 h in 4% buffered formaldehyde at room temperature, mounted in Tissue-Tec OCT compound (Miles Laboratories, Elkhart, IN, USA) and snap frozen in propane chilled with liquid nitrogen. For immunoperoxidase staining, biopsies were fixed for at least 15 h in 4% buffered formaldehyde and paraffin-embedded according to routine procedures.

#### *Antibodies and reagents*

Affinity purified polyclonal rabbit anti-SCCE was provided by Astra-Hässle, Umeå, Sweden. The production and characterization of these antibodies have recently been described (10). The mouse monoclonal antibody (mAb) to keratin 14 (mAb LL001) was a generous gift from Dr. Irene Leigh, London. MAb to keratin 10 (anti-human cytokeratin 10 DE-K10), biotinylated goat anti-rabbit IgG, and fluorescein isothiocyanate (FITC)-labelled streptavidin were obtained from Dakopats, Älvsjö, Sweden. Rhodamine isothiocyanate (TRITC)-labelled goat anti-mouse IgG was from Southern Biotechnology, Birmingham, AL, USA. The detection system for immunoperoxidase staining (Super Sensitive StrAviGen Multi-Link horseradish peroxidase and the Liquid DAB Substrate Pack) were purchased from BioGenex, San Ramon, CA, USA. Rabbit IgG purified from normal rabbit serum by means of protein A affinity chromatography was used as negative control for the rabbit anti-SCCE.

#### *Immunofluorescence staining*

Five  $\mu$ m cryosections were fixed for 10 min in cold acetone and then treated with trypsin 0.33 mg/ml (Boehringer Mannheim GmbH, Germany, cat. no. 109819) in 0.1 M Tris-HCl with 0.002 M CaCl<sub>2</sub> pH 7.6. The sections were then blocked with 5% normal goat serum for 10 min at room temperature and incubated with primary antibodies (anti-SCCE or control antibodies 10  $\mu$ g/ml; mAb anti-keratin 10 1/10; mAb anti-keratin 14 undiluted culture supernatant) for 45 min at 37°C. Each primary antibody was incubated either alone or as a mixture with the anti-SCCE. After being washed in phosphate-buffered saline (PBS) and incubated for 30 min at room temperature with biotinylated goat anti-rabbit IgG, the slides were again washed and incubated with a mixture of FITC-streptavidin (1/50) and TRITC-anti-mouse IgG (1/50) for 30 min at room temperature. All reagents were diluted in 0.1% bovine serum albumin in PBS. After final washings in PBS, the sections were mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA) and analysed using a Zeiss



**Fig. 1.** Immunofluorescence microscopy of sebaceous follicles. (A) Follicle from the chest double-labelled with anti-SCCE (green) and anti-keratin14 (red). (B) Follicle from the scalp double-labelled with anti-SCCE (green) and anti-keratin 10 (red). e=epidermis; pc=pilary canal; sd=sebaceous duct; se=septumlike structures; sg=sebaceous gland. Bars: 100  $\mu$ m. The yellow colour is due to relative overexposure of the FITC-fluorescence (green) and not to overlap of FITC and TRITC.

microscope equipped with double filters for TRITC and FITC epifluorescence.

#### Immunoperoxidase staining

Five  $\mu$ m sections were deparaffinized in xylene and rehydrated in graded ethanol. Blocking of endogenous peroxidase was carried out with 3% hydrogen peroxide in methanol for 5 min at room temperature. For antigen retrieval, slides were incubated for 10 min at room temperature in 0.01 M sodium citrate pH 6.0 which had been preheated to boiling in a microwave oven, rinsed for 2  $\times$  5 min in PBS, and then treated with pepsin 0.04 mg/ml (Sigma, St. Louis, MO, USA, cat. no. P 7000) in 0.2M HCl for 15 min at room temperature. The sections were then blocked with 5% normal goat serum in PBS for 5 min at room temperature and incubated with primary antibodies diluted in 5% normal goat serum in PBS (anti-SCCE or control antibodies 3  $\mu$ g/ml; mAb anti-keratin 10 1/500). For detection of bound primary antibodies, the protocol provided by BioGenex was followed. The sections were counterstained with hematoxylin, dehydrated, and mounted in Histomount (CIAB, Lidingö, Sweden).

#### In situ hybridization

This was carried out with digoxigenin-labelled sense and anti-sense RNA probes corresponding to a segment spanning base pairs 155–498 of the human SCCE cDNA. The protocol of Panoskaltsis-Mortari & Bucy (20) was used, except for the blocking, where goat serum was used. Bound probes were detected with alkaline phosphatase conjugated anti-digoxigenin Fab fragments and 4-nitroblue tetrazolium chloride (NBT)/5-bromo-4-chloro-3-indolyl-phosphate (BCIP) (Boehringer Mannheim, Germany) as chromogenic substrate.

## RESULTS

#### Immunofluorescence microscopy

Figure 1 shows immunofluorescence microscopy after double labelling of a section of a sebaceous follicle from the chest and adjacent interfollicular epidermis, stained with antibodies

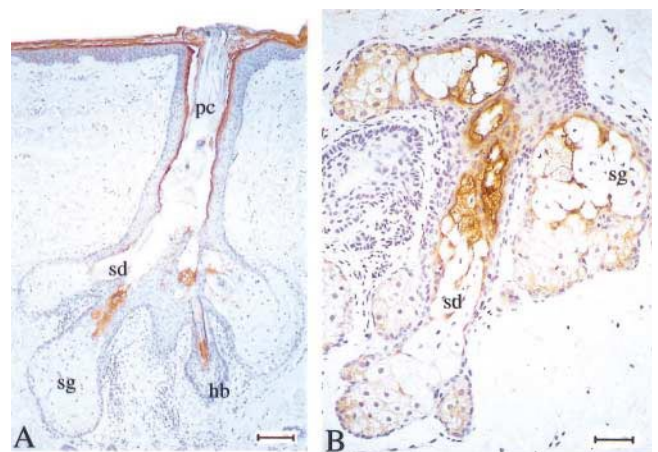
to SCCE and keratin 14 (Fig. 1A), and of a part of a sebaceous follicle from the scalp, stained with antibodies to SCCE and keratin 10 (Fig. 1B). SCCE-positive staining could be detected in high suprabasal cells of the epidermis, in the luminal part of the pilary canal, and in the sebaceous ducts. In addition, there was staining of septum-like structures apparently localized within glandular lobuli (Fig. 1A). Analysis of a large number of sections with longitudinally and transversely sectioned sebaceous glands showed that these structures were localized in the distal parts of the glandular lobuli close to the beginning of the sebaceous ducts (see also below). In the sebaceous ducts, the SCCE-positive staining was of high intensity, and the stained structures sometimes had a mesh-like appearance.

Antibodies to keratin 14 stained basal cells of the epidermis, the pilary canal, the sebaceous ducts, and the sebaceous glands. No overlap in staining with these antibodies and antibodies to SCCE was found (Fig. 1A).

Keratin 10 antibodies stained suprabasal cells in the epidermis (not shown) and in the pilary canal (Fig. 1B). At these sites, the staining with keratin 10 antibodies partially overlapped with SCCE staining. No staining for keratin 10 could be detected in the sebaceous ducts or within the sebaceous glands (Fig. 1B).

#### Immunoperoxidase staining

Results of immunoperoxidase staining with SCCE antibodies after paraffin embedding are shown in Figs. 2, 3A and 4. These results corroborated the findings with immunofluorescence labelling of cryo sections, and allowed a more detailed analysis of the structures being stained. In sebaceous follicles from the chest, SCCE-specific staining was found in a narrow zone facing the lumen of the pilary canal, which was continuous with the SCCE-positive suprabasal parts of the interfollicular epidermis (Fig. 2A). SCCE-positive staining was also seen in the sebaceous ducts, either as a thin luminal zone continuous with the lining of the pilary canal (Fig. 2A), or in a mesh-like pattern within the ducts (Figs. 2A, B). SCCE-positive septum-like structures could be seen in the distal parts of the glandular lobuli (Figs. 2B, 3A) and in the proximal parts of the sebaceous ducts (Figs. 2B). At higher magnification, these structures



**Fig. 2.** (A) and (B). Immunoperoxidase staining with anti-SCCE of sebaceous follicles from the chest. pc=pilary canal; sd=sebaceous duct; sg=sebaceous gland; hb=hair bulb. Bars: (A) 100  $\mu$ m, (B) 50  $\mu$ m.

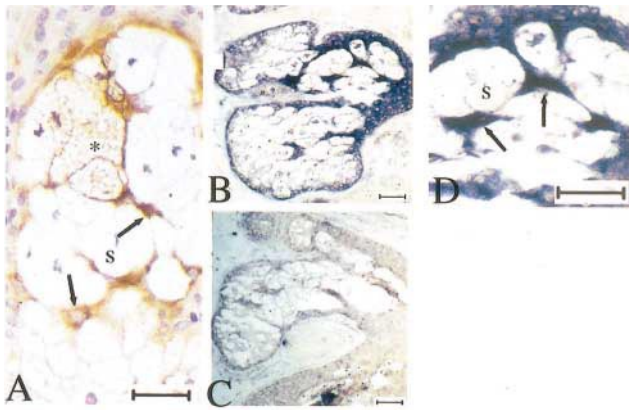


Fig. 3. SCCE-expression in intralobular structures of sebaceous follicles from the chest. (A) Immunoperoxidase staining with anti-SCCE. (B), (C) and (D) *In situ* hybridization with SCCE-specific RNA-probes. (B) Anti-sense. (C) Sense (neg. control). (D) Anti-sense, a part of the sebaceous gland shown in (B), at higher magnification. Arrows: elongated, nucleated SCCE-positive cells interspersed between sebocytes. \* = sebocyte-like SCCE-positive cells; s = sebocyte. Bars: (A) 27  $\mu$ m. (B), (C) and (D) 50  $\mu$ m.

could be seen to consist of elongated, nucleated cells interspersed between sebocytes (Fig. 3A). In the proximal parts of the sebaceous ducts, sebocyte-like cells with a fine intracellular network, which was stained by the SCCE antibodies but not with control IgG, were seen. These sebocyte-like cells were often found in close proximity to the septum-forming SCCE-positive cells (Fig. 3A; negative results with control IgG not shown).

Immunoperoxidase staining of sebaceous follicles after paraffin embedding with antibodies to keratin 10 was positive in suprabasal cells in the pilary canal and in the common sebaceous ducts. No staining was seen in proximal sebaceous ducts or of any structures in distal glandular lobuli (not shown).

Sebaceous follicles in scalp skin were more elongated than in chest skin, but were found to show a similar pattern of SCCE staining (Fig. 4A), with a distinct mesh-like pattern of the staining in the sebaceous ducts. Fig. 4B shows a sebaceous follicle from the scalp with early comedo formation. The most distal part of the follicle was found to be filled with SCCE-positive material with the appearance of cornified keratinocytes, whereas the amorphous material filling up the infrainfundibulum showed no or only weak staining.

#### *In situ* hybridization

The results of *in situ* hybridization with an SCCE-specific anti-sense RNA probe are shown in Fig. 3B and D, and the corresponding sense probe as a negative control in Fig. 3C. At the mRNA level, SCCE expression could also be demonstrated in elongated cells interspersed between sebocytes (Figs. 3B and D) and in cells facing the lumen of the sebaceous duct and the pilary canal (not shown).

#### DISCUSSION

Based on enzymatic properties (1–4), *in vitro* studies (6–8), and ultrastructural localization (5), the proposed function of



Fig. 4. Immunoperoxidase staining with anti-SCCE of sebaceous follicles from the scalp. (A) Normal follicle. (B) Follicle with early comedo formation. v = vellus follicle; sd and arrows = sebaceous duct; sg = sebaceous gland. Bars: 100  $\mu$ m.

SCCE has been to catalyse the degradation of intercellular cohesive structures in the stratum corneum, i.e. one step in an only partially understood series of events which eventually lead to desquamation. In this work, we present findings compatible with this suggested function in the sebaceous follicles as well.

Desquamation may be described as a process specifically taking place where cornified epithelial structures are being continuously formed, and where there must be a continuous turnover of these structures in order to maintain a normal anatomy. The anatomy of sebaceous follicles was described by Montagna (21), and a detailed ultrastructural description of the epithelium lining the pilosebaceous canal and the various parts of the system of ducts in the sebaceous glands was given by Knutson (12). The glandular lobules have, in their distal portions, their own ductal structures, so-called "secondary ducts". Ducts from adjacent lobules merge and form wider ducts, eventually forming the common sebaceous duct ending in the pilary canal. In a pilosebaceous unit, there are several sets of glandular lobules with their own common sebaceous duct (22). The most luminal parts of the ducts, at all levels of the pilosebaceous unit, are made up of cornified cells. These cells are continuously being shed and becoming constituents of the secreted material (12). It may thus be concluded that a desquamation-like process takes place not only in the superficial parts of the epithelium lining the pilary canal and the common duct, but also at the luminal surfaces of the intralobular ducts at all levels of the sebaceous glands.

The SCCE-specific antibodies stained structures at all luminal surfaces of the pilosebaceous unit, i.e. in the pilary canal, the common sebaceous duct, and the secondary sebaceous ducts. Thus, SCCE expression could be demonstrated at all sites in the pilosebaceous unit where epithelial cells are known to go through terminal differentiation, form a cornified surface layer, and eventually be shed to the lumen. The distribution of

keratin 10, however, was confined to suprabasal cells in the pilary canal and in the sebaceous duct, i.e. to a much more restricted part of the follicle. The findings give support for a role of SCCE in desquamation.

It is likely that at least a part of the SCCE-positive material apparently localized within the lumina of sebaceous ducts, which was strongly stained especially by means of immunofluorescence, emanates from shed surface cells. This is supported by findings that the number of intraluminal structures seen in electron microscopy and believed to be derived from shed surface cells is especially high in the more proximal parts of the sebaceous ducts (12).

The nature and function of the SCCE-positive cells apparently interspersed between sebocytes, and sometimes forming septum-like structures in the distal parts of the glandular lobules, remain to be explained. The localization and appearance of these cells suggest that they are truly localized between sebocytes in the intact tissue, and that they are not shed surface cells. This is also supported by the fact that they often contained a nucleus, and that they can be shown to express SCCE also at the RNA level. The cells may represent precursors of the cornified intralobular septae described by others (23). One explanation that we can offer at present is that these cells represent thin proximal extensions of intralobular ducts. It is possible that the type of scaffold that could be formed in this way in the distal parts of a lobule may facilitate the secretory process. The possible function of SCCE at this level of the pilosebaceous unit remains to be elucidated.

Another interesting finding which is not obviously linked to the proposed function of SCCE in desquamation-like processes is the apparent localization of SCCE within sebocyte-like cells in the distal parts of glandular lobules and in proximal sebaceous ducts. Knutson (12) found a significant fraction of the cells facing the lumen of proximal ducts to be filled with small lipid droplets. Since these surface cells are also likely to be shed, they may possibly be identical to the SCCE-positive sebocyte-like cells observed here. If this is true, SCCE expression by these cells would also be compatible with a role of SCCE in desquamation.

The possible role of SCCE or disturbances in SCCE function in the various steps of the evolution of the acne lesion needs further study. Early comedo-formation is the result of disturbances in the turnover of keratinized surface cells in the pilary canal (11, 12), possibly involving disturbances of desquamation. As shown in this work, the keratinous plug in the orifice of a pilosebaceous unit with early comedo-formation, as well as the ductal epithelium facing the comedo, were found to be strongly stained with SCCE antibodies. This suggests that the formation of the comedo could not be explained by a decreased production of the SCCE protein. SCCE is produced as an inactive precursor which has to be activated by means of tryptic cleavage (24) by an as yet unknown epidermal enzyme. A disturbance in SCCE activation could thus contribute to a decreased rate of desquamation. Whether this type of disturbance is involved in comedo-formation remains to be elucidated.

Another possibility is that SCCE and related enzymes may be involved in the initiation and propagation of the inflammatory reaction in acne. It has been shown that SCCE can catalyse the production of biologically active IL-1 $\beta$  from the inactive IL-1 $\beta$  precursor (13). By means of immunohistochemistry, this pro-inflammatory cytokine has been shown to be

widely distributed in the normal pilosebaceous unit (25). Being a protease with broad substrate specificity, SCCE may possibly also catalyse the formation of other inflammatory active products in conditions under which its normal tissue distribution has been disturbed.

To summarize, the pattern of SCCE expression in the sebaceous follicle gives further support to the proposed function of SCCE in desquamation-like processes. We cannot, however, exclude the possibility that SCCE has other as yet unknown functions in sebum secretion. The possible role of SCCE in acne and other pathological processes of the sebaceous follicle is an area for future studies.

#### ACKNOWLEDGEMENTS

This work was supported by the Swedish Medical Research Council (Grant K97-12X-11206-03A), the Medical Faculty, Umeå University and Astra-Hässle AB.

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