Refined Characterization of Corneodesmosin Proteolysis during Terminal Differentiation of Human Epidermis and Its Relationship to Desquamation*

Received for publication, January 10, 2001, and in revised form, February 16, 2001
Published, JBC Papers in Press, February 16, 2001, DOI 10.1074/jbc.M100201200

Michel Simon‡, Nathalie Jonca‡§, Marina Guerrin‡, Marek Haftek§, Dominique Bernard¶, Cécile Caubet***, Torbjörn Egelrud‡‡, Rainer Schmidt††, and Guy Serre§§§

From the ‡Department of Biology and Pathology of the Cell, INSERM Contrat Jeune Formation 96–02, Toulouse-Purpan School of Medicine, University of Toulouse III (Institut Fédératif de Recherche 30, INSERM-CNRS-Université P. Sabatier-Centre Hospitalier Universitaire), 31073 Toulouse, France; §§§INSEM U346-CNRS “Human Skin and Immunity”, 69437 Lyon, France, L’Oreal, Life Science Research, Centre Charles Zviak, 92583 Clichy, France, and the ‡‡Department of Dermatology, University Hospital, S-901 85 Umeå, Sweden

Corneodesmosin is a putative adhesion glycoprotein located in the extracellular part of the desmosomes in the upper layers of the epidermis. Synthesized by granular keratinocytes as a 52–56-kDa protein, corneodesmosin is progressively proteolysed during corneocyte maturation. This processing is a prerequisite for desquamation. Two glycine- and serine-rich domains of the protein might take on the conformation of adhesive secondary structures similar to glycine loops.

Corneodesmosin proteolysis was further characterized. Deglycosylation experiments and reactivity with lectins demonstrated that the corneodesmosin carbohydrate moiety does not prevent the proteolysis. Immunoblotting, immunohistochemistry, and immuno-electron microscopy experiments using affinity-purified anti-peptide antibodies raised to four of the five structural domains of corneodesmosin and a monoclonal antibody against its fifth central domain showed that the first step in corneodesmosin processing is the cleavage of its extremities and probably occurs before its incorporation into desmosomes. Then the glycan loop-related domains are cleaved, first the N-terminal and then part of the C-terminal domain. At the epidermis surface, the multistep proteolytic cleavage leaves intact only the central domain, which was detected on exfoliated corneocytes and probably lacks adhesive properties. Importantly, corneodesmosin was demonstrated to be a preferred substrate of two serine proteases involved in desquamation, the stratum corneum trypsin and chymotryptic enzymes.

Keratinocytes constitute the major cellular population in epidermis, where they proliferate in the innermost basal layer. During their transit through the spinous and granular layers toward the skin surface, keratinocytes express a specific program of terminal differentiation that culminates in the formation of corneocytes (1, 2). These dead “mummified” cells accumulate and form the outermost cornified layer of epidermis or stratum corneum, (SC)1, which plays a critical role in the physical protection of the body. To maintain a constant SC thickness, as observed in normal epidermis, the continuous generation of corneocytes is balanced by cell shedding at the external surface in the tightly regulated process of desquamation (3).

Cohesion of the SC is largely dependent on modified desmosomes or corneodesmosomes. In fact, at the transition between the granular layer and the SC, profound changes are observed in desmosome morphology. The cytoplastic plaque, which provides anchorage for cytokeratin intermediate filaments, is no longer visible, and a homogeneous electron-dense plug occurs instead of the characteristic symmetrical tri-lamellar structure of the extracellular core (4–9). Corneodesmosome degradation is of major importance in the desquamation process (6–9). In xerosis and various hyperkeratotic states, including psoriasis, accumulation of scales is observed, and the number of corneodesmosomes persisting over the corneocyte surface in the upper SC is greatly increased (10–13). Several serine proteases, including the stratum corneum chymotryptic enzyme (SCCE) and the stratum corneum trypsin enzyme (SCTE), are thought to be involved in corneodesmosome proteolysis (14–18). SCCE and SCTE belong to the kallikrein family, a subfamily of serine proteases whose genes are located in a single locus at 19q13.3–13.4 (19).

At a molecular level, the two major adhesive transmembrane components of corneodesmosomes are desmoglein 1 (Dsg1) and desmocollin 1 (Dsc1) (20, 35). Desmogleins and desmocollins are glycoproteins belonging to the family of cadherins, calcium-dependent cell adhesion molecules. Each desmosomal cadherin is known to exist in three different isoforms encoded by three different genes. Their expression is tissue-specific and differentiation-dependent, Dsg1 and Dsc1 being expressed in the uppermost layers of the epidermis (for a review see (21)).

We identified another protein, corneodesmosin (Cdsn), located in the corneodesmosome core (9). Cdsn is synthesized in the upper spinous and/or lower granular layers in the form of a 52–56-kDa phosphorylated basic glycoprotein. It is ex-

* This study was supported in part by grants from the Université Paul Sabatier-Toulouse III, from L’Oreà©al (Paris, France) and from INSERM. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

§ Supported by a postdoctoral fellowship from the Societé de Secours des Amis des Sciences and from the Singer Polignac Foundation.

** Recipient of a grant from the French Ministry of Research and Technology.

†† To whom correspondence should be addressed: Laboratoire de Biologie Cellulaire et Cytologie, CHU Purpan, Place du Dr Baylac, 31059 Toulouse Cedex, France. Tel.: 33-5-61-77-23-95; Fax: 33-5-61-77-76-20; E-mail: serre.g@chu-toulouse.fr.

‡‡ Recipient of a grant from the French Ministry of Research and Technology.

¶§§§ The abbreviations used are: SC, stratum corneum; SCCE, stratum corneum chymotryptic enzyme; SCTE, stratum corneum trypsin enzyme; Dsc, desmocollin; Dsg, desmoglein; Cdsn, corneodesmosin; MoAb, monoclonal antibody; PAGE, polyacrylamide gel electrophoresis.
ported by cytoplasmic vesicles called keratinosomes into the extracellular space where it interacts with the core of the desmosomes just before their transformation into corneodesmosomes (9, 13, 22, 23). Cdsn is a 529-amino acid-long, glycine- and serine-rich protein. These amino acids have been proposed to form peculiar secondary structures, the so-called glycine loop-related domains at the termini of the protein where they are particularly frequent, i.e. amino acids 65–175 and 375–450 (24). The suggested function of these structural motifs, which are found in other epidermal proteins such as cytokeratins and loricrin, is to interact with identical loops on the same or neighboring proteins (25). Some experimental evidence was obtained in favor of a role for Cdsn in corneocyte cohesion (26). During the maturation of the SC, the protein is progressively proteolysed. We previously proposed that the process results in the suppression of the two glycine loop-related domains, thus eliminating the adhesive parts of the molecule and allowing desquamation (23, 24). Until now, the enzymes involved, and the precise steps, in this proteolysis were not known.

Here, we show that Cdsn deglycosylation is unnecessary for proteolysis to occur in vivo, and we characterize the Cdsn cleavage steps and their sequence. Finally, we show that Cdsn is a good substrate in vitro for both SCTE and SCCE.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—The anti-Cdsn monoclonal antibodies (MoAbs) G36-19 and F28-27 are part of a series of antibodies produced and characterized in our laboratory (9, 23). DG3.10, a MoAb directed to Dsg1 and Dsg2, and PG5.1, a MoAb directed to plakoglobin, were purchased from Progen Biotechnik GmbH (Heidelberg, Germany). An anti-γ-catenin MoAb from Transduction Laboratories (Lexington, KY) was also used. The ascites fluid of MOPC-21 (Sigma) was used as a negative control. An anti-involucrin antiserum was purchased from Biomedical Technologies Inc. (Stoughton, CA).

Antibodies to the N- and C-terminal domains of Cdsn were elicited in rabbits by injection of synthetic peptides conjugated via a C-terminal cysteine residue to keyhole limpet hemocyanin. The peptides used were synthesized according to the predicted amino acid sequence (GenBank™ accession number AF030130) as follows: peptide A, DPCKDP-TRITSPNDPC (amino acids 40–55); peptide B, SAGSFKPGTG-TRITSPA (amino acids 213–228); peptide C, GSPYHPGCSQSPC (amino acids 409–423); and peptide D, DGSHPDPDASAGKPC (amino acids 472–486). Anti-peptide antibody titers were determined by enzyme-linked immunosorbent assays (CovalAb, Lyon, France). The antiserum was affinity-purified on their corresponding peptide coupled to agarose-activated affinity columns (SulfoLink® kit) essentially as described by the manufacturer (Pierce). Elution fractions obtained when the antiserum were loaded on an affinity column coupled with a peptide different from that used for their production were used as negative controls.

**Extraction of Proteins from Human Epidermis and Superficial SC**—As previously described (23), dermo-epidermal cleavage of breast skin (obtained from patients undergoing plastic surgery) was performed by heat treatment, and the epidermis was sequentially extracted in a Tris-EDTA buffer containing Nonidet P-40 (10% (vol/vol) buffer extract), 8 M urea (TEU buffer extract), or urea and dithiothreitol (TEUDTT buffer extract). Superficial SC extracts were obtained from volunteers using varnish stripping as previously reported (24). Proteins were solubilized in the presence of 2% SDS (or 8 M urea for two-dimensional gel electrophoresis) and 50 mM dithiothreitol, and their concentration was measured using the Bio-Rad protein assay (Bio-Rad Laboratories GmbH, Munich, Germany).

**Protein Electrophoresis and Immunoblotting**—Proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) or by two-dimensional electrophoresis in the presence of urea as previously described (23). After electrophoresis, proteins were either stained with Coomassie Blue or electrotransferred to reinforced nitrocellulose membranes. The membranes were stained with either Ponceau Red or Pro
togold (British BioCell International, Cardiff, UK) and probed with antibodies as previously reported (23). The anti-γ-catenin MoAb, G36-19, F28-27, and MOPC-21 were diluted to 0.1 μg/ml, and DG3.10 and PG5.1 were diluted to 0.5 μg/ml. Affinity-purified anti-peptide antibodies were diluted to 1/250 or 1/500. Immunoreactivities were revealed with the ECL® Western blotting kit as described by the manufacturer, Amersham Pharmacia Biotech.

**Blocking with Lectins**—Proteins of the SC extracts were separated by two-dimensional gel electrophoresis, electrotransferred to nitrocellulose membranes, and probed with biotinylated lectins as previously described (23).

**Deglycosylation Experiments**—In SC extracts (10 μg of protein), Nonidet P-40 and EDTA concentrations were adjusted to 1% and 20 mM, respectively. To the extracts, 2.4 units of N-glycosidase F (EC 3.5.1.52, Oxford GlycoSystems Ltd., Abingdon, UK) were added, and the reaction mixture was incubated at 37 °C for 6 h. The reactions were stopped by boiling for 2 min in sample buffer. Treated and mock samples were separated by SDS-PAGE and analyzed by immunoblotting as described above. Deglycosylation of a TENP-40 buffer extract was used as a positive control.

**Immunohistochemistry and Immunoelectron Microscopy**—Immunohistochemistry was performed on Bouin’s fixed samples of breast skin embedded in paraffin using the peroxidase-labeled streptavidin-biotin amplification method and on cryosections of breast skin using indirect immunofluorescence. G36-19, F28-27, and MOPC-21 were diluted to 2.5 μg/ml, and the affinity-purified anti-peptide antibodies were diluted to 1/20 or 1/50. On controls, where parallel sections were reacted in the absence of primary antibody, no significant immunoreactivity was observed.

Immunoelectron microscopy was performed on normal skin fragments using postembedding methods or labeling of cryosections essentially as described previously (9, 27, 28). A mild fixation of the tissue in 3% paraformaldehyde for 3 h was used prior to low-temperature embedding in Lowycril K4M (Polysciences Europe GmbH, Eppelheim, Germany), whereas cryofixation in liquid propane preceded the embedding in Lowycril K11M (Polysciences). For ultracyrosectioning, the fragments were fixed in 2% paraformaldehyde overnight at 4 °C, soaked for 30 min in 64% sucrose, and plunged-frozen in liquid propane. Cryosections of 80 nm were obtained at −110 °C. In all cases, the ultrathin sections were harvested on Formvar-coated nickel grids and subjected to immunostainings. The primary antibodies were revealed using either a 5-nm immunogold conjugate (goat anti-rabbit or anti-mouse, Amersham Pharmacia Biotech), or AuroProbe One, 1-nm colloidal gold conjugate (Amersham Pharmacia Biotech) followed by silver enhancement (Aurion, Epe, The Netherlands). Immunoreactivities were visualized using 1-nm colloidal gold (British BioCell International, Cardiff, UK) and probed with the secondary antibody, with and without amplification method and on cryosections of breast skin using indirect immunofluorescence. G36-19, F28-27, and MOPC-21 were diluted to 2.5 μg/ml, and the affinity-purified anti-peptide antibodies were diluted to 1/20 or 1/50. On controls, where parallel sections were reacted in the absence of primary antibody, no significant immunoreactivity was observed.

**Proteolysis Experiments Using a Cdsn-containing Epidermal Extract**—Recombinant ProSCCE was produced using murine C127 cells, purified and activated with agarsone-bound trypsin as previously described (14). Epidermal extracts enriched in SCCE were obtained as published (17). In proteolysis experiments, 50 ng of recombinant ProSCCE or SCCE, 100 ng of enriched epidermal SCCE, or an equal volume of protease buffer (10 mM sodium phosphate buffer, pH 7.2, 0.5 μM NaCl) were added to the TENP-40 buffer extract (70 μg of proteins) together with 10 times the concentrated proteolysis buffer. The reaction mixture was incubated at 37 °C for 0–4 h. The reactions were stopped by boiling for 2 min in sample buffer. Treated and mock samples were separated by SDS-PAGE and analyzed by immunoblotting as described above.

**RESULTS**

**Glycosylation of the 46–43 and 36-kDa Cdsn Fragments**—To examine whether Cdsn deglycosylation is necessary for proteolysis to occur, proteins of superficial SC extracts obtained by varnish stripping from six different individuals were treated with N-glycosidase F and analyzed by immunoblotting with the anti-Cdsn MoAb G36-19 diluted to 200 ng/ml, and the reaction mixture was incubated at 37 °C for 6 h. The reactions were stopped by boiling for 2 min in sample buffer. Treated and mock samples were separated by SDS-PAGE and analyzed by immunoblotting as described above. Glycosylation of a TENP-40 buffer extract was used as a positive control.
apparent molecular mass of the 36-kDa Cdsn fragments in
some of the extracts as shown for individuals 1 and 2. However,
the smaller fragments did not show any modification in their
migration rate. These results strongly suggest persistence of
N-glycosylation of the larger Cdsn fragments.

To confirm these data, proteins of the SC extracts were
separated by two-dimensional gel electrophoresis and analyzed
with biotinylated lectins and with G36-19. Concanavalin A and
Pisum sativum agglutinin strongly bound to proteins that prob-
ably correspond to proteolysis products of desmosomal cad-
herins. These lectins also bound the high but not the low
molecular weight fragments of Cdsn. The other lectins tested
(wheat germ and Ricinus communis agglutinins) did not stain
any proteins (data not shown).

As a whole, these results indicate that the 46–43- and 36-
kDa forms of Cdsn are N-glycosylated, like the 52–56-kDa
precursor form.

**Further Characterization of Cdsn Processing**—To precisely
determine the proteolysis steps of Cdsn maturation and to
examine the fate of Cdsn fragments at the SC surface, four
antibodies were developed and affinity-purified against pep-
tides corresponding to different domains of the protein: serum
A40–55 specifically recognizes the N terminus between the sig-
nal peptide and the N-terminal glycine loop-related domain
(amino acids 40–55), sera B102–115 and C409–423 recognize the
N- and C-terminal glycine loop-related domains, respectively
(amino acids 102–115 and 409–423, respectively), and serum
D472–486 is specific to the C terminus of Cdsn (amino acids
472–486). The specificity of the affinity-purified sera was
tested against Cdsn produced in *Escherichia coli* as a His-tag
recombinant protein.

The antibody reactivity was then investigated by immuno-
blotting analysis of sequential extracts of human breast epi-
dermis and compared with the reactivity of the anti-Cdsn
MoAb, F28-27, directed to amino acids 349–353 (Fig. 2A). The
epidermis was sequentially extracted in equal volumes of a
detergent-containing buffer (TENP-40 buffer extract), 8M urea-
containing buffer (TEU buffer extract), and finally an 8M urea-
and dithiothreitol-containing buffer (TEUDTT buffer extract).

**Fig. 1. Treatment of Cdsn fragments with N-glycosidase F.**
Superficial SC obtained by varnish stripping from three different indi-
viduals (1–3, respectively) was solubilized in the presence of SDS and
dithiothreitol. Equal amounts of extracted proteins were either not
incubated (NI) or incubated in the absence (NT) or presence of N-
glycosidase F (PNGase). After stopping the reactions, proteins were
separated by SDS-PAGE and immunoblotted with G36-19. The ob-
served shifts in the apparent molecular weight of Cdsn fragments are
shown by curved arrows. Note the interindividual variations in the
pattern of Cdsn fragments. The position of molecular mass standards
(kDa) is indicated on the left.

**Fig. 2. Immunoblotting analysis of**
sequential extracts of human epider-
mis and of Cdsn fragments extracted
from the outermost SC with affinity-
purified anti-peptide sera directed to
four different domains of Cdsn. A, hu-
mnan epidermis was serially extracted with equal volumes of buffer containing Nonidet-P40 (1), urea (2), and urea and
dithiothreitol (3). Proteins from an equal
volume of each fraction were then sepa-
rated by SDS-PAGE and immunoblotted
with the anti-Cdsn MoAb F28-27 and
with the affinity-purified sera A40–55,
B102–115, C409–423, and D472–486 as indi-
cated on the top of the blots. B, superficial
SC obtained by varnish stripping from
five different individuals (1–5, respec-
tively) was solubilized in the presence of
SDS and dithiothreitol. Equal amounts of
extracted proteins were analyzed by im-
munoblotting with F28-27 and with the
affinity-purified sera as indicated on the
top of the blots. The position of molecular
mass standards (kDa) is indicated on the
left.
keratinocytes with a weaker reaction on the lower SC. In addition, and in agreement with its reactivity against K14 on immunoblots, serum C\textsuperscript{409–423} stained the cytoplasm of the basal keratinocytes.

We also immunohistochemically analyzed corneocytes spontaneously detached from human skin and collected on microscope slides. Only one of the purified sera, namely serum C\textsuperscript{409–423} and MoAb F28-27 labeled the desquamated corneocytes. Both antibodies showed a granular staining of the corneocyte surface (data not shown) similar to the previously observed labeling produced by the anti-Cdsn MoAb G36-19 on corneocytes scraped from hard palate (9).

The histological observations were confirmed and extended using immunoelectron microscopy (Fig. 4). Various pre- and postembedding indirect immunogold labeling methods were performed to make sure that the observed reactivities indeed reflected the presence or absence of Cdsn. Keratinosomes were the only structures decorated by purified serum A\textsubscript{40–55} (Fig. 4A). The labeling was specific and reproducible but very faint even without chemical fixation or with use of small gold particles and silver enhancement on non-embedded ultracycosections. In addition to staining these secretion vesicles, purified serum B\textsubscript{102–115} decorated the extracellular part of both the desmosomes in the granular layer and of the corneodesmosomes in the lowermost part of the SC (Fig. 4, B and C). Purified serum C\textsubscript{409–423} labeled intermediate filaments of the basal keratinocytes, keratinosomes in the upper spinous and granular keratinocytes, desmosomes of the upper granular keratinocytes, and corneodesmosomes. As previously observed with G36-19 (9, 12), the immunolabeling persisted on corneodesmosomes up to the SC surface (Fig. 4D). The purified serum D\textsubscript{472–486} labeled keratinosomes and faintly decorated some desmosomes (Fig. 4, E and F).

Cdsn Is a Preferred Substrate of Two Proteases Implicated in Desquamation—Two proteases present in the SC, namely SCTE and SCCE, have been suggested to be involved in desquamation. To test whether Cdsn actually is a substrate of these enzymes, TENP-40 buffer extracts were treated for 2 h with SCTE or with recombinant SCCE. Proteins of the extracts were then analyzed by immunoblotting with various antibodies (Figs. 5 and 6). Staining with Protogold did not show any apparent degradation of the major epidermal proteins during incubation with the enzymes (data not shown).

SCTE totally degraded the 52–56-kDa Cdsn and generated Cdsn fragments of 48 and 35 kDa that were stained by G36-19 and F28-27. Incubation in the absence of the enzyme was without effect on the protein (Fig. 5A). Immunoblotting analysis of the same aliquots with PG5.1, a MoAb directed to plakoglobin, demonstrated this desmosomal plaque component to be proteolysed by SCTE and revealed the generation of 70- and 65-kDa fragments (Fig. 5B). Immunoblotting analysis of the same aliquots with DG3.10, a MoAb directed against Dsg1 and 2, and with a serum directed against involucrin, another epidermal protein, indicated that neither Dsgs nor involucrin are SCTE substrates (Fig. 5B).

Incubation of the extract with SCCE induced complete degradation of the 52–56-kDa Cdsn and generated several Cdsn fragments: a fragment of 48 kDa that was stained by G36-19 and F28-27, a doublet of ~30 kDa that was recognized only by G36-19 and low molecular weight fragments that were recognized only by F28-27. Incubation with recombinant inactive Pro-SCCE or in the absence of enzymes was without effect on Cdsn (Fig. 6A). Immunoblotting analysis of the same aliquots with PG5.1 demonstrated that SCCE treatment induced plakoglobin proteolysis and generated a stable fragment of 70 kDa. Incubation of the TENP-40 buffer extracts without enzyme did not induce plakoglobin proteolysis. Immunoblotting analysis of the same aliquots with DG3.10 and with a
**FIG. 4.** Immunoelectron microscopy analysis of human skin with affinity-purified anti-peptide sera directed to four different domains of Cdsn. Normal human skin was analyzed using pre- and postembedding indirect immunogold labelings with the anti-peptide antibodies. Silver enhancement of 1-nm immunogold labeling is shown in all micrographs except in D where indirect labeling was performed with 5-nm gold particles. A and B correspond to ultracryosections of paraformaldehyde-fixed, non-embedded tissue; C and E are Lowicryl K4M-embedded, paraformaldehyde-fixed sections; D and F are Lowicryl K11M embeddings of plunge-frozen skin. A, serum A40–55 gives only a weak but precisely located labeling of keratinosomes in the upper spinous layer and in the lower granular layer. B and C, serum B102–115 labels keratinosomes in the upper spinous and granular layers and desmosomes at the limit between the granular layer and the SC; the labeling of corneodesmosomes does not persist beyond the lowermost SC. D, in the SC, serum C409–423 labels corneodesmosomes. The labeling persists throughout the SC up to the epidermal surface. E and F, serum D472–486 labels keratinosomes in the upper spinous and granular layers and, infrequently, some desmosomes of the granular layer. Arrowheads show desmosomes or corneodesmosomes; arrows indicate keratinosomes. All bars, 200 nm.
serum directed against involucrin, indicated that neither Dsg nor involucrin are substrates for SCCE (data not shown, and Fig. 6B).

**DISCUSSION**

In this study, we characterized the various proteolysis steps of human Cdsn during the late stages of terminal epidermis differentiation. Cdsn is a 529-amino acid-long glycosylated component of corneodesmosomes considered to have adhesive properties and to play a major role in corneocyte cohesion. Its proteolysis seems to be a major prerequisite for desquamation (26).

Our data indicate that the larger fragments of human Cdsn produced by proteolysis in the SC are still glycosylated and contain N-linked oligosaccharides that comprise more than 5% of their mass. Indeed, treatment of the fragments with N-glycosidase F (a glycosidase specific for N-linked sugars) induced a 2–4-kDa decrease in the apparent molecular mass of the larger fragments (46–43 and 36 kDa). Because the fragments reacted with biotinylated *P. sativum* agglutinin and concanavalin A, they may contain α-D-mannose and/or α-D-glucose, like the 52–56-kDa precursor form of Cdsn. Moreover, they seem to contain little or no galactose or N-acetyl-D-galactosamine because they did not react with lectins specific for these carbohydrates. We nevertheless cannot exclude that the sugars linked to the 52–56-kDa Cdsn are modified in the SC.

The results indicate that the oligosaccharide residues do not protect Cdsn against proteolysis in the lower SC unlike what was previously proposed (23). The sugar moiety is thought rather to participate in stabilization or in the adhesion properties of the protein.

Although immunoelectron microscopy experiments performed with MoAbs clearly indicated that Cdsn is located in corneodesmosomes of all cell layers in the SC, the protein is usually not detected in the upper SC when immunohistochemical methods are used (9, 12, 22). As previously discussed (22), this is the consequence of both the poor accessibility of the antigen in corneodesmosomes and the lower sensitivity of the methods. This also explains why the protein was only detected in the upper SC by anti-peptide serum C409–423 using immunoelectron microscopy.

With MoAbs directed against the central domain of the protein, we previously showed that Cdsn is progressively cleaved during SC maturation. At the skin surface, this generates fragments of 15 kDa that, as we previously reported, do not contain at least the first 200 amino acids at the N terminus of the protein. Our present results, using anti-peptide sera, indicate that in fact, Cdsn processing begins in the stratum spinosum and granulosum. The first proteolysis step(s) give rise to a 48–46-kDa fragment lacking both extremities of the protein because it is not recognized by sera directed against amino acids 40–55 (A40–55 serum) or 472–486 (D472–486 serum), respectively, but is still endowed with the glycine loop-related domains (Fig. 7). This step seems to occur either in keratinosomes that were the only structures consistently stained by serum A40–55 or very soon after Cdsn secretion. The 48–46-

---

**FIG. 5.** Effect of SCTE on epidermal proteins. Proteins extracted from human epidermis in the presence of a detergent (TENP-40 buffer extract) were incubated with (+) or without (−) SCTE for increasing periods of time as indicated on top of the plates. After addition of Laemmli’s sample buffer to stop the reaction, proteins were immunoblotted with G36-19 and F28-27 (A) and with PG5.1 directed to plakoglobin, DG3.10 directed to Dsg1 and 2, and a serum directed against involucrin (B). Arrows indicate the 52–56-kDa Cdsn. Open arrowheads show the immunodetected Cdsn fragments. The position of molecular mass standards (kDa) is indicated on the left.

**FIG. 6.** Effect of SCCE on epidermal proteins. Proteins extracted from human epidermis in the presence of a detergent (TENP-40 buffer extract) were incubated with the active enzyme (+ SCCE) or with its inactive precursor (+ ProSCCE) for increasing periods of time as indicated on top of the plates. After addition of Laemmli’s sample buffer to stop the reaction, proteins were immunoblotted with G36-19 and F28-27 (A) and with PG5.1 directed to plakoglobin and a serum directed against involucrin (B). Arrows indicate the 52–56-kDa Cdsn. Open arrowheads show the immunodetected Cdsn fragments. The position of molecular mass standards (kDa) is indicated on the left.
Corneodesmosin Proteolysis and Desquamation

Until now, the proteases involved in Cdsn processing remained unknown. Here, we show that in vitro Cdsn is a preferred substrate of both SCCE and SCTE, two proteases proposed to be involved in corneodesmosome degradation and in desquamation. According to the size and immunoreactivities of the fragments generated, a SCCE-cleavage site is necessarily located between the epitopes recognized by G36-19 (amino acid position 306–309) and F28-27 (amino acid position 349–353). In agreement, the region contains several aromatic amino acids, the amino acids preferentially located (in P1 position) in SCCE cleavage sites (22). Moreover, both enzymes produce a Cdsn immunoreactive fragment of apparent molecular mass similar to that of the Cdsn form associated with desmosomes, i.e. 48–46-kDa. This suggests that SCTE and/or SCCE could be the enzyme(s) involved in the first step of Cdsn proteolysis. Because almost all fragments disappeared after a 2–4-h incubation with SCTE, this enzyme may also be involved in further processing of Cdsn.

SCCE and SCTE were proposed to be actors of a cascade of activated proteases and inactive pro-enzymes that could regulate the rate of desquamation (18). Our results suggest that SCCE and SCTE may cooperate to completely degrade Cdsn and possibly other corneodesmosomal proteins. However, whether or not the two enzymes act on Dsg1 and Dsc1 is not clear and has to be further studied. This would suppress corneocyte cohesion and induce desquamation.

CDSN (or S gene) is a highly polymorphic gene, and many of the coding single nucleotide polymorphisms detected in CDSN induce amino acid substitutions (29–32). Do sequence variations in Cdsn influence the protein function? Alteration of proteolysis sites constitutes one type of the possible dysfunction. For example, the reported CT transition at nucleotide position 619 leads to a Ser202/Phe202 exchange that could alter a potential chymotrypsin cleavage site and therefore the susceptibility of Cdsn to SCCE. Such modifications could be involved in an individual’s susceptibility to xerosis. Similarly, Cdsn polymorphism may also be the cause of the hyperkeratosis observed in psoriasis. Psoriasis is a human multifactorial skin disease characterized by T-cell infiltration, keratinocyte hyperproliferation, and epidermal differentiation abnormalities resulting in impaired desquamation. The involvement of Cdsn in the pathogenesis of the disease has been suggested based on its putative function and on genetic studies including association of various forms of psoriasis with a particular allele of CDSN (31–34). Therefore, it appears of particular importance to determine the primary substrate specificity of SCCE and SCTE. Such studies, currently being performed in our laboratory, will certainly highlight the role of Cdsn in the physiology of desquamation and allow its involvement in the pathophysiology of psoriasis to be precisely determined.

Acknowledgments—We thank Profs. M. Costagliola and J.-P. Chavoine (Service de Chirurgie Plastique, CHU Rangueil, Toulouse, France) for providing us with normal human skin. The technical assistance of C. Pons is gratefully acknowledged. The electron microscopy samples were observed at the Centre de Microscopie Electronique Appliquée à la Biologie et à la Géologie, Claude Bernard University, Lyon, France.

REFERENCES

1. Holbrook, K. A. (1994) in The Keratinocyte Handbook (Leigh, I. M., Lane, E. B., and Watt, F. M., eds), pp. 255–292, Cambridge University Press, Cambridge
2. Roop, D. (1995) Science 267, 474–477
3. Egelrud, T. (2000) in Dry Skin and Moisturizers: Chemistry and Function (Loden, M. and Maibach, H. I., eds), pp. 109–117, CRC Press, New York
4. Allen, T. D., and Potten, C. S. (1975) J. Ultrastruct. Res. 51, 34–105
5. Skerrow, C. J., Clelland, D. G., and Skerrow, D. (1980) J. Cell Sci. 92, 667–677
6. Lundström, A., and Egelrud, T. (1990) J. Investig. Dermatol. 94, 216–220
7. Chapman, S. J., and Walsh, A. (1990) Arch. Dermatol. Res. 282, 304–310
8. Serre, G., Mills, V., Haefek, M., Vincent, C., Creuze, F., Réano, A., Oubayouen,
Corneodesmosin Proteolysis and Desquamation 20299

J.-P., Bettinger, S., and Soleilhavoup, J.-P. (1991) *J. Invest. Dermatol.* 97, 1061–1072.

Ghadially, R., Williams, M. L., Hou, S. Y. E., and Elias, P. M. (1992) *J. Invest. Dermatol.* 99, 1061–1072.

Haftek, M., Simon, M., Kanitakis, J., Maréchal, S., Claude, A., Serre, G., and Schmitt, D. (1997) *Br. J. Dermatol.* 137, 864–868.

Simon, M., Bernard, D., Minondo, A.-M., Canus, C., Piat, F., Corrucc, P., Schmidt, R., and Serre, G. (2001) *J. Invest. Dermatol.* 116, 23–30.

Hansson, L., Strömquist, M., Backman, A., Wallbrant, P., Carlstein, A., and Egelrud, T. (1994) *J. Biol. Chem.* 269, 19420–19426.

Suzuki, Y., Nomura, J., Koyama, J., and Horii, I. (1994) *Arch. Dermatol. Res.* 286, 249–253.

Skytt, A., Strömquist, M., and Egelrud, T. (1995) *Biochem. Biophys. Res. Commun.* 211, 586–589.

Brattsand, M., and Egelrud, T. (1999) *J. Biol. Chem.* 274, 30033–30040.

Ekholm, E., Brattsand, M., and Egelrud, T. (2000) *J. Invest. Dermatol.* 114, 56–63.

Diamandis, E. P., Youssef, G. M., Luo, L.-Y., Magklara, A., and Obiezu, C. (2000) *Trends Endocrinol. Metab.* 11, 54–60.

Arnsen, J., Sullivan, K. H., Magee, A. I., King, I. A., and Buxton, R. S. (1993) *J. Cell Sci.* 104, 741–759.

Kowalczyk, A. P., Bornslaeger, E. A., Norvell, S. M., Palka, H. L., and Green, K. L. (1999) *Int. Rev. Cytol.* 185, 237–302.

Haftek, M., Serre, G., Milz, V., and Thivolet, J. (1991) *J. Histochem. Cytochem.* 39, 1531–1538.

Simon, M., Montézin, M., Guerrin, M., Durieux, J.-J., and Serre, G. (1997) *J. Biol. Chem.* 272, 31770–31776.

Steinert, P. M., Mack, J. W., Korge, B. P., and Gann, S.-Q. (1991) *Int. J. Macromol.* 13, 130–139.

Lundström, A., Serre, G., Haftek, M., and Egelrud, T. (1994) *Arch. Dermatol. Res.* 286, 369–375.

Haftek, M., Zone, J. J., Taylor, T. B., Kowalewski, C., Chorzelski, T. P., and Schmitt, D. (1994) *J. Invest. Dermatol.* 103, 656–659.

Mommaas, A. M., Wijsman, M. C., Mulder, A. A., van Praag, M. C. G., Vermeer, B. J., and Koning, F. (1992) *Hum. Immunol.* 34, 99–106.

Zhou, Y., and Chaplin, D. D. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 9470–9474.

Enerback, C., Enlund, F., Inerot, A., Samuelsson, L., Wahlström, J., Swanbeck, G., and Martisson, T. (2000) *J. Invest. Dermatol.* 114, 1158–1163.

Jenisch, S., Koch, S., Henseler, T., Nair, R. P., Elder, J. T., Watts, C. E., Westphal, E., Voorhees, J. J., Christophers, E., and Kronke, M. (1999) *Tissue Antigens* 54, 439–449.

Guerrin, M., Vincent, C., Simon, M., Tazi Ahnini, R., Port, M., and Serre, G. (2001) *Tissue Antigens* 57, 32–38.

Allen, M. H., Veal, C., Faassen, A., Powis, S. H., Vaughan, R. W., and Trembath, R. C. (1999) *Lancet* 353, 1589–1590.

Chakrabarti, R., Camp, N. J., Cork, M. J., Mee, J. B., Keohane, S. G., Duff, G. W., and di Giovine, F. S. (1999) *Hum. Mol. Genet.* 8, 1135–1140.

Long, S., Banks, J., Watkins, A., Harding, C., and Rawlings, A. V. (1996) *J. Invest. Dermatol.* 106, 872, (abstr.)