Characterization and Purification of Human Corneodesmosin, an Epidermal Basic Glycoprotein Associated with Corneocyte-specific Modified Desmosomes*

(Received for publication, July 17, 1997, and in revised form, September 19, 1997)

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Using monoclonal antibodies, we identified a new protein in mammalian epidermis, which we called corneodesmosin. It is located in the extracellular part of the modified desmosomes in the cornified layer of the tissue, and its proteolysis (from 52–56 to 33 kDa) is thought to be a major prerequisite of desquamation. We have now further characterized human corneodesmosin. Proteolysis of purified cornified cell envelopes produced immunoreactive fragments, confirming the covalent linkage of the protein to these structures. Sequential extraction of epidermal proteins indicated that the 52–56-kDa precursor form of the protein exists in two distinct pools, one extracted with a non-detergent hypoionic buffer, and the other with urea. Two-dimensional gel analysis and reactivity with phosphoserine-specific antibodies showed that it is a basic phosphoprotein. Deglycosylation experiments, reactivity with lectins, and chromatography on concanavalin A-Sepharose indicated that corneodesmosin is N-glycosylated. Partial sequences, 10 and 15 amino acids long, of the purified 52–56-kDa corneodesmosin showed identity with sequences predicted from a previously cloned gene, proved to be expressed in the epidermis and designated S. This indicates that corneodesmosin is probably encoded by the S gene, the function of which was unknown until now. A model of corneodesmosin maturation during cornification is proposed.

Corneocytes are anucleated “mummified” cells derived from keratinocytes during the late stages of terminal differentiation in cornified squamous epithelia such as the epidermis. They mainly consist of a cytokeratin-containing fibrous matrix surrounded by a highly resistant 15-nm thick protein structure called the cornified cell envelope. Stacking of the corneocytes at the outermost layer of the tissue, namely the stratum corneum (SC),1 plays a critical role in the epidermal barrier function and in the mechanical protection of the body (1–3). During the normal desquamation process, the most superficial corneocytes are shed from the skin surface. The structures involved in

the cohesion between individual corneocytes, and the mechanisms that lead to the detachment of these cells are poorly understood.

Although intercellular structures, thought to be derived from desmosomes (4, 5), and called corneosomes or corneodesmosomes (6, 7), have been described in the SC, they were initially considered as non-functional remnants. Recent studies have shown, however, their major importance in corneocyte cohesion, and there is now growing evidence that their degradation is a key event in the desquamation process (6–11). In particular, a tight correlation seems to exist between cell dissociation and proteolysis of some corneodesmosomal components (12–14). Moreover, in plantar SC, where cell cohesion is very strong, corneodesmosomes can be detected over the whole corneocyte surface up to the top of the layer. In non-plantar SC, where cohesion is weaker, they only persist at the periphery of the cells (6, 7, 9). In psoriasis, various ichthyoses, and skin xerosis, characterized by an accumulation of corneocytes and by scaling, the number of corneodesmosomes is increased throughout the SC including the upper part (11, 15, 16). Several trypsin-like and chymotrypsin-like serine proteases, including the stratum corneum chymotryptic enzyme, are thought to be involved in the corneodesmosome proteolysis (17–19).

Using three different monoclonal antibodies (mAbs), we recently identified a new corneodesmosome protein antigen expressed in various mammals including human, and we called it corneodesmosin (7, 20). Corneodesmosin is only expressed in the cornified squamous epithelia, i.e. in man: epidermis, hard palate, epithelium, and inner root sheath of the hair follicle (7, 21). In human non-plantar epidermis, the protein was shown to be located first in cytoplasmic vesicles, termed keratinosomes or lamellar bodies, of the upper spinous keratinocytes, then in the intercellular part of the desmosomes of the granular keratinocytes, these living cells being just beneath the SC. Lastly, it was detected in the core of corneodesmosomes (21) in psoriasis, various ichthyoses, and skin xerosis, characterized by an accumulation of corneocytes and by scaling, the number of corneodesmosomes is increased throughout the SC including the upper part (11, 15, 16). Several trypsin-like and chymotrypsin-like serine proteases, including the stratum corneum chymotryptic enzyme, are thought to be involved in the corneodesmosome proteolysis (17–19).

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The obtained sequences were contained in the product of the S gene, recently identified and located 160 kilobase pairs telomeric to HLA-C (24). They confirmed our recent cloning of the human corneodesmosin cDNA.

EXPERIMENTAL PROCEDURES

Monoclonal Antibodies—The murine IgG1 mAbs G36-19, F28-27, and B17-21 are part of a series of mAbs directed against epidermal differentiation antigens, produced and characterized in our laboratory, after immunization of mice with homogenate of human plantar SC (7, 29). The ascites fluid of the IgG1 mAb MOPC-21 (Sigma) was used as a negative control. The anti-phosphotyrosine mAb PY20 was purchased from Transduction Laboratories (Lexington, KY).

Preparation and Analysis of Cornified Cell Envelopes—Human corneocytes were purified from planter SC and from breast epidermis, as described under “Experimental Procedure,” by extensive extraction in the presence of SDS, dithiothreitol, and urea, and by electrodialysis. A, plantar envelopes were digested with V8 protease for 0 h (lane 1), 24 h (lane 2), 48 h (lane 3), and 72 h (lane 4). Solubilized fragments were separated by SDS-PAGE, transferred to nitrocellulose membranes, and analyzed by immunoblotting with G36-19, a mAb specific for corneodesmosin (lane 1) and breast epidermis (lane 2) envelopes were digested for 72 h, and analyzed in the same way. The position of molecular mass standards (kDa) is indicated on the left. C, plantar (upper) and breast epidermis (lower) envelopes were analyzed by indirect immunofluorescence with G36-19. Bars, 35 μm.

EXPERIMENTAL PROCEDURES

Preparation and Analysis of Cornified Cell Envelopes—Human cornified cell envelopes were purified from planter SC and from breast epidermis, as described earlier (25). Briefly, the samples were extracted by heat treatment for 5 min in phosphate-buffered saline at 56 °C. The epidermis was sequentially homogenized on ice in equal volumes of the following buffers (three times in each buffer): 40 mM Tris·HCl, pH 7.5, 10 mM EDTA, 0.25 mM phenylmethylsulfonyl fluoride, and 2 μg/ml each of aprotinin, pepstatin A, and leupeptin (TE buffer); TE buffer containing 0.5% Nonidet P-40 (TE-Nonidet P-40 buffer). The obtained pellet was then divided into three parts that were extracted in one-third of the original volume of TE buffer containing various concentrations of urea (4, 6, and 8 M) (TEU buffers). After each extraction, the homogenates were centrifuged for 15 min at 15,000 × g, and the supernatants were kept at −30 °C until used. Finally, the pellet corresponding to the last extraction in 8 M urea was homogenized in 35 mM Tris·HCl, pH 6.8, 8 M urea, 50 mM dithiothreitol, 5% glyceral, 0.25 mM phenylmethylsulfonyl fluoride, and 2 μg/ml each of aprotinin, pepstatin A, and leupeptin (TUDTT buffer), incubated at 95 °C for 30 min, and centrifuged as described above. Protein concentrations were measured using the Coomassie Plus protein assay (Pierce Chemical Co., Rockford, IL).

Preparation and Analysis of Cornified Cell Envelopes—Human cornified cell envelopes were purified from plantar SC and from breast epidermis, as described earlier (25). Briefly, the samples were extracted by repeated boiling with vigorous agitation in a solution containing 2% (w/v) SDS and 25 mM dithiothreitol, then at 37 °C for 72 h in a solution containing 8 M urea and 25 mM dithiothreitol. The urea-extracted envelopes were pelleted, resuspended in 0.1% SDS, 192 mM glycine, and 125 mM Tris, and electrodialyzed against 20 mM Tris·HCl, pH 7.5, 0.5% SDS (w/v), 1 M NaCl, and electrotransferred to nitrocellulose membranes. The purified cornified cell envelopes were collected by centrifugation, then washed with distilled water and counted. They were analyzed by indirect immunofluorescence as described previously (7). Peptides were produced by digestion of the envelopes with V8 protease and analyzed by immunoblotting as previously reported (25). To produce envelope fragments, intact plantar envelopes were sonicated for 30 s at 30 watts and re-extracted.

Protein Electrophoresis and Immunoblotting—Proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) or by two-dimensional electrophoresis in the presence of urea, exactly as described previously (20).

After electrophoresis, proteins were either stained with Coomassie Blue or electrotransferred to reinforced nitrocellulose above. Membranes were stained with either Ponceau Red or Protogold (British BioCell International, Cardiff, United Kingdom) and probed with mAbs as previously reported (25). Immunoreactivities were revealed with the ECL™ Western blotting kit, as described by Amersham International (Aylesbury, UK), the manufacturer.

Immunoprecipitation—The 52–56-kDa corneodesmosin was immunoprecipitated from a TE-Nonidet P-40 buffer extract using a pull-down assay (see “Experimental Procedures” for details). The precipitated 52–56-kDa corneodesmosin were loaded onto SDS gels, and electrotransferred to nitrocellulose membranes. The membranes were incubated, as described previously (20), in 5% gelatin blocking buffer, then with biotinylated lectins purchased from Pierce Chemical Co. and diluted to 1 μg/ml. After washing, the lectins were detected with peroxidase-labeled streptavidin (diluted to 1/400,000) and the ECL™ Western blotting kit.

Deglycosylation Experiments—The TE-Nonidet P-40 buffer extract (10 μg of protein) was boiled for 3 min in 20 μl of 1% SDS (w/v) and 0.1 M sodium phosphate buffer, pH 7.2. Then Nonidet P-40 and EDTA were added to give final concentrations of 1% and 20 mM, respectively. To the extract, 2.4 units of N-glycosidase F (EC 3.2.1.18, Boehringer Mannheim) were added, and the reaction mixture was incubated at 37 °C for 6 h. Proteins (34 μg) of the TE-Nonidet P-40 buffer extract were also incubated with 5 milliunits of endo-α-N-acetylgalactosaminidase (EC 3.2.1.97) at 37 °C for 6 h, in the presence or absence of N-glycosidase F and/or neuraminidase (EC 3.2.1.18), in the conditions described by Oxford GlycoSystems Ltd. (Abingdon, UK), the manufacturer. The reactions were stopped by boiling for 2 min in sample buffer. As a positive control, the deglycosylation of fetuin was tested by SDS-PAGE and with biotinylated lectins. Treated and mock samples were separated by SDS-PAGE and analyzed by immunoblotting and affinoblotting as described above.

Concanavalin A-Sepharose Chromatography—The TE-Nonidet P-40 buffer extract was loaded directly at a flow rate of 0.5 ml/min onto a 5-ml column of concanavalin A-Sepharose 4B (ConA-Sepharose; Sigma) which had been equilibrated with 20 mM Tris·HCl, pH 7.5 (washing buffer), containing 0.2 M NaCl. After washing, the adsorbed proteins were eluted at a flow rate of 0.5 ml/min with 0.5 mM a-methyl-d-mannopyranoside (Sigma) in the washing buffer. Proteins were then separated by SDS-PAGE and analyzed as described above.

Corneodesmosin Purification—After dermo-epidermal cleavage, the epidermis was homogenized in TEA buffer: 40 mM Tris·HCl, pH 7.5, 10 mM EDTA, 10 μg/ml aprotinin, and 0.8 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride (Interchim, Paris, France), and the homogenate was centrifuged for 15 min at 15,000 × g. The supernatant was cleared by filtration and loaded at a flow rate of 0.3 ml/min on an anion exchange HiTrap Q column (Pharmacia LKB), previously equilibrated in washing buffer. Nonretained proteins were then directly injected at a flow rate of 0.3 ml/min onto an affinity column prepared as follows. Two mg of the anti-corneodesmosin mAb F28-27 were grafted onto the matrix of a 1-ml column of N-hydroxysuccinimide-activated Sepharose 4B (HiTrap- NHS) as described by Pharmacia LKB, the manufacturer. The column was extensively washed at a flow rate of 1 ml/min with 1 M NaCl. The anti-corneodesmosin mAb was electrotransferred from nitrocellulose membranes. The membranes were incubated, as described previously (20), in 5% gelatin blocking buffer, then with biotinylated lectins purchased from Pierce Chemical Co. and diluted to 1 μg/ml. After washing, the lectins were detected with peroxidase-labeled streptavidin (diluted to 1/400,000) and the ECL™ Western blotting kit.
RESULTS

Human Corneodesmosin Is a Component of Cornified Cell Envelopes—To confirm that corneodesmosin is cross-linked to cornified cell envelopes, fragments generated by proteolysis of these structures, purified from plantar epidermis, were analyzed with the anti-corneodesmosin mAb G36-19. The envelopes were incubated for increasing periods of time with V8 protease, and the resulting fragments were separated by SDS-PAGE and analyzed by immunoblotting (Fig. 1A). G36-19 strongly stained multiple bands from 50 kDa to high molecular mass bands migrating at the top of the gel. This may indicate that corneodesmosin is incorporated into large, highly cross-linked heteropolymers released during proteolysis of the envelopes. Several bands stained with Protagold were not immunodetected, further confirming the specificity of the reaction (data not shown). No clear immunoreactive bands were detected using antibodies to corneodesmosin in the absence of protease cleavage (Fig. 1A, lane 1), showing a covalent association between the protein and other envelope components. After immunoblotting of fragments produced by proteolysis of the same number of cornified cell envelopes purified from breast epidermis, only faint immunoreactive bands of high molecular mass were observed (Fig. 1B, lane 2).

Consistently, using indirect immunofluorescence, most plantar envelopes (or washed sonicated envelope fragments) were strongly stained by G36-19, with a regular and punctate labeling of almost their whole surface, whereas most of the envelopes purified from breast epidermis were not. However, a few of the latter were stained but slightly and only around their edge (Fig. 1C).

Sequential Extraction of Human Corneodesmosin—To analyze the solubility properties of corneodesmosin, human epidermis was sequentially extracted in equal volumes of a Tris-HCl buffer (TE-buffer extract), a detergent-containing buffer (TE-Nonidet P-40 buffer), and finally 8 M urea/dithiothreitol- (TEU buffer extracts), and finally an 8 M urea/dithiothreitol-containing buffer (TEUDTT buffer extract). Extracted proteins

Fig. 2. Solubility properties of human corneodesmosin as determined by sequential extractions. Human epidermis was serially extracted with equal volumes of a hypotonic buffer (TB), and a detergent-containing buffer (TE-Nonidet P-40); the pellet obtained was then divided into three parts that were extracted, in one-third of the original volume, with urea at a concentration of 4, 6, or 8 M. Finally the pellet obtained after the extraction in 8 M urea was incubated in a buffer containing 8 M urea and dithiothreitol (urea-DTT). Proteins from an equal volume of each fraction were then separated by SDS-PAGE, stained with Coomassie Blue (GEL), or transferred to nitrocellulose membranes and immunoblotted with G36-19 (BLOT). The position of molecular mass standards (kDa) is indicated on the left. K indicates the cyto-keratins, and the arrowhead shows the 52–56-kDa corneodesmosin.

Amino Acid Sequencing—The proteins were digested directly in the gel with endoproteinase Lys-C (EC 3.4.99.30), and the peptides generated were eluted and resolved by high performance liquid chromatography using a DEAE-C18 column. Selected peptides were then sequenced for 10–15 Edman degradation cycles on an Applied Biosystems Procise Sequencer, following the manufacturer’s specifications. This was performed in the Laboratoire de Microsequencing des Protéines at the Institut Pasteur (Paris, France).

Fig. 3. Two-dimensional immunoblotting analysis of human epidermal proteins. Proteins directly extracted from human epidermis in TE-Nonidet P-40 buffer (1), and the immunoprecipitated epidermal corneodesmosin (2) were separated by SDS-PAGE and analyzed by immunoblotting with a control mAb (MOPC), with G36-19, and with mAbs specific for phosphoserine (4A3 and 4A9), as indicated on the top of the blot. The arrowhead shows the 52–56-kDa corneodesmosin. The position of molecular mass standards (kDa) and pI references is indicated on the left and at the bottom of the gels, respectively.
were then separated by SDS-PAGE and analyzed by immuno-
blotting with G36-19 (Fig. 2). The 52–56-kDa corneodesmosin
was detected in both the TE and the TEU buffer extracts with
6 and 8 M urea, but not in the TE-Nonidet P-40 and TEUDTT
buffer extracts. In some blots, the protein migrated as a doublet
differing by about 2 kDa, the immunoreactivity of the upper
band always being far less intense. Moreover, when the TE
buffer extract was centrifuged for 30 min at 100,000 × g,
corneodesmosin was totally recovered in the supernatant (re-
sult not shown). G36-19 also recognized some proteins of lower
molecular mass, from 46 to 40 kDa, that were extracted partly
in the presence of 6 or 8 M urea, and partly in the presence of
the reducing agent (Fig. 2, lanes 4–6). Overexposition of the
blots or overloading of the gels showed, in these fractions, small
immunoreactive bands with molecular masses below 36 kDa. These low molecular mass immunoreactive bands have
previously been shown to be proteolysis fragments of the 52–
56-kDa corneodesmosin (23). Identical results were obtained
when the extracted proteins were analyzed by immunoblotting
with two mAbs directed against two other epitopes of cor-
neodesmosin, F28-27 and B17-21 (results not shown).

Each extraction step was complete as shown by the observa-
tion that no proteins were immunodetected in the third Tris-
HCl or urea-containing buffer extracts. Control immunoblot-
tings omitting the primary antibody or using MOPC-21 were
always negative (data not shown). It is highly probable that the
low Mr immunodetected proteins were not degradation prod-
ucts generated during the extraction steps, since the extrac-
tions were performed in the presence of several protease inhib-
itors, and since the proteins were also detected when the
epidermis was directly extracted in sample buffer (data not
shown).

FIG. 5. The 52–56-kDa corneodesmosin is a glycoprotein. A,
proteins directly extracted from human epidermis in TE-Nonidet P-40
buffer were either not treated (NT) or incubated with N-glycosidase F
(PNGase), separated by SDS-PAGE, and immunoblotted with a control
mAb (MOPC) or with anti-corneodesmosin mAbs (G36-19 and F28-27).
B, proteins extracted from human epidermis in TE-Nonidet P-40 buffer
were either not incubated at all (ND) or not treated (NT), or treated with
N-glycosidase F, with endo-α-acetylgalactosaminidase (Endo) and with
both enzymes (P+E). The proteins were then separated by SDS-PAGE,
transferred to membranes, and either analyzed with biotinylated wheat
germ agglutinin (WGA) or immunoblotted with G36-19. C, proteins
extracted from human epidermis in TE-Nonidet P-40 buffer (T), and the
immunoprecipitated corneodesmosin (2) were separated by SDS-PAGE,
transferred to membranes, and immunoblotted with G36-19, or anal-
alyzed with biotinylated lectins (wheat germ agglutinin (WGA); D. bifu-
lorus agglutinin (DBA); Ricinus communis agglutinin (RCA); P. sati-
vum agglutinin (PSA) and peanut agglutinin (PNA)). The position of
molecular mass standards (kDa) is indicated on the left.

were then separated by SDS-PAGE and analyzed by immuno-
blotting with G36-19 (Fig. 2). The 52–56-kDa corneodesmosin
was detected in both the TE and the TEU buffer extracts with
6 and 8 M urea, but not in the TE-Nonidet P-40 and TEUDTT
buffer extracts. In some blots, the protein migrated as a doublet
differing by about 2 kDa, the immunoreactivity of the upper
band always being far less intense. Moreover, when the TE
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corneodesmosin was totally recovered in the supernatant (re-
sult not shown). G36-19 also recognized some proteins of lower
molecular mass, from 46 to 40 kDa, that were extracted partly
in the presence of 6 or 8 M urea, and partly in the presence of
the reducing agent (Fig. 2, lanes 4–6). Overexposition of the

FIG. 6. Binding of the 52–56-kDa corneodesmosin to ConA-
Sepharose. Proteins were directly extracted from human epidermis in
TE-Nonidet P-40 buffer and loaded onto a column of ConA-Sepharose.
After washing in 0.2 M NaCl, adsorbed proteins were eluted with 0.5 M
methyl-α-D-mannopyranoside. Absorbance at 280 nm was recorded
(PROFILE). Proteins of the total extract (T) and the indicated fractions
were separated by SDS-PAGE, Coomassie Blue stained, or analyzed by
immunoblotting with G36-19. The arrowhead shows the 52–56-kDa
corneodesmosin. The position of molecular mass standards (kDa) is
indicated on the left.

Human 52–56-kDa Corneodesmosin Is a Basic Phosphopro-
tein—To determine the pl of the 52–56-kDa human corneodes-
mosin, human epidermis was directly extracted in a detergent-
containing nondenaturing buffer (TE-Nonidet P-40 buffer); the
extract obtained was similar to the previous TE buffer extract,
but the extraction was facilitated by the presence of the det-
gerent. Extracted proteins were then separated by two-dimen-

The 52–56-kDa corneodesmosin was purified from a non-denaturing hypotonic buffer extract (TEA buffer extract) of human epidermis, by anion exchange and affinity chromatography (Fig. 7). All the extracted 52–56-kDa corneodesmosin bound onto the affinity column, and was eluted with glycine. Protogold staining of the eluted proteins showed how pure the 52–56-kDa corneodesmosin was (middle panel, fractions number 82–84). In particular, two-dimensional gel analysis indicated that no proteins co-migrated with the 52–56-kDa corneodesmosin (data not shown). Some anti-corneodesmosin mAb immunoreactive fragments were observed, indicating that the protein was somehow proteolysed during the purification procedure (lower panel, fraction number 83). A non-immunoreactive protein of roughly 50 kDa, that could be associated with corneodesmosin, was also recovered in the eluted fractions.

The eluted fractions of the affinity column were pooled, lyophilized, and separated by SDS-PAGE. The 52–56-kDa corneodesmosin and a 45-kDa G36-19 immunoreactive fragment were excised and characterized by internal and NH2-terminal amino acid sequence analysis. No amino acid was obtained from the NH2 terminus, suggesting blocking of the proteins. The internal sequences obtained: KSYGGYEVVGGSSDSY and KIYPVGYFTK, matched perfectly with fragments 275–290 and 301–310, respectively, of the predicted product of a previously identified gene, the S gene (24, GenBank accession number L20815; amino acid sequence numbering as given in this reference).

**DISCUSSION**

In the studies presented above, we biochemically characterized and partially sequenced human corneodesmosin, a protein specific to cornified squamous epithelia where it is thought to play a major role in corneocyte cohesion and whose proteolysis seems to be a major prerequisite of desquamation.

Our data indicate that human corneodesmosin is a glycoprotein containing mainly N-linked oligosaccharides that comprise...
FIG. 8. Cornification-related processing of corneodesmosin, a model.
Schematic representation illustrating synthesis, transport, assembly, extraction properties, and proteolysis processing of corneodesmosin, as deduced from immunoultrastructural and biochemical data previously obtained or presented in this paper. For details, see “Discussion.” SC, stratum corneum; SG, stratum granulosum; TE, Tris-EDTA buffer; 6 M urea, TE buffer containing 6 M urea; DTT, TE buffer containing 8 M urea and dithiothreitol, see “Experimental Procedures” for detailed composition of these buffers. The apparent molecular mass (kDa) of the various corneodesmosin forms is indicated on the right.

—10% of the protein mass. Indeed, treatment with N-glycosidase F (a glycosidase specific for N-linked sugars) of the 52–56-kDa corneodesmosin induced a 5-kDa decrease in its apparent molecular weight, whereas extensive endo-α-N-acetylglactosaminidase digestion (an enzyme specific for O-linked sugars) was without effect, even in the presence of neuraminidase and/or N-glycosidase F. Lectins were used to characterize the corneodesmosin carbohydrate moieties. Since the protein bound to ConA-Sepharose, and reacted with biotinylated P. sativum agglutinin, it may contain α-D-mannose and/or α-D-glucose. In addition, corneodesmosin strongly reacted with wheat germ agglutinin and, as expected for an N-glycosylated protein, may therefore contains β-N-acetylgalactosamine groups. However, it seems to contain few or no galactose and N-acetylgalactosamine, since it did not react (or barely) with lectins specific for these carbohydrates. More extensive experiments will be necessary to precisely identify the oligosaccharide residues linked to corneodesmosin, and to characterize their linkage. In fact these residues may participate in the adhesion properties of the protein, as described for other corneodesmosomal adhesive proteins, desmogleins and desmocollins (26). Alternatively, the oligosaccharide residues may transiently protect corneodesmosin against proteolysis, during maturation of the SC. Indeed, sugars have been proposed to prevent premature desquamation by protecting the desmosome and corneodesmosome core against extracellular proteases (27). Differences in glycosylation between species may also explain, at least in part, differences in corneodesmosin size previously observed in various mammals (20).

Internal peptide sequences of both the purified 52–56-kDa corneodesmosin and an immunoreactive fragment derived from it, indicated 100% matching over 25 amino acids with related sequences of the predicted product of the S gene. This gene, identified by CpG island analysis of the class I region of the human major histocompatibility complex, was shown to be located 160 kilobase pairs telomeric to HLA-C (24), but its function was unknown. Like corneodesmosin, the S gene had been shown to be highly expressed in the epidermal granular keratinocytes (24). Therefore, our results strongly suggest that corneodesmosin is the product of the S gene. This was confirmed by the recent cloning of the human corneodesmosin cDNA we performed by immunoscreening of an expression library. Consistently with the results reported here, the corneodesmosin cDNA sequence predicts a pl of 8.5, one N-glycosylation site and several protein kinase phosphorylation sites.

Furthermore, in light of the strong association of the Cw6 HLA-C allele with Psoriasis vulgaris (28), corneodesmosin or allelic variants of the protein may be directly involved in the pathogenesis of this common skin disorder. Indeed, psoriasis is a chronic cutaneous disease involving inflammation, hyperproliferation, and a defective program of differentiation of epidermal cells, with in particular hyperkeratosis and impaired desquamation. No direct role for the MHC peptide has been shown yet. Moreover, abnormal corneodesmosomes have been observed in psoriasis (15), reinforcing the hypothesis of corneodesmosin involvement in the pathogenesis of the disease.

In view of our previously published results (7, 20–23) and of the present data, an overall scheme of human corneodesmosin processing during SC maturation can be proposed (Fig. 8). The protein is synthesized in the upper spinous and/or lower granular keratinocytes under the form of a 52–56-kDa basic precursor that is extracted with nondenaturing hypotonic buffers. As observed by immunoelectron microscopy (7), it is exported via keratinosomes, probably into the extracellular space (or less likely to the plasma membrane) where it is associated to the desmosome core. Then, the presence of urea, at a concentration of at least 6 M (or SDS), becomes necessary for the protein to be solubilized. During corneocyte maturation, corneodesmosin is progressively proteolysed, a molecular form of 33 kDa being the major form extracted from the most superficial and less firmly attached corneocytes (7, 23). At the same time, the corneodesmosin fragments produced are more firmly

2 M. Guerrin, M. Simon, M. Montézin, C. Vincent, and G. Serre, unpublished observations.

3 M. Simon, M. Montézin, M. Guerrin, and G. Serre, unpublished observations.
bound to corneodesmosomes by intermolecular disulfide bonds and therefore require a reducing agent to be extracted. The fragments and/or the 52–56-kDa form are also cross-linked to cornified cell envelopes, on the external face of the structures, by other covalent bonds whose nature is unknown. Preliminary experiments suggest that corneodesmosin is not a substrate of epidermal transglutaminases, the intracytoplasmic enzymes responsible for the cornified cell envelope formation. Therefore, corneodesmosin could be ester-linked to the hydroxyacyl sphingosines bound to the outside of the cornified cell envelopes. In that case, the enzymes responsible for this linkage remain to be discovered. This linkage to the envelopes may enhance corneocyte cohesion and participate in SC resistance. Since human, pig, guinea pig, mouse, and rat corneodesmosins show similar locations in epidermis, biochemical characteristics and processing (20), the model of human corneodesmosin maturation may probably be extended to these mammals.

Maturation by proteolysis is not particular to corneodesmosin. Indeed, processing of filensin and other lens-specific proteins by proteolysis during lens fiber cell differentiation has been extensively described (Ref. 29 and references therein). More closely related, desmocollin 1 undergoes limited cleavage during the later stages of epidermal differentiation, resulting in the accumulation of stable NH₂-terminal fragments in the SC (12). How corneodesmosin processing is important for the function of the molecule is not clear. However, we recently proposed that desmosomal proteins may be protected and desquamation inhibited until corneodesmosin is proteolysed to 33 kDa (23).

In conclusion, our results indicate that human corneodesmosin is closely related to the product of the S gene, a gene expressed in epidermis but whose function was unknown until now. Our data also show that corneodesmosin is a basic phosphorylated and glycosylated protein. During SC maturation it is covalently linked to the corneocyte-specific structures, i.e., the corneodesmosome core and the cornified cell envelopes. This association to the superstructure (formed by the envelopes linked to the intracellular matrix, and joined together by corneodesmosomes) responsible for SC cohesion clearly indicates the involvement of corneodesmosin in this process. We think that total degradation of corneodesmosome components and in particular corneodesmosin, at the skin surface, is required to allow cell detachment, i.e., desquamation. This hypothesis is now being tested in our laboratory.

Acknowledgments—We thank Professor M. Costagliola (Service de Chirurgie Plastique, CHU Rangueil, Toulouse, France) for providing us with human skin. We are indebted to Doctor C. Vincent for line drawings. The technical assistance of C. Pons, M. Gousampis, M.-F. Isaia, and M.-P. Rué is gratefully acknowledged.

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