A new human 33-kDa serine protease was purified from human epidermis, and its cDNA was cloned from a keratinocyte library, from mRNA from a human keratinocyte line (HaCat) and from mRNA from human skin. Polyclonal antibodies specific for the new protein detected three groups of proteins in partially purified extracts of cornified epithelium of human plantar skin. The three components are proposed to correspond to proenzyme, active enzyme, and proteolytically modified active enzyme. After N-deglycosylation, there was a decrease in apparent molecular mass of all detected components. Expression of the cloned cDNA in a eukaryotic virus-derived system yielded a recombinant protein that could be converted to an active protease by treatment with trypsin. Polymerase chain reaction analyses of cDNA from a number of human tissues showed high expression of the new enzyme in the skin and low expression in brain, placenta, and kidney. Homology searches yielded the highest score for porcine enamel matrix protease (55% amino acid sequence homology). High scores were also obtained for human and mouse neutrophin and for human stratum corneum chymotryptic enzyme. The function of this new protease, tentatively named stratum corneum tryptic enzyme, may be related to stratum corneum turnover and desquamation in the epidermis.

In order to fulfill its role as an efficient physical-chemical barrier, the outermost layer of the skin, the stratum corneum, must have a high mechanical as well as chemical resistance. The cellular building blocks of the stratum corneum, the corneocytes, are well adapted to their function. The integration of the properties of individual corneocytes into the tissue as a functioning unit depends on strong intercellular adhesive structures. There is now strong evidence that the modified desmosomes of the stratum corneum play a crucial role in this context (1–3).

The stratum corneum is continuously being formed in the process of epidermal differentiation, in which a fraction of the basal, proliferating keratinocytes leave the basal membrane and migrate, while differentiating, toward the skin surface. Eventually, the cells become mature, anucleated, anabolically dead corneocytes. The cells stay in the stratum corneum for 2–4 weeks and are then shed from the skin surface in the process of desquamation.

The mechanisms involved in desquamation and their regulation are poorly understood. Since corneocytes are anabolically dead and not responding to common cellular stimuli, the mechanisms responsible for desquamation must be present in the tissue when the deepest parts of the stratum corneum are being formed. In order not to interfere with stratum corneum function, these mechanisms must, however, be “programmed” to be more or less inactive for several weeks and then become activated.

During the last several years, it has become clear that one of the mechanisms involved in desquamation is a well regulated proteolysis of intercellular adhesive structures (4, 5). There is evidence that one of the enzymes involved may be stratum corneum chymotryptic enzyme (SCCE) (6–9). This serine protease with chymotrypsin-like primary substrate specificity (10), which has been cloned and expressed in mammalian cells (9), has enzymatic properties (6) and a tissue localization (11–12) compatible with it being responsible, at least partially, for the degradation of intercellular parts of desmosomes, a process that is a prerequisite for desquamation.

There is also evidence, however, suggesting that other proteases than SCCE, possibly with trypsin-like primary substrate specificity, are involved in desquamation. One such piece of evidence is that SCCE is formed as an inactive precursor with a seven-amino acid residue-long propeptide. The propeptide can be removed in vitro by means of tryptic cleavage of a Lys–Ile bond, yielding proteolytically active SCCE (9). In the stratum corneum, the SCCE precursor constitutes a significant fraction of the total SCCE protein present. This suggests that SCCE activation catalyzed by an hitherto unknown trypsin-like protease may play a regulatory role in desquamation.

In addition to SCCE activation, trypsin-like proteases may also be involved directly in the degradation of intercellular adhesive structures in the stratum corneum. By means of zymography of extracts of stratum corneum, components with caseinolytic activity with apparent molecular mass around 30 kDa can be detected in addition to SCCE. The 30-kDa protease(s) can be inhibited with leupeptin, but not by chymostatin (7–8), suggesting a trypsin-like primary substrate specificity. Suzuki et al. (13–15) have presented evidence that the...
spontaneous dissociation of corneocytes that can be induced in stratum corneum samples is dependent upon the activity of trypsin- as well as chymotrypsin-like enzymes, and they also suggested that the trypsin-like protease involved was the 30-kDa component detected on zymograms.

In order to further elucidate the mechanisms of stratum corneum turnover and desquamation, we have in this work purified the 30-kDa protease detectable by zymography of stratum corneum extracts. The amino acid sequence data thus obtained were used to carry out molecular cloning of a cDNA representing a new, skin-derived serine protease that from the deduced amino acid sequence is predicted to have a trypsin-like primary substrate specificity. The enzyme is tentatively named “stratum corneum tryptic enzyme” (SCTE).

EXPERIMENTAL PROCEDURES

Plantar stratum corneum was collected as described previously (8). Healthy human skin was obtained from plastic surgery and prepared as described (9). HaCat-cells (a spontaneously immortalized human keratinocyte line (16)) was a generous gift from Dr. N. E. Fuseni (Heidelberg, Germany). An epidermal cDNA act11 library (source of mRNA was keratinocytes from human adult foreskin) was obtained from Clontech (Palo Alto, CA).

Purification of SCTE—In all purification steps, protease activity was detected by means of zymography (17) after polyacrylamide gel electrophoresis as presence of SDS in gel containing 1% heat-denatured casein. Samples were diluted in electrophoresis sample buffer without reducing agent and were not heated before application.

The same starting material as for the purification of SCCE (8) was used, i.e., a RCI extract of plantar corneocytes that had undergone spontaneous dissociation during incubation at 37 °C of plantar stratum corneum in 0.1 M Tris-HCl, pH 8.5, 5 mM EDTA. Extracts corresponding to 50 g of dried tissue, approximately 250 ml, were filtered through a 0.45-μm Minisart N membrane (Sartorius, Göttingen, Germany) and equilibrated with 0.1% trifluoroacetic acid in a total volume of 100 ml, flow rate 2 ml/min. Fractions containing SCTE and SCCE, which were pooled (4 ml) and again subjected to reversed phase chromatography of column (Amersham Pharmacia Biotech) connected to FPLC equipment (Amersham Pharmacia Biotech) and equilibrated with 0.1% trifluoroacetic acid. The column was eluted with a linear gradient consisting of 0–100% acetonitrile in 0.1% trifluoroacetic acid in a total volume of 100 ml, flow rate 2 ml/min. Fractions containing SCTE and SCCE, which were pooled (1–2 ml), diluted with 5 ml of 0.1 M sodium acetate, pH 5.0, and applied on a 1-ml Resource RPC reversed phase chromatography column (Amersham Pharmacia Biotech) equilibrated with 0.1% sodium acetate, pH 5.0. The column was eluted with a linear gradient consisting of 0–1 M NaCl in equilibrating buffer in a total volume of 100 ml, flow rate 1 ml/min. SCTE-containing fractions were pooled (4 ml) and again subjected to reversed phase chromatography as above. The fraction (1 ml) containing peak SCTE activity was lyophilized in portions and subjected to further purification by means of electrophoresis in polyacrylamide gels containing SDS (SDS-PAGE) (18) with and without reduction prior to electrophoresis. Separated proteins were transferred to polyvinylidene difluoride membranes (Immobilon P, Millipore AB, Sundbyberg, Sweden) and stained with Coomassie Blue (Fig. 1). Bands were cut out, wetted in methanol, and subjected to reduction and carboxamylation with 5.5 mg/ml diithiobreitol for 2 h at 60 °C followed by 28 mg/ml sodium iodoadacetate for 45 min at room temperature, all in 6 M guanidinium hydrochloride, pH 8.5. The membrane strips were then subjected to amino acid sequence analysis (19) in a Procise 494 protein sequencing system (Applied Biosystems, Foster City, CA). Sequencing was performed with cycle protocols adapted to our reaction cartridges and chemicals from the manufacturer.

Cloning and Sequencing of sct cDNA—Two degenerated oligomers, rD1 and rD2, were designed from the amino acid sequence obtained for the purified stratum corneum tryptic enzyme. rD1 and rD2 were amplified from the act11 library by phenol/chloroform extraction. A nested polymerase chain reaction (PCR) was set up where 1/50 of the product from the first PCR reaction was used as a template in the second round. PCR was performed according to standard protocols using Taq DNA polymerase (Roche Molecular Biochemicals). PCR 1 was run for 15 cycles, and PCR 2 was run to amplify the cDNA segment for the N-terminal sequencing of the purified SCTE, the primer pair rD1-F1 (see Table I) was used followed by a PCR using rD2-P2. The quality of the products was checked on agarose gels before cloning into the PCR2.1 or PCR2 vector, using the Original TA Cloning Kit (Invitrogen BV, Leek, The Netherlands) as recommended by the manufacturer. Plasmid DNA was isolated using the Qiagen QIAprep Spin Plasmid Kit (Qiagen, Chatsworth, CA). Nucleotide sequencing was performed using the ABI Prism DNA sequencing kit (Amersham Pharmacia Biotech) and DNA sequencing was performed by the manufacturer. From the obtained nucleotide sequence, specific primers were designed that were used in the subsequent cloning reactions by means of which act11 clones were obtained that covered the whole coding sequence.

To verify the sequence, total RNA was extracted from HaCat cells and CHO-K1 cells (Life Technologies, Inc., Täby, Sweden), as recommended by the manufacturer. Reverse transcriptase PCR (RT-PCR) was performed according to standard protocols with oligo(dT) (Perkin-Elmer) in the RT reaction and sct-specific primer pairs in the PCRs. To obtain sequence down to the poly(A), the oligomers (dT)17-Ad and Ad (see Table I) were designed (20). The (dT)17-Ad was used to bind to long stretches of adenosines in the RT reaction. In the following PCR, the Ad primer binding to the end of the oligomer used in the RT reaction was used in combination with the sct specific primer P4. Using the sequence information obtained, primer P7 could be designed and used in the subsequent PCRs to verify the 3′-end sequence. Reversed transcriptase and RNAsin were purchased from Promega (Falkenberg, Sweden). Cloning and sequencing was performed as described above. In order to avoid PCR mismatches, all primers were verified on at least three independently cloned products. Homology searches were carried out with the Gapped Blast 2.0.6 On-net program (National Center for Biotechnology Information), and prediction of the signal cleavage site was performed with the SignalP Version 1.1 On-net program (Epsys Tools, Center for Biological Sequence Analysis).

Northern Blot Analyses— Messenger RNA was recovered from total RNA from HaCat cells using the Oligotex Midi kit (Qiagen) as recommended by the manufacturer. Two μg of mRNA was denatured with glyoxal and dimethyl sulfoxide and electrophoresed on a 1% agarose gel in 10 mM sodium phosphate buffer, pH 7, followed by capillary transfer to a positively charged nylon filter (Roche Molecular Biochemicals) in 10× SSC according to standard procedures (21). After UV cross-linking, detection was performed by washing the membrane in 20 mM Tris-HCl, pH 8, at 65 °C for 15 min. Prehybridization for 30 min and hybridization overnight at 68 °C was performed in DIG Easy Hyb solution (Roche Molecular Biochemicals). For this purpose, a sct-specific digoxigenin-labeled riboprobe, covering positions ~62 to 343 in the sct cDNA nucleotide sequence (Fig. 2), was prepared with the DIG RNA labeling kit (Roche Molecular Biochemicals). The membrane was washed three times in 2 × SSC with 0.1% SDS at 68 °C for 3 × 15 min before detection reactions were performed as recommended (DIG wash and Block Buffer Set and CDP-Star; Roche Molecular Biochemicals).

Antibodies—A polyclonal rabbit serum (antibody Br-1) and affinity-purified polyclonal rabbit antibodies specific for pro-SCTE (antibody Br-C10) and the catalytically active part of SCTE (antibody Br-B1) were produced against bacterial fusion proteins from plasmid constructs that were based on the nucleotide sequence shown in Fig. 2. The production and characterization of these antibodies will be described in detail elsewhere.

SCCE-specific antibodies (22) were obtained from Symbol AB (Umeå, Sweden).

Expression and Purification of Recombinant Pro-SCTE—For expression of recombinant pro-SCTE (r-pro-SCTE) in mammalian cells, the Semliki Forest Virus system (Life Technologies, Inc., Täby, Sweden) was used as described (23). A PCR fragment corresponding to positions ~207 to 684 in the sct cDNA (Fig. 2) was amplified using the XbaI-containing oligomers xP8 and rP9x (see Table I) with Pfu Taq polymerase (Stratagene, La Jolla, CA) as recommended. The fragment obtained was ligated into the XbaI site of the pSFV vector (Life Technologies, Inc.). Messenger RNA from the recombinant pSFV1 and the pSPV-Helper 2 plasmids were in vitro transcribed and electroporated into BHK-21 cells as described (22). Virus particles containing recombinant SCTE cDNA were collected and used for infection of CHO-K1 cells, cultured in DMEM/NUT.MIX F-12 W/Glutamax I (Life Technologies, Inc.). For the subsequent purification, r-pro-SCTE was detected by SDS-PAGE and immunoblotting with antisera Br-1. Conditions for purification of r-pro-SCTE and scce were optimized by washing the membrane in 20 mM Tris-HCl, pH 7.4, and subjected to ion exchange chromatography on a 1-ml Resource S column eluted with 0–1 M NaCl in 0.1 M Tris-HCl, pH 7.4, total volume 50 ml. r-pro-SCTE-containing fractions were pooled

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and further purified, either by reversed phase chromatography as above, or by gel exclusion chromatography on two serially coupled Superdex 75HR 10/30 columns (Amersham Pharmacia Biotech) equilibrated with 0.1 M Tris-HCl, pH 7, 0.2 M NaCl. N-terminal amino acid sequence analyses were performed as above on fractions from reversed phase chromatography after lyophilization, SDS-PAGE, and transfer to polyvinylidene difluoride membranes.

Biochemical Analyses—For activation of r-pro-SCTE, 250 μl of a fraction from gel exclusion chromatography was made 0.5 M in NaCl and incubated at room temperature for 90 min with 25 μl of sedimented trypsin-agarose (Sigma-Aldrich, Stockholm; catalog no. T-4019). After centrifugation, the supernatant was recovered and analyzed by zymography as above and immunoblotting.

For glycosidase treatment r-pro-SCTE containing fractions from gel exclusion chromatography or preparations of partially purified native SCTE (see below) were dialyzed against 0.1 M acetic acid, lyophilized, and treated with N-glycosidase F (Roche Molecular Biochemicals) according to the protocol provided by the manufacturer. Treated samples were analyzed by immunoblotting.

Partially purified preparations of native SCTE used for glycosidase treatment and comparative studies were prepared from plantar stratum corneum. Ten g of tissue was ground in a mortar and mechanically homogenized in 200 ml of 0.1 M Tris-HCl, pH 7. The suspension was left at room temperature for 1 h, the insoluble material was recovered by centrifuging, and the pellet was extracted with 40 ml of 1 M acetic acid. The extract was cleared by centrifuging and filtration before being either dialyzed against 0.01 M acetic acid, lyophilized, or reduced with N-glycosidase F as above. Preparations containing SCTE, as detected by zymography and immunoblotting, were further purified by gel exclusion chromatography as above.

Analyses of SCTE Expression in Human Tissues—cDNA was synthesized from 1 μg of mRNA, recovered as described above, from human skin using Ready to Go T-primed First Strand Kit (Amersham Pharmacia Biotech). PCR was performed on cDNA from epidermis and Human Multiple Tissue Panel I (CLONTECH), using the Advantage cDNA Polymerase Mix (CLONTECH) as recommended. For sccte, human sccte and g3pdh primer pairs P4-rP7, S1-rS2, and G1-rG2 (Table I), respectively, were used in the amplification reactions. Samples were collected after 25, 30, and 35 PCR cycles, and the products were visualized by EtBr staining after agarose gels.

### RESULTS

**Purification of Native Human SCTE**—Electrophoretic analyses of a preparation obtained after the three chromatography steps (reversed phase chromatography, cationic ion exchange chromatography, and reversed phase chromatography) are shown in Fig. 1. Zymography (Fig. 1B) in gels containing casein showed one strong proteolytic band with apparent molecular mass around 25 kDa and one weaker component with slightly lower mobility. These two bands correspond to unglycosylated and glycosylated SCCTE, respectively (9). In addition, a zone of proteolysis, apparently consisting of one stronger band with molecular mass around 30 kDa, and two weaker bands, one with slightly higher mobility and one with slightly lower mobility than the major band, were seen. Coomassie staining of a membrane with transferred proteins after SDS-PAGE is shown in Fig. 1B. Samples that had not been reduced prior to electrophoresis (right part of Fig. 1B) were found to contain two groups of proteins with molecular mass apparently similar to the two groups of proteolytic components seen on zymograms with apparent molecular mass of about 30 and 25 kDa, respectively. Samples that had been reduced prior to electrophoresis contained several components: one group with apparent molecular mass around 33 kDa, one group with molecular mass around 30 kDa, and several components with lower molecular mass. Immunoblotting with SCCTE-specific antibodies (not shown) showed that the 25-kDa components (nonreduced samples), the 30-kDa components, and some of the smaller components (reduced samples) were related to SCCTE.

Amino acid sequence analyses of the Coomassie-stained components were carried out on membrane strips as outlined in Fig. 1B, and the results are summarized in Table II. Strips from samples that had not been reduced prior to electrophoresis yielded two N-terminal amino acid sequences. Strips taken from the middle portion and strips taken from the edges of the diffuse 30-kDa band gave identical amino acid sequences. The
The major sequence in nonreduced samples was identical with the only sequence that could be found in strips from reduced samples. The minor sequence in nonreduced samples could later be identified as part of the amino acid sequence for SCTE deduced from its cDNA (see below).

Amino acid sequence analyses were carried out on three independent preparations all with identical results as regards the five N-terminal amino acid residues in reduced samples, i.e., Ile-Ile-Gly-Gly-Ser-Asp (see below on the difference between amino acid and nucleotide sequence data for residue 3). Homology searches based on the major amino acid sequence obtained strongly suggested that the sequence was derived from the N terminus of the catalytically active part of a serine protease.

Cloning of scte cDNA—PCR was carried out on DNA extracted from the keratinocyte library with primer pairs consisting of one primer derived from the bacteriophage λ sequence and one degenerated primer derived from the N-terminal amino acid sequence of the purified protein. PCR products with nucleotide sequences encoding the expected amino acid sequences were used to design new primers, which were used in new PCRs. The information obtained from the keratinocyte library was subsequently used to set up RT-PCR reactions with RNA prepared from HaCat cells and human epidermis. In this way, the nucleotide sequence of an apparently close to full-length cDNA, shown for HaCat cells in Fig. 2, could be obtained. With the exception of a part of the noncoding 3′-end, which was not found in the λgt11 library, the nucleotide sequence shown in Fig. 2 was verified for overlapping PCR products from HaCat cells, human skin, and a commercially available keratinocyte library (see legend to Fig. 2 for position 221).

The cDNA contained an open reading frame encoding 293 amino acid residues. The deduced amino acid sequence contained one part that was identical to the N-terminal amino acid sequence of reduced samples of the purified SCTE, with the exception of residue 3, which was deduced to be N instead of G in the purified protein. In addition, the second amino acid sequence found in nonreduced samples of purified SCTE could be identified (positions 62–80 in the deduced sequence).

As shown in Fig. 2, we propose that the protein encoded by the scte cDNA consists of a 29-amino acid residue-long signal peptide, a 37-amino acid residue-long propeptide, and a 227-amino acid-long catalytically active enzyme protein. The propeptide contains one single cysteine residue. The deduced amino acid sequence of the active enzyme contains the conserved sequences of the catalytic triad of serine proteases. Italics in the amino acid sequence denote the proposed propeptide of SCTE. Boldface letters in the amino acid sequence show amino acid residues consistent with amino acid sequence data for purified SCTE. The underlining at positions 994–999 in the nucleotide sequence denotes a proposed polyadenylation site.
served sequences of the catalytic triad of serine proteases (23) (underlined in Fig. 2) and four potential N-glycosylation sites (residues 3, 107, 142, and 186). The aspartate at position 173 corresponds to the bottom of the primary substrate specificity pouch in serine proteases (24) and is compatible with a trypsin-like primary substrate specificity. The molecular mass calculated from the amino acid sequence of the active enzyme is 25.2 kDa. The observed apparent molecular mass (about 33 kDa) of the purified protein suggests posttranslational modifications, most likely glycosylation, of the mature protein (see below).

Homology searches, protein as well as DNA, yielded high scores for a large number of serine proteases. The five proteins showing the highest amino acid sequence homologies are shown in Table III. Among these proteins are porcine enamel matrix serine protease, human and murine neuropsin, and human SCCE. No significant homologies were found when searches were performed for the amino acid sequence of the proposed propeptide.

Northern blot analysis of mRNA from HaCat cells with a riboprobe derived from the scte cDNA showed one strong hybridization signal corresponding to a size of the mRNA of 1.6 kilobases (Fig. 3). Due to very weak signals, results from analyses of mRNA prepared from human skin were not conclusive, but they were suggestive of an mRNA with the same size as in HaCat cells (results not shown).

Comparison of Native and Recombinant SCTE—Polyclonal rabbit antibodies were prepared against bacterial fusion proteins with polypeptides corresponding to pro-SCTE (antibody Br-C10) and active SCTE (antibody Br-1). On immunoblots of crude extracts of plantar stratum corneum (reduced samples), antibody Br-B1 labeled three groups of proteins: one group with molecular mass of about 37 kDa, proposed to be pro-SCTE; one group with molecular mass of about 33 kDa, suggested to correspond to catalytically active SCTE; and one group with molecular mass of about 25 kDa, suggested to be propro-SCTE (Fig. 4, lane 1). In samples that had been N-glycosidase-treated, there was a shift toward increased electrophoretic mobility of all labeled components, and the detected bands had a sharper appearance (Fig. 4B, lane 2). Br-C10 labeled only the 37-kDa component (proposed to be pro-SCTE; Fig. 4B, lane 1), which also showed a decrease in apparent molecular mass after deglycosylation (Fig. 4B, lane 2). An immunoblot of a glycosidase-treated sample labeled with SCCE-specific antibodies is shown for comparison (Fig. 4C, lane 1). All components labeled by the two SCTE-specific antibodies co-chromatographed on reversed phase chromatography. On gel exclusion chromatography, there was a partial separation of the 37- and 33-kDa components, whereas the 33- and 25-kDa components and the 30-kDa proteolytic activity revealed by zymography appeared to co-elute (chromatography results not shown).

Conditioned media from CHO-K1 cells transfected with scte cDNA (but not media from untransfected cells) contained components with apparent molecular mass of about 40 kDa that were labeled by the SCTE-specific antibodies on immunoblots. The amounts expressed were quite low but still sufficient to yield material for analyses. Purified material contained one detectable N-terminal amino acid sequence, which was found to be Asn-Asn-Asp-Val-Ser-Cys, in agreement with the predicted signal cleavage site (see Fig. 2). When compared with the proposed native pro-SCTE (i.e. the component that could be detected on immunoblots with antibody Br-C10) the recombiant protein had a molecular mass that was around 3 kDa higher. This difference in molecular mass seemed to be due to differences in carbohydrate contents, since it disappeared after deglycosylation (Fig. 5). As for the native protein, the apparent heterogeneity of the recombinant protein seen on immunoblots was abolished by N-glycosidase treatment.

By trypsin treatment, it was possible to convert r-pro-SCTE to an active protease with apparent molecular mass of about 31–35 kDa, as revealed by zymography (Fig. 6). In the same way as was found for native SCTE, the proteolytic activity of recombinant SCTE (r-SCTE) was separated on zymograms into several components that differed slightly in electrophoretic mobility. As compared with native SCTE, r-SCTE had a higher apparent molecular mass (approximately 31–35 kDa versus approximately 28–33 kDa in reduced samples). Immunoblot analyses of trypsin-treated r-pro-SCTE, as compared with a preparation containing native SCTE and pro-SCTE, are shown in Fig. 7. Trypsin treatment lead to a decrease in apparent molecular mass from about 40 to 35 kDa (reduced samples) of the components detected by antibody Br-B1 (specific for the catalytically active part of SCTE; Fig. 7A, lanes 2 and 3).

| TABLE III |

The five proteins showing the highest degree of homology to SCTE

| Sequences producing significant alignments | Score | E value | Identities | Positives |
|------------------------------------------|-------|---------|------------|-----------|
| gnl:2737921 (U76256) enamel matrix serine protease 1 precursor... | 258 | 3e–68 | 125/227 (55%) | 158/227 (69%) |
| gnl:PID1034370 (AB012917) serine protease (TLSP) (Homo sapiens) | 252 | 1e–66 | 117/231 (50%) | 158/231 (67%) |
| gnl:PID1029613 (AB009849) neuropsin [H. sapiens] | 246 | 1e–64 | 112/226 (49%) | 151/226 (66%) |
| pir:156559 neuropsin-mouse >-gi 1020C9|gnl:PID107022 (D30... | 242 | 1e–63 | 108/226 (47%) | 152/226 (68%) |
| gnl:PID1319201 (AJ005641) serine protease [Rattus rattus] | 241 | 3e–63 | 108/226 (47%) | 152/226 (68%) |
| sp:949862/SCTE HUMAN STRATUM CORNEUM CHYMOTRYPIC ENZYME PRECUR... | 233 | 6e–61 | 109/225 (48%) | 149/225 (65%) |
molecular mass markers were from Bio-Rad (Sundbyberg, Sweden). Performance on samples in lane 2 Sweden) and were detected as described (28). Untreated reduced sample with insolubilized trypsin incubated in buffer only. t treated with insolubilized trypsin; t a new batch. as in Fig. 4; prestained markers are from same source as in Fig. 4 but N glycosidase treatment, N neum: Effect of catalytically active part of SCTE) (Procedures”). Primary antibodies were Br-B1 (suggested specificity for samples prepared from a crude extract in acetic acid (see “Experimental Procedures”). Primary antibodies were Br-B1 (suggested specificity for pro-SCTE) (B), and anti-SCCE (C). Secondary antibodies were alkaline phosphatase-conjugated goat anti-rabbit IgG (DAKOPATTS, Alvsjö, Sweden) and were detected as described (28). Untreated reduced samples are shown in lane 1, A and B. Glycosidase treatment had been performed on samples in lane 2, A and B, and in lane 1, C. Prestained molecular mass markers were from Bio-Rad (Sundbyberg, Sweden).

Expression of scte in Human Tissues—Results from expression studies of scte in comparison with scce by means of PCR on cDNA derived from human skin and a commercially available panel of cDNA from a number of human tissues are shown in Fig. 8 and Table IV. Semiquantitative information was obtained by means of comparing the intensities of the PCR products on agarose gels as a function of the number of PCR cycles run. After 25 cycles, visible products were obtained from skin only. With an increasing number of PCR cycles, products could be detected also in other tissues, especially brain, placenta, and kidney. Evidence of expression in lung was found for scce but not for scte.

DISCUSSION

In this work, we present the nucleotide sequence for a cDNA encoding a new human serine protease. The sequence was obtained by means of PCR cloning from a keratinocyte library, from HaCat cells, and from normal human skin with degenerated primers based on amino acid sequence data for a protein purified from human plantar stratum corneum. The cDNA from all three sources had open reading frames with identical nucleotide sequences. The protein was purified from three different batches of pooled starting material. The amino acid sequence deduced from the cDNA contained two stretches, 20 and 19 residues long, respectively, which could be identified by amino acid sequence analyses of the purified protein. This is with the exception of residue 3 in the purified protein, which in all preparations analyzed was found to be a glycine, while all preparations (from three different sources) of cDNA encoded an asparaginyl at this position. At present, we have no explanation for this aberration (see below). On the basis of comparisons of amino acid sequence data for purified proteins and the amino acid sequence deduced from cDNA, we consider it very unlikely, however, that we cloned the “wrong” cDNA. The purified protein had an apparent molecular mass and a distribution pattern on Coomassie-stained membranes that was similar to a group of components with proteolytic activity that could be detected in the purified preparation on zymograms with casein as substrate. This group of proteolytic components with apparent molecular mass of about 30 kDa is one of two groups of major proteolytic enzymes that can be detected on zymograms after SDS-PAGE of crude extracts of human plantar and non-palmar-plantar stratum corneum (6, 7, 13). The other group, with apparent molecular mass of about 25 kDa, is SCCE. Taken together, these facts strongly suggest that we have cloned the cDNA for the 30-kDa serine protease of human stratum corneum with trypsin-like substrate specificity and

FIG. 5. Comparison of native pro-SCTE and r-pro-SCTE: Effect of N-glycosidase treatment. An immunoblot (reduced samples) was labeled with antibody Br-C10. Secondary antibodies and detection are as in Fig. 4; prestained markers are from same source as in Fig. 4 but as a new batch. Lanes 1 and 2, samples partially purified from plantar stratum corneum as described under “Experimental Procedures.” Lane 3, r-pro-SCTE. Asterisks indicate samples as in lanes 1–3, treated with N-glycosidase.

FIG. 6. Comparison of native SCTE and r-SCTE by zymography in polycrylamide gels containing casein. Lane 1, partially purified sample from plantar stratum corneum; lane 2, r-pro-SCTE treated with insolubilized trypsin; lane 3, untreated r-pro-SCTE; lane 4, control sample with insolubilized trypsin incubated in buffer only. t, trypsin released from the trypsin-agarose gel.

difference in molecular mass of around 2–3 kDa between native SCTE and r-SCTE, as suggested by zymography, could be demonstrated also by immunoblot analyses (cf. Fig. 7A, lanes 1 and 3). As expected, trypsin-treated r-pro-SCTE was not labeled by antibody Br-C10 (specific for pro-SCTE; Fig. 7B, lane 3).
Trypsin-like Serine Protease from Human Stratum Corneum

Table IV

Expression studies by RT-PCR (human skin) and PCR (other human tissues) of scte and scce

| Tissue       | scte PCR cycles | scce PCR cycles |
|--------------|-----------------|-----------------|
| Skin         | ++              | ++              |
| Heart        | − −             | (+ +)           |
| Brain        | − −             | − −             |
| Placenta     | − −             | (+ +)           |
| Lung         | − −             | (+ +)           |
| Liver        | − −             | − −             |
| Skeletal muscle | − −           | − −             |
| Kidney       | − (+)           | − −             |
| Pancreas     | − −             | (+ +)           |
| Negative control | − −       | − −             |

Shown is a summary of results after different numbers of PCR cycles. The staining intensities were estimated visually with the products obtained for scte and scce as references, arbitrarily set as +, + +, and + + + after 25, 30, and 35 PCR cycles, respectively. In the negative control, no cDNA was added to the reaction mixtures.

Fig. 8. Expression studies by RT-PCR (human skin) and PCR (other human tissues) of scte (I), scce (II), and g3pdh (III). EtBr-stained agarose gels are shown. The number of PCR cycles was 30 in I and II and 25 in III. Lanes 1–10, skin, heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, and negative control (no cDNA in PCR mixture), respectively.

The calculated molecular mass for SCTE, based on the deduced amino acid sequence, is 25.2 kDa. This, in combination with an apparent molecular mass of the purified protein of about 30 kDa without reduction and about 33 kDa after reduction, the appearance of multiple bands with the same detectable N-terminal amino acid sequences after electrophoresis, and the results from deglycosylation experiments, may be that recombinant pro-SCTE is more heavily glycosylated than native SCTE. The asparagine in the sequence Ile-Ile-Asn-Gly-Ser-Asp-Cys (positions 1–7 in the deduced SCTE amino acid sequence) constitutes a N-glycosylation site not found in the N-terminal amino acid sequence data for native SCTE and the catalytically active recombinant SCTE had an active protease by trypsin treatment. The recombinant pro-SCTE as well as the catalytically active recombinant SCTE had apparent molecular masses that were higher than their counterparts in plantar stratum corneum. A likely explanation for these differences, which was supported by results from deglycosylation experiments, may be that recombinant pro-SCTE is more heavily glycosylated than native SCTE. The asparagine in the sequence Ile-Ile-Asn-Gly-Ser-Asp-Cys (positions 1–7 in the deduced SCTE amino acid sequence) constitutes a N-glycosylation site not found in the N-terminal amino acid sequence of native SCTE (Ile-Ile-Gly-Gly-Ser-Cys). The conflict between the amino acid sequence data for native SCTE and the amino acid sequence deduced from scte-cDNA may thus be real and of relevance. As stated above, we cannot resolve this problem at present.

Previous studies have shown that high expression of SCCE is found in the skin only (9). On the protein level, this enzyme has so far been detected only in squamous epithelia undergoing cornification (11, 22, 26, 27). This is compatible with the proposed function of SCCE in desquamation. In the present work, we found that expression of SCTE may be at least as skin-specific as the expression of SCCE. The function of SCTE in the skin is not known. It remains to be elucidated whether it is involved in desquamation, as has been suggested (13–15). Most likely, being a protease with trypsin-like substrate specificity, SCTE is a possible candidate for being responsible for activation of zymogens such as pro-SCCE. The results presented in this work lay the ground for future search of answers to these questions.
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Note Added in Proof—After submission of this paper, we have become aware of a reported genomic DNA sequence (accession number AF135028) which has combined exon sequences 100% homologous with our cDNA sequence.

REFERENCES
1. Lundström, A., and Egelrud, T. (1990) J. Invest. Dermatol. 94, 216–220
2. Chapman, S. J., and Walsh, A. (1990) Arch. Dermatol. Res. 282, 304–310
3. Egelrud, T., and Lundström, A. (1989) Acta Dermato-Venereol. 69, 470–476
4. Lundström, A., and Egelrud, T. (1988) J. Invest. Dermatol. 91, 340–343
5. Egelrud, T., and Lundström, A. (1990) J. Invest. Dermatol. 95, 456–459
6. Egelrud, T., and Lundström, A. (1991) Arch. Dermatol. Res. 283, 108–112
7. Lundström, A., and Egelrud, T. (1991) Acta Dermato-Venereol. 71, 471–474
8. Egelrud, T. (1993) J. Invest. Dermatol. 101, 200–204
9. Hansson, L., Stroemqvist, M., Bäckman, A., Wallbrandt, P., Carlstein, A., and Egelrud, T. (1994) J. Biol. Chem. 269, 19429–19436
10. Skutt, A., Stroemqvist, M., and Egelrud, T. (1995) Biochem. Biophys. Res. Commun. 211, 565–569
11. Sondell, B., Thornell, L. E., Stigbrand, T., and Egelrud, T. (1994) J. Histochem. Cytochem. 42, 459–465
12. Sondell, B., Thornell, L. E., and Egelrud, T. (1995) J. Invest. Dermatol. 104, 819–823
13. Suzuki, Y., Nomura, J., Hori, J., Kayama, J., Takahashi, M., and Horii, I. (1993) Arch. Dermatol. Res. 285, 372–377
14. Suzuki, Y., Nomura, J., Kayama, J., and Horii, I. (1994) Arch. Dermatol. Res. 286, 249–253
15. Suzuki, Y., Kayama, J., Moro, O., Horii, I., Kikuchi, K., and Tanida, M. (1996) Br. J. Dermatol. 134, 460–464
16. Boukamp, P., Petrussevska, R. T., Breitkreutz, D., Hornung, J., Markham, A., and Fusenig, N. E. (1988) J. Cell Biol. 106, 761–771
17. Horie, H., Fukuyama, K., Ito, Y., and Epstein, W. L. (1984) Comp. Biochem. Physiol. 77B, 349–354
18. Laemmli, U. K. (1970) Nature 227, 680–685
19. Matsudaira, P. (1987) J. Biol. Chem. 262, 10035–10038
20. Frohman, M. A., Dush, M. K., and Martin, G. R. (1988) Proc. Natl. Acad. Sci. 85, 8998–9002
21. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
22. Sondell, B., Dyberg, P., Anneroth, G. K. B., Östman, P.-O., and Egelrud T. (1996) Acta Dermato-Venereol. 76, 177–181
23. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1995) Current Protocols in Molecular Biology, Vol. 2, p. 16.20 Massachusetts General Hospital, Harvard Medical School, Boston
24. Rawlings, N. D., and Barret, A. J. (1994) Methods Enzymol. 244, 19–61
25. Polgar L. (1987) in Neuberger, A., Brocklehurst, K. (eds) Hydrolytic Enzymes: New Comprehensive Biochemistry, Vol. 16, pp. 159–200, Elsevier Science Publishers B.V., Amsterdam
26. Ekholm, E., and Egelrud, T (1998) Br. J. Dermatol. 138, 585–590
27. Ekholm, E., Sondell, B., Dyberg, P., Jonsson, M., and Egelrud, T. (1998) Acta Dermato-Venereol. 78, 343–347