Trichohyalin: A Structural Protein of Hair, Tongue, Nail, and Epidermis

Edward J. O'Keefe,* Elizabeth H. Hamilton,* Seung-Chul Lee,† and Peter Steinert†

*Department of Dermatology, University of North Carolina, Chapel Hill, North Carolina; and †Skin Biology Branch, National Institute of Arthritis Metabolic and Skin Disease, National Institutes of Health, Bethesda, Maryland, U.S.A.

In the course of studies of desmosomes, we found trichohyalin, a 200-kDa protein of the inner root sheath and medulla, in a citric acid–insoluble fraction ("desmosome preparation") from tongue epithelium. Pig tongue epithelium yielded milligram quantities of pure trichohyalin from about 100 g of keratomed epithelium. The protein has an extended shape as determined by gel filtration, ultracentrifugation, and electron microscopy, with a rod domain and a globular domain at one end and overall dimensions of about 85 nm. Crosslinking studies suggest that the protein may be dimeric in solution. The protein is a doublet in some animals but apparently is a single polypeptide of 220 kDa in humans. Immunofluorescence studies showed that it is a major protein of the filiform papillae of the tongue of mammals and is present in isolated cells of the stratum granulosum of some regions of epidermis in a subset of cells containing filaggrin and in the nail matrix. Similarly, in filiform papillae some cells contain granules that stain for both trichohyalin and filaggrin. Immunoblotting confirmed that trichohyalin is present in tongue and epidermis. Polymerase chain reaction with human genomic DNA using oligonucleotide primers based on sheep trichohyalin resulted in synthesis of multiple DNAs, from which a 504-bp fragment was subcloned and sequenced and found to resemble closely the carboxyl terminus of sheep trichohyalin. Studies with antibody to the carboxyl-terminal 14 amino acids of the human sequence show that, whereas the carboxyl-terminal epitope is present only in the stratum granulosum, in epidermis epitopes detected by a monoclonal antibody are demonstrated in both the stratum granulosum and stratum corneum, suggesting that the carboxyl terminus is cleaved in the stratum corneum. *J Invest Dermatol 101:65S–71S, 1993*

We have purified a structural protein from a pig tongue epithelium desmosome preparation initially thought to be a desmosome protein but that we now know is trichohyalin. Trichohyalin is the name given in 1903 to granules of the inner root sheath of hair follicles [1] and later to a protein extracted from the inner root sheath granules [2] and also present in the medulla of the hair fiber. The inner root sheath, a narrow sheath that forms a rigid insoluble tube that may guide the direction of growth of the hair fiber, has three distinct cell layers, which differentiate at different levels relative to the hair matrix. All three layers contain trichohyalin, which appears first near the base of the follicle in non-membrane bound granules that become larger in size as the cells of the internal root sheath grow further from the hair matrix and then abruptly disappear at a distinct zone (the "transformation zone") in association with the development of filaments, at which point the inner root sheath becomes birefringent [3].

The biochemistry of trichohyalin has been elucidated primarily by George Rogers' laboratory and has been reviewed in recent publications [4,5]. The protein was the first described to contain citrulline bound in peptide linkage and is a substrate for a hair-follicle transglutaminase. Although it is soluble when located in granules, it becomes insoluble in concert with modification of some arginine residues to become citrulline and with crosslinking by transglutaminase to form ϵ-(γ-glutamyl)lysine crosslinks. The inner root sheath contains filaments, which are presumably intermediate filaments composed of keratin to which trichohyalin is tightly bound (discussed by Fietz *et al* [4] and reviewed by Hamilton *et al* [5]). The morphologic data of O'Guin *et al* [6] describe a trichohyalin epitope identified by a monoclonal antibody that suggests that trichohyalin binds to keratin filaments at regular intervals, in which case it should be classified as a keratin-binding protein, a member of the class of intermediate filament associated proteins (IFAPs). Trichohyalin protein was extracted and purified from sheep wool follicles by Rothnagel and Rogers [2], and antibodies were used to screen a cDNA library. A partial clone was found to contain 23 amino acid repeats and was thought to be capable in theory of forming filamentous aggegates, but the heptad repeat characteristic of alpha-helices and present in intermediate filaments was absent [4].

We have devised a purification procedure for trichohyalin from tongue epithelium involving a citric acid–insoluble pellet first used by Skerrow and Matoltsy [7] to study desmosomes as starting material. About 120 g of epithelium yielded 800 mg of pellet, which in turn yielded about 0.9 mg of pure trichohyalin after thirtyfold purification from the insoluble pellet [5]. Unlike the protein from humans and sheep, the pig protein is a doublet of about 195 and 210 kDa as determined by pulverizing and extracting the snap-frozen epithelium (to minimize proteolysis) and examining the size of the protein by immunoblotting; both polypeptides of the doublet have similar two-dimensional peptide maps. The protein as visualized by rotary shadowing electron microscopy is an extended rod with a single terminal globular domain; an extended configuration is also consistent with data from ultracentrifugation (Svedberg constant = 6) and gel filtration (Stokes radius = 12.4 nm). Crosslinking with a bifunctional reagent suggested that the protein may be dimeric in solution. In the course of these earlier studies, antibodies were raised and used to study the protein in tissues. This report

Reprint requests to: Dr. Edward J. O'Keefe, Department of Dermatology, 137 NCMH, CB 7600, University of North Carolina, Chapel Hill, NC 27514.

Abbreviations: IFAP, intermediate filament associated proteins.

0022-202X/93/$06.00

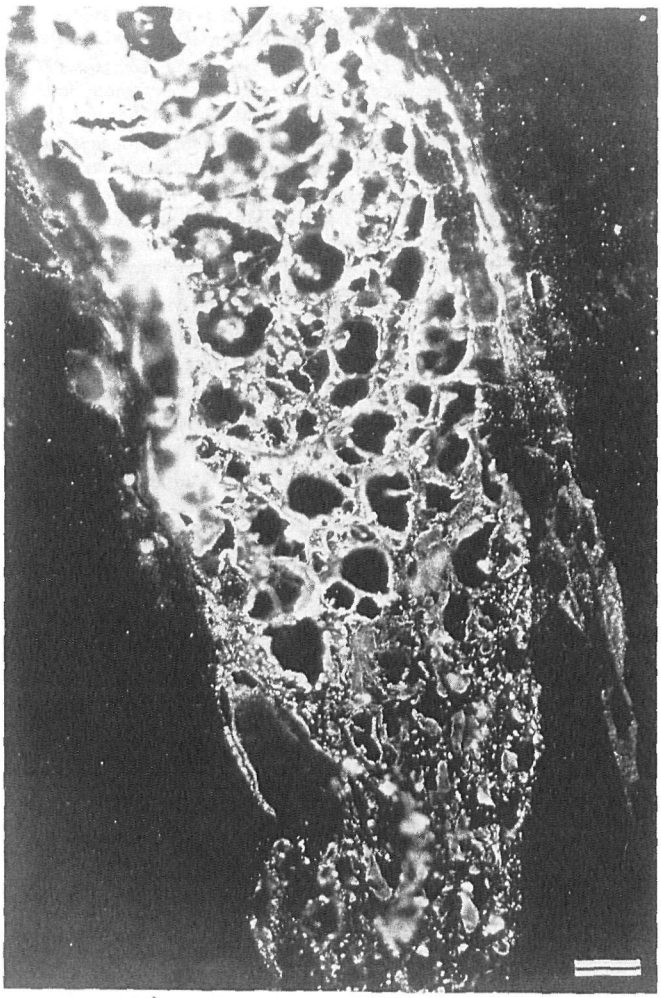

Figure 1. Granular-to-amorphous progression of trichohyalin staining in pig tongue filiform papilla. Pig tongue epithelium was stained with monoclonal antibody to trichohyalin and then with fluorescein-conjugated anti-mouse IgG. *Scale bar,* 25 μm.

describes some new aspects of the distribution and localization of trichohyalin in tongue and nail.

MATERIALS AND METHODS

Materials Antibodies to purified pig trichohyalin were raised in rabbits and affinity purified on a column of trichohyalin-Sepharose, and a monoclonal antibody was obtained with mice immunized with purified trichohyalin; both antibodies have been described [5,8]. A peptide corresponding to the carboxyl terminus of trichohyalin was synthesized by use of the f-MOC (9-fluorenylmethyloxycarbonyl) technology on an Applied BioSystems 431A peptide synthesizer using the manufacturer's protocol as modified [9]. The carboxyl-terminal epitope was used because secondary structural analyses [10] suggested that this sequence adopts a random coil conformation and is therefore likely to be antigenic. We have found for other epidermal differentiation products that the carboxyl-terminal sequences provide highly chain-specific antibodies [11]. Antibody to the carboxyl-terminal 14 amino acids of trichohyalin [4] was raised in rabbits and gave the characteristic staining of the inner root sheath and filiform papilla in pig and human tongue (not shown). Monoclonal anti-human filaggrin was obtained from Biomedical Technologies, Inc., Stoughton, MA. Secondary antibodies were from Kirkegaard and Perry (Gaithersburg, MD) and were used at 1:50 dilution. Chemicals were reagent grade or better.

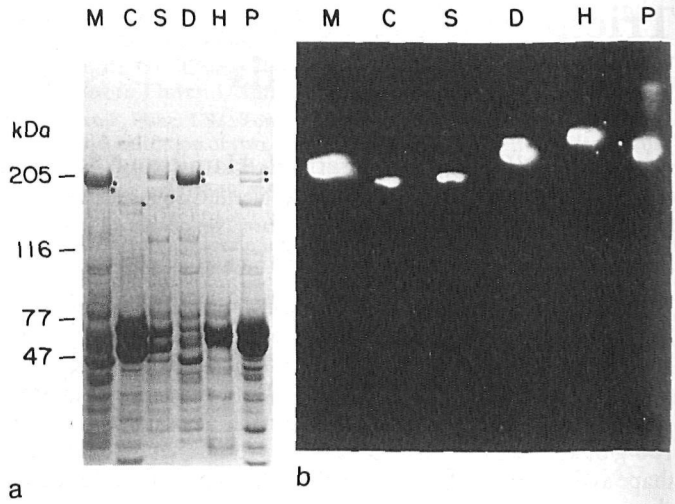

Figure 2. Trichohyalin is present in tongue epithelium of diverse mammals. Comparison of Coomassie blue-stained SDS-polyacrylamide gels and immunoblots containing extracts of tongue epithelium from various mammals. Freshly keratomed epithelium was frozen in liquid nitrogen, pulverized, and solubilized in Fairbanks' sample buffer, and samples were analyzed by SDS polyacrylamide gel electrophoresis. Additional samples were diluted in Fairbanks' sample buffer to reduce the amount of protein in each and transferred to nitrocellulose. The blots were incubated with antibody to trichohyalin and then with ^{125}I-labeled protein A and processed for autoradiography. M, mouse; C, cow; S, sheep; D, dog; H, human; P, pig. *(a)* Coomassie blue-stained gel; *(b)* immunoblot. Molecular weight standards shown at left were myosin, beta-galactosidase, bovine serum albumin, and ovalbumin. Dots on *(a)* indicate location of trichohyalin bands.

Immunofluorescence and Immunoblotting These were performed by standard techniques, as previously described [8]. Immunofluorescence was performed with monoclonal anti-trichohyalin and affinity-purified rabbit anti-pig trichohyalin and with rhodamine- or fluorescein-labeled second antibody as described. Sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis was performed with 3–17.5% gradient gels in Fairbanks' buffer system [12].

Digestion of Trichohyalin Twenty micrograms of trichohyalin purified as described previously [8] was incubated with 1.25 μg of chymotrypsin for 20 or 40 min in 10 mM sodium phosphate buffer, pH 7.4, containing 10% glycerol, 1 M NaBr, 1 mM dithiothreitol, 1 mM ethylene diamine tetra-acetic acid, 1 mM sodium azide, 0.05% Tween-20, and 1.5 mM $CaCl_2$ in a volume of 0.125 ml on ice. The digestion was terminated by addition of 200 μg of phenylmethyl sulfonyl fluoride per ml (final concentration), and the sample was solubilized in Fairbanks' sample buffer for analysis by SDS-polyacrylamide gel electrophoresis.

Cloning and Sequencing of cDNA Normal human genomic DNA was used as a template for PCR amplification of the end of the coding region of the human trichohyalin gene. The primers used were based on published sequences of sheep trichohyalin and were +primer, 5′-GACAGAAAGTTCCGCGAGGAGGAACAGCT; and −primer, 5′-GGGCGGTACTGAGATCTCTGCTCTTGGATGTA. The conditions for PCR were about 400 ng of genomic DNA in a 0.1 ml reaction with 1.5 mM $MgCl_2$, 200 μM dNTPs, and 0.4 μM of each of the primers. Amplifications were done for 30–35 cycles (95°C, 1 min; 65°C, 30 seconds; 72°C, 1 min) in a DNA Thermal Cycler (Perkin Elmer-Cetus, Norwalk, CT). The amplified DNA products were resolved on a 1.5% agarose gel, and the band of about 500 bp was excised, purified by the GeneClean kit (Bio101, La Jolla, CA), reacted to fill in the ends [13], and blunt-end ligated into the pGEM-3z vector for sequencing by the dideoxy

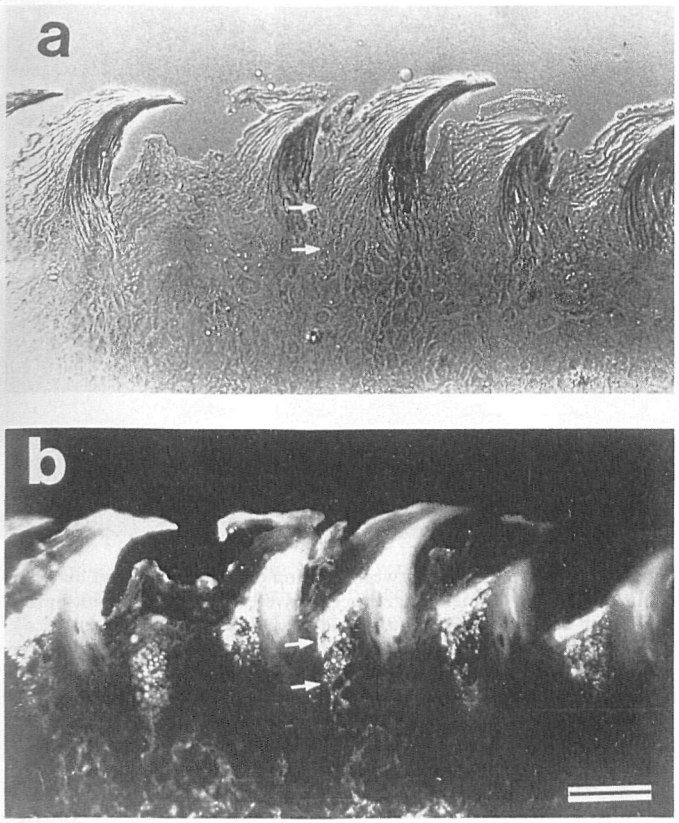

Figure 3. Trichohyalin in mouse tongue. *(a)* Phase-contrast view of tongue epithelium with papillae; *(b)* immunofluorescence with anti-trichohyalin. *Arrows,* location of trichohyalin. *Scale bar,* 100 μm.

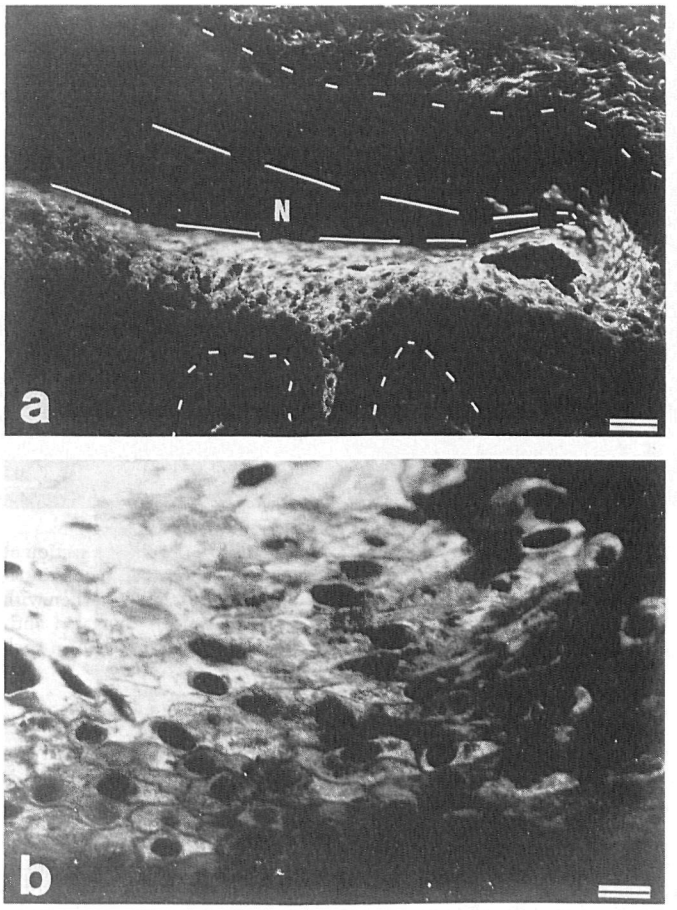

Figure 4. Trichohyalin is present in the ventral matrix of the human nail. Toenail was sectioned and stained with monoclonal antibody to trichohyalin and with fluorescein-conjugated goat anti-mouse IgG. *(a)* Lower-power view of the nail showing staining for trichohyalin in ventral and apical regions of matrix but not in dorsal matrix (above nail plate). *Dotted lines,* location of basement membrane separating epithelium of ventral and dorsal matrix from connective tissue. *Broken line,* outline of nail plate. Region of separation at posterior ventral matrix is an artifact of sectioning; *(b)* higher power view of cells of ventral matrix staining for trichohyalin showing apparent absence of granules. *Scale bars,* 40 μm *(a)*, 120 μm *(b)*.

chain termination method (Sequenase II kit, United States Biochemicals, Cleveland, OH).

RESULTS

Localization of Trichohyalin in Tongue Immunfluorescence microscopy demonstrated intense staining for trichohyalin in the filiform papillae of the tongue, the source of the purified protein (Fig 1). As described in the inner root sheath, the trichohyalin was found in granules that were small at the base of the papilla and then became larger. At a point about midway to the top of the papilla, the staining for trichohyalin appeared to become more diffuse throughout the cells of the papilla, and the granular quality disappeared, reminiscent of the "transformation zone" in the inner root sheath, where trichohyalin apparently becomes cross-linked by transglutaminase, some of its arginine residues become converted to citrullines, and it can no longer be extracted; antibody may fail to recognize trichohyalin above this point [2]. Whether trichohyalin in the filiform papilla undergoes this transformation has not been determined.

Trichohyalin is a major component of the filiform papillae of mouse, cow, sheep, dog, human, and pig, as judged by immunofluorescence. Trichohyalin can be detected as a major band by Coomassie blue staining of SDS gels of extracts of tongue epithelium in mouse and dog; is visible in extracts from cow, sheep, and pig; and is barely visible in the extracts of human tongue epithelium (Fig 2). Trichohyalin is a single polypeptide on one-dimensional SDS–polyacrylamide gel electrophoresis in the human, cow, and sheep, but in mouse, dog, and pig it is a doublet (Fig 2). The two isotypes appear to be present in varying amounts in the dog, in which the upper band is barely visible on Coomassie blue–stained gels but is clearly visible in immunoblots, and in the mouse, in which the lower band is very fine on stained gels and gives a weak signal on immunoblots that is not apparent in the contact print of the autoradiogram.

The "hard" keratins of hair, nail, quill, hoof, and claw, the baleen of whales, and the horn of cattle, goats, and rhinoceroses [14] have been found to be present also in filiform papillae of tongue [15,16]. Whereas hard keratins are present in the hair fiber, trichohyalin is absent in the hair fiber but is present in the inner root sheath, which has not been shown to contain hard keratins [15,16]. Although the precise keratin complement of the inner root sheath is not agreed upon, evidence has been proposed that the filaments of the inner root sheath are composed of keratins [17–20]. A similar situation is found in the tongue. The hard keratins comprise the spine of the filiform papilla but are not present in the region of the papilla containing trichohyalin. In Fig 3, the spine in the mouse is shown to be located in the posterior region of the papilla, pointing posteriorly (towards the pharynx and towards the right side of the figure). Trichohyalin is found anterior to the base of the spine in the papilla, close to the region containing filaggrin, which is also present in a specific region of the filiform papilla of the mouse [21]. Examination of another structure containing hard keratins, the human nail, by immunofluorescence showed that trichohyalin was present in the ventral matrix (Fig 4) but not in the nail bed. A few scattered

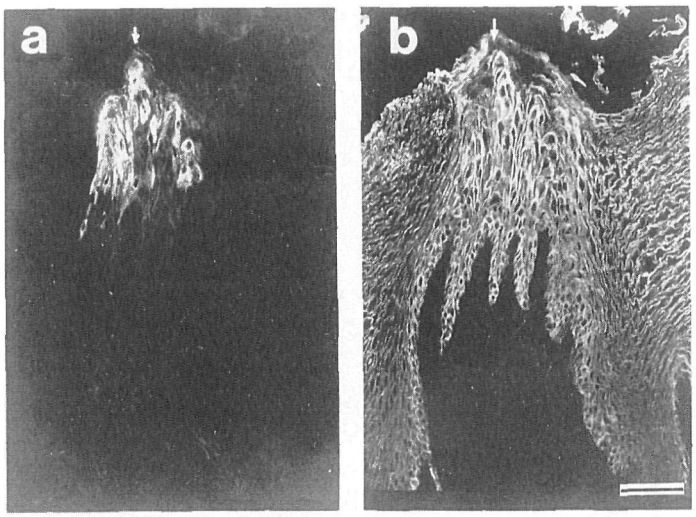

Figure 5. Trichohyalin in the human tongue filiform papilla. A section of tongue epithelium was incubated with mouse monoclonal antibody to trichohyalin *(a)* and rabbit antibody to desmoplakin I and II *(b)* and then with fluorescein-conjugated anti-mouse IgG and rhodamine-conjugated anti-rabbit IgG as in *Materials and Methods. Arrows,* identical location in each photomicrograph. *Scale bar,* 100 μm.

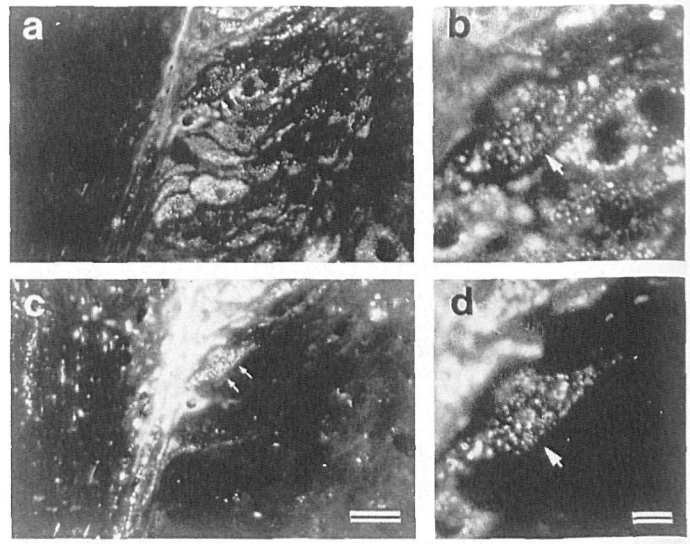

Figure 7. Correspondence between location of trichohyalin and filaggrin in filiform papilla. Human tongue sections were stained with affinity-purified rabbit anti-trichohyalin *(a,b)* and with mouse anti-filaggrin *(c,d)*. Higher-power views *(b,d)* indicate a trichohyalin-containing cell *(b)* in which identical granules also stain for filaggrin *(d)*. *Arrows,* same cell in all four panels. *Scale bars, (a,c)* 20 μm; *(b,d)* 10 μm.

cells staining for trichohyalin were present within the nail plate (not shown).

The tongue epithelium is complicated with respect to both structure and keratin content; the keratins associated with the papilla differ from those in the interpapillary regions [21], and the filiform papilla, especially the spine, has different configurations in different animals. Trichohyalin is present in a symmetric distribution in the human tongue (Fig 5*a*), and the whole papilla usually appears symmetrical, unlike that of some other mammals.

Localization of Filaggrin and Trichohyalin in the Filiform Papilla Trichohyalin is present in scattered cells in the granular layer of some regions of the epidermis [8]. Filaggrin, a major protein of the granular layer, is also present in these trichohyalin-containing cells in epidermis; the trichohyalin-containing cells of the stratum granulosum are a subset of the filaggrin-containing cells [8]. Filaggrin is also known to be present in the filiform papilla [21]. When we compared the distribution of trichohyalin (Fig 6*a*) and filaggrin (Fig 6*b*) in human tongue epithelium, we found that filaggrin, unlike trichohyalin, is widely distributed throughout the epithelium and does not appear to be located exclusively in the papilla, although it is found concentrated in some regions of the papilla. Figure 6 shows filaggrin granules present both densely at the edge of the papilla and also in the interpapillary epidermis. More detail of these regions of the papilla containing both trichohyalin and filaggrin shows that one region of a filiform papilla from human tongue is stained for both trichohyalin (Fig 7*a,b*) and filaggrin (Fig 7*c,d*). In the papilla, many cells appear by immunofluorescence to contain trichohyalin but not filaggrin, whereas some contain both (shown by *arrows*). The same granules appear to contain both proteins, but there are small differences in the exact size and shape of the stained granules (Fig 7*b,d*), suggesting that there may be some heterogeneity in the composition of the granules.

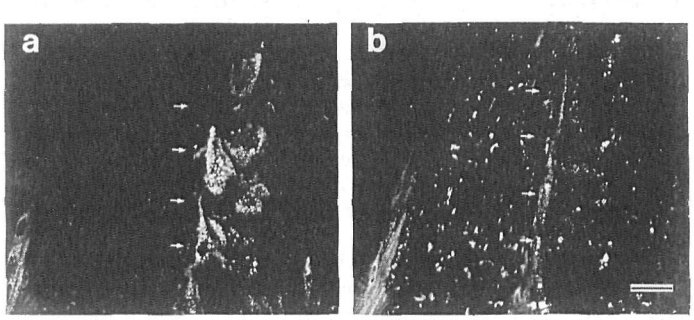

Figure 6. Location of trichohyalin and filaggrin in human tongue epithelium. A single section was incubated with affinity-purified rabbit antibody to trichohyalin and with mouse monoclonal antibody to filaggrin and then with fluorescein- and rhodamine-conjugated second antibodies. *Arrows,* division between interpapillary epithelium (to left of *arrows*) and filiform papilla (to right of *arrows*). *(a)* Trichohyalin; *(b)* filaggrin. *Scale bar,* 20 μm.

Cloning of a cDNA for Human Trichohyalin and Studies of the Carboxyl-Terminal Epitope We used polymerase chain reaction (PCR) amplification methods to isolate and characterize a portion of the human trichohyalin gene using primers based on the published sequences of a partial sheep cDNA clone [4], assuming that there would be considerable sequence homology with the

```
  E  E  Q  L  R  Q  G  R  E  E  Q  Q  L  R  S  Q  E  S  D  R  K  L  R  E  E  25
GAGGAGCAGCTCCGCCAGGGAAGGGAGGAACAGCAGCTGCGCAGCCAAGAGTCTGACAGAAAATTCCGCGAGGAG  75

  E  Q  L  R  Q  E  R  E  E  Q  Q  L  R  P  Q  Q  R  D  G  K  Y  R  W  E  E  50
GAACAGCTACGCCAGGAAAGGGAAGAACAGCAGCTGCGCCCCCAACAGCGTGACGGAAAGTATCGCTGGGAAGAA 150

  E  Q  L  Q  L  E  E  Q  E  Q  R  L  R  R  S  E  T  G  S  T  G  A  E  E  Q  75
GAGCAGCTCCAACTTGAGGAACAAGAGCAGAGGCTGCGCAGGAGCGAGACCGGCAGTACCGGTGCGGAGGAGCAG 225

  F  A  T  Q  E  K  S  R  R  E  E  Q  E  L  W  Q  E  E  E  Q  K  R  R  Q  E 100
TTTGCCACGCAGGAGAAGAGTCGTCGTGAGGAACAAGAACTATGGCAAGAAGAGGAGCAGAAACGTCGCCAGGAA 300

  R  E  R  K  L  R  E  E  H  I  R  R  Q  Q  K  E  E  Q  R  H  R  Q  V  G  E 125
CGGGAAAGGAAATTACGGGAAGAACACATCCGCCGCCAGCAGAAGGAGGAACAGAGGCACCGCCAAGTCGGGGAG 375

  I  K  S  Q  E  G  K  G  H  G  R  L  L  E  P  G  T  H  Q  F  A  S  V  P  G 150
ATAAAATCCCAAGAAGGGAAGGGCCATGGGCGGCTTCTGGAGCCCGGCACTCATCAGTTTGCCAGTGTCCCAGTG 450

  R  S  S  P  L  Y  E  Y  I  Q  E  Q  R  S  Q  Y  R  P                     168
CGCTCCAGCCCTCTCTATGAGTACATCCAAGAGCAGAGATCTCAGTACCGCCCC                     504
```

Figure 8. Nucleic acid and deduced amino acid sequences of the carboxyl-terminal end of the human trichohyalin gene. The single letter code is used; *underlined* nucleotides indicate positions of the primers.

```
                .         .         .         .         .
Human   EEQLRQG REEQQLRSQES DRKFREEEQ LRQEREEQQLRPQQRDGKYR
        :::::   :: : : :   :::: :: : : ::::::  : : : :.
Sheep   EEQLRRREQEEEQ RRQRQRDRKFLEEGQSL QEREEEKRRQEQ DRKF

Human   WE EEQLQLEEQEQRLRRSE TGSTGA  EEQFATQEKSRREEQELWQEE
        .: ::::  ::::  :::         .  :::::  :: :: :::: :::
Sheep   LEQEEQLHREEQEE LRRRQQ LDQQYRREEQFAREEKERRQEQELRQEE

Human   EQKRRQERERKLREE HIRRQQKEEQRHRQ  VGEIK SQ     EGKGH
         :.::::::::: :::  .:::: :::..::    ..  :      : ::.
Sheep    QRRRQERERK REEEQLRRQQQEEQKRRQER  DVQQSRRQVWEEDKGR

Human   GR LLEPGTHQFASVPVRSSPLYEYIQEQRSQYRP
         : .:: : .::::.:::::::::::::::::::::
Sheep    RQVLERGKRQFASAPVRSSPLYEYIQEQRSQYRP
```

Figure 9. Comparison of the amino acid sequences of sheep [4] and human trichohyalins. The two deduced sequences are aligned to maximize sequence identities (:) or homologies (.).

human gene. We have noted high degrees of sequence homology of the carboxyl-terminal ends of mammalian loricrins [22] and profilaggrin [23]. After the PCR reaction, we obtained two principal products of about 500 and 575 bp; the former was successfully subcloned and sequenced (Fig 8) and was found to have significant sequence homology with the published sheep cDNA clone [4] (Fig 9). Most notably, there is marked sequence homology with a repeating sequence motif within the sheep trichohyalin, and the carboxyl-terminal end was indeed precisely conserved. Comparison of the amino acid sequences of the sheep and human sequences demonstrated 58% identity; the carboxyl-terminal 20 amino acids are identical.

We made antibody to a synthetic peptide corresponding to the 14 carboxyl-terminal amino acids and used this antibody to examine proteolytic digests of purified pig trichohyalin by immunoblotting and also to examine trichohyalin in human filiform papillae and epidermis by immunofluorescence. Immunoblots against limited proteolytic digests of trichohyalin showed that although affinity-purified rabbit antibody to whole trichohyalin and monoclonal anti-trichohyalin [8] identified similar digested fragments of relatively large size (Fig 10*a,b*), the antibody to the carboxyl terminus identified a smaller fragment of about 15 kDa but did not detect any larger fragments (Fig 10*c*), suggesting that the carboxyl-terminal domain was cleaved. When staining with a monoclonal anti-trichohyalin was compared by double immunofluorescence with that produced by the antibody to the carboxyl terminus, the carboxyl-terminal epitope was detected throughout the filiform papillae and in the epidermis. Both antibodies detected cells in the granular layer, but the carboxyl-terminal epitope was absent in cells staining for trichohyalin in the stratum corneum (Fig 11), indicating that trichohyalin is probably cleaved in the stratum corneum, losing its carboxyl-terminal epitope.

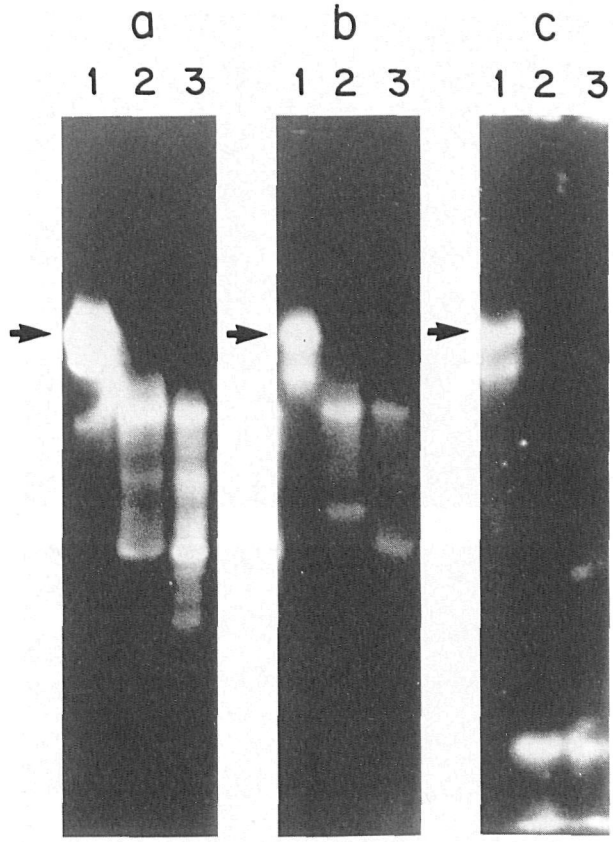

Figure 10. Digestion of purified trichohyalin cleaves the carboxyl-terminal epitope. Purified trichohyalin was digested with chymotrypsin as in *Materials and Methods,* analyzed by SDS–polyacrylamide gel electrophoresis, and transferred to nitrocellulose. Immunoblots were made using affinity-purified polyclonal rabbit antibody *(a)*, monoclonal antibody *(b)*, or antibody to the carboxyl terminus *(c)* and were developed using ^{125}I-labeled protein A. *Lanes 1,* undigested purified pig trichohyalin; *lanes 2,* 20-min digestion; *lanes 3,* 40-min digestion. *Arrows,* location of 210-kDa upper band of the pig trichohyalin doublet.

DISCUSSION

Although trichohyalin has been known since early in the century to be present in the inner root sheath, it has only recently been found to be a major protein of filiform papillae [5] and also present in small amounts in the epidermis [8]. Studies of the nail showed that it also appears to be present in part of the nail matrix, although the staining is amorphous rather than granular. We have characterized a few features of the distribution and localization of trichohyalin and have purified trichohyalin in a form that may be functional, because it retains an apparently native configuration in solution as judged by rotary shadowing electron microscopy and is not aggregated as judged from ultracentrifugation and gel filtration. These studies provide a basis on which to study possible functions of trichohyalin such as binding to intermediate filaments.

A recent review of intermediate filaments [24] lists trichohyalin as well as desmoplakin, plectin, and filaggrin as keratin IFAPs. The evidence for such a role for trichohyalin as a keratin-binding protein, or IFAP, are presented in the two studies cited above [2,6], which demonstrate an intimate association of the proteins and the periodic decoration of keratin by trichohyalin as defined by a monoclonal antibody. It will be important to confirm that trichohyalin is an IFAP by demonstrating binding to keratin filaments *in vitro.* It may be important in this respect that trichohyalin remains tightly associated with keratins during purification; when tongue keratins are solubilized in citric acid buffer and then assembled, trichohyalin remains associated with keratins and cannot be separated except after solubilization, for example, with high concentrations of urea [5]. It is possible that trichohyalin binds to specific type of keratins; if so this may be demonstrated by colocalization of trichohyalin and specific keratins and by binding only to certain keratins *in vitro.*

Our studies indicate that trichohyalin is a major protein of the filiform papilla of mammals, is localized in the tongue exclusively to the papillae, and is limited to a specific region of the papilla. The transition from granular to amorphous staining suggests that trichohyalin may undergo crosslinking similar to that known to occur in the inner root sheath. Trichohyalin differs in molecular weight on SDS gels in different species, but antibodies to porcine trichohyalin cross-react with these different polypeptides. Trichohyalin clearly has two isotypes in three of six mammals examined; whether there are two polypeptides that comigrate in the other species is not known. Other data have suggested that tricho-

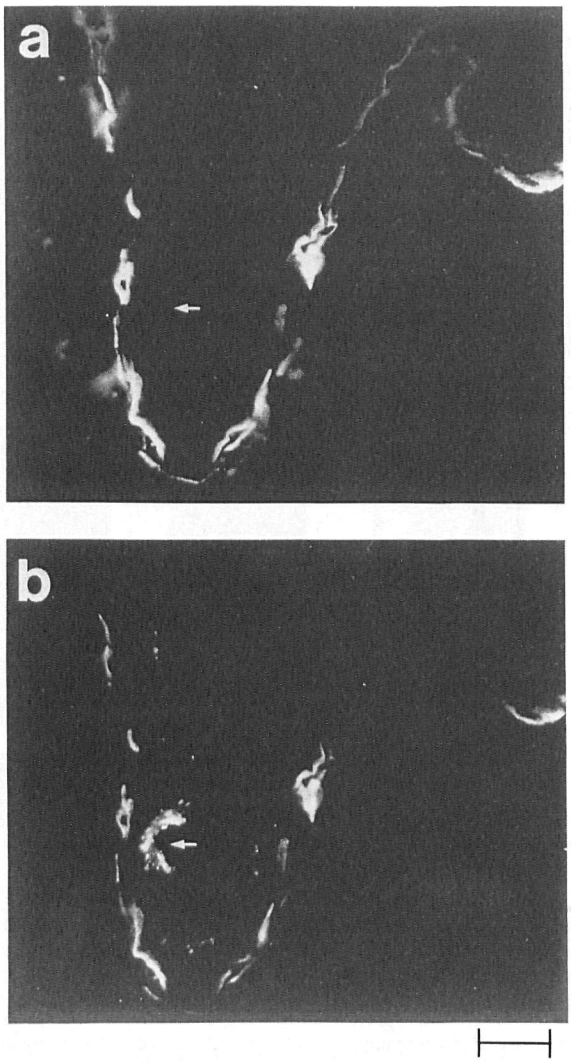

Figure 11. Loss of the carboxyl-terminal epitope in the stratum corneum. Comparison of staining in human scalp skin epidermis with antibody to carboxyl-terminal 14 amino acids *(a)* and monoclonal antibody to whole trichohyalin *(b)* shows that a cell stained by the monoclonal anti-trichohyalin antibody *(b)* is not stained by antibody to the carboxyl terminus *(a)*. *Arrow,* location of cell in stratum corneum that fails to stain with antibody to carboxyl terminus. Trichohyalin stained cells in *(a)* identify the stratum granulosum. *Scale bar,* 50 μm.

hyalin is a dimer, which could contain the two isotypes found on gels.

Filaggrin, which can be shown to be present in the interpapillary epithelium as well as in the papilla in humans, is present in some trichohyalin-containing granules. This has also been demonstrated in the cells of the granular layer of epidermis that contain trichohyalin [8].

Antibodies to different epitopes of trichohyalin (Fig 10) should be useful for determining whether processing of the protein occurs as it becomes crosslinked by transglutaminase and modified by deiminase as well as for determining the structure of trichohyalin. For example, studies with antibody to the carboxyl terminus indicate that this epitope is cleaved in the stratum corneum (Fig 11), although it is unclear whether this represents proteolytic processing in the dead cells or biologically relevant processing. It may be possible to determine whether the carboxyl terminus is the rod domain, rather than the globular end of the molecule, as is suggested by the cDNA cloning studies, which indicate that the carboxyl terminus is rod shaped. Additional cDNA clones will be useful for generation of other highly specific antibodies for these types of studies.

This work was supported by grant AR 25871 from the National Institutes of Health to EJO'K. The technical assistance of Mr. Richard E. Payne, Jr., is gratefully acknowledged.

REFERENCES

1. Voerner H: On trichohyalin. A study of the anatomy of the hair root. Dermatol Zeitschr (Berlin) 10:357–376, 1903
2. Rothnagel JA, Rogers GE: Trichohyalin, an intermediate filament-associated protein of the hair follicle. J Cell Biol 102:1419–1429, 1986
3. Rogers GE: Some aspects of the structure of the inner root sheath of hair follicles revealed by light and electron microscopy. Exp Cell Res 14:378–387, 1958
4. Fietz MJ, Presland RB, Rogers GE: The cDNA-deduced amino acid sequence for trichohyalin, a differentiation marker in the hair follicle, contains a 23 amino acid repeat. J Cell Biol 110:427–436, 1990
5. Hamilton EH, Sealock R, Wallace NR, O'Keefe EJ: Trichohyalin: purification from porcine tongue epithelium and characterization of the native protein. J Invest Dermatol 98:881–889, 1992
6. O'Guin WM, Sun T-T, Manabe W: Interaction of trichohyalin with intermediate filaments: three immunologically defined stages of trichohyalin maturation. J Invest Dermatol 98:24–32, 1992
7. Skerrow CJ, Matoltsy AG: Chemical characterization of isolated epidermal desmosomes. J Cell Biol 63:524–530, 1974
8. Hamilton EH, Payne RE Jr, O'Keefe EJ: Trichohyalin: presence in the granular layer and stratum corneum of normal human epidermis. J Invest Dermatol 96:666–672, 1991
9. Yajima H, Fujii N, Funakoshi S, Watanabe T, Murayama E, Otaka A: New strategy for the chemical synthesis of proteins. Tetrahedron 44:805–819, 1988
10. Devereux J, Haeberli P, Smithies O: A comprehensive set of sequence programs for the VAX. Nucleic Acids Res 12:387–394, 1984
11. Roop DR, Cheng CK, Titterington L, Meyers CA, Stanley JR, Steinert PM, Yuspa SH: Synthetic peptides corresponding to keratin subunits elicit highly specific antibodies. J Biol Chem 259:8037–8040, 1984
12. Fairbanks G, Steck TL, Wallach DFH: Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10:2606–2617, 1971
13. Sambrook EG, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989
14. Marshall RC, Orwin DFG, Gillespie JM: Structure and biochemistry of mammalian hard keratin. Electron Microsc Rev 4:47–83, 1991
15. Dhouailly D, Xu C, Manabe M, Schermer A, Sun T.-T: Expression of hair-related keratins in a soft epithelium: subpopulations of human and mouse dorsal tongue keratinocytes express keratin markers for hair-, skin-, and esophageal-types of differentiation. Exp Cell Res 181:141–158, 1989
16. Heid HW, Moll I, Franke WW: Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. II. Concomitant and mutually exclusive synthesis of trichocytic and epithelial cytokeratins in diverse human and bovine tissues (hair follicle, nail bed and matrix, lingual papilla, thymic reticulum). Differentiation 37:215–230, 1988
17. Heid HW, Moll I, Franke WW: Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. I. Human and bovine hair follicles. Differentiation 37:137–157, 1988
18. Ito M, Tazawa T, Ito K, Shimizu N, Katsuumi K, Sato Y: Immunological characteristics and histological distribution of human hair fibrous proteins studied with anti-hair keratin monoclonal antibodies HKN-2, HKN-4, and HKN-6. J Histochem Cytochem 34:269–275, 1986
19. Lynch MH, O'Guin WM, Hardy C, Mak L, Sun T-T: Acidic and basic

hair/nail ("hard") keratins: their colocalization in upper cortical and cuticle cells of the human air follicle and their relationship to "soft" keratins. J Cell Biol 103:2593–2606, 1986

20. Lane EB, Bartek J, Purkis PE, Leigh IM: Keratin antigen in differentiating skin. Ann NY Acad Sci 455:241–258, 1985
21. Dale BA, Salonen J, Jones AH. New approaches and concepts in the study of differentiation of oral epithelia. Crit Rev Oral Biol Med 1:167–190, 1990
22. Yoneda K, Hohl D, McBride OW, Wang M, Cehrs KU, Idler WW, Steinert PM: The human loricrin gene. J Biol Chem 267:18060–18066, 1992
23. Gan SQ, McBride OW, Markova N, Idler WW, Steinert PM: Structure, organization, and polymorphisms of the human profilaggrin gene. Biochemistry 29:9432–9440, 1990
24. Steinert PM, Roop DR: Molecular and cellular biology of intermediate filaments. Annu Rev Biochem 57:593–625, 1988