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Biochemistry of Transglutaminases and Cross-Linking in the Skin

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Transglutaminase is a calcium-dependent enzyme found widely in nature. It catalyzes the formation of ϵ-(γ-glutamyl)lysine bonds that participate in processes varying from fibrin clot formation to epidermal cell envelope formation.

Epidermal transglutaminase is localized to the granular layer of the epidermis. It catalyzes the covalent cross-linking of a soluble cytoplasmic substrate into large polymers to form the cornified envelope that lines the inner membrane of keratinocytes in the stratum corneum. The soluble precursor from epidermis has been named *keratolinin*, and from keratinocyte culture, it has been named *involucrin*.

Hair follicle transglutaminase is biochemically and immunochemically distinct from its epidermal counterpart. It has been localized to the inner root sheath and medulla of the hair follicle. The substrate of hair follicle transglutaminase has been poorly defined but appears to be rich in the amino acid citrulline.

Transglutaminase has been shown to be an important marker of normal differentiation. There is a rise in its activity at the time of keratinization, and transglutaminase activity has been shown to be greatly decreased in basal cell epithelioma and in psoriasis. Keratinocyte cell culture has proven most helpful in delineating the processes of normal differentiation and keratinization, since the formation of the cell envelope in culture appears to parallel the formation in vivo.

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Three types of covalent cross-links occur between proteins. They include the disulfide bridge, the aldol and aldimide bonds,

and the ϵ-(γ-glutamyl)lysine cross-link. The latter is catalyzed by the calcium-dependent enzyme transglutaminase. The transglutaminases are a group of enzymes classified by immunochemical and biochemical differences. They include (1) hair follicle transglutaminase, (2) tissue transglutaminase, including epidermal transglutaminase, and (3) protransglutaminases (factor XIII) of plasma, liver, uterus, placenta, prostate gland, and platelet.

As shown in Fig. 1, transglutaminase in the presence of calcium catalyzes covalent cross-linking in which the γ-carboxamide group of peptide-bound glutamine residues are the acyl donors and the ϵ-amino group of peptide-bound lysine residues function as acyl acceptors. Ammonia is released in the reaction.

The ϵ-(γ-glutamyl)lysine cross-link is essentially a universal bond, being found in a range of organisms from humans to bacteria and plasmodium. It is also found in a variety of proteins, including clotted vesicular protein, collagen, fibrin, and keratin. In the last decade, the ϵ-(γ-glutamyl)lysine cross-link catalyzed by transglutaminase has been shown to be of major importance in the cornification of epidermis and hair structures. The cornified envelope found in the stratum corneum and the medulla and inner root sheath of the hair follicle are composed of proteins linked by this bond. The end result is the production of an extremely insoluble protein polymer that is relatively impermeable and resistant to denaturation.

The substrates of epidermal and hair follicle transglutaminase, besides containing lysine and glutamine residues, also contain citrulline (Fig. 2). This amino acid is an intermediate in arginine synthesis and was previously thought not to be associated with proteins.

Our review will be limited to epidermal and hair follicle transglutaminase and their substrates, in keeping with the theme of this Symposium.

THE ϵ-(γ-GLUTAMYL)LYSINE BOND AND HAIR FOLLICLE TRANSGLUTAMINASE

Substrates of Hair Follicle Transglutaminase

Research by wool biochemists in the early sixties led to the discovery of the ϵ-(γ-glutamyl)lysine isodipeptide cross-link in hair follicle proteins, and identification of a hair follicle transglutaminase soon followed. In 1962, Rogers [1] reported a citrulline-rich protein located in the inner root sheath of hair follicles, medulla from hair, and related structures such as porcupine quills. This citrulline-rich protein was also characterized by a very high glutamic acid and low cysteine content, which was very different from the surrounding keratin proteins of the cortex. In 1969, Steinert et al. [2] demonstrated that the citrulline-rich protein was covalently cross-linked, which prompted a search for the nature of this covalent bond. Unlike keratin, these citrulline-rich proteins were easily digested by proteolytic agents [3].

Evidence that the citrulline-rich protein was also high in glutamic acid led some investigators to question the possible role of glutamine in covalent-bond formation. In 1969, Pisano et al. [4] had shown that the cross-linking of glutamine and lysine in the fibrinogen-fibrin transformation was catalyzed by coagulation factor XIII, a transglutaminase, and the bond formed was an ϵ-(γ-glutamyl)lysine bond. In 1970, Asquith et al. [5] discovered ϵ-(γ-glutamyl)lysine bonds in digests of wool keratin. They estimated the amount (micromoles per gram of protein) of the isodipeptide in Australian Merino wool extracts to be the following: keratin, 15; alpha keratose, 9; beta keratose, 30; and gamma keratose, 8. Subsequently, Harding and Rogers [6] reported the presence of the ϵ-(γ-glutamyl)lysine cross-link in the citrulline-containing protein of medulla cells from hair and quills. Some of the values [7], expressed as moles of cross-links per 1000 moles of amino acids, were rat, 29.5; porcupine, 2.3; guinea pig, 28.1; rabbit, 25.9; and guinea pig keratin, 0.8. This value for keratin was about half the value obtained by Asquith et al., but confirmed a small amount of ϵ-(γ-glutamyl)lysine bonds in keratins. The vast majority, though, were found in medulla and inner-root-sheath proteins. Further delineation of the nature of these citrulline-rich proteins was achieved by Harding and Rogers [8] in 1976 by isolating cross-linked proteins that contained citrulline from the medulla and inner root sheath. Amino acid analysis revealed that approximately two-thirds of the cross-linked peptides were composed of glutamine and citrulline residues. Labeled antisera to these tryptic peptides stained the inner-root-sheath and medulla cells in a pattern coincidental with trichohyalin [9]. The exact nature of hair follicle transglutaminase substrates from medulla and inner root sheath remains to be defined. Advancement has been hindered by the extreme insolubility of the proteins, once they are polymerized.

Hair Follicle Transglutaminase

In 1972, Harding and Rogers [7] reported an enzyme in hair follicle homogenates that incorporated [^{14}C]glycine ethyl ester into casein by forming ϵ-(γ-glutamyl) derivatives. The reports of ϵ-(γ-glutamyl)lysine bonds [5,7] led Chung and Folk [10] to the first isolation of hair follicle transglutaminase from the guinea pig. Purification of the inner root sheaths of hair follicles yielded two distinct transglutaminases. The first was similar to the liver transglutaminase they had previously isolated and contained esterase activity. The other enzyme they named *hair follicle transglutaminase* because it demonstrated properties that were characteristic only of that enzyme, including no esterase activity. The enzyme had a molecular weight of 54,000 on gel filtration, and it dissociated into two apparently identical subunits at 27,000 daltons on polyacrylamide gel electrophoresis containing sodium dodecyl sulfate. Antiserum to the liver enzyme gave no precipitin band upon immunodiffusion with the hair follicle enzyme. The liver and hair follicle enzyme gave no indication of having a zymogen form. Its dependence on calcium was also demonstrated.

Buxman and Wuepper [11] found that hair follicle transglutaminase and epidermal transglutaminase were immunochemically distinct. Antiserum to bovine snout epidermal transglutaminase failed to precipitate in agar or to functionally inhibit transglutaminase activity in hair follicle extracts. These observations were substantiated by the discovery that fluorescein-conjugated antiserum to bovine epidermal transglutaminase

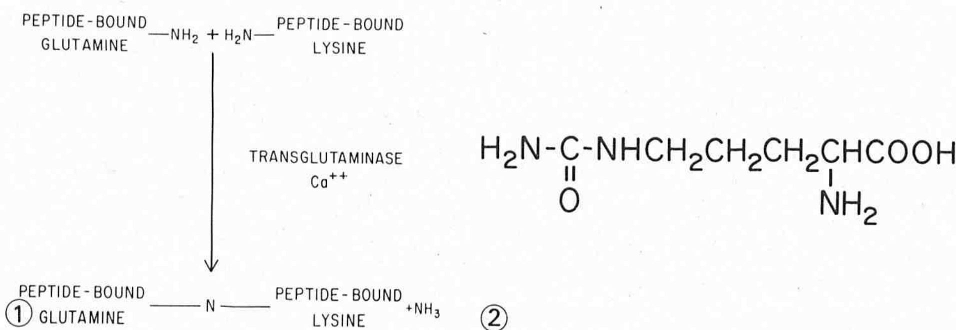

FIG 1. Reaction catalyzed by transglutaminase forming the ϵ-(γ-glutamyl)-lysine bond.

FIG 2. Structure of the amino acid citrulline.

localized the enzyme in epidermis but not follicles [12]. Ogawa and Goldsmith [13] reported similar results with antiserum to human epidermal transglutaminases.

Differential purification, characterization, and immunochemical localization of epidermal and hair follicle transglutaminase was finally achieved by Peterson and Buxman [14] in 1979 using epidermal extracts from rats 4 to 5 days old. Epidermal transglutaminase had a molecular weight of approximately 57,000 by gel filtration and in gels containing sodium dodecyl sulfate, while hair follicle transglutaminase had a molecular weight of 54,000 and was reduced to two apparently identical subunits of 27,000 daltons by denaturing media. Purified hair follicle transglutaminase and epidermal transglutaminase had specific activities of 160 and 312 amine-incorporating units per milligram per hour, respectively. Antiserum specific to each enzyme was produced in chickens, and no cross-reaction was seen between transglutaminases with human or bovine epidermis or with rat plasma factor XIII. When conjugated to fluorescein, these antisera localized epidermal transglutaminase and hair follicle transglutaminase to the granular layer of the epidermis (Fig. 3) and the medulla and inner root sheath of hair follicles (Fig. 4), respectively. Table I summarizes the hair follicle transglutaminases that have been characterized.

EPIDERMAL TRANSGLUTAMINASE AND ITS SUBSTRATES

Epidermal Transglutaminase

The discovery of ϵ-(γ-glutamyl)lysine bonds and hair follicle transglutaminase in hair structures led to the investigation of glabrous epidermis for an epidermal transglutaminase.

Buxman and Wuepper [15] found transglutaminase activity in cow snout epidermis, a source of hair-free epidermis, and were the first to purify the enzyme and produce a monospecific antibody to epidermal transglutaminase. The antisera did not cross-react with bovine or human factor XIII. Additionally, localization of epidermal transglutaminase was achieved by direct histochemical staining using dansyl cadaverine. The fluorescence was restricted mainly to the granular layer. Fluorescein-labeled antiserum to bovine epidermal transglutaminase produced similar results. In 1976, they further characterized their purified enzyme [11]. Analytical ultracentrifugation produced a molecular weight of 55,800 from sedimentation equilibrium data. The purified enzyme had a specific activity of 3000 amine incorporating units per milligram per hour.

In 1974, Goldsmith et al. [16] most extensively investigated cow snout epidermis, but also found transglutaminase activity in skin extracts from rat, turtle, toad, and frog. The bovine epidermal transglutaminase had a relative molecular weight between 50,000 and 60,000 by gel filtration. It was similar to many of the transglutaminases previously studied, demonstrating heat stability, inhibition by EDTA, stimulation by reducing agents, and the ability to cross-link fibrin. Enzyme activity was highest in the stratum corneum and granular layer. Detection of the ϵ-(γ-glutamyl)lysine bond was achieved by using the indirect technique of Pisano et al. [4], but actual isolation of depeptides containing this bond was unsuccessful.

Ogawa and Goldsmith [17] then purified human epidermal transglutaminase to homogeneity and reported its molecular weight to be 50,000 by sodium dodecyl sulfate electrophoresis. Their immunologic studies of human epidermal transglutaminase [13] showed that the enzyme was immunologically distinct from other transglutaminases. Crude extracts of skin from frog, rat, and mouse and of hair follicles did not cross-react with antisera to human epidermal transglutaminase.

Buxman et al. [18] have shown that transglutaminase is an important marker for terminal epidermal differentiation. Fetal rat epidermis and hair follicle inner root sheath undergo terminal keratinization late in gestation and 4 to 5 days after birth, respectively. Concomitant with the onset of keratinization is a rise in transglutaminase activity in both structures, as shown biochemically and histochemically. Their conclusions were consistent with earlier studies showing transglutaminase activity to be greatly decreased in poorly differentiated cells such as basal cell epithelioma and psoriatic cells [12]. A summary of epidermal transglutaminases is presented in Table II.

The Cornified Envelope and Substrates of Epidermal Transglutaminase

The chemical and electron microscopic studies by Matoltsy and Balsamo [19] demonstrated that the plasma membrane undergoes many chemical and structural changes as the epidermal cell reaches maturity. The formation of a cornified envelope lining the inner surface of the mature keratinocytes was evident in the upper granular layers. The thickness of the membrane

TABLE I. *Hair follicle transglutaminases*

Author:	Chung and Folk [10]	Peterson and Buxman [14]
Tissue:	Guinea pig	Rat
Metal requirements:	EDTA inhibits, Ca^{2+} activates	EDTA inhibits, Ca^{2+} activates
Substrates tested:	[^{14}C]putrescine, [^{14}C] methylamine	Dansyl cadaverine
Molecular weight:	54,000 (gel filtration); 27,000 (SDS electrophoresis)	54,000 (gel filtration); 27,000 (SDS electrophoresis)
Immunologic identity:	No inhibition with Ab to liver T-gase	No cross-reaction with rat anti-factor XIII, rat or bovine epidermal T-gase

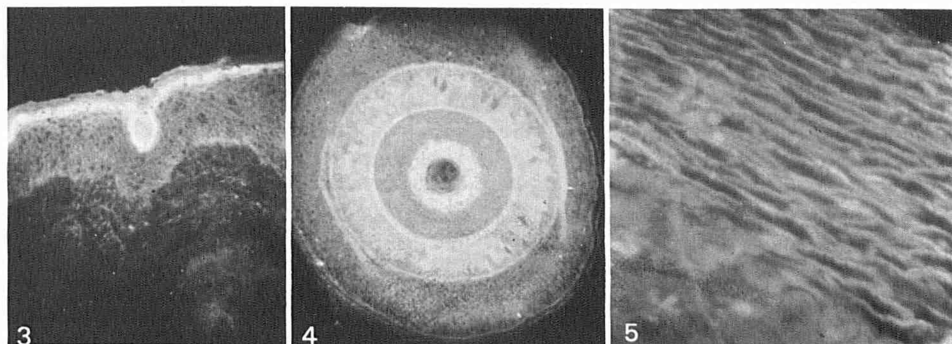

FIG 3. Direct staining of human epidermis with dansyl cadaverine and calcium (reduced from ×62.5). A similar pattern was seen in rat epidermis stained with fluorescein-conjugated antibody to rat epidermal transglutaminase. The granular layer is fluorescing.

FIG 4. Direct staining of a cross-section of a human hair with dansyl cadaverine and calcium (reduced from ×600). A similar pattern was seen in rat hair stained with fluorescein-conjugated antibody to rat hair follicle transglutaminase. The medulla and inner root sheath are fluorescing.

FIG 5. Direct staining of bovine snout epidermis with fluorescein-conjugated antibody to bovine keratolinin showing fluorescence located on the cell membrane of the stratum corneum (reduced from ×200).

TABLE II. *Epidermal transglutaminases*

Author:	Ogawa and Goldsmith [13, 16]	Buxman and Wuepper [15]	Peterson and Buxman [14]
Tissue:	*Human callus*	*Cow snout epidermis*	*Rat epidermis*
Metal requirements:	Ca^{2+} activates, EDTA inhibits	Ca^{2+} activates, EDTA inhibits	Ca^{2+} activates, EDTA inhibits
Substrates tested:	Putrescine, fibrin	Dansyl cadaverine	Dansyl cadaverine
Molecular weight:	50,000 (gel filtration); 51,000 (SDS electrophoresis)	55,000 (gel filtration and SDS electrophoresis); 55,800 (sedimentation equilibrium)	56,000 (gel filtration and SDS electrophoresis)
Immunologic identity:	No cross-reaction with human factor XIII, frog, rat, mouse, chicken, or human hair follicle transglutaminase crude extracts	No cross-reaction with bovine or human factor XIII	No cross-reaction with bovine or rat hair follicle transglutaminase or rat factor XIII

increased from 80 to 150 Å, and the envelope appeared electron-dense. It was also noticed that the cornified cells were much more rigid and impervious to keratinolytic agents, e.g., urea, dithiothreitol. This was attributed to the extremely insoluble proteins of the envelope.

Identification in the early seventies of ϵ-(γ-glutamyl)lysine bonds [5] and transglutaminases [10,11,16] from epidermis and hair prompted the investigation of substrates for epidermal transglutaminase. Significant advances were made by Buxman et al. [20] in 1976. Bovine epidermal extracts containing endogenous epidermal transglutaminase were incubated with dansyl cadaverine and calcium. Substrates of epidermal transglutaminases were identified by this method. A soluble substrate was isolated and had a molecular weight of 150,000. A highly insoluble substrate was also identified and solubilized by chemical cleavage with cyanogen bromide. Neither of the substrates precipited in agar with bovine antifibrinogen, but were quite strongly bound by a sepharose antifibrinogen column, being released by thiocyanate. Antisera to the insoluble substrate produced two separate precipitation lines in agar with either the dialyzed cyanogen bromide extract or purified soluble substrate. The insoluble substrate was determined to be neither an α-helical fibrous protein nor a sulfur-rich matrix protein. Preliminary amino acid analysis of the purified substrate showed a very low half-cystine content and a high serine, glutamic acid, and lysine content. They proposed that the natural substrate for epidermal transglutaminase was a soluble precursor protein enzymatically cross-linked by transglutaminase into large polymers that formed the envelope of the keratinocyte.

ϵ-(γ-glutamyl)lysine cross-links were now considered a major characteristic of the envelope, like the disulfide bond was to keratin. Even epidermal keratin, like hair keratin [5], was found to contain some ϵ-(γ-glutamyl)lysine bonds.

In 1976, Hanigan and Goldsmith [21] confirmed the presence of two high-molecular-weight substrates similar to those reported by Buxman et al. They investigated human stratum corneum and newborn rat epidermis. In 1979, Rice and Green [22] isolated from epidermal keratinocyte culture a soluble precursor of the cross-linked envelope. It was identified by its ability to bind dansyl cadaverine and to purified to homogeneity. The precursor had a molecular weight on gel filtration of 500,000 and on sodium dodecyl sulfate gel electrophoresis of 92,000. Faint fluorescent bands were also seen at 45,000 and 20,000. The 92,000 molecular weight protein had an isoelectric point at pH 4.5 ± 0.3, and it was biochemically and immunochemically distinct from keratin. Immunofluorescent staining was consistent with the precursor being cytoplasmic in the upper epidermal layers and membrane-bound in the envelope in the stratum corneum. An interesting finding was the amino acid composition of the envelope precursor compared with the cross-linked envelope. The former had a glutamine content of almost 46 percent, while the latter had a content of 15 percent. They have named this soluble precursor *involucrin* (from the Latin, *involucrum*—"an envelope").

In 1980, Buxman et al. [23] proposed that their previously described relative high-molecular-weight substrates of epidermal transglutaminase were actually intermediate polymers of a lower-molecular-weight protein. Until this time, substrates had been identified by their ability to incorporate dansyl cadaverine. The problem with this method is that at the same time the substrate proteins are incorporating dansyl cadaverine, they are also, to a limited extent, cross-linking to each other and forming polymers. With the antibody to the 150,000 molecular weight substrate described earlier [20], they were able to identify a low-molecular-weight soluble substrate from bovine snout epidermis that cross-reacted with it. This soluble substrate demonstrated a molecular weight of 36,000 by gel filtration, and in the presence of calcium and transglutaminase, it was converted to higher-molecular-weight polymers ranging in molecular weight from 75,000 to greater than 200,000. An insoluble polymer was also formed that was not solubilized by urea, sodium dodecyl sulfate, or beta-mercaptoethanol. Cross-linking did not occur in the absence of transglutaminase or in the presence of putrescine or EDTA. The 36,000 molecular weight substrate dissociated in sodium dodecyl sulfate gels to considerably smaller subunits. The ϵ-(γ-glutamyl)lysine isodipeptide was identified in high-molecular-weight products of cross-linking, but not in the 36 K precursor. They proposed that the 36 K protein was the precursor protein that cross-linked to form the epidermal envelope. Amino acid analysis of this purified substrate [24] revealed a content per 100 amino acids of glutamic acid 13.46 and citrulline 2.38 and low levels of methionine and cystine. This analysis is somewhat similar to that reported by Rice and Green for the envelope protein [22], and a comparison is shown in Table III. Electron microscopy demonstrated the polymer to be a globular, amorphous substance lacking a fibrillar structure. Further studies showed that the high-molecular-weight polymer was immunologically identical

TABLE III. *Amino acid composition of keratolinin and involucrin and of envelopes after cross-linking*

	Involucrin; Rice and Green [22]	Keratolinin; Buxman [24]	Cross-linked envelopes; Rice and Green [22]
Asx	2.80	8.71	9.20
Thr	1.60	9.22	4.90
Ser	1.60	10.43	6.70
Glx	45.80	13.46	14.90
Pro	5.70	4.50	7.50
Gly	6.70	8.80	7.70
Ala	1.50	8.44	6.90
Val	3.70	6.32	5.40
Met	0.90	0.63	2.40
Ile	0.40	3.14	3.80
Leu	14.60	8.19	8.60
Tyr	0.80	1.63	2.90
Phe	0.60	2.67	3.30
His	4.70	1.13	2.00
Lys	7.40	5.65	8.10
Trp	0.20	0.70	1.10
Arg		2.46	
Cit		2.38	
	100.00	100.00	100.00

TABLE IV. *Comparison of involucrin and keratolinin*

Author:	Rice and Green [22]	Buxman, et al. [23]; Zettergren et al. [25]
Tissue:	Human epidermal cell culture (involucrin)	Bovine snout and human epidermis (keratolinin)
Molecular weight:	500,000 (gel filtration) 92,000 + traces 40,000 and 20,000 (SDS gel electrophoresis)	36,000 (gel filtration); 6000 (SDS electrophoresis)
Immunologic identity (antisera of substrate):	No identity with keratolinin or epidermal extracts	Bovine and human show no identity
Isoelectric point:	4.5	Bovine: A 6.3, B 6.0; Human: A 5.4, B 5.0

to the 36 K protein. By indirect immunofluorescence, substrate was located to the cytoplasm and inner cell membrane of the granular layer and to the inner membrane of the stratum corneum (Fig. 5).

We recently have extended these studies of bovine snout epidermis to include human epidermis [25]. The predominant human and bovine substrates purified from epidermis each demonstrated a molecular weight of 36,000 on gel filtration. By using several low-molecular-weight standards, we have shown in both species the substrate to dissociate in sodium sulfate gels to a molecular weight of 6000 ± 200. When the substrates were incubated with their respective enzymes, dansyl cadaverine and calcium, fluorescent bands at 6000, 12,000, 24,000, and 150,000 were apparent. No band was seen at 92 K, where involucrin from cultured keratinocytes has been identified. Isoelectric focusing of human or bovine substrate revealed two moieties (human: 5.4/50; bovine: 6.3/6.0). The two proteins were readily resolved by chromatofocusing, and each of the moieties of the bovine substrate incorporated dansyl cadaverine and precipitated in a line of identity with antiserum to the soluble bovine substrate.

The origin of the heterogeneity of the bovine protein was next determined, since many animals were pooled for a single purification procedure. Five cow snouts were individually processed and the crude protein extract was subjected to isoelectric focusing. Half the gel was stained for protein with Coomassie G-250. The corresponding half of the gel was electrophoretically blotted and the immobilized proteins were exposed to FITC-conjugated antibody to bovine keratolinin. Numerous proteins were seen on the dye-stained gel, but only two bands on the nitrocellulose paper reacted with the bovine antibody and fluoresced under ultraviolet light. These bands had the same pI values as pure bovine keratolinin (pI 6.3, 6.0). The heterogeneity seen in bovine keratolinin is present in each individual and does not represent allelic differences. We have named the soluble 36 K molecular weight envelope precursor *keratolinin* (Greek, *keratos*—"horny tissue"; Greek *linios*—"to cover the inner surface of"). Antiserum to purified human keratolinin failed to cross-react with its bovine counterpart and vice versa. Since that report, we have received from Dr. Rice antiserum to involucrin. This antiserum does not cross-react by double immunodiffusion with human epidermal extracts or keratolinin. Human and bovine keratolinins appear biochemically similar, but they are immunochemically distinct proteins. Keratolinin from epidermis and involucrin from cell culture are also immunochemically distinct. It appears that involucrin is a substrate of transglutaminase present primarily in cell culture. A comparison of some of the properties of involucrin and keratolinin are presented in Table IV.

CONCLUSION

Transglutaminase and the bond it forms are found in a wide variety of organisms. Its role in clot formation is well understood, but its importance in many tissues remains unclear. While much is known about the formation of the cross-links, very little is known about their catabolism. Presumably, polymer degradation in skin is inconsequential, since keratinocytes are sloughed off in the stratum corneum.

The ϵ-(γ-glutamyl)lysine bond is now known to be an important component of the epidermis and hair. The presence of a cornified envelope has become a marker for normal differentiation. Characteristics of the skin, including stability and strength, can be attributed in part to the presence of epidermal and hair follicle transglutaminase.

Much has been learned about epidermal and hair follicle transglutaminase and the substrate of epidermal transglutaminase. Both enzyme and substrate appear to be species- and tissue-specific. The relative insolubility of the hair follicle has prevented extensive biochemical analysis of the substrate of hair follicle transglutaminase. The fact that hair follicle transglutaminase has two subunits and epidermal transglutaminase has none is somewhat surprising, since both are so closely related embryologically. Even more surprising is the paucity of involucrin in normal epidermis. Regardless, cell culture has provided us with a model for envelope formation. In the future, cell culture will be an important tool in further understanding the process of keratinization.

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Involucrin and Other Markers of Keratinocyte Terminal Differentiation

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Human epidermal keratinocytes in culture form stratified colonies that retain many of the properties of intact epidermis. Mitosis is restricted to the basal layer, and cells that leave it undergo terminal differentiation, increasing in size and synthesizing a range of specialized molecules as they pass through the different cell layers.

One useful marker for an early stage in the pathway of terminal differentiation is involucrin, a soluble protein precursor of the cross-linked envelope. Involucrin is synthesized after keratinocytes have left the basal layer and begun to enlarge, but some time before the onset of envelope cross-linking, which occurs only in the outermost cell layers.

Small, involucrin-negative cells can be isolated and cultured in medium containing a low concentration of calcium ions, to prevent stratification, but not cell division and enlargement. Under these conditions, involucrin synthesis is initiated by some cells in the monolayer, indicating that attainment of a suprabasal position is not required for this step in terminal differentiation. Furthermore, if the level of calcium is raised to induce stratification, involucrin-positive cells are selectively expelled from the basal layer. This suggests that migration from the basal layer may be a consequence, not a cause, of terminal differentiation.

Changes in cell-surface properties during terminal differentiation might explain the observed sorting out of involucrin-positive and -negative cells. We have therefore investigated lectin binding to different cell layers in natural and cultured human epidermis. Some lectins bind only to nonbasal keratinocytes, and the binding of peanut agglutinin, in particular, shows interesting correlations with involucrin expression, both in stratified colonies and in monolayers.

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Abbreviations:
PNA: peanut agglutinin

Under optimal conditions, human epidermal keratinocytes in culture form stratified colonies that retain many of the properties of intact epidermis. Mitosis is confined to the basal layer [1], and when cells leave it, they undergo terminal differentiation. Migrating outward through the different cell layers, keratinocytes increase in size and protein content [2] and synthesize a range of new molecules. In the outermost layers, an insoluble envelope of cross-linked protein is formed beneath the plasma membrane, and destruction of the nucleus and cytoplasmic organelles begins.

Several properties of the keratinocyte culture system make it an attractive model for studying terminal differentiation. Cells at all stages in the differentiation pathway are present simultaneously in culture, and the temporal sequence of events has a spatial correlation in the migration of cells outward from the basal layer. One practical advantage is that large numbers of cells can be grown for biochemical analysis. Finally, since epidermal biopsies are easy to obtain, cell behavior in culture can be compared directly with the properties of the intact tissue.

This article reviews recent studies on terminal differentiation in which involucrin, a precursor of the cross-linked envelope, has been used as a marker for an early stage in the process. In addition, new information is presented on changes in lectin binding during keratinocyte differentiation, both in intact epidermis and in culture.

CELL CULTURE

Using the technique of Rheinwald and Green [1], keratinocytes from different species and a variety of body sites can be cultured successfully for many generations [3, 4]. The most common starting material, however, is newborn human foreskin epidermis. Cells are grown on a feeder layer of 3T3 mouse fibroblasts that have been lethally irradiated or treated with mitomycin c. The culture medium is Dulbecco's modified Eagle's medium supplemented with fetal calf serum, hydrocortisone [1], cholera toxin [5], and epidermal growth factor [6]. The concentration of fetal calf serum required for optimal growth can be reduced by adding insulin, transferrin, and triiodothyronine [7]. Under these conditions, epidermal cells give rise to