Proteolytic release of keratinocyte transglutaminase

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Human keratinocytes express a particulate transglutaminase that can be released from the membrane by limited proteolysis with trypsin or plasmin to yield a form that is ≈ 80 kDa. The enzyme from cultured cells was also releasable by endogenous proteolysis to yield a catalytically active fragment of ≈ 80 kDa. Endogenous release was strongly dependent upon temperature and Ca^{2+} concentration and was inhibited by iodoacetate, but not by leupeptin, antipain or phenylmethanesulphonyl fluoride. These phenomena raise the possibility of partial translocation of transglutaminase activity to the cytoplasm by proteolysis to which the enzyme is subject during terminal differentiation. In addition, hydrodynamic measurements showed that the endogenously released enzyme was monomeric in solution (79 kDa), whereas that solubilized by hydroxylamine without proteolysis appeared dimeric (190 kDa). The latter dimeric state may reflect either an altered conformation of the enzyme or post-translational modification beyond fatty acid esterification.

INTRODUCTION

Terminal differentiation in the epidermis results in a layer of non-living, biochemically inert, barrier cells (corneocytes) in which the organelles have been removed by digestive processes and the remaining proteins have been stabilized by cross-linking reactions (Green, 1979). It is evident that a proper balance of proteolysis and cross-linking in these cells is important for their proper maturation. Cross-linking of the major protein component, the keratins, occurs primarily through oxygen-mediated disulphide bonding upon permeabilization of the plasma membrane (Sun & Green, 1978). However, a substantial fraction of the remaining protein, insoluble even in strong denaturants, is extensively cross-linked by isopeptide bonding mediated by transglutaminase action, which is also stimulated by plasma-membrane permeabilization (Rice & Green, 1979). Among the substrates of the enzyme reported in cells of the epidermis or appendages are involucrin (Rice & Green, 1979), keratolinin (Zettergren *et al.*, 1984), several membrane-bound proteins of high molecular mass (Simon & Green, 1984), trichohyalin (Rothnagel & Rogers, 1986), polyamines (Piacentini *et al.*, 1988) and certain polypeptides, utilization of which may vary with the physiological state or treatment of the cells (Michel *et al.*, 1987; Nagae *et al.*, 1987). Most of the enzymically cross-linked protein is associated with envelope structures, which can be isolated from the periphery of mature epidermal cells in skin (Matoltsy & Balsamo, 1955) and in culture (Sun & Green, 1976). However, substantial transglutaminase mediated cross-linking occurs in the cytoplasm of inner root sheath and medullary cells of the hair follicle (Rothnagel & Rogers, 1986), and isopeptide bonding has been detected in the keratin fraction of epidermis as well (Abernathy *et al.*, 1978).

One approach to studying enzymic cross-linking during terminal differentiation has been to elucidate the structure of keratinocyte transglutaminase, thereby providing a biochemical basis for its interactions with

substrate proteins in the membrane or cytoplasm. This enzyme is found primarily anchored in the plasma membrane by esterified fatty acid and accounts for virtually all the particulate activity in cultured human epidermal cells (Chakravarty & Rice, 1989). However, a small fraction of it which is soluble and lacks the acylation plausibly could contribute to cytoplasmic isopeptide bonding. Moreover, the membrane-bound (particulate) transglutaminase becomes soluble to a small extent in cell extracts (Schmidt *et al.*, 1988; Rice *et al.*, 1988) and is readily solubilized by trypsin (Thacher & Rice, 1985), suggesting that release of significant activity could occur during the proteolytic remodelling phase of terminal differentiation. Thus clarifying the possible processing of this transglutaminase could help in evaluating the enzyme's participation in cytoplasmic cross-linking as well as its relation to other transglutaminase activities reported in the skin and appendages (for reviews, see Peterson & Wuepper, 1984; Rothnagel & Rogers, 1984). That such processing can occur *in vivo* is indicated by the observed serine-proteinase conversion of a 72 kDa to a 50 kDa form of transglutaminase during extraction of human callus (Negi *et al.*, 1985).

EXPERIMENTAL

Cell culture

Keratinocytes from normal epidermis were cultured in 10 cm-diameter dishes with support from feeder layers of lethally irradiated 3T3 cells by standard methods (Allen-Hoffman & Rheinwald, 1984) in a 3:1 mixture of Dulbecco-Vogt Eagle's and Ham's F-12 media supplemented with fetal-bovine serum (5%), cortisol (0.4 $\mu\text{g/ml}$), epidermal growth factor (10 ng/ml), adenine (0.18 mM), insulin (5 $\mu\text{g/ml}$), transferrin (5 $\mu\text{g/ml}$), 3,3',5-tri-iodothyronine (20 pM) and antibiotics. The cells were inoculated in the presence of cholera toxin (10 ng/ml) and held at confluence for 1 week before harvest. SCC-9 human squamous carcinoma cells (Rheinwald &

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Beckett, 1981) were cultured in the same fashion, except that epidermal growth factor and cholera toxin were omitted from the medium.

Proteolytic release

Frozen keratinocyte cultures were thawed, homogenized in 20 mM-Tris/HCl (pH 7.4)/1 mM-EDTA and centrifuged at high speed (100 000 *g* for 45 min). For measurement of released activity, aliquots of the pelleted (referred to as 'particulate') material, each corresponding to approx. $\frac{1}{10}$ of culture, were resuspended in this buffer, incubated under the conditions described for given experiments, again subjected to high-speed centrifugation and assayed in parallel with activity remaining in the pellets. The latter were solubilized before assay by stirring at 4 °C for 2 h in 20 mM-Tris/HCl (pH 7.4)/1 mM-EDTA/0.3 % Emulgen 911 (non-ionic detergent) and clarified by high-speed centrifugation. Release by endogenous proteolysis was generally performed in the presence of antipain and leupeptin (each 10 μ g/ml).

Human skin

Newborn-human foreskins (from which subcutaneous fat and vascular connective tissue had been removed by dissection) were stored frozen. The particulate fraction was prepared from thawing samples (usually a pool of three to ten) by homogenization in Tris buffer and high-speed centrifugation as described for cultured keratinocytes. Aliquots corresponding to one to five foreskins were resuspended in 2 ml of 20 mM-Tris buffer and treated with 1 % Emulgen for 2 h at 4 °C, 50 μ g of tosylphenylalanylchloromethane ('TPCK')-treated trypsin for 15 min at 23 °C or 0.3 unit of human plasmin for 15 min at 23 °C. The released material was then immunoprecipitated. In some cases an aliquot was resuspended in 1 M-hydroxylamine for 2 h at 23 °C and immunoprecipitated directly or subjected to gel filtration on a Bio-Gel P-6 column in Tris buffer before immunoprecipitation.

Immunoprecipitation

To the solubilized enzyme in 20 mM-Tris/HCl (pH 7.4)/0.2 M-NaCl/1 mM-EDTA/0.3 % Emulgen buffer were added typically 2 μ g of B.C1 monoclonal antibody (Thacher & Rice, 1985). After incubation at 4 °C for 2 h, the immune complexes were incubated with gentle agitation for 45 min at room temperature with 4 μ g of rabbit anti-mouse IgG (Cappel, Cochranville, PA, U.S.A.) and 2 mg of Protein A-Sepharose, recovered by centrifugation, rinsed three times in the immunoprecipitation buffer and subjected to SDS/10 %-(w/v)-polyacrylamide-gel electrophoresis (Laemmli, 1970). The gels were stained with Coomassie Blue or with silver (Wray *et al.*, 1981).

Gel filtration

The particulate material from one or two dishes was isolated by high-speed centrifugation, solubilized by extraction with non-ionic detergent [1 % Emulgen 911 in 0.02 M Tris (pH 7.4)/1 mM-EDTA buffer] for 2 h at 4 °C, neutral hydroxylamine (1 M for 2 h at 23 °C) or by endogenous proteolysis, and clarified by high-speed centrifugation. Each sample was then passed through a 15 ml column of Sephadex G-25 maintained in 50 mM-Tris/HCl (pH 7.5)/100 mM-NaCl/1 mM-EDTA/0.5 mM-dithioerythritol/0.1 % Emulgen. To the excluded

peak fraction (1 ml) was added 0.1 mg of bovine liver catalase, and the sample was applied to a column (1.5 cm $\times$ 42 cm) of Sepharose CL-4B in the above buffer (4 °C). For molecular-mass estimations, diffusion coefficients ($D_{20,w}$) of transglutaminase solubilized by hydroxylamine or endogenous release were estimated by gel filtration performed as described above. In this case, the buffer in the Sephadex G-25 and Sepharose CL-4B columns did not contain Emulgen. The Sepharose column (1.5 cm $\times$ 48 cm) was calibrated by using the following proteins [values for $D_{20,w}$ (10^{-7} cm²/s) in parentheses]: horse spleen ferritin (1.8), bovine liver catalase (4.5), bovine lactate dehydrogenase (5.1), bovine serum albumin (5.9) and horse myoglobin (11.3).

Sedimentation coefficients ($s_{20,w}$)

Samples passed through Sephadex G-25 without exposure to non-ionic detergent as described above were applied to linear 5–20 % (w/v) sucrose gradients (Martin & Ames, 1961) in 0.1 M-NaCl/0.05 M-Tris/HCl (pH 7.5)/1 mM-EDTA/1 mM-dithioerythritol. Samples (0.2 ml) contained bovine liver catalase ($s_{20,w}$ 11.3S), bovine lactate dehydrogenase (7.0S) and, in some cases, human haemoglobin (4.5S) in addition to hydroxylamine-solubilized or endogenously released transglutaminase. After centrifugation at 35 000 rev./min (SW 50.1 rotor) for 17 h at 4 °C, the 5 ml gradient tubes were punctured at the bottoms and 6-drop (approx. 0.2 ml) fractions were collected.

Calibration proteins

The diffusion and sedimentation coefficients employed for the various markers were obtained from the following sources: bovine serum albumin (Wagner & Sheraga, 1956); equine myoglobin and human haemoglobin (Svedberg & Petersen, 1940); lactate dehydrogenase (Markert & Appella, 1961); ferritin (Rothen, 1944); catalase (Sumner & Gralen, 1938; Sumner *et al.*, 1940).

Assays

Transglutaminase activity was measured by incorporation of [³H]putrescine into dimethylcasein (Means & Feeney, 1968) as previously described (Thacher & Rice, 1985). Catalase activity was assayed at 240 nm by consumption of H₂O₂, and lactate dehydrogenase at 340 nm by oxidation of NADH according to standard methods (Decker, 1977) with minor modification. Haemoglobin was localized by absorption at 407 nm and ferritin at 340 nm.

Materials

Human plasmin, human thrombin (1000 units/mg), tosyl-lysylchloromethane ('TLCK')-treated bovine pancreatic α -chymotrypsin (type VII), bovine liver catalase (type C-100), horse spleen ferritin (type I), bovine heart lactic dehydrogenase (type XV), bovine serum albumin (fraction V), dipeptidyl peptidase I ('cathepsin C'), cathepsin D, calpain, equine myoglobin, rabbit muscle phosphorylase *a*, phenylmethanesulphonyl fluoride and soybean trypsin inhibitor were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Tosylphenylalanylchloromethane-treated-trypsin was purchased from the Worthington Biochemical Co. (Freehold, NJ, U.S.A.), and human haemoglobin was from Calbiochem (La Jolla, CA, U.S.A.). Proteinase inhibitor EP475 was kindly provided by Dr. Paul Billings (Harvard School of

Public Health, Boston, MA, U.S.A.). *Bacillus thuringiensis* phosphatidylinositol-specific phospholipase C was generously given by Dr. Martin G. Low (Department of Physiology and Cellular Biophysics, Columbia University, New York, NY, U.S.A.).

RESULTS

The aqueous solubility and molecular mass of keratinocyte transglutaminase in human skin were examined by immunoprecipitation with the specific monoclonal antibody B.C1 (Thacher & Rice, 1985). Initial experiments showed that the soluble fraction of crude buffer homo-

Fig. 1. Proteolytic release of keratinocyte transglutaminase from human skin

In addition to the transglutaminase which was specifically immunoprecipitated (indicated by arrows), samples contained IgG heavy chain (50 kDa), which served as an internal size marker. Aliquots of the particulate fraction of skin (five foreskins/lane) were treated with human plasmin (d) or tosylphenylalanylchloromethane-treated trypsin (e), immunoprecipitated and stained with silver. Control immunoprecipitates without added B.C1 monoclonal antibody exhibited goat IgG and two non-specifically precipitating bands of high molecular mass (c). Immunoprecipitates of transglutaminase solubilized from cultured cell particulates by the non-ionic detergent Emulgen (a) or mild trypsin treatment (b) are shown for reference (Chakravarty & Rice, 1989).

genes did not contain transglutaminase detectable in this fashion. However, as illustrated in Figs. 1(d) and 1(e), immunoprecipitable transglutaminase was clearly demonstrable in the soluble fraction after treatment of particulate material with trypsin or human plasmin. The released enzyme had a molecular mass of ≈ 80 kDa and matched in mobility that previously observed to be released from cultured keratinocytes (Chakravarty & Rice, 1989) and shown in Fig. 1(b). In these experiments, several bands of protein from the skin samples precipitated non-specifically (Fig. 1c). Thus, although Emulgen extraction or hydroxylamine treatment were found by this technique to solubilize a transglutaminase matching in mobility that from cultured keratinocyte particulates treated in the same fashion (92 kDa), the detection was often hampered by non-specifically precipitating protein of slightly lower mobility (result not shown). These observations are compatible with transglutaminase anchorage within 10 kDa of a proteinase-sensitive site *in vivo* as well as in cultured epidermal cells.

Further characterization of proteolytic release of the transglutaminase was performed by using cultured cells to provide larger amounts of enzyme and to avoid the contamination arising presumably from residual dermis. First, the susceptibility to release by plasmin was tested. In several experiments, 0.1 unit of plasmin activity solubilized the transglutaminase to an extent and with a time course equivalent to 1 μ g of trypsin. As shown in Fig. 2(c), the released transglutaminase exhibited a mobility in SDS/polyacrylamide gels equal to that of the trypsin-released form (Fig. 2e) and greater than that of the native (Emulgen-solubilized) form (Figs. 1a and 1d). By contrast, thrombin [up to 0.6 NIH (National Institutes of Health) units] and α -chymotrypsin (5 μ g) were inactive in several experiments conducted in parallel with trypsin, where 5 μ g of the latter released most of the particulate activity in 15 min at 37 °C. Under these conditions, dipeptidyl peptidase I (0.16 and 0.47 unit, pH 5) and cathepsin D (0.1 and 0.35 unit, pH 4 and 5) or *B. thuringiensis* phosphatidylinositol-specific phospholipase C at various concentrations neither released transglutaminase activity nor solubilized immunoprecipitable

Fig. 2. Proteolytic release of keratinocyte transglutaminase from cultured epidermal-cell particulates

Lanes (a)–(c) and (d)–(h) illustrate the results from two independent experiments. In addition to the 92 and 80 kDa bands of the enzyme (indicated by arrows), bands of IgG heavy chain (50 kDa) with greater mobility are visible. The particulate transglutaminase was solubilized by brief treatment with plasmin (c) or trypsin (e), by the non-ionic detergent Emulgen (d) or by endogenous proteolysis (90 min at 37 °C) in the presence of antipain and leupeptin (10 μ g/ml each) in Tris/HCl buffer (f) or Tris buffer containing either 1 mM-EDTA (g) or 1 mM- CaCl_2 (h). For reference, lane (a) is a mixture of native (Emulgen-solubilized) and proteolytically released forms, and lane (b) is a control immunoprecipitate of Emulgen-extracted particulate material lacking B.C1 monoclonal antibody. In the experiments illustrated, 1–2 ml suspensions of particulate material (from the number of cultures given in parentheses) were treated with 0.3 % Emulgen (one), 25 μ g of trypsin for 15 min at 23 °C followed by 75 μ g of soybean trypsin inhibitor (one), 0.2 units of plasmin for 15 min at 23 °C (eight) or buffer with 1 mM-EDTA, 1 mM- Ca^{2+} or no additives (six each) before immunoprecipitation.

enzyme. In addition, treatment with calpain (0.01–1 unit) in the presence of 5 mM- CaCl_2 at 30 °C for 30 min did not release measurable transglutaminase activity.

In such experiments a small but significant fraction of the insoluble transglutaminase from cultured keratinocytes reproducibly became soluble upon incubation of the resuspended particulate fraction at neutral pH without added proteinases. (In occasional experiments this was even evident in Emulgen-extracted samples, as seen in Fig. 1a.) The extent of enzyme release into the supernatant was strongly temperature-dependent, as illustrated in Fig. 3. Thus approx. 10% of the total activity became soluble in 90 min at 37 °C, or twice as much as at 23 °C (5%) and 10-fold that at 0 °C (1%). The rate of release was linear with time for 80–90 min at 23 or 37 °C, after which little increase in total activity was noted (results not shown). In several experiments, inclusion of 1 mM-EDTA in the incubation buffer decreased the degree of release by half, whereas addition of 1 mM- CaCl_2 instead increased the enzyme release by 2–4-fold. Investigation of this phenomenon has focused on the normal cells, but it was clear in this work that cultures of SCC-9 squamous carcinoma cells displayed transglutaminase release to the same extent.

Inclusion of a variety of proteinase inhibitors such

Fig. 3. Temperature-dependence of endogenous release

Isolated particulate material from cultures of normal human epidermal (▨) or SCC-9 cells (▤) were incubated at the temperatures indicated for 90 min. After clarification by high-speed centrifugation, the transglutaminase remaining in the particulate material was extracted with non-ionic detergent and assayed in parallel with the endogenously released enzyme to calculate the percentage released. Illustrated are the mean and range for two trials with normal epidermal cells (hEpC) and one trial with SCC-9. The results are representative of six experiments with the former and three with the latter. An activity of 100% represents the incorporation of 4–10 nmol of putrescine/h.

Fig. 4. Gel filtration of keratinocyte transglutaminase

Particulate material from approximately half a culture was extracted for 2 h with (a) 1% Emulgen at 4 °C or (b) 1 M-hydroxylamine at 23 °C or was incubated (c) for 90 min at 23 °C upon resuspension in 0.1 M-Tris buffer (pH 7.4). After clarification by high-speed centrifugation and passage through Sephadex G-25, the samples were applied to Sepharose CL-4B maintained in 0.1% Emulgen. The elution position of catalase added as an internal marker in each sample is indicated by the vertical arrow. Aliquots (0.1–0.2 ml) of column fractions were assayed for transglutaminase activity.

as antipain and leupeptin (10 µg/ml each) or EP475 (10 µg/ml) had no effect on the endogenous release of transglutaminase activity from particulate material. Nevertheless, to determine more definitively whether the release involved proteolysis, the transglutaminase solubilized in several ways was immunoprecipitated and compared by SDS/polyacrylamide-gel electrophoresis. For reference, Figs. 2(d) and 2(e) show the mobility difference of transglutaminase solubilized by Emulgen non-ionic detergent (92 kDa) or by mild trypsin treatment of particulates (80 kDa) as previously demonstrated (Chakravarty & Rice, 1989). Upon endogenous release without added EDTA or CaCl_2 , the enzyme showed similar amounts of protein of each mobility (Fig. 2f). However, inclusion of Ca^{2+} in the buffer led almost completely to release of the form with greater mobility (Fig. 2h). In the presence of EDTA, the little transglutaminase that was solubilized migrated with the slower mobility and much less of the lower-molecular-mass form was evident (Fig. 2g). Inclusion of 10 mM-iodoacetate [but not 10 mM-iodoacetamide or phenylmethane-

Fig. 5. Estimation of diffusion and sedimentation coefficients of keratinocyte transglutaminase

The enzymes released by hydroxylamine (H) or endogenous proteolysis (EP) were submitted to Sepharose CL-4B gel filtration (a) or sucrose-gradient sedimentation (b). The closed circles (●) represent the positions of internal standard proteins normalized to the top (0) and bottom (1.0) of the gradient.

sulphonyl fluoride (0.3 mg/ml)] also prevented appearance of the latter form (results not shown).

The hydrodynamic properties of the transglutaminase released by endogenous proteolysis were examined in comparison with that solubilized by hydroxylamine. In gel-filtration studies, as shown in Fig. 4(c), the proteolytically released enzyme was eluted from Sepharose 4B considerably later than the internal marker catalase. The native enzyme solubilized by Emulgen extraction, by contrast, was eluted considerably ahead of catalase (Fig. 4a), whereas that solubilized with hydroxylamine was eluted slightly earlier than the internal marker (Fig. 4b). These samples were chromatographed in the presence of non-ionic detergent to maintain the solubility of the Emulgen-extracted enzyme. However, preliminary experiments with the same column showed that the elution positions of the hydroxylamine and proteolytically released forms were not altered by omission of the detergent, suggesting that they bound relatively little Emulgen. When the elution positions were compared with those of standard proteins of known diffusion coefficients in a second column maintained in the absence of the detergent, values for $D_{20,w}$ of 4.1×10^{-7} and 6.4×10^{-7} cm²/s were obtained for the hydroxylamine and proteolytically released forms respectively, as shown in Fig. 5(a).

Sedimentation coefficients for these two forms of transglutaminase were also determined in sucrose gradients. As indicated in Fig. 5(b), the proteolytically released form sedimented considerably more slowly than the hydroxylamine released form, with respective values

of 5.5 S and 8.6 S obtained by comparison with known proteins as internal standards. Assuming partial specific volumes ($\bar{v}$) of 0.735 and solution densities (ρ) of 1 g/cm³, these sedimentation ($s_{20,w}$) and diffusion ($D_{20,w}$) coefficients were substituted into the Svedberg relationship:

$$s_{20,w} = \frac{M_r(1 - \bar{v}\rho)}{RT}$$

to calculate M_r values (Siegel & Monty, 1966). Thus the proteolytically and hydroxylamine released enzymes displayed calculated M_r values of 79000 and 190000 respectively. Since the latter corresponds to a dimer, the possibility of a disulphide dimerization as a consequence of the hydroxylamine treatment was investigated. However, unlike human tissue factor (Bach *et al.*, 1988), only monomers and no evidence of dimers were obtained by SDS/polyacrylamide-gel electrophoresis of this released form of the enzyme when the reducing agent was omitted during sample preparation.

DISCUSSION

Previous studies of keratinocyte transglutaminase in cultured cells (Thacher & Rice, 1985; Chakravarty & Rice, 1989) have established that the particulate form can be solubilized by treatment with non-ionic detergent or hydroxylamine or by removal of an anchorage region (≈ 10 kDa) upon limited trypsin treatment. The present results indicate this enzyme is present in epidermis, as indicated recently by Thacher (1989), and is proteolytically releasable similarly to that in culture. Although the differentiation programme in cultured keratinocytes resembles closely that in the natural epidermis (Green, 1979), this result is important in view of discrepancies in expression of certain markers (e.g. keratins, filaggrin) noted in epidermis as against epidermal cells in culture.

Proteolytic activities play important roles in the epidermis, not only in extracellular but also in intracellular function, such as filaggrin processing (Resing *et al.*, 1989). In the present work it is clear that keratinocyte transglutaminase can be solubilized by proteolytic activity to which it is likely to be exposed during terminal differentiation in the epidermis. For example, plasmin from the circulation appears responsible for much of the digestion of cellular organelles in the permeabilized mature keratinocyte (Green, 1977) and could simultaneously release substantial transglutaminase. Moreover, permeabilization of the plasma membrane and the consequent increase in cytosolic Ca²⁺, which leads to cross-linked envelope formation (Rice & Green, 1979), could also activate intracellular Ca²⁺-dependent proteolysis. The activity detected in the particulate fraction in these experiments has been termed 'endogenous', since none was added. This activity is distinct from plasmin, since it (unlike the latter) was not inhibited by phenylmethanesulphonyl fluoride. Several plausible intracellular enzymes appeared not to release the transglutaminase. For instance, cathepsin D has been reported to increase the activity of the purified soluble transglutaminase from callus (Negi *et al.*, 1981), but under present conditions did not elicit release of the particulate enzyme. Similarly, the Ca²⁺-dependent intracellular regulatory proteinase calpain (Bond & Butler, 1987) also was ineffective.

Release by endogenous proteolysis resulted in a soluble

monomeric transglutaminase. The observed values for its sedimentation and diffusion coefficients were quite similar to those obtained previously for the soluble form of native keratinocyte transglutaminase, which was also found to be monomeric (Thacher & Rice, 1985). The latter has a molecular mass of 92 kDa but lacks the fatty acid acylation present in the particulate form (Chakravarty & Rice, 1989). The possibility that a small fraction of the endogenously released material in the present experiments could have been solubilized by enzymic deacylation is not excluded, but if so, it was much less than that due to proteolysis, or it was rapidly converted into the 80 kDa form.

By contrast, although hydroxylamine deacylates and thereby releases the particulate form, the hydrodynamic properties of the enzyme solubilized in this manner indicated it behaves as a dimer. A conformational change arising from its exposure to, and then removal from, the membrane environment is possible, by analogy to the membrane trigger phenomenon (Wickner, 1979). Alternatively, this finding may reflect post-translational modification beyond esterified (or thioesterified) fatty acid. For example, some fatty acid might be *N*-linked and thus resistant to hydroxylamine treatment. Also, the possibility cannot be dismissed of chemical modification of the protein by hydroxylamine if thioesters of cysteine and acidic amino acid residues occur, as reported for apolipoprotein B (Huang *et al.*, 1988). Moreover, the anchorage region is subject to phosphorylation at multiple sites (R. Chakravarty, X. Rong & R. H. Rice, unpublished work), which could conceivably result in a conformational change inducing dimerization. Identification of the proteolytically released anchorage region has not been possible with the B.C1 antibody, in part because this monoclonal antibody was originally raised against the 80 kDa trypsin-solubilized form (Thacher & Rice, 1985). In addition, however, cleavage of the 10 kDa anchorage region into at least three peptides is evident upon trypsin digestion of phosphate- and fatty acid-labelled transglutaminase (R. Chakravarty, X. Rong & R. H. Rice, unpublished work).

Keratinocyte transglutaminase, by far the most of which ordinarily is membrane-bound, is responsible for the formation of cross-linked envelopes at the cell periphery upon its activation by Ca^{2+} during terminal differentiation (Rice & Green, 1979; Simon & Green, 1985; Thacher & Rice, 1985). Participation of the solubilized particulate transglutaminase in envelope formation (Thacher, 1989) and in stabilizing internal structures during terminal differentiation has not been established, but appears likely. For example, the proteolytic-release process could be primarily responsible for effecting the isopeptide bonding which has been reported in the keratin fraction of callus extracts (Abernathy *et al.*, 1978). In addition, cross-linking in this fashion probably accounts for the persistence of involucrin in the cytoplasm of squames of the stratum corneum (Warhol *et al.*, 1985). Indeed, the possibility merits consideration that involucrin, as a transglutaminase substrate, may provide greater rigidity or cohesiveness to the cytoplasm of the mature cells, analogous to trichohyalin in the inner-root-sheath and medulla cells of the hair follicle (Rothnagel & Rogers, 1986). Detection of keratinocyte transglutaminase immunoreactivity in the inner-root-sheath cells (Parenteau *et al.*, 1986) raises the possibility of its participation in trichohyalin cross-linking. Although

keratinocytes are capable of synthesizing the soluble tissue transglutaminase in culture (Lichti *et al.*, 1985; Thacher & Rice, 1985; Rubin & Rice, 1986), this enzyme is not likely to play such a role *in vivo*, since it appears not to be expressed in the epidermis in the absence of exogenous retinoic acid (Lichti & Yuspa, 1988).

A peripheral-membrane transglutaminase from rat chondrosarcoma tissue reportedly can be decreased in molecular mass from an estimated 95 kDa to 50 kDa by thrombin treatment, prompting speculation that the 50 kDa transglutaminase observed in human callus, cow snout and hair follicle could arise by proteolytic processing of a similarly large form (Chung *et al.*, 1988; Martinet *et al.*, 1988). Supportive of this reasoning, the soluble 50 kDa form initially identified in human callus is now known to arise from a larger 72 kDa form which is also soluble (Negi *et al.*, 1985). A correspondence between the ≈ 80 kDa form we have observed with the soluble ≈ 72 kDa precursor of the 50 kDa form is not ruled out by our data. However, a recent preliminary account indicates the soluble form isolated from skin, which is a zymogen (≈ 77 kDa) and is not synthesized in cultured keratinocytes, is indeed distinct (Park *et al.*, 1988).

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