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ISOLATION, PURIFICATION AND CHARACTERIZATION OF BOVINE EPIDERMAL TRANSGLUTAMINASE *

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Summary

A crosslinking enzyme, epidermal transglutaminase, was isolated from soluble proteins of glabrous cow snout epidermis. This enzyme stabilized fibrin clots rendering them insoluble in 2% acetic acid. It also catalyzed the incorporation of the fluorescent amine, dansyl cadaverine, into casein. Epidermal transglutaminase was purified by chromatography upon DEAE-Sephadex A-50, zone electrophoresis in Pevikon, and Sephadex G-200 gel permeation chromatography. The highly purified substance, which had a specific activity of 3267 amine-incorporating units/mg per h and a molecular weight of 55 000, behaved as a single molecular species in the analytical ultracentrifuge. It had a sedimentation coefficient of 4.4 S and migrated as a γ-globulin at pH 8.6; it displayed anomalous migration in polyacrylamide gels containing sodium dodecyl sulfate.

The enzyme was dependent upon free calcium ions and a reduced sulfhydryl group for activity. The apparent K_m for dansyl cadaverine was $1.2 \cdot 10^{-4}$ at pH 7.5.

Monospecific antiserum to bovine epidermal transglutaminase precipitated with the enzyme in agar. The antiserum prevented fibrin crosslinking but enhanced incorporation of dansyl cadaverine into casein by the enzyme.

The epidermal enzyme differed biochemically and immunochemically from bovine plasma transglutaminase (Factor XIII).

Introduction

Covalent intermolecular crosslinks serve to stabilize many structural proteins and biopolymers. The identification of ϵ-(γ-glutamyl) lysine dipeptides in

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native wool keratin by Asquith et al. [1] and confirmation of this work by others [2,3] implied that this mode of crosslinking may stabilize structural hair proteins. Support for this hypothesis was gained when Harding and Rogers [4] and Chung and Folk [5,6] reported the isolation of a transglutaminase which catalyzed the formation of inter-chain γ-glytamyl bonds from guinea pig hair bulbs. The hair follicle transglutaminase differed in many respects from plasma or liver transglutaminases and appeared unique to these appendages [7].

Recently, transglutaminases were partially isolated from extracts of non-hairy epidermis [8—12], and shown to crosslink fibrin by γ-γ dimers [11]. This suggested that transglutaminases may subserve a general crosslinking role in keratinization of hair and epidermis. In order to elucidate the function of transglutaminases in epidermal tissues and, further, to localize these enzymes within epidermal cells, we have undertaken an analysis of the transglutaminases of bovine epidermis. We now describe the isolation, purification and characterization of an epidermal transglutaminase from bovine snout epidermis.

Materials and Methods

Monodansyl cadaverine (Cyclo Chemical Company, Los Angeles, Calif.) was dissolved to a concentration of 0.002 M in 0.15 M sodium chloride containing 0.05 M Tris, pH 7.5 (Tris-buffered saline). Purity was confirmed by thin-layer chromatography.

Hammersten casein (Schwartz-Mann, Orangeburg, N.Y.), prepared as a 0.2% solution, was dialyzed against Tris-buffered saline to remove low molecular weight contaminants, clarified by high-speed centrifugation, and stored at —20° C until use.

Human fibrinogen (95% clottable, Lot # 65292, Kabi Products, Stockholm, Sweden) was dissolved at a concentration of 0.2% in 0.15 M ammonium acetate, pH 7.0. Following overnight dialysis at 4° C, during which an initial cold-insoluble fraction precipitated, the solution was clarified by centrifugation and stored at —20° C.

Coagulation Factor XIII was partially purified from fresh bovine plasma according to the method of Loewy [14] by a series of precipitations in 16—20% saturated ammonium sulfate followed by heating at 56° C and filtration to remove fibrinogen.

Bovine thrombin was purchased from Parke-Davis (Detroit, Mich.) and freshly prepared to contain 50 NIH units/ml in 0.15 M KCl 0.1 M calcium chloride.

Behring Diagnostics, Woodbury, N.J., supplied rabbit antisera to whole bovine serum proteins and human Factor XIII-A; Hyland Laboratories (Oakland, Calif.) provided agar diffusion plates.

Dithiothreitol and *p*-chloromercuribenzoate were obtained from Calbiochem, San Diego, Calif. The latter was further purified before use [13].

Pharmacia, Uppsala, Sweden, produced the diethylaminoethyl (DEAE) Sephadex A-50, Sephadex G-200, and AH-Sepharose 4B used in these experiments. Reagents for acrylamide gel electrophoresis (acrylamide, methylene bisacrylamide, *N,N,N',N'*-tetramethylethylene diamine, ammonium persulfate)

were obtained from Eastman Organic Chemicals, Rochester, N.Y.

All other chemicals and reagents were the finest grade available.

Enzyme Assays

The qualitative assay described for Factor XIII by Loewy et al. [14] provided a model for the clot solubility test for the epidermal enzyme. To 0.5 ml fibrinogen substrate, 50 or 100 μl of enzyme or buffer and 10 μl bovine thrombin (to initiate clotting) were added; the mixture was incubated at room temperature for 60 min. Addition of 2.0 ml of 2% acetic acid stopped the reaction, and the formed clot was gently loosened from the sides of the tube with a thin spatula. Clot solubility was graded visually at 1 and 24 hours as soluble, partially soluble, or insoluble.

Partially purified Factor XIII, preactivated with thrombin for 20 min at 37°C, served as positive control. Negative controls contained Tris-buffered saline in place of enzyme.

The dansyl cadaverine assay was patterned according to Lorand and Gotoh [15]. Casein solution 0.5 ml was combined with 0.25 ml dansyl cadaverine solution and 0.25 ml enzyme or buffer in the presence of 1 mM ethylenediamine tetraacetic acid (EDTA), 10 mM calcium chloride, and 2 mM dithiothreitol, and incubated at 37°C for 60 min. Initial (and final) pH of the reaction mixture was 7.5. Reaction products were precipitated with an equal volume of 10% trichloroacetic acid and washed with 5 changes of 5 ml ethanol/ether (v/v). The precipitate was dried overnight and solubilized in 2 ml of 0.5 M Tris, pH 8.0 containing 8 M urea and 0.5% (w/v) sodium dodecyl sulfate.

The extent of dansyl cadaverine incorporation into casein was then read as "relative fluorescence" against a reagent blank containing all components of the reaction precipitated with trichloroacetic acid at time 0, on an Aminco-Bowman spectrophotofluorometer with excitation and emission wavelengths at 355 nm and 525 nm respectively.

Specific activity, calculated at each step of protein purification, was expressed as amine incorporating units per mg of protein per hour (AIU/mg per h). The following formula was employed:

$$\text{AIU} = F_i - F_b / F_r - F_s$$

F_i, Fluorescence of test sample after 60 min of incubation; F_b, Fluorescence of blank containing all components of the test sample at time 0; F_r, Fluorescence of reference standard of dansyl cadaverine, 1 μM in Tris/urea/sodium dodecyl sulfate solvent; F_s, Fluorescence of Tris/urea/sodium dodecyl sulfate solvent alone.

Protein was measured by the Lowry et al. method with crystalline bovine albumin (Schwartz-Mann, Orangeburg, N.Y.) as standard [16].

Purification Procedures

A Storz keratotome set at 0.2 mm was used to remove epidermis from 50 to 70 cow snouts obtained from a local abbatoir immediately after slaughter. After snap-freezing in liquid nitrogen and pulverization in a stainless steel

cylinder, the epidermal powder was ground in a Polytron (Brinkman Instruments, Westburg, N.Y.) with 100 ml cold 0.05 M Tris buffer containing 0.001 M EDTA, pH 8.0. (starting buffer). Centrifugation at 20 000 rev./min for 1 h in a Sorvall RC2-B refrigerated centrifuge, followed by dialysis overnight completed preparation of a crude epidermal extract. This and all subsequent steps were carried out at 4° C.

In preparation for Anion Exchange Chromatography (Step I), hydrated DEAE Sephadex A-50 beads were placed in a 2.5 × 60 cm column. A Varioperpex pump (LKB Instruments, Rockville, Md.) maintained constant flow rate of 40 ml/h thereafter. The sample (approx. 70—100 ml) was layered onto the exchange medium, and washed with 1000 ml wash of 0.025 M Tris/0.001 M EDTA, pH 8.0. The column was then developed with a 600 ml linear gradient to 0.3 M NaCl in the Tris buffer.

The conductivity of fractions eluting from the column was monitored with a Beckman conductivity bridge (Beckman Instruments, Cedar Grove, N.Y.); protein content was expressed as absorbance at 280 nm in a Zeiss PMQ II spectrophotometer with a 1 cm light path.

Step II of purification was Zone Electrophoresis in the supporting medium Pevikon (Mercer Chemical Company, New York, N.Y.) as described previously [17]. The sample (5—6 ml in 0.05 M Tris/0.001 M. EDTA, pH 7.6) was introduced into a 2 mm wide slit at the origin of a 20 × 40 × 2 cm block and subjected to electrophoresis at 10 V/cm for 18—20 h at 4° C. 1-cm segments of the bed were cut and proteins eluted by aspiration.

Gel permeation chromatography [18] was performed on a 0.9 × 90 cm Sephadex G-200 column equilibrated with Tris-buffered saline, pH 7.5, which had been previously calibrated with blue dextran, human IgG globulin, bovine albumin, ovalbumin, cytochrome *c* (horse heart) and bacitracin. The 1.0 ml sample was layered onto the gel bed in 10% sucrose, and flow was maintained at 4.5 ml/h with a Varioperpex pump.

Where necessary, protein solutions were concentrated by nitrogen pressure ultrafiltration in special chambers designed for this purpose (Amicon Corp., Lexington, Mass.) using a PM 30 membrane.

Characterization Studies

Highly purified enzyme 300 μg/ml in 0.1 M NaCl was studied by approach to sedimentation equilibrium in a Beckman Model E Analytical Ultracentrifuge equipped with interference optics. Centrifugation was carried out at 9284 rev./min for 72 h at 20° C. The data obtained were used to calculate molecular weight, assuming a partial specific volume of 0.74 ml/gm, according to the method of DiCamelli et al. [19].

Sedimentation velocity was determined in a Beckman Model E Analytical Ultracentrifuge with an AN-E rotor and 30 mm standard cell. Epidermal transglutaminase at a concentration of 3 mg/ml in Tris-buffered saline, centrifuged at 50 740 rev./min for 2 h at 20° C.

Sucrose gradient ultracentrifugation was performed in a Beckman Model L5-65 Ultracentrifuge with a SW-50 rotor (Beckman Instruments, Palo Alto, Calif.), at 45 000 rev./min for 16 h at 5° C. External and internal standard proteins (human IgG, bovine serum albumin, cytochrome *c*) were run simul-

taneously. Fractions were extracted dropwise from the bottom of the gradient tube (37% sucrose) and assayed for transglutaminase activity.

Acrylamide gel electrophoresis in sodium dodecyl sulfate followed a modification [20] of the method described by Shapiro et al. [21]. The purified enzyme was either dialyzed against 1% sodium dodecyl sulfate/1% β-mercaptoethanol or 1% sodium dodecyl sulfate/2 mM dithiothreitol/8 M urea at room temperature for 18 h or heated at 96°C for 3—5 min in 1% sodium dodecyl sulfate/1% β-mercaptoethanol/8 M urea prior to electrophoresis. Marker proteins were treated similarly.

Dependence of enzyme activity upon divalent cations was assessed by serially dialyzing the purified enzyme first against 1 mM EDTA or 1 mM ethyleneglycol bis(β-aminoethylether)-*N*,*N*'-tetraacetic acid (EGTA) and then against Tris-buffered saline. Standard dansyl cadaverine assays were performed with calcium concentration varying from 0 to 0.025 M.

In order to determine dependence of crosslinking on the presence of free sulfhydryl groups, purified enzyme was incubated with varying concentrations of *p*-chloromercuribenzoate, with or without 10 mM calcium, at room temperature for 120 min prior to dansyl cadaverine or fibrin crosslinking assays (dithiothreitol was not present in these studies).

A K_m value for dansyl cadaverine was obtained by varying the concentration of that substrate from 0 to 2 mM while holding constant all other components of the reaction.

Optimum pH for the enzyme was determined by separate adjustment of enzyme and substrate solutions to desired pH with dilute NaOH or HCl followed by combination of the two. Initial and final pH of the reaction was recorded; the range of pH studied was 5.5—10.0 (pH range of Tris buffer).

Immunochemical Studies

Goat immunization was carried out by subcutaneous injections of 200 μg highly purified epidermal transglutaminase emulsified in complete Freund's adjuvant. Two booster injections, each of 200 μg enzyme in incomplete Freund's adjuvant, were given at weekly intervals thereafter. Serum was obtained one week after the final injection.

Goat immune sera were precipitated with 33% cold neutral saturated ammonium sulfate. Following a 30 min equilibration period, the precipitate was removed by centrifugation and taken up in 1/5 the original serum volume of Tris-buffered saline, extensively dialyzed against the same, and stored at −20°C until used. Preimmunization (control) serum, was prepared in the same way.

For enzyme inhibition studies, goat anti-transglutaminase was diluted with preimmunization serum or Tris-buffered saline and incubated for 1 h at 4°C with enzyme prior to substrate addition. Rabbit anti-human Factor XIII-A was studied similarly.

Antibodies against epidermal transglutaminase were isolated by adsorption to a 0.4 × 5 cm column of purified epidermal transglutaminase insolubilized by carbodiimide coupling [22] to Sepharose 4B. Washing with 0.5 M NaCl removed contaminant protein; specific antibodies were then eluted with 3 M NaSCN and dialyzed into Tris-buffered saline.

Results

Fragments of bovine snout epidermis removed at 0.2 mm showed minimal dermal contamination on routine histologic examination. Typical sections contained 90% stratum malpighii, stratum granulosum, and keratinized epithelium.

After homogenization and centrifugation of the pulverized fragments, crosslinking activity resided entirely in the supernatant; time and concentration-dependent incorporation of dansyl cadaverine into casein was demonstrated. Although the pellet was tested for residual activity, none was found. Addition of thrombin to the supernatant did not increase crosslinking activity. Small (50 μl) aliquots of the supernatant were able to insolublize fibrin clots in concentration-dependent fashion. This reaction did not require addition of thrombin. Fibrin clots prepared with buffer instead of epidermal supernatant were uniformly soluble at 1 and 24 h.

The enzyme bound to DEAE Sephadex A-50 at pH 8.0 and low ionic strength. It was subsequently eluted with the linear NaCl gradient at approx. 14 $m\Omega^{-1}$, as shown in Fig. 1.

When pooled fractions containing crosslinking activity (fractions 130—145 in Fig. 1) were concentrated by ultrafiltration, all activity was recovered in the retentate. The second purification step, Pevikon electrophoresis, is depicted in Fig. 2. The enzyme migrated as a gamma globulin in this medium; all activity was found in a single peak 6 cm from the origin after 20 h. Examination of this peak by sodium dodecyl sulfate gel electrophoresis revealed three protein bands in fractions 2—7, which were then concentrated by ultrafiltration. The three

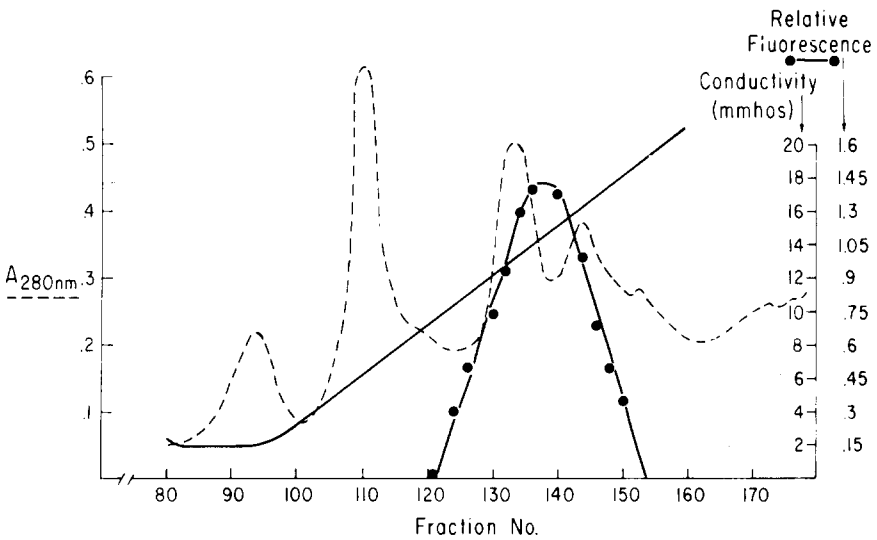

Fig. 1. Chromatography of bovine epidermal homogenate on DEAE Sephadex A-50 in Tris-EDTA buffer at pH 8.0. Epidermal transglutaminase, identified by the dansyl cadaverine assay, bound to the column at low ionic strength and eluted with the salt gradient. Fractions 130 through 145 were combined for the next step in purification.

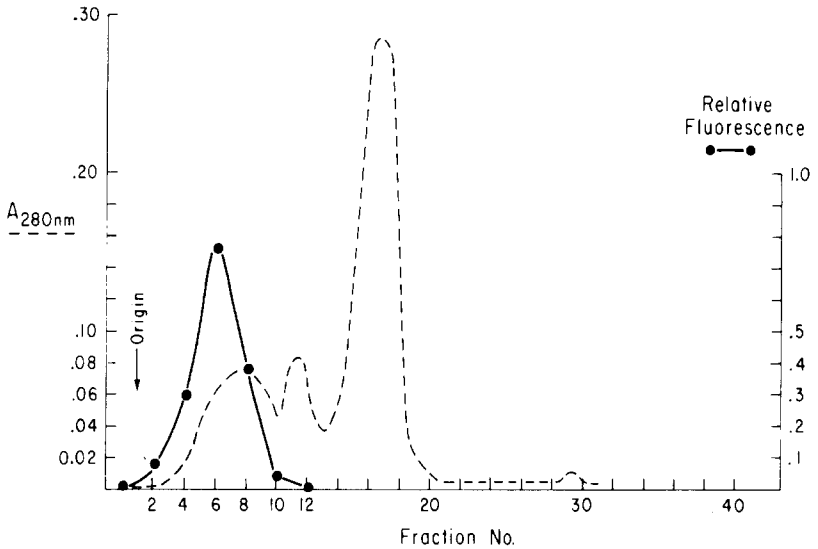

Fig. 2. Electrophoresis of partially purified epidermal transglutaminase in Pevikon in Tris-EDTA buffer at pH 8.6. After 20 h at 10 V/cm, enzyme is found as a single peak 6 cm from the origin. Fractions 2—7 were combined for the next purification step.

proteins were subsequently resolved into three separate peaks by Sephadex G-200 gel filtration (Fig. 3). Transglutaminase activity was symmetric about the third peak, which corresponded to a mol. wt. of 54 600 (mean of 6 determinations) when plotted on a graph of elution volume vs. log mol. wt. of the standard proteins. Fractions 38—42 were concentrated and used for characterization studies.

A typical purification yielded 300—500 μg of enzyme with a specific activity of approx. 3000 AIU/mg and represented a 10—15% yield (Table I). The purified protein was stable at 4°C for a week and at —70°C for several months.

Preliminary experiments with partially purified epidermal transglutaminase showed a pH optimum of 8.8 and maximal activity at calcium concentrations greater than 2 mM; the purified protein exhibited the same properties. Enzyme relieved of associated metal ions by serial dialysis had a low level of crosslinking activity (although it had none in the presence of 1 mM EDTA or EGTA) and responded to addition of calcium by the increase in activity seen in Fig. 4. Magnesium did not substitute for calcium.

Rapid and complete inactivation of the enzyme was seen upon addition of *p*-chloromercuribenzoate to a solution containing 24 μg enzyme either in the presence or absence of calcium. Inactivation was linear to 0.01 mM concentration of inhibitor and complete with higher concentrations.

The apparent K_m for dansyl cadaverine substrate was $1.2 \cdot 10^{-4}$ at pH 7.5 when casein substrate was in excess, and the assay medium contained 7 mM $CaCl_2$, 1 mM EDTA and 1 mM dithiothreitol.

The enzyme behaved as a single homogeneous species in the analytical ultracentrifuge (Fig. 5). A mol. wt. of 55 800 was calculated from sedimentation equilibrium data, in close agreement with results from Sephadex G-200 (54 600).

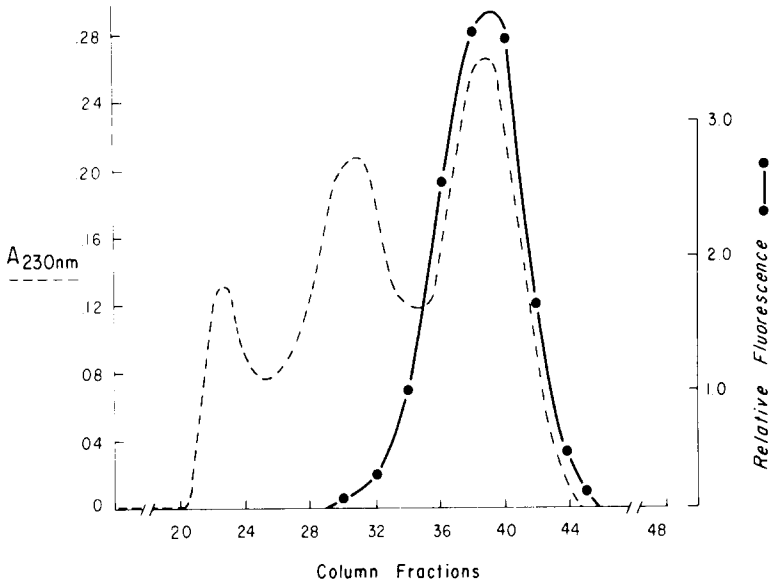

Fig. 3. Gel filtration of partially purified epidermal transglutaminase on Sephadex G-200 in Tris-buffered saline, pH 7.5. Each fraction contained 2.1 ml. Enzyme activity is symmetric about a peak of protein eluting at a position equivalent to a molecular weight of 54 600 when compared with marker proteins of known molecular weight. Fractions 38—42 gave a single protein band on sodium dodecyl sulfate gel electrophoresis and were used as purified enzyme.

Sedimentation velocity of epidermal transglutaminase in the analytical ultracentrifuge was 4.4 S, consistent with a protein of mol. wt. 50 000—60 000.

As shown in Fig. 6, the enzyme traveled into the gradient of sucrose 10—37% as a single homogeneous activity peak. Based on known sedimentation rates for marker substances (see legend to Fig. 6), epidermal transglutaminase was observed to sediment at 4.0 S. Assuming a partial specific volume of 0.73, the molecular weight of the enzyme was calculated by the method of Martin and Ames [23] and determined to be 53 200.

Sodium dodecyl sulfate gel electrophoresis of the purified protein yielded a single stainable band of protein at a mol. wt. of 110 000 when compared with

TABLE I

PURIFICATION BOVINE EPIDERMAL TRANSGLUTAMINASE

Step	Volume (ml)	Protein (mg)	AIU *	Specific activity (AIU/mg)	Purification	%Yield
Crude homogenate (Supernatant)	70	854	7980	9.3	1	100
DEAE sephadex A-50	4.6	62.1	3100	49.9	5.4	38.9
Pevikon	1.0	2.5	1800	720	77	22
Sephadex G-200	1.2	0.36	1176	3267	351	14.7

* AIU, Amine-incorporating units. Refer to text for definition.

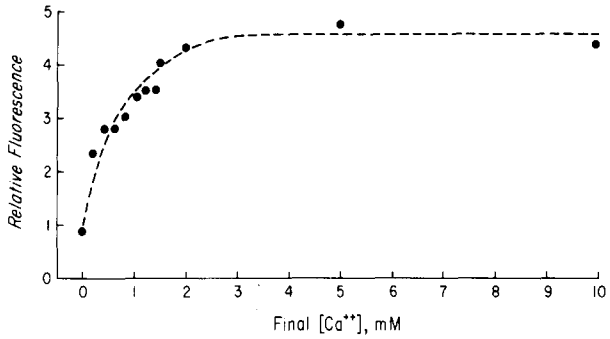

Fig. 4. Concentration-dependent activation of epidermal transglutaminase by calcium. The purified enzyme had previously been dialyzed serially against EDTA and Tris-buffered saline to remove associated calcium ions.

standard proteins. Identical results were obtained with enzyme from several different purifications, and with the enzyme previously run in the analytical ultracentrifuge either in sucrose density centrifugation or sedimentation equilibrium studies. Addition of 6 M urea to electrophoresis gels resulted in dissociation of part or all of the high molecular weight material to a position of 55 000 on the gels. Marker proteins gave sharp single bands of protein in urea-containing gels, the bands occupied positions corresponding to the monometer protein; multimeter present on plain sodium dodecyl sulfate gels were absent.

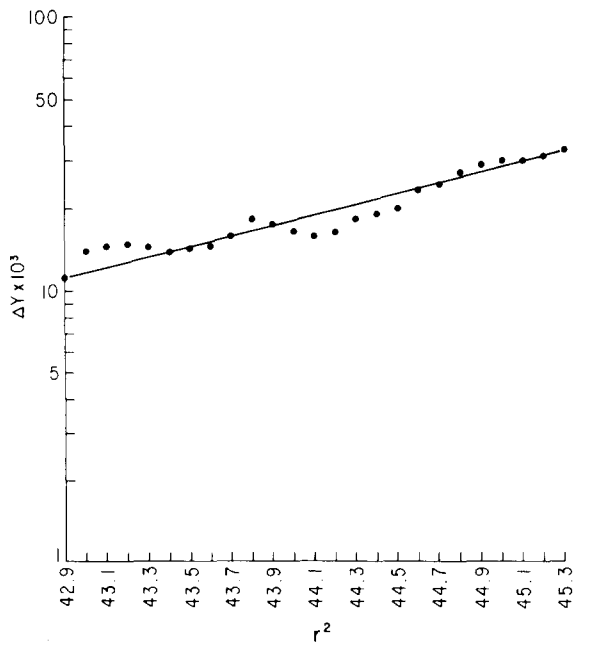

Fig. 5. Data from sedimentation equilibrium study plotted as lot of fringe deviation (ΔY) versus square of radius of rotation.

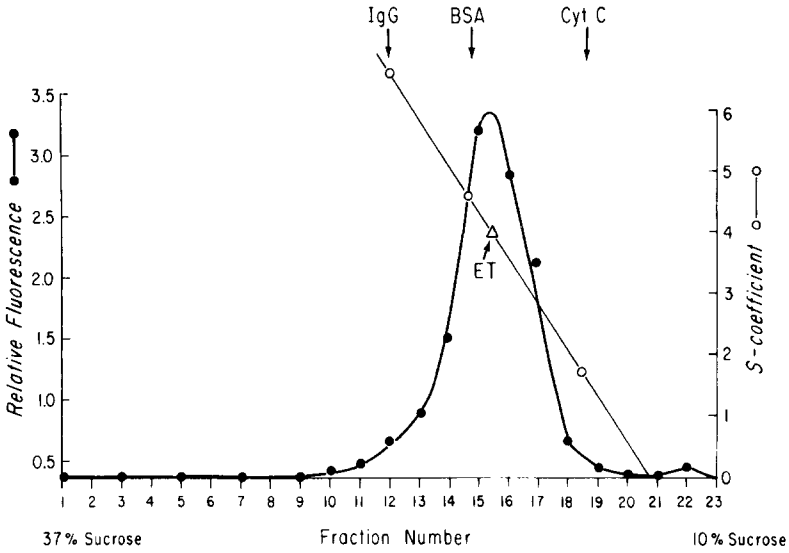

Fig. 6. Sucrose gradient ultracentrifugation of purified epidermal transglutaminase. 0.9 mg of enzyme in 0.2 ml. Tris-buffered saline was applied to the top of gradient and centrifuged as described in the text. Fractions of 0.2 ml squentially extracted from the bottom were assayed for enzyme activity. When compared to external marker proteins, epidermal transglutaminase had an *s*-coefficient of 4.0. Coefficients of markers proteins were as follows: IgG, 6.7 S; bovine serum albumin (BSA), 4.5 S, cytochrome *c*, 1.7 S.

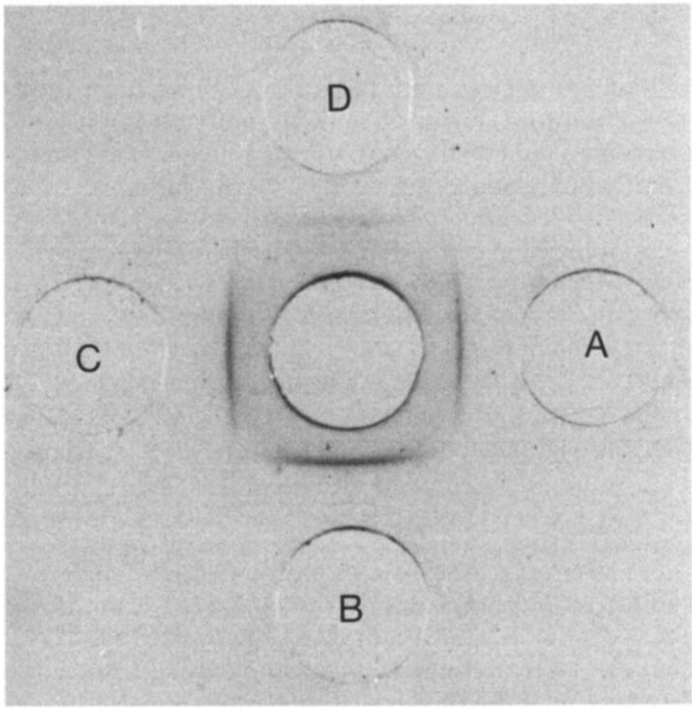

Fig. 7. Single precipitin line of identity produced by antiserum to epidermal transglutaminase (center well) diffused against the enzyme at various stages of purity. From R, clockwise, A-crude epidermal supernatant, B-concentrate from Sephadex A-50, C-concentrate from Pevikon electrophoresis, D-concentrate from G-200 gel filtration.

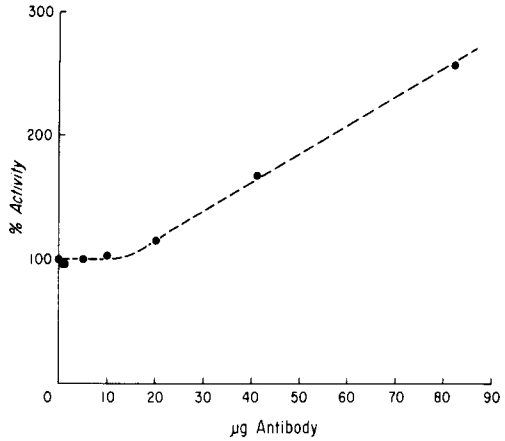

Fig. 8. Concentration-dependent activation of purified epidermal transglutaminase by antibodies to the enzyme purified by enzyme adsorption chromatography.

Agar diffusion of crude or highly purified epidermal enzyme against dilutions of antiserum to bovine serum proteins or Factor XIII-A yielded no precipitate. However, a single precipitin line was produced when samples of enzyme from each stage of purification were diffused against anti-transglutaminase serum (Fig. 7). Bovine Factor XIII did not precipitate in agar with antisera to epidermal enzyme, but did precipitate with anti-Factor XIII-A (Fig. 8). Thus, no antigenic cross-reactivity was observed between bovine epidermal and plasma transglutaminases.

Antiserum to epidermal transglutaminase inhibited the enzyme from crosslinking fibrin monomers. Inhibition was not seen when nonimmune goat serum was used in place of antiserum. Preincubation of purified enzyme with antiserum enhanced (rather than inhibited) incorporation of dansyl cadaverine into casein. This enhancement occurred whether antibody was diluted with Tris-buffered saline or with preimmunization serum. Bovine Factor XIII was neither enhanced nor inhibited by incubation with antiserum to epidermal transglutaminase. Specific antibodies to transglutaminase, isolated by enzyme affinity to chromatography, retained their ability both to inhibit fibrin stabilization and to enhance dansyl cadaverine-casein crosslinking (Fig. 8) demonstrating that the enhancement phenomenon was associated with the antigen-antibody interaction and was not due to serum factors.

Discussion

Purification and characterization of a transglutaminase specific to epidermis constitutes an important step toward elucidating the role of transglutaminases in keratinization. Although a fibrin-stabilizing enzyme functionally similar to plasma Factor XIII is easily detectable in homogenates of epidermal tissues, the possibility that one is observing activity derived from plasma contamination could only be ruled out when the epidermal enzyme was isolated and its biophysical and immunologic attributes studied in detail.

The three-step purification scheme presented here has given reproducible

results in over fifteen separate purifications. Always, the first step results in a large apparent decrease in yield. The "crude enzyme" loses activity rapidly, even at 4°C, presumably due to proteolytic digestion by lysosomal enzymes released during tissue homogenization. The partially purified or purified enzyme is more durable; this is reflected by more predictable losses in steps 2 and 3.

Purified epidermal transglutaminase is a calcium and sulfhydryl-dependent enzyme, as shown by chelation and calcium addition studies, and by *p*-chloromercuribenzoate inhibition. Maximal activity was observed in the presence of dithiothreitol. Rapid and complete inactivation with *p*-chloromercuribenzoate is a phenomenon reported for other transglutaminases [24,25].

The molecular weight of purified epidermal transglutaminase was 53 000–55 000 by three techniques; approach to sedimentation equilibrium, sucrose density centrifugation, and Sephadex G-200 chromatography. Its anomalous behavior in sodium dodecyl sulfate is reproducible, and is partially corrected by including urea in the electrophoresis medium. Since the anomalous behavior is characteristic not only of freshly purified material, but of enzyme subjected to ultracentrifugation as well, and is reversed by the presence of urea in gels, we believe it may result from failure of sodium dodecyl sulfate to react completely with the enzyme. Further experimentation is in progress to solve this problem.

Specificity of our antiserum to epidermal transglutaminase was demonstrated by its ability to give a single precipitin line against both crude and highly purified enzyme in agar, and by its ability to block fibrin crosslinking by epidermal transglutaminase but not by Factor XIII. The enhancement of activity seen with antibody-complexed enzyme in the dansyl cadaverine assay is comparable to phenomena described for other enzymes, when small synthetic substrates are used [26]. No attempt was made to remove antigen-antibody complexes from solution by centrifugation.

As noted in the introduction, a similar enzyme was isolated from guinea pig hair follicles by Harding and Rogers and highly purified by Chung and Folk. Hair follicle transglutaminase shares many properties of the epidermal enzyme, namely, calcium and sulfhydryl dependence, recovery of a fully active enzyme in the crude homogenate, crosslinking of fibrin monomers, molecular weight (hair follicle transglutaminase 54 000; epidermal transglutaminase 53 000–55 000). However, Harding and Rogers found that crosslinking functions of their enzyme were inhibited by rabbit antiserum to Factor XIII, although precipitation between the two in agar was not observed. Chung and Folk reported no immunologic cross-reactivity between hair follicle transglutaminase and antiserum to the well-characterized liver transglutaminase, but did not comment on antiserum to Factor XIII. Their molecular weight findings in sodium dodecyl sulfate gels vary from ours. After pretreatment with sodium dodecyl sulfate, urea, and dithiothreitol, purified hair follicle transglutaminase subjected to electrophoresis in the presence of sodium dodecyl sulfate gave an identifiable protein band at 27 000 mol. wt., in addition to trace contaminants at higher molecular weights. These results were interpreted to mean that hair follicle transglutaminase consisted of two identical subunits which were separable under their conditions. We have been unable to find any material of less than 55 000 mol. wt. in any of our preparations.

Homogenization of bovine vibrissa follicles in our laboratories has yielded measurable amounts of a transglutaminase biochemically similar to bovine epidermal transglutaminase. However, this enzyme fails to precipitate in agar with anti-epidermal transglutaminase, nor does incubation of this enzyme with anti-epidermal transglutaminase result in enhancement of dansyl cadaverine incorporation into casein in a more sensitive assay of immunologic interaction [10]. Preliminary evidence thus indicates that hair follicle transglutaminase and epidermal transglutaminase may be isoenzymes.

The enzyme purified here is thus a unique one, immunologically distinct from transglutaminase of hair follicle origin, and biochemically and immunochemically distinct from plasma Factor XIII. The physiologic function of epidermal transglutaminase is not known, although immunochemical studies with fluorescein-labeled antiserum to epidermal transglutaminase have localized the enzyme to upper Malpighian and granular layers of cow snout epidermis [27], and this indicates a role for the enzyme in the final, stabilizing, phases of keratinization.

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