SUMMARY

Evidence is presented that transglutaminase is composed of a single polypeptide chain of molecular weight 80,000 to 90,000. (a) Polyacrylamide gel electrophoresis in the presence of dodecyl sulfate and mercaptoethanol gave a single band with a mobility corresponding to a molecular weight of approximately 85,000. (b) Gel filtration in guanidine HCl of the 14C-carbamidomethyl carboxymethylated enzyme protein showed a single peak of absorbance and radioactivity from which a molecular weight of approximately 85,000 was estimated. (c) Amino-terminal analysis by conventional methods showed no evidence of free α-amino groups. A peptide, believed to contain the amino-terminal residue, was obtained by Pronase digestion and was isolated at levels of 0.75 and 0.8 mole/90,000 g of enzyme. The sequence of this peptide was determined as pyroglutamylalanylaspartylleucine. (d) Digestion by carboxypeptidase A of the carboxymethylated enzyme protein in denaturing solvents released glycine and serine at equal rates to the level of 1 mole/90,000 g of protein. Hydrazinolysis gave approximately 1 mole of glycine. These findings, together with earlier evidence that the molecular weight of the native enzyme is 80,000 to 90,000 and that the enzyme protein contains 17 or 18 —SH groups, but no disulfide bonds, form the basis for the view of an unbridged monomeric structure of transglutaminase.

Indication that transglutaminase performs its catalytic functions in the monomeric form was obtained from a comparison of the gel filtration patterns for the enzyme protein in the presence and absence of calcium ion. The identical functions in the monomeric form was obtained from a comparison of the gel filtration patterns for the enzyme protein in the presence and absence of calcium ion. The identical patterns are in accord with the suggestion that this metal, which is essential for activation of transglutaminase, does not affect a change in enzyme molecular weight.

A revised enzyme purification procedure is presented. Rabbit antiserum against transglutaminase has been prepared and used to characterize the enzyme purified by this procedure as immunologically homogeneous.

The Ca++-activated transglutaminase of guinea pig liver, first described by Sarkar, Clarko, and Waelsch (1) and Clarke et al. (2), has been shown to have a molecular weight in the range of 90,000 (3). The enzyme protein contains 17 or 18 free sulfhydryl groups per molecule and is devoid of disulfide bonds (3, 4). Selective alkylation of the —SH group of a single cysteine residue results in complete loss in enzymatic activities (3, 6). This has been taken as evidence that the enzyme contains one active site per molecule.

In early attempts to determine the monomeric or polymeric nature of the enzyme protein, chromatography and gel filtration of the carboxymethylated enzyme protein showed a single component.1 We were unable, however, to detect any amino-terminal residue by the use of the usual procedures under various reaction conditions. Further, treatment of the enzyme with carboxypeptidases in the absence of denaturants yielded only small fractional amounts of amino acids.

The results reported here, obtained by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, gel filtration in guanidine, and amino- and carboxyl-terminal analyses, supply strong evidence for a single chain structure of transglutaminase.

EXPERIMENTAL PROCEDURE

Materials—Ammonium sulfate, urea, and guanidine HCl were ultra pure products from Mann. Sodium dodecyl sulfate (Matheson, Coleman and Bell) was recrystallized from ethanol. Hydrazine (95+%), hydrazine sulfate, and 1-fluoro-2,4-dinitrobenzene (Eastman) were used without further purification. L-Pyroglutamic acid (L-2-pyrrolidone-5-carboxylic acid) was obtained from Aldrich; L-pyroglutamyl-L-alanine was from Cycle; aminobenzylglutamate was from Mann; Bio-Gels A-0.5M (200 to 400 mesh) and A-15M (200 to 400 mesh) were obtained from Bio-Rad. Ultrasulfate (J. T. Baker) diluted with deionized water was used for hydrolysis. Carboxypeptidase A (disisopropyl fluorophosphate-treated), chymotrypsinogen A, and β-galactosidase and phosphorylase a were from Worthington; L-amino acid oxidase and bovine serum albumin were from Mann; Pronase was from Calbiochem. The α1 chain and the β component of collagen were kindly supplied by Dr. K. A. Piez; β-lactoglobulin was supplied by Dr. E. W. Davies and methylated bovine serum albumin was from Dr. J. L. London.

Pyroglutamic acid peptides from Aerobacter cloacae, purified through the Sephadex G-200 step according to Armentrout and Doolittle (7), was a gift from Dr. A. Peterkosky.

Other material and reagents have been described in a previous publication (3).

Transglutaminase Activity—This was measured by the col-
orimeic hydroxamate procedure (3, 5) with 30 mM benzyl-
oxycarbonyl-L-glutaminylglycine, 1 mM EDTA, 5 mM CaCl₂, and 0.1 M hydroxylamine in 0.1 M Tris-acetate at pH 6.0 and 37°. Protein concentrations were determined on impure preparations by the method of Lowry et al. (8). On pure preparations and for all quantitative experiments protein concentrations were determined by the use of an absorbance index, A₁₆₅ = 15.8 (3).

**Polyacrylamide Gel Electrophoresis in Sodium Dodecyl Sulfate**—The polypeptide chain molecular weight of transglutaminase was estimated by a modification (9) of the dodecyl sulfate-gel electrophoresis procedure of Shapiro, Vinuela, and Maizel (10). Electrophoresis was conducted in gels prepared with 0.135 g of methylenebisacrylamide and 10 g of acrylamide per 100 ml of solution (9).

**Gel Filtration in Guanidine**—The polypeptide chain molecular weight of transglutaminase was also estimated by gel filtration on 4% agarose (BioGel A-15M) in 6 M guanidine HCl by a method similar to that of Fish, Mann, and Tanford (11). The sample of transglutaminase, which had been alkylated with ¹⁴C-iodoacetamide at the single essential —SH group and with non-radioactive iodoacetic acid at the remaining —SH group (4), and the protein standards were applied in 6 M guanidine HCl, pH 5.0, to a column, 1.5 × 120 cm, of agarose that had been equilibrated with the same guanidine solution. The fractions from the column were monitored in the conventional manner by absorbance at 280 nm and, in the case of ¹⁴C-labeled transglutaminase, also by radioactivity.

**Analysis for Amino Acids**—These analyses were carried out by an ion exchange procedure with an automatic amino acid analyzer. Acid hydrolysis of peptide materials was carried out at 108° for 22 hours in 6 N HCl in sealed, evacuated tubes which had been flushed with nitrogen.

**Examination for Amino-terminal Amino Acids**—Transglutaminase was examined for amino-terminal acids by the fluorodinitrobenzene procedure of Sanger (12). Careful examination was made for bisdinitrophenylhistidine and di-fluorodinitrobenzene. Short time hydrolysates (2 and 4 hours) were prepared and examined for dinitrophenylarginine. Digestions of peptide material (approximately 0.1 pmole per mg) were carried out with ¹⁴C-carboxymethylating agent (4) (1.5 mg per ml) by carboxypeptidase A were carried out in 6 mM urea or in 0.25% sodium dodecyl sulfate at pH 8.0 (0.1 M NH₄HCO₃ buffer) and 37° with 1.20 molal ratios of enzyme to protein substrate. Appropriate aliquots were removed at various times and acidified with glacial acetic acid. Free amino acids were absorbed on Dowex 50-X8 (20 to 50 mesh) and eluted with 5 N NH₄OH in the usual manner, and finally analyzed on an amino acid analyzer.

**Carboxy-terminal Residues**—Digestions of carboxymethylated transglutaminase protein (4) (1.5 mg per ml) by carboxypeptidase A were carried out in 6 M urea or in 0.25% sodium dodecyl sulfate at pH 8.0 (0.1 M NH₄HCO₃ buffer) and 37° with 1.20 molal ratios of enzyme to protein substrate. Appropriate aliquots were removed at various times and acidified with glacial acetic acid. Free amino acids were absorbed on Dowex 50-X8 (20 to 50 mesh) and eluted with 5 N NH₄OH in the usual manner, and finally analyzed on an amino acid analyzer.

Digestions of peptide material (approximately 0.1 µmole per ml) by carboxypeptidase A were carried out as above except in the absence of denaturants. Aliquots were acidified with glacial acetic acid and taken to dryness under vacuum and analyzed as above.

**Hydrazinolysis of transglutaminase**—Hydrazinolysis of transglutaminase was carried out on 0.1-µmole samples of protein in 0.2 ml of hydrazine containing 25 mg of hydrazine sulfate in sealed tubes for 16 hours at 60°. Hydrazine and hydrazides were removed and samples were prepared for analysis by a published procedure (15). Amino acids were determined by the use of an amino acid analyzer. No corrections were applied for losses.

**Immunization Procedure**—New Zealand White rabbits were immunized according to the following schedule. The animals were given four intradermal injections in the back at 1-week intervals of an emulsion prepared from 0.32 ml of Freund's complete adjuvant, 0.37 ml of transglutaminase (1.1 to 1.5 mg per ml in 10 mM Tris-acetate buffer, pH 6.0, containing 1 mM EDTA and 0.16 M KCl), and 0.01 ml of a 0.1% (w/v) aqueous solution of methylated bovine serum albumin. On the 10th day following the last intradermal injection a mixture of 0.59 ml of the transglutaminase solution and 0.01 ml of the methylated bovine serum albumin solution was administered intravenously. Four weeks after the booster injection blood was collected by cardiac puncture. The antisera were stored in 1-ml portions at -20°.

**Immunoelectrophoresis**—Immunoelectrophoresis was conducted by the procedure of Scheidegger (10) with agarose in place of agar.

**RESULTS**

**Purification of Transglutaminase**

Difficulties were frequently experienced with the published procedure (3,17) because of inconsistent and unexplained losses in enzyme activity during the last DEAE-cellulose chromatography step. The procedure has been modified as follows in order to overcome these difficulties and to obtain as much as 100 mg of enzyme from a single preparation.

**Preparation of Crude Extract**—Unfrozen guinea pig livers were obtained, packed in ice, from Pel-Freez Biologicals, Inc. As in the earlier procedure, only unfrozen livers yield satisfactorily pure enzyme preparations. Livers used as long as 5 days after removal from the animals have given excellent yields of pure enzyme. A portion of 200 g of the tissue in enough cold 0.25 M sucrose to give a final volume of 450 to 500 ml was homogenized for about 2 min with a Polytron PT 10 OD homogenizer (Brinkmann Instruments) at intermediate speed. This and all further operations were carried out below 5°. The homogenate was centrifuged for 1 hour at 105,000 × g in a Spinco preparative ultracentrifuge with the No. 30 rotor.

**Chromatography on DEAE-cellulose**—The supernatant fluid was filtered through four layers of cheesecloth and pumped rapidly into a column, 3.5 × 20 cm, of DEAE-cellulose equilibrated with 5 mM Tris-chloride, pH 7.5, containing 2 mM EDTA. Following a 200-ml wash with the equilibrating buffer, the protein was eluted at the rate of 5 to 10 ml per min by the use of a 1.5-liter linear gradient of 0 to 1.0 M NaCl in the same buffer. The fractions rich in enzyme activity, eluted between about 0.25 and 0.4 M NaCl as determined by assay, were combined.
tion for 1 min at 2,500 × g. Three extractions, each with 40 ml of 0.05 M ammonium sulfate in 5 mM Tris-chloride, pH 7.5, containing 2 mM EDTA, were performed on the washed protamine precipitate. These were carried out in a Duvall No. 24 homogenizer vessel by the use of the Polytron homogenizer at low speed. For each extraction three 0.5-min homogenizations were used, followed in each case by a 0.5-min cooling period in an ice water bath. After each extraction the precipitate was recovered by centrifugation for 1 min at 2,500 × g. The combined extracts were filtered through a column, 2 × 5 cm of carboxymethyl cellulose, equilibrated with 5 mM Tris-succinate, pH 6.0, containing 2 mM EDTA, in order to remove the protamine. A portion of 2.4 ml of 1 M EDTA, pH 8.0, and 47.4 g of solid ammonium sulfate were added to the filtrate with stirring.

Gel Filtration on Agarose—The ammonium sulfate suspensions of partially purified enzyme from two preparations described in the previous step were combined and the precipitate was collected by centrifugation for 10 min at 15,000 × g. This precipitate was dissolved in 5 to 7 ml of 10 mM Tris-acetate, pH 6.0, containing 1 mM EDTA and 0.16 M KCl. A small amount of insoluble material that usually remained was removed by centrifugation for 30 min at 27,000 × g. The supernatant fluid was transferred to a column, 5 × 100 cm (Pharmacia, Inc.), of 10% agarose (Bio-Gel A-0.5 M) equilibrated with the Tris-EDTA-KCl buffer and filtration was carried out at the rate of 35 to 40 ml per hour using the same buffer. A typical gel filtration pattern is shown in Fig. 1. Fractions were combined into two pools on the basis of their specific activity. Those of Pool A were ones containing enzyme with specific activity of 12 or above, as indicated by the horizontal arrow in Fig. 1. Fractions containing enzyme with specific activity from 8 to 12 were designated Pool B. The protein concentration of each pool was increased to 10 to 20 mg per ml by the use of a 50-ml Diaflo ultra-filtration cell with the UM-10 filter. The concentrated enzyme solutions were stored frozen at −20°. Table I gives a summary of the purification procedure.

Pool B preparations from several runs were combined and rechromatographed on DEAE-cellulose as outlined above to give material of specific activity 11 to 12. Certain preliminary experiments were conducted with enzyme from this pool. However, all studies reported here were confirmed with Pool A enzyme.

**Immunochemical Characterization of Transglutaminase**

Two samples of antisera were prepared with two separate preparations of Pool A transglutaminase. Each of these antisera showed a single sharp precipitin line in diffusion and electrophoresis experiments with Pool A and with Pool B enzyme over a wide range of enzyme concentration. The results of an immunoelectrophoretic examination are shown in Fig. 2. In this case enzyme in which the essential —SH group had been alkylated with ¹⁴C-labeled iodoacetamide (3, 6) was also tested for reactivity with antibody. A positive reaction of labeled enzyme protein is evident from the shape and position of the precipitin band as visualized by radioautography. Control serum, obtained from animals that had been injected with emulsion prepared without transglutaminase, gave no precipitin bands. An antiserum prepared with Pool B enzyme showed a single sharp precipitin line with Pool A enzyme. This antiserum showed in addition to the major line, a minor precipitin band in diffusion experiments with Pool B enzyme.

**Table I**

| Purification step                  | Protein | Specific activity | Yield |
|-----------------------------------|---------|------------------|-------|
| Homogenate                        | 100     | 0.06             |       |
| Supernatant fluid                 | 40      | 0.11             | 90    |
| DEAE-cellulose chromatography     | 1.0     | 2.5              | 50    |
| Protamine extracts                | 0.225   | 8.0              | 36    |
| Agarose gel filtration Pool A     | 0.072   | 14.0             | 20    |
| Pool B                            | 0.030   | 11.0             | 6.6   |

* One unit is defined as the amount of enzyme that catalyzes formation of 1 pmole of hydroxamate per min in the test given under "Experimental Procedure" (17).

**Fig. 1.** Gel filtration of transglutaminase on Bio-Gel A-0.5M. Fractions of 8.6 ml were collected. See text for details.

**Fig. 2.** Immunoelectrophoresis of transglutaminase and the ¹⁴C-iodoacetamide-labeled enzyme. *Left,* precipitin line observed when native enzyme was subjected to electrophoresis followed by application of antiserum to center trough. *Right,* radioautograph prepared following an experiment in which ¹⁴C-iodoacetamide-inactivated transglutaminase was used in place of native enzyme.
Structural Properties of Transglutaminase

Gel Electrophoresis in Sodium Dodecyl Sulfate—When transglutaminase was subjected to gel electrophoresis in this denaturant a single band was obtained. The same was observed when the enzyme protein was first incubated for 6 hours at 37° in 1% sodium dodecyl sulfate and 1% mercaptoethanol at pH 7.0. A molecular weight of approximately 85,000 was estimated from the migration of this band relative to those of several other proteins of known polypeptide chain molecular weight (Fig. 3).

Gel Filtration in Guanidine—The 14C-carbamidomethyl carboxymethylated transglutaminase derivative was eluted from 4% agarose in guanidine HCl as a single peak. The specific radioactivity, based on the 280 nm absorbance, was found to be the same in all fractions across the peak. Comparison of the distribution coefficient (Kd) of the enzyme derivative with those of several standards shows that the molecular weight of this derivative is greater than that of bovine serum albumin and probably is in the range of 80,000 to 90,000 (Fig. 4).

Amino-terminal Analysis—Analysis for amino-terminal amino acids by the fluorodinitrobenzene method, both in the presence and absence of 5 mM guanidine HCl, by the paper strip phenylisothiocyanate method and by the dansyl method showed only trace amounts (less than 0.05 mole per mole of enzyme protein) of terminal amino acids. Therefore, we decided to attempt isolation of a terminal peptide with the aim of characterizing this region of the molecule. To this end a digestion and isolation procedure, similar to that used by Press, Piggot, and Porter on human immunoglobulin IgG (15), was used.

To 27 mg (0.3 μmoles) of native transglutaminase in 1 ml of 10 mM Tris-chloride buffer, pH 8.1, containing 1 mM EDTA was added 0.3 mg of Pronase. Digestion was carried out at 37°C. The pH was maintained between 8 and 8.1 by the periodic addition of dilute NaOH. After 2 hours an additional 0.2 mg of Pronase and 2 drops of toluene were added and digestion was allowed to proceed for 20 hours longer. The pH at this time was 7.9. A small amount of precipitate that formed during the digestion was removed by centrifugation. The digest was cooled to 2°C and passed into a column (0.7 X 18 cm) of Dowex 50 × 2 (200 to 400 mesh, H⁺ form) that had been washed free of acid with water and was maintained at 2°C. After application of the digest, the column was washed with cold water. Unretained material was located in the eluant by measuring the absorbance at 230 μm. The solution of this material was reduced in volume to 0.2 to 0.3 ml and subjected to gel filtration on a Bio-Gel P-2 (100 to 200 mesh) column (0.7 X 22 cm) in 0.1 M acetic acid. The 230 μm absorbing material was eluted as a single peak in the position of the salt fraction. This material, which failed to react with ninhydrin or fluorodinitrobenzene, showed a positive reaction with ninhydrin after acid or alkaline hydrolysis. It appeared as a single elongated spot that moved toward the anode in high voltage paper electrophoresis when visualized by means of the chlorine-o-tolidine-KI test (18).

Amino acid analysis showed that this peptide was composed of equimolar amounts of aspartic acid, glutamic acid, alanine, and leucine (Table II). The results of carboxypeptidase A digestion (Table II) gave substantial support for an Asp-Leu carboxy-terminal sequence. That the peptide was composed of only four amino acids was indicated by the fact that it appeared in

| Amino acid | Acid hydrolysat | Carboxypeptidase A digest: amount released in |
|------------|----------------|---------------------------------------------|
|            | mg/100,000 μg  | 0.5 hr | 5 hrs |
| Aspartic acid | 0.80 | 1.0 | 0 | 0.3 |
| Glutamic acid | 0.96 | 1.2 | 0 | 0 |
| Alanine | 0.80 | 1.0 | 0 | 0 |
| Leucine | 0.64 | 0.7 | 0.4 | 0.8 |

* Based on alanine.
Evidence for pyroglutamic acid as amino-terminal residue in peptide isolated from Pronase digest of transglutaminase

Peptides (0.6 mm) were incubated with pyrrolidonecarboxylyl peptidase (~100 units (defined according to Reference 7) per ml) for 2 hours at 20° in 0.01 m phosphate buffer, pH 7.3, containing 2 mm mercaptoethanol and 0.2 mm EDTA. After the incubation period samples were applied directly to paper for high voltage electrophoresis at pH 6.5 (pyridine-acetic acid-water, 200:8:1800, v/v, 1 hour at 50 volts per cm) and to thin layer plates for chromatography (1-propanol-H_2O, 7:3, v/v). The chloroform-tolidine-KI test (18) was used to locate standards and products of digestion.

| Peptide or amino acid derivative | Electrophoresis: distance migrated from origin | Chromatography: R_F |
|----------------------------------|-----------------------------------------------|---------------------|
| Pyroglutamic acid                | 12.0                                          | 0.5                 |
| N-Acetylglutamic acid            | 16.5                                          |                     |
| Pyroglutamylalanine              | 8.3                                           | 0.5                 |
| Pyroglutamylalanine + pyrrolidonecarboxylyl peptidase | 12.0^a |                     |
| Tetrapeptide from transglutaminase | 5.0                                           | 0.38                |
| Tetrapeptide from transglutaminase + pyrrolidonecarboxylyl peptidase | 5.0 and 12.0^b | 0.35 and 0.5^b |

^a Complete digestion was evidenced by the disappereance of the peptide.
^b Digestion was judged, from the size and intensity of the spots, to have proceeded only to between 25 and 50%.

The recovery of peptide in two preparations was calculated to be 0.80 and 0.75 mole/90,000 g of enzyme protein.

Evidence that the amino-terminal residue of this tetrapeptide was L-pyroglutamic acid was obtained by the use of the enzyme, pyrrolidonecarboxylyl peptidase. Incubation with this peptidase resulted in the release of free pyroglutamic acid as shown in detail in Table III.

From these findings it may be concluded that a peptide with the probable sequence pyroglu-Ala-Asp-Leu is released from transglutaminase during digestion with Pronase. Earlier evidence that the conditions used here for digestion and for peptide isolation do not lead to a significant amount of conversion of amino-terminal glutamine to amino-terminal pyroglutamic acid (15) substantiates the conclusion that this pyroglutamyl sequence was present in transglutaminase before digestion. Since no amino-terminal residue was detectable by conventional methods, and on the basis of recovery of essentially 1 mole of this peptide per mole of enzyme protein, we conclude that transglutaminase contains the single NH_2-terminal sequence pyroglu-Ala-Asp-Leu.

Carboxyl-terminal Analysis—The results of digestion of carboxymethylated transglutaminase protein with carboxypeptidase A in denaturing solvents are summarized in Table IV. Both glycine and serine were released in almost stoichiometric amounts. Hydrazinolysis experiments served to define glycine as the carboxyl-terminal residue. In one experiment the following moles of amino acid per 90,000 g of enzyme protein were found: glycine, 0.04; alanine, 0.36; aspartic acid, 0.24; tyrosine, 0.1; phenylalanine, 0.1. In a second experiment only glycine, 1.1 moles per mole, and alanine, 0.4 mole per mole, were found. The source of alanine is not known. These results provide evidence that the carboxyl-terminal sequence of transglutaminase is Ser-Gly.

Gel Filtration of Native Transglutaminase in Presence and Absence of Ca++

Gel filtration was carried out on a column, 2.5 x 94 cm, of 10% agarose (Bio-Gel A-0.5M) in 10 mM Tris-acetate buffer containing 0.5 mM EDTA and 0.16 M KCl at pH 6.0 and 4° with and without the addition of 10 mM CaCl_2. The proteins were equilibrated with column buffer and applied in a total volume of 2 ml. Elution was carried out at a flow rate of 60 ml per hour. Fractions were monitored by absorbance at 230 or 280 nm and by enzyme assay in the case of transglutaminase. The over-all elution patterns appeared the same in the presence and absence of CaCl_2. The distribution coefficients (K_x values) were determined as described in the legend to Fig. 4.

Discussion

The results reported here, together with earlier findings, provide strong evidence that transglutaminase is composed of a single unbridged polypeptide chain. These include the close agreement in the values for molecular weight in denaturing solvents with those determined for the native enzyme (9), the finding of only one amino- and one carboxyl-terminal residue...
per molecule, and the fact that the enzyme protein contains no disulfide bonds (4).

Kinetic studies support a mechanism of calcium ion activation of transglutaminase wherein metal ion functions by combining with enzyme, rather than by combining with substrates (5, 19, 20). Further, spectrophotometric studies have shown that binding of Ca\(^{2+}\) results in a conformational alteration of the enzyme protein (19). The dissociation constant for Ca\(^{2+}\) with enzyme, rather than by combining with substrates (5, 6).

The dissociation constant for Ca\(^{2+}\) with enzyme, rather than by combining with substrates (5, 6, 7).

The gel filtration carried out here in the presence and absence of Ca\(^{2+}\) shows that the molecular weight of the enzyme is not significantly changed by binding of this metal ion. It seems reasonable to postulate on the basis of this finding that transglutaminase performs its enzymatic functions in the monomeric form.

The amino-terminal analyses reported here are in accord with the proposition that transglutaminase possesses the single amino-terminal residue, pyroglutamic acid. With this enzyme, the terminal pyroglutamic acid are indeed prevalent in mammalian tissues. The implication suggested by these findings is that proteins and peptides having amino-terminal pyroglutamic acid have a wide distribution of the enzyme, pyrrolidonecarboxylyl peptidase, in mammalian tissues, and those findings do not contribute to resolving this question. The recent work of Toda's careful examination of transglutaminase for amino-terminal residues by the dansyl procedure (15, 21), the question arises as to whether it is formed as an artifact of isolation. The present results do not contribute to resolving this question. The recent finding of a wide distribution of the enzyme, pyrrolidonecarboxylyl peptidase, in mammalian tissues gives some support to the proposition that transglutaminase possesses the single amino-terminal residue, pyroglutamic acid. With this enzyme, the terminal pyroglutamic acid are indeed prevalent in mammalian tissues (22).

The antiserum elicited by purified transglutaminase and the antiserum elicited by a partially purified preparation (Pool B enzyme) have been used to define the purified enzyme as immunologically homogeneous. Preliminary studies with native transglutaminase and with several chemically modified derivatives of the enzyme, including the iodoacetamide-inactivated derivative (Fig. 2), suggest that antigenic reactivity is confined to a site in the molecule that is structurally separate from the catalytic site. The details of these findings will be given in a subsequent report.

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