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 $^{14}$C-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 nature of these patterns is 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.
Guinea Pig Liver Transglutaminase

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orimetric hydroxamate procedure (3, 5) with 30 mm benzoyl-

oxycarbonyl-l-glutamylglycine, 1 mM EDTA, 5 mM CaCl₂,

and 0.1 M hydroxylamine in 0.1 M Tris-acetate at pH 6.0 and

37°C. Protein concentrations were determined on impure

preparations by the method of Lowry et al. (8). On pure

preparations and for all quantitative experiments protein con-

centrations were determined by the use of an absorbance index,

\[ A_{158} = 15.8 \times (3). \]

**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, Vithala, 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, that had been alkylated with ¹⁴C-

iodoacetamide at the single essential —SH group and with non-

radioactive iodoacetic acid at the remaining —SH groups (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 trans-

glutaminase, 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°C for 22 hours in 6 M hydrochloric acid containing 25 mg of methylenebisacrylamide and 100 mg of acrylamide per 1 ml of solution (9).

**Examination for Amino-terminal Amino Acids—** Trans-

glutaminase was examined for amino-terminal amino acids by the fluorodinitrobenzene procedure of Sanger (12). Careful ex-

amination was made for bisdinitrophenylhistidine and di-

nitrophenyllysine. Short time hydrolysates (2 and 4 hours)

were prepared and examined for dinitrophenylglycine. Thin

layer chromatography was used in these studies. The phenyl-

isothiocyanate method of Edman with the paper strip procedure

(12, 13) was also used for this examination, as was the "dansyl" method (14). Hydrolysates were examined for dansyl-amino acids by thin layer chromatography (15).

**Carboxyl-terminal Residues—** Digestions of carboxymethyl-

ated transglutaminase protein (4) (1.5 mg per ml) by carboxy-

peptidase A were carried out in 6 M urea or in 0.25% sodium dodecyl sulfate at pH 8.0 (0.1 M CH₃CO₂ buffer) and 37°C with 1.20 molar 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 M 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 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°C. Hy-

drazine 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 correc-
tions 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 0.18 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°C.

**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 chroma-
tography 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 give 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 (Brink-
mann Instruments) at intermediate speed. This and all further operations were carried out below 5°C.

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 equili-

brated 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.

**Protamine Precipitation and Extraction—** The pooled fractions from two DEAE-cellulose chromatograms performed on the

same day were combined and a 40-ml portion of a freshly pre-

pared 1% (w/v) solution of protamine sulfate (El Lilly and

Company) was added gradually with stirring. The precipitat,

which contains all of the enzyme activity, was collected by

centrifugation for 15 min at 14,600 × g. This precipitate was

washed by suspending it in 20 ml of 0.2 M Tris-acetate buffer,

pH 6.0, and homogenizing with the Polytron homogenizer for 1

min at low speed. The precipitate was recovered by centrifuga-
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-0.5M) 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**

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Protein</th>
<th>Specific activity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>100</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Supernatant fluid</td>
<td>40</td>
<td>0.11</td>
<td>90</td>
</tr>
<tr>
<td>DEAE-cellulose chromatography</td>
<td>1.0</td>
<td>2.5</td>
<td>50</td>
</tr>
<tr>
<td>Protamine extracts</td>
<td>0.225</td>
<td>8.0</td>
<td>36</td>
</tr>
<tr>
<td>Agarose gel filtration Pool A</td>
<td>0.072</td>
<td>14.0</td>
<td>20</td>
</tr>
<tr>
<td>Pool B</td>
<td>0.030</td>
<td>11.0</td>
<td>6.6</td>
</tr>
</tbody>
</table>

* 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. 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-carbamidoethyl 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 47,000 rnp absorbance, contained within and without the gel matrix (11) converted 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).

The void volume and tritiated water to measure the solvent contained within and without the gel matrix (11) was determined with blue dextran to measure the solvent contained within and without the gel matrix (11). 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).

Molecular Weights—The pI of 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).

The molecular weights of these marker proteins were taken from Table I of Reference 9. The points shown for transglutaminase represent the variation observed in six runs.

Fig. 3. Determination of the molecular weight of the polypeptide chain of transglutaminase by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. All marker proteins were run on duplicate gels. The molecular weights of these marker proteins were taken from Table I of Reference 9. The points shown for transglutaminase represent the variation observed in six runs.

Fig. 4. Estimation of the molecular weight of 14C-iodoacetamide-inactivated, carboxymethylated transglutaminase by gel filtration on 4% agarose in guanidine. The distribution coefficients (Kd values) were determined with blue dextran to measure the void volume and tritiated water to measure the solvent contained within and without the gel matrix (11).

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 m 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 µmole) 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°. 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° and passed into a column (0.7 x 15 cm) of Dowex 50 x 2 (200 to 400 mesh, H+ form) that had been washed free of acid with water and was maintained at 2°. After application of the digest, the column was washed with cold water. Unretained material was located in the effluent by measuring the absorbance at 230 nm. 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 nm 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 carboxyl-terminal sequence. That the peptide was composed of only four amino acids was indicated by the fact that it appeared in the 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.

Table II

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Acid hydrolysate</th>
<th>Carboxypeptidase A digest: amount released in 0.5 hr</th>
<th>5 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>0.80</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.96</td>
<td>1.2</td>
<td>0</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.80</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.64</td>
<td>0.7</td>
<td>0.4</td>
</tr>
</tbody>
</table>

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

Peptides (0.6 nm) were incubated with pyrrolidonecarboxyl peptidease (~10 units) for 2 hours at 30° 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 chro-
matography (1-propanol-H2O, 7:3, v/v). The chloroform-
tolidine-KI test (18) was used to locate standards and products of digestion.

<table>
<thead>
<tr>
<th>Peptide or amino acid derivative</th>
<th>Electrophoresis: distance migrated from origin (cm)</th>
<th>Chromatography: RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyroglutamic acid</td>
<td>12.0</td>
<td>0.5</td>
</tr>
<tr>
<td>N-Acetylglutamic acid</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td>Pyroglutamylalanine</td>
<td>8.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>
| Pyroglutamylalanine + pyrro-
lidonecarboxyl peptidease         | 12.0b                                            |                   |
| Tetrapeptide from transgluta-
minase                            | 5.0                                              | 0.38              |
| Tetrapeptide from transgluta-
minase + pyrrolidonecarboxyl peptidase | 5.0 and 12.0b                      | 0.35 and 0.5b     |

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

d the low molecular weight fraction from Bio-Gel P-2 (see above). 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, pyrrolidonecarboxyl peptidease. Incubation with this pepti-
dase 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 con-
version of amino-terminal glutamine to amino-terminal pyro-
glutamic acid (15) substantiates the conclusion that this pyro-
glutamyl 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 NH2-terminal se-
quence 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++ Transglutaminase functions catalytically only in the presence of calcium ion (2, 19). In order to determine whether this essential metal ion alters the molecular weight, i.e. causes polymerization, of the enzyme protein, we examined the gel filtration characteristics of the native enzyme in the presence and absence of CaCl2. The finding of the identical gel filtration pattern in each case (Table V) is evidence that Ca++ does not induce a gross change in the size or shape of the enzyme molecule.

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 (2), 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++ results in a conformational alteration of the enzyme protein (19). The dissociation constant for Ca++ with enzyme, rather than by combining with substrates (5, disulfide bonds (4).

Per molecule, and the fact that the enzyme protein contains no amino-terminal residue, pyroglutamic acid. With this enzyme, filtration carried out here in the presence and absence of Ca++ 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, as is true in each case in which pyroglutamic acid has been assigned as the amino terminus (15, 21), the question arises as to whether this cyclic residue exists under physiological conditions or whether it is formed as an artifact of isolation. The present findings do not contribute to resolving this question. The recent finding of a wide distribution of the enzyme, pyrrolidonecarboxyl peptidease, in mammalian tissues gives some support to the implied suggestion that proteins and peptides having amino-terminal residues by the dansyl procedure. We also attribute whether it is formed as an artifact of isolation. The present findings 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.

Acknowledgments—The authors wish to acknowledge Dr. H. Toda's careful examination of transglutaminase for amino-terminal residues by the dansyl procedure. We also attribute to this investigator the first suggestion that the enzyme protein possesses a blocked amino terminus. We express thanks to Dr. E. Rossomando and Mrs. C. E. Sullivan for carrying out the gel filtrations in guanidine.

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