Comparison of the Size and Physical Properties of \( \gamma \)-Glutamyltranspeptidase Purified from Rat Kidney Following Solubilization with Papain or with Triton X-100*

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\( \gamma \)-Glutamyltranspeptidase is associated with the brush border membrane of kidney proximal straight tubule cells. It can be solubilized quantitatively by treatment with papain or Triton X-100. Neither procedure affects its catalytic activity but the two resulting forms of the enzyme differ considerably in their physical properties. The papain-solubilized transpeptidase is soluble in aqueous buffers and was purified 430-fold. It has an \( s_{20,w} \) of 4.9 S, a Stokes radius of 36 Å, and a calculated molecular weight of 69,000. It appears homogeneous by sedimentation equilibrium centrifugation (\( M_r = 66,700 \)). In contrast, the Triton-solubilized transpeptidase is soluble only in the presence of detergents and was purified 300-fold. This form of the enzyme has a Stokes radius of \( 70 \) Å but an \( s_{20,w} \) of only 4.15 S. Aggregation of the enzyme just below the critical micelle concentration of Triton X-100 and its ability to bind 1.16 mg of Triton X-100/mg of protein suggest that it binds to micelles of Triton X-100. The molecular weight of the Triton X-100-protein complex was calculated to be 169,000, but the glycoprotein portion of the complex is 52% of the total mass (87,000). The mass of Triton X-100 (82,000) is consistent with its reported micelle molecular weight. Treatment of the Triton-purified transpeptidase with papain or bromelain results in a form of the enzyme identical in all respects with the papain-purified enzyme. Both the Triton- and papain-purified transpeptidase exhibit two protein bands on sodium lauryl sulfate-polyacrylamide gel electrophoresis. The smaller subunits of the two forms appear identical (\( M_r = 27,000 \)), while the larger subunits of the Triton- and papain-purified enzyme have apparent molecular weights of 54,000 and 51,000, respectively. These data suggest that a peptide (3,000 to 19,000) in the larger subunit of \( \gamma \)-glutamyltranspeptidase is responsible for its binding to Triton micelles and probably for holding the enzyme in the brush border membrane.

\( \gamma \)-Glutamyltranspeptidase is a broad specificity transferase which catalyzes the transfer of \( \gamma \)-glutamyl groups from a large variety of peptide donors to a wide range of amino acid and peptide acceptors. This lack of substrate specificity and its localization in membrane structures principally involved in absorption or secretion have led to the proposal that this enzyme participates in amino acid transport (1) and more recently to its participation in the \( \gamma \)-glutamyl cycle (2, 3). But the ability of water to act as an acceptor has suggested other possible physiological functions such as degradation of extracellular glutathione (4–6) or bioynthesis of mercapturic acid (7, 8). Information regarding the mechanism of interaction of this protein with membranes might help in understanding its physiological function.

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\( \gamma \)-Glutamyltranspeptidase was first extensively purified from beef (9) and from hog kidney (10) following acetone precipitation and extraction with deoxycholate. A similar procedure has been recently reported for the purification of this enzyme from human kidney (11). In addition, \( \gamma \)-glutamyltranspeptidase can be solubilized by proteolytic treatment of membranes (12–16). This approach has no effect on the catalytic properties of the enzyme. It has recently been used to obtain apparently homogeneous preparations of the transpeptidase from rat (15) and sheep kidney (16). Comparative studies have suggested that the proteolytically solubilized form of \( \gamma \)-glutamyltranspeptidase may be significantly smaller than that solubilized with detergents (13, 15).

We have extensively purified \( \gamma \)-glutamyltranspeptidase from rat kidney following solubilization with either papain or with Triton X-100. This represents the first report where this enzyme has been purified from the same source and to comparable specific activities using both approaches. Characterization of the size and physical properties of these two forms of \( \gamma \)-
glutamyltranspeptidase indicate that this enzyme is held in the membrane by a small peptide which is released by proteolytic treatment.

**EXPERIMENTAL PROCEDURES**

Enzyme Assay—γ-Glutamyltranspeptidase was assayed at room temperature using γ-glutamyl-p-nitroanilide as substrate (17). The reaction mixture contained 75 mM NaCl, 2.5 mM γ-glutamyl-p-nitroanilide, 20 mM glycylglycine, and 50 mM Tris/Cl, pH 8.0. Catalase (18), fumarase (19), alcohol dehydrogenase (20), glutamate oxalacetate transaminase (21), alkaline phosphatase (22), and horseradish peroxidase (23) were assayed as reported previously. Hemoglobin and blue dextran were detected by absorbance at 410 nm and 660 nm, respectively. Protein was assayed by the procedure of Lowry et al. (24). Radioactivity was counted in a Triton X-100 scintillation fluid.

Polycrylamide Gel Electrophoresis—Electrophoresis of native protein was performed according to the procedure of Davis (25) except that a 50 mM Tris/acetate solution, pH 8.6, was used as the reservoir buffer. Sodium lauryl sulfate-polycrylamide gel electrophoresis was performed according to the procedure of either Laemmli (26) or of Weber and Osborn (27) where indicated. Duplicate gels were stained for carbohydrate as described by Zschauer et al. (28).

**Determination of Molecular Weights**—Stokes radius was determined on a calibrated Sephadex G-200 column as described by Siegel and Monty (29) using the correlation of Laurens and Killander. Details and standard proteins are described in the legend to Fig. 2. Sedimentation coefficients were estimated by sucrose gradient centrifugation as described by Martin and Ames (30). Linearity of gradients was checked by reading refractive index of selected fractions with a Bausch and Lomb refractometer. Details and standard proteins are described in the legend to Figs. 3 and 4. Sedimentation coefficients were also determined in the model E ultracentrifuge as described by Chervenka (31). Sedimentation equilibrium analysis was performed in a model E ultracentrifuge according to the method of Yphantis (32). Triton binding assays were carried out according to the method of Clarke (33).

**RESULTS**

γ-Glutamyltranspeptidase was purified following solubilization with either papain or Triton X-100 as described in the miniprint supplement. The two purified forms of transpeptidase behaved differently during polyacrylamide gel electrophoresis (Fig. 1). The papain-purified enzyme produced a single protein staining band, but the Triton-purified enzyme would not enter the gel unless the sample buffer and gel contained 0.1% Triton X-100. Proteolytic treatment of the Triton-purified transpeptidase with either papain or bromelain produced a water-soluble protein which was electrophoretically indistinguishable from the papain-purified transpeptidase. This suggests that γ-glutamyltranspeptidase is equally susceptible to limited proteolysis before or after removal from the membrane and that a similar cleavage product is produced by treatment with either papain or bromelain.

Sodium lauryl sulfate-polycrylamide gel electrophoresis indicates that the papain-purified transpeptidase is composed of two nonidentical subunits. This is consistent with a similar subunit structure reported for the enzyme from sheep kidney (16). Staining of a duplicate gel with periodic acid-Schiff's reagent indicates that both subunits are glycoproteins.

If the papain-purified transpeptidase were a cleavage product of the Triton-purified enzyme, then the two forms would be expected to differ in molecular weight. Evidence of this possibility was first observed during the purification procedure. The Triton-solubilized enzyme eluted earlier during Sephadex chromatography than the papain-solubilized enzyme. Therefore, the two forms of purified enzyme were subjected to gel filtration on a calibrated Sephadex G-200 column (Fig. 2). A Stokes radius of 36 Å and 70 Å was determined for the papain- and Triton-purified transpeptidase, respectively. This 2-fold difference in Stokes radius converts to greater than a 300,000 difference in molecular weight. But treatment of the Triton-purified enzyme with papain produced a protein with Stokes radius of 34 Å; indistinguishable from the papain-purified enzyme.

To confirm this large difference in molecular weight, we next estimated sedimentation coefficients of the two forms of transpeptidase by sucrose gradient centrifugation (Fig. 3). The papain-purified enzyme has an observed $s_{20,w}$ of 5.0 $S$ and 4.9 $S$ in the absence and presence of Triton X-100, respectively. The Triton-purified enzyme had an observed $s_{20,w}$ of 4.5 $S$, which is inconsistent with the large Stokes radius. As expected, the Triton-purified enzyme aggregates in the absence of Triton and under these conditions it has an observed $s_{20,w}$ of 24 $S$. Treatment of this form with papain produced an enzyme with an observed $s_{20,w}$ of 5.0 $S$; again identical with the papain-purified transpeptidase. Determination of sedimentation coefficient in the ultracentrifuge confirmed these values. An $s_{20,w}$ of 4.9 $S$ and 4.15 $S$ were determined for the papain-purified enzyme in buffer and the Triton-purified enzyme in buffer containing 1% Triton, respectively.

Electrophoresis of the bromelain-treated Triton-purified transpeptidase suggests that our papain-purified enzyme is nearly identical with the bromelain-purified transpeptidase...
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Fig. 2. Determination of Stokes radius of \( \gamma \)-glutamyltranspeptidase by gel filtration. A Sephadex G-200 column (45 \( \times \) 1.2 cm) was pre-equilibrated and run in either buffer (TRT) or buffer containing 1% Triton X-100 (+TRT). Then 250 \( \mu \)g of standard proteins and 50 \( \mu \)g of either Triton-purified (T-\( \gamma \)GT), papain-purified (P-\( \gamma \)GT), or papain-treated Triton-purified (PT-\( \gamma \)GT) transpeptidase were applied in a total volume of 200 \( \mu \)l to the top of the column and 600-\( \mu \)l portions were pumped off from the bottom and collected in 40 to 50 fractions. Standard proteins were hemoglobin (Hb) (29); alkaline phosphatase (AP) (33); glutamate oxalacetate transaminase (GOT) (35); alcohol dehydrogenase (ADH) (29); fumarase (FUM) (37), and catalase (CAT) (29).

prepared by Tate and Meister (15). Their preparation was reported to contain 18.8% carbohydrate. From their reported value of 0.711 cm\(^3\)/g was calculated using the partial specific volume of 0.711 cm\(^3\)/g was calculated using the partial specific volume of individual amino acids and sugars (38, 39). Using this partial specific volume, \( \theta_{20,W} \) and Stokes radius the molecular weight of the papain-purified transpeptidase was calculated to be 69,000. This value was confirmed by sedimentation equilibrium analysis in the ultracentrifuge, which indicated that this form of transpeptidase was homogenous with respect to size and had a molecular weight of 65,700.

The observed aggregation of the Triton-purified transpeptidase in the absence of detergent suggested that the inconsistency in its measured \( s_{20,W} \) and Stokes radius may be due to the binding of a very large amount of Triton X-100. If this were true, the Triton-purified transpeptidase would be expected to have an abnormally high partial specific volume. Fig. 4 presents data consistent with this idea. Standard proteins with normal partial specific volumes will migrate slower by a constant factor in sucrose gradients prepared in deuterium oxide than in aqueous gradients (40). But a protein with an abnormally high partial specific volume will migrate slower relative to standard proteins in D\(_2\)O. The Triton-purified enzyme has an observed \( s_{20,W} \) in aqueous gradients of 4.5 S while in D\(_2\)O the observed \( s_{20,W} \) is only 2.5 S. In contrast, the papain-purified enzyme (not shown) migrates slightly faster relative to the standard proteins in D\(_2\)O, as would be expected for a glycoprotein.

This evidence for Triton binding by the Triton-purified transpeptidase means that the sedimentation coefficient and Stokes radius determined previously were really parameters of the Triton-protein complex and not of the protein alone. Molecular weight calculations of the Triton-purified enzyme therefore depend on knowing the exact proportions of Triton and protein in the complex. This information was obtained by using the detergent binding assay developed by Clarke (33) (Fig. 5). The upper panel is a control experiment using the papain-purified transpeptidase. No binding of \(^{3}H\)Triton is

Fig. 3. Determination of observed sedimentation coefficients of \( \gamma \)-glutamyltranspeptidase by sucrose gradient centrifugation. Linear sucrose gradients (5 ml) were prepared from solutions of 5 and 20% sucrose dissolved in either buffer (TRT, open circles) or buffer containing 1% Triton X-100 (+TRT, solid circles). Then, 250 \( \mu \)g of standard proteins and 50 \( \mu \)g of either Triton-purified (T-\( \gamma \)GT), papain-purified (P-\( \gamma \)GT), or papain-treated Triton-purified (PT-\( \gamma \)GT) transpeptidase in 100 \( \mu \)l were layered on top of gradients and centrifuged for 15 h, at 35,000 rpm in an SW 50.1 rotor at 20°. Gradients were pumped off from the bottom and collected in 40 to 50 fractions. Standard proteins were horseradish peroxidase (HRP) (34), glutamate oxalacetate transaminase (GOT) (35), alkaline phosphatase (AP) (36), and fumarase (FUM) (37). The data are plotted as percentage of migration into the gradient versus reported \( s_{20,W} \) values.

Fig. 4. Sedimentation of Triton-purified \( \gamma \)-glutamyltranspeptidase (T-\( \gamma \)GT) in H\(_2\)O and D\(_2\)O sucrose gradients. The 250 \( \mu \)g of standard proteins and 50 \( \mu \)g of transpeptidase in 100 \( \mu \)l were applied to the top of 5-ml linear gradients prepared from 5 and 20% sucrose dissolved in either H\(_2\)O (open circles) or D\(_2\)O (solid circles) buffer solutions containing 1% Triton X-100. Gradients and standard proteins were handled the same as described in the legend to Fig. 3 including alcohol dehydrogenase (ADH) (40). Data was plotted as percentage of migration into the gradient versus reported \( s_{20,W} \) values. HRP, horseradish peroxidase; AP, alkaline phosphatase; FUM, fumarase.
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**FIG. 5.** Binding of γ-glutamyltranspeptidase to [3H]Triton X-100. Linear 5-ml gradients were prepared from 5 and 20% sucrose dissolved in buffer containing 0.05% [3H]Triton X-100. Either 650 μg of papain-purified transpeptidase (PyGT) dissolved in buffer containing 0.1% [3H]Triton X-100 or 180 μg of Triton-purified transpeptidase (TyGT) dissolved in buffer containing 0.05% [3H]Triton X-100 and 0.5% unlabeled Triton X-100 were layered on the gradients, centrifuged for 15 h at 34,000 rpm in an SW 50.1 rotor at 20°C and pumped off from the bottom in 35 to 40 fractions. Aliquots (50 μl) from each fraction were used for protein determination (open circles) and for determining radioactivity (solid circles).

The data in the lower panel indicates that the Triton-purified enzyme extensively binds [3H]Triton. Both gradient profiles had corresponding protein and activity peaks (not shown) and an unexplainable small peak of radioactivity at the top of the plateau. This was also present in the control gradient layered with only buffer. The binding ratio obtained from averaging across the protein peak is 1.16 mg of Triton/mg of protein. Assuming the partial specific volume of Triton X-100 is 0.908 cm³/g (41) and using the same partial specific volume and percent of carbohydrate as used for the papain-purified enzyme for the glycoprotein portion, the partial specific volume of the complex was calculated to be 0.806 cm³/g. Using this value, the Stokes radius of 70 Å and the $s_{20, w}$ of 4.15 S, the molecular weight of the Triton-purified transpeptidase in Triton X-100 is 169,000. The glycoprotein portion would be 51.5% of this weight or 87,000 and the Triton X-100 would contribute 82,000 to the molecular weight of the complex. This value is in good agreement with the reported value for the molecular weight of a Triton X-100 micelle.

If the Triton-purified transpeptidase protein was binding a micelle its solubility in a detergent solution would depend on the presence of micelles. A direct test of this possibility is shown in Fig. 6, where constant amounts of protein were resuspended in solutions containing various concentrations of Triton X-100 and then centrifuged through gradients containing the same concentration of Triton X-100. The sharp transition from a 15 S species in 0.01% Triton X-100 to a 5 S species in 0.05% Triton X-100 is consistent with the reported critical micelle concentration of Triton X-100 of 0.016% (41). This evidence supports the conclusion that the Triton-purified transpeptidase binds to micelles of Triton X-100. Assuming a monomer molecular weight of Triton X-100 to be 640, this corresponds to about 130 molecules of Triton X-100 bound to every molecule of enzyme.

Comparing the molecular weights of the papain-purified transpeptidase (68,000) and the Triton-purified enzyme (87,000) the initial assumption of the former being a degradation product of the latter seems reasonable. If the two forms of enzyme differ by 19,000 molecular weight, this should be detectable by sodium lauryl sulfate-polyacrylamide gel electrophoresis. As shown in Fig. 7, the larger subunit of the papain-purified enzyme is smaller than the corresponding subunit from the Triton-purified enzyme. Again, treatment of the Triton-purified transpeptidase with papain or bromelain produced a form of the enzyme indistinguishable from the papain-purified transpeptidase on sodium lauryl sulfate-polyacrylamide gel electrophoresis (Fig. 8). Using standard proteins to determine molecular weight on sodium lauryl sulfate-polyacrylamide gels (Fig. 9) the smaller identical subunits were determined to be 51,000 and 54,000 for the papain-
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FIG. 7 (left). Sodium lauryl sulfate-polyacrylamide gel electrophoresis of Triton- and papain-purified γ-glutamyltranspeptidase. A slab of 10% polyacrylamide containing 0.1% sodium lauryl sulfate (27) was layered with alternating 15-μg samples of Triton-purified (T) and papain-purified (P) transpeptidase containing 1% sodium lauryl sulfate, electrophoresed for 8 h with a 75-mA current, and stained for protein as described under "Experimental Procedures."

FIG. 8 (right). Sodium lauryl sulfate-polyacrylamide gel electrophoresis of various forms of γ-glutamyltranspeptidase. Electrophoresis of Triton-purified (T), papain-purified (P), papain-treated (PT), and bromelain-treated (BT) Triton-purified transpeptidase was carried out as described in legend to Fig. 7.

purified and the Triton-purified enzyme, respectively. The sum of subunit molecular weights (81,000) is less than the calculated molecular weight for the Triton-purified enzyme (87,000). Whereas, the combined molecular weights for the subunits of the papain-purified transpeptidase (78,000) is significantly larger than its estimated molecular weight (68,000). Thus the difference in molecular weight of the papain- and Triton-purified transpeptidase glycoprotein portion is estimated to be between 3,000 and 19,000. A summary of these data is given in Table 1.

DISCUSSION

The data presented regarding the behavior of the two forms of γ-glutamyltranspeptidase during gel filtration, sucrose gradient centrifugation in water and deuterium oxide, and Triton X-100 binding assays indicate that the Triton-purified transpeptidase binds micelles of Triton X-100 approximately equivalent to its own weight and that it undergoes aggregation in the absence of detergents. Proteolysis by papain or bromelain removes the micelle binding site leaving the catalytic site intact and the protein soluble in aqueous buffer. This micelle binding site appears to be a small peptide from the larger of the two subunits of the enzyme which is equal in size to the difference in the molecular weights of the papain- and Triton-purified transpeptidases (3,000 to 19,000).

The large range of molecular weights for this peptide exists because all of the methods used for molecular weight determinations have some inherent error. The papain-purified transpeptidase molecular weight of 66,700 obtained from sedimentation equilibrium analysis is probably the most accurate value. The only uncertain term in its calculation is the partial specific volume which was determined from the amino acid and carbohydrate composition reported by Tate and Meister (15). But, the value of 0.711 cm³/g is a reasonable estimate for a protein containing 18.8% carbohydrate. The molecular weight of 69,000 calculated from Stokes radius, ɛ\textsubscript{par}, and partial specific volume is in good agreement. The higher molecular weight of 78,000 obtained from sodium lauryl sulfate-polyacrylamide gel electrophoresis probably reflects the high content of carbohydrate in the transpeptidase. Glycoproteins are known to yield anomalously high molecular weights on sodium lauryl sulfate gels (42).

In contrast, the molecular weight of 81,000 for the Triton-

physiological distribution of the enzyme and its subunit molecular weights (27).

FIG. 9. Estimation of subunit molecular weight of γ-glutamyltranspeptidase by sodium lauryl sulfate-polyacrylamide gel electrophoresis. The 10- to 15-μg samples of standard proteins, Triton-purified (T-γGT), and papain-purified (P-γGT) transpeptidase in 1% sodium lauryl sulfate were layered separately on a 10% polyacrylamide slab gel with a 4% polyacrylamide stacking gel both containing 0.1% sodium lauryl sulfate (26) and electrophoresed for 4 h with a 25-mA current. The slab was stained for protein as described under "Experimental Procedures" and RF values were determined by measuring the distance to the leading edge of the protein staining bands and the dye front (bromphenol blue). The standard proteins were: 1, bovine serum albumin; 2, catalase; 3, pyruvate kinase; 4, glutamate oxalacetate transaminase; 5, fumarase; 6, ovalbumin; 7, aldolase; 8, yeast alcohol dehydrogenase; 9, lactate dehydrogenase; 10, malate dehydrogenase; 11, chymotrypsinogen; 12, trypsin; 13, papain; and 14, myoglobin. The data are plotted as log of reported molecular weights (27) versus RF.
purified transpeptidase obtained from sodium lauryl sulfate-polyacrylamide gel electrophoresis is slightly smaller than the calculated molecular weight of 87,000. Both of these methods for estimating molecular weight have greater possibilities for error associated with them. Estimation of the molecular weight of the Triton-purified transpeptidase from sodium lauryl sulfate gels is complicated by the presence of carbohydrate which the percentage of acrylamide was varied from 5 to 15% resulted in the same estimates of molecular weight for the individual subunits. Therefore, the only reasonable conclusion which can be made from the sodium lauryl sulfate gels is that the two forms differ in one subunit and that the Triton-form of the enzyme is probably larger.

The calculated molecular weight of 87,000 for the Triton-purified transpeptidase is probably more accurate. The estimated binding ratio of Triton X-100 to protein is probably the potential source of the greatest error in this calculation. But the fact that the estimated molecular weight of the Triton X-100 component of the complex (82,000) is very similar to the reported molecular weight of a Triton X-100 micelle suggests that the calculated molecular weight for the glycoprotein portion of the complex is reasonably accurate. The molecular weight of a micelle of sized homogeneous Triton X-100 was reported by Robinson and Tanford (41) to be 64,000 to 76,800. A more accurate determination of the size of the Triton X-100-binding peptide will require its isolation and characterization.

The range of the estimated molecular weight of the Triton X-100 micelle-binding peptide (3,000 to 19,000) is reasonable in comparison to studies with well characterized membrane proteins. Cytochrome b₅ has a molecular weight of 16,200. Tryptic cleavage produces an Mₛ = 10,300 protein containing the active site and a 40 amino acid peptide of Mₛ = 4,600 containing 60% hydrophobic amino acids (43). Studies by Robinson and Tanford (41) have shown that cytochrome b₅ binds micelles of various detergents including Triton X-100, deoxycholate, and sodium lauryl sulfate and aggregates in the absence of detergent. The Mₛ = 10,300 protein produced by trypsin cleavage no longer binds micelles and is soluble in aqueous buffers. Spatz and Strittmatter (44) have also shown that intact cytochrome b₅ can bind to lecithin vesicles and to microsomal membranes. A similar example is cytochrome b₆, reductase, which has a molecular weight of 45,000. Lysosomal treatment produces a catalytically active protein of Mₛ = 33,000. Of the 99 amino acids removed, 65% are hydrophobic (44). The intact protein has also been purified and it binds to lecithin vesicles (45).

The data presented here indicate that γ-glutamyltranspeptidase contains a peptide sequence that acts as a nucleus for formation of or insertion into Triton X-100 micelles. The similarity of these results to those obtained with cytochrome b₅ and cytochrome b₆ reductase suggest that γ-glutamyltranspeptidase is inserted into the brush border membrane using this peptide as an anchor. The remaining heavily glycosylated hydrophilic portion of the enzyme which the active site would then be free to move in the aqueous environment beyond the membrane lipids. Due to the high content of carbohydrate and its solubility in aqueous buffers it is unlikely that the active site ever diffuses across the membrane or acts within the membrane. Therefore, the catalytic activity of γ-glutamyltranspeptidase is probably restricted to one side of the membrane. Previous experiments from our laboratory (46) using intact kidney cells and by Wendel et al. (6) using isolated kidney tubules suggest that γ-glutamyltranspeptidase is localized on the brush border membrane in the lumen of the proximal straight tubule.

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Preparation of γ-Glutamyltransferase

The Triton-X-100 solubilized enzyme was routinely converted to a water-soluble form by incubation with either 0.04 mg papain per mg protein for 3 hours at 37°C or 0.04 mg bromelain per mg protein for 3 hours at 37°C in 10 mM mercaptoethanol. These incubations had no effect on enzymatic activity and these new forms of transpeptidase could be quantitatively recovered without detergent by acetone precipitation as described above.

Solubilization of γ-Glutamyltransferase from rat kidney brush border membranes by incubation with either papain or Triton X-100 has no effect on its catalytic activity but the two preparations had noticeably different physical properties. The papain treatment produced a transpeptidase that remained soluble in aqueous buffers throughout its subsequent purification (457-fold). In contrast, the Triton-X-100 extracted transpeptidase was soluble only in buffers which contained detergent even after removal of contaminating lipids by acetone precipitation. This form of the enzyme was purified 300-fold using similar procedures but carried out in the presence of detergent. A summary of the purification scheme is presented in Table 1a.

Table 1a

| Step | Volume | Protein Activity | Specific Activity |
|------|--------|------------------|------------------|
|      | ml     | mg               | umoles·min⁻¹·mg⁻¹ |
| Solubilization by Triton |        |                  |                  |
| Crude homogenate | 1,500   | 30,000           | 48,000           |
| Microsomes | 100     | 2,920            | 12,000           |
| Acesone extraction | 13      | 429              | 30,500           |
| Sephadex G-200 column | 110     | 126              | 17,600           |
| Con A-Sepharose column | 140     | 74               | 17,600           |
| Hyaluronic acid column | 116     | 20               | 8,920            |

Solubilization by Papain

Crude homogenate | 1,500   | 30,000           | 48,000           |
Microsomes | 100     | 2,920            | 12,000           |
Sephadex G-200 column | 150     | 205              | 21,350           |
Con A-Sepharose column | 88      | 29               | 11,590           |
Ammonium sulfate | 2       | 17               | 8,920            |

The Triton-X-100 solubilized transpeptidase was routinely converted to a water-soluble form by incubation with either 0.04 mg papain per mg protein for 3 hours at 37°C or 0.04 mg bromelain per mg protein for 3 hours at 37°C in 10 mM mercaptoethanol. These incubations had no effect on enzymatic activity and these new forms of transpeptidase could be quantitatively recovered without detergent by acetone precipitation as described above.

Solubilization of γ-Glutamyltransferase from rat kidney brush border membranes by incubation with either papain or Triton X-100 has no effect on its catalytic activity but the two preparations had noticeably different physical properties. The papain treatment produced a transpeptidase that remained soluble in aqueous buffers throughout its subsequent purification (457-fold). In contrast, the Triton-X-100 extracted transpeptidase was soluble only in buffers which contained detergent even after removal of contaminating lipids by acetone precipitation. This form of the enzyme was purified 300-fold using similar procedures but carried out in the presence of detergent. A summary of the purification scheme is presented in Table 1a.

This preparation of a detergent solubilized transpeptidase represents a significantly higher fold purification of this enzyme than reported previously. Previous attempts to purify γ-glutamyltransferase from rat kidney using deoxycholate (17) or Lubrol (18) extraction of membranes resulted in less than a 100-fold purification. The Triton-X-100 solubilized transpeptidase is of approximate 95% purity (25% SDS polyacrylamide gel electrophoresis assuming that the high molecular weight contaminant stains equally well for proteins (Fig. 6). Our papain-solubilized enzyme appears homogenous on native and SDS polyacrylamide gel electrophoresis (Figs. 1, 2, 3) and is probably very similar to the homogenate-solubilized transpeptidase purified 500-fold from rat kidney by Tate and Meister (15).
Comparison of the size and physical properties of gamma-glutamyltranspeptidase purified from rat kidney following solubilization with papain or with Triton X-100.

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