Purification and Partial Characterization of Transglutaminase from *Physarum polycephalum*

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An intracellular form of calcium ion-dependent transglutaminase (R-glutaminylpeptide:amine γ-glutaminyltransferase, EC 2.3.2.13) was purified 818-fold to apparent homogeneity from acetone powder preparations of spherules of the acellular slime mold *Physarum polycephalum*. The enzyme was purified by combined methods of precipitation with 15% (wt/vol) polyethylene glycol, DEAE-cellulose chromatography, and isoelectric focusing in a pH 5 to 7 gradient. The isoelectric point of the enzyme was 6.1. The molecular mass of the denatured enzyme was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to be 39.6 kDa. A molecular weight of 77,000 was found by gel filtration of the native enzyme on a Superose 12 fast protein liquid chromatography column, indicating that the native functional protein is a dimer. The purified transglutaminase catalyzed the incorporation of [14C]putrescine into protein substrates including casein, N,N′-dimethylcasein, actin purified from *P. polycephalum*, and actin purified from bovine muscle. Actin was the preferred substrate for the enzyme, both as a purified protein and in crude extracts prepared from *P. polycephalum*. With N,N′-dimethylcasein as the amine acceptor substrate, [14C]putrescine, [14C]spermine, and [14C]permine were all effective amine donor substrates with *Km* values of 49, 21.4, and 31.7 μM, respectively. All three of these polyamines demonstrated strong substrate inhibition of the enzyme activity between 100 and 200 μM. Upon starvation induced by depletion of a carbon source for growth, the specific activity of this enzyme increased sixfold during the differentiation of *P. polycephalum* microplasmodia to spherules. This suggests a role for transglutaminase in the construction of spherules, which have the capacity to survive starvation and dessication.

Transglutaminases (TGase; R-glutaminylpeptide:amine γ-glutaminyltransferase, EC 2.3.2.13) enzymes catalyze a number of conjugation and cross-linking reactions at glutaminyl amino acid side chains in select proteins (9). TGases have been investigated extensively in animals, where they are widely distributed in various tissues, organs, and extracellular fluids (9). A number of biological functions have been proposed for extracellular TGases in animal systems. These functions include formation of the fibrin clot as a terminal step in the blood clotting cascade (6), ionophore-induced hardening of the erythrocyte membrane (32), keratinization of the epidermis and hair structures (38), and participation in receptor-mediated endocytosis (7). In some cases, specific physiological protein substrates for TGases have been identified. Loricin (18), involucrin (35), fibronectin (2), vitronectin (43), keratinin in the human epidermis (47), fibrin in animal blood sera (47), and the large subunit of ribulose 1,5-bisphosphate carboxylase-oxygenase in plants (33) are known targets of TGase.

The important roles of TGases in cellular processes involving blood clotting, wound healing, envelope formation of the stratum corneum, coagulation of semen, neuronal differentiation, and stabilization of the cell cytoskeletal network have prompted extensive efforts to determine the structures of various TGase enzymes. The primary structure of human plasma factor XIIIa (a TGase [22]), guinea pig liver TGase C (20), and human epidermal TGase K (23) have all recently been established by combined cDNA cloning and amino acid sequence analysis. These investigations have made it possible to analyze and compare the structures of these proteins and their corresponding genes (21). TGase C, factor XIIIa, and TGase K have 690, 731, and 813 amino acids with molecular masses of 76.6, 83.1, and 89.3 kDa, respectively. As expected, these proteins all demonstrate major degrees of homology in their respective sequences. Interestingly, a pentapeptide sequence, Tyr-Gly-Gln-Cys-Trp, is conserved around the active-site cysteine in all TGases sequenced to date.

Few works have been published on TGases from microbial sources. A recent presumptive report suggested that the enzyme may exist in *Bacillus subtilis* (40). The purification of a calcium ion-independent extracellular TGase from the culture filtrate of *Streptoverteicillium* sp. strain S8112 (1) was reported during the course of the present study.

The purification of TGase from *Physarum polycephalum* was undertaken because all previously isolated TGases were derived from mammalian sources. In addition, the functional role of TGases in stiffening of cellular or membrane structures such as the erythrocyte membrane, semen coagulation, and cornification of epithelial cells suggested a possible similar function in the differentiation of vegetative plasmodia to spherules by *P. polycephalum* (42). The purified enzyme would provide the means to obtain antibodies and nucleotide probes to study this process in *P. polycephalum* and the expression of this enzyme in other fungi.

**MATERIALS AND METHODS**

Organism, growth conditions, and acetone powder preparation. The organism used in this study was *P. polycephalum* M2cV. The procedures used for maintaining stock cultures,
growth of vegetative microplasmodia (4), spherulation (24), and sampling and harvesting of microplasmodia during spherulation (24), were described previously. Microplasmodia or spherules (100 ml, wet-packed volume) were suspended in 1 liter of chilled acetone in a cold Waring blender. The suspension was blended at low speed for 2 min and then filtered through a Whatman no. 1 filter on a Büchner funnel. The cell cake was rinsed with chilled acetone on the funnel until the filtrate was colorless. The acetone-dried powder was air dried in a hood overnight and then stored at −50°C in a sealed jar. Approximately 5 g of powder was obtained from 100 ml of wet-packed cell mass. Powders prepared and stored by this procedure retained full TGase enzyme activity for at least 3 years.

Purification of TGase. Crude extracts were prepared from acetone powders derived from 96-h-old shake cultures of spherules. A 5-g sample of powder was suspended with stirring into 200 ml of TCEM buffer (50 mM Tris-HCl, 5 mM CaCl₂, 2 mM free acid EDTA, 5 mM 2-mercaptoethanol [pH 8.5]) at ice temperature for 20 min. The suspension was thoroughly homogenized in a cold glass Potter-Elvehjem homogenizer and then centrifuged at 100,000 × g for 1 h at 2°C. The supernatant fraction was allowed to warm to room temperature and then brought to 15% (wt/vol) polyethylene glycol (PEG; molecular weight, 6,000 to 8,000; 65 ml of 50% [wt/vol] PEG solution prepared in TCEM buffer per 150 ml of protein solution) by dropwise addition over a 1-h period. The suspension was stirred for another 30 min at room temperature. Precipitated protein was collected by centrifugation at 15,000 × g for 45 min at 20°C. The resulting protein pellet could be stored at −20°C for at least 3 years with full retention of TGase activity. All subsequent procedures were conducted at 2°C. TGase was resolubilized by trituration from the PEG pellet in cold TCEM buffer. The redissolved protein was applied to a column of Whatman DE52 DEAE-cellulose (2.5 by 8 cm) equilibrated in TCEM buffer (sample volume, 40 ml; total protein, 35 mg; flow rate, approximately 60 ml/h; fraction volume, 10 ml). Elution was conducted with TCEM buffer. Enzymatic activity was generally found in the initial fractions, indicating that TGase did not bind to the DEAE-cellulose. These fractions were combined and further purifying through a 5- to 7 gradient of sucrose gradient prepared in 1% ampholyte (Ampholine 5-7; LKB Pharmacia, Piscataway, N.J.). The lower electrolyte reservoir contained 450 g of sucrose, 450 g of water, and 30 ml of 1 M NaOH. The upper electrolyte reservoir contained 10 ml of concentrated H₃PO₄ diluted to 1 liter with water. Up to six glass tubes, each containing 90 μl of enzyme preparation from DEAE-cellulose chromatography, were focused simultaneously in a Bio-Rad model 150A electrophoresis chamber at 300 V for 5 h and then at 750 V for 1.5 h. Fractions (0.25 ml) were collected from each focusing tube and assayed for TGase activity. Fractions with a pH centering at 6.1 contained the enzyme. The enzyme recovered after isoelectric focusing was judged to be pure by subsequent electrophoretic analysis.

Biochemical characterization. The molecular mass of de-natured TGase was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by the method of Laemmli (26). The gel contained 12.5% acrylamide and was stained with silver by the method of Morrissey (36). Standard proteins were used as indicated to construct a standard curve relating molecular mass to mobility. The molecular mass of the native enzyme was determined by gel filtration with fast protein liquid chromatography with a Superose 12 gel filtration column (Pharmacia) that was equilibrated with potassium phosphate buffer (50 mM; pH 7) supplemented with 0.1 M KCl. A sample of enzyme preparation purified through DEAE-cellulose was dialyzed against the equilibration buffer and applied to the column (sample volume, 0.2 ml; total protein, 2 mg; flow rate, 0.3 ml/min; fraction volume, 0.5 ml). The molecular mass standards were alcohol dehydrogenase (150 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), trypsin inhibitor (21.5 kDa), and cytochrome c (12.3 kDa).

TGase assays and kinetic studies. TGase assays measured the enzyme-catalyzed incorporation of [14C]putrescine into trichloroacetic acid-insoluble protein adapted to the filter paper assay method of Lorand et al. (29). The final reaction mixture at pH 7.5 and in 105 μl of final volume contained 0.27 mg of N,N'-dimethyl casein, 4.5 μmol of Tris-HCl, 0.25 μmol of CaCl₂, 0.10 μmol of dithiothreitol, 0.0067 μmol of [14C]putrescine (73.9 mCi/mmol; DuPont-New England Nuclear Corp.), and a maximum of 0.5 μg of enzyme preparation. Reactions were initiated by the addition of enzyme protein. The reaction was conducted at 30°C for 45 min and quenched by pipetting a 75-μl sample onto a Whatman 3MM paper disc 23 mm in diameter. The disc was immediately incubated in 10% trichloroacetic acid for 15 min, 5% trichloroacetic acid for 15 min, and acetone for 1 min, dried at 50°C, and counted in 3.5 ml of scintillation cocktail. Enzyme activities were determined in duplicate, triplets, and normalized to protein content (33) and are presented as averages of these determinations. Product formation was always checked for linearity with time and the amount of protein preparation added. Where indicated, [14C]putrescine was substituted by other radiolabeled polyamines or N,N'-dimethyl casein was substituted by other protein substrates to which [14C]putrescine was conjugated by TGase activity. Actin was also purified from 96-h-old shake cultures of spherules of P. polycystum by the method of Hatano and Osawa (14, 15).

Identification of endogenous substrate protein for TGase in crude extracts with monodansylcadaverine and immunodetection of actin. A sample of crude extract proteins from actinomas of several plates was mixed into 1 ml of 12.5% polyacrylamide, 3 mm thick) and electrophoresed at 20 V for 15 h. Detection and recording of protein zones in the gel that were covalently conjugated to monodansylcadaverine were achieved by using UV-activated fluorescence.

A second 50-μl sample of the reaction mixture was similarly fractionated as described above and then transferred to a nitrocellulose filter (0.2-μm pore size; Bio-Rad Laboratories, Richmond, Calif.) essentially by the method of Towbin et al. (46). Transfers were conducted with 25 mM Tris–192 mM glycine–20% (vol/vol) methanol–0.1% (wt/vol) SDS. Electrophoretic transfer was conducted for 20 h at about 20 V and 20°C. Actin thus transferred to the nitrocellulose sheet was detected immunologically with a commercial polyclonal antibody prepared in mice against actin that
FIG. 1. Specific activity of TGase during differentiation of plasmodia of *P. polypephalum* to spherules. An inoculum of vegetative microplasmodia was transferred into six 500-ml culture flasks containing 100 ml of growth medium per flask (24). At each of the times indicated (△), the cell mass from one flask was harvested, washed, and assayed for TGase activity; the data are presented as micro- moles of [14C]putrescine incorporated into N,N’-dimethylcasein per minute per milligram of protein versus hours of incubation of the cell culture in the growth medium. At each of the times indicated (●), a 5-ml sample of culture suspension was removed from a single culture flask, the cell mass was removed by centrifugation, and the supernatant culture medium was analyzed colorimetrically at 490 nm for its residual content of glucose by the phenol-sulfuric acid method (1).

was purified from *Amoeba proteus* clone KJ 43A (Sigma Chemical Co., St. Louis, Mo.). Enzyme-linked immunosorbent assays were conducted on the transblotted nitrocellulose with the primary mouse actin antibody at 30 μg/ml in 10 mM sodium phosphate (pH 7.5)-0.1% Tween 20 (Sigma) (PBST). The secondary antibody, alkaline phosphatase-linked rabbit anti-mouse antibody (Tago Inc., Burlingame, Calif.), was diluted to 2.5 μg/ml in PBST. The alkaline phosphatase substrate solution of 4-chlorophenol-1-iodophosphate and the chromogen nitroblue tetrazolium were prepared and used as prescribed previously (27).

**RESULTS**

**TGase expression in *P. polypephalum***. Assayable TGase enzymatic activity was induced in *P. polypephalum* during differentiation of microplasmodia to spherules. A predictable schedule of increased intracellular TGase activity accompanied the starvation of vegetative microplasmodia in spent growth medium in which the carbon source, glucose, had been depleted during the first 30 h of growth. A nearly sixfold increase in specific activity of TGase accompanied this differentiation (Fig. 1). During the 96-h period during which TGase activity accumulated, the soluble protein isolated from harvested cell material decreased by no more than 40%. Thus, the rise in specific activity of TGase could not be ascribed to the selective loss of other cellular proteins that are normally utilized by starving microplasmodia. No TGase activity was detected in the extracellular growth medium throughout these experiments, thus indicating that enzymatically active TGase was not excreted during differentiation.

Acetone powders were prepared from 96-h cultures of *P. polypephalum* that had formed spherules as described in the legend to Fig. 1. These powders were the starting material for purification of TGase.

**Purification of TGase**. The purification scheme for TGase from 5 g of acetone powder-treated *P. polypephalum* is given in Table 1. SDS-PAGE under denaturing conditions indicated that the protein preparation obtained after isoelectric focusing was free of detectable contaminants in a 0.6-μg sample (Fig. 2A). Measurements of enzymatic activity were conducted on sectioned gel slices after PAGE of the purified enzyme under nondenaturing conditions (Fig. 2B). The protein band in Fig. 2B migrated in a gel slice that showed enzyme activity and thus represented the TGase enzyme. The enzyme was purified 818-fold with an overall yield of 54%. This implies that the enzyme represents about 0.12% of the total soluble cellular protein in acetone powder preparations of *P. polypephalum* spherules, assuming that the purified enzyme retains the specific activity of the enzyme in crude extracts.

**Molecular mass**. The molecular mass of the TGase was estimated to be 39.6 kDa by SDS-PAGE (Fig. 2A). A molecular weight of 77,000 was found by gel filtration of the native enzyme on a Superose 12 column (fast protein liquid chromatography) (data not shown). These combined results indicated that TGase from *P. polypephalum* was a dimeric protein consisting of two monomers with identical electrophoretic mobilities.

**Substrate and cofactor requirements**. The purified TGase was totally dependent on calcium ions for catalytic activity (Table 2). Conditions or treatments that efficiently sequestered calcium ions from TGase preparations, such as EDTA, dialysis, or EGTA, irreversibly inactivated the enzyme (Table 2).

As reported for other TGases (20, 22, 23), reagents such as N-ethylmaleimide and p-chloromercuribenzoate that alkylate free sulfhydryl groups inactivated the purified enzyme.

Three protein substrates were identified to which purified TGase catalyzed conjugation of [14C]putrescine. Both casein and N,N’-dimethylated casein could serve as amine acceptors. N,N’-Dimethylation of the ε-amino groups on lysine residues in casein improved its capacity to serve as a substrate fivefold (Table 2), suggesting that free lysine side chains competed with putrescine as amine donors in unmodified casein. Actins isolated from *P. polypephalum* and from bovine muscle were the best protein substrates found. Bovine serum albumin, bovine myosin, histone mixture, human serum fibronectin, and ribulose 1,5-bisphosphate carboxyl-
cross-reacted with a polyclonal mouse antibody prepared against actin (Fig. 3D).

The purified TGase catalyzed incorporation of the polyamines \([^{14}\text{C}]\text{putrescine}\) (Fig. 4), \([^{14}\text{C}]\text{ spermidine}\) (data not shown), and \([^{14}\text{C}]\text{spermine}\) (data not shown) into \(N,N'\)-dimethylcasein. The \(K_m\) values for these three polyamines were 49 \(\mu\text{M}\) for putrescine, 21.4 \(\mu\text{M}\) for spermidine, and 31.7 \(\mu\text{M}\) for spermine. The ratio of the respective maximum rates of metabolism was 4.92:1.00:1.57 for putrescine/spermidine/spermine (data not shown). Figure 4 (inset graph) also illustrates substrate inhibition of TGase at putrescine concentrations exceeding about 100 \(\mu\text{M}\). Spermidine and spermine also elicited similar incipient inhibition at about 100 \(\mu\text{M}\).

**DISCUSSION**

Spherule development in *P. polycephalum* is completed within about 35 h after the depletion of a carbon source in the growth medium (42). The results reported here demonstrate that TGase began to accumulate in microplasmodia as glucose became limiting and continued to increase in spherules to at least six times the level found in growing vegetative microplasmodia. No detectable TGase enzymatic activity was found in the extracellular culture medium throughout spherule formation. Numerous enzymes associated with protein (12, 39), amino acid (19), and carbohydrate (24) catabolism and complex polysaccharide biosynthesis (16) have been reported to increase in specific activity during spherule formation in *P. polycephalum*. The sixfold increase in TGase specific activity observed in the current work ranks among the larger accumulations of these enzymes. The coincidence of an increase in TGase activity with the initial depletion of the carbon source, glucose, in the growth medium and the subsequent continuous accumulation of the enzyme throughout the period of spherule formation suggest a role for protein cross-linking activity in the conversion of microplasmodia to spherules. During the formation of spher-

![FIG. 2. PAGE of 0.6 \(\mu\text{g}\) of purified TGase under denaturing conditions in a 12.5% acrylamide gel containing SDS and silver stained by the method of Morrissey (36) (lane A), 10 \(\mu\text{g}\) of purified TGase under non-denaturing conditions in a 7.5% acrylamide gel (8) and silver stained (lane B), and standard molecular weight marker proteins (2 \(\mu\text{g}\) of each) stained with silver reagent (phosphorylase B [top arrow], 92.5 kDa; bovine serum albumin, 66.2 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 31 kDa; soybean trypsin inhibitor, 21.5 kDa; and lysozyme [bottom arrow], 14.4 kDa) (lane C).](http://jb.asm.org/)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of maximum activity</th>
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<tr>
<td>1. Complete*</td>
<td>100</td>
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<tr>
<td>2. Complete minus Ca**</td>
<td>0</td>
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<tr>
<td>3. Complete plus 5 mM EDTA</td>
<td>0</td>
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<tr>
<td>4. Complete (enzyme dialyzed against 2 mM EGTA)</td>
<td>0</td>
</tr>
<tr>
<td>5. Complete plus 0.10 mM N-ethylmaleimide</td>
<td>7</td>
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<tr>
<td>6. Complete plus 0.10 mM p-chloromercuribenzoate</td>
<td>0</td>
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<tr>
<td>7. Complete minus dimethylcasein plus 0.27 mg of casein</td>
<td>14</td>
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<tr>
<td>8. Complete minus dimethylcasein plus 0.27 mg of actin from <em>P. polycephalum</em></td>
<td>282</td>
</tr>
<tr>
<td>9. Complete minus dimethylcasein plus 0.27 mg of actin from bovine muscle</td>
<td>230</td>
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* The complete reaction mixture contained 0.27 mg of \(N,N'\)-dimethylcasein, 4.5 \(\mu\text{mol}\) of Tris-HCl, 0.25 \(\mu\text{mol}\) of \(\text{CaCl}_2\), 0.10 \(\mu\text{mol}\) of dithiothreitol, and 0.0067 \(\mu\text{mol}\) of \([^{14}\text{C}]\text{ putrescine}\) (73.9 mCi/mmol). Each assay contained 0.13 \(\mu\text{g}\) of purified TGase dissolved in TCEM buffer except treatments 2 and 4. In treatment 2 the enzyme was purified and then dialyzed into TCEM buffer lacking calcium ion. In treatment 4 the enzyme was dialyzed for 8 h against TCEM buffer containing 2 mM ethyleneglycol-bis-(\(\beta\)-aminoethyl ether)\(N,N'\)-tetraacetic acid (EGTA) in place of 2 mM EDTA. The specific enzyme activity of purified TGase was 4.96 nmol of \([^{14}\text{C}]\text{putrescine}\) incorporated per min per mg of protein.
FIG. 3. Fluorescent detection of TGase-directed dansylation of endogenous proteins in a crude extract from acetone powder preparations of spherules of *P. polycephalum*. Lane A shows fluorescent protein bands (100 μg) in an SDS-PAGE gel (12.5% polyacrylamide) viewed by illumination of the polyacrylamide gel electrophoretogram from behind with a model TM-36 UV-transilluminator lamp (Chromato-Vue, San Gabriel, Calif.). Fluorescence was recorded by photography with technical pan film 2415 (Kodak) through a no. 504 red filter lens. The whitened zone in lane A indicates fluorescence by the dansyl moiety from monodansylcadaverine. Lane B shows crude extract proteins (100 μg) prepared in TCEM buffer electrophoresed in an SDS-PAGE gel (12.5% polyacrylamide, 3 mm thick) for 15 h at 20 V. Protein zones were stained with Coomassie blue dye. Lane C shows actin (ca. 10 μg) purified from spherules of *P. polycephalum* and treated as for lane B. Lane D shows crude extract proteins similar to those in lane B that were transblotted onto nitrocellulose and developed by an enzyme-linked immunosorbent assay to show stained gel zone that cross-reacted with antibody against actin. Lane E shows standard molecular weight marker proteins (Fig. 2).

ules, TGase may incorporate important biochemical modifications into select proteins that are critical to survival of the spherule against starvation and eventual dessication.

The purification procedure reported here has features that were important to isolating TGase in an enzymatically active form. First, the protocol does not include lengthy dialysis steps, which were found to yield large losses in TGase activity, especially in the early stages of the procedure. Second, precipitation with PEG as an early selective step in the protocol and collection of the precipitate by centrifugation yielded protein pellets that could be stored indefinitely at −20°C with retention of enzymatic activity. Third, the entire purification protocol could be completed within about 24 h beginning with acetone powder preparations of spherules. The purified enzyme was moderately stable when stored frozen at −20°C. The purification scheme for TGase from *P. polycephalum* is uncommonly efficient, since there are no procedures that require adsorption of the enzyme onto a chromatographic medium.

Both the precipitation of TGase by 15% PEG and subsequent treatment with DEAE-cellulose provided recoveries of enzyme activities that exceeded 100%. The enhanced recovery of TGase enzyme activity did not strictly correlate with removal of actin (data not shown). Actin coprecipitated with TGase upon addition of 15% PEG, but actin was removed by DEAE-cellulose treatment. Thus, the enhanced recovery of enzyme units was perhaps due to removal of inhibitory substances present in lesser purified preparations of the enzyme; these inhibitory substances could include proteins that were more efficient substrates than *N,N*-dimethylcasein but that did not precipitate with trichloroacetic acid in the enzyme assay protocol.

It should be noted that the specific activity reported here for the purified enzyme is a minimum value. The concentration of [14C]putrescine used in all assays during purification of TGase was about 64 μM. The *K*ₐ for putrescine was 49 μM. The submaximizing substrate conditions were necessitated by substrate inhibition, which was demonstrated by all polyamine substrates (Fig. 4).

The TGase from *P. polycephalum* shares several properties with TGases isolated from other sources, both mammalian and microbial. First, the molecular mass of 39,600 Da for the enzyme from *P. polycephalum* is similar to that of 40,000 Da reported for the enzyme isolated from another fungus, *Streptoverticillium* sp. strain 58112 (1), and that of 39,000 Da reported for the enzyme from the plant *Medicago sativa* L. (alfalfa) (25). However, unlike the latter two enzymes, which are calcium independent, the TGase from *P. polycephalum*  

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has an absolute requirement for the calcium ion. Thus, this enzyme is the smallest protein purified to date that demonstrates a calcium-dependent TGase activity. All TGases that have been purified and characterized from mammalian sources are calcium ion dependent and have molecular weights that are approximately double that of the nonmammalian enzymes (20, 22, 23). Second, TGase characterized from guinea pig liver (10), guinea pig hair follicle (5), bovine epidermis (3), and plasma factor XIII (5) all have a sulfhydryl group at the active site that is essential for activity. The enzyme from P. polycephalum also has a presumptive free sulfhydryl group that must be free for enzymic activity, since N-ethylmaleimide and p-chloromercuribenzoate, each at 0.1 mM, caused 93 and 100% loss of enzymatic activity, respectively. A conserved active site pentapeptide sequence Tyr-Gly-Gln-Cys-Trp may very well prove to be a feature of all TGases that exhibit inhibition by sulfhydryl alkylating agents (20, 22, 23).

Monodansycadaverine has been used successfully as a convenient fluorescent tracer of TGase-directed amine conjugation to substrate proteins in many types of biological tissues (31). The reagent has proved to be an efficient substrate for all known TGases by virtue of its capacity to function as a lysine substrate analog. The reagent was designed by Lorand et al. (30) for the specific purpose of demonstrating TGase-directed labeling of reactive-glycamyl- nyl sites in proteins with a dansylcadaverine fluorophore. Detection of protein-bound monodansycadaverine in SDS-polyacrylamide gels by fluorescence of the dansyl moiety suggested dominant conjugation to actin in crude extracts from P. polycephalum. The finding that the gel zone containing protein-bound monodansycadaverine also cross-reacted with an antibody prepared against actin (Fig. 3A and D) supports this hypothesis. The significance of this observation is not clear from the present work. However, covalently cross-linked matrices have been reported in plasmodia of P. polycephalum (11) in which the matrix has been shown to consist of superfine filaments 2 to 3 nm in width. Superfine filaments are cytoskeletal structures (41) that have been implicated as cross-linkers that interconnect microtubules, intermediate filaments, and actin filaments (17, 34, 37, 44). Indeed, e-(γ-glutamyl)lysine cross-links that are products of the catalytic action of TGase have been reported in the cytoskeleton proteins of P. polycephalum (28, 45). Thus, changes in the organization of the cytoskeleton of P. polycephalum that accompany spherule formation may involve extensive cross-linking processes that require actin or actin filaments.

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