Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 3442–3446, June 1982
Biochemistry

Post-translational modification of neuronal proteins: Evidence for transglutaminase activity in R2, the giant cholinergic neuron of *Aplysia*

(polyamines/γ-glutamylputrescine/microinjection/γ-glutamylamine cyclotransferase/microtubules)

RICHARD T. AMBRON* AND LEON T. KREMZNER†

*Departments of Anatomy and Cell Biology and †Rehabilitation Medicine, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Communicated by Seymour S. Cohen, February 26, 1982

ABSTRACT [^{3}H]Putrescine injected into the cell body of the giant neuron R2 of *Aplysia* was readily converted to γ-aminobutyric acid, acetylputrescine, spermidine, and spermine. In addition, labeled putrescine and spermidine were found covalently linked to protein through the action of an intracellular transglutaminase. This was shown by exhaustively treating the acid-insoluble fraction from injected cells with Pronase, aminopeptidase M, and carboxypeptidases A and B. High-performance liquid chromatography of the digest revealed labeled γ-glutamylputrescine and γ-glutamylspermidine, the products expected from the transglutaminase-catalyzed post-translational modification of intracellular proteins. *In vitro* assays of *Aplysia* nervous tissue showed the presence of transglutaminase as well as γ-glutaminyl cyclotransferase, an enzyme that cleaves the γ-glutamylpolyamine bond. Incorporation of polyamine into proteins in R2 is a specific process because only a few ^{3}H-labeled polypeptides were found after injection.

Transglutaminases (EC 2.3.2.13) are a family of calcium-dependent enzymes that crosslink proteins by catalyzing the formation of ε-(γ-glutamyl)lysine bonds: γ-carboxamide groups of peptide-bound glutamine are acyl donors and primary amino groups of peptide-bound lysine serve as acyl acceptors (1). It is the action of extracellular transglutaminases, for example, that mediates the covalent polymerization of fibrin during hemostasis (2). Transglutaminases also exist inside of cells (see ref. 1 for a review), and work on erythrocytes (3, 4) has shown that an intracellular form of the enzyme covalently links integral proteins of the surface membrane with those of the underlying spectrin network. The possibility that transglutaminase mediates interactions between membrane proteins and the cytoskeleton (5) is also indicated by the fact that actin (6) and myosin (7), as well as HLA-A and HLA-B histocompatibility antigens (5), are all substrates for the enzyme.

The role(s) of transglutaminase has now been extended by the finding that primary amino groups of the polyamines putrescine and spermidine are also able to act as acyl acceptors. In this case the polyamine becomes covalently linked to a glutamine moiety on an existing protein via a γ-glutamylpolyamine bond (8). As substrates, therefore, polyamines may modulate the cross-bridging activity of transglutaminase.

A biological system suitable for exploring the relationship between transglutaminase activity, cell surface proteins, and polyamines is the giant neuron R2 of *Aplysia californica*. The external membrane of R2 is known to contain several types of voltage-dependent calcium channels (9), and these can be manipulated electrically, thereby regulating the flow of calcium through the membrane. The external membrane also contains two partially characterized integral glycoproteins that have their carbohydrate moieties exposed on the surface and that are potential substrates for transglutaminase (10). Recently, we have shown that R2 activity metabolizes putrescine injected directly into the cell body (11). In the present paper we report that transglutaminase activity is present in *Aplysia* nervous tissue and that [^{3}H]putrescine injected into R2 can be used by transglutaminase to modify proteins by a post-translational covalent addition of the polyamine. A brief report of some of this work has already appeared (12).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

EXPERIMENTAL PROCEDURE

Aplysia californica, weighing between 50 and 250 g, were purchased from Pacific Biomarine (Venice, CA). In experiments designed to examine polyamine incorporation into total nervous tissue, the abdominal ganglion was excised, placed in 0.5 ml of a supplemented sea water (13) containing 50 μCi of [^{3}H]putrescine (New England Nuclear; 20.6 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) and incubated at 15°C for 24 hr. The tissue was then homogenized at 4°C in a ground glass grinder containing either 0.4 M perchloric acid or 5% trichloroacetic acid and the precipitate was collected by centrifugation. The pellet was dissolved in 0.1 M NaOH and precipitated again with acid. This cycle was repeated until no radioactivity appeared in the supernatant.

The metabolism of polyamines in single neurons was examined by using the identified cholinergic neuron designated R2 (14). [^{3}H]Putrescine was purified and micro-injected intrasomatically into R2 within the abdominal ganglion as described (11, 15). The tissue was maintained at 15°C for 24 hr and then the ganglion, containing R2's cell body, and the right connective, containing R2's major axon, were homogenized in trichloroacetic acid. Because injected putrescine is metabolized only in the injected cell (11), it was not necessary to separate R2 from surrounding tissue.

The washed ^{3}H-labeled protein fraction obtained after incubation or injection was dissolved in 0.1 M NaOH and the pH was adjusted to 8.0 at 37°C by addition of Tris·HCl. In order to show that labeled polyamine had been incorporated covalently, the protein fraction was digested at 37°C by sequential treatment with Pronase (18 hr) (Calbiochem), additional Pronase (8 hr), aminopeptidase M (65 hr), and carboxypeptidases A and B (24 hr) (Boehringer Mannheim) (2). After digestion, an equal volume of 10% trichloroacetic acid was added, the precipitate was removed, and the supernatant was analyzed for *N*-(γ-glutamyl)polyamines by using a column (0.5 × 7 cm) of HPAN 90 cation-exchange resin (Hamilton, Whittier, CA) eluted with a gradient of sodium chloride in sodium citrate (11).

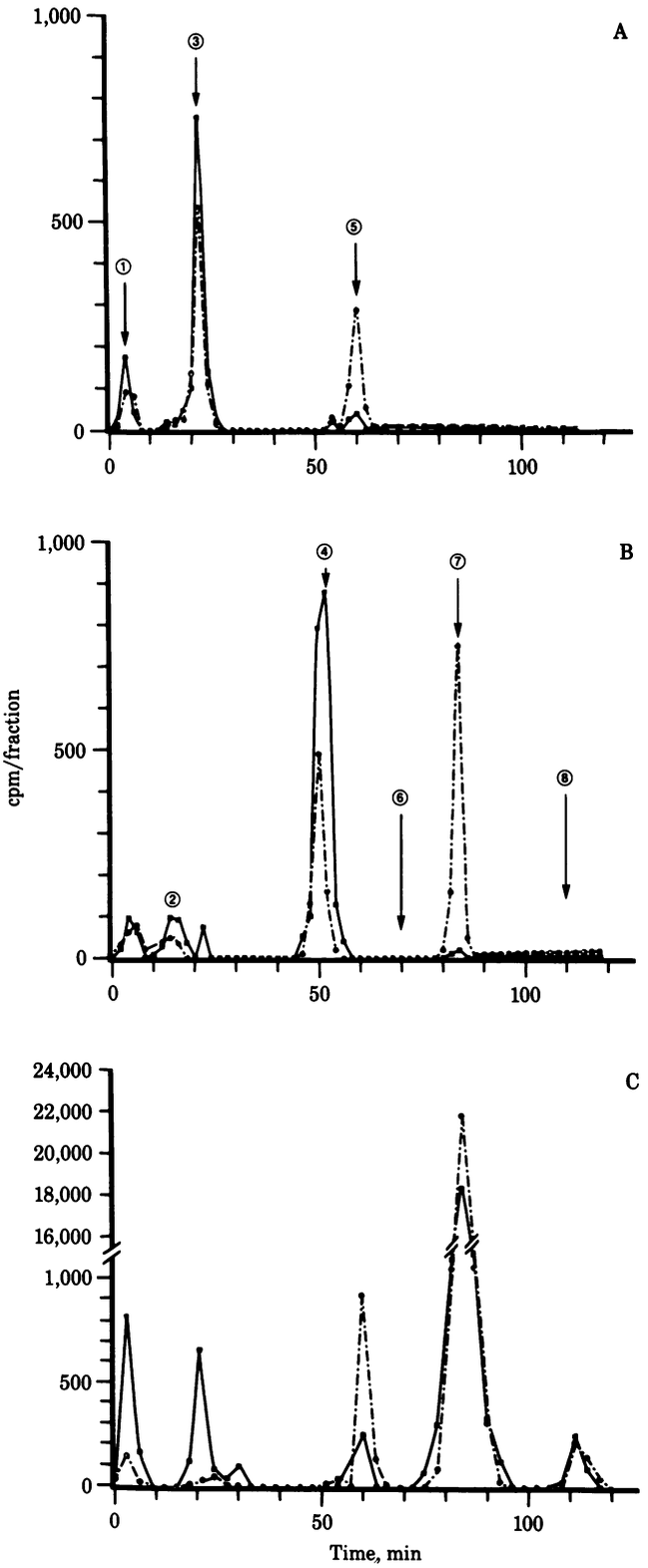

FIG. 1. Transglutaminase-catalyzed incorporation of polyamines into protein. Partially purified guinea pig liver transglutaminase (10 μg) was incubated for 10 min at 37°C in 0.280 ml of 50 mM Tris·HCl (pH 8.0) containing 100 μg of *Aplysia* muscle extract, 5 mM $CaCl_2$, 5 mM dithiothreitol, and 25 μCi of either [^{3}H]putrescine (*A*) or [^{3}H]spermidine (*B*). The reaction was stopped by adding ice-cold perchloric acid and the washed precipitate was treated with proteolytic enzymes. One-half of the digest was analyzed directly by ion-exchange chromatography (■——■); the other half was treated with γ-glutamylamine cyclotransferase and then analyzed (●·–·●). Alternatively, in some experiments the digest was treated with 6 M HCl at 105°C for 24 hr. Under these conditions all of the radioactivity was found as the free labeled polyamine. In *C*, an abdominal ganglion was incubated in 25 μCi of [^{3}H]putrescine. Twenty-four hours later the tissue was homogenized in perchloric acid and the washed precipitate was treated as above. ■——■, Direct analysis of the digest; ●·–·●, analysis after treatment with HCl. Comparable results were obtained by digestion with γ-glutamylamine cyclotransferase (not shown). The arrows indicate the positions of the authentic compounds: 1, bis(γ-glutamyl)-putrescine; 2, bis(γ-glutamyl)spermidine; 3, γ-glutamylputrescine; 4, N^1- and N^8-γ-glutamylspermidine; 5, putrescine; 6, γ-glutamylspermine; 7, spermidine; 8, spermine.

The column was standardized with authentic *N*-(L-γ-glutamyl)putrescine (Vega–Fox, Tucson, AZ), putrescine, and spermidine (Calbiochem): a mixture of the N^1 and N^8 forms of (L-γ-glutamyl)spermidine, N^1,N^4-bis(L-γ-glutamyl)putrescine, and N^1,N^8-bis(L-γ-glutamyl)spermidine standards were generous gifts of J. Folk. They were also prepared by proteolytic digestion of *N*,*N*′-dimethylcasein that had been incubated with [^{3}H]-putrescine or [^{3}H]spermidine (Amersham; 20 Ci/mmol) in the presence of transglutaminase partially purified from guinea pig liver (16). The identity of all derivatives formed *in vivo* or *in vitro* was verified by treatment of the enzymic hydrolyates with 6 M HCl or by digestion using γ-glutamylamine cyclotransferase (17). Both procedures liberate the labeled polyamine from the glutamyl conjugate.

Transglutaminase activity was assayed in the 105,000 × *g* supernatant from *Aplysia* nervous tissue by measuring the incorporation of [^{3}H]putrescine into *N*,*N*′-dimethylcasein in the presence of saturating levels of Ca^{2+} and the reducing agent dithiothreitol (16). In some experiments the activity of the enzyme was tested by using a crude high-salt extract of *Aplysia* body wall muscle that contained myosin heavy and light chains, as well as actin (D. Harris, A. Sherbany, and J. Schwartz, personal communication). The activity of γ-glutamylamine cyclotransferase in *Aplysia* nervous tissue was determined by using 10 mM γ-glutamylputrescine, 100 μl of a crude tissue extract (50 μg of protein) in 300 μl 200 mM sodium phosphate buffer (pH 7.5) for 3 hr at 24°C. Activity was measured chromatographically by assessing the amount of putrescine liberated from γ-glutamylputrescine per unit time of incubation (17).

RESULTS

The unequivocal demonstration of intracellular transglutaminase activity using polyamines as substrates requires the isolation of the γ-glutamylpolyamine products from the protein and the separation of the products from other possible metabolites. This was initially accomplished in a defined test system by using guinea pig liver transglutaminase to incorporate [^{3}H]putrescine and [^{3}H]spermidine into *Aplysia* muscle proteins. Prolonged proteolytic digestion of the labeled protein (see *Experimental Procedure*) released [^{3}H]polyamine derivatives and, as shown in Fig. 1 *A* and *B*, γ-glutamylputrescine was separated from γ-glutamylspermidine by ion-exchange chromatography.

The next objective was to determine if transglutaminase activity is present in *Aplysia* nervous tissue. Homogenates of the nervous system were examined and were found to contain an enzyme that, in the presence of Ca^{2+}, incorporated labeled putrescine or spermidine into exogeneous protein (Table 1). The rate of incorporation was linear for approximately 20 min but then diminished, possibly as a result of activation of calcium-dependent proteases (18). All of the radioactivity incorporated under these conditions was found as the appropriate γ-glutamylpolyamine derivative. Other studies showed that the nervous system also has γ-glutamylamine cyclotransferase, an enzyme that cleaves the covalent linkage between the polyamine and glutamate moieties (17). The activity of the enzyme in

Table 1. Transglutaminase activity in the nervous system of *Aplysia*

Time, min	Addition	Incorporation cpm	Incorporation Activity, mol/mg protein per hr
10	—	488	124
20	—	948	240
30	—	740	188
20	2 mM EDTA	262	67
20	1 mM iodoacetamide	0	0

Activity was measured at 24°C in 0.350 ml of 50 mM Tris·HCl (pH 8.0) containing 100 μg of nervous tissue extract, 5 mM $CaCl_2$, 5 mM dithiothreitol, and 5 μCi of [^{3}H]spermidine (30 μM final concentration). The reaction, run in duplicate, was terminated at the times indicated by adding an equal volume of ice-cold 0.8 M perchloric acid. The precipitate was washed five times and digested with proteolytic enzymes, and the digest was analyzed by HPLC (see Fig. 1). Control values (0 time) were subtracted from each subsequent time.

Aplysia averaged 6 mol/mg of protein per hr at 22°C, which is the same order of magnitude as that reported for rabbit brain (17) when corrected for temperature.

The existence of transglutaminase activity in extracts does not necessarily imply that the enzymes are active in the tissue under normal physiological conditions. For example, transglutaminase activity in lymphocyte protein could be detected only after treatment of the cells with mitogen (8). Evidence for an active intracellular transglutaminase in the *Aplysia* nervous system was obtained when excised abdominal ganglia were incubated in [^{3}H]putrescine for 24 hr. Not only was radioactivity found associated with the acid-insoluble, protein fraction, but also treatment of the protein according to the protocol described above revealed labeled γ-glutamylputrescine (Fig. 1*C*). The identity of this derivative was further established by its susceptibility to acid and enzymatic hydrolysis (Fig. 1*C* and *Experimental Procedure*).

It is important to note that in every experiment in which endogenous proteins were labeled the proteolytic digest also contained considerable amounts of free labeled putrescine (Fig. 1*C*). This stands in contrast to experiments in which polyamines were incorporated into casein or other purified proteins by using liver transglutaminase: under these conditions no free polyamine was found in the digest (Fig. 1 *A* and *B*). Endogenous proteins retained noncovalently bound polyamine even after extensive washing with trichloroacetic acid, perchloric acid, acetone, or treatment with detergents under a variety of conditions.

Aplysia ganglia are heterogenous, consisting of neurons, glial cells, and connective tissues (14). That the covalent incorporation of polyamine occurs in neurons was shown directly by injecting [^{3}H]putrescine into the cell body of R2, the giant cholinergic neuron found in the abdominal ganglion. As shown in Table 2, injected putrescine is converted to spermidine, spermine, and other metabolites in the acid-soluble fraction of the neuron. No labeled γ-glutamylpolyamine derivatives are found in this fraction. Approximately 15% of the total radioactivity in injected cells was associated with the acid-insoluble fraction. When analyzed for covalently bound polyamines, about one-half of the radioactivity was found in two compounds that eluted with the γ-glutamylputrescine and γ-glutamylspermidine standards. Smaller amounts of radioactivity were associated with peaks corresponding to bis(γ-glutamyl)putrescine and bis(γ-glutamyl)spermidine (Table 3).

Table 2. Distribution of radioactivity in the acid-soluble fraction after intrasomatic injection of [^{3}H]putrescine into R2

Tissue	Total cpm	Metabolites, % of total cpm GABA	AcPut	Put	Spd	Spm	Other
Cell body	20,000	15	5	50	23	3	4
Axon	1,500	20	9	37	23	8	3

Twenty-six hours after injection, the abdominal ganglion, containing R2's cell body, and the right connective, containing R2's axon, were homogenized separately in 0.4 M perchloric acid and the acid-soluble radioactivity was analyzed by ion-exchange chromatography (11). The identity of the metabolites was established by comparison with the authentic compounds. A small percentage of the radioactivity (other) was not identified. GABA, γ-aminobutyric acid; AcPut, monoacetylputrescine; Put, putrescine; Spd, spermidine; Spm, spermine.

The identification of the latter two derivatives must be considered tentative, however: whereas treatment of the proteolytic digest with acid completely eliminated radioactivity associated with the γ-glutamylputrescine peak and reduced that of γ-glutamylspermidine by more than 80%, the two other derivatives were reduced to a lesser extent (Table 3). This strongly suggests that the peaks containing the latter compounds also contained other components. Moreover, treatment of the putative bis derivatives with γ-glutamylamine cyclotransferase released only a small amount of labeled polyamine (not shown). Authentic bis(glutamyl)spermidine and bis(glutamyl)putrescine are good substrates for this enzyme (17). As expected, concomitant with the reduction in labeled γ-glutamylpolyamine was an increase in the amount of labeled putrescine and spermidine. The acid-resistant radioactivity in the γ-glutamylspermidine peak may be hypusine, an unusual amino acid that on HPLC behaves like the spermidine derivative (19, 20).

Some indication of the nature of the proteins that become labeled by polyamines was obtained by using the vinca alkaloid vinblastine, an agent commonly used to precipitate tubulin (21). Vinblastine is not specific for tubulin, however, but will react with a wide variety of proteins that bind calcium (22). When an extract of *Aplysia* nervous tissue that had been incubated in [^{3}H]putrescine was treated with vinblastine, a broad range of

Table 3. Distribution of radioactivity in the acid-insoluble fraction after intrasomatic injection of [^{3}H]putrescine into R2

Preparation	Total cpm	Hydrolysis products, % of total cpm N^1,N^4-Bis-(γ-Glu)-Put	γ-Glu-Put	N^1,N^8-Bis-(γ-Glu)-Spd	N^1- + N^8-γ-Glu-Spd*	Put	Spd	Spm	Ptr	Other
Enzyme hydrolysate	2,300	8	16	11	33	24	3	0	2	3
Acid hydrolysate	700	3	0	3	6	32	30	6	6	14

Twenty-four hours after injection of [^{3}H]putrescine into R2, the abdominal ganglion and the right connective were combined and homogenized in 0.4 M perchloric acid. The precipitate was washed five times and digested with proteolytic enzymes. Three-quarters of the digest was analyzed chromatographically. The remaining material was first hydrolyzed with 6 M HCl at 105°C for 24 hr and then examined. Ptr, putreanine; other abbreviations as in Table 2.

* N^1- and N^8-γ-glutamylspermidine are incompletely resolved: a mixture of the two compounds may be present.

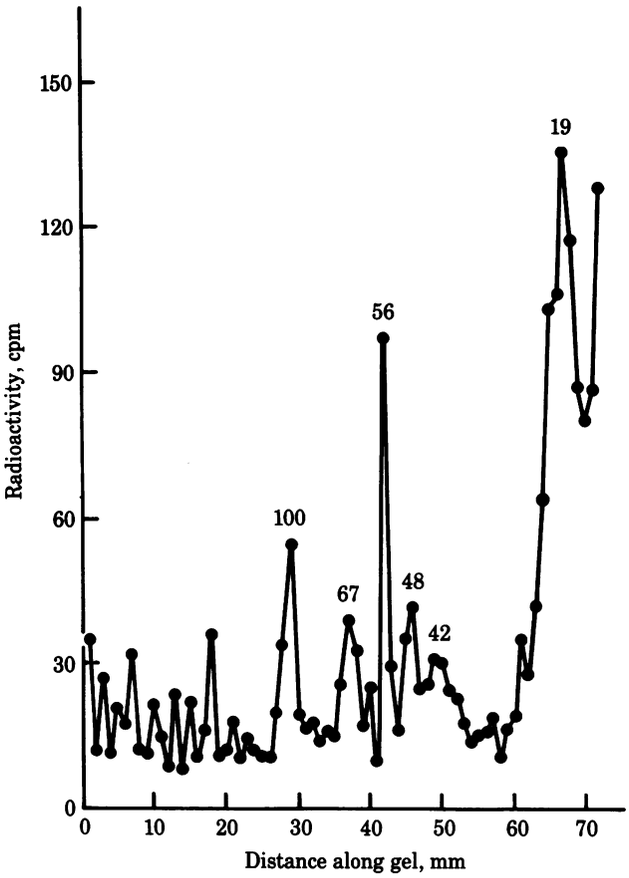

FIG. 2. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of vinblastine-precipitated proteins. The central nervous system was removed from the animal and incubated in 1.0 ml of supplemented sea water containing 50 μCi of [^{3}H]putrescine. After 20 hr the tissue was washed in the same medium without putrescine at 0°C, homogenized, and centrifuged at 105,000 × *g* for 45 min (10). The pellet was then extracted with 50 mM Tris·HCl (pH 7.6) containing 30 mM putrescine, and the combined supernatants were lyophilized and reconstituted in 1.0 ml of buffer containing 900 μg of vinblastine sulfate (Velban; Eli Lilly). Incubation at 37°C for 1 hr resulted in a white flocculent precipitate, and this was collected by low-speed centrifugation. The precipitate was dissolved in buffer containing sodium dodecyl sulfate and mercaptoethanol and electrophoresed to equilibrium on a 4–30% gradient of polyacrylamide (10). The gel was frozen and sectioned into 1-mm segments, and the radioactivity was measured by scintillation counting (10). The ability of vinblastine to precipitate *Aplysia* tubulin was tested in separate experiments in which [^{3}H]colchicine (New England Nuclear) was added to the 105,000 × *g* supernatant from unlabeled nervous tissue. Addition of vinblastine precipitated all of the protein-bound radioactivity; after sodium dodecyl sulfate gel electrophoresis of the precipitated material, staining with Coomassie blue revealed a number of proteins, including a prominent band at 56,000 daltons (not shown). Peaks are labeled in kilodaltons.

proteins was precipitated reproducibly, including several that were radioactive (Fig. 2). The major labeled bands included a 56,000-dalton protein that migrated with *Aplysia* tubulin and a smaller (19,000-dalton), more heavily labeled polypeptide that is similar in size to the protein that is labeled in lymphocytes (8). We do not know at present which bands contain covalently bound polyamine and which contain polyamines tightly bound by electrostatic interactions.

DISCUSSION

We have been examining the metabolism and axonal transport of the polyamines putrescine, spermidine, and spermine in the giant neuron R2 with the objective of gaining insight into the role of these compounds in the nervous system. [^{3}H]Putrescine injected into R2 is readily metabolized to spermidine and spermine (Table 2), and the pathways appear similar to those in mammalian tissues (11). We now have evidence that putrescine and spermidine, but not spermine, are covalently bound to protein via the action of an intracellular transglutaminase (Table 3). The presence of the active enzyme in R2 was shown by isolating and characterizing labeled γ-glutamylputrescine and γ-glutamylspermidine obtained from digests of cellular proteins after injection.

We consistently observed that ^{3}H-labeled polyamines were bound to endogenous proteins with such great affinity that they were not dissociated by trichloroacetic acid, perchloric acid, or other agents commonly used to separate small ligands from proteins (Fig. 1*C* and Table 3). Because this binding is probably not unique to *Aplysia* proteins, the unequivocal demonstration of transglutaminase activity absolutely requires the separation and identification of the γ-glutamylpolyamine derivatives. Two other labeled products found associated with R2's proteins after injection behaved like bis(γ-glutamyl)putrescine and bis(γ-glutamyl)spermidine, respectively (Table 3). The presence of these compounds would imply that transglutaminase is crosslinking proteins by means of a polyamine bridge. Such bridges occur in extracellular proteins (2, 8) but have not been found inside cells (8). Although ^{3}H-labeled products from R2 and the authentic compounds cochromatographed, the peaks that should contain the labeled bis compounds were contaminated with as-yet-unidentified labeled products that were resistant to acid hydrolysis (Table 3) and to treatment with γ-glutamylamine cyclotransferase, the enzyme that specifically hydrolyzes these compounds (17). It is likely that some of the radioactivity in the peaks is present as the bis conjugates, but they must be completely separated from the acid-resistant contaminants before their identity can be conclusively established.

For years polyamines were believed to act primarily as organic cations that maintain the conformation of certain proteins and nucleic acids (23, 24). The finding in lymphocytes (8) and in *Aplysia* R2 that putrescine and spermidine are substrates for intracellular transglutaminase now suggests a role for these compounds as modulators of transglutaminase activity: high levels of the polyamines will preclude formation of γ-glutamyl–lysine crossbridges by competing with peptide-bound lysine for the active site of the enzyme. Moreover, the covalent incorporation of polyamine may alter the conformation of the protein, thereby providing a signal that influences the fate of the protein, perhaps by making it more susceptible to proteolysis. If these ideas are correct, then calcium would have a pivotal role, because not only does calcium activate transglutaminase (2) but also it can initiate selective proteolysis (18). *Aplysia* neurons have several types of calcium channels in their membranes (9). Because calcium flux can be regulated electrically in these cells, it should be possible to examine the relationship between calcium entry, transglutaminase activity, and protein turnover.

A major advance in elucidating the function of transglutaminase in R2 would be to identify the protein substrates. There is evidence in fibroblasts (25) that transglutaminase acts on membrane proteins and that in erythrocytes it crosslinks cell surface proteins to spectrin (3, 4). The possibility that membrane protein–cytoskeletal interactions are mediated by transglutaminase is also supported by work showing that actin (6), myosin (7), and the histocompatibility antigens HLA-A and -B (5) are all substrates for the enzyme *in vitro*. In the latter case, transglutaminase specifically recognizes glutamine moieties on the carboxy-terminal region of the antigens—i.e., the portion of the membrane complex that extends into the cytoplasm (5).

As a preliminary step toward identifying transglutaminase substrates in *Aplysia* neurons, we have assumed that the proteins to which polyamines are added are the same as those that are crosslinked when polyamine levels are low. When vinblastine was used to precipitate proteins from an extract of *Aplysia* nervous tissue that had been incubated in [^{3}H]putrescine (Fig. 2), several labeled proteins were found, including a band that corresponded to *Aplysia* tubulin. We do not know which of the proteins contain covalently bound polyamines, but, coupled with a recent report implicating putrescine and spermidine in microtubule assembly (26), our results provide additional support for the hypothesis that polyamines are involved in cellular events that depend on cytoskeleton interactions.

The authors thank Mr. David Weissman and Ms. Donna Futterman for their able technical assistance. This work was supported by Grant NS 14555 and Career Development Award NS 00350 from the National Institutes of Health (to R.T.A), and by Grant NS 11766 from the National Institutes of Health, a grant from the Muscular Dystrophy Association, and Grant PDT-162 from the American Cancer Society (to L.T.K.).

1. Folk, J. E. (1980) *Annu. Rev. Biochem.* **49,** 517–532.
2. Folk, J. E. & Finlayson, J. S. (1977) *Adv. Protein Chem.* **31,** 1–133.
3. Anderson, D. R., Davis, J. L. & Carraway, K. L. (1977) *J. Biol. Chem.* **252,** 6617–6623.
4. Lorand, L., Weissmann, L. B., Epel, D. L. & Brunner-Lorand, J. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4479–4481.
5. Pober, J. S. & Strominger, J. L. (1981) *Nature (London)* **289,** 819–821.
6. Derrick, N. & Laki, K. (1966) *Biochem. Biophys. Res. Commun.* **22,** 82–88.
7. Hinney, P. & Eccleston, J. F. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40,** 1419 (abstr.).
8. Folk, J. E., Park, M. H., Chung, S. I., Schrode, J., Lester, E. P. & Cooper, H. L. (1980) *J. Biol. Chem.* **255,** 3695–3700.
9. Hagiwara, S. & Byerly, L. (1981) *Annu. Rev. Neurosci.* **4,** 69–126.
10. Ambron, R. T. (1982) *Brain Res.*, in press.
11. Kremzner, L. T. & Ambron, R. T. (1982) *J. Neurochem.* **38,** 1719–1727.
12. Kremzner, L. T. & Ambron, R. T. (1981) *Trans. Am. Soc. Neurochem.* **12,** 186 (abstr.).
13. Eisenstadt, M., Goldman, J. G., Kandel, E. R., Koike, H., Koester, J. & Schwartz, J. H. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3371–3375.
14. Frazier, W. T., Kandel, E. R., Kupfermann, I., Waziri, R. & Coggeshall, R. E. (1967) *J. Neurophysiol.* **30,** 1288–1351.
15. Ambron, R. T., Goldman, J. G., Thompson, G. B. & Schwartz, J. H. (1974) *J. Cell Biol.* **61,** 649–664.
16. Connellan, J. M., Chung, S. I., Whetzel, N. K., Bradley, L. M. & Folk, J. E. (1971) *J. Biol. Chem.* **246,** 1093–1098.
17. Fink, M. L., Chung, S. I. & Folk, J. E. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4564–4568.
18. Baudry, M., Bundman, M. C., Smith, G. K. & Lynch, G. S. (1981) *Science* **212,** 937–938.
19. Imaoka, N. & Nakajima, T. (1973) *Biochim. Biophys. Acta* **320,** 97–103.
20. Park, M. H., Cooper, H. L. & Folk, J. E. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2869–2873.
21. Shelanski, M. L. & Taylor, E. W. (1968) *J. Cell Biol.* **38,** 304–309.
22. Wilson, L., Bryan, J., Ruby, A. & Mazia, D. (1970) *Proc. Natl. Acad. Sci. USA* **66,** 807–814.
23. Bachrach, U. (1973) *Function of Naturally Occurring Polyamines* (Academic, New York).
24. Cohen, S. S. (1971) *Introduction to the Polyamines* (Academic, New York).
25. Birckbichler, P. J., Carter, H. A., Orr, G. R., Conway, E. & Patterson, M. K. (1978) *Biochem. Biophys. Res. Commun.* **84,** 232–237.
26. Pohjanpelto, F., Virtanen, I. & Holtta, E. (1981) *Nature (London)* **293,** 475–477.