Isolation and characterization of brain-specific transglutaminases from rat

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Abbreviations: TGase, transglutaminases; FBS, fetal bovine serum

Abstract

The relevance of transglutaminases with neural function and several disorders has been emphasized recently. Especially, many polypeptides associated with neurodegenerative diseases are suggested to be putative transglutaminase substrates such as β amyloid protein of Alzheimer's disease, microtubule-associated proteins and neurofilaments, etc. In addition, the CAG repeated gene products with probable polyglutamine tract, putative transglutaminase substrates, were identified in several neuro-degenerative disorders. However, the identity of the brain transglutaminase has not been confirmed, because of enzymic stability and low activity. In the present experiment, we have isolated brain-specific transglutaminases, designated as TGase NI and TGase NII, which are different from other types of transglutaminases in respects of molecular weights (mw. 45 kDa, 29 kDa respectively), substrate affinity, elution profile on ion-exchange chromatography, sensitivity to proteases and ethanol, and immuno-logical properties. The enzymes were localized specifically in the brain tissues but not in the liver tissue. And neural cells such as pheochromocytoma cell, glioma cell, primary neuronal and glial cells were shown to be enriched with TGase NI and TGase NII. The possible biological roles of the enzymes were discussed not only on the aspect of crosslinking activity but also of signal transducing capacity of the enzyme in the brain.

Keywords: TGase NI, TGase NII, brain, rat, localization

Introduction

The transglutaminases (TGase), protein crosslinking enzymes, has been suspected to be associated with the pathogenesis of neurodegenerative diseases, notably of Alzheimer's disease, where the crosslinkings of neuro-filaments (Selkoe *et al.*, 1982), microtubule-associated tau proteins (Dudek and Johnson, 1993) or amyloid β protein (βA_4) (Ikura *et al.*, 1993, Rasmussen *et al.*, 1994) were probably involved. And the recent findings that TGase-mediated formation of cytokine dimers such as interleukin 2 (Eitan and Schwartz, 1993) and midkine (Mahony *et al.*, 1996) could affect the neuronal function suggest strongly the significant roles of the enzyme in brain.

Moreover, it was found that Huntington's disease and several other neurodegenerative disorders are associated with CAG repeat expansion encoding a polyglutamine tract (Ross, 1995). The glutamine residue can be possibly involved in formation of cross-linked supramolecular structure both within and between proteins through reactions catalyzed by TGase. Relevantly, it would be natural to assume the participation of the enzyme in the pathogenesis of neurodegenerative disorders.

In addition, the enzyme has GTP-binding and GTPase activity (Lee *et al.*, 1989), related with signal transduction system as Gh protein between α_1 adrenergic receptor and phospholipase C, influencing the neural functions (Nakaoka, 1994, Whang *et al.*, 1995). Therefore, the regulation of TGase activity either as a crosslinker or as a signal transducer would be very important in functional, developmental and pathogenetic aspects of nervous system.

The presence of transglutaminase C (TGase C) in the brain has been suggested but debated because of low activity and poor evidence of identity. Possible existence of other types of TGase has been implied from the studies of tetanus-toxin activatable synaptic TGase (Facchiano *et al.*, 1993), substrate-specificity to IL-2 (Schwartz, 1993), and acetylcholine receptor aggregation (Hucho and Bandini, 1990), which showed a quite distinct property different from TGase C. Therefore, it is necessary to investigate the identity and properties of brain TGase. In the present study, we tried to isolate and characterize TGases from rat brain tissues and to elucidate their distribution.

Materials and Methods

Materials

[1,4-¹⁴C] putrescine hydrochloride (118 mCi/mmol) was purchased from Amersham Corp.; Phenylmethyl sulfonyl fluoride, aprotinin, dansylcadaverine and dithiothreitol from Sigma; benzamidine hydrochloride from Aldrich; dispase, proteinase K, trypsin, chymotrypsin and leupeptin from Boehringer Mannheim; Q-sepharose, DEAE-sephacel, CM cellulose from Pharmacia Fine Chemical Co. Other chemicals of analytical grade were obtained from the local commercial sources.

Methods

TGase assay

A modification of a published assay (Folk and Chung, 1985) was used. Reactions were conducted at 37°C in 0.5 ml of 0.1 M Tris acetate buffer, pH 7.5, containing 1% dimethylated casein, 1 mM CaCl₂, 0.5% Lubrol PX, 5 mM DTT, 0.15 M NaCl, 0.5 µCi of [1,4-¹⁴C] putrescine, and 50 µl portions of enzyme solution. Assays were carried out for 1 h and were terminated by the addition of 4 ml of cold 7.5 % trichloroacetic acid. After 30 min in the cold, the precipitates were collected on GF/A glass filters (Whatmann) and washed with several 30 ml portions of 5% trichloroacetic acid. The radioactivity was measured in a Beckman liquid scintillation spectrometer. Enzyme activity is expressed as cpm incorporated/h.

Preparation of TGase from rat brain

The brains were collected from 100 heads of Sprague-Dawley male rats, which were homogenized in buffer A (50 mM Tris-acetate, 1 mM EDTA, 1 mM PMSF, 1 mM benzamidine, 2 mg of leupeptin and 200 trypsin inhibitor unit of aprotinin/liter, pH 7.5) by polytron homogenizer (Brinkman Instruments). The homogenate was centrifuged for 1 hour at 10,000 *g* and 4°C. The supernatant was filtered by cheese cloth. The filtrate was subjected to sequential column chromatography and gel filtration. The samples were loaded on DEAE-Sephacel column (7.5 × 14 cm), equilibrated with buffer A. The fractions with high transglutaminase activity eluted by NaCl concentration gradient from 0 to 1.0 M were combined. The combined fractions after dialysis against buffer B (50 mM Tris-acetate, 1 mM EDTA, pH 7.5) were loaded on CM-cellulose column (3.2 × 15 cm). When the CM-cellulose column was eluted with Buffer B, the enzyme activity was found in the washing fractions. The pooled enzyme fractions were dialyzed against Buffer B, and loaded on Q-Sepharose column (2.6 × 10 cm). The loaded enzyme fractions on Q-Sepharose column were eluted by NaCl concentration gradient from 0 to 0.6 M. The two peaks of the enzyme activity were separately pooled and were subjected to 2nd Q-sepharose column chromatography, respectively. The enzymic fractions after 2nd Q-sepharose column were concentrated,

separately by Amicon conflow 10, which were designated as NI and NII fractions. The concentrated samples were dialyzed against buffer A, which were subjected to FPLC gel filtration (Waters Co.). The samples were eluted with Buffer C (50 mM Tris-acetate, 1 mM EDTA, 0.15 M NaCl, pH 7.5) using Glass SW 300 gel filtration column. The fractions of high enzymic activities were collected and concentrated in Amicon conflow 10.

Properties of the enzyme

The biochemical characteristics of the enzyme fraction NI and NII were studied for *K_m* value toward putrescine, and Ca²⁺ requirement. For the sensitivity to protease, batches of TGase NI and NII were incubated with 0.2 units of dispase (Boehringer-Mannheim)/ml of Tris-acetate buffer (pH 7.5, 0.05 M) or with trypsin in a similar manner. The sensitivity of the respective enzyme to ethanol was determined by addition of ethanol to the reaction mixture during the incubation time.

Antibody preparation

Antibodies to purified TGase NI or NII were prepared in rabbits. The enzyme (0.1 mg in 1 ml PBS) was emulsified with an equal volume of Freund's complete adjuvant and administered intramuscularly at the area near the foreleg lymph nodes. The same procedure was followed 2 weeks later. After two additional weeks, emulsified antigen solution composed of 0.1 mg of enzyme in 1.0 ml PBS and an equal volume of Freund's incomplete adjuvant was administered in a similar fashion. The rabbit was bled 2 weeks after the last injection and the antiserum was stored at -20°C.

Immunoblot analysis

Protein samples were transferred electrophoretically onto nitrocellulose strips. These nitrocellulose strips were saturated with 3% gelatin in 20 mM Tris, pH 7.4 containing 0.5 M NaCl by incubation for 1 h at room temperature. The strips were then washed with this buffer containing 0.05% Tween 20 and incubated overnight with immunoglobulin in a 1% gelatin solution. The strips were washed with the Tween-containing buffer and further incubated for 1 hr with horse radish peroxidase-conjugated protein A (Bio-Rad) (1:2000 dilution). The strips were washed again with the Tween-containing buffer, and the blots were developed for 2-15 min with a 4 chlor-1-naphthol/hydrogen peroxide reagent. After the completion of the reaction, the strips were washed with distilled water and photographed.

Immunocytochemical analysis

For the immunocytochemical analysis, the midbrain was dissected out from the fetal brain of 15 days of pregnant rat. The nerve cells were collected by centrifugation and pipetting procedure, which were cultured in DMEM/F12

(1:1) medium including 10% FBS, 2 mM glutamine, 50 IU/ml penicillin and 50 µg/ml streptomycin. The 10,000~20,000 cells were cultured on Aclar coverslip. To inhibit the growth of astrocyte, the defined media without FBS was used. After 20 hours of incubation, the media was shifted to FBS-free media but containing 20 µg/ml insulin, 100 µg/ml transferrin, 20 mM selenium, 0.1 M putrescine, 1 mM pyruvate, 2 mM glutamine, 50 µg/ml penicillin, 50 µg/ml streptomycin and 0.5% glucose. PC12 and C6 glioma cell lines were maintained in 10% FBS-DMEM. All the plastic dishes were coated with poly-L-lysine (10 µg/ml). After culture, the cells were fixed for 30 minutes with 4% paraformaldehyde and washed twice with PBS buffer. Sections were then incubated for 45 min with 1 : 100 dilution of rabbit polyclonal antiTGase NI, antiTGase NII or antiTGase C antibody, respectively. The slides were washed in PBS and incubated with a second antibody consisting of biotin-conjugated swine antirabbit immunoglobulin (LSAB kit, DAKO) and normal swine serum for 30 min, followed by a TBS wash. Finally, the sections were incubated with HRP-conjugated avidin biotin complex (ABC complex). Antibody localization was performed using diaminobenzidine reaction. Negative

controls consist of substitution of primary antibody with non-cross reacting rabbit serum.

Results

Enzyme isolation from rat brain

By the sequential treatments of homogenization, chromatography on DEAE-Sephacel, CM-cellulose, double Q-sepharose and the final FPLC gel filtration, the transglutaminase activities from rat brain tissues had been found in two different fractions. The salt gradient on DEAE-Sephacel chromatography eluted the major TGase activity at 0.35 M NaCl, while minor fraction at 0.28 M NaCl. After dialysis of the combined fractions, pH of the mixture was adjusted to 6.0 to eliminate other denatured proteins by centrifugation. The CM-cellulose chromatography indicated the elution profile of the enzymic activities in the washing fractions, indicating the low pI value of the brain TGase. After Q-sepharose chromatography, the enzymic activities were observed to be eluted at 0.28 M and 0.35 M of NaCl concentration (Figure 1). The respectively pooled fractions were designated as TGase NI with lower NaCl elution profile and TGase NII with higher NaCl elution profile fraction. Each fraction was again purified by FPLC gel filtration, which was concentrated by Amicon conflow 10.

Figure 1. The elution profile of TGase activity through Q-Sepharose column chromatography. The enzyme preparation purified up to first Q-Sepharose column chromatography was dialyzed against buffer B, and was rechromatographed on the same column. Two peaks of enzyme activity were separated and they were designated as NI and NII. Closed dotted line indicates the enzyme activity and broken line for NaCl concentration. TGase activity was measured according to the method describe in Material and Method.

Figure 2. Molecular sizes of TGase NI and NII by SDS-polyacrylamide gel electrophoresis. Number 1 indicates phosphorylase B (mw.); 2, bovine serum albumin (mw.); 3, ovalbumin (mw.); 4, carbonic anhydrase (mw.); 5, soybean trypsin inhibitor (mw.); 6, lysozyme (mw.); fraction NI, TGase NI (mw. = 45 kDa); NII, TGase NII (mw. = 29 kDa)

Figure 3. Sensitivity of partially purified TGase toward dispase and trypsin treatment. Partially purified NI and NII TGase was treated with 0.2 units each of dispase (▲ for NI and △ for NII) or trypsin (● for NI and ○ for NII), and the remaining enzyme activities were determined.

Figure 4. Dependency of NI and NII TGase activity on calcium concentration. TGase NI (closed circle) and NII (open circle) activities were measured in the presence of different concentration of calcium from 1nM to 100 mM.

Figure 5. Effect of ethanol on partially purified brain TGase. TGase NI (closed circle) and NII (open circle) activities were measured in the presence of different concentration of ethanol.

Figure 6. Western blot analysis of brain and liver tissue homogenates with the TGase type-specific antibodies. B and L represent for brain and liver homogenate, respectively. Panels NI, NII and C represent different type-specific antibodies utilized, TGase NI, TGase NII, TGase C, respectively.

Enzyme properties

The purified enzyme was very labile, though its purification fold was approximately 200 fold to the control homogenate, it was very difficult to stabilize the enzymic activity, of which TGase NII lost its activity completely after two week storage at 4°C. The activity staining by dansylcadaverine fluorescence illustrated the TGase activity in the respective protein bands, confirming the enzymic fraction (data not shown). The molecular weights of the enzyme were estimated by SDS-polyacrylamide gel electrophoresis to be 45 kDa for TGase NI and 29 kDa for TGase NII with a minor additional 80 kDa protein (Figure 2). But by western blot analysis with the respective antibody, only the 29 kDa protein was detected in TGase NII fraction (Figure 6). Both TGase NI and TGase NII lost their activities by proteolytic attack either of dispase or trypsin (Figure 3) or thrombin (data not shown). TGase NI required 100 µM calcium for optimum activity while TGase NII did 10 µM. Interestingly, the high concentration of Ca^{2+} over 10 µM rather inhibited TGase NII activity (Figure 4). These two types of brain TGases showed different sensitivity to ethanol, that is, TGase NII was more sensitive to ethanol than TGase NI (Figure 5). The K_m values of the enzymes toward putrescine were 0.28 mM for TGase NI and 0.17 mM for TGase NII, indicating the high affinity of these enzymes to polyamine. The N-terminal for TGase NI and TGase NII were blocked.

Western blot analysis revealed that TGase NI and NII were present in brain tissue but not in liver tissue (Figure 6). The molecular sizes of the antibody-responsive

proteins in the brain homogenate were compatible with the purified enzymes, 45 kDa and 29 kDa, respectively. The antibody against TGase C could recognize the intact enzyme in the liver homogenate as well as the probable proteolytic fragment (30 kDa) of TGase C in the brain homogenate. This result suggested that the TGase NI and NII would be immunologically different from TGase C, and that TGase C in the brain tissue might be fragmented.

Immunocytochemical Analysis

Immunocytochemical analysis showed that both of neuronal cells and astrocytes at 4th and 9th day of culture positively reacted with anti TGase NI, TGase NII antibodies. Both cells were also positive to TGase C antibody. But the anti-TGase NI, and anti-TGase NII antibodies showed the much stronger reaction than anti-TGase C antibody (Figure 7).

Anti-TGase E antibody showed the very weak but positive response in both types of nerve cells. The cell lines showed the similar pattern of TGase presence. PC12 cell showed positive reaction to anti TGase NI, NII and C antibodies, while anti TGase E antibody showed the very weak response (Figure 8). In case of C6 glioma cell, only anti TGase NI and NII antibodies showed the positive response, while anti TGase E and TGase C antibodies reacted negligibly (Figure 9).

Discussion

Five different types of TGases from the mammalian

Figure 7. Immunocytochemical staining of neurons and astrocytes cultured from fetal rat brain with the subtypes of TGase. Mesencephalic neurons were cultured from 14 days-old fetal rat brain and immunostained with rabbit polyclonal antibodies specific to subtype of TGase (A, TGase NI; B, TGase NII; C, TGase C; D, TGase E) as described 'Materials and Methods'. Transglutaminase NI, NII, C were expressed in most of neurons and astrocytes, but TGE was very weakly expressed in these cells. Original magnification $\times 200$.

Figure 8. Immunocytochemical staining of PC12 pheochromocytoma cells (cultured for 4 days) with polyclonal antibodies specific to subtype of TGases (A, TGase NI; B, TGase NII; C, TGase C; D, TGase E). Original magnification $\times 200$.

Figure 9. Immunocytochemical staining of C6 glioma cells with polyclonal antibodies specific to subtypes of TGases (A, TGase NI; B, TGase NII; C, TGase C; D, TGase E). Original magnification $\times 200$.

tissues have been isolated and characterized on their genetic and biochemical properties, such as TGase 1 (keratinocyte TGase, particulate TGase, TGase K), TGase 2 (tissue TGase, liver TGase, cytosolic TGase, TGase C), TGase 3 (epidermal TGase, TGase E), TGase 4 (prostate TGase) and Factor XIII. Each enzyme has been found to be expressed organ-specifically and associated with a variety of biological processes (Folk and Cole, 1966; Takagi and Doolittle, 1974; Thatcher, 1989; Kim *et al.*, 1990; Seitz *et al.*, 1991).

Recently, the probable involvement of a TGase in the neurodegenerative diseases attracted the special concern on several aspects. The devastating Alzheimer's disease might be derived from the crosslinking of the neurofilaments, microtubule-associated tau proteins, or amyloid β proteins (Selkoe *et al.*, 1982; Miller and Anderton, 1986; Lorand, 1996). The gene products with polyglutamine tracts whose length is determined by the number of CAG repeats could be the putative substrates for TGase, associated with Huntington's disease, spinocerebellar ataxia

type I, dentarubral-phalidoluysian atrophy, spinobulbar muscular atrophy and spinocerebellar ataxia type 3 (Cariello *et al.*, 1996; Kahlem *et al.*, 1996). The midkine, a highly basic heparin-binding cytokine, dimerized by a TGase can stabilize the growth factor, endowing its neurite-promoting activity amplification (Mahoney *et al.*, 1986). In contrast, TGase-mediated dimers of interleukin 2, unlike monomer, induce apoptosis in oligodendrocytes (Schwartz, 1993).

These effects of TGase activity in brain disorders are assumed under the presence of the enzyme in the brain tissues, but without success in their isolation and characterization. The induction of neural apoptosis was suggested to be TGase-associated. Transfection of TGase C gene into neuroblastoma cells sensitized the retinoic acid-induced apoptosis, while that of antisense oligonucleotide to TGase C caused a pronounced inhibition (Melino *et al.*, 1994). Furthermore, depolarizing agents such as acetylcholine or carbachol, a muscarinic receptor agonist, caused a significant activation of a TGase in superior cervical ganglion, which might be related with nitric oxide (Nagata and Ando, 1994). In the vicinity of acetylcholine receptor, plenty of TGase activities have been observed, probably for receptor aggregation (Hucho and Bandini, 1990). Some of neurotransmitters were post-translationally modified by polyamine such as substance P and β -endorphin, which inactivated the respective functions (Porta *et al.*, 1988; Pucci *et al.*, 1988; Kowall *et al.*, 1991; Persico *et al.*, 1992). These activities were functionally increased by neurodifferentiation and development (Maccioni and Seeds 1986, Gilad and Varon 1985), long-term potentiation of memory (Friedrich *et al.*, 1991), and stimulation by gangliosides or acetylcholine (Ando *et al.*, 1991a, b).

However, most of the studies for TGase-related phenomena in brain or neural cells were carried out by use of TGase C under the assumption that it would be the brain enzyme. Actually, some characteristics of the brain TGase are similar to those of TGase C. Nevertheless, the identity of brain TGase as TGase C has not been confirmed. Several studies to isolate and characterize TGase from brain tissue or nerve cells showed a variety of enzymes, but none have been successfully isolated and characterized. From the pheochromocytoma cells, two types of TGases were suggested (Byrd and Licht 1987), probably of TGase C-like one and TGase E-like one on the basis of elution profile, thermolability and cytosolic localization. And from human brain, erythrocyte TGase (identical to TGase C, tissue TGase)-like enzyme was characterized (Selkoe *et al.*, 1982).

The difficulty of isolating brain TGases might be due either to its instability or extreme low activity. In our preliminary study with electroconvulsive shock, we could observe the temporal and spatial changes in TGase activity, but the activity was very low and unstable to

compare with those of other organs (Park, unpublished). With aging process, the enzymic activity was increased in brain tissue, indicating the possible involvement of the enzyme in neurodegenerative changes associated with senescence (Park *et al.*, paper submitted)

In the present experiment, we also experienced the difficulty in maintaining the stability of the enzyme for purification. The brain TGases isolated partially in the present study, however, showed the some different characteristics from other types of previously known TGases (Figure 1, 2). The sensitivities of the enzyme to proteases such as thrombin, dispase or trypsin were similar to those of TGase C but totally different from those of TGase E and Factor XIII (Figure 3). Between TGase NI and NII, the sensitivities to protease or ethanol and K_m values toward putrescine were different (Figure 5). The elution profiles from DEAE-cellulose chromatography of brain TGases were different from other TGases (Figure 1). TGase NI fraction was eluted at 0.35 M NaCl, while TGase NII fraction at 0.28 M NaCl. But the other TGases showed the distinct elution profiles; TGase K, at 0.2 M NaCl; TGase C at 0.3 M NaCl; TGase E, at 0.05 M NaCl (Goldsmith and Martin, 1975; Chung, 1986; Park, 1988). And the elution behavior of brain TGases in washing fraction from CM-cellulose chromatography indicated that the enzymes might be negatively charged. But amino acid sequence analysis could not identify the N terminal amino acids, probably because of N terminal block of the enzyme. Moreover, the immunologic properties of TGase NI and NII were distinct from TGase C, TGase E and Factor XIII. As shown in Figure 6, antibodies to TGase NI and NII recognized the brain-specific TGases, while antibody to TGase C would detect liver TGase C and the probable proteolytic fragment of TGase C in the brain tissue. The unique tissue distribution pattern of TGase NI and TGase NII again illustrated the specificity of the enzyme as the brain TGases (Figure 6). TGase NI and TGase NII were localized only to brain tissue but not to liver tissue, while the amount of TGase C in brain tissue was very restricted or the enzyme could be in proteolytic form to be compared with that in liver tissue. The presence of the unique TGase in the brain tissue would attract the special concern for the role of enzyme in the neural system. In addition, the proteolytic fragment responsive to anti TGase C antibody would explain the biochemical difficulty in identification of TGase C as the brain enzyme.

The immunocytochemical analysis with the respective antibody showed that TGase NI localized at process-bearing and polygonal neuronal cells of rat brain-derived primary nerve cell culture, while TGase NII localized mostly at process-bearing neurons (Figure 7, 8 and 9). Also in the previous studies, TGase activity was present ubiquitously in all the fractions of brain tissue, but at the

synaptosomal fraction, the highest activity was detected (Gilad and Varon, 1985, Pastuszko *et al.*, 1986, Korner and Bachrach, 1987, Reichelt and Poulsen, 1992, Campisi *et al.*, 1992).

In most of TGase work with brain or neural system, the crosslinking activity has been focused for its probable association with neurodegeneration and apoptosis. For that work, it is very important to identify the brain-specific TGases and to characterize their regulatory mechanism and natural physiological substrates. If the β amyloid protein, microtubule-associated proteins, neurofilaments or the polyglutamine-rich neural proteins were identified to be the biological substrates for brain-specific TGases the drugs for the various neurodegenerative diseases would be developed from the specific inhibitors to brain TGases. Moreover, the TGase activity increase by tetanus or botulinum toxin was suggested to be a mechanism of neurotoxicity by blocking exocytosis of the neurotransmitters (Facchiano and Luini, 1992), important for to development of antineurotoxic drugs.

In addition to crosslinking activity, the enzyme has some other properties such as GTP binding and GTP hydrolysing activities, which are closely related with the signal transduction system (Lee *et al.*, 1989). In cardiac myocyte, TGase C was identified to be Gh protein mediating the α_1 adrenergic receptor signal to phospholipase C (Nakaoka *et al.*, 1994). But this activity was found to be independent of crosslinking activity of the enzyme from active-site mutant analysis.

Moreover, the TGase-gene transfected cell showed the delaying at the progression of cell cycle from S phase to G2/M phase, connoting the role of TGase in cell cycle control. But the cell cycle control activity of the enzyme was found not to be associated with its crosslinking activity, either. These results strongly suggested that TGase protein has another important cellular regulatory function apart from its crosslinking activity. Therefore, it is important to consider the role of the TGase protein in the biological phenomenon not necessarily as the crosslinker but as the non-crosslinking cellular modulator.

This additional activity of TGase as a signal transducer stimulated us to consider TGase protein as an active regulatory molecule for cell cycle and signal transduction. The latter function of the enzyme would solve many mysteries concerning its high appearance in growth factor stimulated proliferating phase, endogenous high level of expression in endothelial and smooth muscle cells, and its active role for cellular survival with drug-resistance toward toxic compounds, etc, which could not be readily explained in terms of crosslinking of the proteins (Park *et al.*, 1994).

In conclusion, we have isolated novel brain TGases, which are different from other types of TGases in biochemical and immunological properties, and have confirmed its localization in neural cell and brain tissues. For the analysis of their physiological roles, it is required to

investigate the mechanism of the enzyme with multiple functional aspects not only from macromolecular modification or stabilizing activity from its crosslinking ability, but also from signal transducing activity from GTP binding or GTP hydrolyzing property and cell cycle controlling activity.

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References

- Ambron, R. T. and Kemzner, L. T. (1982) Posttranslational modification of neuronal proteins : evidence for transglutaminase activity in R2, the giant cholinergic neuron of *Aplysia*. *Proc. Natl. Acad. Sci. USA* 79: 3442-3446
- Ando, M., Nakashima, Y. and Nagata, Y. (1991a) Stimulation by acetyl-choline and inhibition by norepinephrine of transglutaminase activity in superior cervical ganglia excised from adult rats. *Neurosci. Res.* 12: 356-365
- Ando, M., Nakashima, Y. and Nagata, Y. (1991b) Stimulation of transglutaminase activity by GMI-gangliosides and α -sialocholesterol in superior cervical and nodose ganglia excised from adult rat. *J. Neurochem.* 57: 1848-1854
- Ando, M., Tatematsu, T., Kunii, S. and Nagata, Y. (1994) Blockade effect of nerve growth factor on GMI ganglioside-induced activation of trans-glutaminase in superior cervical sympathetic ganglia excised from adult rat. *Neurosci. Res.* 19: 373-378
- Byrd, J. C. and Licht, U. (1987) Two types of transglutaminase in the PC12 pheochromocytoma cell line : stimulation by sodium butyrate. *J. Biol. Chem.* 262(24): 11699-11705
- Campisi, A., Renis, M., Russo, A., Sorrenti, V., Di Giacomo, C., Castorina, C. and Vanella, A. (1992) Transglutaminase activity in primary and subcultured rat astroglial cells. *Neurochem. Res.* 17: 1201-1205
- Cariello, L., Cristofaro, T. D., Zanetti, L., Cuomo, T., Maio, L. D., Campanella, G., Rinaldi, S., Zanetti, P., Lauro, R. D. and Varrone, S. (1996) Trans-glutaminase activity is related to CAG repeat length in patients with Huntington's disease. *Hum. Genet.* 98: 633-635
- Dudek, S. M. and Johnson, G. V. (1993) Transglutaminase catalyzes the formation of SDS-insoluble Alz 50-reactive polymers of tau. *J. Neurochem.* 61: 1159-1162
- Eitan, S. and Schwartz, M. (1993) A transglutaminase that converts interleukin-2 into a factor cytotoxic to oligodendrocytes. *Science* 261: 106-108
- Facchiano, F. and Luini, A. (1992) Tetanus toxin potently stimulates tissue transglutaminase: a mechanism of neurotoxicity. *J. Biol. Chem.* 267(19): 13267-13271
- Folk, J. E. and Chung, S. I. (1985) Transglutaminases. *Methods Enzymol.* 113: 358-375
- Folk, J. E. and Cole, P. W. (1996) Mechanism of action of guinea pig liver transglutaminase. *J. Biol. Chem.* 241: 5518-5529
- Friedrich, P., Fesus, L., Tarsa, E. and Czech, G. (1991) Protein crosslinking by transglutaminase induced in long-term potentiation in the CA1 region of hippocampal slices. *Neuroscience* 43: 331-334
- Gilad, G. M. and Varon, L. E. (1985) Transglutaminase activity in rat brain: characterization, distribution and changes with age. *J. Neurochem.* 45: 1522-1526
- Goldsmith, L. A. and Martin, G. M. (1995) Human epidermal transglutaminase. *J. Invest. Dermatol.* 64: 316-321

- Gray, M. H., Smoller, B. R., McNutt, N. S. and Hsu, A. (1990) Immuno-histochemical demonstration of factor XIII a expression in neurofibromas. *Arch. Dermatol.* 126: 472-476
- Hucho, F. and Bandini, G. (1986) Ca^{2+} dependent inactivation of acetyl-choline receptors by an endogenous transglutaminase. *FEBS Lett.* 200: 279-282
- Ikura, K., Takahara, K. and Sasaki, R. (1993) Crosslinking of a synthetic partial length(1-28) peptide of Alzheimer B/A4 amyloid protein by trans-glutaminase. *FEBS Lett.* 326: 109-111
- Kahlem, P., Terre, C., Green, H. and Djian, P. (1996) Peptides containing glutamine repeats as substrates for transglutaminase-catalyzed cross-linking: Relevance to diseases of the nervous system. *Proc. Natl. Acad. Sci. USA* 93: 14580-14585
- Kim, H. C., Lewis, M. S., Gorman, J. J., Park, S. C., Girad, J. E., Folk, J. E. and Chung, S. I. (1990) Protransglutaminase E from guinea pig skin. *J. Biol. Chem.* 265: 21971-21978
- Korner, G. and Bachrach, U. (1987) Intracellular distribution of active and inactive transglutaminase in stimulated cultured C6 glioma cells. *J. Cell. Physiol.* 130: 44-50
- Kowall, N. W., Beal, M. F., Busciglio, J., Duffey, L. K. and Yanker, B. A. (1991) An *in vivo* model for the neurodegenerative effects of β -amyloid and protection by substance P. *Proc. Natl. Acad. Sci. USA* 88: 7247-7251
- Lee, K. N., Birckbichler, P. J. and Patterson, M. K. Jr. (1989) GTP hydrolysis by guinea pig liver transglutaminase. *Biochem. Biophys. Res. Comm.* 162: 1370-1375
- Lorand, L. (1996). Neurodegenerative diseases and transglutaminase. *Proc. Natl. Acad. Sci. USA* 93: 14310-14313
- Maccioni, R. B. and Seeds, N. W. (1986) Transglutaminase and neuronal differentiation. *Mol. Cell Biochem.* 69: 161-168
- Mahoney, S. A., Perry, M., Seddon, A., Bohlen, P. and Haynes, L. (1996) Transglutaminase forms midkine homodimers in cerebellar neurons and modulates the neurite-outgrowth response. *Biochem. Biophys. Res. Comm.* 224: 147-152
- Melino, G., Annicchiarico-Petruzzelli, M., Piredda, L., Candi, E., Gentile, V., Davies, P. J. A. and Piacentini, M. (1994) Tissue transglutaminase and apoptosis: sense and antisense transfection studies with human neuroblastoma cells. *Mol. Cell. Biol.* 14(10): 6584-6596
- Miller, C. C. J. and Anderton, B. H. (1986) Transglutaminase and the neuronal cytoskeleton in Alzheimer's disease. *J. Neurochem.* 46: 1912-1922
- Nakaoka, H., Perez, D. M., Baek, K. J., Das, T., Husain, A., Misono, K., Im, M. J. and Graham, R. M. (1994) Gh: a GTP-binding protein with transglutaminase activity and ras signaling function. *Science* 264: 1593-1596
- Park, S. C., Kim, W. H., Lee, M. C., Seong, S. C., Song, K. Y. and Choe, M. A. (1994) Modulation of transglutaminase expression in rat skeletal muscle by induction of atrophy and endurance training. *J. Kor. Med. Sci.* 9: 490-497
- Paschen, W., Rohn, G., Schmidt-Kastner, S. (1990) Transglutaminase activity in reversible cerebral ischemia in the rat. *Neurosci. Lett.* 110: 232-236
- Pastuszko, A., Wilson, D. F. and Erecinska, M. (1986). A role for trans-glutaminase in neurotransmitter release by rat brain synaptosome. *J. Neurochem.* 46: 499-508
- Persico, P., Calignano, A., Fancuso, F., Marino, G., Pucci, P., Esposito, C., Mariniello, L. and Porta, R. (1992) Substance P inactivation by transglutaminase *in vivo*. *Peptides* 13: 151-154
- Porta, R., Esposito, C., Metafora, S., Pucci, P., Malorni, A. and Marino, G. (1988) Substance P as a transglutaminase substrate: identification of the reaction products by fast atom bombardment mass spectrometry. *Anal. Biochem.* 172: 499-503
- Pucci, P., Malorni, A., Marino, G., Metafora, S., Esposito, C. and Porta, R. (1988) β -endorphin modification by transglutaminase *in vitro*: identification by FAB/MS of glutamine-11 and lysine-29 as acyl donor and acceptor sites. *Biochem. Biophys. Res. Comm.* 154: 735-740
- Rasmussen, L. K., Sorenson, E. S., Peterson, T. E., Glieman, J., Jensen, P. H. (1994) Identification of glutamine and lysine residues in Alzheimer amyloid beta A4 peptide responsible for transglutaminase catalyzed homopolymerization and crosslinking of $\alpha_2\text{M}$ receptor. *FEBS Lett.* 358, 161-166
- Reichelt, K. L. and Poulsen, E. (1992) γ -Glutamylaminotransferase and transglutaminase in subcellular fractions of rat cortex and in cultured astrocytes. *J. Neurochem.* 59: 500-504
- Ross, C. A. (1995) When more is less: pathogenesis of glutamine repeat neurodegenerative diseases. *Neuron* 15: 493-496
- Seitz, J., Keppler, C., Huntemann, S., Rausch, U. and Aumuller, G. (1991) Purification and molecular characterization of a secretory transglutaminase from coagulating gland of the rat. *Biochim. Biophys. Acta.* 1078: 139-146
- Selkoe, D. J., Abraham, C. and Ihara, Y. (1982) Brain transglutaminase: *In vitro* crosslinking of human neurofilament proteins into insoluble polymers. *Proc. Natl. Acad. Sci. USA* 79: 6070-6074
- Takagi, T. and Doolittle, R. F. (1974) Amino acid sequence studies on factor XIII and the peptide released during its activation by thrombin. *Biochemistry* 13: 750-756
- Thacher, S. M. (1989) Purification of keratinocyte transglutaminase and its expression during squamous differentiation. *J. Invest. Dermatol.* 92: 578-584.
- Whang, K. C., Gray, C. D., Sivasubramanian, N. and Im, M. J. (1995) Interaction site of GTP binding Gh(transglutaminase II) with phospholipase C. *J. Biol. Chem.* 270: 27158-27062