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Differential incorporation of biotinylated polyamines by transglutaminase 2

Ju-Hong Jeon[a], Chai-Wan Kim[a], Dong-Myung Shin[a], Kyu-il Kim[b], Sung-Yup Cho[a], Joon-Cheol Kwon[a], Kyung-Ho Choi[a], Heun-Soo Kang[b], In-Gyu Kim[a,*]

[a] *Department of Biochemistry and Molecular Biology/Aging and Apoptosis Research Center (AARC), Seoul National University College of Medicine, 28 Yongon Dong, Chongno Gu, Seoul 110-799, South Korea*
[b] *Metabolic Engineering Laboratories, Seoul 110-510, South Korea*

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Abstract Polyamine incorporation or cross-linking of proteins, post-translational modifications mediated by transglutaminase 2 (TGase 2), have been implicated in a variety of physiological functions including cell adhesion, extracellular matrix formation and apoptosis. To better understand the intracellular regulation mechanism of TGase 2, the properties of biotinylated polyamines as substrates for determining in situ TGase activity were analyzed. We synthesized biotinylated spermine (BS), and compared the in vitro and in situ incorporation of BS with that of biotinylated pentylamine (BP), which is an artificial polyamine derivative. When measured in vitro, BP showed a significantly higher incorporation rate than BS. In contrast, in situ incorporation of both BS and BP was not detected even in TGase 2-overexpressed 293 cells. Cells exposed to high calcium showed a marked increase of BP incorporation but not of BS. These data indicate that the in situ activity of TGase 2 gives different results with different substrates, and suggest the possibility of overrepresentation of in situ TGase 2 activity when assayed with BP. Therefore, careful interpretation or evaluation of in situ TGase 2 activity may be required.

Key words: Transglutaminase 2; Biotinylated spermine; Polyamine conjugation; Post-translational modification

1. Introduction

Transglutaminase 2 (TGase 2, or tissue TGase) is a calcium-dependent enzyme that catalyzes polyamine conjugation or cross-linking of proteins through the transamidation reaction between the γ-carboxyamide group of a glutamine residue in a polypeptide chain and the primary amine group of a polyamine or a lysine residue in a polypeptide chain [1]. Of eight isoforms of TGase, which share a common amino acid sequence at the active site (GQCWV) and a strict calcium dependence for their transamidation activity [2], TGase 2 is characterized by ubiquitous tissue distribution, cytosol and nuclear localization [3], and by exhibiting GTP binding and hydrolysis activity [4]. Although TGase 2 null mice showed no distinct phenotype [5], TGase 2 has been implicated in a wide variety of physiological functions including cell adhesion [6], formation of extracellular matrix [7] and apoptosis [8]. Recently, much attention has focused on the role of proteins modified by TGase 2 in various diseases. For instance, deamidated gluten is implicated in celiac disease [9], and polyamine-conjugated tau and cross-linked mutant huntingtin have also been thought to contribute to the pathological progression of Alzheimer's disease and Huntington's disease, respectively [10,11]. However, the precise molecular mechanism(s) involved in activation of TGase 2 are not fully understood.

The transamidation activity of TGase 2 is regulated by calcium and GTP [12]. Previous studies showed that the increased expression level of TGase 2 by retinoic acid is not correlated with its increase of in situ activity [13], suggesting that TGase 2 activity is tightly regulated in the intracellular milieu, and derangement of the regulation mechanism is likely involved in the disease processes. Elucidation of the intracellular regulation mechanisms requires the accurate measurement of protein–polyamine conjugates resulting from in situ activity of TGase 2. The determination of [^{14}C]putrescine incorporated into *N*,*N*′-dimethylcasein has been used for assays of in vitro TGase 2 activity [14]. However, the application of this classical method for assaying in situ activity is limited by the need for extensive proteolysis and subsequent HPLC analysis [15]. Recently, a fluorescent amine or biotinylated amine has been used as an alternative acyl acceptor substrate for the rapid and sensitive measurement of in situ activity of TGase 2 [16]. In contrast to in vitro assays which use *N*,*N*′-dimethylcasein as a acyl donor, the in situ assay relies on unknown intracellular proteins for conjugation of intracellular polyamines. Moreover, in situ TGase 2 activity could be affected by the intracellular concentration of endogenous polyamines, such as putrescine, spermidine, and spermine, which reflect their metabolic status.

Biotinylated pentylamine (BP) is commonly used as a biotinylated amine for the measurement of in situ activity of TGase. However, BP is not involved in the biosynthesis of spermidine and spermine by *S*-adenosylmethionine decarboxylase or degraded by polyamine oxidase because pentylamine,

*Corresponding author. Fax: (82)-2-744 4534.
E-mail address: igkim@plaza.snu.ac.kr (I.-G. Kim).

Abbreviations: TGase, transglutaminase; BP, biotinylated pentylamine; BS, biotinylated spermine; THF, tetrahydrofuran; TFA, trifluoroacetate; MeOH, methanol; EtOAc, ethylacetate; DCC, 1,3-dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; DMF, dimethylformamide; TLC, thin layer chromatography

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a structural analog of putrescine, is an artificial substrate. Therefore, there is a possibility of the introduction of a bias in the representation of the physiological polyamine conjugation by in situ TGase activity when assayed with BP. In this report, we synthesized biotinylated spermine (BS), a derivative of natural polyamine, and compared the conjugation of BP and BS into cellular proteins by TGase 2 in order to assess the validity and efficacy of in situ TGase 2 activity assayed with BP.

2. Materials and methods

2.1. Synthesis of biotinylated spermine

The principal details of biotinylated spermine synthesis are illustrated in Fig. 1. A solution of ethyl trifluoroacetate (TFA, 0.59 ml, 4.94 mmol) in tetrahydrofuran (THF, 5 ml) was added dropwise for 5 min to a solution of spermine **1** (0.5 g, 2.47 mmol) in THF (30 ml) at 0°C. The reaction was monitored by thin layer chromatography (TLC) (solvent A) and, upon completion (approx. 10 min), the mixture was concentrated in vacuo to afford the crude product. *N*,*N*-di-TFA spermine **2** was obtained as a white crystal by recrystallization with MeOH/EtOAc. For amidation, *N*,*N*-di-TFA spermine **2** (0.32 g, 0.82 mmol), biotin (0.20 g, 0.82 mmol), 1,3-dicyclohexylcarbodiimide (DCC, 0.25 g, 1.23 mmol) and 1-hydroxybenzotriazole (HOBt, 0.02 g, 0.16 mmol) were added to the 30 ml portion of anhydrous dimethylformamide (DMF) into a flame-dried round bottom flask. The reaction was stirred for 24 h at room temperature and extracted with 20% MeOH/CH_2Cl_2. The organic mixture was washed with brine (2×30 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The pure product **3** was obtained as a syrup by column chromatography with solvent B. Deprotection of compound **3** (0.20 g, 0.32 mmol) was performed with 2 M NH_3/MeOH (20 ml). The reaction was maintained at room temperature with vigorous stirring, until TLC examination showed total conversion into the lower moving product **4** (R_f 0.43, solvent C). The solvent was removed under reduced pressure. The crude mixture was dissolved in THF and 1 M ethereal HCl was added slowly with stirring. The white solid which was formed by adding ethereal HCl was filtered and washed with diethyl ether (3×30 ml). *N*1-(4-Aminobutyl)-*N*1-{4-[(3-aminopropyl)-amino]-butyl}-5-[(3a*S*,6a*R*)-5-oxoperhydrocyclopenta[*c*]thiophen-1-yl] pentanamide **4** was successfully obtained as a white solid by column chromatography with solvent C. TLC was performed using pre-coated silica gel plates (60 mesh, Merck) with fluorescence indicator, and column chromatography was carried out on silica gel (230–400 mesh, Merck) with the following systems: (A) CH_2Cl_2:MeOH:NH_4 OH(20:5:1); (B) CH_2Cl_2:MeOH:NH_4OH (70:10:1); (C) CH_2Cl_2:MeOH:NH_4OH (4:3:1). Non-radioactive compounds were detected by spraying the plates with phosphomolybdic acid (10%) in ethanol and heating them.

2.2. Purification of human TGase 2

TGase 2 was purified from human red blood cells (RBCs) as previously described [17]. Human RBCs were lysed by osmotic pressure in distilled water, centrifuged and the supernatants were collected. TGase 2 was purified from these crude extracts by sequential column chromatography steps using DEAE-cellulose and a heparin column. Purification profiles were obtained from the protein quantitation and enzyme activity assays. Purity was evaluated by 12% SDS–PAGE and by Coomassie staining. Purified TGase 2 was stored in storage buffer (50 mM Tris–HCl, pH 8.0, 1 mM EDTA, 5 mM 2-mercaptoethanol, 150 mM NaCl, 50% glycerol, 2 μg/ml phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 2 μg/ml pepstatin A, 2 μg/ml trypsin inhibitor) at −70°C.

2.3. Establishment of stable cell line that expresses TGase 2

293 cells were plated at 5×10^5 cells per 60-mm dish in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum). After 16 h of incubation in a humidified atmosphere with 5% CO_2 at 37°C, cells were transfected with 2 μg of full length cDNA of human TGase 2 in pcDNA3 (Invitrogen), or empty vectors, using Lipofectamine (Invitrogen). Forty-eight hours after transfection, cells were split and subjected to geneticin (Invitrogen) selection (400 μg/ml) for 3 weeks. Geneticin-resistant clones derived from single cells were isolated and grown up for further analysis. To test stable expression of TGase 2, pcDNA3(TGase 2) and empty vector-transfected clones (2×10^6 cells) were lysed in 500 μl of lysis buffer (50 mM Tris–HCl, pH 7.5, 0.5% NP-40, 1 mM EDTA, 1 mM dithiothreitol (DTT), 150 mM NaCl, 2 μg/ml phenylmethylsulfonyl fluoride, 2 μg/ml pepstatin, 2 μg/ml leupeptin, 2 μg/ml pepstatin A, 2 μg/ml trypsin inhibitor). Protein quantitation was performed using the BCA protein assay kit (Pierce). The expression and enzyme activity of TGase 2 were confirmed by Western blotting and transamidation assay using anti-human TGase 2 antibody and [^{14}C]putrescine, respectively.

2.4. Transamidation assay

Transamidation activity was determined by measuring the incorporation of [^{14}C]putrescine into *N*,*N*′-dimethylcasein as previously described [14]. In brief, 100–200 μg of total cell lysates from TGase 2-overexpressing and control cell lines were incubated with 1 mg of *N*,*N*′-dimethylcasein and 500 nCi of [^{14}C]putrescine (NEN) in reaction buffer (50 mM Tris-acetate, pH 7.5, 10 mM $CaCl_2$, 5 mM DTT, 150 mM NaCl, 0.5% Triton X-100, 2 μg/ml phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 2 μg/ml pepstatin A, 2 μg/ml trypsin inhibitor). After 1 h of incubation at 37°C, the reaction was stopped by the addition of 7.5% trichloroacetic acid. Radioactivity bound to *N*,*N*′-dimethylcasein was measured by liquid scintillation counting. Background radioactivity was evaluated with bovine serum albumin (BSA), a negative control, as an acceptor protein.

2.5. Well plate transamidation assay

Well plate format assay for the measurement of transamidation activity was performed as previously described [18] with the following modifications. 50 μl of *N*,*N*′-dimethylcasein (0.1 mg/ml, Sigma) in coating buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 5 mM EGTA, 5 mM EDTA) was coated to each well of a 96-well microtiter (Nunc), and the plate was incubated for 2 h at 37°C, followed by three washes with 0.1% Tween 20 in phosphate-buffered saline (PBS). After subsequent incubation with 200 μl of 5% BSA in PBS for 2 h at 37°C, the wells were washed three times with 0.1% Tween 20 in PBS. Purified human TGase 2 and indicated amounts of BP (Pierce) or BS in 50 μl of reaction buffer (50 mM Tris-acetate, pH 7.5, 10 mM $CaCl_2$, 5 mM DTT, 150 mM NaCl, 0.5% Triton X-100, 0.5 mM EDTA, pH 8.0) were added to each well, and further incubated for 1 h at 37°C. Incorporated BP or BS was probed using 1:2500 diluted horseradish peroxidase (HRP)-conjugated streptavidin (Jackson Laboratory). The coloring reaction was performed with 50 μl of substrate solution (0.4 mg/ml of *O*-phenylenediamine dihydrochloride in 50 mM sodium citrate phosphate, pH 5.0). The reaction was stopped by the addition of 1 M H_2SO_4 and quantitated by measuring the absorbance at 490 nm on a microplate spectrophotometer (Molecular Devices).

2.6. In situ transamidation assay

The measurement of in situ TGase 2 activity was performed as previously described [13] with the following modifications. The 293 cell line overexpressing TGase 2 and control cells (2×10^6) were treated with 1 mM of BP or BS for 1 h with indicated concentrations of calcium. Each cell lysate was prepared and dialyzed against coating buffer. 10 μg of cell lysates was coated and incorporated biotin was probed as the procedures described for the well plate transamidation assay. To visualize the BP- or BS-incorporated proteins, 20 μg of cell lysates were subjected to 12% SDS–PAGE and transferred to nitrocellulose membranes. After blocking and probing with 1:2000 diluted HRP-conjugated streptavidin, the blots were developed by use of a standard ECL protocol (Pierce). To compare transport into cells of BP and BS, 293 cells were plated at 1×10^4 cells in 96-well plates. About 16 h later, cells were treated with 1 mM of BP or BS for the indicated times. After washing with PBS twice, ethanol fixation was performed and the cells were allowed to dry in the air. Then, cells were treated with 0.25% glutaraldehyde (ICN) in PBS at room temperature for 15 min, followed by 0.5% sodium borohydride (Sigma) at room temperature for 10 min. After a subsequent incubation with 200 μl of 5% BSA in PBS at room temperature for 2 h, transported BP or BS was probed using HRP-conjugated streptavidin and chromogenic substrate *o*-phenylenediamine dihydrochloride (Sigma). Assays were quantitated by measuring the absorbance at 490 nm on a microplate spectrophotometer (Molecular Devices). In parallel, to visualize transported BP or BS, immunocytochemical staining was performed using Texas red-conjugated streptavidin (Jackson Laboratory).

3. Results and discussion

3.1. Synthesis of biotinylated spermine

Protection of NH_2 groups with ethyl TFA afforded compound **2** in a nearly quantitative yield. The diagnostic NMR signals for compound **2** were observed at 2.44 ppm ($-CH_2NHCOCF_3$) in the ^1H NMR spectrum and 157.10 ppm, 156.74 ppm (–CO–) and 117.59 ppm, 114.73 ppm ($-CF_3$) in the ^{13}C NMR spectrum (DMSO-d_6). In this case, the four protons appear as a singlet at the same chemical shift. For the amidation step, the reaction of compound **2** with biotin using DCC and HOBt gives the expected amide **3** in moderate yield (52%). Amidation was a critical step, because only one of the two NH groups has to react with biotin. Compound **3** showed a triplet at 4.17 ppm and 4.28 ppm corresponding to the ring proton of biotin in the ^1H NMR spectrum ($CDCl_3$). Deprotection of NH_2 affects the chemical shifts of $-CH_2NH_2$ in the ^1H and ^{13}C NMR spectra of compound **4** (DMSO-d_6). In addition, the signals corresponding to $-CH_2NH_2$ are shifted downfield to 2.74 ppm and 55.48 ppm, in comparison with the same signal in compound **3** (2.32 ppm and 41.03 ppm), due to the β effect.

3.2. In vitro transamidation of biotinylated spermine

One or both of the primary amino groups of polyamines serve as natural acyl acceptor substrates for TGase [15]. In contrast to the limited substrate specificity of the acyl donor, the γ-carboxamide group of peptide-bound glutamine residues, TGases display broad specificity for acceptor substrates [1]. Although determination of [^{14}C]putrescine or BP incorporation has been used for in vitro TGase activity assay, [^{14}C]putrescine, when added to cultured cells, is converted into spermidine or spermine for the formation of protein–polyamine conjugates [19]. Moreover, the intracellular concentration of putrescine was much lower than that of spermidine or spermine in eukaryotic cells (~1%) [20]. Thus, we chose spermine for synthesizing the biotinylated derivative, and compared the acyl acceptor substrate specificity between BP and BS. Incorporation of BP or BS into *N*,*N*′-dimethylcasein by purified TGase 2 was measured using the well plate transamidation assay method. As shown in Fig. 2, BP showed

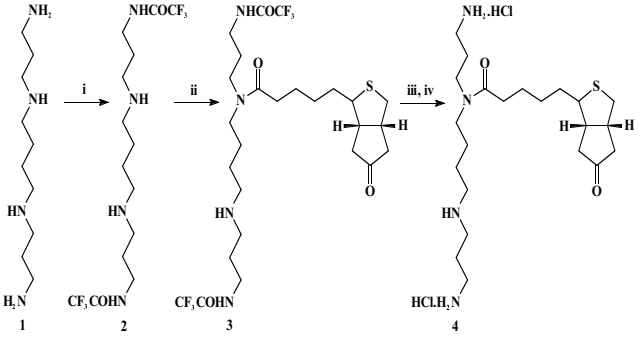

Fig. 1. Synthesis scheme of biotinylated spermine. i, CF_3COOEt, THF, 0°C, 10 min; ii, biotin, DCC, HOBt, DMF, rt 24 h; iii, 2 M NH_3, MeOH, rt 48 h; iv, 1 M ethereal HCl, THF, rt 1 h. Each reaction was monitored by TLC using pre-coated silica gel plate (60 mesh, Merck) with fluorescence indicator, and reaction products were purified by column chromatography (silica gel, 230–400 mesh, Merck). ^1H and ^{13}C NMR spectra of biotinylated spermine were recorded with a JEOL JNM EX-400 spectrometer. Chemical shifts were referenced to internal TMS or solvent signals for ^1H and ^{13}C NMR.

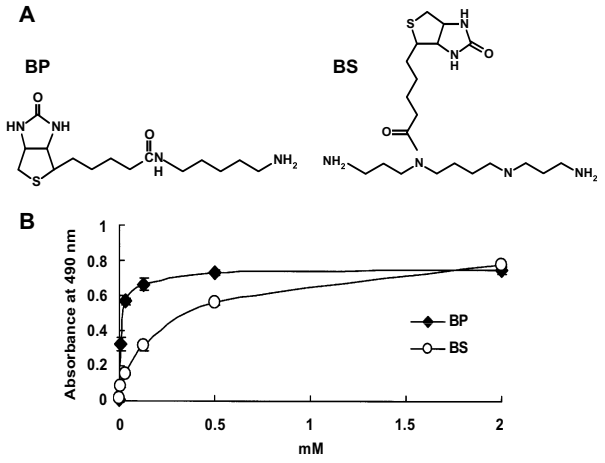

Fig. 2. Comparison of substrate specificity between BP and BS in vitro. A: Schematic presentation of BP (left) and BS (right). B: Incorporation of BP or BS into *N*,*N*′-dimethylcasein by TGase 2. Transamidation activity was measured using the well plate transamidation assay. Substrate concentrations were 0.0078, 0.0316, 0.125, 0.5, and 2 mM. Incorporated BP and BS were determined using HRP-conjugated streptavidin and *o*-phenylenediamine dihydrochloride. Results are presented as the mean ± S.D. of at least three independent experiments.

significantly higher incorporation than BS when the substrate concentration was below 2 mM, and calculated K_m values are 0.01 mM and 0.09 mM, respectively. These results indicate that BP has a higher affinity for TGase 2 than BS, and could be used as a better acyl acceptor substrate for in vitro TGase 2 assays.

3.3. In situ transamidation of biotinylated spermine

It had been demonstrated that exogenous radiolabeled polyamines, when incubated with mitogen-stimulated lymphocytes [15] or partially hepatectomized rat liver [21], were incorporated into cellular proteins at different rates. To compare the incorporation between two substrates in situ, we generated a TGase 2-overexpressing cell line, using 293 cells that have no detectable expression of TGase 2. Overexpression of TGase 2 was identified by Western blotting using anti-TGase 2 antibody (Fig. 3A, upper), and further confirmed by the measurement of incorporated [^{14}C]putrescine into *N*,*N*′-dimethylcasein. Cell lysates of the TGase 2-overexpressing cell line showed a more than 30-fold increase in transamidation activity compared to the control cell line (Fig. 3A). Next, we compared in situ TGase 2 activity using BP and BS. In contrast to in vitro activity of TGase 2, in situ activity was not detected in either cell line (Fig. 3B,C). Under physiological conditions, the concentration of intracellular free calcium ions (~10^{-7} M) is not sufficient to activate TGase 2. Therefore, we added calcium to the culture media and adjusted the final concentration to 5 mM or 10 mM. Cell viability was not affected at these calcium concentrations (data not shown). In situ activity of TGase 2 assayed with BP showed a more than 10-fold increase in high calcium concentration (Fig. 3B). But when assayed with BS, in situ TGase 2 activity was increased slightly (Fig. 3C), thus resulting in a differential incorporation between BP and BS. We confirmed this result by visualizing the proteins conjugated with BP or BS with streptavidin-HRP. Proteins conjugated with BP were increased in TGase 2-overexpressing as well as control cells at the high calcium condi-

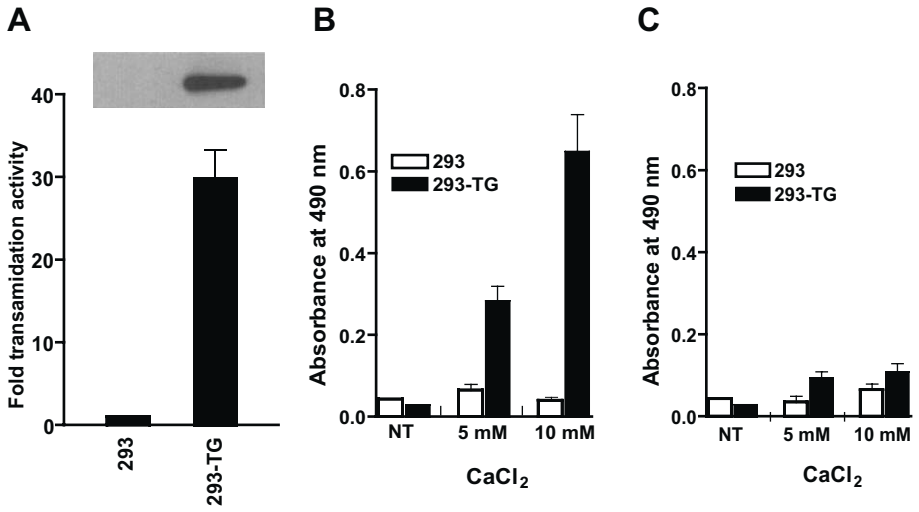

Fig. 3. Comparison of in situ transamidation activity using BP and BS as amine substrates in TGase 2-overexpressing 293 cells. A: Establishment of TGase 2-overexpressing cell line. Full length of TGase 2 cloned in pcDNA3 or empty vector was transfected into 293 cells. Geneticin-resistant clones were isolated and grown up for further analysis. Stable expression of TGase 2 was validated by Western blotting using anti-TGase 2 antibody (upper) and further confirmed by the quantitation of [^{14}C]putrescine incorporation (lower). 293 and 293-TG indicate empty vector-derived control cell line and TGase 2-overexpressing cell line, respectively. B,C: Measurement of incorporated BP (B) and BS (C). 293 and 293-TG cells (2×10^6) were treated with 1 mM BP or BS in the presence of indicated calcium concentrations for 1 h. Cells were then lysed by sonication, and 10 μg of cell lysate was used for in situ transamidation assay. The BP- or BS-incorporated proteins of each cell line were probed using HRP-conjugated streptavidin. Results are presented as the mean ± S.D. of at least three independent experiments. NT, no treatment.

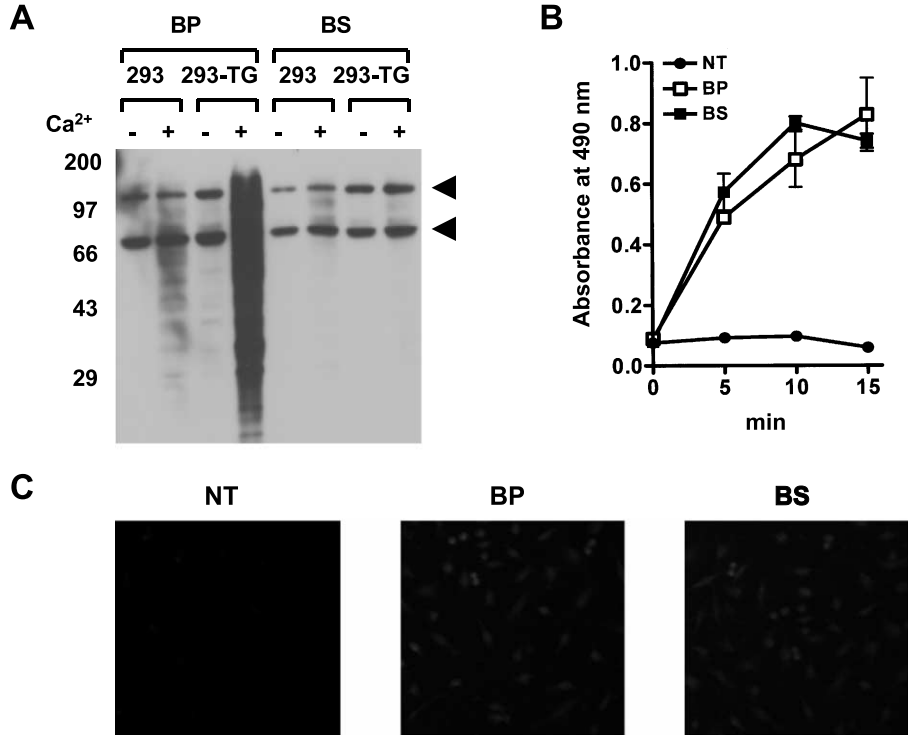

Fig. 4. A: Visualization of incorporated BP or BS. BP- or BS-treated cells in the absence or presence of calcium were lysed by sonication. 20 μg of each cell lysate was subjected to 12% SDS–PAGE and transferred to nitrocellulose membrane. After blocking and probing with HRP-conjugated streptavidin, the blots were developed by standard ECL protocol. Arrows indicate endogenous biotin-conjugated proteins. B: Uptake of BP and BS by 293 cells. 293 cells were incubated with 1 mM of BP or BS for the indicated times. Cells were washed and fixed immediately. After treatment with glutaraldehyde and sodium borohydride at room temperature, transported BP or BS was probed using HRP-conjugated streptavidin. NT, no treatment. C: Microscopic visualization of transported BP and BS in 293 cells. BP or BS was probed using Texas red-conjugated streptavidin.

tion. By contrast, no protein–BS conjugate was detected even in TGase 2-overexpressing cells at high calcium concentration (Fig. 4A).

In order to test whether differential incorporation of biotinylated polyamines results from a difference in polyamine transport rates, we measured the relative concentrations of intracellular BP and BS. 293 cells stably expressing TGase 2 were incubated with BP or BS over a period of 20 min. Cells were then washed, and uptake of polyamine was determined. However, free BP or BS was not held in the intracellular location because of their low molecular weight. We thus used the chemical cross-linking method to quantitate the free intracellular polyamines. As demonstrated in Fig. 4B, both BP and BS enter the cell immediately and maintain a constant level after 15 min. We found no difference in transport between BP and BS. In addition, we further confirmed polyamine uptake by the microscopic visualization of intracellular biotinylated polyamines using immunocytochemistry (Fig. 4C). Therefore, these results indicate that in situ TGase 2 activity may be assayed differently depending on the substrates supplied.

The presence of polyamine-conjugated or cross-linked protein is evidence of in situ TGase activity. The reaction rate of intracellular TGase largely depends on the physiological concentration of polyamines. Eukaryotic cells contain polyamines at abundant levels, but most of the polyamines, which are positively charged at physiological pH, are sequestered by interacting non-covalently with negatively charged cellular components, such as DNA, RNA or phospholipids [22]. Thus, only a small fraction of polyamines, free polyamine, would be available for TGase-mediated acyl transfer reactions. Most polyamine derivatives, even with large substituents on the secondary amino group of spermidine and spermine, are taken up by most cells [23]. BP used in this study, however, could not interact with negatively charged cellular constituents because one of two primary amino groups is protected by a biotin residue (Fig. 2A). This suggests a substantial in vivo accumulation of free BP. By contrast, for BS biotin was amidated on the secondary amino group of spermine and could be a substrate of spermidine/spermine *N*1-acetyltransferase, the rate-limiting enzyme in polyamine catabolism [24]. Additionally, studies with transgenic mice overexpressing ornithine decarboxylase, a regulatory enzyme in polyamine biosynthesis [25], or spermidine/spermine *N*1-acetyltransferase [20] showed excessive accumulation of putrescine but minimal derangement in spermidine and spermine concentrations, indicating that spermidine and spermine are more strictly maintained by complex regulatory mechanisms than putrescine. Previous studies showing that exogenous spermine might act as a growth inhibitor of prostate cancer cells [26], and polyamine-induced programmed cell death [27], further support the importance of spermidine and spermine regulation on cell viability. Taken together, these observations suggest that higher in situ incorporation of BP than BS might be due to differences in free substrate concentrations.

The measurement of physiological activity of TGase 2 is an essential step to understand its biological function. Enzyme assays with high sensitivity may lead to overestimation of the physiological value or false-positives. Thus, the selection of a suitable method and substrate is one of the keys to exactly represent enzyme activity or function. Although TGase 2 is overexpressed in the cell line used, its activity is dormant inside the cells. Together, these results show that the expression level of TGase 2 cannot represent enzymatic activity. Moreover, our results indicate the possibility of overrepresentation of in situ activity of TGase 2 as a consequence of the choice of substrates. Therefore, to evaluate the role of TGase 2 in several pathologic situations, careful interpretation of in situ activity may be required.

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References

[1] Folk, J.E. (1980) Annu. Rev. Biochem. 49, 517–531.
[2] Grenard, P., Bates, M.K. and Aeschlimann, D. (2001) J. Biol. Chem. 276, 33066–33078.
[3] Lesort, M., Attanavanich, K., Zhang, J. and Johnson, G.V. (1998) J. Biol. Chem. 273, 11991–11994.
[4] Nakaoka, H., Perez, D.M., Baek, K.J., Das, T., Husain, A., Misono, K., Im, M.J. and Graham, R.M. (1994) Science 264, 1593–1596.
[5] De Laurenzi, V. and Melino, G. (2001) Mol. Cell. Biol. 21, 148–155.
[6] Gentile, V., Thomazy, V., Piacentini, M., Fesus, L. and Davies, P.J. (1992) J. Cell Biol. 119, 463–474.
[7] Aeschlimann, D., Kaupp, O. and Paulsson, M. (1995) J. Cell Biol. 129, 881–892.
[8] Oliverio, S., Amendola, A., Di Sano, F., Farrace, M.G., Fesus, L., Nemes, Z., Piredda, L., Spinedi, A. and Piacentini, M. (1997) Mol. Cell. Biol. 17, 6040–6048.
[9] Anderson, R.P., Degano, P., Godkin, A.J., Jewell, D.P. and Hill, A.V. (2000) Nature Med. 6, 337–342.
[10] Tucholski, J., Kuret, J. and Johnson, G.V. (1999) J. Neurochem. 73, 1871–1880.
[11] Green, H. (1993) Cell 74, 955–956.
[12] Achyuthan, K.E. and Greenberg, C.S. (1987) J. Biol. Chem. 262, 1901–1906.
[13] Zhang, J., Lesort, M., Guttmann, R.P. and Johnson, G.V. (1998) J. Biol. Chem. 273, 2288–2295.
[14] Folk, J.E. and Chung, S.I. (1985) Methods Enzymol. 113, 358–375.
[15] Folk, J.E., Park, M.H., Chung, S.I., Schrode, J., Lester, E.P. and Cooper, H.L. (1980) J. Biol. Chem. 255, 3695–3700.
[16] Lesort, M., Tucholski, J., Zhang, J. and Johnson, G.V. (2000) J. Neurochem. 75, 1951–1961.
[17] Jeon, J.H., Cho, S.Y., Kim, C.W., Shin, D.M., Kweon, J.C., Choi, K.H., Park, S.C. and Kim, I.G. (2002) Biochem. Biophys. Res. Commun. 294, 818–822.
[18] Slaughter, T.F., Achyuthan, K.E., Lai, T.S. and Greenberg, C.S. (1992) Anal. Biochem. 223, 166–171.
[19] Piacentini, M., Martinet, N., Beninati, S. and Folk, J.E. (1988) J. Biol. Chem. 263, 3790–3794.
[20] Pietila, M., Alhonen, L., Halmekyto, M., Kanter, P., Janne, J. and Porter, C.W. (1997) J. Biol. Chem. 272, 18746–18751.
[21] Haddox, M.K. and Russell, D.H. (1981) J. Cell Physiol. 109, 447–452.
[22] Igarashi, K. and Kashiwagi, K. (2000) Biochem. Biophys. Res. Commun. 271, 559–564.
[23] Holley, J.L., Mather, A., Wheelhouse, R.T., Cullis, P.M., Hartley, J.A., Bingham, J.P. and Cohen, G.M. (1992) Cancer Res. 52, 4190–4195.
[24] Dezeure, F., Sarhan, S. and Seiler, N. (1988) Int. J. Biochem. 20, 1299–1312.
[25] Soler, A.P., Gilliard, G., Megosh, L.C. and O'Brien, T.G. (1996) J. Invest. Dermatol. 106, 1108–1113.
[26] Smith, R.C., Litwin, M.S., Lu, Y. and Zetter, B.R. (1995) Nature Med. 1, 1040–1045.
[27] Facchiano, F., D'Arcangelo, D., Riccomi, A., Lentini, A., Beninati, S. and Capogrossi, M.C. (2001) Exp. Cell Res. 271, 118–129.