J Ind Microbiol Biotechnol (2013) 40:317–325
DOI 10.1007/s10295-012-1221-y

BIOTECHNOLOGY METHODS

Enhancement of *Streptomyces* transglutaminase activity and pro-peptide cleavage efficiency by introducing linker peptide in the C-terminus of the pro-peptide

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Received: 25 July 2012 / Accepted: 30 November 2012 / Published online: 24 January 2013
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Abstract *Streptomyces* transglutaminase (TGase) has been widely used in food, pharmaceutical and textile industries. *Streptomyces* TGase is naturally synthesized as zymogen (pro-TGase), which is then processed to produce active enzyme by removing its N-terminal pro-peptide. Although the pro-peptide is essential for TGase folding and secretion, few studies have been reported on improving the properties of TGase by pro-peptide engineering. In this study, we developed a new approach to improve the properties of TGase based on pro-peptide engineering. When the α-helix$^{37G-42S}$ in pro-peptide was substituted with three glycines and three alanines respectively, the mutants exhibited higher specific activity and the efficiency of pro-peptide cleavage was enhanced. To further improve the properties of TGase, relevant mutations were constructed by introducing linker peptides in the C-terminus of the pro-peptide. Mutants with GS (GGGGS) and PT (PTPPTTPT) linker peptide exhibited 1.28 fold and 1.5 fold higher specific activity than the wild-type enzyme, respectively. This new method could be used to improve the properties of TGase by pro-peptide modification, which is a promising technology for creating unique TGase with various beneficial properties.

Keywords Transglutaminase · Pro-peptide · Protein folding · Specific activity · Linker peptide

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Introduction

Transglutaminase (TGase, EC 2.3.2.13) is a family of enzymes that exhibit several catalytic activities: the crosslinking of proteins by forming N^{ϵ}-(γ-glutamyl) lysine bonds, the incorporation of polyamines into protein, the deamidation of protein-bound glutamines, and the covalent attachment of proteins to long-chain ω-hydroxyl of lipid by ester bond formation [20, 21]. This capacity of TGase has been used in attempts to improve the functional properties of food. It is able to catalyze crosslinking of whey proteins, soy proteins, wheat proteins, beef myosin, casein and crude actomysin, leading to their texturization [10, 29]. TGase is also used in tissue engineering and leather processing, for example, collagen scaffolds and gelatin scaffolds [32]. TGase is widely distributed in various organisms, including plants [4], microorganisms [33], and invertebrates [5]. Among these TGases, TGase from *Streptomyces* is advantageous for industrial applications because of the

Ca^{2+}-independent, higher reaction rate, broad substrate specificity for acyl donor and smaller molecular size [12, 27].

Streptomyces TGase is naturally synthesized as zymogen (pro-TGase), which is then processed to produce active enzyme by removing its N-terminal pro-peptide. The pro-peptide, which consists of a short one-turn helix and a long α-helix, covers the active-site cleft and subsequently blocked the substrate from accessing the active sites [27]. The pro-peptide works as an intramolecular chaperone and is essential for the folding and secretion of the functional protein [16, 17]. The direct expression of TGase without the pro-peptide resulted in inclusion bodies in *Escherichia coli* [13]. However, the expression of pro-TGase followed by processing to TGase in vitro could produce a large amount of soluble pro-TGase [17, 19]. Although the pro-peptide of TGase is essential for its efficient secretion and folding, few studies have reported on improving the properties of TGase by pro-peptide engineering. Most studies about improving TGase properties focused on the residues in TGase. For example, random and rational mutageneses were adopted to improve the specific activity and thermostability of TGase [18, 30]. Moreover, methods of saturation mutagenesis and DNA-shuffling were used to enhance thermostability of TGase [2]. Comparing with the mutations in mature enzyme, the pro-peptide could also present as a candidate for modification to improve the properties of TGase.

To date, a large number of pro-peptides from various proteins, such as subtilisin and α-lytic protease [3], have been identified to function as intramolecular chaperones to assist the folding of the respective functional domains. Moreover, pro-peptide engineering was proposed to investigate the molecular mechanism of protein folding with an intramolecular chaperone [25]. Pro-peptide engineering, such as site-directed and/or random mutagenesis, chimeras and gene shuffling between members of the family, were used to improve the properties of proteins [7, 15]. For example, the extracellular production of subtilase was enhanced by random mutagenesis of its pro-peptide [6]. The pro-peptide from subtilisin homologies was also found to chaperone the intramolecular folding of denatured subtilisin [25]. Pro-peptide engineering is thus a useful method to improve the properties of proteins.

In our previous studies, the pro-TGase from *S. hygroscopicus* was efficiently secreted by *E. coli* [17]. The molecular weights of *S. hygroscopicus* pro-TGase and mature TGase were 43 kDa and 38 kDa, respectively. The pro-peptide and pro-TGase consisted of 57 and 389 amino acid residues, respectively [31]. We also found that co-expression of *S. hygroscopicus* TGase and its pro-peptide as independent polypeptides under a single T7 promoter resulted in active TGase in *E. coli* [16]. This approach for TGase expression indicates that the pro-peptide of TGase could function as a separate protein to help TGase folding. The C-terminal loop of the pro-peptide could be recognized as a linker peptide and the pro-peptide as a fusion protein with TGase through this linker peptide. It is well known that a linker peptide could play an essential role in maintaining cooperative inter-domain interaction [9]. Many linker peptides in various protein families lack a regular secondary structure, and they display varying degrees of flexibility to match their particular biological purpose. In general, altering the length and composition of linker peptides connecting different domains affect protein stability, folding rates and domain–domain orientation [8]. Poly-glycine, serine-rich flexible sequences (GS linker peptide), and proline-threonine-threonine (PT linker peptide) have been frequently used as linker peptides [26, 28]. Compared with methods of site-directed and/or random mutagenesis in pro-peptide engineering, we developed a new approach to improve TGase properties by adding a linker peptide to the C-terminus of the pro-peptide.

Materials and methods

Strains and plasmids

Escherichia coli JM109 and *E. coli* BL21(DE3) were purchased from TaKaRa (TaKaRa, Dalian, China). pBB1-1011 is the pro-TGase expression vector [17].

Site-directed and linker peptide mutagenesis

All basic recombinant DNA procedures, such as isolation and purification of DNA, and transformation of *E. coli* were performed as described previously [17]. Mutations of TGase were accomplished by TaKaRa mutanBEST kit (TaKaRa, Dalian, China) using the following primers: pro-37-42 (5′-GCGGAATTGCCGCCGAGCGTC-3′ and 5′-ACCCGCAGTCAGGGCCCTTTTGTTC-3′); pro-37-42AAA (5′-GCCGCCGCCGCGGAATTGCCGCCGAGCGTC-3′ and 5′-ACCCGCAGTCAGGGCCCTTTTGTTC-3′); pro-37-42GGG (5′-GGCGGCGGCGCGGAATTGCCGCCGAGCGTC-3′ and 5′-ACCCGCAGTCAGGGCCCTTTTGTTC-3′); pro-TG-W-D58A, pro-37-42AAA-D58A and pro-37-42GGG-D58A (5′-CCCCCGCCGCTGCCGACGAGAGGGT-3′ and 5′-CCCGGAAGAGCGCACTGACGCTCGG-3′); pro-52G (5′-GGCCTCTTCCGGGCCCCCGACGCTG-3′ and 5′-CGCACTGACGCTCGGCGGCAATTC-3′); pro-52GG (5′-GGCGGCCTCTTCCGGGCCCCCGACGCTG-3′ and 5′-CGCACTGACGCTCGGCGGCAATTC-3′); pro-52GGG (5′-GGCGGCGGCCTCTTCCGGGCCCCCGACGCTG-3′ and 5′-CGCACTGACGCTCGGCGGCAATTC-3′); pro-52GGGG (5′-GGCGGCGGCGGCCTCTTCC

GGGCCCCCGACGCTG-3′ and 5′-CGCACTGACGCTC GGCGGCAATTC-3′); pro-52GS (5′-GGCGGCGGCGGC AGCCTCTTCCGGGCCCCCGACGCTG and 5′-CGCAC TGACGCTCGGCGGCAATTC-3′); pro-52PT (5′-CCGAC GCCGCCGACGACGCCGACGCTCTTCCGGGCCCCCG ACGCTG and 5′-CGCACTGACGCTCGGCGGCAATTC-3′). The PCR amplification conditions were as follow: initial denaturation at 95 °C for 5 min followed by 30 cycles each consisting of 98 °C for 15 s, 60 °C for 15 s, 72 °C for 6 min. These recombinant plasmids were individually transformed into *E. coli* BL21 (DE3) for expression of the TGase and its derivatives.

The α-helix$^{37G-42S}$ was deleted and substituted with AAA and GGG in mutants pro-37-42, pro-37-42AAA and pro-37-42GGG, respectively. The residue Asp58 was substituted with Ala in mutants pro-TG-W-D58A, pro-37-42AAA-D58A and pro-37-42GGG-D58A. The mutants with linker peptides (G, GG, GGG, GGGG, GGGGS and PTPPTTPT) were named as pro-52G, pro-52GG, pro-52GGG, pro-52GGGG, pro-52GS and pro-52PT, respectively.

Expression and purification of recombinant proteins in *E. coli*

A seed culture of each recombinant strain was prepared by growing cells in Luria–Bertani medium containing ampicillin (100 μg/mL) at 37 °C for 12 h. The seed culture was inoculated into Terrific Broth medium containing ampicillin (100 μg/mL) and cultivated at 37 °C until the optical density (OD) at 600 nm reached 2.0. Isopropyl-β-D-thiogalactopyranoside was added to the culture at a final concentration of 0.4 mM, followed by incubation at 20 °C. The pro-TGase and the mutants were solubly expressed in *E.coli* BL21 (DE3). The recombinant enzymes were purified with nickel affinity chromatography (HisTrap™ FF crude, GE Healthcare) and gel filtration (Superdex™ 75 10/300GL, GE Healthcare). The proteins of higher than 90 % purity measured by SDS-PAGE and coomassie blue staining were used to analyze profiles of enzymatic activities and kinetic parameters as described previously [17].

Pro-TGase activation in vitro

The pro-TGase activation was carried out by dispase (final concentration 2 μg/mL) at 37 °C for 5–60 min.

Assay of TGase activity

TGase activity was measured by a colorimetric procedure, in which N–CBZ–Gln–Gly (Sigma, Shanghai, China) was used as the substrate. A calibration curve was obtained using L-glutamic acid γ-monohydroxamate (Sigma, Shanghai, China). One unit of TGase is defined as that required to generate 1 μmol of γ-glutamic acid γ-monohydroxamate per minute at 37 °C. The results are the averages of triplicate assays [11].

Characterization of selected variants

The protein concentrations were measured with the Bradford reagent. The values of Michaelis–Menten constant (K_m) and k_{cat} were determined as previously described [17].

Homology modeling

Homology modeling via SWISS-MODEL (http://swissmodel.expasy.org/) was employed to build structures of the wild-type enzyme and its mutants [1, 14]. The template structure is *S. mobaraensis* pro-TGase (PDB code: 3IU0), which has the highest sequence identity (79 %) with the wild type enzyme.

Results and discussion

Deletion and substitution of the α-helix$^{37G-42S}$ in pro-peptide of TGase

The α-helix$^{37G-42S}$ in pro-peptide contains six amino acids (GQPGNS), but its function in TGase folding is still unclear. To investigate its function, the amino acid residues in α-helix$^{37G-42S}$ were deleted. On the other hand, there were two glycines in α-helix$^{37G-42S}$. Glycine and alanine have short side chains and have few interactions with nearby residues. To check if the glycine and alanine in this region had any influence in the properties of TGase, the α-helix$^{37G-42S}$ was substituted with three alanines and glycines, respectively. All the mutants could be expressed successfully by *E.coli* (Fig. 1, lanes 2, 3 and 4) and were active after dispase cleavage (Table 1). To verify if the

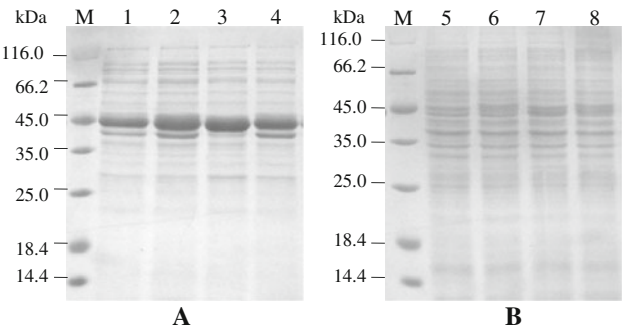

Fig. 1 SDS-PAGE analysis of pro-TG-W and its mutants expressed in *E.coli*. **a** SDS-PAGE analysis of extracellular pro-TG-W and its mutants; **b** SDS-PAGE analysis of whole cells of recombinant strains. *M*: protein molecular weight marker; *1*, pro-TG-W; *2*, pro-37-42; *3*, pro-37-42AAA; *4*, pro-37-42GGG

Table 1 Enzymatic properties of pro-TG-W and its mutants

	Activity in supernatant (U/mL)	Specific activity (U/mg)	K_m (mmol/L)
pro-TG-W	1.7 ± 0.2	15.3 ± 0.9	54.6 ± 1.5
pro-37-42	2.1 ± 0.1	17.4 ± 1.1	46.7 ± 2.2
pro-37-42AAA	2.4 ± 0.1	18.7 ± 1.2	44.8 ± 1.9
pro-37-42GGG	2.2 ± 0.3	19.1 ± 0.6	42.9 ± 2.6
pro-TG-W-D58A	1.7 ± 0.3	15.1 ± 0.6	54.1 ± 1.7
pro-37-42AAA-D58A	1.6 ± 0.1	15.9 ± 1.0	54.8 ± 0.9
pro-37-42GGG-D58A	1.7 ± 0.3	15.4 ± 0.8	53.9 ± 2.1

mutations in α-helix$^{37G-42S}$ made any imprint onto the mature domain, the enzyme properties were characterized after protein purification. It was found that the specific activities of all mutants increased compared with the wild-type enzyme (pro-TG-W) (Table 1). These results indicated that the α-helix$^{37G-42S}$ in the pro-peptide was not essential for TGase expression. However, the α-helix$^{37G-42S}$ had a negative influence on the activity of TGase. The deletion or substitution of α-helix$^{37G-42S}$ was beneficial to improve TGase activity. The interaction between α-helix$^{37G-42S}$ and TGase may have played an important role in TGase activity.

Homologous structures of pro-TG-W and its mutants

To analyze the results caused by the mutations in pro-peptide, the structural models of pro-TG-W and its mutants were simulated via SWISS-MODEL. Homologous modeling is a reliable method to generate a structure based on a known crystal structure of a homologue with a high sequence identity [14]. In the absence of crystal structure of the pro-TGase and its mutants, homology modeling was used to generate the three-dimensional structures of these enzymes via SWISS-MODEL to understand why the mutants have shown the higher activity. The template structure was pro-TGase from *S. mobaraensis* (PDB code 3IU0) [27]. As shown in Fig. 2a, the pro-peptide of pro-TGase from *S. hygroscopicus* contained two helices in its N-terminus and a disordered loop in its C-terminus. When the α-helix$^{37G-42S}$ was deleted or substituted, the C-terminal loop in pro-peptide turned into a helical structure in all mutants (Fig. 2b–d). A short loop between the two helices (α-helix$^{12Y-30N}$ and α-helix$^{40L-54P}$) appeared in both deletion and substitution mutants. Furthermore, some differences were found in pro-peptides of the mutants. The loop between the two helices (α-helix$^{12Y-30N}$ and α-helix$^{40L-54P}$) in pro-peptide were prolonged in pro-37-42AAA and pro-37-42GGG compared with that of pro-37-42. The prolonged loop made the C-terminal helix far from the N-terminal helix in the pro-peptide. The movement of the C-terminal helix in the pro-peptide had an effect on the location of the N-terminal residues in TGase (Fig. 2, colored in red). It has been reported that the N-terminal residues in TGase could affect the activity of TGase by influencing the interaction between the active sites and substrates [22, 24]. Thus, the movement of the N-terminal loop in TGase may reduce the hindrance of substrate binding that resulted in high activity.

According to the homologous structures of TGase, the hydrogen bonds between pro-peptide and TGase were analyzed. New hydrogen bonds were found in the mutants compared with that of pro-TG-W (Fig. 3a–c). Notably, the N-terminal residue 58 (asparagine) in TGase formed a new hydrogen bond with pro-peptide in all mutants. The Asp 58 was located in the N-terminus of TGase, which was important for TGase activity. To determine a possible role of the new hydrogen bond in the increased activity, the residue Asp58 in pro-TG-W and its mutants was substituted with Ala. When the Asp58 was substituted with Ala in pro-TG-W, no change in the specific activity was observed (Table 1). However, the mutants pro-37-42AAA-D58A and pro-37-42GGG-D58A exhibited decreased activity compared with those of pro-37-42AAA and pro-37-42GGG. These results indicated that the increased activity was caused by the new hydrogen bonds.

Proteolytic cleavage of the pro-peptide from pro-TG-W and pro-37-42GGG

The pro-peptide has to be cleaved if TGase is to become an active form. As described previously, the simulation of pro-TGase revealed that the structure of the pro-peptide had been altered. To investigate the efficiency of pro-peptide cleavage, we performed the proteolysis of pro-TG-W and pro-37-42GGG. Both of them could be converted into mature TGase by the removal of the N-terminal pro-peptide with dispase (Fig. 4). It is noteworthy that the highest activity of pro-37-42GGG and pro-TG-W appeared at 5 min and 15 min, respectively (Fig. 4a). The pro-peptide cleavage process was then analyzed by SDS-PAGE. The cleavage efficiency of pro-37-42GGG was increased as compared with pro-TG-W (Fig. 4b, c). The band of pro-TGase of pro-37-42GGG was turned into mature TGase in 5 min (Fig. 4c). The increased cleavage efficiency of the pro-peptide was influenced by amino acid substitutions. The C-terminal helix in the mutant was likely to expose the cleavage sites of proteolysis on the surface, and thus more susceptible to processing. These results confirmed that the mutant pro-37-42GGG caused a structural change in pro-

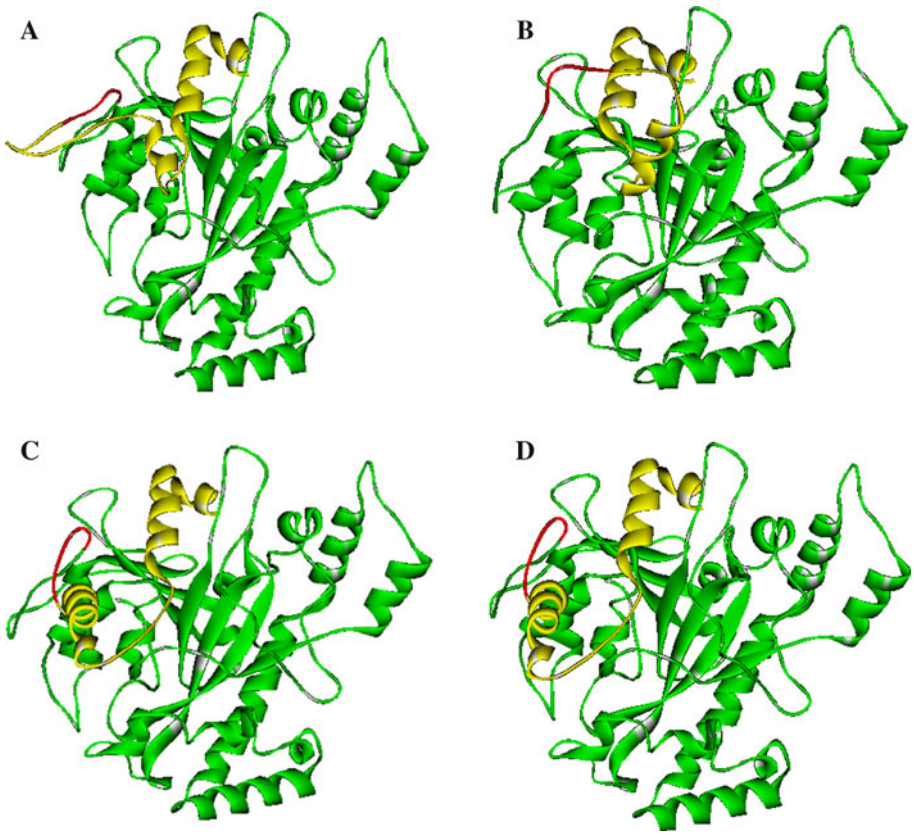

Fig. 2 Homology modeling on the structure of pro-TG-W and its mutants via SWISS-MODEL. **a** pro-TG-W; **b** pro-37-42; **c** pro-37-42AAA; **d** pro-37-42GGG. The pro-peptide is colored in *yellow* and the six residues in N-terminus of TGase are colored in *red* (color figure online)

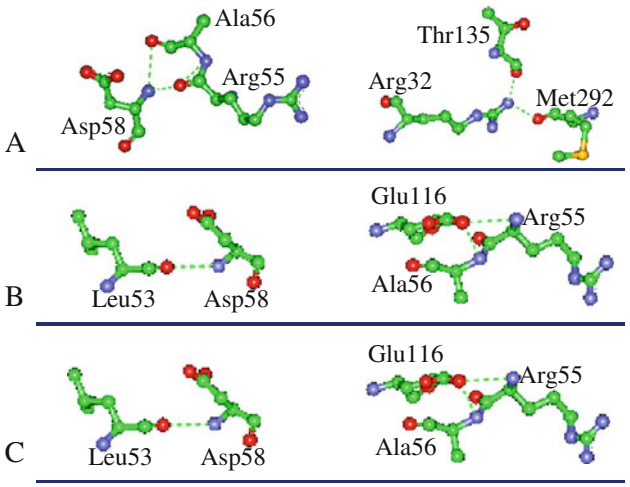

Fig. 3 The new hydrogen bonds between pro-peptide and TGase in the mutants **a** pro-37-42; **b** pro-37-42AAA; **c** pro-37-42GGG

peptide that was consistent with the results of homologous structural analysis.

Construction of the mutants with linker peptide between pro-peptide and TGase

By taking the aforementioned investigation into account, the α-helix$^{37G-42S}$ substituted mutant with GGG or AAA

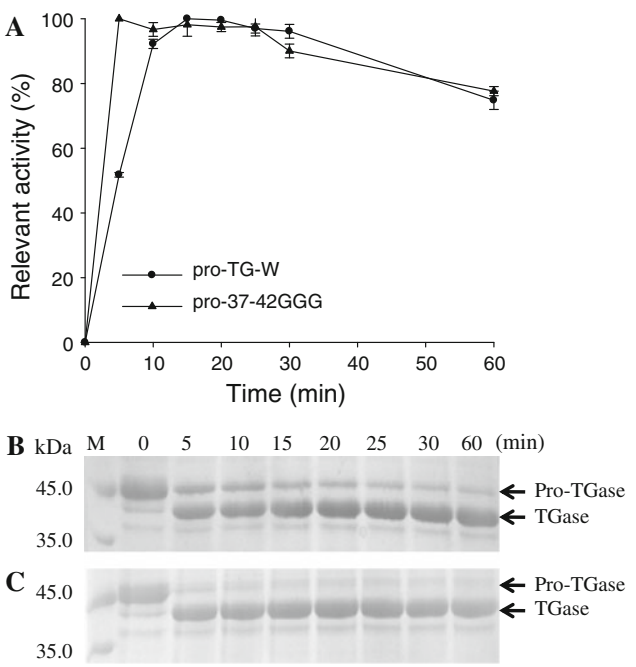

Fig. 4 Proteolysis of pro-TG-W and pro-37-42. **a** activity of pro-TG-W and pro-37-42 cleaved by dispase; **b** SDS-PAGE analysis of the proteolysis of pro-TG-W; **c** SDS-PAGE analysis of the proteolysis of pro-37-42. Purified preforms (200 μg/ml) were mixed with dispase (final concentration, 2 μg/ml) and incubated at 37 °C. At the time intervals indicated, samples were taken and subjected to activity assay and SDS-PAGE analysis

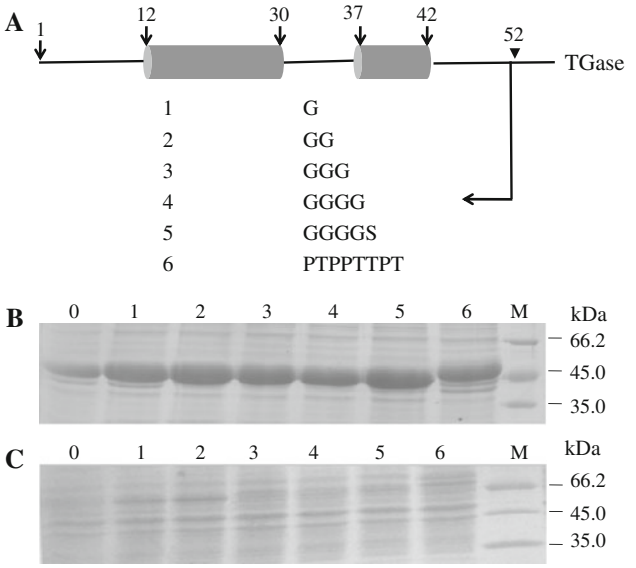

Fig. 5 Expression of pro-TGase and its mutants in *E.coli*. **a** schematic representation of pro-TG-W and its mutants with the linker peptides; **b** SDS-PAGE analysis of extracellular pro-TGase and its mutants; **c** SDS-PAGE analysis of whole cells of recombinant strains. *0*, pro-TG-W; *1*, pro-52G; *2*, pro-52GG; *3*, pro-52GGG; *4*, pro-52GGGG; *5*, pro-52GS; *6*, pro-52PT, M: protein molecular weight marker

Table 2 Enzyme properties of pro-TG-W and its mutants

	Specific activity (U/mg)	K_m (mmol/L)	K_{cat} (s^{-1})	K_{cat}/K_m (mmol/L S)
pro-TG-W	15.3 ± 0.9	54.6 ± 1.5	31.3 ± 1.7	0.57
pro-52G	17.3 ± 1.3	50.6 ± 2.9	32.3 ± 1.1	0.64
pro-52GG	17.3 ± 1.5	49.1 ± 1.9	30.3 ± 2.1	0.62
pro-52GGG	17.1 ± 0.6	49.5 ± 1.5	35.3 ± 1.2	0.71
pro-52GGGG	17.3 ± 1.5	50.1 ± 1.4	45.3 ± 2.2	0.90
pro-52GS	19.3 ± 0.8	40.3 ± 2.3	63.3 ± 1.5	1.57
pro-52PT	22.7 ± 2.1	30.1 ± 2.1	81.3 ± 2.4	2.70

exhibited higher specific activity compared with pro-TG-W. The C-terminal loop of pro-peptide was found to have an important effect on the properties of TGase. Therefore, the C-terminal loop was modified to improve the properties of TGase by inserting the poly-glycine and linker peptides (GS and PT) into the C-terminus of the pro-peptide (Fig. 5a). The linker peptides were added before the recognition sites of dispase (Leucine-Phenylalanine) in the C-terminal loop of the pro-peptide. All the mutants with linker peptides were expressed successfully by *E. coli* (Fig. 5b, c). These results indicated that the GS or PT linker peptides in the C-terminus of pro-peptide had no influence in pro-TGase expression.

Characteristics of TGase and its mutants with linker peptides

To confirm if the linker peptides between pro-peptide and TGase had any impact on the folding of the mature domain, proteins were purified and characterized. The specific activity of all mutants increased as compared with pro-TG-W. Especially for the mutants with GS (pro-52GS) and PT (pro-52PT) linker peptides, showed the highest specific activities, 1.28 fold and 1.5 fold higher than pro-TG-W, respectively (Table 2). When the values of K_m and K_{cat} were assayed and calculated, most of mutants had little effects on the K_m, K_{cat} and K_{cat}/K_m values, except for the mutants pro-52GS and pro-52PT. The increased K_{cat}/K_m values of mutants pro-52GS and pro-52PT suggested that the catalytic efficiency had increased compared with that of pro-TG-W. The linker peptide was near the activity sites, in which some interactions between the linker peptide with the catalytic region might lead to the increased catalytic efficiency. The activation processes of pro-TGase were further analyzed by TGase activity (Fig. 6a) and SDS-PAGE (Fig. 6b–d). The pro-TGase was cleaved into mature TGase in 5 min, which is more efficient than pro-

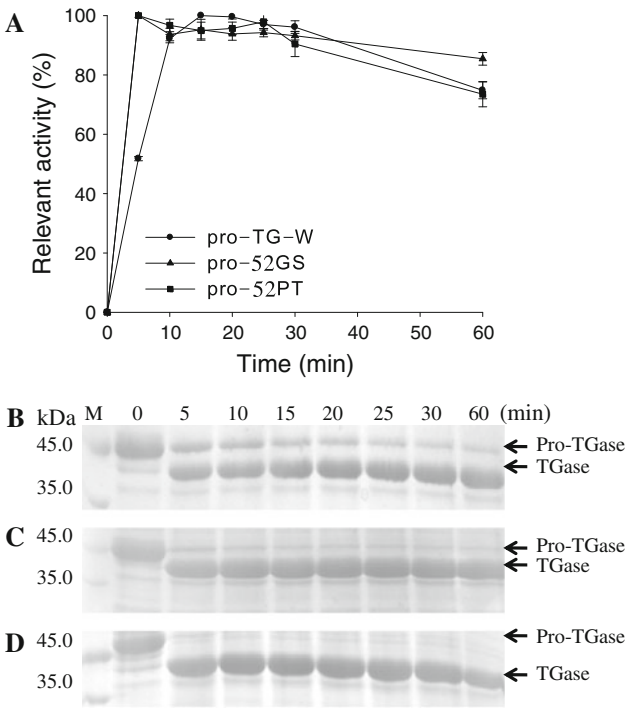

Fig. 6 Limited proteolysis of pro-TG-W, pro-52GS and pro-52PT. **a** activity of pro-TG-W and its mutants cleaved by dispase; **b** SDS-PAGE analysis of the proteolysis of pro-TG-W; **c** SDS-PAGE analysis of the proteolysis of pro-GS; **d** SDS-PAGE analysis of the proteolysis of pro-PT. Purified proforms (100 μg/ml) were mixed with dispase (2 μg/ml) and incubated at 37 °C. At the time intervals indicated, samples were taken and subjected to activity assay and SDS-PAGE analysis

TG-W. These results are similar with the pro-37-42GGG and pro-37-42AAA mutants. Both specific activity and cleavage efficiency of mutants were enhanced by the mutations in pro-peptide.

Homologous structures of pro-TG-W and its mutants

Three mutants (pro-52GGGG, pro-52GS and pro-52PT)that exhibited highly specific activity were chosen to simulate the structures via SWISS-MODEL. As shown in Fig. 7, the N-terminal α-helix$^{12-30}$ maintained the helical structure in both pro-TG-W and its mutants. The major changes occurred in the C-terminus of the pro-peptide. GS linker peptide formed a loop structure in pro-52GS (Fig. 7c, colored in blue), whereas PT linker peptide formed a helical structure in pro-52PT (Fig. 7d, colored in blue). Although both mutants contained a new helix in the C-terminus of the pro-peptide, the helix in mutant pro-52PT was longer than that in mutant pro-52GS. The interaction between pro-peptide and TGase could be varied because of the C-terminal helices. The new C-terminal α-helix was close to the left wall of catalytic active sites of TGase, which could help the left wall folding to enhance the catalytic ability [12]. The N-terminal residues in TGase following the pro-peptide were moved, which could be beneficial for TGase activity.

In our previous studies, the pro-peptide was found to be essential for its efficient secretion and solubility in *E. coli* by deletion of the N-terminal amino acids in pro-peptide [17]. In this study, we found that adding linker peptides in C-terminus of pro-peptide could improve the activity and cleavage efficiency of TGase. It is well known that linker peptides are frequently used to fuse two different domains of protein and are likely to facilitate the folding of multi-domain proteins, and that the composition and the lengths of linker peptides are very important for protein stability, folding rates and domain–domain orientation [8]. Unlike the functions of linker peptides in fusion proteins, linker peptides used in this study could enhance the activity and the pro-peptide cleavage efficiency of TGase by altering the structure of the pro-peptide. The linker peptide was near the N-terminus and active sites of TGase. The N-terminal residues have been recognized as an important region for TGase modification through many studies [2, 18]. The new interactions between the pro-peptide and N-terminus of TGase that were caused by linker peptide could contribute the increased activity.

Furthermore, the improvement of TGase properties indicated that the pro-peptide functioned as an intramolecular chaperone for the correct folding of the enzymes. A theory of "protein memory" indicates that an identical polypeptide can fold into an alter conformation with different secondary structure, stability and specificities through a mutated pro-peptide [23]. Once the function of the pro-peptide is completely deciphered, it could be possible to design a novel pro-peptide to improve the

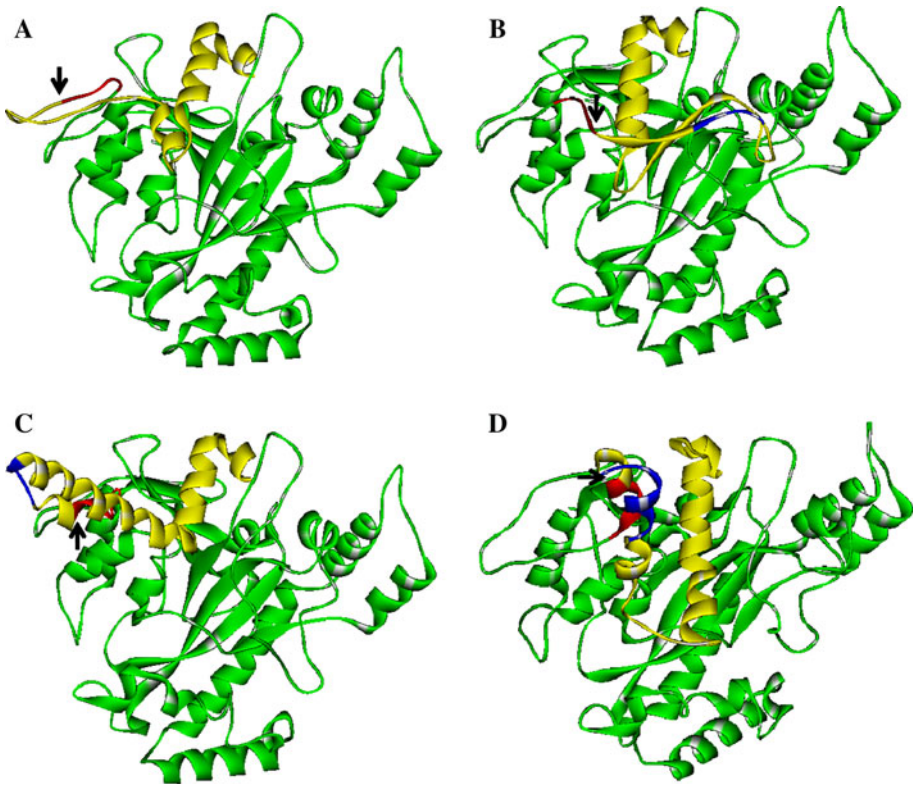

Fig. 7 Homology modeling on the structure of pro-TG-W and its mutants via SWISS-MODEL. **a** pro-TG-W; **b** pro-52GGG; **c**, pro-52GS; **d** pro-52PT. The pro-peptide is colored in *red*. The amino acids of linker peptides were labeled with *ball* and *stick*. The N-terminal loop in TGase is marketed with *arrows* (color figure online)

properties of enzymes. The modification of pro-peptide has become an efficient approach to improve properties of enzymes. Compared to the methods of site-directed and/or random mutagenesis in pro-peptide engineering, we developed a new approach to improve TGase properties by adding polypeptide in the C-terminus of pro-peptide. The composition and the length of linker peptides should be optimized and studies on the mechanism of folding involved in TGase are underway.

Conclusions

We have described a new approach to alter TGase function based on pro-peptide engineering. Linker peptides introduced in the C-terminus of the pro-peptide in the pro-TGase could improve TGase properties. This new approach is not only an important tool for studying the mechanism of protein folding, but also a promising technology for creating unique TGase with various beneficial properties.

Acknowledgments We would like to thank Professor Byong H. Lee for critical reading and suggestions on the manuscript. This work was supported by the National Natural Science Foundation of China (No. 31171639, No. 31000031, and No. 31070711), the National High Technology Research and Development Program of China (No. 2011AA100905), the NCET-10-0461, the Natural Science Foundation of Jiangsu Province (No. BK2010147) and the Independent Innovation Program of Jiangnan University (JUSRP11215).

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