Appl Microbiol Biotechnol (2004) 64: 447–454
DOI 10.1007/s00253-003-1539-5

MINI-REVIEW

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Properties and applications of microbial transglutaminase

Received: 22 August 2003 / Revised: 1 December 2003 / Accepted: 5 December 2003 / Published online: 22 January 2004
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Abstract Some properties and applications of the transglutaminase (TGase) referred to as microbial TGase (MTGase), derived from a variant of *Streptomyces mobaraensis* (formerly classified as *Streptoverticillium mobaraense*), are described. MTGase cross-linked most food proteins, such as caseins, soybean globulins, gluten, actin, myosins, and egg proteins, as efficiently as mammalian TGases by forming an ε-(γ-glutamyl)lysine bond. However, unlike many other TGases, MTGase is calcium-independent and has a relatively low molecular weight. Both of these properties are of advantage in industrial applications; a number of studies have illustrated the potential of MTGase in food processing and other areas. The crystal structure of MTGase has been solved. It provides basic structural information on the MTGase and accounts well for its characteristics. Moreover, an efficient method for producing extracellular MTGase has been established using *Corynebacterium glutamicum*. MTGase may be expected to find many uses in both food and non-food applications.

Introduction

Transglutaminase (TGase; protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) catalyzes an acyl transfer reaction between the γ-carboxyamide group of a peptide-bound glutaminyl residue (acyl donors) and a variety of primary amines (acyl acceptors), including the amino group of lysine. In the absence of amine substrates, TGase catalyzes the hydrolysis of the γ-carboxyamide group of the glutaminyl residue, resulting in deamidation. When the ε-amino group of a peptide-bound lysyl residue is the substrate, peptide chains are covalently connected through ε-(γ-glutamyl)lysine (G-L) bonds (Fig. 1). TGases are present in most animal tissues and body fluids, and are involved in several biological processes, including blood clotting, wound healing, epidermal keratinization, and stiffening of the erythrocyte membrane (Aeschlimann and Paulsson 1994). A typical example of a TGase-catalyzed protein crosslinking reaction is the termination of bleeding in wound healing (blood coagulation) by Factor XIIIa, an activated form of plasma TGase (Chung et al. 1974; Hornyak et al. 1989). Another example is the setting phenomenon—suwari—of salted, ground fish protein pastes in Japanese kamaboko manufacture, where the contribution of the fish TGase intrinsic in suwari has been demonstrated by Seki et al. (1990). TGase is thought to modify the behavior of protein substrates (Whitaker 1977), as demonstrated in the above cases. In our attempts to develop novel proteins and processing methodologies, we have been interested in the changes in behavior of food proteins resulting from the formation of G-L bonds by TGase.

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Feasibility study on protein modification by TGase

In the early 1980s, in parallel with our own work, the possibility of modifying the behavior of milk caseins and soybean globulins was demonstrated using TGase derived from guinea pig liver (Ikura et al. 1992) and bovine plasma (Kurth and Rogers 1984). In these studies, the cross-linking of proteins of different origins, as well as the incorporation of amino acids or peptides to counteract nutritional deficiencies, was demonstrated. At that time we were investigating the feasibility of modifying food protein in industrial applications using the guinea pig liver enzyme (Motoki and Nio 1983; Motoki et al. 1984, 1986, 1987a, 1987b; Nio et al. 1985, 1986; Nio and Motoki 1986), and we used whey proteins and actomyosin from beef, pork, chicken or fish as substrates that could be gelled. Subsequently, improvements in the solubility, water-holding capacity and thermal stability of food proteins were demonstrated. Proteins in an oil-in-water

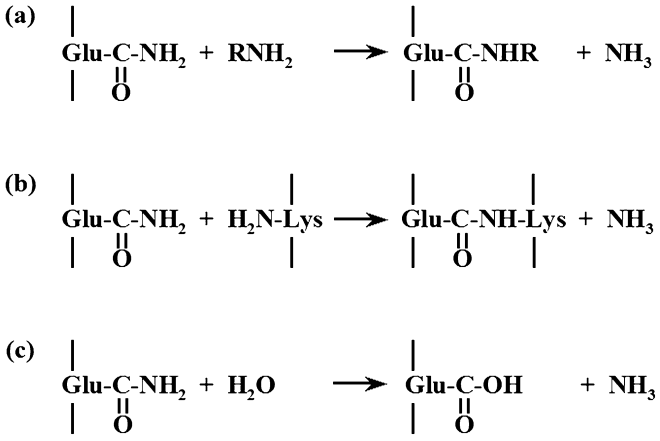

Fig. 1a–c Reactions catalyzed by transglutaminase (TGase). **a** Acyl transfer. **b** Crosslinking of Gln and Lys residues in proteins or peptides. The resulting bridge is called an ε-(γ-glutamyl)lysine (G-L) bond. **c** Deamidation

type of emulsion were also gelled by TGase. We were able to prepare a transparent, water-resistant, and slowly digestible protein film by spreading protein solutions on a flat surface. On the basis of these results, TGase was considered potentially useful for creating proteins with novel properties. However, the limited supply of guinea pig liver and the unacceptability of its use in food manufacture hindered commercialization. The critical issue was the mass production of TGase.

Search for a TGase for industrial application

In general, there are three approaches to developing industrially useful TGases. The first is to extract and purify the enzyme from the tissues or body fluids of food-use animals, such as cattle, swine, and fish. In Europe, factor XIII, a type of TGase, is extracted commercially from the blood of cattle and swine at slaughter (Wilson 1992). The blood enzyme is, however, rarely utilized in food manufacture, since the red pigmentation is often detrimental to the appearance of the product. In addition, thrombin, a specific protease, is required to activate the enzyme. The second approach is to obtain the enzyme by means of genetic manipulation using host microorganisms such as *Escherichia coli*, *Bacillus*, yeast, or *Aspergillus*. Many researchers, including Ikura et al. (1998; guinea pig liver TGase in *E. coli*), Bishop et al. (1990; human factor XIIIa in yeast), Takehana et al. (1994; *Streptomyces* TGase in *E. coli*), Washizu et al. (1994; *Streptomyces* TGase in *Streptomyces lividans*), Yokoyama et al. (2000; *Streptomyces* TGase in *E. coli*) and Yasueda et al. (1995; fish TGase in *E. coli*), have attempted to obtain large amounts of TGase at low cost. Recently, we succeeded in obtaining efficient secretion of *Streptomyces* TGase by *Corynebacterium glutamicum*, a bacterium much used for the industrial production of amino acids such as glutamate and lysine (see below). However, none of these TGases has been commercialized, due in large part to the problem of public acceptance. The third approach is to screen for TGase-producing microorganisms. If an appropriate microorganism could be found, it should be possible to mass-produce TGase by traditional fermentation technology.

Microbial TGase derived from a variant of *Streptomyces mobaraensis*

In order to achieve commercialization of TGase, a constant supply is an absolute prerequisite. We therefore screened about 5,000 microorganisms for TGase in collaboration with Amano Enzyme Co. (Nagoya, Japan) and identified some microorganisms that produce TGase-like enzymes using the hydroxamate assay (Ando et al. 1989). These microorganisms excreted the enzyme, and one of them produced a high activity. The enzyme in the latter strain was shown to form G-L bonds in proteins, the critical property of a TGase (Nonaka et al. 1989); it was named microbial transglutaminase (MTGase), and the source was classified as a variant of *S. mobaraensis* (Washizu et al. 1994).

Characteristics of MTGase

Since the MTGase was secreted into the culture medium, cell disruption was unnecessary. Its purification thus proved rather easy and its subsequent commercialization has therefore been rapid. Its physicochemical properties, such as molecular weight and secondary structure, and its enzymatic properties, had already been reported (Ando et al. 1989; Nonaka et al. 1989; Kanaji et al. 1993). Its molecular weight (MW) was reported to be approximately 38,000 kDa, by both SDS-polyacrylamide electrophoresis (SDS-PAGE) and gel-permeation chromatography (Ando et al. 1989), and its isoelectric point (pI), 9. Protein sequencing by the automated Edman method, and mass spectrometry, revealed its primary structure to contain 331 amino acids (Kanaji et al. 1993). Sequencing of the cDNA from the producing microorganism gave consistent results (Washizu et al. 1994). MTGase has a single cysteine residue and a calculated MW of 37,842 kDa, close to the experimentally obtained value of 38,000 kDa. Therefore MTGase is a monomeric, simple protein (not a glycoprotein, lipoprotein, etc.), although it has two potential glycosylation sites (-Thr-Xxx-Asn-).

Enzymatic properties of MTGase

The optimum pH for MTGase activity was found to be between 5 and 8. However, MTGase showed some activity at pH 4 or 9 (Ando et al. 1989), and was thus considered to be stable over a wide pH range. The optimum temperature for enzymatic activity was 55°C (for 10 min at pH 6.0); it maintained full activity for 10 min at 40°C, but lost activity within a few minutes at 70°C. It was active at 10°C, and retained some activity at near-freezing temperatures. With respect to substrate specificity, most food

proteins, such as legume globulins, wheat glutens, egg yolk and albumin proteins, actins, myosins, fibrins, milk caseins, α-lactalbumin, and β-lactoglobulin, as well as many other albumins, could be crosslinked (Nonaka et al. 1992; Kang et al. 1994; Seguro et al. 1995a; Nonaka et al. 1997).

TGases, including the well-characterized guinea pig liver enzyme, generally have an absolute requirement for Ca^{2+} (Enzyme Nomenclature 1992; Aeschlimann and Paulsson 1994). However, MTGase is totally independent of Ca^{2+}, and in this respect is quite different from the mammalian enzymes. This property is very useful in modifying food proteins, as many food proteins—such as milk caseins, soybean globulins and myosins—are sensitive to, and easily precipitated by, Ca^{2+}. The sensitivity of MTGase to other cations in the absence of reducing agents has been investigated. Cu^{2+}, Zn^{2+}, Pb^{2+}, and Li^{+} were found to be strongly inhibitory. Because heavy metals such as Cu^{2+}, Zn^{2+}, and Pb^{2+} are expected to bind the thiol group of the single cysteine residue, this strongly supports the idea that this residue is part of the active site of MTGase.

MTGase is capable of gelling concentrated solutions of proteins such as soybean proteins, milk proteins, beef, pork, chicken and fish gelatin and myosins (Nonaka et al. 1992, 1997; Kang et al. 1994; Nielsen 1995; Seguro et al. 1995a; Zhu et al. 1995), in a manner similar to that of guinea pig liver TGase. When gelled soybean globulin is further hardened by heating at 100°C for 15 min, it acquires novel gel properties. Milk caseins, which are non-heat setting proteins, are also gelled by MTGase without heating, as is gelatin, a cold-setting protein. However, the gelled gelatin no longer melted on heating at 100°C. Different proteins can be covalently linked by MTGase to produce combinations with novel functionalities. For instance, conjugation of milk caseins or soybean globulins with ovomucin, an egg white glycoprotein, improved emulsifying activity compared to both the starting proteins (Kato et al. 1991). Casein-gelatin conjugation by MTGase also yielded novel proteins that were highly soluble at acidic pH (Neilsen 1995).

MTGase is able to incorporate amino acids or peptides covalently into substrate proteins, and this reaction can improve the nutritional value of food or feed proteins, because covalently incorporated amino acids or peptides behave like endogenous amino acids. For instance, milk caseins and soybean proteins, in which methionine and lysine are limiting, could be improved by this type of MTGase reaction. In practical applications, all common amino acids, except lysine, should have their α-carboxyl group either amidated, esterified or decarboxylated to eliminate their negative charge. Lysine, whose ε-amino group is a primary amine, is a good substrate of MTGase. In such reactions, proteins act as acyl donors, while amino acids, including lysine, act as acyl acceptors.

Both lysine- and glutamine- containing peptides are capable of serving as substrates without modification. The lysine-containing peptides act as acyl acceptors, while the proteins act as acyl donors, whereas glutamine-containing peptides act as acyl donors, and the proteins as acyl acceptors. For instance, lysylmethionine (or methionyllysine) could be incorporated into caseins to counteract methionine deficiency. Likewise, lysylarginine (or arginyllysine) can be incorporated into caseins to combat arginine deficiency. In the case of glutamine-containing peptides, hydrophobic moieties must be placed on the amino group of the glutamine residue, since the substrate specificity of the glutamine residue is much higher than that of primary amines. Consequently, the application of glutamine-containing peptides in the food industry is at present complex and impractical.

Crystal structure of TGase

The crystal structure of MTGase has been determined at 2.4 Å resolution (Kashiwagi et al. 2002). It forms a single, compact domain with overall dimensions 65×59×41 Å, and folds into a plate-like shape with a deep cleft at the edge of the molecule (Fig. 2). The catalytic residue, Cys^{64}, is at the bottom of the cleft. MTGase belongs to the α+β folding class, with 11 α-helices and 8 β-strands; one of the β-sheets is surrounded by α-helices, which are clustered into three regions. The central β-sheet forms a seven-stranded anti-parallel structure, although it is severely twisted between the β_5 and β_6 strands and there is only one hydrogen bond between the main chains of these strands (Trp^{258} and Thr^{273}). The first cluster of α-helices is on the left side of the front view of the MTGase molecule and is composed of the α_1, α_2, and α_3 helices, and the Cys^{64} residue is in the loop between the α_2, and α_3 helices. The second cluster, comprising the α_4, α_5, and α_{10} helices, and the third, comprising the α_6, α_7, α_8, and α_9 helices, are on the right and bottom side of the front

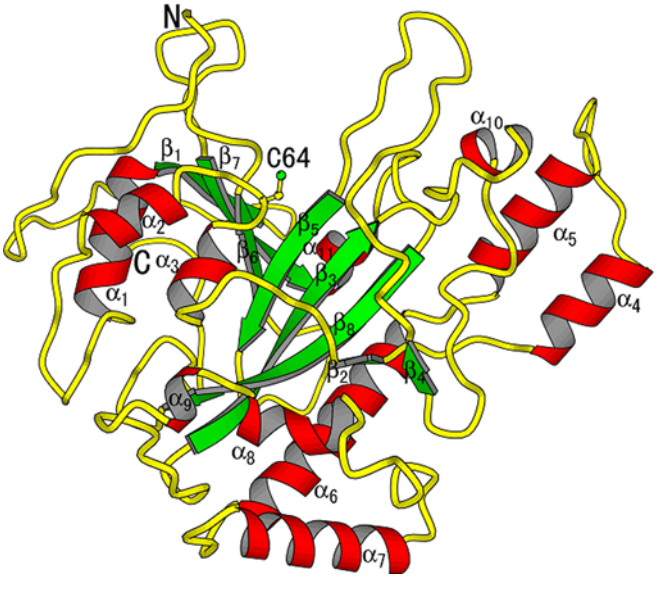

Fig. 2 Overall structure of microbial TGase (MTG): schematic ribbon drawing of the MTG molecule viewed from above the plate face. The secondary structure is numbered. *Ball-and-stick model* Side chain of Cys^{64}

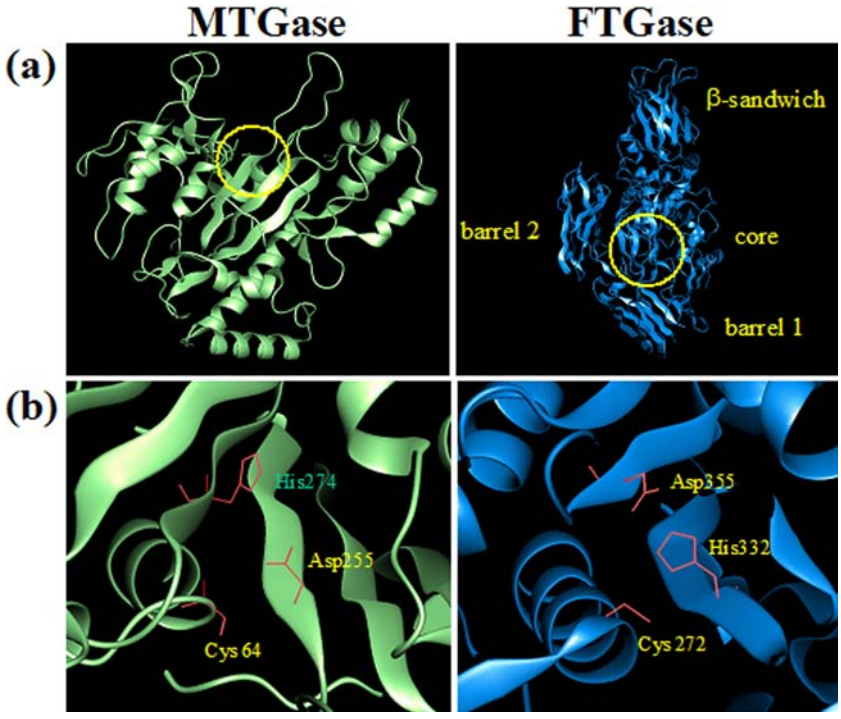

Fig. 3a, b Structural comparison of MTGase and red sea bream liver TGase (FTGase). **a** Overall structures, **b** structures around the active sites of MTGase and FTGase. *Regions in yellow circles* Active sites. *Red wire model* Catalytic triad of FTGase (Cys^{272}, His^{332}, Asp^{355}) and the corresponding position in MTGase (Cys^{64}, Asp^{255}, His^{274})

view of MTGase, respectively. We could not find any proteins similar in structure to MTGase in databases.

The crystal structures of two other TGases, human factor XIII and red sea bream liver TGase (FTGase), have already been determined (Yee et al. 1994; Noguchi et al. 2001). The overall structures of FTG and human factor XIII resemble each other. Figure 3 is a structural comparison between the active site structures of MTGase and FTGase. The overall structure of MTGase is completely different from that of the factor XIII-like TGase, which possesses a cysteine protease-like catalytic triad (Fig. 3a). In contrast to the compact, single domain structure of MTGase, FTGase, like human factor XIII, consists of four sequential domains, named β-sandwich, core, barrel 1, and barrel 2 by Yee et al. (1994). The active site of FTGase is in the core domain, which has 334 amino acid residues.

However, the relationship between the active site structures of MTGase and FTGase is of interest. In MTG, the Asp^{255} residue occupies a position nearest to Cys^{64} and is also adjacent to His^{274}, and Cys^{64}, Asp^{255}, and His^{274} superimpose well on the catalytic triad Cys-His-Asp of the factor XIII-like TGase, in that order (Fig. 3b). The secondary structure frameworks around these residues are also similar. These results imply that the two TGases are related by convergent evolution; however, MTGase has developed a novel catalytic mechanism specialized for the cross-linking reaction.

In the three-dimensional structure of the factor XIII-like TGase, the Sγ atoms of the catalytic Cys residue hydrogen bonds with the Oη atoms of the Tyr residue and are solvent inaccessible (Yee et al. 1994; Noguchi et al. 2001). The Tyr residue is in the loop of the barrel 1 domain and covers the active site of the core domain. Binding of Ca^{2+} and the acyl donor probably causes a conformational change in which the Tyr residue is released from the catalytic Cys residue and the acyl-enzyme intermediate is formed. In contrast to the restricted solvent accessibility of the active site and the complicated activation process of the factor XIII-like TGase, Cys^{64} of MTGase is sufficiently exposed to the solvent to react rapidly with substrates. Moreover, the flexibility of the right side wall of the active site cleft (Fig. 2) may decrease steric hindrance between enzyme and substrates. These structural differences between MTG and the factor XIII-like TGases underlie the differences in their substrate specificity and reaction rate. The structure of MTG accounts well for its catalytic mechanism, in which Asp^{255} is considered to be essential, as well as for the higher reaction rate and broader substrate specificity. A recent NMR study revealed that the reaction rate of MTG for the acyl donor is higher than that of the factor XIII-like TGases such as guinea pig liver TGase, while its specificity requirements are lower (Shimba et al. 2002).

Bioavailability of crosslinked proteins

Because of the various applications of MTGase-catalyzed modifications of food proteins, careful attention must be paid to the nutritional value of such crosslinked proteins. MTGase-modified and native proteins differ only with respect to G-L bonds, and the digestibility and bioavailability of the G-L moiety of the proteins have been investigated. First, the distribution of naturally occurring G-L bonds were measured in raw materials and processed foods (Sakamoto et al. 1995). Raw food materials such as meats, tongues and fish and shellfish, and most processed

foods, including kamaboko, ham, stewed beef, fried chicken, grilled pork, and hamburger were found to contain a certain number of G-L bonds; the exception to these findings was dairy products. The G-L level was rather higher in processed foods, especially cooked foods, than in raw food materials. G-L bonds are also present naturally in the eggs of various fishes, such as red salmon, lumpfish, herring, sardine, and Alaska Pollack (Kumazawa et al. 1996a). Of these, caviar and salted fish eggs are popular in Western countries.

It is likely that an intrinsic TGase forms the G-L bonds in proteins during cooking, since many living organisms contain TGase in their tissues and organs (Aeschlimann and Paulsson 1994; Ando et al. 1989). The rise in temperature in food materials during cooking is frequently very slow, so that intrinsic TGases could remain active for some time. It is thus quite reasonable to conclude that cooked or processed foods contain increased numbers of G-L moieties due to the catalytic activity of intrinsic TGases. That dairy products contain no G-L bonds tends to support this idea, since milk does not contain any intrinsic TGase. Heating has also been reported to induce G-L bonds as a consequence of chemical dehydration between the γ-carboxyl group of glutamate and ε-amino group of lysine. Since cooking itself results in G-L formation in proteins, mankind has been ingesting G-L moieties ever since the discovery of fire and cooking, a conclusion that supports the idea that their consumption must be safe.

After ingestion of cross-linked proteins, normal mammalian gastrointestinal digestive enzymes cleave them into amino acids but leave the G-L dipeptide intact. The resistant G-L dipeptide may be absorbed through the intestinal brush-border and transported to the kidney. Two kinds of enzymes have been found to degrade the G-L bond in vivo. One is a kidney enzyme, γ-glutamylamine cyclotransferase, which cleaves the G-L dipeptide yielding free lysine and 5-oxo-proline (synonym, pyroglutamate; Fink et al. 1980). Since lysine is an essential amino acid, this pathway could help to satisfy nutritional requirements. However, as 5-oxo-proline is converted to glutamate by an ATP-dependent enzyme, 5-oxo-prolinase, ingestion of a large quantity of cross-linked proteins would lead to the consumption of a great deal of ATP in the kidney and this could be a burden on kidney function. The other enzyme that degrades the G-L bond in vivo is γ-glutamyltransferase (EC 2.3.2.2), present mainly in intestinal brush-border membranes, kidneys, and blood (Meister et al. 1981). It cleaves the G-L dipeptide into lysine and glutamate (Seguro et al. 1995b) without consuming ATP. The G-L moiety, therefore, could be effectively metabolized to lysine by these two enzymes; hopefully, the lysine generated would be nutritionally beneficial.

Many researchers have demonstrated that G-L dipeptides can be metabolized in rats, and that the lysine produced is incorporated into tissues (Finot et al. 1978; Iwami and Yasumoto 1986; Friedman and Finot 1990; Seguro et al. 1994). On the other hand, the bioavailability of the G-L moiety in cross-linked proteins remained unproven, perhaps because of the impracticality of using guinea pig liver TGase for large-scale production of cross-linked proteins. When large amounts of MTGase became available, cross-linked caseins were prepared on a kilogram scale, and were fed to rats to evaluate the nutritional value of the lysine in the G-L moiety. Rats fed the crosslinked caseins grew normally, unlike those fed native caseins (Kuraishi et al. 1996), indicating that the G-L moiety in the crosslinked caseins is cleaved and the lysine is incorporated into proteins.

Applications of MTGase in food processing

As already mentioned, many food proteins are gelled upon incubation with MTGase. The following examples illustrate some of the industrial uses of MTGase in food processing.

Meat products

MTGase can produce restructured meat by binding together small pieces of meat. Kuraishi et al. (1996) developed a novel meat binding system using MTGase and caseinate simultaneously. Caseinate, when reacted with MTGase, becomes viscous, and functions as a glue to bind different foodstuffs together. Using this system, large pieces of restructured meat such as beefsteaks or fish fillets can be produced from fragments (Fig. 4). Pieces of meat, including minced meat, can be bound together without any need for sodium chloride or phosphates, yielding 'healthy' meat products.

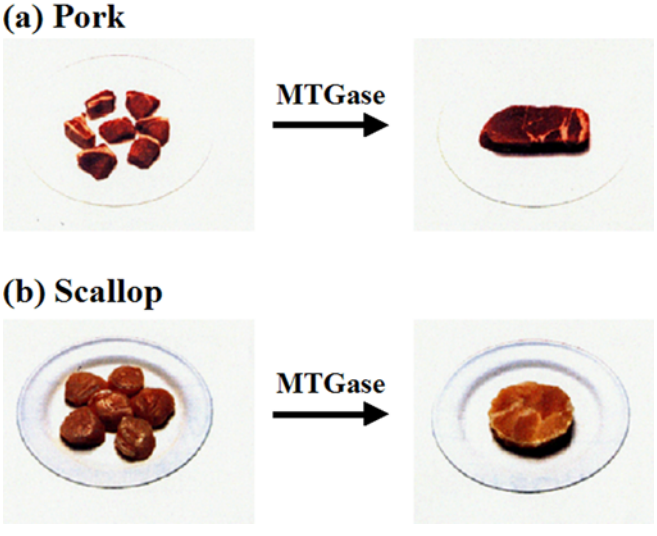

Fig. 4a, b Applications of MTGase. **a** Steak reconstituted from small chunks of pork, **b** new type of seafood from scallops

Fish products

Seki et al. (1990) found that endogenous fish TGase caused swari setting, or hardening fish protein paste at low temperature by crosslinking. TGase from walleye Pollack, used to produce surimi, has been purified and characterized by Kumazawa et al. (1996b). There is no doubt that both endogenous fish TGase and exogenous MTGase could improve the efficacy of fish raw materials by increasing crosslinking. However, there is still controversy over whether the endogenous fish TGase is the only factor in suwari setting. Kumazawa et al. (1996a) measured the G-L bonds in several fish eggs, and suggested that the texture of raw and processed egg products is related to the level of endogenous TGase. It seems that TGase treatment maintains and improves the texture of fish products, which is strictly dependent on the freshness of the raw materials.

Dairy products

Milk casein, which does not gel even when heated, is a very good substrate for TGases, which convert it into a heat-resistant, firm gel. Yogurt, a milk gel formed by acidic fermentation with lactic starter, has the disadvantage of serum separation upon change of temperature, or physical impact. The addition of MTGase can overcome this problem, because MTGase improves the water-holding capacity of the gel. MTGase also makes it possible to produce dairy products, such as ice cream and cheese, with low fat content or reduced content of non-fat solids.

Soybean products

Soya proteins, such as 11S and 7S globulins, are good substrates for the MTGase reaction. Tofu, a typical soybean curd product, is prepared by the coagulation of soybean proteins with the addition of Ca^{2+} and Mg^{2+} and/or glucono-δ-lactone. It is very difficult to produce long-life tofu, since its soft, smooth texture is easily destroyed by retort sterilization. However, the addition of MTGase enables the smooth texture of retorted tofu to be maintained for a long time.

Wheat products

Sakamoto et al. (1996) found that treatment of noodles and pasta with MTGase prevented the deterioration of texture upon cooking, and improved the strength of the products, even when low-grade flours were used. They also suggested that the loaf volume of several breads might be increased or maintained by the addition of MTGase when certain ingredients were substituted or reduced during mixing of the dough.

Production of MTGase

The nucleotide and amino acid sequences of pre-pro-MTGase from *S. mobaraensis* have been determined (Washizu et al. 1994; Pasternack et al. 1998; Kikuchi et al. 2003). The gene contains a 1,221-nucleotide open reading frame encoding a 407-amino acid protein, corresponding to the predicted pre-region of 31 amino acids, the pro-region of 45 amino acids, and the mature protein of 331 amino acids. The pro-region, which is processed by proteases in the culture medium of *S. mobaraensis*, inhibits enzyme activity and increases enzyme thermostability (Pasternack et al. 1998).

Currently, MTGase is produced from *S. mobaraensis* by conventional fermentation. However, a more efficient system is desirable, and a number of reports have described the expression and production of MTGase in host-vector systems such as *S. lividans* and *E. coli*. In the *S. lividans* system, MTGase, under the control of the tyrosinase promoter, was secreted to a level of no more than 0.1 mg/l (Washizu et al. 1994), while the level was about 5 mg/l using the *E. coli* OmpA signal peptide (Takehana et al. 1994). A level of about 250 mg/l has been achieved in *E. coli* but the product formed an inclusion body that could be refolded in vitro (Yokoyama et al. 2000). The levels of expression in these studies were low, and it would be very difficult to produce MTGase on an industrial scale via an inclusion body.

Recently, MTGase has been efficiently secreted using *C. glutamicum* as host system (Kikuchi et al. 2003; Date at al. 2003). *C. glutamicum* is a Gram-positive, non-sporulating bacterium with a DNA content of about 56% GC (Malumbres et al. 1993), whose genome has been sequenced (Ikeda and Nakagawa 2003). It is used in the industrial production of amino acids such as glutamate and lysine that have been employed in human food and animal feed throughout the world for several decades; it is nonpathogenic and produces no hazardous toxins (Krämer 1994; Liebl 1991). For the reasons mentioned above, *C. glutamicum* should be suitable for producing a food enzyme such as MTGase.

In a recent study, *C. glutamicum* secreted pro-MTGase efficiently when it was coupled to signal peptides derived from the cell surface proteins of Corynebacteria (Kikuchi et al. 2003), and improvements in the fermentation process yielded 930 mg/l pro-MTGase (Kikuchi et al. 2002). In order to convert it to the active form, a subtilisin-like serine protease (SAM-P45) (Suzuki et al. 1997) from *Streptomyces albogriseolus* was co-secreted by the *C. glutamicum* (Kikuchi et al. 2003). However, the N-terminal amino acid sequence of the MTGase differed from the native *S. mobaraensis* enzyme. In a second experiment, site-directed mutagenesis was performed to generate an optimal SAM-P45 cleavage site in the C-terminal region of the pro-region (Date et al. 2003). Finally, native-type MTGase was successfully secreted, and the specific activity of the purified enzyme was similar to that of native MTGase (Date et al. 2003). It was thus shown that *C. glutamicum* can efficiently secrete native-

type MTGase, and that it has great potential as a host for industrial-scale production of the enzyme.

Conclusions

MTGase can form G-L bonds in many food proteins, and this crosslink drastically alters protein behavior. The application of G-L cross-linking should lead to the development of novel foods and processing methodologies. The G-L moiety in the crosslinked proteins is cleaved by both γ-glutamylamine cyclotransferase and γ-glutamyltransferase. The fates of the crosslinked proteins and of the lysine in the G-L moiety are of much concern, and both were found to be metabolized in the body, while the safety of the G-L moiety is proven by the long-established, habitual intake of cooked foods by mankind. The crystal structure of MTGase has been solved and has helped to clarify the structure-function relationships of MTGase and its substrates. An efficient method for achieving the secretion of MTGase has been developed and the enzyme promises to permit the development of powerful alternative technologies.

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