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Microbial transglutaminase – a review of its production and application in food processing

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Abstract Transglutaminase (EC 2.3.2.13) catalyses an acyl-transfer reaction in which the γ-carboxamide groups of peptide-bound glutaminyl residues are the acyl donors. The enzyme catalyses in vitro cross-linking in whey proteins, soya proteins, wheat proteins, beef myosin, casein and crude actomyosin refined from mechanically deboned poultry meat. In recent years, on the basis of the enzyme's reaction to gelatinize various food proteins through the formation of cross-links, this enzyme has been used in attempts to improve the functional properties of foods. Up to now, commercial transglutaminase has been merely obtained from animal tissues. The complicated separation and purification procedure results in an extremely high price for the enzyme, which hampers a wide application in food processing. Recently studies on the production of transglutaminase by microorganisms have been started. The enzyme obtained from microbial fermentation has been applied in the treatment of food of different origins. Food treated with microbial transglutaminase appeared to have an improved flavour, appearance and texture. In addition, this enzyme can increase shelf-life and reduce allergenicity of certain foods. This paper gives an overview of the development of microbial transglutaminase production, including fermentation and down-stream processing, as well as examples of how to use this valuable enzyme in processing foods of meat, fish and plant origin.

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Introduction

Transglutaminase (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) is an enzyme capable of catalysing acyl-transfer reactions introducing covalent cross-links between proteins (Nonaka et al. 1989) as well as peptides and various primary amines. When the ε-amino groups of lysine residues in proteins act as acyl acceptors, ε-(γ-Glu)-Lys bonds are formed both intra- and inter-molecularly. Without primary amines in the reaction system, water becomes the acyl acceptor and the γ-carboxyamide groups of glutamine residues are deaminated, becoming glutamic acid residues (Ando et al. 1989). The transglutaminase-catalysed reactions are schematically shown in Fig. 1 (Folk 1980; Ikura 1988; Motoki and Seguro 1994).

These transglutaminase-catalysed reactions can be used to modify the functional properties of food proteins. Transglutaminase has been used to catalyse the cross-linking of a number of proteins, such as whey proteins, soya proteins, gluten, myosin and actomyosin. The modification of food proteins by transglutaminase may lead to textured products, help to protect lysine in food proteins from various chemical reactions, encapsulate lipids and/or lipid-soluble materials, form heat- and water-resistant films, avoid heat treatment for gelation, improve elasticity and water-holding capacity, modify solubility and functional properties, and produce food proteins of higher nutritive value through cross-linking of different proteins containing complementary limiting essential amino acids (Matheis and Whitaker 1987; Kitabatake and Doi 1993; Motoki and Seguro 1994).

Transglutaminase has been found in animal and plant tissues (Folk 1980; Falcone et al. 1993; Yasueda et al. 1994) and microorganisms (Ando et al. 1989). Since the 1960s, the purification, characterization and application of Ca^{2+}-dependent transglutaminase of animal origin, mainly guinea-pig liver, have been intensively studied (Folk and Cole 1965, 1966; Connellan

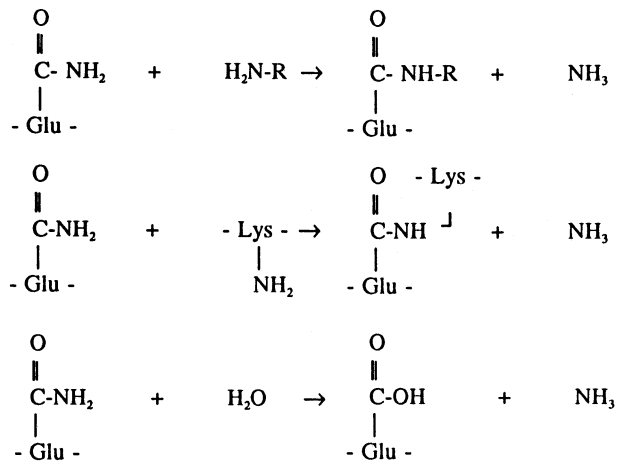

Fig. 1 Transglutaminase-catalysed reactions

et al. 1971; Folk 1980; Brookhart et al. 1983; Matheis and Whitaker 1987; Ikura 1988; Miwa 1989; Singh 1991; Larre et al. 1992, 1993a, b). A process chart of transglutaminase production from different sources is shown in Fig. 2.

Guinea-pig liver has been the sole source of commercial transglutaminase for decades. The scarce source and the complicated separation and purification procedure for obtaining tissue transglutaminase have resulted in an extremely high price of the enzyme, about U.S. $80 for one unit. It is thus not possible to apply such tissue transglutaminase in food processing on an industrial scale. Separation and purification of transglutaminase from fish tissue and plant tissue are still in their infancy. Recently efforts have been made to search for transglutaminases derived from microorganisms. Transglutaminases were found in cultures of *Streptoverticillium* sp. and *Streptomyces* sp. (Motoki et al. 1989; Ando et al. 1989, 1992). Microbial fermentation makes it possible to achieve mass production of transglutaminase from cheap substrates. A number of examples of the application of microbial transglutaminase in food processing have been announced. However, the potential for using microbial transglutaminase in food processing, as well as in cosmetics, pharmaceutical products and medical treatment, remains uncertain for commercial reasons and because of communication difficulties. This paper gives an overview of the production of microbial transglutaminase and its application in food processing.

Production of microbial transglutaminase

The production of transglutaminase derived from microorganisms was not reported until the late 1980s. Motoki et al. (1989) and Ando et al. (1989) explored the possibility of producing transglutaminase from microorganisms. Ando et al. (1989) screened about 5000 strains isolated from soil collected from a variety of locations. Among these strains, *Streptoverticillium* S-8112 was found to have the capability of producing transglutaminase. Motoki et al. (1989) reported that other *Streptoverticillium* strains, such as *S. griseocarneum*, *S. cinnamoneum* subsp. *cinnanoneum* and *S. mobaraense*, also have the ability to produce transglutaminase. Transglutaminase activity has also been found in a culture of *Streptomyces* sp. (Ando et al. 1992).

The fermentation procedure for the production of transglutaminase is in principle the same for the various microorganisms mentioned (Ando et al. 1989, 1992; Motoki et al. 1989). Glucose, sucrose, starch, glycerine and dextrin can be used as carbon source. Inorganic as well as organic nitrogen sources can be used, for instance NH_4NO_3, $(NH_4)_2SO_4$, urea, $NaNO_3$, NH_4Cl, soya, rice, maize, wheat or wheat flour, bran, defatted soya bean, maize-steep liquid, peptone, meat extract, casein, amino acids and yeast extract. Necessary minerals and trace elements are phosphate, magnesium, potassium, iron, copper, zinc and vitamins; non-ion surfactant and antifoam can be added if necessary. The culture is an aerobic fermentation so that aeration and agitation are necessary. The temperature for growth and product formation is between 25°C and 35°C, and the fermentation time is dependent on the culture conditions and determined by the highest transglutaminase activity that can be achieved, normally 2–4 days. Microbial transglutaminase is an extracellular enzyme dissolved in the fermentation broth so that it can be recovered through separation of the solid material from the broth. The methods normally used in enzyme purification can be used for microbial transglutaminase. For instance, ethanol, acetone, isopropyl alcohol and other organic solvents can be used in down-stream processing. Salting-out with ammonium sulphate and sodium chloride, dialysis, ultrafiltration, ion-exchange chromatography, absorption chromatography, gel filtration, absorption and isoelectronic point methods can all be used to purify the enzyme. A good combination of the methods can increase efficiency and recovery. The enzyme obtained can then be mixed with enzyme stabilizers such as various salts, sugars, proteins, lipids and surfactant (Sakamoto et al. 1992).

Examples of fermentation and purification have been described by Ando et al. (1989, 1992) and Motoki et al. (1989). The microorganism was precultured in a medium composed of 0.2% peptone, 0.5% glucose, 0.2% K_2HPO_4, 0.1% $MgSO_4$, at pH 7.0. The strain was inoculated in 100 ml medium in a 500-ml Sagakuchi flask and cultured at 30°C for 48 h. The culture broth obtained was added to 20 l fresh medium (pH 7.0) composed of 2.0% polypeptone, 2.0% soluble starch, 0.2% K_2HSO_4, 0.1% $MgSO_4$, 0.2% yeast extract and 0.05% antifoam, and then cultured for 3 days at 30°C under aeration (10 l/min) and agitation (250 rpm). The

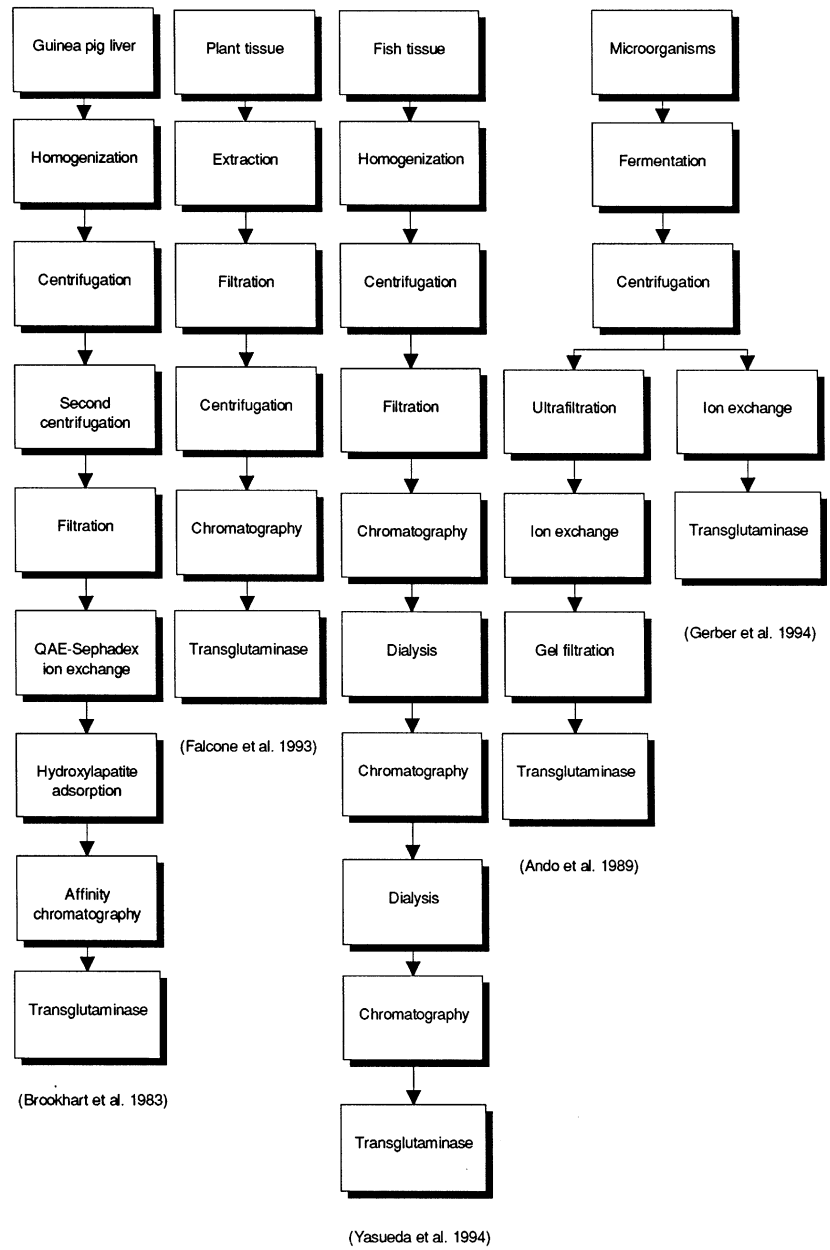

Fig. 2 Process chart of transglutaminase production from different sources

fermentation broth had an enzyme activity ranging from 0.28 U/ml to 2.5 U/ml, dependent upon the strain used. The microorganism was separated by centrifugation at 3000 rpm. The clear liquid thus obtained was concentrated with an ultrafiltration membrane and then treated on a column of Amberlite CG-50 that had been equilibrated with 0.05 M sodium phosphate buffer (pH 6.5). The column was washed with the same buffer and then the active fractions were pooled. The pooled fraction was treated again on the Amberlite CG-50 column under the same conditions. Then the active fraction was diluted to reduce the conductivity and passed through a Blue Sepharose column (Pharmacia). After this treatment, the enzyme was purified 174 times. The total recovery of transglutaminase activity was about 42%.

Recently a modified downstream process for purifying microbial transglutaminase was described by Gerber et al. (1994). After the fermentation broth had been centrifuged and filtered, the enzyme was separated directly with a strong-acid ion exchanger in a single step. According to the authors, this method is simple, rapid and has a transglutaminase recovery of 40%.

Table 1 Overview of application of microbial transglutaminase in food processing

Source	Product	Effect	Reference
Meat	Hamburger, meatballs, stuffed dumplings, shao-mai	Improved elasticity, texture, taste and flavour	Sakamoto and Soeda (1991)
	Canned meat	Good texture and appearance	Seguro and Motoki (1991)
	Frozen meat	Improved texture and reduced cost	Takagaki and Narukawa (1990)
	Moulded meat	Restructuring of meat	Matsui et al. (1990)
Fish	Fish paste	Improved texture and appearance	Ichihara et al. (1990), Wakameda et al. (1990a)
Krill	Krill paste	Improved texture	Wakameda et al. (1990b)
Collagens	Shark-fin imitation	Imitation of delicious food	Tani et al. (1990)
Wheat	Baked foods	Improved texture and high volume	Ashikawa et al. (1990)
Soya bean	Mapuo doufu	Improved shelf-life	Kato et al. (1991)
	Fried tofu (aburaage)	Improved texture	Soeda et al. (1990)
	Tofu	Improved shelf-life	Nonaka et al. (1990)
Vegetables and fruits	Celery	Food preservation	Takagaki et al. (1991)
Casein	Mineral absorption promoters	Improved mineral absorption in intestine	Noguchi et al. (1992)
	Cross-linked protein	Allergenicity reduction	Yamauchi et al. (1991)
Gelatin	Sweet foods	Low-calorie foods with good texture, firmness and elasticity	Yamanaka and Sakai (1992)
Fat, oil and proteins	Solid fats	Pork-fat substitute with good taste, texture and flavour	Takagaki et al. (1990)
Plant proteins	Protein powders	Gel formation with good texture and taste	Soeda et al. (1992)
Seasonings	Seasonings	Improve taste and flavour	Kobata et al. (1990)

Application of transglutaminase

The production of transglutaminase by microorganisms makes it possible to apply this enzyme in a variety of food processes.

An overview of the application possibilities for microbial transglutaminase in food processing is given in Table 1. A few of these examples will be described in some detail below, in order to show the simplicity of the treatment with microbial transglutaminase and the positive effects that can be obtained.

In meat processing it is of great interest to maximize the yield of marketable products. This includes development of methods for re-structuring low-value cuts and trimmings to improve their appearance, flavour and texture and to enhance market value. Re-structuring treatment usually involves size reduction, reforming and binding (Kim 1993). In such a treatment, transglutaminase can have a very important function. Sakamoto and Soeda (1991) developed a method for producing minced-meat products containing transglutaminase. Minced meat and other food ingredients are mixed with transglutaminase, shaped, packed in pressure-resistant containers and retorted to manufacture meat products such as hamburgers, meatballs, stuffed dumplings and shao-mai (a typical Chinese food). The foods show improved elasticity, texture, taste and flavour. Minced beef and pork, flour, onion, skim-milk powder and condiments were mixed with water and microbial transglutaminase, packed with sauce in bags and retorted to make raw hamburgers. Similar methods for meat and meat products treated with transglutaminase can be found in the literature (Seguro and Motoki 1991; Soeda 1992; Takagaki and Narukawa 1990; Muguruma et al. 1990; Miwa 1989).

Ichihara et al. (1990) and Wakameda et al. (1990a) reported methods for manufacturing fish paste containing transglutaminase. Fish paste products are manufactured from material containing fish meat as the main ingredient and 0.1–700 U transglutaminase/g fish meat protein. A mixture of 100 parts of dehydrated walleye pollack (*Theragra chalcogramma*), with 3 parts NaCl, 5 parts potato starch, 10 parts water, 0.5 part monosodium glutamate and 0.01 part transglutaminase was packed in a film, heated at 60°C for 30 min and at 90°C for 20 min, and cooled to manufacture kamaboko (Japanese fish paste) with good texture and whiteness. Another processing method, reported by Tani et al. (1990), was the manufacture of shark-fin imitation food with transglutaminase. Shark-fin is considered to be a delicious and healthy (functional) food in South East Asia. An imitation of shark-fin is prepared by cross-linking gelatins, collagens or a mixture thereof with transglutaminase and making a gel from the product. The collagen ingredient may be collagen fibres, collagen fibrils, collagen solutions or mixtures thereof. The molecular mass of collagens ranges from 500 Da to 50 000 Da. Shark-fin imitation food was prepared by treating a gelatin (jelly strength 244, m.p. 30°C, isoelectric point 9.1) in water at pH 7 with transglutaminase,

extruding the solution through holes, forming a fibrous gel and, finally, drying the product.

Kato et al. (1991) developed a method for manufacturing storage-stable retort mapuo-doufu (doufu is tofu in Chinese). Mapuo-doufu, braised tofu with minced beef and chili pepper, is one of the most typical hot-spiced dishes in Sichuan province, China. In this method, retort mapuo-doufu that can be preserved at room temperature for a long time is manufactured by treating soya bean milk solutions with coagulating agents and transglutaminase at temperatures up to 80°C to manufacture tofu (soya bean curd), optionally cutting the tofu into pieces, putting it in heat-resistant containers with minced beef and seasonings, and retort sterilization. Soya bean milk was mixed with 3 g/l glucono-σ-lactone and transglutaminase at 50°C for 1 h to manufacture tofu, which was sealed in a pouch with sauce and sterilized at 110°C. The mapuo-doufu showed good texture, taste and appearance after 6 months storage at 25°C and 60% relative humidity. Other methods for improving the taste, texture, appearance and shelf-life of tofu were reported by Nonaka et al. (1990) and Soeda et al. (1990).

Takagaki et al. (1991) reported a method for coating vegetables and fruits with transglutaminase and proteins for preservation. Freshness of vegetables and fruits is maintained by coating with a membrane containing transglutaminase and proteins. Cut celery was treated with an aqueous solution containing transglutaminase, proteins, gelatins and Partner-S (natural bactericide from spices) and then heated at 50°C for 5 min to form coating membranes. The coated celery was kept at 20°C for 3 days showing up to 300 bacterial cells/g, compared to 2×10^6 without treatment.

Yamauchi et al. (1991) developed a method for reducing the allergenicity of some food proteins and/or peptides. α_{s1}-Casein (23 kDa) was treated with transglutaminase at 25°C for 20 h in water to manufacture cross-linked casein (approx. 90 kDa), which was less allergenic.

A material promoting absorption of minerals in the human body was developed by Noguchi et al. (1992). It is prepared by deaminating of casein through treatment with transglutaminase. The resulting material promotes absorption of minerals in intestine and can be used in the food industry and for medicines, for instance in mineral supplement formulations for adults, children and infants. The casein is soluble in neutral and slightly acid conditions and can keep minerals solubilized in the intestine.

Perspectives

It is of paramount interest to search world-wide for new protein sources and to broaden the application potentials of existing proteins for human consumption. In developing countries, many people are still suffering from starvation and efforts are being focused on producing acceptable protein foods from non-animal proteins, to solve the problem of protein deficiencies (Steinkraus 1994). On the other hand, in addition to their awareness of health problems caused by obesity, people in developed countries are increasingly aware of the environmental burden caused by surplus livestock (Bol and Paardekooper 1994). Facing a novel food product, consumers are very sensitive to properties such as flavour, nutritional value, appearance, shelf life and palatability. In this respect, protein modification by enzymes, especially by microbial transglutaminase, the mass production of which can be achieved by fermentation from cheap substrates, is one of the most promising alternatives in developing novel protein foods.

With respect to the production of microbial transglutaminase, the microbial process has no doubt an advantage in its independence from regional and climatic conditions, in addition to its reasonable cost. But it is still of great interest to improve fermentation and down-stream processing to reduce production cost and waste further. Modification of strains by genetic engineering is one of the alternatives. However, in Western countries, there is an increasing tendency among consumers not to approve the application of genetically engineered organisms to food and food ingredients (Jank 1995). In this respect, improvements in process technology, adoption of new fermentation technology and/or a combination thereof will offer promising perspectives.

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