Protein-Glutamine Gamma-Glutamyltransferase

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Synonyms

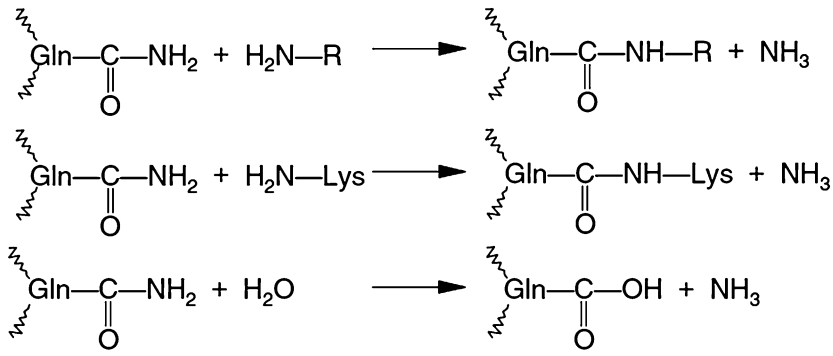
Fibrin stabilizing factor; Fibrinoligase; Glutaminylpeptide gamma-glutamyltransferase; Polyamine transglutaminase; R-glutaminyl-peptide:amine gamma-glutamyl transferase; TG; TGase; Transglutaminase

Historical Background

Transglutaminases (TGs) are a family of enzymes (EC 2.3.2.13) that catalyze the formation of amide bonds between proteins to form insoluble cross-linked protein aggregates that are resistant to chemicals, detergents, and proteases degradation (Griffin et al. 2002; Yokoyama et al. 2004).

TG activity was first observed in 1957 by Clarke et al. (1957), when they found an enzyme with transamidating properties extracted from guinea pig liver. Only in 1959 the name transglutaminase was assigned by Waelsch and collaborators (Mycek et al. 1959), in order to distinguish this enzymatic activity from that of other enzymes with similar activity.

TGs catalyzes posttranslational cross-link reactions between two substrates that can be two proteins or two residues of the same protein, involving lysine and glutamine. More specifically, TGs catalyze acyl-transfer reactions introducing covalent crosslinks between a γ-carboxyamide group of glutamine residue and ε-amino group of lysine residue or other primary amines. Peptides and various primary amines act as acyl donors, and primary amino groups (including ε-amino groups of lysine residues), either as



Protein-Glutamine Gamma-Glutamyltransferase, Fig. 1 Crosslinking reactions catalyzed by TGs

peptide-proteins bound or free lysine, act as the acyl acceptors (Folk and Cole 1966). The formation of a protease-resistant isopeptide bond is the result of these crosslinking reactions (Fig. 1).

For these reactions to occur, temperature and pH are determinant factors. Shi and collaborators (Shi et al. 2011) reported that the optimum conditions of temperature and pH for free, crosslinked, and immobilized TG activity were 40, 45, and 50 °C respectively being the optimal pH of 6.0 not altered by crosslinking treatment or immobilization.

At the NIH, the John E. Folk laboratory was one of the most interested in TG research. They develop the first standardized procedure for successful isolation of TG from tissue extracts and determined the main properties of guinea pig liver TG (Folk and Cole 1966; Folk and Chung 1973).

Guinea pig liver TG, reported to be composed of a single polypeptide chain structure with a molecular weight between 80 kDa and 90 kDa (Connellan et al. 1971), was the only form arriving at the market by the end of the 1980s but not arousing much interest from the industrial point of view, since it had a complicated method of separation and purification resulting in high prices (Zhu et al. 1995). For that reason the search of less expensive and more abundant forms of TGs, increased, namely derived from microorganisms and plants.

Types of TGs

TGs are widely distributed in nature being found in mammalians, vertebrates, invertebrates, plants, and microorganisms, reviewed in (Griffin et al. 2002). As aforementioned, mammalian TGs were the first ones isolated and studied and are divided in nine different groups of Ca^{2+}-dependent isoenzymes, with different functions of most animal cells metabolism. Microbial TGs were also identified and are the most widely used due to lower costs and high yields involved with their extraction and purification when compared to mammal sources. Also in a variety of plants TGs were found but in a much less extent due to their difficult purification.

Despite the vast heterogeneity among the three types of TGs amino acid sequences, their active site region includes a specific catalytic triad of Cys-His-Asp or Cys-His-Asn (Beninati and Piacentini 2004).

Although TG is associated with food industry as a food additive and in wool textiles and biopolymers, they are involved in several biological processes, including blood clotting, wound healing, and epidermal keratinization, reviewed in (Griffin et al. 2002; Lorand and Graham 2003).

Mammalian TGs

Despite the fact that the first mammalian TG identified was derived from guinea pig liver, this enzyme has been identified in many other mammal species and tissues.

In mammals, nine different types of isoenzymes with different molecular mass were described, TG1 – TG7, factor XIII and band 4.2, reviewed in (Lorand and Graham 2003). Although all the nine types present some homology, the amino acid sequence is not highly conserved, having in

common the Ca^{2+}-dependent catalytic triad of Cys-His-Asp or Cys-His-Asn at the active site region (Beninati and Piacentini 2004).

A summary of the main functions of each TG as well as the tissue distribution and expression are represented in Table 1.

Despite the main biological functions described in Table 1, mammalian TGs are involved in the pathology of several diseases, including cancer, inflammatory (wound healing, tissue repairs, and fibrosis), chronic degenerative (rheumatoid arthritis and osteoarthritis), infectious (hepatitis C and AIDS), neurodegenerative and autoimmune diseases (reviewed in Beninati and Piacentini (2004) and Tabolacci et al. (2012)).

Microbial TGs

In the late 1980s, microbial TGs were found in cultures of *Streptoverticillium* sp. and *Streptomyces* sp. (Ando et al. 1989). The first one discovered and the most extensively studied was TG from *Streptomyces mobaraensis*, described as an extracellular monomeric protein of 331 amino acids, containing a single Cys in the catalytic active site (Kanaji et al. 1993).

Microbial fermentation allowed an increased production of TG which is remarkably stable over a wide range of temperatures and pHs. The fact of being extracellular calcium independent enzymes with a wide range of cheap subtracts, allowing a low cost production, is very important for industrial and biotechnological applications, mainly in food processing (Zhu et al. 1995; Yokoyama et al. 2004). Nevertheless, microbial TGs have also different applications besides food processing, namely in tissue engineering, textiles and leather processing, in site-specific protein conjugation, and in wheat gluten allergy reduction (reviewed in Zhu and Tramper 2008). Recently, it was reported the role of a microbial TG to prevent food allergy in children due to ovomucoid, the dominant allergen in hen's egg (Porta et al. 2013).

Plant TGs

TG activity has been found in lower and higher plants being the first one cloned and sequenced from *Arabidopsis thaliana* (Della Mea et al. 2004). It was described that their physiological role is related to photosynthesis, fertilization, response to abiotic and biotic stresses, senescence and programmed cell death (reviewed by Del Duca and Serafini-Fracassini 2005; Serafini-Fracassini and Del Duca 2008).

Plant TGs are Ca^{2+}-dependent, similar to mammalian sharing a possible structural homology,

Protein-Glutamine Gamma-Glutamyltransferase, Table 1 Mammalian TGs (reviewed in Lorand and Graham (2003), Mehta (2005))

TG	Distribution/expression	Function
TG1	Epithelial tissue	Keratinocyte differentiation
TG2	Ubiquitously distributed in many types of tissue, cell membrane, cytosol, nucleus, extracellular	Apoptosis, cell survival signalling, cell differentiation, matrix stabilization, and endocytosis
TG3	Epithelial tissue	Terminal differentiation of keratinocytes, hair follicles
TG4	Prostate gland	Reproduction and fertility, involved in semen coagulation in rodents
TG5	Epithelial tissue	Epidermal differentiation
TG6	Testis, lungs, and brain	Central nervous system development, motor function, and cell envelope formation in the epidermis and hair follicle
TG7	Testis and lungs	
Factor XIII	Blood	Wound healing, blood clotting, and bone growth
Band 4.2[a]	Erythrocyte membranes, bone marrow, fetal liver, and spleen	Maintains erythrocyte shape and mechanical properties

[a]Some authors consider Band 4.2 a member of the TG2 family; others the catalytically inactive ninth member of the TG family

despite showing little sequence homology (Serafini-Fracassini et al. 2009).

TGs Applications

TG biocatalytic activity was first used in food industry and continues having a huge impact to this day to improve the texture, stability, water-holding capacity, elasticity, nutritional value and appearance, and other functional properties of food products (Kuraishi et al. 2001).

Nonetheless, the use of these enzymes outside of the food industry has been expanding due to the development of novel biotechnological applications which allowed researchers to discover new ways to exploit the crosslinking activity of TGs. Examples include other industrial fields namely textile and leather processing (Cortez et al. 2005) and for medical applications.

Mammalian TGs have been reported to play critical roles in blood coagulation, immune responses, endocytosis, cell-matrix assembly, apoptosis, and cellular adhesive processes (Lorand and Graham 2003; Gundemir et al. 2012). The role of TGs in several physiological and biochemical functions increased the interest in studying these enzymes from a medical standpoint to better understand its role in disease (Facchiano et al. 2006; Collighan and Griffin 2009). Examples include, amongst others: tissue engineering and regenerative medicine (Zeugolis et al. 2010; Teixeira et al. 2012), cancer (Lentini et al. 2013), and neurodegenerative diseases as Alzheimer's (Zhang et al. 2016).

Summary

Transglutaminases are a family of enzymes widely distributed in nature, being found in mammalian, plants, and microorganisms, that catalyze posttranslational modifications by inter- or intramolecular crosslinking reactions in many proteins, to form insoluble protein aggregates. This specific crosslinking function has been exploited mainly in food industry and more recently in textile industry as well as in medical field. Due to technological advances in biotechnology, TGs can be effectively applied for novel biomedical applications as a promising therapeutic tool.

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