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Current insight and futuristic vistas of microbial transglutaminase in nutraceutical industry

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ABSTRACT

Microbial transglutaminase (MTGase) has become a driving force in the food industry cross-linking the food proteins. MTGase-the nature's molecular glue is recognized to reorient food protein's functional properties without affecting its nutritive value. The scope and approach of this review is to have insight on the action mechanism of MTGase and impact of molecular linkage on functional proteins in various protein moieties in development of innovative features in food production for better consumer's choice and satisfaction. The study covers a wide range of published work across food industries involving innovative use of MTGase, an environment friendly production approach for commercial utilization to get better outcome in terms of culinary delight. The intrinsic biochemical properties and structural information by sequence analysis and clustering validates the mode of reaction mechanism of the biological glue enzyme. The review singles out how the MTGase emerged as a prime choice in ever evolving food industry.

1. Introduction

Transglutaminase (TGase; protein-glutamine gamma-glutamyltransferase-EC 2.3.2.13) is responsible for acyl transfer, deamidation and cross-linking protein intra- or inter chain glutamine (acyl donor) and lysine (acyl acceptor) peptide moieties (Romeih and Walker, 2017). Further to it, the enzyme catalyzes the addition of free amines into proteins by joining the glutamine residue. Furthermore, in the absence of free amine, water becomes the acyl acceptor and the γ-carboxamide groups are deamidated to glutamic acid residues (Heil et al., 2016). Ever since Clarke et al. (1957) elaborated transamidating activity in guinea pig liver and coined the term 'transglutaminase' (TGase), advances have been made in this realm of research.

The cross-links affect the physico-chemical properties of protein quite significantly (Khare and Gupta, 1987). The advantage of the enzyme transglutaminase (TGase) in polymerization, gelation, film formation, and peptide intake into protein has been explored by many scientists in the past decade (Nawong et al., 2016). These reactions are of utmost interest to food research and bioprocesses. They affect enormously on the molecular structure of proteins in food matrices that improve texture and stability, without impacting the pH, colour, flavour or culinary quality of food, moreover, making it more nutritious on account of incorporation of essential amino acids (Grossmann et al., 2017).

Enzymatic modification has been generally used as a tool for improving the properties of food/proteins. It provides high specificity of the enzymatic reactions occurring at gentle conditions with less toxic products. Understanding the mechanisms of action in altering the properties of protein is the basis of interest in industrial use (Gaspar and Góes-Favoni, 2015).

Transglutaminases (TGases) are widely distributed and isolated from microbes, plants and animals which are used in different industrial applications (Kieliszek and Misiewicz, 2014). However, microbial TGases gained prominence for being cost effective and eco-friendly as it's Ca^{2+}-independency which prevent the chances of by-products formation (calcium-protein complexes), and have greater thermal stability (Kieliszek and Misiewicz, 2014).

The first industrial scale production was done by the Japanese company Ajinomoto Co. in consonance with Amano Enzyme Co. (Nagoya, Japan) using the hydroxamate assay. Large scale production of the enzyme whose critical attribute is to from G-L bonds in proteins was accomplished. TGase-producing microorganisms were discovered first by Ando et al. (1989), after screening of 5000 microbial strains (Jin et al., 2016). The testimony of active research going on utilizing TGase with number of published papers reaching around 785 per year in the past five years (ScienceDirect: January 2018). Microbial TGase gained momentum over other sources (animals or plants origin) after its discovery lately in many applications in the food industry.

This review highlights the importance of microbial TGase, by presenting its biochemical and structural properties. Comparative

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sequence analysis of the MTGase protein sequences available in the database was also deciphered to correlate structure-function relationship. These will aid in its applications in a wide range of food products in the food industries to accomplish value added delicacies to delight consumer's taste. For instance by revamping rheological properties (like in rice flour), encapsulation of lipids or lipid-soluble materials, film production, better protein polymerization, enrichment of gel properties, solubility, foaming properties and water-holding capacity (Hong and Xiong, 2012).

2. Mechanism of action of cross-linking enzyme MTGase

The microbial TGase (MTGase) is known for the modification of proteins in terms of its molecular weight, charge, conformation, and stability leading to enhanced value added culinary attributes. The cross-link formed by the group of acyltransferases (EC: 2.3.2.13) is a ε-(γ-glutamyl) lysine isopeptide bond (G-L bond) (Folk and Finlayson, 1977). The reaction scheme exhibits Ping-Pong model that can be elucidated in three ways:

I. The transitory state (active-site i.e. thiol residue-acyl donor-glutamine residues) forms which is driven by the release of ammonia (one NH_3 release per cross-link).
II. In unfavourable conditions this activated thioester can undergo hydrolysis resulting in release of glutamyl residues.
III. Following alternate route could result in formation of glutamyl-lysine protein cross-link wherein an acyl transfer to primary amine (polyamine) or protein bound (the ε-amino group of a lysine residue) takes place.

MTGase acts in three different steps (Fig. 1) that can be summarized as follow:

- Acyl transfer reaction
- Cross-linking between glutamine and lysine cross-linking via a lysyl residue (Transamidation)
- Deamidation

Acyl transfer reaction takes place when γ-carboxyamide groups of Glutamine (Glu) bound to peptide (acts as acyl donor) reacts with primary amines (acyl acceptor).This results in primary amine incorporation (ε-amino group) of lysine resulting in bridge. Hydrolytic deamidation accepts water as acyl acceptor forming glutamic acid, when free lysine residues or primary amines are absent from reaction system. The reaction of G-L cross-linking is faster than acyl transfer and deamidation in any food protein based system. The detailed description has been discussed in Section 4.

3. Microbial TGases unique importance

With the speculations of MTGase main functionality still needs to be delved upon, it is postulated to impart chemical and physical resilience to cellular structures (such as spore coat) by tweaking the cellular proteins. This sheds light on the formation of an antibiotic shield and protects concerned microrganisms against a plethora of host proteases (Strop, 2014).

The microbial TGases, comparatively newer source are quite different from other animal or plant based sources. In the last decade TGases of microbial origin having a variety of substrate specificity with easier manipulation outside of their natural conditions, have been used as a biotechnological tool (Taylor et al., 2006). The upper hand of MTGase over other sources is by virtue of its less cost and greater yields involved with their extraction and purifications (Kieliszek and Misiewicz, 2014). The MTGase is markedly featured by elevated transamidating activity and minimal peptidylglutamine hydrolase activity.

MTGase was initially isolated in 1989 from *Streptoverticillium* sp. by Ando et al. (1989). Enzyme catalytic site contains residues of cysteine (Cys), histidine (His) and aspartate (Asp). Ando et al. (1989) found that transglutaminase isolated from *Streptomyces mobaraense* (formerly classified as *Streptoverticillium mobaraense*) showed a different reactivity to food proteins depending upon Glu residues available on its secondary and tertiary structure (Date et al., 2004). The enzyme acts in the pH range of 5–8 (5.5 optimum), with isolectric point at pH-8.9 having optimal activity at 40 °C but is inactivated at 70 °C (Romeih and Walker, 2017).

The crystal structure of MTGase has been deciphered; the first report came from *Streptomyces mobaraensis* (Kashiwagi et al., 2002).

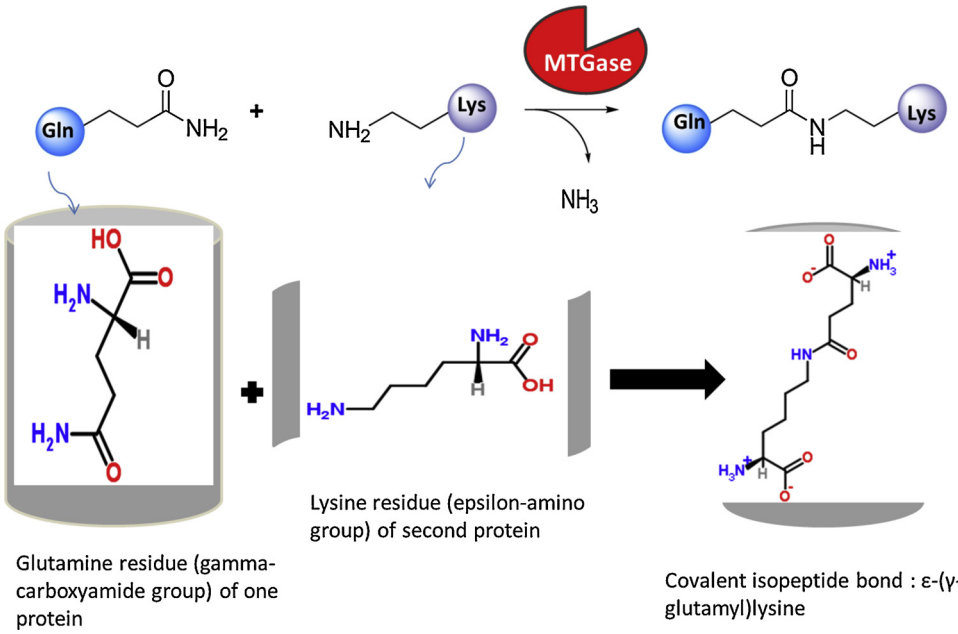

Fig 1. Transglutaminase mode of action: Reaction catalyzed by MTGase.