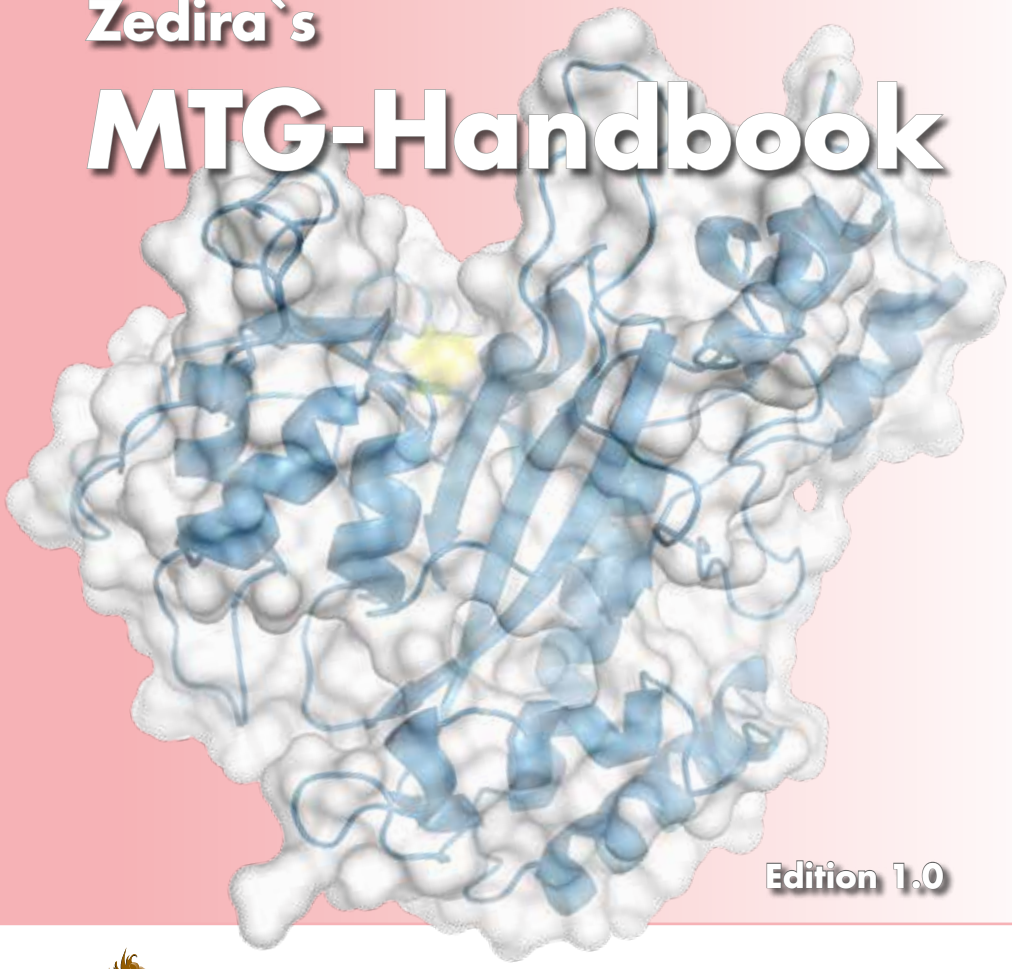
Zedira`s

MTG-Handbook



Edition 1.0

Follow me on a journey on MTG!

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2 MTG – Structure and Numbers

In the late 1980ies the Japanese companies Amano Enzyme® and Ajinomoto® screened 5,000 microorganisms seeking a cheap and stable transglutaminase for food applications. *Streptoverticillium* sp. S-8112, later on determined as *Streptoverticillium mobaraense* and reclassified as *Streptomyces mobaraensis* was found to produce a Ca^{2+} independent and stable transglutaminase, named microbial transglutaminase (MTG or synonymous BTG for bacterial transglutaminase) (Ando et al. 1989; Washizu et al. 1994; Witt and Stackebrandt 1990).

Since then, MTG is produced in an industrial scale and marketed by Ajinomoto® under the brand Activa®. Upon patent expiry additional producers entered the market. Using different formulations, the enzyme is widely used to modulate the texture and properties of protein containing food. Also, innovative non-food applications using protein cross-linking have been described.

The MTG-gene is translated into a prepro-enzyme consisting of 407 amino acid residues. The hydrophobic 31 amino acid long pre-peptide is required for efficient protein secretion. It is followed by the pro-peptide. In case of *Streptomyces mobaraensis* the pro-peptide consists of 45 amino acids rendering the enzyme inactive (zymogen). This protects the strain from uncontrolled cross-linking reactions. The extracellular pro-enzyme is activated by cleavage of the N-terminal pro-peptide (~ 5 kDa). The mature enzyme has a mass of 38 kDa (Pasternack et al. 1998; Washizu et al. 1994) and an isoelectric point at pH 8.9 (Ando et al. 1989). The primary structure is given in Figure 2.

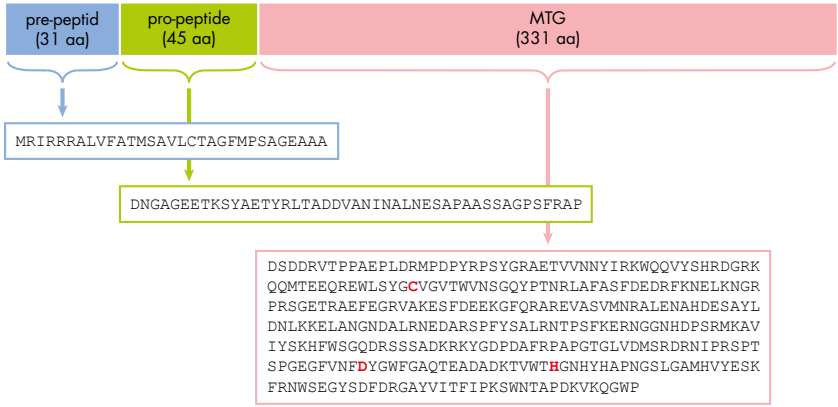


Figure 2: Primary structure of microbial transglutaminase prepro-enzyme. Proposed catalytic triade consisting of amino acids cysteine, aspartate and histidine is marked in red. Figure adapted from Kanaji et al. 1993; Kashiwagi et al. 2002; Pasternack et al. 1998.

In *Streptomyces mobaraensis* MTG is activated in two steps. First, the pro-peptide is cleaved by transglutaminase-activating metalloproteinase (TAMP), yielding an already active MTG with the N-terminus FRAPDSDDR… . In the consecutive step, the tetrapeptide FRAP is cleaved by tripeptidyl aminopeptidase (TAP), resulting in the fully processed MTG with the DSDDR… N-terminus (Zotzel et al. 2003a; Zotzel et al. 2003b). The processing of MTG is shown in Figure 3.

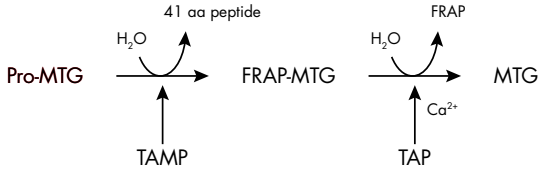


Figure 3: Processing of Pro-MTG from *S. mobaraensis* by proteases TAMP and TAP. After the "transglutaminase-activating metalloproteinase" (TAMP) removed most of the pro-peptide, resulting in an already active MTG, the FRAP tetrapeptide is cleaved by the tripeptidyl aminopeptidase (TAP) to yield the mature MTG. Figure adapted from Zotzel et al. 2003b.

Recombinantly produced Pro-MTG can be processed by several proteases, like Dispase® from *Bacillus polymyxa*, chymotrypsin, trypsin (Pasternack et al. 1998; Pfleiderer et al. 2005) as well as proteinase K, cathepsin B and thrombin (see Figure 4) (Marx et al. 2008).

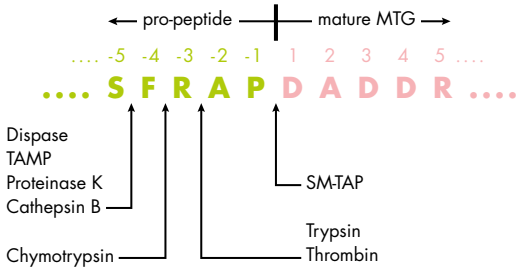


Figure 4: Amino acid sequence in the proteolytic cleavage area between pro-peptide and mature MTG from *S. mobaraensis*. Specific processing sites of proteases are marked with arrows. Figure adapted from Zotzel et al. 2003a.

Table 1 summarizes biochemical and biophysical parameters of MTG.

Table 1: Summary on MTG.

Parameter	Wild type MTG	Recombinant FRAP-MTG
N-terminus	DSDDR…	FRAPDSDDR…
Amino acids	331	335
Size (kDa)	37,861	38,333
Charge (calc)[1]	+0.25	+1.25
pI (calc)[1]	7.09	7.45
pI	8.9[2]	n.d.
Temperature optimum	50°C[3]	n.d.
pH-range	5 – 9[4]	5 – 9[5]
pH-optimum	6 – 7[4]	5 – 8[5]

[1] DNASTAR Editseq
[2] Ando et al. 1989
[3] at pH6
[4] at 37°C
[5] Zedira data

MTG-structure

The crystal structure of MTG has been determined by Kashiwagi and co-workers at 2.4 Å. MTG belongs to the α+β folding class. It consists of 11 α-helices and 8 β-strands (Kashiwagi et al. 2002).

The protein folds into a disc like shape with a deep cleft on the edge of the molecule (Figure 5). The residue Cys64 is located at the bottom of this cleft, named active site cleft, which is essential for catalytic activity of MTG. The residues Asp255 and His274, also important for the catalytic mechanism (see chapter 4), are in proximity of Cys64 (Figure 6B).

The model suggests exposure of Cys64 to the solvent. Thus, it can react promptly with substrates. Furthermore, the flexibility of the right-side wall of the active site cleft minders steric hindrance between enzyme and substrates.

At the entrance of the active site cleft resides the N-terminus. The Pro-MTG structure deposed in 2009 by Yang et al. at the protein data bank PDB revealed that the pro-peptide covers the active site cleft, rendering the zymogen inactive (Figure 5A).

MTGs three-dimensional structure is unique and completely different from human transglutaminases' structures.

Figure 5: Microbial transglutaminase crystal structures. A) Pro-Transglutaminase (PDB-ID: 3IU0). The pro-peptide is shown in blue. B) active Transglutaminase (PDB-ID: 1IU4). Active site cysteine is marked in yellow.

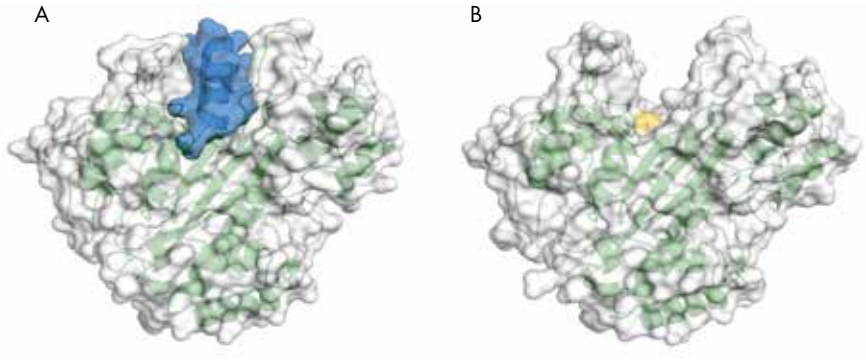
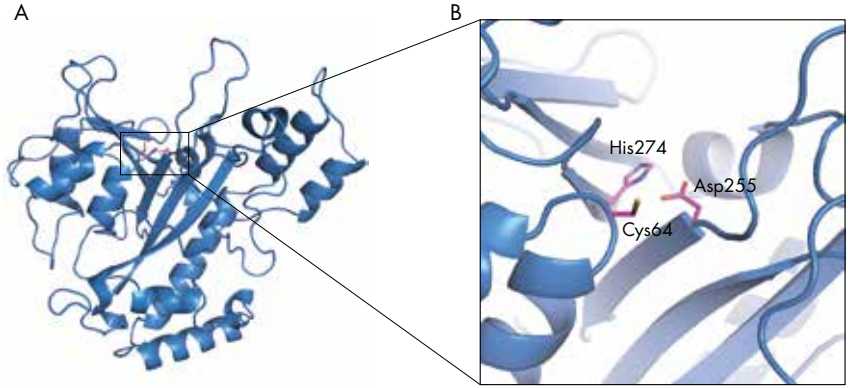


Figure 6: Ribbon model of microbial transglutaminase from *S. mobaraensis*. A) Tertiary structure with highlighted catalytic triade in the active side cleft. B) Enlarged active side with residues Cys64, Asp255 and His274 (PDB code 1IU4).



Targeting
Transglutaminase
for research and medicine!

Microbial transglutaminase (MTG) is an enzyme originally produced by *Streptomyces mobaraensis*. MTG catalyzes cross-linking of proteins in the absence of calcium. Current application field of the enzyme is processesing of meat, sausage, dairy, bakery, and pasta products to improve food properties.

However, MTG cannot only cross-link proteins, it can also incorporate primary amines to proteins in a covalent, irreversible manner. This feature allows linking of a whole bundle of labels to proteins, including biotin, fluorescent dyes, click chemistry substrates and cytotoxins.

MTG's unique properties can be utilized to generate site specific and homogenous antibody drug conjugates (ADCs). Today, ADCs manufactured with MTG reached clinical trials.

Zedira's MTG-Handbook shall provide an overview on this fascinating enzyme. While this first edition is just the beginning – future editions will follow to include latest developments and to cover novel applications.

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