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TRANSGLUTAMINASE – AN ENZYME GROUP OF EXTENDED METABOLIC AND APPLICATION POSSIBILITIES

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The structure, properties and activity directions, catalytic, regulatory and protein modification ability of an enzyme group called transglutaminase (TG-ase – EC. 2.3.2.13) are presented. The basic reaction catalysed by those enzymes is the transfer of primary –ε-aminoacyl residue (*e.g.* Lys) on the specific place of a γ-amide group of peptide bound Gln; the result is the so-called isopeptide bond formation. However TG-ases can catalyse also many other reactions, *e.g.* deamidation of a γ-amide group, the nitrosylation and denitrosylation of –SH groups of Cys, the isopeptide bond proteolysis or by means of the complexing the G_h protein (GDP⇔GTP) they activate some protein kinases and participate in signal transfer through the membranes (cellular or nuclear).

Because of differential functions TG-ases may participate in differently directed posttranslational protein modifications according to multiple mechanisms. In this review the properties and action mechanisms of TG-ases of different origin, including microbial (MTG-ases), are presented. As TG-ases may participate in many metabolic, physiological and regulatory processes bound with protein modification (also those contained in food products), many trials of the application of these enzymes have recently been undertaken to improve in this way the properties of many nutritional products. Therefore much attention is given in this review to present this aspect of TG-ase possibilities. Particularly interesting are those, concerning the cereal, milk, soy and muscle proteins, modification of which would change the structure and properties, such as cross-linking, N-supplementation, gelling, emulsifying, foaming *etc*.

INTRODUCTION

Transglutaminase – more strictly – a group of at least nine types of similarly active enzymes, catalyse mainly the formation of covalent, nondisulfide, cross-linking bonds between peptide bound glutaminyl (Gln) residues and the primary amine groups (except those at the asymmetric C (not α-C), *e.g.* Lys [Chobert *et al.*, 1996]. The correct name of the enzyme is: R-glutaminyl-peptide-γ-glutamyltransferase. TG-ase is an inductive, mainly Ca^{2+}-dependent enzyme, which occurs in many isoforms and in almost all organisms investigated [Dickinson & Yamamoto, 1996]. Because of the substantial protein structure (and properties) modification, when new cross-linking, isopeptide bonds are formed, TG-ase have recently become the object of numerous investigations of theoretical, as well as application character, concerning also some important areas of the food technology.

It was demonstrated recently, that TG-ases play in living organisms, mainly animal, different and important catalytic, regulatory, signaling and immunological functions, which are bound with their localization inside the different cell compartments. As they are able to form the cross-linking, isopeptide bonds, causing the protein polymerization, their activity can modify the secondary, tertiary and quaternary structures of proteins, particularly those being rich in Gln, produced in plants and animals. On the other hand the microbial TG-ases of some *Streptoverticillium* strains are Ca^{2+}-independent and demonstrate significant overproduction, therefore those strains were found to be the excellent biotechnological source, when produced on the industrial scale.

TRANSGLUTAMINASE CLASSIFICATION, STRUCTURE AND PROPERTIES

Transglutaminases (TG-ases – EC. 2.3.2.13) can be Ca^{2}--dependent or independent, the latter being rather of microbial origin. They are S-rich, contain many Cys SH groups (at least 18), and demonstrate catalytic and regulatory properties, particularly in protein modification. They have been found in all living cells investigated and are also taking part in many physiological processes, therefore they can be localized in soluble (cytoplasmic), as well as insoluble (cell membrane or tissue) forms. They have also been found in nervous tissues (both central and peripheral ones) and some plastids (*e.g.* in chloroplasts or erythrocyte membranes) [Kim *et al.*, 1999].

TG-ases exist in multiple forms, expressed at least by 9 genes, differentiated in solubility, localization, quaternary structure, and functions. TG-ase 1 (MW 106 kDa) is bound mainly in epithelial membranes; the most prevailing TG-ase 2

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(MW 80 kDa) is soluble, cytosolic and was investigated most extensively; TG-ase 3 (77 kDa), also soluble and localized mainly in epithelium; TG-ase 4 (80 kDa) and its inactive precursor form 4.2 occurring in some more specific cells; the TG-ase X (80 kDa) is the same with the XIIIa factor of blood clotting. Independently, literature reports on many other, or undetermined forms as concerns the types mentioned.

TRANSGLUTAMINASES 2

The most common and best recognised are TG-ases of type 2 [Fesus & Piacentini, 2002]; it is distinguishing the diverse localization concerning tissue, cell and compartments, as well as various recognized functions. They catalyse the cross-linking, *i.e.* the isopeptide bond formation between the peptide bound to specifically located amide Gln residues and the primary amine groups of Lys (peptide-bound or free) or other amines. Therefore proteins can be modified by: linking the polyamines, deamidation or hydrolysis of the isopeptide bonds (as the Ca-dependent isopeptidase [Parameshwaran *et al.*, 1997]). The other type of modification is the nitrosylation of at least 18 Cys SH groups [Lai *et al.*, 2001]. Moreover the TG-ase 2, when localized on the cell wall external surface, may serve as the integrating factor (extend cross-linking [Nakaoka *et al.*, 1994], as well as may participate in signal transfer across the membrane, as it possesses also the G protein function; the latter is bound with complexing the GDP/GTP system. The mechanisms of processes catalysed by TG-ase 2 are presented in Figures 1 a and b.

The most important activities of TG-ase 2 were localized in the following compartments: in cytosole (C), in nucleus (N), in extracellular space (E), and in cell membrane (M). At appropriate Ca^{2+} concentration, the following TG-ase catalysed reactions can be carried out: (1) the acyl transfer between the determined Gln amide group (peptide bound) and the primary amine group of Lys (peptide bound or free), as well as polyamines (putrescine, cadaverine, histamine, as well as some other primary amines); (2) joining *via* Lys bound NH to form isopeptide bonds; (3) deamidation of specific peptide bound Gln (amide group hydrolysis with NH_3 elimination, Gln→Glu); (4) in the absence of Ca^{2+} TG-ase is able to bind the G_h-GDP/GTP protein system; (5) as a result of P number change and the transfer to the outer membrane surface TG-ase can serve as the signal for receptor stimulation; (6) the intercellular TG-ase form is able to join the protein integrin and fibronectin after Ca^{2+} increase to catalyse the cell integration process and tissue formation. The protein modifications mentioned resulted from both the catalysis and interaction with the receptor activation and complexing with integrating components are connected with TG-ase cooperation as a regulator of multiple metabolic processes.

TG-ase 2 crystallizes in dimeric form as a GDP/GTP complex [Liu *et al.*, 2002] and 4 separate domains are distinguished in its structure: (1) N-terminal containing the fibronectin and integrin binding site; (2) the catalytic core containing two Trp molecules necessary to the acyl transfer; (3) and (4) two β-turn motifs present in C-terminal part, the latter of which (4) contains the phospholipase C binding site [Murthy *et al.*, 2002]. The special guanine nucleotide

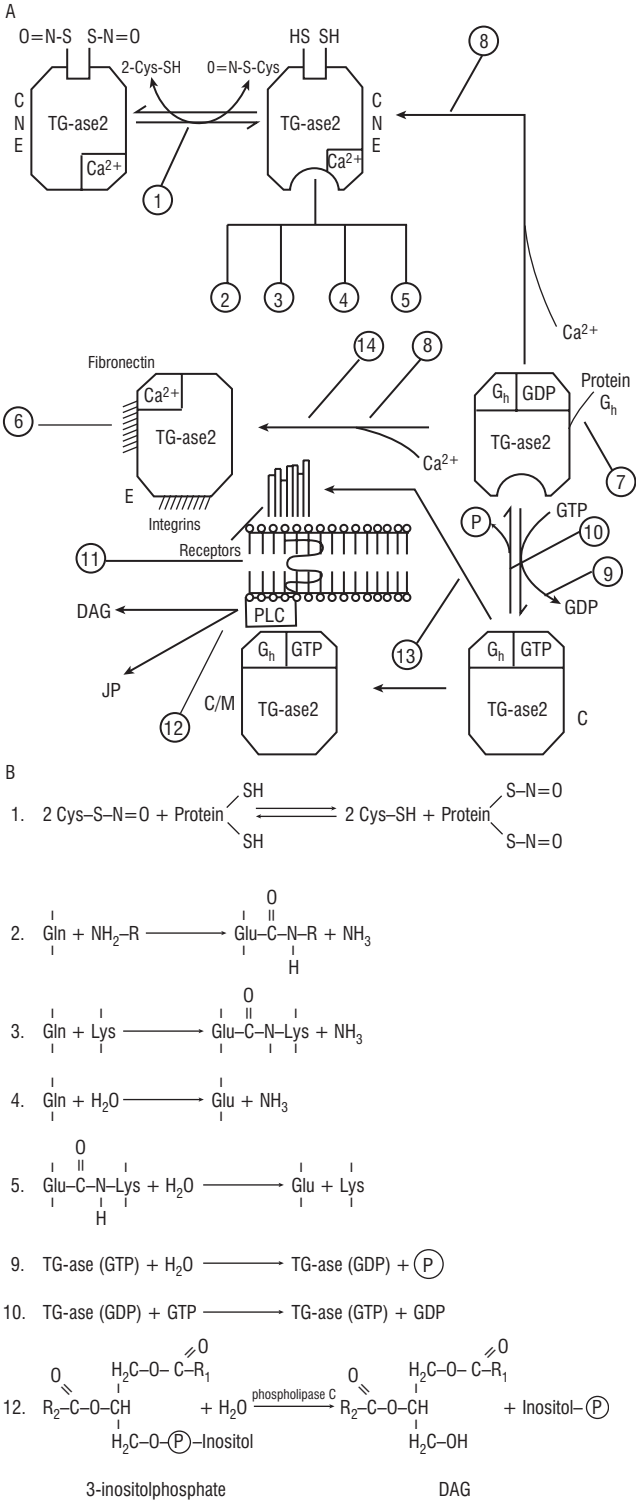

FIGURE 1. Most effective biochemical activities of transglutaminase 2 (mammalian); A – enzyme modifications; B – mechanisms of reactions. (1) –nitrosylation and denitrosylation of Cys-SH groups; (2) – incorporation of amines (free or bound) or basic amino acids into proteins; (3) – cross-link formation (isopeptide bonds); (4) – site specific Gln deamidation; (5) – isopeptide bond hydrolysis; (6) – intercell interaction; (7) – protein G_h complexing; (8) – Ca^{2+} - incorporation; (9) –TG-ase protein kinase activation; (10) – protein phosphatase (GTP-ase); (11) – signal transfer across membrane; (12) – phospholipase C activation to form inositole phosphate; (13) – receptor (oxytocin, TP-α-thromboxane, adreno-) stimulation; (14) – activation transfer; PlC-phospholipase C; DAG – diacylglycerole; PI - phosphoinositole. Localisation of following TG-ase activities: C – cytoplasm; N – nucleus; M – membrane; E – extracellular space.

binding site, absent in other TG-ase proteins is localized in the slit between the catalytic core and the first β-turn motif (Figure 2). The mode of Ca^{2+} binding to TG-ase is not fully elucidated, but comparing to that observed to TG-ase 3 Ca^{2+} binding, it is suggested to be localized close to the start of catalytic site under the Ca^{2+} influence two Trp residues controlling the substrate binding, are exposed. There was demonstrated also, that Ca^{2+} binding decreases the enzyme affinity to the GDP/GTP system (Figure 1) in the case of cooperation with G_h protein. At last, in TG-ase 2 two additional motifs were demonstrated, which take part in the protein transfer to the outer surface of cell membrane (without an accessory signal), to the integrin or (and) fibronectin complexing, as well as to the transfer into the nucleus inside.

The TG-ase 2 seems to be particularly active in processes occurring inside the cell membrane or at its outer surface. The following, more important processes localized there, were demonstrated: (1) signal transfer from the transmembrane helical receptors to the phospholipase C, which is then activated by removing the GDP inhibition from the TG-ase complex (GTP exchange [Murphy *et al.*, 1999]); (2) the activation of the mentioned is strictly connected with the protein nitrosylation and denitrosylation, which was shown to be necessary for vascular neogenesis, but also as such leads to protein modification [Lai *et al.*, 2001]; (3) GTP to GDP hydrolysis, which is localized in two TG-ase amino acid motifs: 34–42 and 63–79; they participate at the retinoic acid derivative RhoA activation (GDP---GTP) and deactivation (GTP→GDP respectively; two enzymes are active in these processes: protein kinase and specific protein phosphatase (RhoA-GTP-ase). The sequence of those events is taking part in stress fibril formation and the cytoskeleton adhesion, *e.g.* actin and myosin. TG-ase 2 cooperates also with tubular and microtubular proteins in cross-linking; (4) of interest seems to be the TG-ase 2 translocation into the nucleus aided by the protein – importin [Peng *et al.*, 1999]; the enzyme is suggested to function inside it as the specific protein G or as the histone cross-linking factor [Ballister *et al.*, 2001]. The suggestion also exists, that TG-ase inhibits the apoptosis (see below); (5) when localized on the outer surface of cell membrane or in the intercellular space, TG-ase bound with integrin protein forms the binding site to join fibronectin (they demonstrate high affinity to each other). TG-ase 2 cooperating with the integrin β subunit facilitates adhesion and spreading mobility of the cells [Akimov & Bełkin, 2001]. It is also worth mentioning that TG-ase function exceedes those mentioned above, as it has been demonstrated to participate directly in the healing of wounds and in vascular wall neogenesis. At these activities the cross--linking and associations of fibrinogen/fibrin (fibronectin) lipoproteins and some other factors may cooperate.

FIGURE 2. Primary structure of human TG-ase 2; domains: N-terminal, catalytic core and two C-terminal ones containing accumulated many Gln-rich motifs typical for elastomeric (β-turn) proteins; I – integrin and fibronectin binding site; II – the 15 amino acid structure able to bind G_h protein; III – Ca^{2+}- binding site; IV – phospholipase C binding site.

THE TG-ASE PATHOGENIC ROLES IN SOME DISEASES

Unfortunately some negative TG-ase activities concerning the specific disease units were also demonstrated. This was shown in the case u.a. of celiac disease (CD), *i.e.* the adsorption incapability of digestion products in the small intestine, because of villi degradation; this occurs in the case of patients sensitive genetically to cereal grain storage proteins. TG-ases influence the formation of peptides able to stimulate the T-cell development, caused by deamidation of specific Gln residues. Those peptides initiating the disease, develop the immunity, which can destroy the intestinal epithelium. Simultaneously the autoimmunological process with the T cell cooperation is started, which causes the antibody IgA formation against TG-ase protein. The level of this antibody has been chosen recently as the measure of the CD level. Deregulation of some TG-ase functions at the pathological background may cause some fibrosis formation (by cross-linking in patients sensitive organs, *e.g.* lungs, liver or kidneys).

The other example of TG-ase negative action is the Huttington disease, *i.e.* the gradual neurotic atrophy, which is based on the accumulation inside the gene encoding the protein – huttingtine (htt) trinucleotides CAG, which results in the inclusion of Gln residues in the form of repeated sequences in htt proteins. Such modified protein presents the excellent TG-ase substrate causing the formation of many cross-links. Also the exone to introne exchange in brain TG-ase mRNA at patients suffering from the Alzheimer disease causes the synthesis of shortened TG-ase form (unable to bind G_h protein GDP/GTP system) and this results in cross-linking activity increase of this enzyme.

MECHANISMS OF ACTION AND BASIC TG-ASE FUNCTIONS

Functions of other TG-ases

Besides TG-ase 2 activities characterized above, those attributed to other enzymes of this group, or those in which no particular TG-ase type is indicated, are described in the literature. Many reports are still of theoretical character and concern the localization, reaction mechanisms and substrate affinity.

Of extreme interest is the recent observation of Oliverio *et al.* [1999], that the inhibiting of extracellular Ca^{2+}--dependent TG-ase, taking part in irreversible cross-linking of concomitant proteins causes the life extension of separate human embryonic U937 cells. Therefore the enzyme is deregulating the normal embryo development and its repression protects the cell against apoptosis, *i.e.* their controlled annihilation. The increase in all-trans-retinoic acid and intercellular Ca^{2+} concentrations induce TG-ases, which are harmful to the programmed embryonic survival. The confirmation of this phenomenon also for other cell types would be of significant biological interest.

As mentioned, the structure of many TG-ases are oligomeric, as they are composed of an catalytic γ amide

transferase cross-linkage formation unit and the regulatory one of an G-protein character (type G_h). The latter function as receptor signals and are bound with an guanosine phosphate system in aequilibrium GDP/GTP forms. They are Ca^{2+}- and Cys-SH-dependent, their GTP forms are almost always active and those with GDP are inactive. The GDP⇔GTP aequilibrium is maintained by two enzymes mentioned: protein kinase and the specific phosphatase – GTP-ase. In the membrane and intercellular space the G_h protein can cooperate in some activation types [Nanda *et al.*, 2001], *e.g.* phospholipase C-activated inositolephosphate liberation from phosphoinositolelipides, mediated by TP-α-thromboxanes A-2 and oxytocine receptors. It can modify also the velocity of ion transport through the maxi-channel K in smooth muscle as well as the cGMP formation. Moreover in the intercellular space the G_h protein can catalyse the cross-linking bond formation and stabilize in that way substrates present therein, *e.g.* fibronectin, fibrinogen, collagen, osteonectin and some components cooperating in cell adhesion, which was demonstrated on fibroblasts and epithelial cells. The G_h protein expression had been confirmed also in the wound healing region, tissue granulation and angiogenesis [Nanda *et al.*, 2001].

The TG-ase protein nitrosylation and denitrosylation, mentioned earlier are taking part in processes connected u.a. in vessel expanding, thrombocyte aggregation, smooth muscle proliferation, as well as maintaining bound GMP. In those cases, protein modification is based on Cys SH group covalent blocking by NO groups [Lai *et al.*, 2001]. Such changes participate in the TG-ase activity regulation, as its protein contains at least 18 Cys residues, close or inside the catalytic site. The Cys nitrosylation mechanism of proteins is mediated by an O=N-nitroso-Cys; when $Ca^{2+}$is absent, 8 Cys are blocked only and TG-ase modification does not occur (Cys SH groups are located outside the catalytic site at the sufficient Ca^{2+} concentration about 15 Cys are blocked, which causes the TG-ase inhibition, whereas at the Ca^{2+} excess – the denitrosylation takes place). The variable TG-ase behaviour at different Ca^{2+} concentrations creates many possibilities of TG-ase regulation. It is also suggested that NO blocking of most Cys residues can modify by itself the structure of other proteins, when they are sufficiently rich in Cys SH groups.

Of interest would be also the TG-ase 1 and 2 mediation in the complexed Ca^{2+}-dependent signal transfer [Ruse *et al.*, 2001], in which also Ca-binding proteins designated as S100 class are taking part. They constitute the multigenic family of components of MW 10–14 kDa and of advanced homology. They do not exhibit their own enzymic activity and contain at least one EF motif (left-handed- β-helical structure) caused by the significant Gln content. The activity of Ca^{2+}-binding allows the structure modification of EF region, which can be transferred into the destined protein conversion. Typical examples of destined proteins in this case were demonstrated to be TG-ases 1 and 2 (*e.g.* keratinocyte TG-ases responsible for the cornification of epithelial cell surfaces). The S100 proteins may participate in the regulation of: cell differentiation, cell cycle process, energy metabolism (particularly that of protein kinases), and some interactions between membrane and cytoskeleton. Both the excessive substrate S100 expression increase and its lowering can lead to various disease disorders.

The another TG-ase substrate of interest is also the thymosine- β_4, which was recently shown to be identical with the factor F_x – the main intracellular peptide, which allows to maintain almost in all mammalian cells the monomeric G actin forms, thus inhibiting its conversion *in vivo* into the polymeric F-actin. The β_4 thymosine high concentration occurs in blood cells (except erythrocytes), but the low one in the serum; in stationary thrombocytes it is the main factor blocking the actin G coagulation. It participates also in the conversion of endothelial cells into capillary tubular forms; this peptide cooperates also in wound healing. In TG-ase interaction, thymosine β4 is the only substrate needed both as the primary-NH_2 donor (Lys-38) and the amide Gln group, which indicates the important role of thymosine β_4 in many TG-ase catalysed processes [Huff *et al.*, 1999].

Until recently TG-ase functions were suggested to be limited to the intracellular processes only (or mainly), but now much attention is given to their localization on the cell surfaces; this enables their adhesive functions, as well as the action outside the cell [Gaudry *et al.*, 1999]. It is suggested therefore, that besides the cross-linking activity, of significance might be TG-ase ability to construct and stabilize the pericellular matrix and to connect it to cell membranes. The so-called tissue TG-ases (tTG-ases) demonstrate the significant affinity to the extracellular matrix and, contrary to some other TG-ases, it can complex the GTP/GDP system; in this form however they demonstrate a very low cross-linking activity. The guanosine phosphate binding inside the cell prevents the Ca^{2+} activation, according to the intercellular enzyme function, nevertheless it maintains properties typical of cytosolic proteins (it does not contain the typical pilot sequence, which might take part in the transport to ER and to the intercellular region). It is assumed, that TG-ases are transferred across the cell membrane passively using a slot which can be formed in the membrane under the influence of some stress. The high TG-ase affinity to fibronectin, as well as the specific complexing site of the enzyme, were also localized. In experiments with tissue cultures, as well as the antisense ones, it was demonstrated that fibronectin polymerization outside the cell occurs by means of cross-linking mediated by external TG-ase and this mechanism seems to be basic for adhesion and cell spreading.

The TG-ase role in apoptosis

It is common knowledge that the tissue TG-ase (tTG-ase) is formed by the induction of one of genes participating in cell turning to the programmed death process. TG-ase place in the event sequence is not strictly elucidated, nevertheless the suggestion exists that the enzyme action causes the protein network formation, which is stabilized by insoluble and hardly hydrolysable isopeptide cross-linkings [Oliverio *et al.*, 1999].

The network is fixing cell contents, particularly some toxins, DNA, lysosomes *etc*., until they are being digested by autolysis or phagocytosis. It was observed indeed, that in cells designed to the programmed death, the TG-ase activity is much higher than in the normal ones.

PLANT TRANSGLUTAMINASES

Transglutaminases of plant origin described until now were shown to use similar substrates as animal TG-ases, *e.g.*

the cereal grain storage proteins. The best characterized enzyme is the chloroplast TG-ase isolated from *Helianthus tuberosus* leaves [Duca del *et al.*, 2000] under the active photosynthesis. The enzyme was identified according to the native chloroplast protein reaction with putrescine and isolated using the monoclonal antibody against TG-ase 4. The presence of strongly activated by Ca^{2+} and inhibited by EGTA SH group was demonstrated in it. The enzyme was highly active at pH 8.0–9.5 and the γ-glutamyl –mono- and di-putrescine were obtained as products. The authors concluded that chloroplast proteins might be regulated by the TG-ase modification in the presence of natural diamines. Also Villalobo *et al.* [2001] investigated the TG-ase localization in various maize cell types bound to polyclonal antibodies formed against TG-ase (58 kDa) and purified from *H. tuberosus* leaves; they demonstrated its prevailed presence in thylacoids of mature chloroplasts when exposed to light. During chloroplast differentiation from germ cell callus the thylakoid TG-ase level depended on grana type, being higher in developing than in mature chloroplasts; two other TG-ase forms were found to accompany the main form – 77 and 34 kDa. In mature leaves the enzyme was well visible in squeezed thylacoids of mesophyll cells, but in those of bundle sheet ones – only in trace amounts and in dispersion (latter are lacking the grana). The suggestion exists, that TG-ases may have the significant role in photosynthesis, *e.g.* at the modification of absorption capacity changes by the polyamine incorporation to chlorophyll anthenne molecules, when the photon excess does exist.

OTHER EVENTS DISCOVERED AS THOSE BOUND WITH TRANSGLUTAMINASE ACTIVITY

Some other cases of TG-ase cooperation in metabolic processes were also described in the literature. They are additionally indicating the importance and possibilities of TG-ase type enzymes and even broader events and effects of protein posttranslational modifications catalysed by them. For example, Fellbrich *et al.* [2002] reported on the species unspecific defence system in plants (parsley, *Arabidopsis*) against *Phytophtora*. It was based on the recognition of elicitors produced by pathogen; in the *Phytophtora* cell wall the TG-ase (42 kDa) was shown to exist, the 13 kDa fragment of which may initiate inside the plants investigated an multistep defence event sequence. Many *Oocyte* species contain a cell wall protein of 24 kDa, which exhibits the similar defence effect; it contains the sequence homology to that of 13 kDa protein fragment mentioned. Some protein fragments of high homology to it were demonstrated also in many other fungi and bacteria, but not in higher plants. Similar defence systems and some details of their induction were also demonstrated at some other plants.

The other example of theoretical interest may be the human monocyte cell infection by bacteria *Ehrlichia chaffensis*, which are unable to exist free, but inside of some eucariotic cells only [Liu *et al.*, 2002]. The infection of monocytal cells by those bacteria was shown to be blocked by some inhibitors, *e.g.* intracellular Ca^{2+} mobilization, the TG-ase cross-linking of some proteins, Tyr phosphorylation, the phospholipase C activation and inositol-3-P liberation. The other fact of interest was the demonstration of an Cys-Gly-His-Cys- motif – the fragment of an active site for transamidation reaction in TG-ase protein [Blasko *et al.*, 2003]. The same motif was formerly shown to be the basic active site fragment of thioredoxin, *i.e.* the natural important protein oxidoreductive transporter [Kączkowski, 2000]. The TG-ase fragment active in this process was found to be identical with the discovered earlier disulfide isomerase [Gobin *et al.*, 1997].

TRANSGLUTAMINASE REACTIONS AND EFFECTS OF WHEAT STORAGE PROTEIN INTOLERANCE

The popular, commonly known illness resulting from the hereditary intolerance to cereal storage proteins (mainly wheat) is celiac disease (CD). Its main symptoms (the digestion disorder in the small intestine) resulted from the villi athrophy, caused by some specific amino acid sequence in peptides formed as the protein digestion products. CD symptoms can be easily cancelled by removing cereal proteins from diet [Vogelsang, 2002]. Those and other experiments (*e.g.* Scorbjerg *et al.* [2002]), showed that wheat prolamines (particularly α and γ fractions) were demonstrated to be an excellent substrate to TG-ases of various origin – animal, microbial and plant (the latter concerning those, extracted from wheat, soy, pea and maize). The protein substrate undergoes cross-linking under the TG-ase action, in which a donor is peptide bound specific Gln amide group donor. The allergenity of prolamine proteins depends on the level and amino acid sequence – particularly Gln and Pro, when sequenced in specific motifs: Gln-Gln-Gln-Pro or Pro-Ser-Gln-Gln- [Kłys, 2003; Rivabene *et al.*, 1996; Osman *et al.*, 1996]. Proteins rich in those or some similar motifs cause the formation of antigens of IgA type.

Contrary to allergens causing CD, wheat proteins contain also other motifs of amino acid sequences, which cause allergies of other types (and symptoms), responsible for the formation of IgE antigens. The symptoms of those prolamine-dependent allergies are: bakers asthma, vessel oedema, nettle rash, atopic skin inflammation, as well as some psychiatric disorders like anaphylactic shock or cerebral ataxia [Burk *et al.*, 2001; Varionen *et al.*, 2000; Visser *et al.*, 2001]. In this case, the allergenic factors contain amino acid motifs: Ser-Gln-Gln-Gln or Pro-Pro-Phe-. Similarly, also some glycoproteins Mw 60 kDa and specific monoglucans as well as some germ proteins called germines, isolated from seeds of cereals or other plants, may function as allergens [Tanabe, 2002; Jensen-Jarolin *et al.*, 2002]; the latter are formed as the answer to biotic or abiotic stresses.

TRANSGLUTAMINASES AS PROTEIN MODIFYING FACTORS FOR FOOD TECHNOLOGY

General information

Information reviewed above demonstrate that TG-ases are present in all the living cells investigated and that they are localized in various cell compartments (cytosol, chloroplasts, cell membranes and both surfaces thereof, as well as intercellular space). Because of different structure TG-ases can take part in protein modifications of various types: amine incorporation, polypeptide chain cross-linking, deamidation, isopeptide bond hydrolysis, cell adhesion, receptor stimulation, signal transfer; hence the list of their

metabolic or physiological (*e.g.* regulatory) function mechanisms is extended. Some of those mechanisms have been reported even 40 years ago [Fesus *et al.*, 2002] but in the eighties the first application trials of TG-ase purified preparations or tissue extracts for food modification, were reported [Ivami & Yamamoto, 1986]. At present, the interest of food technologists in TG-ase enzymes is extremely high and concerns both, the theoretical investigation (reported shortly above) as well as enzyme application for food protein structure (and properties) modification.

Protein modification is performed with multiple mechanisms, *e.g.* oxidative processes, catalysed by glucose oxidase [Rassel *et al.*, 2003] or protein reoxidation following the prior partial S-S bond reduction [Mueller & Wieser, 1997; Meyer *et al.*, 1999; Antes & Wieser, 2000], by genetic substitution of subunits bearing motifs of special features (*e.g.* elastomeric [Tatham & Shewry, 2000]) and some others.

The modification of interest dependent on TG-ase function is the additional cross-linking, however it is limited to proteins containing high Gln level (cereal grain, milk, meat) or those containing a primary NH_2 group (putrescine, cadaverine, Lys). Similar modification occurs also when the free Lys or polyamide are incorporated, which increases the positive charge and also influences protein properties. Therefore the interest of cereal or milk technologists in TG-ase protein modification seems to be quite natural. Also some other branches of food technologists of meat, fish, soy isolates) are interested in this technique.

Finally, it is necessary to notice that in all branches of TG-ase application, the enzymes of microbial origin are used to modify food proteins. Most common are the reactor produced *Streptoverticillium* preparations or their genetic modifications.

Relatively early and in most experiments TG-ases have been used for cereal storage protein modification (particularly for wheat) and the baking industry. This results from the knowledge that those proteins have been demonstrated to be the excellent TG-ase substrates and to display very high Gln level.

Transglutaminases as cereal protein modificators

There has been recognized relatively early that some prolamine fractions (or their digestion products) are the excellent TG-ase substrates, and under the enzyme's influence the protein's MW and cross-linking bond level increase [Moliberg *et al.*, 1998]. It was also demonstrated that TG-ase activity was somehow connected with patients suffering from celiac disease (CD) by means of the recognition of T-cells in the small intestine (see above). The main allergenic motif of amino acid sequence causing the other sensitizations bound with IgE formation is: Gln-Gln-Gln--Pro-Pro-, which was shown to be the epitope binding this antibody [Tanabe *et al.*, 1996]. It is a common knowledge that the baking industry utilizes wheat seed storage proteins because of their extensive cross-linking ability and the expanded HMW structure formation of elastomeric character [Tatham & Shewry, 2000; Parchment *et al.*, 2001]. Such protein structure promotes particular visco-elastic properties of dough enabling the formation of the proper porous and elastic bread consistency after baking. Parchment *et al.* [2001] confirmed the presence and function of protein β-helical structure by their synthetic modeling, as well as their cross-linking ability, when the motifs were localized as repetitive and gathered in central peptide domains. Wheat prolamines, particularly HMW ones were shown to be very adequate substrates to undergo the cross-linking caused also by TG-ase activity, but when originated from *Streptoverticillium* sp. in spite of small Lys contents and water solubility [Larre *et al.*, 2000], but the cross-linking degree depended on the enzyme activity and incubation time. The network structure became more stable against peptidases (which in most cases do not hydrolyse isopeptide bonds) and against heating. The TG-ase application was shown to be useful for the harmful effect elimination to wheat baking quality of pathogen infection by *Eurygaster* spp. or *Aelia* spp., which occurs frequently in Middle East and Northern Africa [Sivri *et al.*, 1999; Koksal *et al.*, 2001]. In this case the enzyme applied was active at rebuilding up the cross-linking structure of HMW-prolamines degraded earlier by proteinases introduced by the infecting pathogens. At last the experiments of increasing the Lys contents in proteins by its attachment to the Gln amide group in TG-ase catalysed reaction was found to be of interest [Ivami & Yasumoto, 1989]. Similar effect could be also obtained by complexing prolamines with protein rich in Lys, but also in other exogenous amino acids (*e.g.* milk, soy), which is based on joining together these protein types by γ/ε-isopeptide bond formed by TG-ase [Kurth & Rogers, 1989]. The improvement of N-balance in wheat prolamines by the polyamine (putrescine, cadaverine) incorporation to Gln isopeptide bound was also endeavored with some success. The HMW-prolamines originating from wheat samples as substrates, of farther genetic lines did not show specificity differences to some TG-ases applied [Bassman *et al.*, 2002]. It has been sufficiently proved recently that TG-ases (particularly of microbial origin) are extremely specific in modification of wheat prolamine II (specially HMW-), by additional cross-linking formation, which can improve significantly dough rheological properties and flour baking quality [Bauer *et al.*, 2003; Mujoo & Ng, 2003; Russell *et al.*, 2003]. The latter authors tried also to obtain the positive modification of wheat proteins in oxidoreductive reaction catalysed by glucose oxidase, but contrary to TG-ase, they did not achieve the desired effect. On the other hand, the positive effect was obtained at confectionary TG-ase application to the frozen (-18°C) dough [Hozova *et al.*, 2002], where not only the structure, but also the taste properties were improved.

Of interest is also wheat improvement, proposed by Mujoo and Ng [2003] based on the addition of some probiotic fructooligosaccharides (FOS) to flour, which offers positive effects to the consumer, like stimulation of bifidobacterium, inhibition of pathogens able to form amines (amino acid decarboxylation), increasing the immunological function and digestion capacity. A rich FOS source was shown to be the immature wheat grains harvested two weeks PA (*post anthesis*). However, the immature wheat addition to flour needs the non-disulfide cross-linking protein (which are not fully polymerized [Huebner *et al.*, 1990] and that function is fulfilled excellently by the microbial TG-ase, which was applied with success by the authors cited above [Mujoo & Ng, 2003]).

Summarising, it should be pointed out, that TG-ase application to additive cereal prolamine cross-linking mod-

ification, not only improves their structural and rheological properties needed in baking, but also the nutritional value of the product by Lys supplementation or probiotic FOS addition. TG-ase application to flour from pathogen-infected grains may substitute the destroyed S-S bonds by the formation of isopeptide ones, thus to restore the technological value, but blocking some specific Gln residues at isopeptide bond formation; this could probably decrease the allergenic properties of peptides obtained during prolamine digestion. It seems, however, to be still too early for the economical analysis of events bound with TG-ase application in the technological procedures.

Modification of milk proteins

The TG-ase application for milk protein modification was described earlier, than those concerning cereal grains. Also demonstrated earlier was the conclusion that globular protein cross-linking is more efficient, when Ca^{2+} independent TG-ase of microbial origin is applied, contrary to enzymes of other origin (animal or plant [Motoki *et al.*, 1987; Nio *et al.*, 1985; 1986; Zhu & Damodaran, 1994]. In those investigations also the transglutamination activity of MTG-ase (M – microbial) between two peptide groups: γ-Gln and ε-NH_2-Lys was utilized. Such investigations were carried out between both the milk proteins or their complexes with those of other origin (muscle, soy and others).

The *Streptoverticillium* TG-ase is able to link most efficiently proteins of similar tertiary structures, however also in the case of more differentiated components to be complexed, some cross-linking degree could be also obtained. In this case however the intermediary components would be sufficient. This concerns *e.g.* milk albumin and casein with lactoglobulin intermediary ones [Han & Damodaran, 1996]. TG-ase applied in those experiments is Ca^{2+}-independent and was isolated and purified at first by Ando *et al.* [1989] and surely it should be soon the main gelling and cross-linking factor in the milk industry. It is expected to be used for protein conjugate formation of wanted proteins, *e.g.* soy conjugates, as well as of increased stability, but even those similar to rennet activity [Lorenzen, 2000]. TG-ase of this type has a significant affinity to the opened macromolecular casein structure, whereas the globular ones of whey proteins need some preliminary modification (*e.g.* heat denaturation [Han & Damodaran, 1996; O'Sullivan *et al.*, 2002]).

The investigation of TG-ase influence on milk protein demonstrated until now its direct action on the cross-linking of micellar casein and lactoalbumin, whereas the β-lactoglobulin could become its proper substrate after heat denaturation only [Rayan-Sharma *et al.*, 2001; Schorsch *et al.*, 2000], or the previous incubation in the lowered pH; the other direction concerned the increased heat and acid stability. Some other investigations concerned the milk protein modification for increasing the viscosity [Lorenzen *et al.*, 2002], gelling properties [Schorsch *et al.*, 2000], clotting and activity similar to rennet [Lorenzen, 2000], as well as emulsifying and foaming ability. The emulsion foaming ability of milk protein and its stability increased only when cross-linking after emulsifying has been carried out, because the level of protein absorbed in those conditions was higher [Rayan-Sharma *et al.*, 2002]. The need of TG-ase application was shown to be useful in the low-fat milk powder production used as the half-product to yoghurt manufacturing; the product was then more compact and had better sensoric properties [Wieland & Eberhard, 2002]. Worth of mention is also the procedure of Na-caseinate modification by the 0.5% hydrolysis using *Bacillus* proteinase, following by MTG-ase treatment, which caused the two-fold foaming increase of protein [Flanagan & Fitzgerald, 2003].

Vasbinder *et al.* [2003] presented the mechanism of milk casein gelling, when temperature or pH dependent conditions were applied. They demonstrated, that the gelling process proceeds as a result of electrostatic interactions occurring between micellar and soluble casein forms. The suggested model is now the background for the optimization and control of some technological processes, *e.g.* pasteurization of acidified milk products.

Modification of soy protein isolates

As soy proteins demonstrate a particularly high nutritional value, their modification to improve the structural properties by the TG-ase reaction, was also investigated. The soy proteins treated directly with the enzyme (or following the limited proteolysis – to 5%) caused an increase in soluble N (particularly at lower pH), the emulsifying ability, the compactness of gel formation, as well as the contribution instead of non-disulfide, the γ/ε-isopeptide crossings in the network form [Soeda, 2003; Walsh *et al.*, 2003]. The $NaHSO_3$ addition caused the accessory gel strengthening [Tsao *et al.*, 2002], which was shown to be significant for the formation of the product with particularly hard gel structure. The implementation of soy proteins to this programme gave many possibilities in their applications having the valuable components in food production. Worth of interest are the trials of TG-ase participation in cross-linking of soy with the muscle proteins, but this complexing needs a limited denaturation of the latter [Ramirez-Suarez & Xiang, 2003].

Attention should also be paid to information concerning the muscle protein modification [Ramirez-Sanchez & Xiang, 2003; Ching & Chung, 2003; Ervanto *et al.*, 2003], *e.g.* collagen for sausage production, with increased gelling ability, as well as fish proteins to obtain more compact structure and ability to maintain more water [Armbrust *et al.*, 2003].

Technical transglutaminase

It has been mentioned earlier that TG-ase applied for food protein modification is almost exclusively of microbial origin (MTG-ase). It displays different properties, compared to the best known tTG-ase (tissue) 2 or others of animal or plant origin, the activities of which are Ca^{2+}-dependent. The MTG-ase is manufactured using the *Streptoverticillium* bacteria [Ando *et al.*, 1989]. The commercial preparation purchased by Ajimoto (Japan) is supplied in the form of polysaccharide carrier powder, which contains about 4.5% water, 0.8% ash and 7.9% crude protein (Nx5.7); the latter was demonstrated to be almost 100% TG-ase protein. The enzyme activity is 890–900 u/g and is routinely determined by the hydroxamate method [Folk & Cole, 1966]; 1 TG-ase unit = 1 μmol/L hydroximate/min, which corresponds to 9.9 u/mg enzyme protein of MW=40 000 Da [Bauer *et al.*, 2003].

As it was mentioned earlier, the MTG-ase of *Streptoverticillium* sp. is Ca^{2+}-independent and is now almost exclusively applied for food protein modification, as a not expensive and easily available product of reactor processes. Most

effective was shown to be the *Streptoverticillium mobaraense* var., which produces the extracellular MTG-ase [Anda *et al.*, 1989]; this enzyme was demonstrated also to be the most suitable for food protein modification [Nakahara *et al.*, 1999; Matsumura *et al.*, 2000; 2003]. Moreover, the primary product of this strain is proenzyme form which needs to be converted into the active one. Yokogama *et al.* [2003] transferred the MTG-ase into *E. coli* cells, in which the transfolding of the proenzyme form produced *in vitro* in acid milieu to the active one, could be achieved. This maturation enabled the valuable technical improvement and the production of a high-purity enzyme.

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