Possible Role of the Transglutaminase Activity in Molecular Mechanisms Responsible for Human Neurodegenerative Diseases

Martina Iannaccone, Nicola G. Gatta, Vittorio Gentile

Transglutaminases are Ca²⁺-dependent enzymes which catalyze post-translational modifications of proteins. The main activity of these enzymes is the cross-linking of glutaminyl residues of a protein/peptide substrate to lysyl residues of a protein/peptide co-substrate. In addition to lysyl residues, other second nucleophilic co-substrates may include monoamines or polyamines (to form mono- or bi-substituted/crosslinked adducts) or –OH groups (to form ester linkages). In absence of co-substrates, the nucleophile may be water, resulting in the net deamidation of the glutaminyl residue. Transglutaminase activity has been suggested to be involved in molecular mechanisms responsible for both physiological or pathological processes. In particular, transglutaminase activity has been shown to be responsible for a widespread human autoimmune disease, the Celiac Disease. Interestingly, neurodegenerative diseases, such as Alzheimer's Disease, Parkinson's Disease, supranuclear palsy, Huntington's Disease and other polyglutamine diseases, are characterized in part by aberrant cerebral transglutaminase activity and by increased cross-linked proteins in affected brains. This review focuses on the possible molecular mechanisms responsible for such diseases and on the possible therapeutic effects of transglutaminase inhibitors for patients with diseases characterized by aberrant transglutaminase activity.

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Key words: Transglutaminases; Post-translational modifications of proteins; Protein aggregation; Neurodegenerative diseases; Transglutaminase inhibitors


THE BIOCHEMISTRY OF THE TRANSGLUTAMINASES

Transglutaminases (TGs, E.C. 2.3.2.13) are Ca²⁺-dependent enzymes which catalyze post-translational modifications of proteins. Examples of TG-catalyzed reactions include: (1) acyl transfer between the γ-carboxamide group of a protein/polypeptide glutaminyl residue and the ε-amino group of a protein/polypeptide lysyl residue; (2) attachment of a polyamine to the γ-carboxamide of a glutaminyl residue; (3) deamidation of the γ-carboxamide group of a protein/polypeptide glutaminyl residue (Figure 1)². The reactions catalyzed by TGs occur by a two-step mechanism (ping-pong type), (Figure 2). The transamidating activity of TGs is activated by the binding of Ca²⁺, which exposes an active-site cysteine residue. This cysteine residue reacts with the γ-carboxamide group of an incoming glutaminyl residue of a protein/polypeptide substrate to yield a thioacyl-enzyme intermediate and ammonia, (Figure 2, Step 1). The thioacyl-enzyme intermediate then reacts with a nucleophilic...
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THE MOLECULAR BIOLOGY OF THE TRANSGLUTAMINASES

To date at least eight different TGs, distributed in the human body, have been identified (Table 1)\textsuperscript{[14-19]}. Complex gene expression mechanisms regulate the physiological roles that these enzymes play in both the intracellular and extracellular compartments.

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**Figure 1** Examples of reactions catalyzed by TG: I) acyl transfer between the γ-carboxamide group of a protein/polypeptide glutaminyl residue and the ε-amino group of a protein/polypeptide lysyl residue; II) attachment of a polyamine to the γ-carboxamide of a glutaminyl residue; III) deamidation of the γ-carboxamide group of a protein/polypeptide glutaminyl residue; IV) GTPase activity; V) protein disulfide isomerase activity; VI) protein kinase activity.

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Step 1: In the presence of Ca\(^{2+}\), the active-site cysteine residue reacts with the \(\gamma\)-carboxamide group of an incoming glutaminyl residue of a protein/peptide substrate to yield a thioacyl-enzyme intermediate and ammonia. Step 2: The thioacyl-enzyme intermediate reacts with a nucleophilic primary amine substrate, resulting in the covalent attachment of the amine-containing donor to the substrate glutaminyl acceptor and regeneration of the cysteinyl residue at the active site. If the primary amine is donated by the \(\varepsilon\)-amino group of a lysyl residue in a protein/polypeptide, a \(\text{N}^\alpha\text{-}L\text{-glutamyl}-L\text{-lysine (GGEL) isopeptide bond is formed.}\)

In the Nervous System, for example, several forms of TGs are simultaneously expressed\(^{[20-22]}\). Moreover, several alternative splice variants of TGs, mostly in the 3'-end region, have been identified in these last years\(^{[23]}\). Interestingly, some of them are differently expressed in human pathologies, such as Alzheimer’s Disease (AD) \(^{[24]}\). On the basis of their ubiquitous expression and their biological roles, we may speculate that the absence of these enzymes would be lethal. However, this does not always seem to be the case, since, for example, null mutants of the TG2 are usually phenotypically normal at birth\(^{[15,25,26]}\). This result may be explained by the multiple expressions of other TG genes that may substitute the TG2 missing isoform, although other TG isof orm mutations have been associated to severe phenotypes, such as lamellar ichthyosis for TG1 isoform, although other TG isoform mutations have been associated to disease progression. For example, several experimental findings reported that TG2 activity in vitro leads to the formation of soluble aggregates of \(\alpha\)-synuclein\(^{[47]}\) or polyQ proteins\(^{[48,49]}\). To date, as previously reported, at least ten human CAG-expansion diseases have been described (Table 2)\(^{[49-51]}\) and in at least eight of them their neuropathology is caused by the expansion in the number of residues in the polyglutamine domain to a value beyond 35-40. Remarkably, the mutated proteins have no obvious similarities except for the expanded polyglutamine domain. In fact, in all cases except SCA 12, the mutated proteins have no obvious similarities except for the expanded polyglutamine domain. In fact, in all cases except SCA 12, the mutation occurs in the coding region of the gene. However, in SCA12, the CAG triplet expansion occurs in the untranslated region such as the active site and the calcium binding regions. However, high sequence conservation and, therefore, a high degree of preservation of secondary structure among TG2, TG3 and FXIIIa indicate that these TGs all share four-domain tertiary structures which could be similar to those of other TGs\(^{[52]}\).

### Table 1: TGs and their physiological roles when known.

<table>
<thead>
<tr>
<th>TG</th>
<th>Physiological role</th>
<th>Gene map location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor XIIIa</td>
<td>Blood clotting</td>
<td>6p24-25</td>
<td>[14]</td>
</tr>
<tr>
<td>TG 1 (Keratinocyte TG, kTG)</td>
<td>Skin differentiation</td>
<td>14q11.2</td>
<td>[15]</td>
</tr>
<tr>
<td>TG 2 (Tissue TG, tTG, cTG)</td>
<td>Apoptosis, cell adhesion, signal transduction</td>
<td>20q11.2</td>
<td>[16]</td>
</tr>
<tr>
<td>TG 3 (Epidermal TG, eTG)</td>
<td>Hair follicle differentiation</td>
<td>20p11.2</td>
<td>[17]</td>
</tr>
<tr>
<td>TG 4 (Prostate TG, pTG)</td>
<td>Suppression of sperm immunogenicity</td>
<td>3q21-2</td>
<td>[18]</td>
</tr>
<tr>
<td>TG 5 (TG X)</td>
<td>Epidermal differentiation</td>
<td>15q15.2</td>
<td>[19]</td>
</tr>
<tr>
<td>TG 6 (TG Y)</td>
<td>Central Nervous System development</td>
<td>20p13</td>
<td>[19]</td>
</tr>
<tr>
<td>TG 7 (TG Z)</td>
<td>Unknown function</td>
<td>15q15.2</td>
<td>[19]</td>
</tr>
</tbody>
</table>

### POSSIBLE ROLES OF THE TRANSGLUTAMINASE ACTIVITY IN NEURODEGENERATIVE DISEASES

Numerous scientific reports suggest that the transglutaminase activity is involved in the pathogenesis of neurodegenerative diseases, but, to date, however, also controversial experimental findings about the role of the TGs enzymes in these diseases have been obtained\(^{[28-30]}\). Protein aggregates in affected brain regions are histopathological hallmarks of many neurodegenerative diseases\(^{[10]}\). More than 30 years ago Selkoe et al\(^{[21]}\) suggested that TG activity might contribute to the formation of protein aggregates in AD brain. In support of this hypothesis, tau protein has been shown to be an excellent in vitro substrate of TGs\(^{[13,34]}\), and GGEL cross-links have been found in the neurofibrillary tangles and paired helical filaments of AD brains\(^{[20]}\). Interestingly, a recent work showed the presence of bis \(\gamma\)-glutamyl putrescine in human CSF, which was increased in Huntington’s Disease (HD) CSF\(^{[25]}\). This is an important evidence that protein/polypeptides crosslinking by polyamines does indeed occur in brain, and that this is increased in HD brain. TG activity has been shown to induce also amyloid \(\beta\)-protein oligomerization\(^{[33]}\) and aggregation at physiologic levels\(^{[34]}\). By these molecular mechanisms, TGs could contribute to AD symptoms and progression\(^{[35]}\). Moreover, there is evidence that TGs also contribute to the formation of proteinaceous deposits in Parkinson’s Disease (PD)\(^{[36,40]}\), in supranuclear palsy\(^{[21,42]}\) and in HD, a neurodegenerative disease caused by a CAG expansion in the affected gene\(^{[36]}\). For example, expanded polyglutamine domains have been reported to be substrates of TG2\(^{[38,40]}\) and therefore aberrant TG activity could contribute to CAG-expansion diseases, including HD (Figure 3). However, although all these studies suggest the possible involvement of the TGs in the formation of deposits of protein aggregates in neurodegenerative diseases, they do not indicate whether aberrant TG activity per se directly determines the disease progression. For example, several experimental findings reported that TG2 activity in vitro leads to the formation of soluble aggregates of \(\alpha\)-synuclein\(^{[47]}\) and polyQ proteins\(^{[48,49]}\). To date, as previously reported, at least ten human CAG-expansion diseases have been described (Table 2)\(^{[49-51]}\) and in at least eight of them their neuropathology is caused by the expansion in the number of residues in the polyglutamine domain to a value beyond 35-40. Remarkably, the mutated proteins have no obvious similarities except for the expanded polyglutamine domain. In fact, in all cases except SCA 12, the mutation occurs in the coding region of the gene. However, in SCA12, the CAG triplet expansion occurs in the untranslated region...
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at the 5' end of the PPP2R2B gene. It has been proposed that the toxicity results from overexpression of the brain specific regulatory subunit of protein phosphatase PP2A\(^{(59)}\). Most of the mutated proteins are widely expressed both within the brain and elsewhere in the body. A major challenge then is to understand why the brain is primarily affected and why different regions within the brain are affected in the different CAG-expansion diseases, i.e., what accounts for the neurotoxic gain of function of each protein and for a selective vulnerability of each cell type. Possibly, the selective vulnerability\(^{(58)}\) may be explained in part by the susceptibility of the expanded polyglutamine domains in the various CAG-expansion diseases to act as cosubstrates for a brain TG (Figure 4). To strengthen the possible central role of the TGS in neurodegenerative diseases, a study by Hadjivassiliou \emph{et al}\(^{(61)}\) showed that anti-TG2 IgA antibodies are present in the gut and brain of patients with gluten ataxia, a non-genetic sporadic cerebellar ataxia, but not in ataxia control patients. Recently, anti-TG2, -TG3 and -TG6 antibodies have been found in sera from CD patients, suggesting a possible involvement also of other TGS in the pathogenesis of dermatitis herpetiformis and gluten ataxia, two frequent extra intestinal manifestations of gluten sensitivity\(^{(52,60)}\).

**Figure 3** Possible physiopathological effects of the mutated huntingtin. Some of the physiopathological roles of mutated huntingtin, including the formation of nuclear inclusions, have been described in the figure.

**Figure 4** Possible mechanisms responsible for protein aggregate formation catalyzed by TGS. Transglutaminase activity could produce insoluble aggregates both by the formation of N\(^{-}\gamma\)-L-glutamyl)polyamine bridges (left side of the figure) and by the formation of N,N-bis-(\(\gamma\)-L-glutamyl)polyamine bridges (right side of the figure) in the mutated huntingtin.

**Table 2** List of polyglutamine (CAG-expansion) diseases.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Sites of neuropathology</th>
<th>CAG triplet number</th>
<th>Gene product (Intracellular localization of protein deposits)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corea Major or Huntington’s Disease (HD)</td>
<td>cerebellar cortex (Purkinje cells), dentate nucleus and brain stem</td>
<td>5-40</td>
<td>Huntingtin (n, c)</td>
<td>[50]</td>
</tr>
<tr>
<td>Spinocerebellar Ataxia Type 1 (SCA1)</td>
<td>cerebellum, pontine nuclei, substantia nigra</td>
<td>5-40</td>
<td>Ataxin-1 (n, c)</td>
<td>[51]</td>
</tr>
<tr>
<td>Spinocerebellar Ataxia Type 2 (SCA2)</td>
<td>substantia nigra, globus pallidus, pontine nucleus, cerebellar cortex</td>
<td>5-40</td>
<td>Ataxin-2 (c)</td>
<td>[52]</td>
</tr>
<tr>
<td>Spinocerebellar Ataxia Type 3 (SCA3) or Machado-Joseph disease (MJD)</td>
<td>cerebellum and mild brainstem atrophy</td>
<td>5-40</td>
<td>Ataxin-3 (c)</td>
<td>[53]</td>
</tr>
<tr>
<td>Spinocerebellar Ataxia Type 6 (SCA6)</td>
<td>photoreceptor and bipolar cells, cerebellar cortex, brainstem</td>
<td>5-40</td>
<td>Calcium channel Subunit (q1A) (m)</td>
<td>[54]</td>
</tr>
<tr>
<td>Spinocerebellar Ataxia Type 7 (SCA7)</td>
<td>cortical, cerebellar atrophy</td>
<td>5-40</td>
<td>Brain specific regulatory subunit of protein phosphatase PP2A (?</td>
<td>[55]</td>
</tr>
<tr>
<td>Spinocerebellar Ataxia Type 12 (SCA12)</td>
<td>gliosis and neuronal loss in the Purkinje cell layer</td>
<td>5-40</td>
<td>TATA-binding protein (TBP) (n)</td>
<td>[56]</td>
</tr>
<tr>
<td>Spinocerebellar Ataxia Type 17 (SCA17)</td>
<td>motor neurons (anterior horn cells, bulbar neurons) and dorsal root ganglia</td>
<td>5-40</td>
<td>Androgen receptor (n, c)</td>
<td>[57]</td>
</tr>
<tr>
<td>Authophagy of Huntington’s Disease</td>
<td>globus pallidus, dentato-rubral and subthalamic nucleus</td>
<td>5-40</td>
<td>Atrophin (n, c)</td>
<td>[58]</td>
</tr>
</tbody>
</table>

Cellular localization: c, cytosol; m, membrane; n, nucleus.

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In support to the hypothesis of the toxic effect of TG activity in other neurodegenerative diseases, such as Alzheimer’s disease and Parkinson’s Disease, TG activity has been shown to induce amyloid beta-protein and α-synuclein oligomerization and aggregation at physiologi levels. In fact, TG activity induces protofibril-like amyloid beta-protein assemblies that are protease-resistant and inhibit long-term potentiation. Therefore, by these molecular mechanisms, TG activity could also contribute to Alzheimer’s disease symptoms and progression. Finally, a recent work by Bass et al. found that in addition to TG2, TG1 gene expression level is significantly induced following stroke in vivo or due to oxidative stress in vitro. Moreover, structurally diverse inhibitors, used at concentrations that inhibit TG1 and TG2 simultaneously, are neuroprotective. Together, these last studies suggested that multiple TG isoforms, not only TG2, participate in oxidative stress-induced cell death signalling, and that isoform nonselective inhibitors of TG will be most efficacious in combating oxidative death in neurological disorders. These are interesting and worthwhile studies, suggesting that multiple TG isoforms can participate in neuronal death processes. Therefore, all these studies suggest that the involvement of brain TGs could represent a common denominator in several neurological diseases, which can lead to the determination of pathophysiological consequences through different molecular mechanisms.

POSSIBLE ROLE OF THE TRANSGlutAMINASE ACTIVITY IN NEUROINFLAMMATION

Neuroinflammation plays an important role in various chronic neurodegenerative diseases, characterized also by the pathogenetic accumulation of specific protein aggregates. Several of these proteins have been shown to be substrates of transglutaminases. Interestingly, it has recently been demonstrated that transglutaminase 2 (TG2) may also be involved in molecular mechanisms underlying inflammation. In the central nervous system, astrocytes and microglia are the cell types mainly involved in this inflammatory process. The transcription factor NF-κB is considered the main regulator of inflammation and it is activated by a variety of stimuli including calcium influx, oxidative stress and inflammatory cytokines. Under these conditions, the over-expression of TG2 results in the sustained activation of NF-κB. Several findings emphasize the possible role of the TG2/NF-κB activation pathway in neurodegenerative diseases, including Alzheimer’s disease, Parkinson’s disease, multiple sclerosis and amyotrophic lateral sclerosis. Together, evidences suggest that TG2 could play a role in neuroinflammation and could contribute to the production of compounds that are potentially deleterious to neuronal cells.

TRANSGlutAMINASE INHIBITION AS POSSIBLE THERAPEUTIC APPROACH

In consideration to the fact that up to now there have been no long-term effective treatments for the human neurodegenerative diseases previously reported, then the possibility that selective TG inhibitors may be of clinical benefit has been seriously considered. In this respect, some encouraging results have been obtained with TG inhibitors in preliminary studies with different biological models of CAG-expansion diseases. For example, cystamine (Figure 5) is a potent in vitro inhibitor of enzymes that require an unmodified cysteine at the active site. Inasmuch as TGs contain a crucial active-site cysteine, cystamine has the potential to inhibit these enzymes by a sulfide-disulfide interchange reaction. A sulfide-disulfide interchange reaction results in the formation of cysteamine and a cysteamine-cysteine mixed disulfide residue at the active site. Recent studies have shown that cystamine decreases the number of protein inclusions in transfected cells expressing the atrophin (DRPLA) protein containing a pathological-length polyglutamine domain. In other studies, cystamine administration to HD-transgenic mice resulted in an increase in life expectancy and amelioration of neurological symptoms. Neuronal inclusions were decreased in one of these studies. Although all these scientific reports seem to support the hypothesis of a direct role of transglutaminase activity in the pathogenesis of the polyglutamine diseases, cystamine is also found to act in the HD-transgenic mice by mechanisms other than the inhibition of TGs, such as the inhibition of caspases, suggesting that this compound can have an additive effect in the therapy of HD. Currently, cystamine is already in phase I studies in humans with HD, but several side effects, such as nausea, motor impairment and dosing schedule have been reported as reasons for non-adherence during phase II studies in human patients affected by cystinosis. Another critical problem in the use of TG inhibitors in treating neurological diseases relates to the fact that, as previously reported, the human brain contains at least four TGs, including TG1, 2, 3 and TG6, and a strong non-selective inhibitor of TGs might also inhibit plasma Factor XIIa, causing a bleeding disorder. Therefore, from a number of standpoint it would seem that a selective inhibitor, which discriminates between TGs, would be preferable to an indiscriminate TG inhibitor. In fact, although most of the TG activity in mouse brain, at least as assessed by an assay that measures the incorporation of radioactive putrescine (amine donor) into N,N-dimethyl casein (amine acceptor), seems to be due to TG2, no conclusive data have been obtained by TG2 gene knock-out experiments about the involvement of this TG in the development of the symptoms in HD-transgenic mice. Moreover, a recent scientific report showed that cystamine reduces aggregate formation in a mouse model of oculopharyngeal muscular dystrophy (OMPD), in which also the TG2 knockdown is capable of suppressing the aggregation and the toxicity of the mutant protein PABPN1, suggesting this compound as a possible therapeutic for OMPD.

CONCLUSIONS

In conclusion, numerous scientific reports have implicated aberrant TG activity in neurodegenerative diseases, but still today we are looking for experimental findings which could definitely confirm the direct involvement of TGs in the pathogenetic mechanisms responsible for these diseases. However, as result of the putative role of specific TG isoforms, such as TG2, in some human diseases, there is a considerable interest in developing inhibitors of these enzymes. Of those currently available, cystamine is the most commonly used experimentally to inhibit TG2 activity. In addition to cystamine, several types of TG2 inhibitors have been developed up to now. Interestingly, some of these inhibitors have shown promising results in experimental diabetic models. Therefore, the use of these inhibitors of TGs could be then useful also for other clinical approaches. To minimize the possible side effects, however, more selective inhibitors of the TGs should be required in the future. Progress in this area of research could be achieved also through pharmacogenetic techniques.
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CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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