Possible Role of the Transglutaminases in the Pathogenesis of Neurodegenerative Diseases

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Abstract: Transglutaminases are ubiquitous enzymes which catalyze posttranslational modifications of proteins. Recently, transglutaminase-catalyzed post-translational modifications of proteins have been shown to be involved in molecular mechanisms responsible for human diseases. Transglutaminase-catalyzed post-translational modifications of proteins have been hypothesized to be involved also in the pathogenetic mechanisms responsible for several human neurodegenerative diseases. Alzheimer's disease and other neurodegenerative diseases, such as 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 by which transglutaminase activity could be involved in the pathogenesis of Alzheimer's disease and other neurodegenerative diseases, and on the possible therapeutic effects of selective transglutaminase inhibitors for the cure of patients with diseases characterized by aberrant transglutaminase activity.

Keywords: Neurodegenerative diseases, post-translational modifications of proteins, protein aggregation, transglutaminases, transglutaminase inhibitors.

BIOCHEMISTRY

Transglutaminases (TGs, E.C. 2.3.2.13) are a family of Ca^{++}-dependent enzymes (Table **1**) catalyzing covalent post-translational modifications of proteins. Examples of TG-catalyzed reactions include: 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 (Figure **1**) [1, 2]. The reactions catalyzed by TGs occur by a two-step mechanism (Figure **2**). The transamidating activity of TGs is activated by the binding of Ca^{2+}, which exposes an active-site cysteine residue. This cysteine residue reacts with the γ-carboxamide group of an incoming glutaminyl residue of a protein/peptide substrate to yield a thioacyl-enzyme intermediate and ammonia (Figure **2**, Step 1). The thioacyl-enzyme intermediate then 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 (Figure **2**, Step 2). If the primary amine is donated by the ε-amino group of a lysyl residue in a protein/polypeptide, a N^{ε}-(γ-L-glutamyl)-L-lysine (GGEL) isopeptide bond is formed (Figure **1**, example I). On the other hand, if a polyamine or another primary amine (e.g. histamine) acts as the amine donor, a γ-glutamylpolyamine (or γ-glutamylamine) residue is formed (Figure **1**, example II). It is also possible for a polyamine to act as an N,N-bis-(γ-L-glutamyl)polyamine bridge between two glutaminyl acceptor residues either on the same protein/polypeptide or between two proteins/polypeptides [3]. If there is no primary amine present, water may act as the attacking nucleophile, resulting in the deamidation of glutaminyl residues to glutamyl residues (Figure **1**, example III). It is worthwhile noting that two of these reactions, in particular, the deamidation of peptides obtained from the digestion of the gliadin, a protein present in wheat, and the N^{ε}-(γ-L-glutamyl)-L-lysine (GGEL) isopeptide formation between these peptides and "tissue" Transglutaminase (TG2 or tTG), have been shown to cause the formation of new antigenic epitopes which are responsible of immunological reactions during the Celiac Disease (CD), one of the most common human autoimmune diseases [4, 5]. The reactions catalyzed by TGs occur with little change in free energy and hence should theoretically be reversible. However, under physiological conditions the cross linking reactions catalyzed by TGs are usually irreversible. This irreversibility partly results from the metabolic removal of ammonia from the system and from thermodynamic considerations resulting from altered protein conformation. Some scientific reports suggest that TGs may be able to catalyze the hydrolysis of N^{ε}-(γ-L-glutamyl)-L-lysine cross-links (GGEL) isopeptide bonds in some soluble cross-linked proteins. Furthermore, it is likely that TGs can catalyze the

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Table 1: TG Enzymes and their Biological Functions when Known

TG	Physiological role	Gene map location	Reference
Factor XIIIa	Blood clotting	6p24-25	[59]
TG 1(Keratinocyte TG, kTG)	Skin differentiation	14q11.2	[60]
TG 2(Tissue TG, tTG, cTG)	Apoptosis, cell adhesion, signal transduction	20q11-12	[61]
TG 3 (Epidermal TG, eTG)	Hair follicle differentiation	20p11.2	[62]
TG 4 (Prostate TG, pTG)	Suppression of sperm immunogenicity	3q21-2	[63]
TG 5 (TG X)	Epidermal differentiation	15q15.2	[64]
TG 6 (TG Y)	Nervous System development	20p13	[64]
TG 7 (TG Z)	Unknown function	15q15.2	[64]

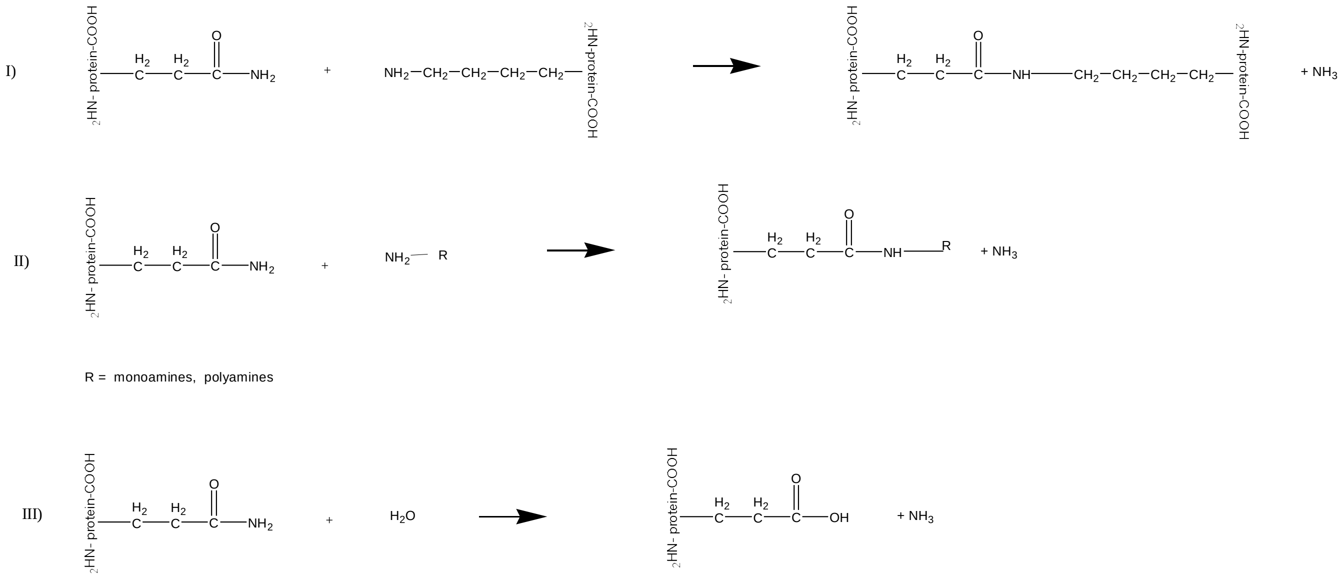

Figure 1: Transglutaminase-catalyzed reactions. Examples of TG-catalyzed reactions: I) acyl transfer between the γ-carboxamide group of a protein/polypeptide glutaminyl residue and the ε-amino group of a protein/polypeptide lysyl residue; II) attachment of a polyamine to the γ-carboxamide group of a glutaminyl residue; III) deamidation of the γ-carboxamide group of a protein/polypeptide glutaminyl residue.

exchange of polyamines onto proteins [2]. In some TGs other catalytic activities, such as the ability to hydrolyze GTP (or ATP) into GDP (or ADP) and inorganic phosphate, a protein disulfide isomerase activity, a serine/threonine kinase activity and an esterification activity, are often present [6-9]. In fact, experimental evidences indicate that some TGs are multifunctional proteins with distinct and regulated enzymatic activities. For example, under physiological conditions, the transamidation activity of TGs is latent [10], while other activities, recently identified, could be present. Morever, in some pathophysiological states, when the concentration of Ca^{2+} increases, the crosslinking activity of TGs may contribute to important biological processes. As previously described, one of the most intriguing properties of some TGs, such as TG2, is the ability to bind and hydrolyze GTP and furthermore, to bind to GTP and Ca^{2+}. GTP and Ca^{2+} regulate its enzymatic activities, including protein cross-linking, in a reciprocal manner: the binding of Ca^{2+} inhibits GTP-binding and GTP-binding inhibits the transglutaminase cross-linking activity of the TG2 [6]. Interestingly, TG2 shows no sequence homology with heterotrimeric or low-molecular-weight G-proteins, but there is evidence that TG2 (TG2/Ghα) is involved in signal transduction, and, therefore, TG2/Ghα should also be classified as a large molecular weight G-protein. Other studies, along with ours, showed that TG2/Ghα can mediate the activation of phospholipase C (PLC) by the α_{1b}-adrenergic receptor [11] and can modulate adenylyl cyclase activity [12]. TG2/Ghα can also mediate the activation of the δ1 isoform of PLC and of maxi-K

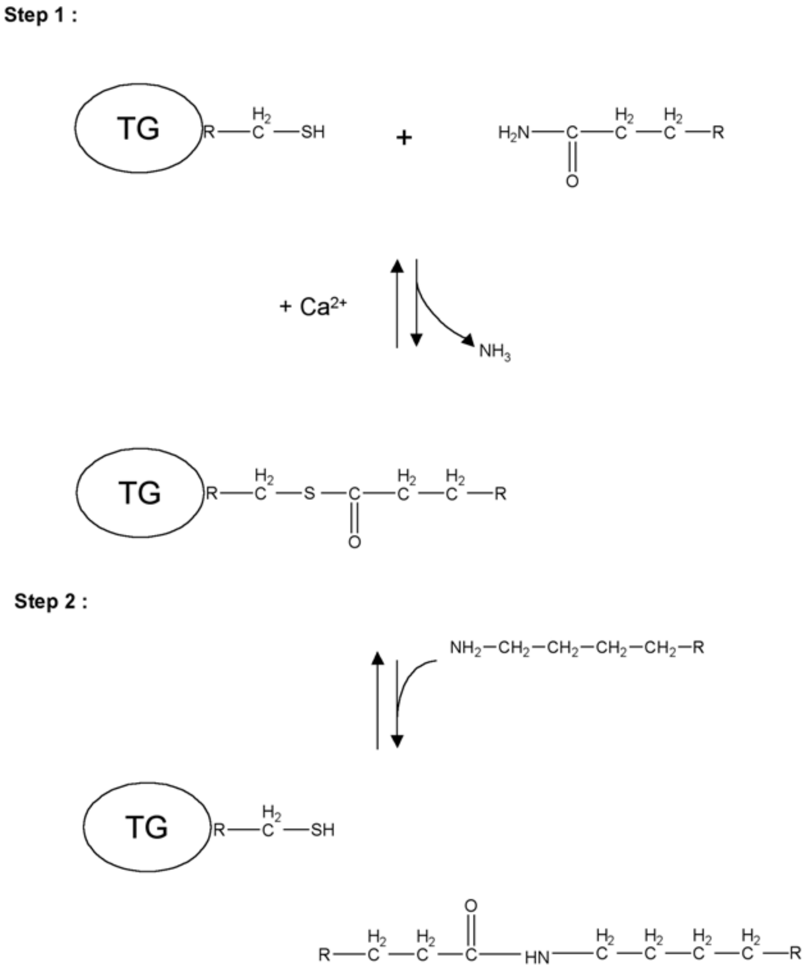

Figure 2: Schematic representation of a two step transglutaminase reaction. Step 1: In the presence of Ca^{2+}, the active-site cysteine residue reacts with the γ-carboxamide group of an incoming glutaminyl residue of a protein/peptide substrate to yield a thioacyl-enzyme intermediate and ammonia. Step 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 ε-amino group of a lysyl residue in a protein/polypeptide, a N^{ε}-(γ-L-glutamyl)-L-lysine (GGEL) isopeptide bond is formed.

channels [13]. Interestingly, the signaling function of TG2/Ghα is preserved even with the mutagenic inactivation of its crosslinking activity by the mutation of the active site cysteine residue [14]. Evidence of a pathophysiological role of the TGs in cell signaling and in disulfide isomerase activity is lacking to date.

MOLECULAR BIOLOGY

At least eight different TGs, distributed in the human body, have been identified (Table **1**). Complex mechanisms regulating the gene expression of TGs, both at transcriptional and translational levels, determine a complex but precise distribution of these enzymes in a cell and/or a tissue [15]. Such complex gene expression reflects the physiological roles that these enzymes play in both the intracellular and extracellular compartments. In the Nervous System, for example, several forms of TGs are simultaneously expressed [16-18]. Moreover, several alternative splice variants of TGs, mostly in the 3'-end region, have been identified. For example, Figure **3** shows some splice variants of TG2, up to now described in the literature. Interestingly, some of them are differently expressed in human pathologies, such as Alzheimer's disease (AD) [22] and cancer [23]. 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 [24]. This result may be explained by the multiple expressions of other TG genes that could be substituting the missing isoform.

Bioinformatic studies have shown that the primary structures of human TGs share some identities in only few regions, such as the active site and the calcium binding regions. However, high sequence conservation

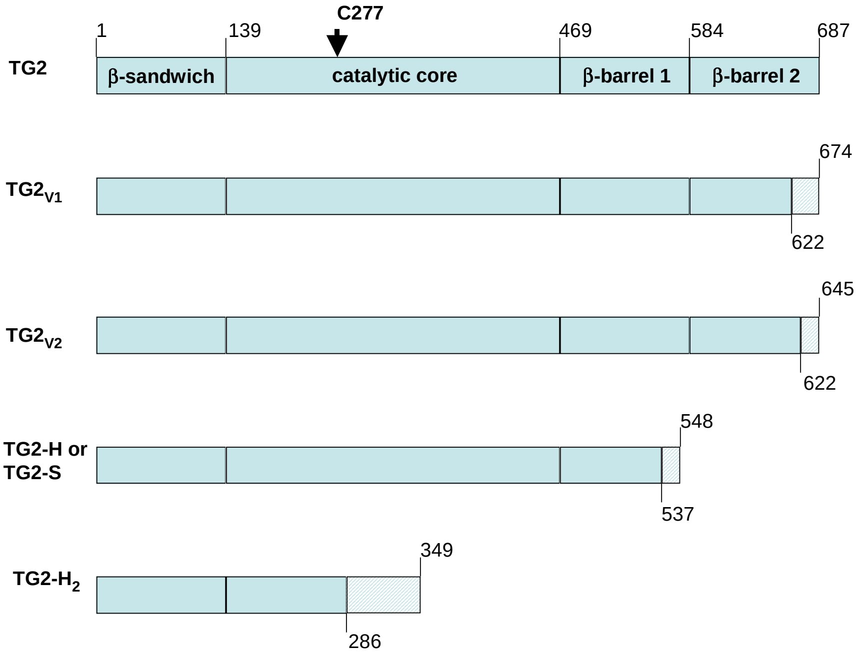

Figure 3: Schematic diagram of TG2 and its different isoforms. Gray boxes represent TG2 protein sequences, and shaded boxes represent alternate aminoacid sequences due to changes in reading frames (see Ref. 19-22).

and, therefore, a high degree of preservation of residue 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 [25].

TRANSGLUTAMINASES AND NEURODEGENERATIVE DISEASES

A large number of scientific reports suggests that TG activity is involved in the molecular mechanisms responsible for the pathogenesis of neurodegenerative diseases, but, to date, definitive experimental findings regarding the role of these enzymes in the development of these human diseases have not yet been obtained. Protein aggregates in affected brain regions are histopathological hallmarks of Alzheimer's disease and many other neurodegenerative diseases [26]. More than 20 years ago Selkoe *et al*. [27] 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 [28-31] and GGEL cross-links have been found in the neurofibrillary tangles and paired helical filaments of AD brains [32, 33]. In addition to these experimental findings, it has been shown that TGs and transglutaminase-catalyzed cross-links co-localize with pathological lesions in Alzheimer's disease brain [33-35]. Interestingly, a recent work showed the presence of bis γ-glutamyl putrescine in human CSF, which was increased in Huntington's disease (HD) CSF [36]. These are important experimental data which demonstrate that protein/peptides cross-links and protein/peptides cross-linking by polyamines do indeed occur in brain, and that these transglutaminase-catalyzed reaction products are increased in AD and HD brains. More recently, TG activity has been shown to induce amyloid β-protein oligomerization and aggregation at physiologic levels *in vitro* [37, 38]. By these molecular mechanisms, TGs could contribute to AD symptoms and progression [38]. Moreover, there is evidence that TGs also contribute to the formation of proteinaceous deposits in Parkinson's disease (PD) [39, 40] and in supranuclear palsy [41, 42]. To support the role of the TG activity in the pathogenesis of neurodegenerative diseases, expanded polyglutamine domains, present in HD and other neurodegenerative diseases caused by a CAG expansion in the affected gene (Table **2**) [43], have been reported to be substrates of TG2 *in vitro* [44-46]. Therefore, aberrant TG activity could contribute to the pathogenesis of neurodegenerative diseases, including Alzheimer's disease and other neurodegenerative diseases, by different molecular mechanisms, as described in Figure **4**. 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's progression. In support of the hypothesis of a pathophysiological role for protein aggregates in neurodegenerative diseases,

Table 2: List of Polyglutamine (CAG-Expansion) Diseases

Disease	Sites of Neuropathology	CAG triplet number		Gene product (Intracellular localization of protein deposits)	Reference
		Normal	Disease		
Corea Major or Huntington's Disease (HD)	Striatum (medium spiny neurons) and cortex in late stage	6-35	36-121	Huntingtin (n, c)	[65]
Spinocerebellar Ataxia Type 1 (SCA1)	Cerebellar cortex (Purkinje cells), dentate nucleus and brain stem	6-39	40-81	Ataxin-1 (n, c)	[66]
Spinocerebellar Ataxia Type 2 (SCA2)	Cerebellum, pontine nuclei, substantia nigra	15-29	35-64	Ataxin –2 (c)	[67]
Spinocerebellar Ataxia Type 3 (SCA3) or Machado-Joseph disease (MJD)	Substantia nigra, globus pallidus, pontine nucleus, cerebellar cortex	13-42	61-84	Ataxin-3 (c)	[68]
Spinocerebellar Ataxia Type 6 (SCA6)	Cerebellar and mild brainstem atrophy	4-18	21-30	Calcium channel Subunit (α1A) (m)	[69]
Spinocerebellar Ataxia Type 7 (SCA7)	Photoreceptor and bipolar cells, cerebellar cortex, brainstem	7-17	37-130	Ataxin-7 (n)	[70]
Spinocerebellar Ataxia Type 12 (SCA12)	Cortical, cerebellar atrophy	7-32	41-78	Brain specific regulatory subunit of protein phosphatase PP2A (?)	[71]
Spinocerebellar Ataxia Type 17 (SCA17)	Gliosis and neuronal loss in the Purkinje cell layer	29-42	46-63	TATA-binding protein (TBP) (n)	[72]
Spinobulbar Muscular Atrophy (SBMA) or Kennedy Disease	Motor neurons (anterior horn cells, bulbar neurons) and dorsal root ganglia	11-34	40-62	Androgen receptor (n, c)	[73]
Dentatorubral-pallidoluysian Atrophy (DRPLA)	Globus pallidus, dentato-rubral and subthalamic nucleus	7-35	49-88	Atrophin (n, c)	[74]

Cellular localization: c, cytosolic; m, transmembrane; n, nuclear.

it is worth noting that the aggregate formation has been shown to inhibit the proteasome degradation of expanded polyglutamine proteins [47].

TRANSGLUTAMINASES AS POTENTIAL THERAPEUTIC TARGETS IN NEURODEGENERATIVE DISEASES

In light of a lack of long-term effective treatments for human neurodegenerative diseases, 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 [48]. Inasmuch as TGs contain a crucial active-site cysteine, cystamine has the potential to inhibit these enzymes by disulfide interchange reactions. Recent studies have shown that cystamine decreases the number of protein inclusions in transfected cells expressing the atrophin protein containing a pathological-length polyglutamine domain, responsible for the Dentato-Rubro-Pallido-Luysian Atrophy (DRPLA) [49]. In other studies, cystamine administration to HD-transgenic mice resulted in an increase in life expectancy and amelioration of neurological symptoms [50, 51]. Neuronal inclusions were decreased in one of these studies [51]. Although all these scientific reports seem to support the hypothesis of a direct role of TG 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 [52], suggesting that this compound can have an additive effect in the therapy of

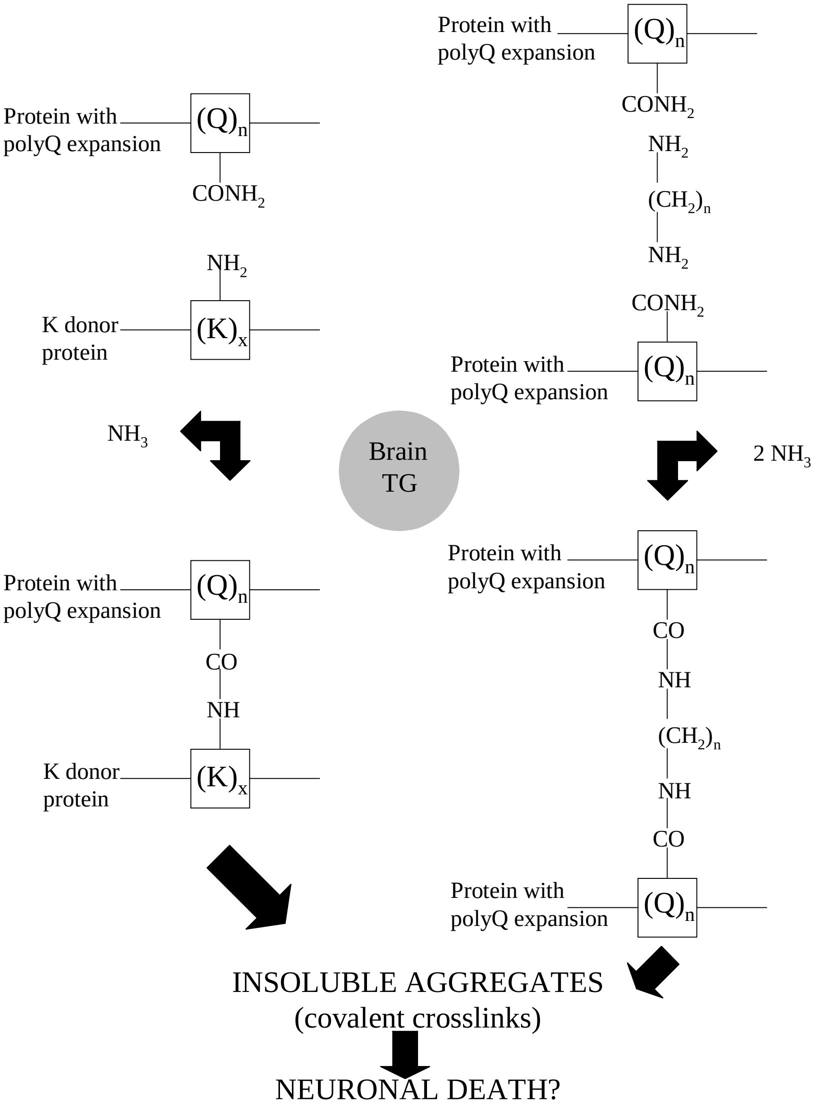

Figure 4: Possible mechanisms responsible for protein aggregate formation catalyzed by TGs.

HD. Currently, cystamine is already in phase II studies in humans, but several side effects, such as nausea, motor impairment and dosing schedule have been reported as reasons for non-adherence during these studies in humans [53, 54]. The pharmacodynamics and the pharmacokinetics of cystamine, therefore, should be carefully investigated in order to confirm the same effectiveness in patients with neurodegenerative diseases. 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 [17] and TG6 [55], and a strong non-selective inhibitor of TGs might also inhibit plasma Factor XIIIa, causing a bleeding disorder. Therefore, from a number of standpoints 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 [56], no conclusive data has been obtained by TG2 gene knock-out experiments about the involvement of this TG in the development of the symptoms in HD-transgenic mice [57]. However, 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 to suppress the aggregation and the toxicity of the mutant protein PABPN1 [58],

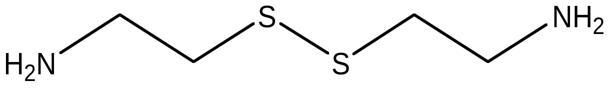

Figure 5: Chemical structure of cystamine.

suggesting this compound as a possible therapeutic for OMPD.

CONCLUSIONS

Although many scientific reports have implicated aberrant TG activity in neurodegenerative diseases, still today we are looking for data which could definitely confirm the direct involvement of TGs in the pathogenetic mechanisms responsible for these diseases. The use of inhibitors of TGs could be then useful for experimental approaches. To minimize the possible side effects, however, selective inhibitors of the TGs should be required in the future. Progress in this area of research may be achieved also through pharmaco-genetic techniques.

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