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Biochemical mechanisms for a possible involvement of the transglutaminase activity in the pathogenesis of the polyglutamine diseases: Minireview article

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Summary. Transglutaminases are a family of enzymes which show the common capacity to catalyse the cross-linking of protein substrates. Some members of this family of enzymes are also capable to catalyse other chemical reactions for the cell life. The distribution and the role of these enzymes have been studied in numerous cell types and tissues, but only recently their expression and functions started to be investigated in the Nervous System. One of the main biochemical properties of the Transglutaminase enzymes is to form large protein aggregates that are insoluble in all known protein detergents. Recently, the Transglutaminase activity has been hypothesised to be involved in the pathogenetic mechanisms responsible for the formation of cellular inclusions present in the Corea Major and in other polyglutamine diseases. In this review we describe the biochemical mechanisms by which the Transglutaminases could play a critical role in the physiopathology of the polyglutamine diseases.

Keywords: Transglutaminases – Polyglutamine diseases – Protein aggregates – Nervous system

Introduction

Post-translational modifications of proteins are fundamental steps in the maturation process of many proteins. Among these important processes of protein metabolism, Transglutaminase (TGase, E.C. 2.3.2.13) enzymes catalyse specific covalent post-translational modifications of proteins. In prokaryotic and eukaryotic cells several forms of this large family of enzymes have been identified and numerous genes have been isolated in mammalian cells: Factor XIII-a subunit, keratinocyte TGase (kTGase, type 1), tissue TGase (tTGase, type 2), epidermal TGase (eTGase, type 3), prostate TGase (pTGase, type 4), TGY and TGX from human keratinocytes, and band 4.2, a non catalytic TGase homologue present in the erythrocyte plasma membrane (Griffin et al., 2002).

TGases are widely distributed enzymes in the human body (Thomazy and Fesus, 1989). Gene expression regulatory mechanisms, not yet fully understood, determine a complex but precise distribution of each enzymatic isoform in a cell and/or a tissue (Thomazy and Fesus, 1989). Such complex gene expression pattern must be dependent upon the importance of the biological and physiological roles that these enzymes play in both the intra-cellular and extracellular compartments, where the enzymes and their catalytic products have been identified (Folk, 1983; Lorand and Conrad, 1984). The physiological roles of several TGase isoforms have been investigated and clarified in the last years. Critical cellular functions, such as cell proliferation and differentiation, extracellular matrix organisation, and physiological processes, such as blood coagulation and sperm immuno-suppression, have been strictly linked to the TGase activity (Griffin et al., 2002). Interestingly, a large series of studies showed that the activity of the tTGase (type 2) isoform is critical for a very diffuse human pathology, the coeliac disease (CD) (Dietrich et al., 1997). This disease is due to the intolerance to a food protein, the gliadin, and is characterised by a very complex clinical syndrome, including gastrointestinal pathological manifestations, often associated with dermatitis herpetiformis, dental enamel defects, IgA glomerulonephritis, liver diseases, connective tissue disorders, malignant diseases, neuropsychiatric complications (epilepsy, brain atrophy, brain calcifications, etc.), and other extra-intestinal manifestations (Corazza et al., 1997). Recent experimental works, carried out by the transfection of

the gene of tTGase (type 2) in several cell types, including neuroblastoma cell lines, showed that this enzyme is involved in important biological processes such as programmed cell death or apoptosis (Melino et al., 1994). In this present review, we will discuss the biochemistry and the molecular biology of TGases in relationship to the role played by these enzymes in the Nervous System, with particular reference to the biochemical mechanisms by which these enzymes could be responsible of the formation of protein aggregates, insoluble in detergents such as urea, guanidinium, formic acid and sodium dodecylsulfate, a typical hallmark of the polyglutamine (PolyQ) diseases.

Biochemistry and molecular biology of TGases in the nervous system

TGase activity is present in isolated synaptosomes (Pastuzko et al., 1986) and in astrocytes in culture (Riechelt and Poulsen, 1992). Rat brain contains at least two TGases type 2 that react with antibodies to guinea pig liver TGase (type 2) (Facchiano et al., 1993). One isoform is present in synaptosomal vesicles and the other in the cytosol. The cytosolic form is less stimulated by tetanus toxin than the vesicular form (Facchiano et al., 1993). Ohashi and co-workers (Ohashi et al., 1995) purified a TGase from rat brain that was only weakly immunoreactive to polyclonal and monoclonal antibodies to guinea pig liver TGase (type 2). Two TGase isoforms have been purified and characterised from rat brain (Kwak et al., 1999), with molecular weight of 45 and 29 kDa, respectively, confirming the presence in the rat brain of brain tissue-specific TGases. To date, at least three different TGases (type 1, 2 and 3) have been identified in the human brain (Kim et al., 1999) and the existence of alternatively spliced mRNA for TGase type 2 was recently shown in human brains affected by Alzheimer's disease (Citron et al., 2001). This is an extremely interesting finding which supports the possibility that the expression or the transcriptional or post-translational regulation of the TGase type 2 could be altered in the brain in some pathological conditions.

The physiological role of the brain TGase(s) is, to date, not yet fully understood, although some evidence suggests that the enzyme(s) may be involved in neurotransmitter release (Pastuzko et al., 1986; Facchiano et al., 1993). The relationships of the brain-type TGase(s) with the widely distributed liver type tTGase

Table 1. Polyglutamine (PolyQ) diseases to date identified

Disease	Sites of neuropathology	Q residue number		Gene product (intracellular localization)
		normal	*disease*	
Corea Major or Huntington's Disease (HD)	Striatum (medium spiny neurons and cortex in late stage)	6–35	36–121	Huntingtin (c)
Spinocerebellar ataxia type 1 (SCA1)	Cerebellar cortex (Purkinje cells), dentate nucleus and brain stem	6–39	40–81	Ataxin-1 (n, c)
Spinocerebellar ataxia type 2 (SCA2)	Cerebellum, pontine nuclei, substantia nigra	15–29	35–64	Ataxin-2 (c)
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)
Spinocerebellar ataxia type 6 (SCA6)*	Cerebellar and mild brainstem atrophy	4–18	21–30	Calcium channel subunit (α1A) (m)
Spinocerebellar ataxia type 7 (SCA7)	Photoreceptor and bipolar cells, cerebellar cortex, brainstem	7–17	37–130	Ataxin-7 (n)
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)
Dentatorubral-pallidoluysian atrophy (DRPLA)	Globus pallidus, dentato-rubral and subthalamic nucleus	7–35	49–88	Atrophin (c)

* SCA6 shows the lowest pathological range of CAG repeats, and comparison with other mutations in the same gene suggests that polyglutamines affects normal function. *c*, cytoplasmic; *m*, transmembrane; *n*, nuclear

Note added: Some familial forms of schizophrenia show a polyglutamine expansion in the potassium channel protein SCA12 shows CAG triplet expansion in a region at the 5′ UTR of gene PPP2R2B

and the properties of the brain-type tTGase(s) should be further investigated.

Physiopathology of TGases in the polyglutamine diseases affecting the nervous system

Green (1993) proposed that increased Q_n size, present in the gene products responsible of the PolyQ diseases (Table 1), may lead to aberrant or increased TGase activity. Peptides containing small Q_n domains (where $n < 18$) were found to provide excellent substrate for tTGase-catalysed attachment to [^{14}C]-labelled glycine ethyl ester (attacking nucleophile) (Kahlem et al., 1996). The $R_5Q_{18}R_5$ polypeptide was reported to form highly insoluble aggregates in the presence of tTGase and brain proteins, and brain homogenates were reported to catalyse incorporation of [^{14}C]-labelled glycine ethyl ester into a Q_{12}-containing peptide (Kahlem et al., 1996). The rate of incorporation of label into involucrin peptide, containing Q_n inserts was $Q_1 < Q_3 < Q_5 = Q_8 = Q_{12}$, but the larger peptides may have non covalently aggregated to some extent (Kahlem et al., 1996). The latter study (Kahlem et al., 1996) is important, because it showed that the brain possesses K-donor proteins capable of forming tTGase-catalysed cross-links to Q_n domains.

Q_n domains in a full-length protein(GST) are substrates of guinea pig liver tTGase (Cooper et al., 1997). Thus, tTGase-catalysed incorporation of [^{14}C]putrescine into various proteins was found to be in the following order: $GSTQ_0 <$ casein (a commonly used Q/K substrate of tTGase) $\ll GSTQ_{10} = GSTQ_{62}$ (Cooper et al., 1997). In a subsequent study, in order to establish a direct correlation between the length of the polyglutamine tracts and the Transglutaminase activity, we investigated whether "naked" Q_n peptides of 20 aa residues (corresponding to physiological sequences) and peptides of 40 or 60 aa residues (corresponding to pathological sequences) were good substrates of tTGase. tTGase-catalysed attachment of labelled specimen to various Q_n domains was found to be in the order: $Q_{20} < Q_{40} \ll Q_{60}$ (Gentile et al., 1998). In this study (Gentile et al., 1998), the activity per Q residue was significantly greater with Q_{60} than with Q_{20} or Q_{40}. In this last study (Gentile et al., 1998), the tTGase-catalysed attachment of polyamines to naked Q_n domains was demonstrated to yield high-molecular-weight polymers on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The latter finding suggested that once the polyamines have become attached to a Q_n domain, the other end of the polyamines becomes an excellent substrate for attachment to another Q_n domain. Polyamines are well represented in the brain, especially conjugated with structural elements (Seiler and Deckard, 1976). The findings that tTGase readily catalyses covalent attachment and the evidence of the cross-linking of the polyamines to Q_n domains may be important in the pathogenesis of $(CAG)_n/Q_n$-expansion diseases.

It has been showed that Q_n domains bind tightly to GAPDH *in vitro* (Burke et al., 1996) and that non covalent interaction of $GSTQ_n$ constructs with GAPDH does not inactivate the enzyme (Cooper et al., 1997), even though association occurs near the NAD^+-binding site (Koshy et al., 1996). However, time-dependent inactivation of GAPDH does occur when tTGase is also present. In the presence of tTGase, inactivation of GAPDH is more pronounced with $GSTQ_{62}$ (or $GSTQ_{81}$) than with $GSTQ_0$ (or $GSTQ_{10}$) (Cooper et al., 1997). This finding agrees with evidences that GAPDH is a K substrate of tTGase and that favourable positioning of a Q (from the

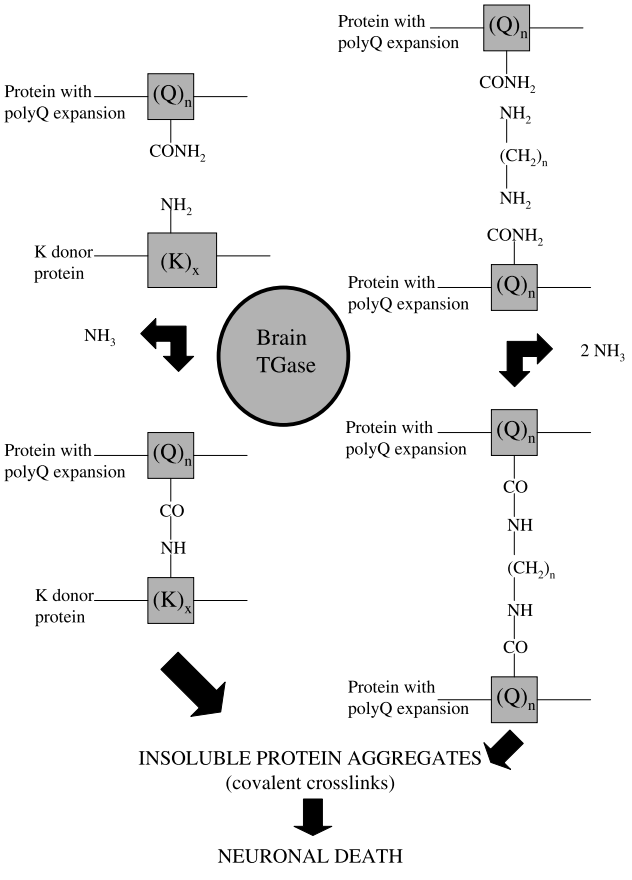

Fig. 1. Biochemical reactions catalysed by transglutaminase activity on polyglutamine proteins, suggested as pathogenetic mechanisms of the polyglutamine diseases

Q_n domain) and a K (from GAPDH) for cross-linking is enhanced with the longer Q_n domains (Cooper et al., 1997; Gentile et al., 1998). In our studies, we showed that the cross-linking of the GAPDH monomer was catalysed when this enzyme was incubated with catalytic amounts of tTGase and either dimethylcasein (Q substrate) or naked Q_n (Gentile et al., 1998). With GAPDH and Q_{60}, high-molecular-weight aggregates were detected, suggesting that more than one GAPDH monomer may be covalently bound to a single Q_n molecule or that a GAPDH monomer may have more than one tTGase-reactive K residue and thereby acts as a bridge between two or more Q domains (Gentile et al., 1998). We also showed that GAPDH in a homogenate of a fibroblast cell line over-expressing human tTGase was cross-linked in the presence of Q_{60} (Gentile et al., 1998). The studies of Cooper and co-workers (Cooper et al., 1997) and of Gentile and co-workers (Gentile et al., 1998) suggest that GAPDH monomer is a possible K substrate for tTGase-catalysed cross-linking reactions to Q_n domains. Figure 1 shows the crosslinking reactions that TGases catalyse on polyQ domains.

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