TRANSGLUTAMINASES AND ENDOCRINE SYSTEM

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Transglutaminases catalyze the posttranslation modification of proteins by catalyzing Ca^{2+} dependent acyl-transfer reaction resulting in the formation of new g-amide bonds between g-carboxamide groups of peptide-bound glutamine residues and various primary amines. Such glutamine residue serves as acyl-donor and the most common acyl-acceptors are e-amino groups of peptide–bound lysine residues or primary amino groups of some naturally occurring polyamines, like putrescine or spermidine. The active site of cysteine reacts first with the g-carboxamide group of glutamine residue to form the acyl-enzyme intermediate under the release of ammonia. In the second step, the complex reacts with a primary amine to form an isopeptide bond and liberate the reactivated enzyme.

The presence of transglutaminases has been observed in various endocrine glands such as human pituitary which was investigated by immunohistochemical methods using specific antibodies. A significant increase in the expression and activity of tissues transglutaminase was observed during involution of thymus. In the genital tract of the male rat two different forms of the enzyme transglutaminase could be identified and characterized. the presence of p53 and tissues transglutaminase gene expressions in human normal and pathologic adrenal tissues. The Ca^{2+}-responsive enzyme transglutaminase, which catalyzes the cross-bridging of proteins, was found in pancreatic islet cells.

1. Biochemical role of transglutaminases

Transglutaminases catalyze the posttranslation modification of proteins. These enzymes catalyze Ca^{2+} dependent acyl-transfer reaction yielding to the formation of new g-amide bonds between g-carboxamide groups of peptide-bound glutamine residues and various primary amines. A glutamine residue serves as acyl-donor and the most common acyl-acceptors are e-amino groups of peptide–bound lysine residues or primary amino groups of some naturally occurring polyamines, like putrescine or spermidine. The reaction is a multistep process, in which the active site cysteine reacts first with the g-carboxamide group of glutamine residue to form the acyl-enzyme intermediate under release of ammonia. In the second step, the complex reacts with a primary amine to form an isopeptide bond and liberate the reactivated enzyme (Folk and Finlaynson 1997; Lorand and Conrand 1980).

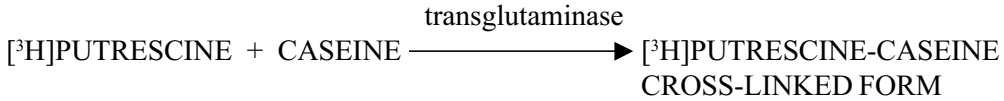

Fig. 1 Reaction scheme of transglutaminase-catalyzed transfer reaction [9]

The activity of transglutaminase was found in many tissues and body fluids of vertebrates. A group of enzyme with a similar function to vertebrate transglutaminases has been found in invertebrates, in plants, in unicellular eukaryotes and in bacteria. Table 1 presents a list of transglutaminases that occur in vertebrates and/or in invertebrates [FOLK 1980].

There are specific differences in molecular forms of transglutaminases. Several transglutaminases are zymogens that need proteolysis for activation to Ca^{2+}-binding, e.g. factor XIII (HORNYAK and SHAFER 1991; KIM et al. 1986). Those of them which have been examined so far display certain similarities in molecular features, e.g.. each contains an essential sulfhydryl group which presumably enables the formation of a thioester acyl-intermediate by an exchange reaction at the g-carboxamide group of a peptide-bound substrate glutamine residue. For the expression of enzymatic activity, transglutaminases require Ca^{2+} which is the physiological activator. Metal ion-induced conformation changes in the enzyme proteins occur and are essential for binding of glutamine substrates and for other steps in catalysis (CHUNG and FOLK 1972).

The physiological function of various transglutaminases is not well understood with the exception of the comparatively well characterized factor XIII (SHAINHOFF et al. 1991). Selected characteristics of transglutaminases are presented in Table1.

Tissue transglutaminase participates in multiple cellular processes e.g. apoptosis, cellular adhesiveness (GRIGORIEV et al. 2001), homeostasis, etc. Several *in vivo* and *in vitro* experimental models demonstrate direct relationship between the expression and activity of tissue transglutaminases and programmed cell death.. Tissue transglutaminases is accumulated in the cytoplasm of hepatocytes undergoing terminal differentiation both *in vivo* and *in vitro* (FESUS et al. 1991). In addition, both the inhibition of cell growth and apoptosis were found to be associated with increased transglutaminase activity in cells from human normal prostate and prostate cancer, and with increased transglutaminase protein and messenger ribonucleic acid levels only in the cells from human normal prostate (PASQUALI et al. 1999). This transglutaminase plays a fundamental role in embryonal development and metamorfosis. While the synthesis an activation of tissue transglutaminases participate in normal protective cellular response contributing to tissues homeostasis, the enzyme has been also implicated in a number of pathological conditions including the fibrosis, atherosclerosis (AESCHLIMANN and THOMAZY 2000), neurodegenerative diseases such Alzheimer‘s and Huntington‘s disease (LESORT et al. 2000), coeliac disease (MOLBERG et al. 2001), cancer metastases (AESCHLIMANN and THOMAZY 2000) and rheumatoid arthritis (WEINBERG et al. 1991).

Several factors have been found to influence the activity and expression of transglutaminases. Thus, increased transglutaminase activity was observed in human lung fibroblasts treated with trypsin (BIRCKBICHLER et al. 1977) and thrombin (CHUNG 1972). Similarly, an increase of the transglutaminase activity was observed in chick embryonic skin several hours after the addition of hydrocortisone (OBINATA and ENDO 1977). Transglutaminase activity was reduced in various cell types following viral and chemical transformations of the cells in tissues culture (BIRCKBICHLER et al. 1977) and in chemically induced rat hepatoma (BIRCKBICHLER et al. 1976). Several metal ions such as Fe^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+} etc. strongly inhibit transglutaminase activity in the presence of Ca^{2+} (FOLK et al. 1967; BOOTHE and FOLK 1969). Similar selected cation dependence has been described for other transglutaminases, e.g. factor XIII (LORAND and CONRAND 1980) and keratinocyte transglutaminase (CHANG and CHUNG 1986). Regulation of transglutaminase activity by guanosine nucleotides appears to be a characteristic feature of the tissue type of enzyme, whereas the activities of other transglutaminases such as factor XIII, keratinocyte transglutaminase, prostate transglutaminase and hemocyte transglutaminase remain unaffected (ACHYUTHANN and GREENBERG 1987).

The expression of tissue transglutaminase was found to be regulated by all-trans retinoic acid. The mediators of retinoic acid-induced transglutaminase expression were shown to occur via nuclear receptors for all-trans retinoic acid (RARs) as well as 9-cis retinoic acid receptors (RXRs) (AESHLIMANN and PAULSSON 1994). Co-regulation of RAR-b and RAR-g, but not RAR-a, with transglutaminase has been observed in many adult tissues, e.g. trachea, lung, liver and bladder (VERMA et al. 1992). The recent cloning of the promoter region of transglutaminase revealed presence of consensus motifs that function as retinoic acid responsive elements (SUTO et al. 1993).

2. Transglutaminases in endocrine system

The presence of transglutaminases has been observed in various endocrine glands. The distribution of transglutaminases in the human pituitary was investigated by immunohistological methods using specific antibodies. Tissues type transglutaminase was specifically localized in ACTH-producing cells, and the cells producing GH, PRL, TSH, FSH, and LH contained no appreciable amount of the enzyme. No detectable plasma-type transglutaminase (coagulation factor XIII) was found in pituitary tissues (KITAHARA et al. 1987). ROHN et al. (1992) measured transglutaminase activity in tissues samples of human brain tumors (pituitary adenomas, meningiomas and gliomas) obtained during neurosurgery. The highest enzyme activity was found in non-glial tumors, but due to a high variability of values, no significant changes were found between the various groups of brain tumors. Transglutaminase activity, although considered to play a role in neoplastic growth, does not represent a biochemical marker of malignancy in human brain tumors.

A significant increase in the expression and activity of tissues transglutaminase was observed during involution of thymus elicited by treatment with either anti-CD3 antibody or dexamethanose or by irradiation. The blood plasma concentration of e-(g-glutamyl)lysine isodipeptide, the end-product of the digestion of transglutaminase cross-linked proteins, was also elevated in each of those cases. Tissue transglutaminase was found to be localized in cells of the cortical layer of the thymus and immunofluorescence double staining revealed that the enzyme appeared in the apoptotic cells. Distinct signaling pathways, which induce apoptosis within the same cell type, can differentially regulate the expression of tissues transglutaminase, and enzyme may be involved in structural stabilization of apoptotic cells (SZONDY et al. 1997).

ADLEFF et al. (1998) investigated the presence of p53 and tissues transglutaminase gene expressions in human normal and pathological adrenal tissues. The results showed a significant positive correlation between both mRNA in all adrenal tissues except adrenocortical carcinomas. These observations may support potentially important roles for p53 and tissues transglutaminase in adrenal pathophysiology, especially in mechanisms which influence the evolution and/or progression of aldosterone-producing and Cushing's adenomas and, most probably, hyperplasias (ADLEFF et al. 1998).

In the genital tract of the male rat two different forms of the enzyme transglutaminase could be identified and characterized. The coagulating gland and the dorsal prostate secrete a glycosylated and acylated transglutaminase (65000 Da), and testicular transglutaminase (82000 Da), which represents a tissues-type transglutaminase (SIETZ et al. 1991). GRASSO et al. (1987) observed the inverse relationship between transglutaminase activity and [^{125}I]hFSH-receptor dissociation. They suggest that transglutaminase activity may be involved in the interaction of FSH with its receptor and that protein cross-linking (via peptide bond formation) may be a mechanism whereby some FSH-receptor complex are stabilized in the bovine testis (GRASSO et al. 1987).

The Ca^{2+}-responsive enzyme transglutaminase, which catalyzes the cross-bridging of proteins, is present in pancreatic islet cells (SENER et al. 1985). Its activity plays a critical role in the process of glucose-induced insulin release (GOMIS et al. 1983). In the pancreatic B cell, transglutaminase participates in the machinery controlling the access of secretory granules to the exocytotic sites (SENER et al. 1985). Pancreatic islet homogenates catalyze, in a Ca^{2+}-dependent fashion, the incorporation of [2,5-^{3}H]histamine, [1,4-^{14}C]putrescine, [1,2-^{3}H]agmatine, [^{14}C]methylamine and L-[U-^{14}C]lysine in N,N-dimethylcasein. The incorporation is inhibited by monodansylcadaverine, which is inhibitor of transglutaminase (GOMIS et al. 1983).

The distribution of transglutaminases was investigated by immunohistological methods (KITAHARA et al. 1987), immunofluorescence method (SZONDY et al. 1997), the expression of transglutaminases was demonstrated by reverse transcription-PCR technique, Western blot analysis (DE LAURENZI and MELINO 2001) and Northern blot analysis)SABER-LICHTENBERG et al. 2000), and the activity of the enzyme by incorporation of labeled diamines and polyamines into cellular protein, which serve as substrates for transglutaminase (FOLK 1980).

A noteworthy modification of the method commonly used for the estimation of transglutaminase activity in liver (PALLET et al. 19970 enabled us to set up that assay in human lymphocytes in which the protein concentration was reduced six times (DVORCAKOVA et al. 2001).

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ENZYME and Mol.wt	**PRIMARY MOL.** FEATURES	**OCCURRENCE** FUNCTION	**PHYSIOL.**
Plasma factor XIII *300-350 kDa*	Exists as zymogen, composed of two catalytic *a* subunits and two noncatalityc *b* subunits; activated proteolytically to $a'_2 b_2$	Blood plasma	Cross-link formation of fibrin
Liver (tissue) transglutaminase *75-80 kDa*	Monomeric	Widely distributed in many tissues and organs	Cross-link formation in programmed cell death
Keratinocyte transglutaminase *89-90 kDa*	Monomeric	Human and rat epidermal keratinocytes	Terminal differentiation of epithelia
Epidermal transglutaminase *77 kDa*	Monomeric	Epidermis	Cross-link formation in epidermal proteins
Prostate Transglutaminase *150 kDa*	Two forms; charge properties different from those of tissue enzyme and factor XIII	Anterior prostate (coagulating) gland of rodents	Vaginal plug formation through cross-linking of proteins in seminal vesicle secretion
Erythrocyte membrane protein band 4.2 *77 kDa*	Monomeric	Cytoskeletal network of the erythrocyte plasma membrane	Component of the cytoskeletal network
Hemocyte transglutaminase **and annulin** *86-87 kDa*	Monomeric	Hemocytes, hepatopancrease and ephitelial tissues of invertebrate	Similar to mammalian factor XIII

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