

Structural aspects of transglutaminase 2: functional, structural, and regulatory diversity

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Abstract Transglutaminase 2 (TG2) is a multi-functional protein that has both protein cross-linking and guanosine 5'-triphosphate (GTP) hydrolysis activities. The activities of this protein are controlled by many cellular factors, including calcium (Ca^{2+}) and GTP, and have been implicated in several physiological activities, including apoptosis, angiogenesis, wound healing, cellular differentiation, neuronal regeneration, and bone development. TG2 is linked to many human diseases such as inflammatory disease, celiac disease, neurodegenerative disease, diabetes, tissue fibrosis, and various cancers and is one of the most dynamic enzymes in terms of its functions, structures, and regulatory mechanisms. The aim of this review was to summarize the functional, structural, and regulatory diversity of TG2, with a particular focus on the structure of TG2.

Keywords Transglutaminase 2 · Apoptosis · Cross-linking · Structure · Inhibitor

Introduction

Transglutaminase 2 (TG2), also known as tissue transglutaminase, is a multi-functional protein containing both protein cross-linking and guanosine 5'-triphosphate (GTP) hydrolysis activities [1, 2]. This protein can also function as

a protein disulfide isomerase [3], protein kinase [4, 5], and as a scaffolding factor [6] (Fig. 1a). Protein cross-linking activity by transamidation, one of the main functions of TG2, is regulated positively by calcium (Ca^{2+}) and negatively by GTP (Fig. 1a) [7]. TG2 is ubiquitously found in the cell, while other TG isoenzymes activated at specific cell types (for example TG1 (skin), TG3 (skin), TG4 (prostate), TG5 (skin), TG6 (unclear), TG7 (testis and lung)) [2].

TG2 is localized both intracellularly and extracellularly, and in some cell types, such as neuroblastoma cells, the enzyme is also found in the nucleus [8]. In the cytosol, TG2 functions as a signal transfer molecule that transmits a receptor signal to an effector by binding GTP and hydrolyzing it to guanosine diphosphate (GDP) and inorganic phosphate (Pi) [9]. When secreted outside the cell, TG2 functions as a cross-linking enzyme in the matrix in a Ca^{2+} -dependent manner [10]. TG2 activity has been implicated in various physiological activities, including apoptosis [11–13], angiogenesis [14, 15], wound healing [15, 16], cellular differentiation [17, 18], neuronal regeneration [19], and bone development [20, 21]. This protein has also been linked to many human diseases such as inflammatory disease [22], celiac disease [23], neurodegenerative disease [24, 25], diabetes [26], tissue fibrosis [27], and cancers [28–30].

Several reports show that TG2 is downregulated in aggressive tumors and metastases [29, 31]. Transfection and expression of recombinant TG2 in several tumor cells led to significant reduction in tumor incidence and progression [14, 32]. On the other hands, elevated TG2 expression in many types of cancer cells, including pancreatic and breast cancer cells, was reported [33, 34]. Several mutations on TG2 that impair its activities have been found in the early stage of type 2 diabetes [26]. Overexpression of TG2 in the pathogenic stage of

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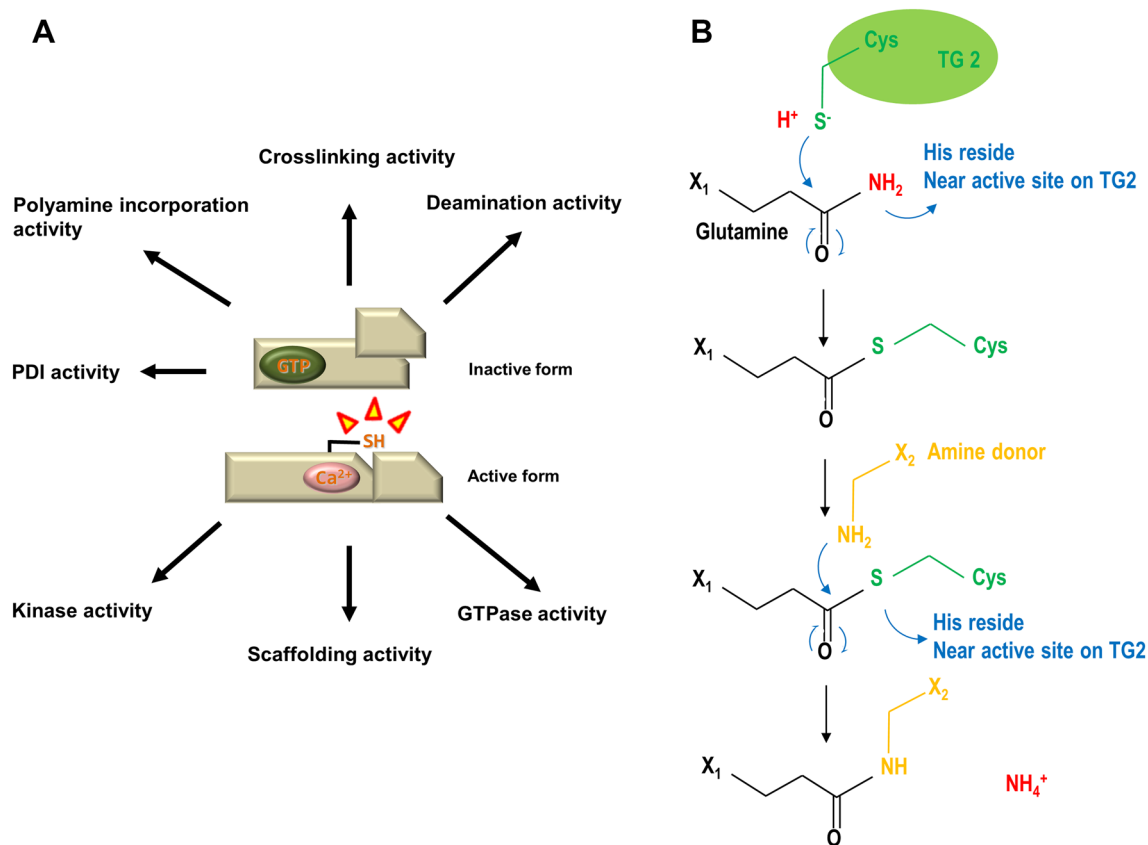


Fig. 1 Functional diversity of transglutaminase 2 (TG2). **a** TG2 is multifunctional protein, possessing transamidation activity (crosslinking, deamination, and polyamine incorporation), GTP hydrolysis activity, protein disulfide isomerase (PDI) activity, protein kinase activity, and scaffolding activity. The transamidation activity of TG2

is regulated positively by Ca^{2+} and negatively by GTP. **b** Transamidation reaction mechanism of TG2. Reaction is started by a nucleophilic attack by the thiol group at catalytically active cysteine on TG2. Second nucleophilic attack from the amine donor transforms the thioester intermediate to complete the reaction

neurodegenerative diseases, such as Alzheimer's disease (AD), causes cross-linking of several AD-related proteins, including tau, $\text{A}\beta$, and α -synuclein. Cross-linking of these proteins causes their accumulation, which has been linked to long-term chronic stimulation and neurodegeneration in AD [35–38]. TG2 is a biologically and therapeutically important target for the treatment of the aforementioned diseases. Several small molecules and peptidomimetics that can inhibit TG2 activities have been developed and used for treatment.

The aim of this review is to summarize the functional, structural, and regulatory diversity of TG2, with a particular focus on its structure. We will discuss various functions, dynamic conformational changes, and regulatory mechanisms that control the activities of multi-functional TG2. This review might provide a better understanding of TG2's functions and activity control mechanisms, and could lead to the identification of new inhibitor target sites to develop the next generation of TG2 inhibitors.

Functional diversity of TG2

Transamidation activity

Among the many activities of TG2, post-translational modification of target proteins by cross-linking, deamination, and amine incorporation, are the main functions of TG2. TG2 can deamidate the γ -carboxamide group of specific glutamine residues on a protein during the cross-linking process with an ϵ -amino group of a lysine residue. The cysteine thiol at the active site of TG2 is used as a nucleophile for the transamidation process (Fig. 1b). The thiol group attacks the carboxamide of a glutamine residue to form a thioester intermediate. This intermediate is then attacked by the ϵ -amino group of a lysine residue, producing a stable isopeptide bond that cross-links the two substrates (Fig. 1b). TG2 can also convert glutamine to glutamic acid via a deamination reaction, which removes the γ -carboxamide group of specific glutamine residues, replacing it with water. In vitro, this deamination activity

was shown by the deamination of gliadin A, which is a component of wheat and a main factor of celiac disease. The transamidation activity of TG2 is positively regulated by Ca^{2+} and negatively by GTP [7].

GTPase activity

Under low Ca^{2+} concentration conditions, TG2 maintains a closed form and acts as a GTPase, indicating that it functions as a G protein, participating in signaling processes [39]. The GTPase activity of TG2 is involved in α_1 -adrenergic receptor-mediated transmembrane signaling with phospholipase C δ in the liver and mediates the proliferation of hepatic cells [40].

Protein disulfide isomerase activity

The protein disulfide isomerase (PDI) activity of TG2 was initially reported in a study of the denaturation and renaturation of RNase A [3]. Reduced, denatured RNase A could be transformed into the native active form by TG2. The PDI activity of TG2 is not affected by the presence of Ca^{2+} and GTP. The cysteine residue in the active site is not important for the PDI activity of TG2, indicating that the domain responsible for the PDI activity is not related to the active site of the transaminase activity. More recently, another substrate for the PDI activity of TG2, adenine nucleotide translocator 1 (ANT1), was identified [41]. ANT1 is a bifunctional protein involved in ADP/ATP exchange and is one of the components of the mitochondrial membrane permeability transition pore [42, 43]. Mitochondrial pore formation by ANT1 is induced by many pro-apoptotic agents such as Ca^{2+} and atractyloside. Upon apoptosis stimulation, ANT1 becomes a substrate for TG2's cross-linking and PDI activities [41]. These activities of TG2 on ANT1 cause oligomerization of ANT1 on the mitochondrial membrane, leading to apoptosis [41]. Thus, the PDI activity of TG2 is implicated in mitochondrial-dependent apoptosis.

Kinase activity

The protein kinase activity of TG2 was initially reported in a functional study of the insulin-like growth factor-binding protein-3 (IGFBP-3) kinase in T47D breast cancer cells [44]. In that study, TG2 on the cell membrane was observed to phosphorylate IGFBP-3 and IGFBP-5 at multiple serine residues, and Ca^{2+} could inhibit this kinase activity [44]. Additional substrates for the kinase activity of TG2 have been identified. These include P53 [5]; histones H1, H2A, H2B, H3, and H4 [4]; and the retinoblastoma protein (Rb) [45]. Phosphorylation of P53 by TG2 decreases its binding with Mdm2, while phosphorylation of Rb by TG2 destabilizes complex formation between Rb and E2F1, indicating

that TG2 might act as a kinase to promote the function of certain tumor suppressor proteins. This hypothesis correlates with the reported positive function of TG2 in the suppression of certain cancers [29, 31, 32]. The most recent TG2 substrate identification study, using a candidate-coated chip, showed that the cytosolic proteins G6PD, CYCS, and CAPN1, the transmembrane protein CDH1, and the extracellular protein MMP-3, were substrates for TG2 kinase [46]. G6PD is involved in the pentose phosphate pathway and the protective mechanism against oxidative stress [46]. CYCS and CAPN1 are involved in TG2-mediated apoptosis [47]. TG2 on the plasma membrane functions as an extracellular stabilizing factor; therefore, identification of the transmembrane protein CDH1 and the extracellular protein MMP-3 as TG2 kinase substrates is not surprising. The involvement of these secreted proteins as TG2 kinase substrates suggests that TG2 kinase regulates the breakdown of the extracellular matrix and cancer metastasis.

Scaffold activity

The scaffolding function of TG2 was initially suggested by TG2's ability to form a ternary complex with fibronectin and collagen, which promotes cell adhesion to fibronectin via direct interaction with integrins [6]. This activity of secreted TG2 on the extracellular matrix (ECM) acts in a cross-linking activity-independent manner, although the cross-linking activity of TG2 is well characterized in the process of ECM turnover [48].

Structural diversity of transglutaminase 2

Human TG2 is the most ubiquitous isoform belonging to the TG family. Fulllength human TG2 comprises 687 amino acids and has four distinct domains: the N-terminal β -sandwich domain, the catalytic domain containing a Cys–His–Asp catalytic triad, and two C-terminal β -barrel domains (β -barrel1 and β -barrel2) (Fig. 2a) [49]. The N-terminal β -sandwich domain and catalytic domain are required for the enzymatic activity of TG2, while the C-terminal β -barrel domains are not [50]. Well-known interacting proteins for extracellular stabilization, such as fibronectin and integrin, bind to the N-terminal β -sandwich domain. A unique GTP-binding site is located in a cleft between the catalytic core and the first β -barrel.

To date, nine crystal structures of TG2 complexed with GDP [49], adenosine triphosphate (ATP) [51], GTP [52], two autoantibody Fab fragments [53, 54], and four covalently bound inhibitors (three are not published but deposited on PDB) [55], respectively, have been elucidated. Structural studies showed that TG2 undergoes an extraordinarily large conformational change upon activation

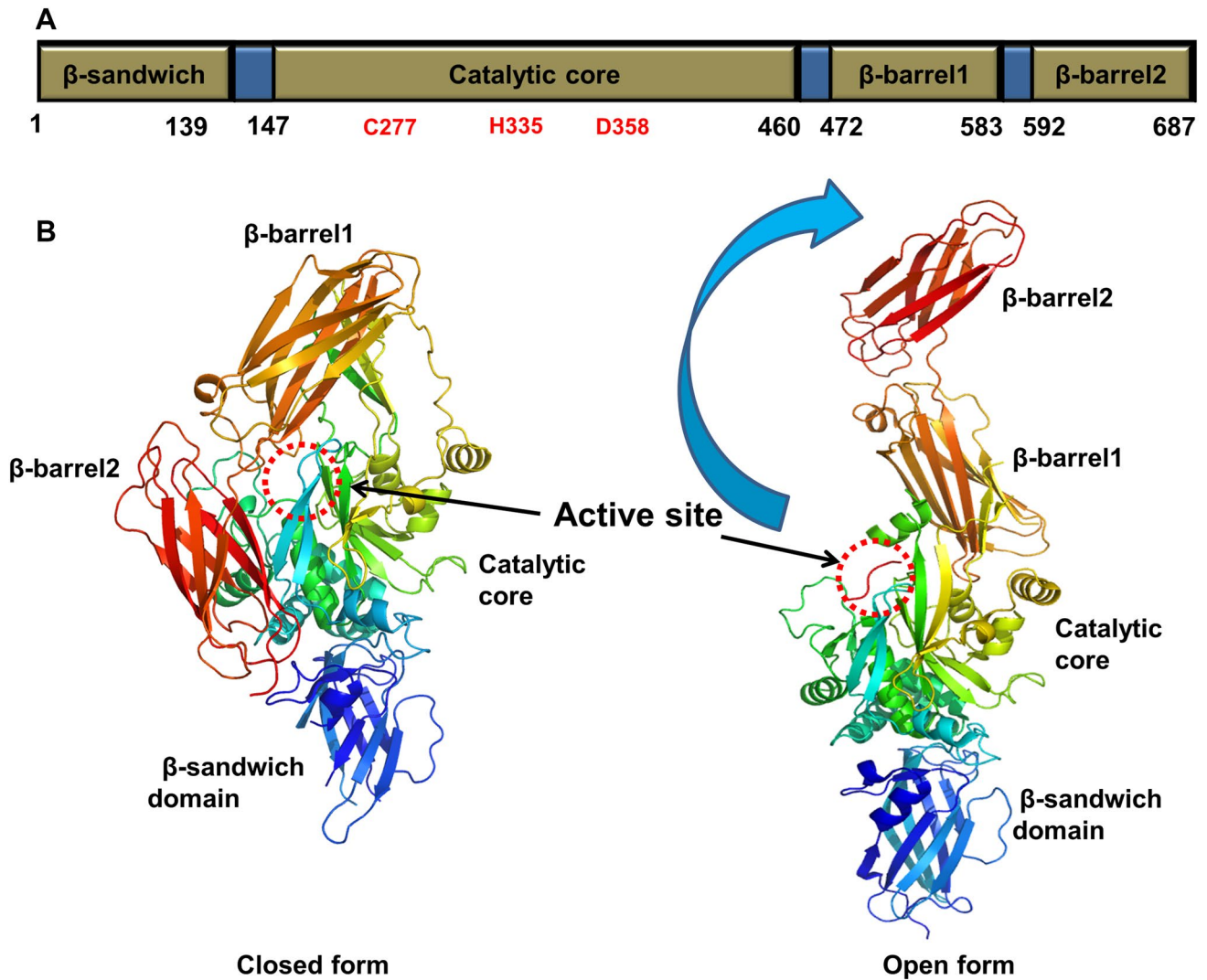


Fig. 2 Structural domain boundary and dynamic three-dimensional structures of transglutaminase 2 (TG2). **a** The four distinct domains are indicated by colored boxes with amino acid positions (below). The catalytic triad comprising C277, H335, and D358, is shown in red. **b** The ribbon structure of the compact, closed form (inac-

tive form) and the expanded, opened form (active form) are shown. A large conformational change upon irreversible inhibitor binding leads to exposure of the active site (marked by red-dotted circle). The chains from the N- to the C-termini are colored from blue to red. (Color figure online)

(Fig. 2b). GTP, GDP, and ATP inactivate TG2 by promoting a transition to the compact conformation (closed form), while Ca^{2+} and the irreversible inhibitor (Ac-P(DON)LPF-NH2 (gluten peptide mimetic)) activate TG2 by promoting an extended ellipsoid structure (open form) (Fig. 2b). The large conformational change detected for the structure of the covalently bound inhibitor/TG2 complex, from the compact to extended form, is a unique feature of TG2 (Fig. 2b). It is thought that intracellular TG2 is maintained in the closed conformation because of the high concentration of GTP. However, chemical and physical injury, and circumstances in which Ca^{2+} is increased, trigger the rapid transition of TG2 into the open conformation, which is the active form for transamidase activity [55]. In the

closed state, the catalytic triad, comprising C227, H335, and D358, is buried in TG2 (Fig. 3a). Once it is activated by the large conformational change, the extended β -barrel domains cause exposure of the catalytic nucleophile (C277) on the surface of TG2 (Fig. 3b, c). An active site study with a covalently attached inhibitor showed that the active site and substrate-binding pocket of TG2 are relatively flat at one side, although opposite side is well-defined, compared with those of other families of enzymes (Fig. 3d). This one side open-shaped active site might be necessary to bind various substrates for its multiple activities. This property of TG2 is one of the reasons why it has been difficult to find chemicals that inhibit TG2 activity specifically for pharmaceutical purposes.

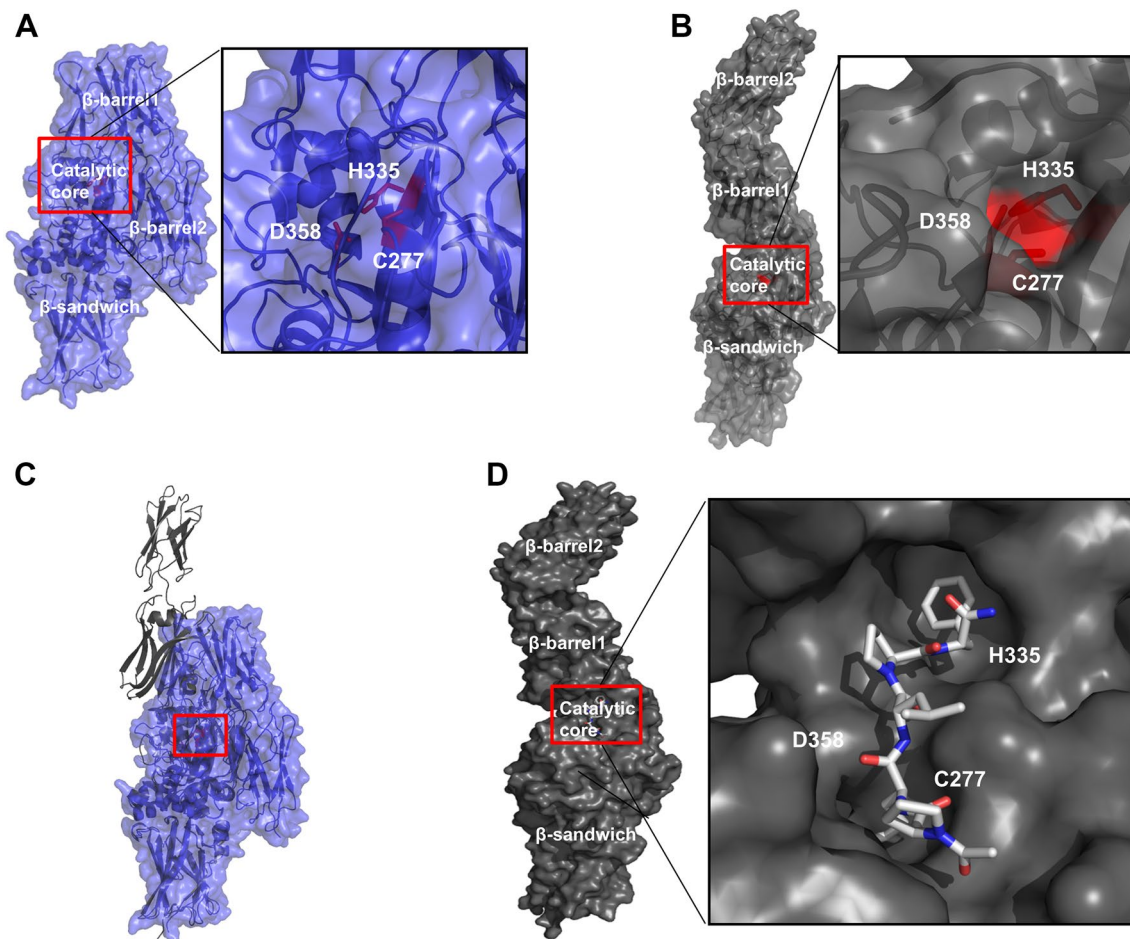


Fig. 3 Extended structure-mediated activation mechanism of transglutaminase 2 (TG2). **a** The closed, inactive form of TG2. The catalytic triad, comprising C277, H335, and D358, is buried in TG2. **b** The extended, active form of TG2. In the opened state, the catalytic nucleophile (C277) is exposed on the surface of TG2. **c** The location of the active site in the opened and closed forms of TG2. Red-rectangles indicate the active site. *Gray ribbon structure and blue surface*

structure indicated opened and closed form of TG2, respectively. **d** The binding pocket of a covalently attached inhibitor. The structural study of the complex of TG2 with the covalently attached inhibitor showed that the substrate-binding pocket is relatively flat at one side, although opposite side is well-defined, compared with other enzyme in this families. (Color figure online)

Structural comparison with other transglutaminases

In mammals, nine different TG isoenzymes have been identified at the genomic level and characterized. Among the characterized proteins, three crystal structures have been determined, including Factor XIII, TG3, and TG2 [49, 56, 57]. Factor XIII, which is activated by thrombin-dependent proteolysis, is involved in blood clotting via stabilization of fibrin clots [58]. TG3 (also known as a epidermal/hair follicle TG) and TG1 (also known as a Keratinocyte TG), which are also activated by proteolysis, are involved in the terminal differentiation of keratinocytes [10, 59, 60]. All members are of the papain-like superfamily of cysteine proteases and might be structurally similar.

Although the substrate specificity and regulation of their mechanisms of action vary significantly within this

enzyme family, all members share high sequence identity and structural similarity. The sequence identities between TG2 and Factor XIII and TG3 are 38% and 39%, respectively. The crystal structure of human TG2 showed that the monomer has four distinct domains that are located similarly to those of Factor XIII and TG3 (Fig. 4a–c). A structural superposition analysis showed that the structure of TG2 superposed well with those of Factor XIII and TG3, which had root mean square deviation (RMSD) values of 2.3 and 2.0 Å, respectively (Fig. 4d). The catalytic triad on TG2 (C277, H335, and D358) is conserved in Factor XIII and TG3 (Fig. 5). The structure of the region containing the catalytic triad is also highly conserved, although the regulation and substrate specificity are distinctly different within the TG family (Fig. 5).

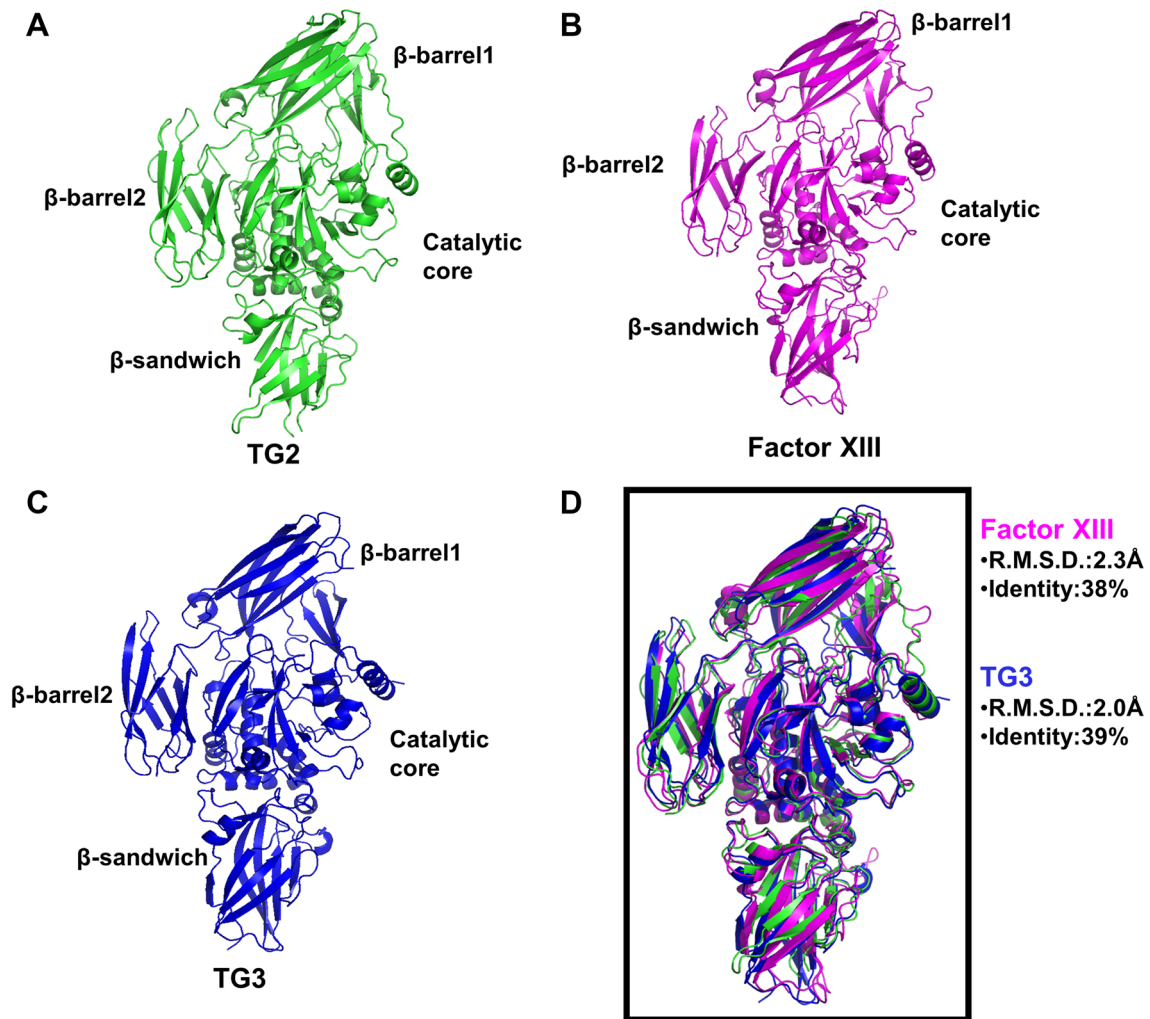


Fig. 4 Structural comparison of transglutaminase 2 (TG2) family members. **a** 2.8 Å crystal structure of TG2 in the GDP bound form is shown in *green* (1KV3) [49], **b** 2.65 Å crystal structure of Factor XIII shown in *magenta* (1GGT) [57], **c** 2.1 Å crystal structure of TG3 shown in *blue* (1L9M) [56], **d** Three different structures of TG were

superposed by using SuperPose Version 1.0 [61] (TG2 (*green*), Factor XIII (*magenta*), and TG3 (*Blue*)). Root mean square deviation (RMSD) and sequence identities based on the structure of TG2 are indicated. (Color figure online)

Structural analysis of GTP specificity for TG2

The unique guanidine nucleotide-binding site on TG2 is located in a cleft between the catalytic core and β -barrel1 (Fig. 6a). Although other GTP binding proteins, such as many small G proteins, use Mg^{2+} to coordinate GTP on the GTP binding proteins, TG2 interacts with GTP in the absence of Mg^{2+} [52]. Several positively charged amino acids (K173, R476, and R580) on TG2 are involved in the interaction with the negatively charged phosphate moieties of GTP [52]. In addition to the charged interaction and hydrogen bonds, a hydrophobic interaction formed by F174, M483, Y583, and V479 from TG2 is important to stabilize the guanine moiety on GTP [52]. The majority of the residues contacting GTP come from the end of the first

β -strand of the first β -barrel domain and the loop that connects it to the next β -strand. Only two residues, K173 and F174, from the catalytic core contribute to the interaction with GTP (Fig. 6b).

Although the TG family shares similar catalytic mechanisms, the activities of individual members are regulated differently. For example, GTP inhibits TG2, TG3, and TG4, but not TG1 and Factor XIII [62–65]. Sequence alignment showed that the amino acids involved in the GTP binding in TG2 are not conserved in Factor XIII (Fig. 6c). In the case of Factor XIII, K173 and F174 are replaced by asparagine and aspartic acid, which are not related at all. In addition, R476 and R478 are replaced by aspartic acid and glutamic acid, which are oppositely charged amino acids. This alignment clearly indicated that GTP cannot bind to or regulate

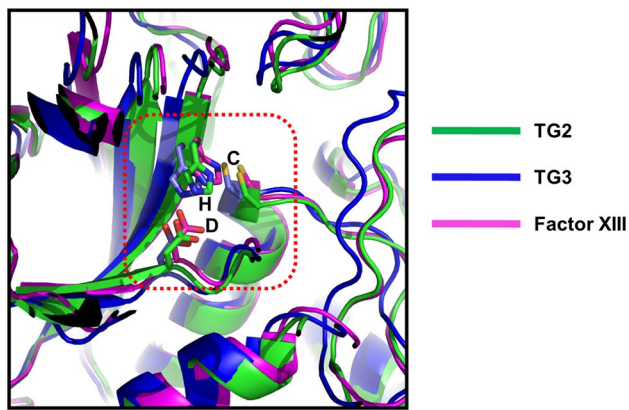


Fig. 5 Conserved catalytic triad among the transglutaminase 2 family. The catalytic triads of three proteins are superimposed by using SuperPose Version 1.0 [61]. Each triad is well conserved at the same structural position. Superimposed triads are boxed

Factor XIII. Further structural studies of other transglutaminase family members with various substrates should be conducted to understand how TG2 can possess multiple functions, can interact with various substrates, and can be regulated by many factors such as GTP, ATP, and Ca^{2+} .

Regulatory diversity of TG2

To prevent random cross-linking and deamidation activity against the many proteins that contain glutamine and lysine residues on their surface, the enzymatic activities of TG2 should be tightly regulated by cofactors, spatial localization, post-translational modification, and endogenous protein regulators. The main TG2 activity regulators are Ca^{2+} and GTP. Transamidase activities, including cross-linking, which are the main activities of TG2, are controlled positively by Ca^{2+} and regulated negatively by nucleotides such as GTP, GDP, and ATP [7]. Although the Ca^{2+} -mediated activation mechanism is not well characterized, it is proposed that Ca^{2+} and irreversible inhibitors activate TG2 by promoting transition to the extended, open conformational structure from the compact inactive form, which reveals the active site. By contrast, GTP, GDP, and ATP inactivate TG2 by maintaining the compact conformation. The binding sites of Ca^{2+} on TG2 are controversial, while the nucleotide-binding site is well-characterized. Upon GTP binding at the GTP-binding site of TG2 (located between catalytic core domain and the first β -barrel), the active site is blocked by two loops, and a hydrogen bond is formed between cysteine in the active site and a tyrosine residue, which blocks the extended conformational change.

Intracellular TG2 is mostly in the inactive state because of the relatively high concentration of GTP, GDP, and ATP,

and the low concentration of Ca^{2+} , inside the cell. Once the Ca^{2+} concentration is increased in the cell by releasing Ca^{2+} into the cytosol from intracellular compartments, or by bringing it in from outside the cell during extreme situations such as cellular injury and apoptosis, Ca^{2+} activates TG2 by promoting transition to the open conformational structure, which exposes the active site to the substrates. In the extracellular space, TG2 is expected to be in the active form because of the low concentration of GTP/GDP and the high levels of Ca^{2+} . However, it was observed that most TG2 in the extracellular matrix was in the inactive form [66]; however, the reason is unknown.

Besides Ca^{2+} and nucleotides, endogenous amine compounds, including cystamine, cystamine, spermidine, and histamine, inhibit TG2 activity by competing with various protein substrates [67, 68]. In this case enzymatically active TG2 can crosslink glutamine(s) in the substrate with amine compound inhibitors. Among the amine compound inhibitors, cystamine might inhibit TG2 activity via multiple mechanisms. It has been reported that cystamine, the reduced form of cystamine, can inhibit TG2 activity by competing with its natural substrates [69]. However, it has been suggested that cystamine might inhibit TG2 activity by forming disulfide bonds with the active site cysteine (C277) or other cysteines that are critical for the activity of TG2 [70, 71]. Recently, an allosteric activity regulation mechanism for TG2 activity by oxidation/reduction of the disulfide bond was proposed, in which extracellular TG2 is inactivated by the formation of a disulfide bond and activated by reducing the disulfide bond by thioredoxin [66, 72]; therefore, cystamine-mediated cysteine modification on TG2 might be a critical regulation point in the control of TG2 activity. In addition, ribosomal proteins RPL7a and RPL13 were identified as endogenous protein inhibitors of TG2 [73]. In that study, the authors showed that RPL7a and RPL13 inhibit the transamidase activity of TG2 specifically by a direct interaction with the β -barrel2 domain at the C-terminus. Indirect activity control mechanisms have also been proposed. Cytokine (interferon- γ)-mediated TG2 activity control was suggested by showing that extracellular TG2 in the small intestine could be activated by interferon- γ via a phosphatidylinositol-3-kinase-dependent pathway [74]. Reactive oxygen species-mediated Ca^{2+} level control was also proposed as a TG2 activation mechanism [75, 76].

Future perspectives

The multiple activities of TG2 are involved in various cellular activities, including apoptosis, differentiation, angiogenesis, and bone development, and are linked to many human diseases, including celiac disease, neurodegeneration,

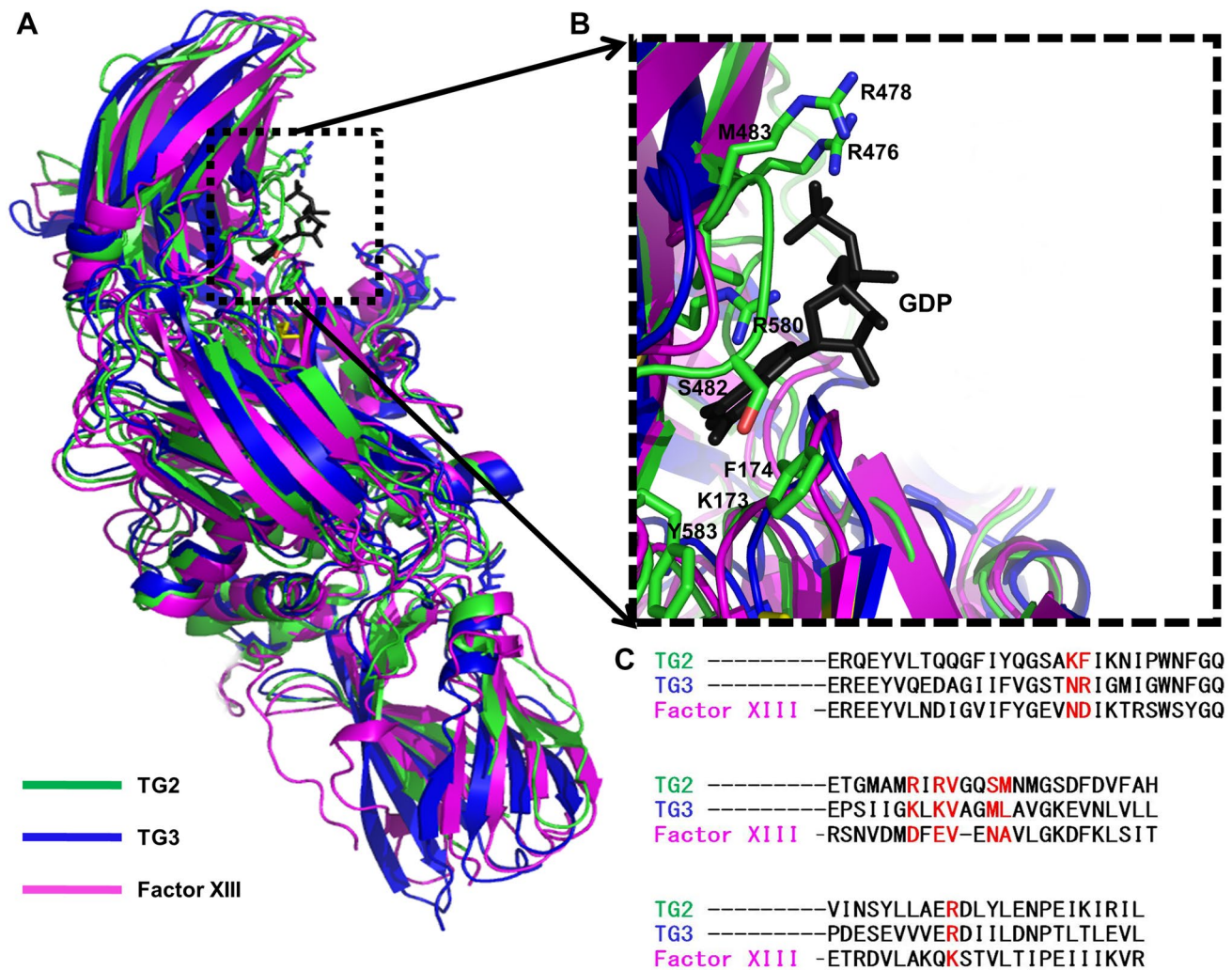


Fig. 6 Comparison of GTP-binding site. **a** Each GTP-binding site of transglutaminase 2 (TG2), TG3, and Factor XIII were identified and compared by structural superposition performed by SuperPose Version 1.0 [61]. **b** GTP-binding sites are shown in close-up the *black box*. GDP bound at GTP-binding site of TG2 is shown by *black sticks*. Amino acids from TG2 that are involved in the interaction with GDP

are shown and labeled. **c** Amino acid sequences of the GTP-binding regions from TG2, TG3, and Factor XIII are compared by alignment. Critical residues for the interaction with GTP on TG2 are shown in *red*, and those aligned residues on TG3 and Factor XIII are also shown in *red*. (Color figure online)

inflammatory diseases, diabetes, tissue fibrosis, and the formation of certain types of cancer. Although knockout mice for this important enzyme are viable and develop normally, follow-up analysis of the TG2-knockout mice indicated that deficient wound healing and phagocytosis, and diabetes appeared in the absence of TG2 [77–80]. The importance of TG2 in human pathogenesis and its potential as a target for therapeutic intervention in human diseases including celiac sprue, neurodegenerative diseases, and several types of cancer, have led to the development and application of its inhibitors [67, 81]. Although TG2 inhibitors that target the active site of TG2 have yielded promising results in a number of different disease models, the effect and application of those inhibitors are limited by their low affinity,

resulting from the absence of specific binding pockets at the active site of TG2 (flat-shaped active site). Therefore, high affinity inhibitors that can target either the active site or different positions on TG2 are desired.

In addition to the two distinct conformations of TG2, the open and closed states, it is possible that multiple conformations of TG2 exist [67, 82, 83]. Although no structural information for the TG2-Ca²⁺ complex is available, a putative Ca²⁺ binding site was proposed by comparing the structure of XIIIa [49] and TG3 [56]. Ca²⁺ binding at the specific site could weaken nucleotide binding and lead to subsequent conformational changes that expose the active site. The structure of TG3 also showed that the Ca²⁺-dependent conformational change would expose two

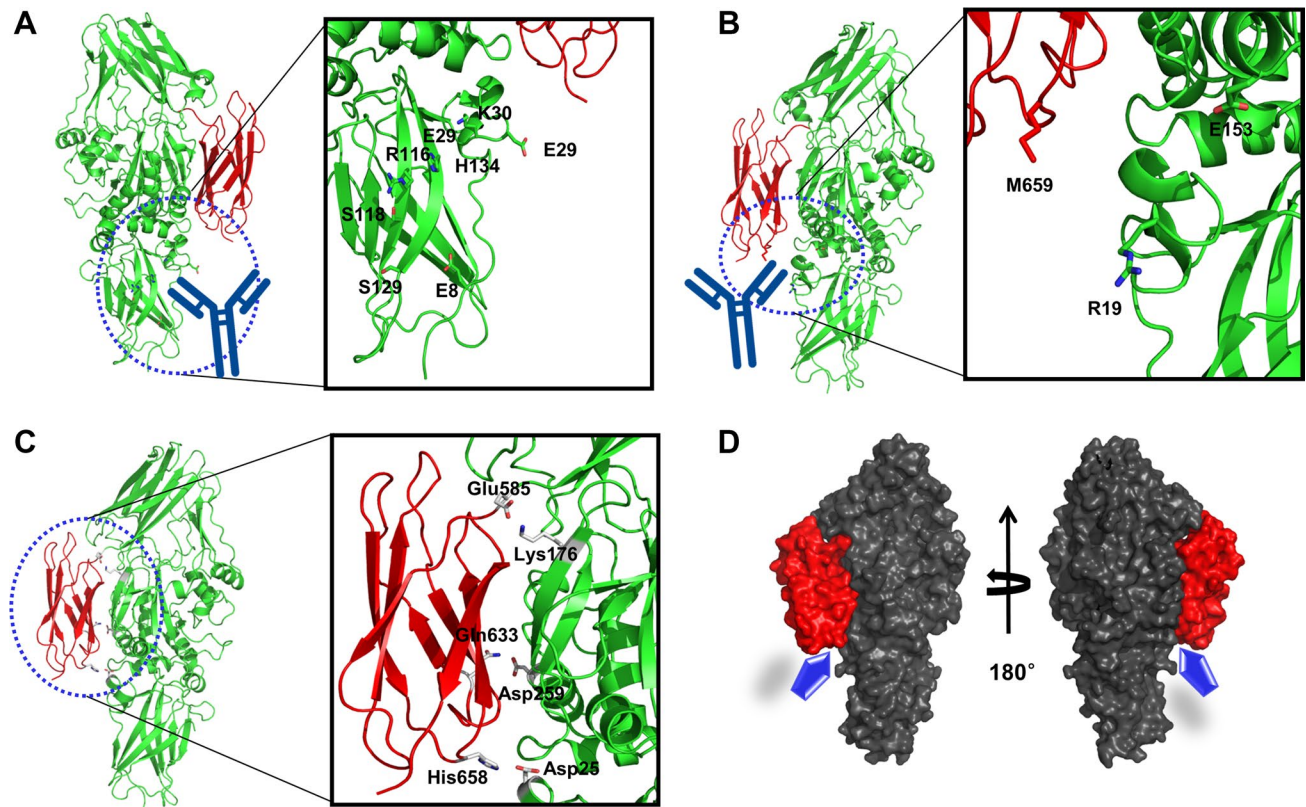


Fig. 7 Innovative inhibitory target site on transglutaminase 2 (TG2). Celiac autoantibody-binding site 1 (a) and site 2 (b). A black box indicates the amino acid residues on TG2 that are critical for the interaction with celiac autoantibodies. c Intermolecular binding interface formed between β -barrel2 domain and the core domain of TG2. A black box shows the amino acid residues on TG2 that are involved

in the interaction. The β -barrel2 domain of TG2 is shown in red. d Surface representation of TG2. Blue arrows indicate the innovative inhibitory target site that might block both autoantibody interaction and conformational changes of TG2. The β -barrel2 domain is shown in red. (Color figure online)

critical Trp residues that control access of substrates to the active site [56]. Therefore, it is possible that TG2 shares a similar structural change upon calcium binding. The structure of the TG2- Ca^{2+} complex should be determined to confirm the Ca^{2+} -dependent TG2 activation mechanism by fine structural changes of TG2. The extended, open conformation has not been detected for other members of the transglutaminase family. However, the active site and the structure are almost perfectly conserved; therefore, the mechanisms of activity control via large structural changes for the other transglutaminase family members must be investigated.

Besides the known TG2 activity regulators, including Ca^{2+} , GTP, and amine compounds, allosteric regulation by oxidation/reduction of disulfide bonds and post-translational modification by glutathionylation were proposed recently [84, 85]. Moreover, inhibition of TG2 activity by the ribosomal proteins RPL7a and RPL13 was also proposed [73]. The authors showed that RPL7a and RPL13 inhibit the transamidase activity of TG2 specifically via direct interaction with the β -barrel2 domain of TG2. How

this interaction inhibits the activity of TG2 should be determined in future studies.

Celiac disease, an autoimmune response to dietary gluten antigens, produces autoantibodies against TG2. Recent studies revealed that the N-terminal β -sandwich domain (R116 and H134) and the region between the β -sandwich and β -barrel2 domains (R19, E153, and M659) might be epitopes that bind the autoantibodies directly [53, 86] (Fig. 7a, b). Blocking autoantibody binding to the celiac epitopes of TG2 represents an ideal strategy for therapeutic intervention in celiac disease. Inhibiting the structural change from the closed to the open state could block the activity of TG2. To open up the β -barrel2 domain to gain active TG2, the β -barrel2 domain interacts loosely with the core domain (Fig. 7c). Thus, to design effective TG2 inhibitors, the surface pocket formed by the N-terminal β -sandwich and β -barrel2 domains might be a tentative target for small molecules that could inhibit both autoantibody binding for celiac disease and the structural changes (Fig. 7d).

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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