Regulation of Human Tissue Transglutaminase Function by Magnesium-Nucleotide Complexes

IDENTIFICATION OF DISTINCT BINDING SITES FOR Mg-GTP AND Mg-ATP

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Tissue transglutaminase (tTG) catalyzes a Ca\(^{2+}\)-dependent transglutaminase (TGase) activity that stabilizes tissues and a GTP hydrolysis activity that regulates cell receptor signaling. The purpose of this study was to examine the true substrates for nucleotide hydrolysis and the effects of these substrates on modulating the dual enzymatic activities of tTG. We found that Mg-GTP and Mg-ATP are the true substrates of the hydrolysis reaction. tTG hydrolyzed Mg-GTP and Mg-ATP at similar rates and interacted with Mg-ATP (K_\text{m} = 38 \pm 10 \mu M) at a 3-fold greater steady-state affinity than with Mg-GTP (K_\text{m} = 130 \pm 35 \mu M). In addition, Mg-ATP inhibited GTP hydrolysis (IC_\text{50} = 24 \mu M), whereas 1 mM Mg-GTP reduced ATP hydrolysis by only 20%. Furthermore, the TGase activity of tTG was inhibited by Mg-GTP, Mg-GDP, and Mg-GMP, with IC_\text{50} values of 9, 9, and 400 \mu M, respectively, whereas the Mg-adenine nucleotides were ineffective. Kinetic analysis of the hydrolysis reaction demonstrates the presence of separate binding sites for Mg-GTP and Mg-ATP. Finally, we found that Mg-GTP protected tTG from proteolytic degradation by trypsin, whereas Mg-ATP was ineffective. In conclusion, we report that Mg-GTP and Mg-ATP can bind to distinct sites and serve as substrates for nucleotide hydrolysis. Furthermore, binding of Mg-GTP causes a conformational change and the inhibition of TGase activity, whereas Mg-ATP is ineffective. The implication of these findings in regulating the intracellular and extracellular function of tTG is discussed.

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The abbreviations used are: tTG, tissue transglutaminase; TGase, transglutaminase; ATP\(_\gamma\)S, adenosine 5'-O-(thiotriphosphate); GTP\(_\gamma\)S, guanosine 5'-O-(thiotriphosphate); PAGE, polyacrylamide gel electrophoresis; GMP-PCP, adenosine 5'β,γ-methylene triphosphate.

EXPERIMENTAL PROCEDURES

Materials—Sodium salts of ATP\(_\gamma\)S, ATP, ADP, AMP, GTP\(_\gamma\)S, GTP, GDP, and GMP and the MgCl\(_2\) stock solution (1 M) were purchased from Sigma. [\(^{32}\)P]GTP and [\(^{32}\)P]ATP (30 Ci/mmol) were purchased from NEN Life Science.
Products. Monoclonal antibody against guinea pig liver transglutaminase (CUB 7401) was kindly provided by Dr. P. Birckbichler (20). All ATP, ADP, AMP, GTP, GDP, and GMP solutions were prepared in 50 mM Tris-Cl, pH 7.0, and stored in aliquots at −80 °C. Restriction enzymes, T4 DNA ligase, LB medium, and yeast extract were obtained from New England Biolabs, Inc. All other reagents used in this investigation were purchased from Sigma unless stated otherwise.

Assembly of Human tTG cDNA and Expression and Purification of Recombinant tTG—The assembly of full-length human tTG cDNA and the purification of glutathione S-transferase-tTG fusion protein were as described (15). The purified glutathione S-transferase-tTG fusion protein was cleaved with factor Xa (11, www. Hematologic Technologies, Inc., Essex Junction, VT) overnight at 4 °C and re-applied to gluthathione one resin to remove the glutathione S-transferase protein. The cleaved tTG migrated as a single band on a Coomassie Blue-stained gel with identical electrophoretic mobility to the tTG in human EAhy926 cells (21). Protein concentrations were quantitated using the Bradford method (22) (Bio-Rad). Preliminary studies demonstrated that recombinant tTG preferentially cross-linked the α-chains of fibrinogen to form high molecular weight α-chain polymers and bound to a fibronectin-coated microrotator plate in a concentration-dependent manner. Based on the SDS-PAGE profile, the fibrin cross-linking pattern, fibronectin binding, TGase activity, and GTP hydrolysis (GTPase) activities, the affinity-purified recombinant human tTG demonstrated properties similar to those of tTG purified from other sources.

Preparation of Mg-Nucleotide Complexes—The concentrations of Mg-nucleotide complexes were prepared according to the procedures described by Morrison (23) and O'Sullivan and Smithers (24). Briefly, the maintenance of a 1–2 mM excess of free Mg2+ over the total nucleotide (GTP or ATP) concentration ensures that the proportion of ATP (or GTP) present as Mg-ATP2− (or Mg-GTP2−) is maximized and remains constant over a large range of ATP (or GTP) concentrations (23, 24). Calculation of the actual concentration of the various free Mg2+ ions, free GTP or ATP, or Mg-GTP or Mg-ATP complexes was made using the computer program developed by Bers et al. (25). Unless otherwise specified, all reactions containing Mg-nucleotide complexes had 1 mM Mg2+ in excess over the total nucleotide concentration to maximize the formation of Mg-nucleotide complexes (23–25).

Transglutaminase Assay—TGase activity was determined by quantitating the incorporation of [3H]putrescine (26) or 5-biotinamidopentylamine into N,N'-dimethylcasein as described previously (27). For inhibition of TGase activity by different Mg-nucleotide complexes, tTG (0.1 μg/ml) was incubated with different concentrations of Mg-GTP, Mg-GDP, Mg-GMP, Mg-ATP, Mg-ADP, or Mg-AMP in the presence of 1 mM GTP or ATP and 2 mM excess Mg2+. TGase activity was measured in triplicate after a 40-min incubation at 37 °C using the 5-biotinamidopentylamine incorporation assay.

\[ γ^3P \text{GTP} \text{ or } γ^3P \text{ATP Hydrolysis Assay—} \text{The assay was performed according to the procedure described (15), with some modifications. For determination of Mg-GTP (or Mg-ATP) as a substrate for GTP (or ATP) hydrolysis, the reaction mixture (50 μl) contained 60 mM Tris-Cl, pH 7.6, 1 mM dithiothreitol, 2 μCi of \( γ^3P \text{GTP} \text{ or } γ^3P \text{ATP} \) (30 Ci/mmol), 250 μM unlabeled GTP (or ATP), and 0–3.2 mM Mg2+. For GTPase competitive inhibition studies (see Fig. 2A), the reaction mixture was as described above, except that 2 mM Mg2+, 250 μM labeled and unlabeled GTP, and 0–100 μM Mg-ATP, Mg-ADP, or Mg-AMP were used. For ATPase competitive inhibition studies (see Fig. 2B), the reaction mixture contained 40 μM labeled and unlabeled ATP and 0–1000 μM Mg-GTP, Mg-GDP, or Mg-GMP. The reactions were initiated by the addition of tTG and allowed to proceed at 37 °C for 30 min. The reaction was terminated by the addition of 750 μl of 50 mM ice-cold monobasic sodium phosphate containing 5% activated charcoal. After centrifugation for 2 min at 12,000 rpm in Sorvall microcentrifuge (Mircospin 24S), 400 μl of the supernatant was used for determination of Pi release by scintillation counting. All the rates of GTP or ATP hydrolysis were as described under “Experimental Procedures.” A, the percentage of GTP hydrolyzed (○) was plotted together with the concentration of free Mg2+ in the reaction. The concentrations of the Mg-GTP complex and free Mg2+ were calculated as described under “Experimental Procedures.” The activity of GTP hydrolysis at 1 mM Mg2+ was used as 100%.

\[ \text{Total Mg GTP (μM)} \]

\[ \text{Total Mg Concentration (μM)} \]

FIG. 1. Mg-GTP is the substrate for GTP hydrolysis. GTP hydrolysis was performed in a reaction containing 250 μM labeled and unlabeled GTP and 0–3.2 mM Mg2+. Total Pi release was measured as described under “Experimental Procedures.” A, the percentage of GTP hydrolyzed (○) was plotted together with the concentration of free Mg2+ in the reaction. The concentrations of the Mg-GTP complex and free Mg2+ were calculated as described under “Experimental Procedures.” The activity of GTP hydrolysis at 1 mM Mg2+ was used as 100%.

RESULTS

Identification of the Mg-Nucleotide Complex as the Substrate for Hydrolysis—It is not known if the requirement for Mg2+ in the tTG-mediated GTPase/ATPase reaction reflects the need for a magnesium-complexed enzyme or a magnesium-GTP/ATP substrate. To address this question, the effect of increasing concentrations of magnesium ions on the rate of GTP hydrolysis was determined in the presence of 250 μM GTP (Fig. 1). If the role of Mg2+ is to provide the enzyme with a Mg-GTP substrate, then the ability of Mg2+ to stimulate GTP hydrolysis should be directly proportional to the formation of a Mg-GTP complex as shown (Fig. 1A). GTPase activity increased and plateaued at 600 μM Mg2+. The increase in GTP hydrolysis activity was proportional to the formation of a Mg-GTP complex (Fig. 1A), suggesting that Mg-GTP was the substrate in this hydrolysis reaction. At 250 μM Mg2+, which correlates with the appearance of free Mg2+ in solution (Fig. 1B), GTP hydrolysis activity was only 71% of the maximum activity. Thus, free
Mg$^{2+}$ has an additional activator effect on GTPase activity (Fig. 1B). Similar experiments performed with ATP demonstrated that Mg-ATP was also a substrate for hydrolysis.

In the reaction containing predominately Mg-GTP (or Mg-ATP; see “Preparation of Mg-Nucleotide Complexes”), the apparent $K_m$ for Mg-GTP was 130 ± 35 μM, with a $k_m$ of 0.06 ± 0.01 min$^{-1}$. The apparent $K_m$ for Mg-ATP was 38 ± 10 μM, with a $k_m$ of 0.08 ± 0.02 min$^{-1}$.

When Ca$^{2+}$ (at 0–4 mM) was substituted for Mg$^{2+}$, GTP or ATP hydrolysis activity was 10% of that obtained in the presence of the optimum Mg$^{2+}$ concentration. When the Ca$^{2+}$ concentration was increased to 8–16 mM, GTPase or ATPase activity was only 20–40% of that obtained in the presence of the optimum Mg$^{2+}$ concentration. The addition of 0–16 mM Ca$^{2+}$ to a GTPase or ATPase reaction containing the optimum Mg$^{2+}$ concentration did not significantly affect hydrolysis activity.

Separate Nucleotide-binding Sites for Mg-GTP and Mg-ATP Hydrolysis—Since tTG can hydrolyze ATP as well as GTP, we investigated which is the preferred substrate. Fig. 2A illustrates the effect of increasing concentrations of Mg-ATP, Mg-ADP, and Mg-AMP on GTP hydrolysis. In the presence of 250 μM GTP, Mg-ATP was able to inhibit GTPase activity (IC$_{50}$ = 24 μM). Substitution of Mg-ATP with Mg-ADP resulted in a more potent inhibition of GTPase activity (IC$_{50}$ = 5 μM) (Fig. 2A). Preincubation of tTG with Mg-ADP on ice for up to 2 h did not further increase this inhibitory effect. Fig. 2B examines the effect of increasing concentrations of Mg-GTP, Mg-GDP, and Mg-GMP on ATP hydrolysis. In the presence of 40 μM Mg-ATP, up to 1 mM Mg-GTP or Mg-GMP inhibited only 20% of ATPase activity, whereas 1 mM Mg-GDP inhibited 50% of ATPase activity (Fig. 2B). Preincubation of tTG with Mg-GTP or Mg-GDP on ice for up to 1 h did not enhance the inhibition.

Fig. 2C illustrates the Lineweaver-Burk plot of GTP hydrolysis in the presence or absence of Mg-ATP to examine the mechanism of inhibition. The kinetic patterns gave lines intersecting the abscissa. Therefore, Mg-ATP was acting as a noncompetitive inhibitor of GTP hydrolysis, with a $K_i$ of 22 ± 4 μM (Fig. 2C). We also found that Mg-ADP acted as a noncompetitive inhibitor of GTP hydrolysis. In addition, Mg-GDP and Mg-ADP acted as competitive inhibitors in GTPase and ATPase reactions, respectively, further supporting the presence of separate binding sites for Mg-GTP and Mg-ATP.

We also performed a TGase reaction to confirm that there were separate binding sites for Mg-GTP and Mg-ATP. If there were separate binding sites for Mg-GTP and Mg-ATP, preincubation of tTG with Mg-ATP or Mg-ADP should not affect the ability of Mg-GTP to inhibit TGase activity. Indeed, we found that preincubation of tTG with Mg-ATP or Mg-ADP did not change the IC$_{50}$ values for Mg-GTP.

Effect of the Mg-Nucleotide Complex on the Modulation of the TGase Activity of tTG—Previous studies using nucleotides as inhibitors of TGase activity did not consider the role of Mg$^{2+}$ in this process (29). Mg$^{2+}$ exists mainly as a Mg-nucleotide complex inside cells (30) and is required for nucleotide hydrolysis by tTG. We therefore examined the effect of Mg-nucleotide complexes on the modulation of the TGase activity of tTG. In a transglutaminase reaction containing 1 mM Ca$^{2+}$, we found that Mg-GTPS, Mg-GTP, and Mg-GDP were equipotent inhibitors, with IC$_{50}$ values of 9.9 μM (Fig. 3A), and that Mg-GMP was less inhibitory, with an IC$_{50}$ of 400 μM. Ca-GTPS, Ca-GTP, Ca-GDP, and Ca-GMP were found to inhibit TGase activity, with IC$_{50}$ values of 4, 4, 4, and 100 μM, respectively.

To determine whether hydrolysis of GTP was required for the inhibition of TGase activity, we preincubated tTG with GTP in the presence of 1 mM excess Mg$^{2+}$ or Ca$^{2+}$ at 37 °C for 30 min. We found that preincubation did not increase the potency of inhibition, demonstrating that hydrolysis of GTP was not required for inhibition. Furthermore, we found that GTP, GTPγS, and GMP-PCP inhibited TGase activity with the
incorporation assay, the substrate (N\textsubscript{-}dimethylcasein) was incubated with 0.1 µg of trypsin plus divalent cations (Ca\textsuperscript{2+} or Mg\textsuperscript{2+}), free GTP or ATP, or Mg-nucleotide or Ca-nucleotide complexes at 37°C for 1 h. Samples were separated by SDS-PAGE and analyzed by immunoblotting using a monoclonal antibody against tTG (CUB 7401). Lane 1, tTG plus 5 mM Ca\textsuperscript{2+}; lane 2, tTG plus 5 mM Mg\textsuperscript{2+}; lane 3, tTG plus 6 µM Mg-GTP; lane 4, tTG plus 5 mM Ca\textsuperscript{2+} and 6 µM GTP; lane 5, tTG plus 5 mM Ca\textsuperscript{2+} and 6 µM ATP; lane 6, tTG plus 6 µM Mg-ATP.

**FIG. 4. Immunoblot of the trypsin digestion pattern in the presence of Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, GTP, ATP, Ca-GTP, or Ca-ATP.** Purified recombinant tTG (1 µg) was incubated with 0.1 µg of trypsin plus divalent cations (Ca\textsuperscript{2+} or Mg\textsuperscript{2+}), free GTP or ATP, or Mg-nucleotide or Ca-nucleotide complexes at 37°C for 1 h. Samples were separated by SDS-PAGE and analyzed by immunoblotting using a monoclonal antibody against tTG (CUB 7401). Lane 1, tTG plus 5 mM Ca\textsuperscript{2+}; lane 2, tTG plus 5 mM Mg\textsuperscript{2+}; lane 3, tTG plus 6 µM Mg-GTP; lane 4, tTG plus 5 mM Ca\textsuperscript{2+} and 6 µM GTP; lane 5, tTG plus 5 mM Ca\textsuperscript{2+} and 6 µM ATP; lane 6, tTG plus 6 µM Mg-ATP.

suggesting that the inhibition by ATP was a chelation effect (data not shown).

**Analysis of tTG Conformational Changes by Trypsin Proteolysis**—We evaluated the possibility that different conformations were induced by divalent cations, free GTP, free ATP, Mg-GTP, Mg-GDP, Mg-ATP, and Mg-ADP. Trypsin digestion patterns of tTG bound to divalent cations and nucleotide complexes were analyzed by SDS-PAGE and immunoblotting using a monoclonal antibody to tTG. The presence of 1 mM Ca\textsuperscript{2+} preserved a 50-kDa fragment of tTG (Fig. 4, lane 1). However, when 5 mM Mg\textsuperscript{2+} was present, the protein was susceptible to proteolysis, and the epitope was completely degraded (Fig. 4, lane 2). Mg-GTP (6 µM) protected tTG from degradation by trypsin (Fig. 4, lane 3). The same pattern was also observed in the presence of 6 µM free GTP or GDP or 6–50 µM Mg-GTP or Mg-GDP. The addition of 5 mM Ca\textsuperscript{2+} in the presence of 6 µM GTP or ATP converted tTG to the Ca\textsuperscript{2+}-protected conformation (Fig. 4, lanes 4 and 5, respectively). In addition, the epitope was completely degraded in the presence of 6 µM Mg-ATP (Fig. 4, lane 6). A similar pattern was also observed in the presence of 6 µM free ATP or ADP or 6–50 µM Mg-ATP or Mg-ADP.

**DISCUSSION**

tTG is present in several different tissues and cellular compartments (1, 2, 16–17). In the extracellular environment, tTG plays a role in extracellular matrix assembly, cell adhesion, and wound healing (1, 2). Inside cells, tTG is associated with the cell-surface membrane and plays an important role in signal transduction and nuclear pore assembly (8, 17). The majority of tTG is present in the cytoplasm, where it can interact with a wide variety of intracellular factors, and is involved in apoptosis and cell cycle arrest (9, 18). In this study, we investigated the effects of divalent cations, nucleotides, and Mg-nucleotide complexes on the modulation of the dual enzymatic activities of tTG.

Calcium and magnesium ions are important regulators of many physiological activities in cells and tissues (31, 32). The fact that TGase activity was observed only in the presence of calcium ions, whereas optimum GTP or ATP hydrolysis occurred in the presence of magnesium ions, suggests that different domains of tTG are involved in these distinct catalytic events. Furthermore, these results suggest that at physiological intracellular and extracellular concentrations of these factors, tTG can display a distinct spectrum of activities. Results from trypsin digestion experiments demonstrate that tTG has distinct conformational states that are dependent on the rela-
tive concentrations of divalent cations and Mg-nucleotide complexes. A protease-susceptible conformation occurred when Mg\(^{2+}\), Mg-ATP, or Mg-ADP was present. Calcium ions alone protected a 50-kDa fragment of tTG, whereas GTP, GDP, Mg-GTP, and Mg-GDP binding made the entire tTG molecule resistant to degradation.

Magnesium ions are relatively abundant inside cells and do not antagonize TGase activity alone. However, when present in a Mg-GTP or Mg-GDP complex, the complex inhibits TGase activity. It is interesting that up to 1 mM Mg-ATP did not have a significant effect on TGase activity. Previous studies using ATP alone as an inhibitor (29) did not examine the role of the Mg-ATP complex in this process. Furthermore, the effects of free ATP on the inhibition of TGase activity might be due to a chelation effect since Ca\(^{2+}\) free ATP on the inhibition of TGase activity might be due to a Mg-ATP complex in this process. Furthermore, the effects of ATP alone as an inhibitor (29) did not examine the role of the Mg-ATP complex in this process. Furthermore, the effects of free ATP on the inhibition of TGase activity might be due to a chelation effect since Ca\(^{2+}\) and Mg\(^{2+}\) completely reversed the inhibition.

The finding that Mg-ATP or Mg-ADP acts as a noncompetitive inhibitor of GTP hydrolysis suggests there are separate binding sites for Mg-GTP and Mg-ATP. This conclusion is further supported by the finding that Mg-ADP (or Mg-GDP) acts as a competitive inhibitor in ATPase (or GTPase) reactions. In addition, demonstrating that preincubation of Mg-ATP or Mg-ADP with tTG did not affect the IC\(_{50}\) for Mg-GTP-mediated inhibition of TGase activity also suggested the presence of separate binding sites. Binding of Mg-ATP to tTG apparently induces a conformational change that inhibits GTPase activity, but not TGase activity. On the other hand, the conformation induced by binding to Mg-GTP inhibits TGase activity, but not ATPase activity. In addition, Mg-ADP is a potent inhibitor of GTPase activity, but not TGase activity, whereas Mg-GDP is a potent inhibitor of TGase activity, but not ATPase activity.

We previously reported that the peptide containing the N-terminal 185 amino acid residues of tTG is the minimal structure required for GTPase/ATPase activity (15). Therefore, the Mg-GTP- and Mg-ATP-binding sites must reside in the N-terminal 185 residues of tTG. Examination of the N-terminal 185 residues of tTG did not reveal an exact match with the conserved sequences for GTP-binding proteins such as \(\text{GXGGXGK(ST)}\), \(\text{DXXGO}\), and \(\text{NTKQDX}\) (33). However, two regions at \(\text{GPGPSQEAGTK}^{174}\) and \(\text{QFYQGSSAK}^{173}\) are homologous to the \(\text{GXGGXGK(ST)}\) sequence and may act as binding sites for ATP and GTP. Further investigations are needed to address this question.

It is clear that the enzymatic activities of tTG are controlled by local concentrations of Mg\(^{2+}\), Ca\(^{2+}\), and nucleotides. Under physiological conditions, intracellular free calcium ion (10\(^{-7}\) M) and GTP (100–150 \(\mu\)M) concentrations are sufficient to keep tTG in a latent state. However, intracellular concentrations of free Mg\(^{2+}\) (approximately millimolar) are sufficient for tTG to express ATPase or GTPase activity. Because GDP and ADP are the major products of the hydrolysis reaction (15), it is conceivable that intracellular tTG is in the Mg-ADP- or Mg-GDP-bound state. Since Mg-ADP is more abundant and is a strong inhibitor of GTP and ATP hydrolysis, one would expect tTG to display minimal hydrolysis activity intracellularly. In addition, there should be no TGase activity because GDP and GTP (both free and complexed forms) are strong inhibitors. For tTG to display TGase activity inside cells, a cofactor must exist to dissociate Mg-GDP or Mg-GTP and/or to reduce the Ca\(^{2+}\) requirement for TGase activity. In support of this hypothesis, we recently discovered that sphingosylphosphocholine can act as a specific cofactor in activating TGase activity at physiological levels of Ca\(^{2+}\) and is able to reverse the inhibition by GTP (19).

It is important to note that the intracellular location of tTG is dependent upon cell types and that the distribution of tTG in the cytoplasm or membrane in the same cell type can also be changed upon retinoic acid treatment (34). Therefore, the enzymatic activities of tTG are modulated by the local environment. Thus, divalent cations, nucleotides, and Mg-nucleotide complexes are not the only factors modulating the enzymatic activities of tTG. The local environment of tTG should also be considered. In the case of rat liver G\(_{ab}\) (also a tissue-type transglutaminase), it plays an important role in transmembrane signaling through the \(\alpha\)-adrenergic receptor complex (8). The GTP binding of G\(_{ab}\) is modulated by a 50-kDa protein called G\(_{ab}\). G\(_{ab}\) accelerates the release of GTP-S from G\(_{ab}\) and changes the affinity of G\(_{ab}\) from GTP to GDP (35). Therefore, modulation of the activity of membrane-bound tTG differs due to the differences in the local environment and the presence of other protein(s) or cofactor(s).

\(G_a\) subunits of heterotrimeric G-proteins hydrolyze GTP at a relatively constant intrinsic rate (\(k_{\text{cat}}/K_{\text{M}}\) = 1–5 min\(^{-1}\)). Other GTPases hydrolyze GTP quite slowly, usually at rates \(\leq 1\) of that of G\(_{a}\) (36), but can be stimulated by GTPase-activating proteins to hydrolyze GTP at rates \(\sim 100\)-fold faster than that of G\(_{a}\) (37, 38). tTG hydrolyzes GTP quite slowly (0.06 min\(^{-1}\)) and may require modification by a protein cofactor like a GTPase-activating protein to stimulate its GTPase activity. In the case of the particulate form of tTG, the cofactor could be membrane components or other proteins like \(G_{ac}\) (35).

Earlier studies, performed in the absence of Mg\(^{2+}\), demonstrated that GTP was a more potent inhibitor of TGase activity than GDP and that GDP had no effect (12). The nature and sensitivity of the prior assay could account for the observed difference in results. The substrate \(N,N'-\text{dimethylcasein} \) remains in solution at high concentrations in the \(^{3}H\)putrescine incorporation assay (26), whereas it was bound to a microtiter plate in the assay used in this study (27). Immobilization of lower concentrations of \(N,N'-\text{dimethylcasein} \) could eliminate interference with the effective concentration of nucleoside phosphates. This was supported by the experiments using 25-fold lower concentrations of \(N,N'-\text{dimethylcasein} \) in the \(^{3}H\)putrescine incorporation assay. We found that the IC\(_{50}\) values for GTP and GDP were similar under these conditions of reduced substrate. When \(N,N'-\text{dimethylcasein} \) is coated on microtiter plates, the interference of \(N,N'-\text{dimethylcasein} \) with GTP and GDP is minimized. Furthermore, using the 5-biotinamidopentylamine incorporation assay, the IC\(_{50}\) values of Mg-GTP, Mg-GDP, and Mg-GMP for guinea pig liver tTG were the same as for recombinant human tTG, demonstrating that it is not a species-dependent effect.

In conclusion, results from this study suggest that local concentrations of Mg-nucleotide complexes play a role in modulating the enzymatic activities of tTG. In the absence of other factors, intracellular tTG is most likely in the Mg-ADP- or Mg-GDP-bound state and displays minimal hydrolysis activity. As calcium levels increase, Mg-ADP- and Mg-GDP-bound tTG can display TGase activity. Further studies are in progress to define the magnesium, NTP, and calcium-binding sites of tTG by site-directed mutagenesis.

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