The Binding Sites on Fibrin(ogen) for Guinea Pig Liver Transglutaminase Are Similar to Those of Blood Coagulation Factor XIII

CHARACTERIZATION OF THE BINDING OF LIVER TRANSGLUTAMINASE TO FIBRIN

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The present study represents detailed investigations into the nature of interactions between an intracellular "tissue" transglutaminase and a plasma protein, fibrinogen. We demonstrate a specific, saturable, and reversible binding of transglutaminase to fibrinogen. The binding was time- and temperature-dependent, was independent of divalent metal ions, did not require the release of either fibrinopeptide A or B, and was partially inhibited by the presence of sodium chloride or plasma proteins, properties similar to Factor XIII binding to fibrinogen. Both Factor XIII and liver transglutaminase also shared similar binding sites on fibrinogen, the Aβ- and the Bβ-chains. The binding characteristics of liver transglutaminase were thus similar to Factor XIII binding to fibrin, but there were also important differences. Scatchard analyses of the binding data indicated that the affinity of liver transglutaminase (Kd = 4.17 x 10^-7 M) was at least 40-fold weaker compared with the affinity of Factor XIII to fibrinogen. Consequently, a 20-fold molar excess of Factor XIII a-chains specifically and completely inhibited the binding of liver transglutaminase to des-Afibrinogen. The association between liver transglutaminase and fibrinogen was also critically controlled by the conformational states of the two proteins. Substances capable of altering the conformation of either transglutaminase (such as guanosine 5'-triphosphate) or of fibrinogen (such as the tetrapeptide Gly-Pro-Arg-Pro and Fragment D) disrupted binding. Excess CaCl2 was counteracted to the effects of guanosine 5'-triphosphate on transglutaminase binding to fibrin. In contrast, Factor XIII binding to fibrin was unaffected by either guanosine 5'-triphosphate, CaCl2, or Gly-Pro-Arg-Pro, suggesting a more stable association between the two proteins. The physiologic implications of transglutaminase-fibrin(ogen) interactions are discussed.

The central role of fibrinogen in hemostatic function has been well established (1). Fibrinogen binds to several proteins including Factor XIII, the protransglutaminase in plasma and platelets (2, 3). Consequent to an interaction between fibrinogen and plasma/platelet transglutaminase (= Factor XIIIa), a cross-linked and stable clot is formed (2-6). However, the role of "vascular" ("tissue") transglutaminase (7) in mediating this reaction has not yet been studied. We have previously shown that vascular transglutaminase (7) is biochemically and immunochemically similar to the well-characterized guinea pig liver transglutaminase (8, 9). The liver transglutaminase also cross-links both fibrinogen and fibrin and also incorporates primary amines into both protein substrates (7-12), properties similar to the vascular transglutaminase (7, 12).

There have been only a few preliminary reports describing the binding of liver transglutaminase to fibrinogen (13-15). It was speculated that the binding of liver transglutaminase to fibrinogen helps to clear the enzyme from the circulation (8). In view of the paucity of data, we undertook a detailed investigation into the nature of interactions between liver transglutaminase and fibrinogen, noncross-linked fibrin and plasmic degradation products of fibrinogen.

In this paper we have extensively characterized the interactions between liver transglutaminase and fibrinogen. We found liver transglutaminase binds to the Aβ- and Bβ-chains of fibrinogen similar to Factor XIII (16). Our results also suggest that the conformational states of transglutaminase and fibrinogen are crucial for binding. Substances capable of altering the conformation of either transglutaminase (such as guanosine 5'-triphosphate (GTP) (17)) or of fibrinogen (such as the tetrapeptide, Gly-Pro-Arg-Pro, (12) or Fragment D, a plasmic degradation product of fibrinogen) disrupted binding.

MATERIALS AND METHODS AND RESULTS

DISCUSSION

There are only a few preliminary reports describing the binding of guinea pig liver transglutaminase to fibrinogen (13-15). The present study therefore represents the first detailed biochemical characterization into the nature of interactions between the two proteins. It must be emphasized these studies were conducted with radiolabeled proteins. This excludes the possibility of artifactual binding due to radio-

Portions of this paper (including "Materials and Methods," "Results," Figs. 1-10, and Tables I and II) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

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Fibrin(ogen)-Transglutaminase Interactions

In the present study we demonstrate a specific, saturable, and reversible binding of guinea pig liver transglutaminase to fibrin(ogen).

The present study revealed an apparent lack of species specificity in liver transglutaminase-fibrinogen interactions. Fibrinogen purified from either guinea pig or human plasma bound equally well to guinea pig transglutaminase. Recently, Lorand et al. (29) reported a similar lack of species specificity in the interactions between human fibronectin and guinea pig liver transglutaminase. Furthermore, guinea pig transglutaminase bound des-A-fibrinogen equally well in either a human or guinea pig plasma milieu. Chung (15) also reported binding of 14C-carboxymethylated liver transglutaminase to plasma, although few experimental details were provided. Therefore, most of the present studies were performed using guinea pig liver transglutaminase and purified human fibrinogen which was readily available in adequate amounts in our laboratory.

The inhibition of transglutaminase binding observed at 50% plasma concentration could be due to interference from such plasma components as Factor XIII and fibronectin. In this context, our data on the competitive inhibition by Factor XIII of transglutaminase binding to fibrin (Fig. 10) is significant. Furthermore, the recent demonstration of fibronectin binding to transglutaminase (29) implies that both fibronectin and Factor XIII could interfere in transglutaminase binding. However, the affinity of fibronectin for transglutaminase is unknown. It is likely that the relative concentrations of transglutaminase, Factor XIII, fibrinogen, and fibrinogen and their relative affinities for each other could regulate the interactions among these proteins. A plasma milieu has earlier been noted to partially inhibit the binding of vitronectin to collagen (30).

The binding characteristics of liver transglutaminase appear similar to Factor XIII binding to fibrin, but there are also important differences. The binding of liver transglutaminase to fibrin did not require cleavage of fibrinopeptides A or B, was a time- and temperature-dependent event, and was independent of divalent cations, characteristics similar to Factor XIII binding to fibrinogen (2, 3). In preliminary experiments we found liver transglutaminase could bind to fibrinogen-agarose beads. Thus, we have demonstrated the specific binding of transglutaminase to various forms of fibrinogen (fibrinogen, des-A-fibrinogen, des-A,B-fibrinogen, and Fragment X). However, there is no information on the relative binding affinities of transglutaminase to the various forms of noncross-linked and cross-linked fibrinogen with the exception of des-A,B-fibrinogen. Future studies will be directed to obtaining this information.

The kinetics of transglutaminase binding to fibrinogen are similar to Factor XIII binding (2). However, the $K_v$ value for transglutaminase binding ($4 \times 10^{-7} \text{ M}$) was at least 40-fold higher than the $K_v$ values reported for Factor XIII binding ($1 \times 10^{-8} \text{ M}$) (2). The binding ratio of liver transglutaminase to fibrin was also one-quarter the value reported for Factor XIII to fibrinogen (2). Our results suggest that a decameric form of fibrinogen may be the minimum structure required to support the binding of one molecule of transglutaminase. In contrast, the binding ratio reported by Greenberg and Shuman (2) suggests a dimer to be the minimum structure required to bind Factor XIII. Indeed according to Lorand (31), one molecule of fibrinogen could even bind two a-chain molecules of Factor XIII. Taken together, these data indicate the vastly weaker interactions of transglutaminase to fibrinogen, relative to Factor XIII. The binding domains on fibrinogen to both Factor XIII (16) and liver transglutaminase (present study) were, however, the same: the Aα- and Bβ-chains. Therefore, Factor XIII a-chains could disrupt the binding of transglutaminase to des-A-fibrinogen (Fig. 10). This inhibitory effect was specific for the a-chains of Factor XIII, since excess b-chains did not modify transglutaminase binding. Our data offer convincing evidence that liver transglutaminase and Factor XIII share similar binding sites on fibrinogen. We have recently isolated and partially sequenced a fibrin-binding fragment from Factor XIII a-chains (37). We are now attempting to localize a similar fibrin-binding domain in liver transglutaminase. Recently, several homologous regions were found by comparing the amino acid sequences of guinea pig liver transglutaminase and Factor XIII a-chains (38). In view of remarkable structural similarities between the two enzymes, it is likely that the fibrin-binding domains in liver transglutaminase and Factor XIII a-chains are homologous as well.

The biphasic effect of sodium chloride on transglutaminase binding to fibrin (Fig. 6) offers an intriguing possibility about the nature of the chemical bond between the two proteins. It is possible that part of the interactions are ionic in nature. However, since the two proteins resist dissociation in presence of higher concentrations of sodium chloride (up to 500 mM), other types of chemical interactions might be responsible for holding the two proteins together. The nature of these chemical bonds are unclear at the moment and might involve conformational changes in both fibrinogen and liver transglutaminase due to the presence of sodium chloride. In this respect, the binding of liver transglutaminase is different from Factor XIII, since Factor XIII-fibrinogen interactions may be completely disrupted by about 300 mM NaCl (2). Sodium chloride has been reported to disrupt other protein-protein interactions as well (30).

Several lines of evidence suggest that conformational states of both fibrinogen and liver transglutaminase are important for the association of the two proteins. We had previously shown that GTP binds to transglutaminase and modulates its enzymatic activity and further that CaCl₂ counteracts the effects of GTP (17). We have extended these findings in the present study. Micromolar concentrations of GTP specifically inhibited the binding of liver transglutaminase to des-A,B-fibrinogen and CaCl₂ could reverse the inhibition (Figs. 7 and 8). These results suggest that in the presence of GTP, liver transglutaminase exists in a conformation unsuited to binding and excess CaCl₂ changes the conformation of the enzyme to one conducive to binding.

Conformational changes in fibrinogen consequent to the binding of Gly-Pro-Arg-Pro (12, 32) and Fragment D (33) have been demonstrated earlier. Our studies offer further, if indirect, evidence for such conformational changes in fibrinogen. Both Gly-Pro-Arg-Pro (Table I) and Fragment D (Table II) significantly inhibited the binding of liver transglutaminase to fibrin. The conformational changes in fibrinogen due to Gly-Pro-Arg-Pro binding appear to specifically affect transglutaminase binding. We previously reported no inhibition of Factor XIII binding to fibrin in presence of Gly-Pro-Arg-Pro (15). Small perturbations in fibrinogen/transglutaminase structures might be sufficient to disrupt the association of the two proteins due to the lower binding affinity of transglutaminase to fibrinogen, in contrast to the relatively higher binding affinity of Factor XIII. The effects of Fragment D on Factor XIII-fibrinogen interactions remains to be determined.

The physiologic significance of the binding of an intracellular transglutaminase to fibrinogen, a plasma protein, re-

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2 K.E. Achuthan, A. Mary, and C.S. Greenberg, unpublished observations.
mains to be elucidated. However, some speculations on this aspect are not out of order. We previously demonstrated that guinea pig liver transglutaminase is biochemically and immunochemically similar to a vascular transglutaminase identified in aortic endothelial cells (7). Preliminary experiments have shown that the vascular transglutaminase also binds to des-A, and des-A,B-fibrinogen. Future studies will be directed at determining whether vascular transglutaminase shares the binding characteristics of liver transglutaminase and Factor XIII. If such is the case, it would offer further evidence regarding the structural and functional similarities between liver and vascular transglutaminases. Finally, such data will strengthen our original hypothesis that the vascular transglutaminase could provide a thrombin-independent alternate pathway toward fibrin stabilization (7).

Recently, Barsigian et al. (35) reported a liver transglutaminase-mediated binding and processing of fibrinogen by hepatocytes. Barsigian et al. (35) speculated this binding could play a role in cell-cell or cell-matrix interactions and possibly even in fibrinogen metabolism by the liver. Our data offer some experimental evidence in support of these hypotheses. In conclusion, the binding of transglutaminase to fibrinogen, either in plasma or in hepatocytes, could have important physiologic consequences.

REFERENCES

Materials, Methods and Results:

Proteins: Guinea pig liver transglutaminase (Mr = 86,000), platelet factor XIII (Mr = 80,000) and human fibrinogen (Mr = 300,000). Purified guinea pig fibrinogen was purchased from Sigma Chemical Co., St. Louis, Mo. Fibrinogen from either source was free of platelets. Neither fibrinogen preparation nor purified guinea pig fibrinogen contained detectable levels of fibrinopeptide A, B or fibrinogen degradation products (FDP) as determined by the Bio-Rad (Mr = 74,000) and Coomassie Blue (Mr = 17,000). Purified guinea pig fibrinogen was purchased from Calbiochem. Other synthetic peptides (Gly-Pro-Arg-Pro, Gly-Pro, Gly-Pro-Arg; Fig. 4A) were obtained from Bachem. Guanine-5'-triphosphate (GTP) (Na-salt) was purchased from Sigma. The synthetic peptide-Hepes buffers (pH 7.5-8.0) were prepared in 50 mM Hepes-NaOH, 100 mM NaCl, 50 mM Tris-HCl, pH 7.5 and incubated at 37°C for 10 minutes. The reaction mixture was then chilled on ice for 5 minutes. At this stage, the reaction mixture had the appearance of an emulsion. Thrombin (1 U/mL) was added to the reaction mixture and non-specific binding or loss of transglutaminase activity due to the operational steps (e.g., mixing of thrombin) was subtracted by re-suspending twice in 100 mM Tris, pH 7.5 and centrifuging down the pellet. The washes precipitated a activity on an anion exchange resin. The supernatant was then eluted with 0.5 M NaCl and the eluted material was used for immunoblotting as described previously (18). The antigen bands were also visualized by SDS-PAGE and immunoblotting. Transglutaminase binding to fibrin was quantitated in two ways. Decrease in the absorbance of a known amount of thrombin activity due to the operational steps (e.g., mixing of thrombin) was subtracted by re-suspending twice in 100 mM Tris, pH 7.5 and centrifuging down the pellet. The washes precipitated a activity on an anion exchange resin. The supernatant was then eluted with 0.5 M NaCl and the eluted material was used for immunoblotting as described previously (18). The antigen bands were also visualized by SDS-PAGE and immunoblotting. Transglutaminase binding to fibrin was quantitated in two ways. First, a decrease in antigen activity due to the reaction mixture had the appearance of an emulsion. Fibrinogen from either source was free of platelets. The amount of transglutaminase antigen in each lane was quantitated in two ways. First, a decrease in the absorbance of the antigen band with increasing concentration of transglutaminase activity was plotted on a graph. Second, a decrease in absorbance with increasing concentration of transglutaminase activity was plotted on a graph.

**Figure 1.** Densitometric Scan of Increasing Concentrations of Transglutaminase (Ab) with increasing concentration of transglutaminase activity. Increasing concentrations of purified guinea pig liver transglutaminase were incubated with a constant amount of fibrinogen as described in the Materials section. The antigen bands were quantitated using laser scanning densitometry. The amount of transglutaminase antigen in each lane was quantitated in two ways. First, a decrease in absorbance with increasing concentration of transglutaminase activity was plotted on a graph. Second, a decrease in absorbance with increasing concentration of transglutaminase activity was plotted on a graph. The antigen bands were quantitated using laser scanning densitometry.

**Figure 2.** Effect of Guineapig Fibrinogen Concentration on the Binding of Purified Guinea Pig Liver Transglutaminase to Fibrin. Increasing concentrations of fibrinogen were mixed with 75 U/mL of transglutaminase in the presence of thrombin (1000 U/mL). Absorbance (A540 nm) and thrombin (1000 U/mL) were added to the mixture and incubated at 37°C for 10 minutes. The samples were then chilled on ice for 5 minutes and then the fibrin pellet was separated from the supernatant by centrifugation. The amount of transglutaminase antigen in each lane was quantitated using laser scanning densitometry. The antigen bands were also visualized by SDS-PAGE and immunoblotting. The antigen bands were quantitated using laser scanning densitometry.

**Figure 3.** Effect of Guineapig Fibrinogen Concentration on the Binding of Purified Guinea Pig Liver Transglutaminase to Fibrin. Increasing concentrations of fibrinogen were mixed with 75 U/mL of transglutaminase in the presence of thrombin (1000 U/mL). Absorbance (A540 nm) and thrombin (1000 U/mL) were added to the mixture and incubated at 37°C for 10 minutes. The samples were then chilled on ice for 5 minutes and then the fibrin pellet was separated from the supernatant by centrifugation. The amount of transglutaminase antigen in each lane was quantitated using laser scanning densitometry. The antigen bands were also visualized by SDS-PAGE and immunoblotting. The antigen bands were quantitated using laser scanning densitometry.

**Figure 4.** Effect of Guineapig Fibrinogen Concentration on the Binding of Purified Guinea Pig Liver Transglutaminase to Fibrin. Increasing concentrations of fibrinogen were mixed with 75 U/mL of transglutaminase in the presence of thrombin (1000 U/mL). Absorbance (A540 nm) and thrombin (1000 U/mL) were added to the mixture and incubated at 37°C for 10 minutes. The samples were then chilled on ice for 5 minutes and then the fibrin pellet was separated from the supernatant by centrifugation. The amount of transglutaminase antigen in each lane was quantitated using laser scanning densitometry. The antigen bands were also visualized by SDS-PAGE and immunoblotting. The antigen bands were quantitated using laser scanning densitometry.

**Figure 5.** Effect of Guineapig Fibrinogen Concentration on the Binding of Purified Guinea Pig Liver Transglutaminase to Fibrin. Increasing concentrations of fibrinogen were mixed with 75 U/mL of transglutaminase in the presence of thrombin (1000 U/mL). Absorbance (A540 nm) and thrombin (1000 U/mL) were added to the mixture and incubated at 37°C for 10 minutes. The samples were then chilled on ice for 5 minutes and then the fibrin pellet was separated from the supernatant by centrifugation. The amount of transglutaminase antigen in each lane was quantitated using laser scanning densitometry. The antigen bands were also visualized by SDS-PAGE and immunoblotting. The antigen bands were quantitated using laser scanning densitometry.

**Figure 6.** Effect of Guineapig Fibrinogen Concentration on the Binding of Purified Guinea Pig Liver Transglutaminase to Fibrin. Increasing concentrations of fibrinogen were mixed with 75 U/mL of transglutaminase in the presence of thrombin (1000 U/mL). Absorbance (A540 nm) and thrombin (1000 U/mL) were added to the mixture and incubated at 37°C for 10 minutes. The samples were then chilled on ice for 5 minutes and then the fibrin pellet was separated from the supernatant by centrifugation. The amount of transglutaminase antigen in each lane was quantitated using laser scanning densitometry. The antigen bands were also visualized by SDS-PAGE and immunoblotting. The antigen bands were quantitated using laser scanning densitometry.

**Figure 7.** Effect of Guineapig Fibrinogen Concentration on the Binding of Purified Guinea Pig Liver Transglutaminase to Fibrin. Increasing concentrations of fibrinogen were mixed with 75 U/mL of transglutaminase in the presence of thrombin (1000 U/mL). Absorbance (A540 nm) and thrombin (1000 U/mL) were added to the mixture and incubated at 37°C for 10 minutes. The samples were then chilled on ice for 5 minutes and then the fibrin pellet was separated from the supernatant by centrifugation. The amount of transglutaminase antigen in each lane was quantitated using laser scanning densitometry. The antigen bands were also visualized by SDS-PAGE and immunoblotting. The antigen bands were quantitated using laser scanning densitometry.

**Figure 8.** Effect of Guineapig Fibrinogen Concentration on the Binding of Purified Guinea Pig Liver Transglutaminase to Fibrin. Increasing concentrations of fibrinogen were mixed with 75 U/mL of transglutaminase in the presence of thrombin (1000 U/mL). Absorbance (A540 nm) and thrombin (1000 U/mL) were added to the mixture and incubated at 37°C for 10 minutes. The samples were then chilled on ice for 5 minutes and then the fibrin pellet was separated from the supernatant by centrifugation. The amount of transglutaminase antigen in each lane was quantitated using laser scanning densitometry. The antigen bands were also visualized by SDS-PAGE and immunoblotting. The antigen bands were quantitated using laser scanning densitometry.

**Figure 9.** Effect of Guineapig Fibrinogen Concentration on the Binding of Purified Guinea Pig Liver Transglutaminase to Fibrin. Increasing concentrations of fibrinogen were mixed with 75 U/mL of transglutaminase in the presence of thrombin (1000 U/mL). Absorbance (A540 nm) and thrombin (1000 U/mL) were added to the mixture and incubated at 37°C for 10 minutes. The samples were then chilled on ice for 5 minutes and then the fibrin pellet was separated from the supernatant by centrifugation. The amount of transglutaminase antigen in each lane was quantitated using laser scanning densitometry. The antigen bands were also visualized by SDS-PAGE and immunoblotting. The antigen bands were quantitated using laser scanning densitometry.
Fibrinogen-Transglutaminase Interactions

Figure 3: Effect of Guinea Pig Plasma Concentration on the Binding of Purified Guinea Pig Liver Transglutaminase

Increasing concentration of plasma (x10) was mixed with 75 μg of transglutaminase in presence of 0.02 M L-Arginine and the mixture was clotted. The supernatant was centrifuged and the activity of each fraction was assayed as described in the text. Transglutaminase activity is expressed in micromoles of putrescine incorporated into casein per 100 μl of supernatant.

We also demonstrated the binding of transglutaminase to fibrin in the presence of plasma proteins by a slightly different method. Factor III deficient plasma depleted of fibrinogen by benzamidine absorption (2.5%) was used to study the effect of plasma proteins on binding. Binding was carried out using various dilutions (1% of plasma, 10% transglutaminase, and 10% fibrinogen) as described in the Methods Section. No inhibition of binding to des-A-β fibrinogen was observed at 0.02 M L-Arginine plasma concentration. Binding was inhibited by over 90% when plasma concentration was increased to 40% and 50%. However, inhibition of binding was not observed at plasma concentration higher than 40%. Guinea pig liver transglutaminase activity however, declined by less than 25% at room temperature or 0°C (unpublished observations). This suggests that binding of enzyme activity was not responsible for the observed inhibition by plasma proteins of transglutaminase binding to fibrin. The inhibition to transglutaminase binding in plasma remains to be characterized.

It was studied the time and temperature dependence of transglutaminase binding to fibrin. It has only recently been shown that varying the temperature (25°C to 37°C) during binding (Figure 4). Nearly 80% of binding was obtained between 0.5°C and 3°C for des-A-β fibrin, and binding was essentially complete by 3 minutes at either temperature. However, at 4°C, guinea pig liver transglutaminase bound fibrin very slowly. Nearly 80% of transglutaminase resisted uptake after 30 minutes at 4°C (Figure 4). Similar results were obtained when binding was analyzed by quantitating the transglutaminase activities bound to the clot at various temperatures (data not shown).

Control experiments demonstrated that under these conditions there was over 95% clumping of fibrinogen within 5 minutes at all three temperatures tested. These data exclude the possibility that the incubation with fibrinogen alone increases the reactivity of fibrinogen for transglutaminase binding at 4°C.

In order to determine the divalent metal ion requirements for transglutaminase binding to fibrinogen, incubations were performed in presence of CaCl₂ (0.1 mM and 0.5 mM), MgCl₂ (1 mM and 0.5 mM) or EDTA (1 mM) in presence or absence of 0.02 M L-Arginine. In these experiments, binding of CaCl₂ to either des-A-β fibrinogen or des-A-β fibrinogen was increased with increasing concentrations of L-Arginine. MgCl₂ and EDTA had no effect on the binding of transglutaminase to either des-A-β fibrinogen or des-A-β fibrinogen. Under these conditions, formation of a fibrin clot was also unaffected.

The effect of sodium chloride concentration on transglutaminase binding to fibrin was also investigated (Figure 5). There was an initial rapid increase in binding with increasing concentrations of sodium chloride, resulting in a plateau curve (Figure 6). Complete inhibition of binding was not observed even at a NaCl concentration of 500 mM.

Figure 4: Effect of Temperature on the Binding of Transglutaminase to Des-A-β Fibrinogen

The reaction mixture consisted of fibrinogen (1.0 μg/ml), transglutaminase (2% activity, 0.1 mg/ml), and 3°C for 30 minutes. The results of these experiments are expressed as percentage of transglutaminase incorporated into clot and measured by quantitative estimation of transglutaminase activity. The binding of transglutaminase to fibrin was quantitated by measuring the enzymatic activity of transglutaminase in the supernatant after incubation. The percent of transglutaminase incorporated into clot, Transglutaminase activity, and the amount of NaCl present are expressed in micromoles of putrescine incorporated into casein per 100 μl of supernatant.

We then investigated the affinity and stoichiometry of transglutaminase binding to fibrin. In the presence of 0.5 mM EDTA fibrinogen, there was increase binding of liver transglutaminase with increasing concentration of the enzyme protein added (Figure 6). Similar results were observed when the binding was analyzed by assaying the transglutaminase activity in the supernatant after incubation. The concentration of transglutaminase that was required for the maximum binding of transglutaminase to fibrin was determined (Figure 7). These results, interpreted as the ratio of 1 binding site/1 fibrinogen for transglutaminase.

The binding of guinea pig liver transglutaminase was inhibited by the addition of micrograms of geniposide (1-5) during binding (Figure 8). Similar results were obtained when the binding was analyzed by assaying the transglutaminase activity in the supernatant after incubation. The binding of guinea pig liver transglutaminase to fibrin was not affected by the presence of 100 μM geniposide. Variation between each experimental value was less than 10%.

Figure 5: Effect of Sodium Chloride Concentration on Transglutaminase Binding to Des-A-β Fibrinogen

The binding of transglutaminase to des-A-β fibrinogen was carried out at 37°C for 30 minutes as described in the legend to Figure 6. Binding was carried out in the absence and presence of indicated concentration of sodium chloride. The binding of transglutaminase to fibrin was quantitated by measuring the enzymatic activity of transglutaminase incorporated into clot, Transglutaminase activity, and the amount of NaCl present are expressed in micromoles of putrescine incorporated into casein per 100 μl of supernatant.

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Figure 6: Effect of Sodium Chloride Concentration on Transglutaminase Binding to Des-A-β Fibrinogen

The binding of transglutaminase to des-A-β fibrinogen was carried out at 37°C for 30 minutes as described in the legend to Figure 6. Binding was carried out in the absence and presence of indicated concentration of sodium chloride. The binding of transglutaminase to fibrin was quantitated by measuring the enzymatic activity of transglutaminase incorporated into clot, Transglutaminase activity, and the amount of NaCl present are expressed in micromoles of putrescine incorporated into casein per 100 μl of supernatant.

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Fibrinogen-Transglutaminase Interactions

![Image](image_url)

**Figure 7:** Effect of Guanosine-5'-triphosphate on the Binding of Transglutaminase to den-A, B Fibrinogen.

The conditions of binding were identical to those described for Figure 6, except that sodium chloride was replaced by the indicated concentrations of guanosine-5'-triphosphate and the amount of transglutaminase during binding assays was increased to 150 ug/ml. The supernatant was decanted and the amount of transglutaminase (determined as described in the methods) measured. The inhibition of binding of GTP was observed in the presence of 1 mM GTP (lane 1) and not 10 mM GTP (lane 2). In contrast, GTP had little effect on the binding to den-A, B fibrinogen (lanes 3 and 4). The concentration of transglutaminase activity was measured as described in the methods.

**Table I**

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<tr>
<th>Peptide</th>
<th>Concentration (M)</th>
<th>Inhibition of Binding (%)</th>
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<tr>
<td>GPR</td>
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<tr>
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<td>GR205PC</td>
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*The clotting of fibrinogen was unaffected by any of the peptides, at the concentrations tested.*

Increasing the GPR concentration up to 100 nM did not cause further inhibition of transglutaminase activity.

*The conditions for binding were: fibrinogen, 1.0 mg/ml; transglutaminase, 0.25 mg/ml; 0.2 M Tris, pH 7.4; 10 mM CaCl2. All inhibition was calculated based on transglutaminase activity in the supernatant. For more details, see text.

**Figure 8:** Effect of Guanosine-5'-triphosphate and CaCl2 on the Binding of Liver Transglutaminase and Platelet Factor XIII to Den-A, B Fibrinogen.

Using the clotting method described previously, we found that the A- and B-chains of fibrinogen were the preferred sites for liver transglutaminase binding (Figure 9). No binding to the A-chains was observed. The bands of fibrinogen either in Coomassie stained gel or immunoblot were detected based on their molecular weights calculated from their migration rates. All bands were resolved at the same electrophoretic mobilities simultaneously. Similar results were obtained using either fibrinogen, den-A, B fibrinogen, or Fragment 1, a plasma degradation product of fibrinogen. Using a slot blot method, we were able to demonstrate binding to the A- and B-chains of fibrinogen. Reduced fibrinogen chains were visualized by Coomassie stain (lane 1) or by a transglutaminase binding immunoassay (lane 2). For details, see text (16). We also tested the effect of several synthetic peptides and fragments D and E-terminal plasma degradation products of supernatant fibrinogen on the binding of guanosine-5'-triphosphate to den-A, B fibrinogen. Our results (Table 11) indicate that Fragment D (Table II) inhibited transglutaminase binding significantly.

**Figure 9:** Transglutaminase Binds to the A- and B-Chains, but not to the C-Chains of Fibrinogen.

Reduced fibrinogen chains visualized by Coomassie stain (lane 1) or by a transglutaminase binding immunoassay (lane 2). For details, see text (16).

**Figure 10:** Effect of Platelet Factor XIII on the Binding of Liver Transglutaminase to Den-A, B Fibrinogen.

Using the clotting method described for Figures 6 & 7 except that purified platelet Factor XIII replaces CaCl2 and GTP and fibrinogen was not clotting by the addition of 0.5 mg/ml Reptilase. The effect of Factor XIII on the binding of liver transglutaminase was quantitated by the amount of transglutaminase antigen bound to the clot (open circles) and by measuring the transglutaminase activity liberated in the supernatant (closed circles). Transglutaminase activity was corrected for the low level of factor XIII activity that was present in the supernatant. Factor XIII activity was inhibited by approximately 50% of the transglutaminase activity present in the supernatant but not in the presence of Factor XIII. Transglutaminase activity is expressed as a percentage of the activity incorporated into calcium by 100 nM of supernatant.