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Plasmin, Substilisin-like Endoproteases, Tissue Plasminogen Activator, and Urokinase Plasminogen Activator Are Involved in Activation of Latent TGF-β_1 in Human Seminal Plasma

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We reported previously that TGF-β_1 is a major immunosuppressive agent in human seminal plasma. TGF-β_1 in seminal plasma is so abundant that it may represent the highest physiologic concentration of TGF-β_1 reported for a biological fluid. The *in vitro* activation of TGF-β_1 is detected at acidic pH. The acidic environment of the vagina is suggested as an *in vivo* physiological condition for the activation of seminal plasma latent TGF-β_1. The present study demonstrates that Pefabloc [4-(2-aminoethyl)-benzenesulfonyl fluoride AEBSF]-inhibitable serine proteases are involved in the activation of latent TGF-β_1. Pefabloc inhibits latent TGF-β_1 activation in a dose- and time-dependent manner. The use of other protease inhibitors and specific antibodies reveals that, in addition to plasmin, substilisin-like endoproteases and tissue- and urokinase-type plasminogen activators participate in the activation of latent TGF-β_1 in human seminal plasma.

TGF-β represents a family of at least five isomers, and three of which are expressed in mammalian cells. The five TGF-β members share up to 80% amino acid homology and the mature active peptide is highly conserved (80–100%) (1). Biologically active TGF-β is a 25-kDa homodimer consisting of two identical disulfide-linked 112 amino acid polypeptides, which are originating from the carboxyl terminal of a 390-amino-acid pre-pro-TGF-β precursor molecule (2,3). TGF-β is secreted as a latent inactive form. Several different forms of latent TGF-β have been identified. Recombinant TGF-β consists of the mature active TGF-β homodimer and an N-terminal remnant of the TGF-β precursor known as latency associated peptide (4). In platelets and erythroleukemia cells, inactive latent TGF-β contains an additional component of 125- to 160-kDa known as TGF-β binding protein, which is linked to latency associated peptide by disulfide bridges forming a 210-kDa complex (5). A third form of inactive latent TGF-β contains the 25-kDa homodimer and α_2-macroglobulin in serum purported for clearance purpose (6). Other forms of latent TGF-β include its complexes with pregnancy zone protein of female reproductive tract, with fibronectin in serum, and with a binding protein found in bovine nuchal ligament (6). The formation of complexes with other proteins generally renders TGF-β inactive. Although latent TGF-β can be activated *in vitro* by extremes of pH, treatment with chaotropic agents, heat, and plasmin, the exact mechanisms involved in the physiological activation of TGF-β are totally unknown (2, 6).

We reported previously that TGF-β_1 is a major immunosuppressive protein in human seminal plasma (7). Seminal plasma TGF-β_1 is predominantly in the latent form and exhibits in a size ranging from 100 to 440 kDa and greater. The total concentration of TGF-β_1 in seminal plasma is fivefold greater than that of TGF-β_1 in human serum, and may represent the highest physiological concentration of TGF-β_1 reported for a biological fluid (8). The *in vitro* activation or release of TGF-β1 from latent TGF-β_1 is detected at acidic pH. Although *in vitro* TGF-β_1 activation mostly occurs at pH of less than 3.5, physiological conditions throughout most of the body will not permit such a harsh pH environment. A mild acid treatment at pH 4 and pH 5 also activates TGF-β_1 but in lesser quantities. The only physiological condition that provides such an acidic condition is that of female genital tract

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Abbreviations used: TGF-β_1, transforming growth factor-β_1; Pefabloc, 4-(2-aminoethyl)-benzenesulfonyl fluoride AEBSF; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator; ELISA, enzyme-linked immunosorbent assay; McAb, monoclonal antibody; PcAb, polyclonal antibody; hk, human kallikrein; PSA, prostate specific antigen.

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(9). The acidic environment of the vagina is therefore suggested to represent an *in vivo* physiological condition for activation of seminal plasma TGF-β_1 (8). One biological function of seminal plasma TGF-β_1 has been proposed to serve as an immunosuppressive factor to protect the integrity of the spermatozoa, since activated TGF-β_1 has been shown to bind to the spermatozoa (10).

The activation of seminal plasma latent TGF-β_1 is not inhibitable by a series of protease inhibitors examined: serine protease- and cysteine protease-inhibiting phenylmethylsulfonyl fluoride (PMSF) and leupeptin, cysteine protease-inhibiting E64, aspartic protease-inhibiting pepstatin, and metalloprotease-inhibiting ethylenediamine tetraacetic acid (EDTA), alone or in combination (8). The present study indicates that a significantly greater amount of activated TGF-β_1 is detected at 37°C than at 4°C, suggesting that enzymes are involved in the activation of seminal plasma latent TGF-β_1. Pefabloc (4-(2-aminoethyl)-benzenesulfonyl fluoride AEBSF) is found to exhibit a profound inhibition in a dose- and time- dependent manner on the activation of TGF-β_1 from its latent form. Pefabloc inhibits the following serine proteases: trypsin, chymotrypsin, thrombin, subtilisin A, kallikrein, plasmin, and tissue plasminogen activator (11). The use of additional protease inhibitors such as $ZnCl_2$, $FeSO_4$, ϵ-aminocaproic acid, pancreatic trypsin inhibitor, trasylol and phenol that differentially inhibit plasminogen activators, plasmin, and substilisins (12–16), and the use of specific anti-tissue plasminogen activator (tPA) and anti-urokinase-type plasminogen activator (uPA) antibodies have led to a new finding revealing that, in addition to plasmin, substilisin-like endoproteases, tPA and uPA are involved in the activation of latent TGF-β_1 in human seminal plasma.

METHODS AND MATERIALS

Materials. Pefabloc (AEBSF), E64, and leupeptin were purchased from Boehringer-Mannheim (Indianapolis, IN). All other protease inhibitors were purchased from Sigma (St. Louis, MO). [^{125}I]-TGF-β_1, at a specificity of 130 μCi/μg, was purchased from New England Nuclear (Boston, MA). Sephacryl S-300 HR was purchased from Pharmacia Biotech (Piscataway, NJ).

Seminal plasma. Seminal plasma, obtained from vasectomized men at their routine clinic visit, was pooled, stored at −20°C within 1 h of collection, and transferred to −70°C within a week until used. The specimens (pool of 50 ml) were thawed and centrifuged at 4000*g* at 4°C for 30 min. The precipitate was discarded and the supernatant was dialyzed (M_r cut-off 6 to 8 kDa) against three changes of 6 liters of phosphate buffered saline 0.01 M, pH 7.2 (PBS) at 4°C over a 28-h period. After centrifugation, the supernatant was used for the experiments.

Gel filtration. Chromatography of the dialyzed seminal plasma specimen was performed at 4°C using a pre-calibrated Sephacryl S-300 HR as described previously (7,8). Briefly, to a column (2.5 × 100 cm) of Sephacryl S-300, 5 ml of dialyzed seminal plasma were applied and eluted with PBS at a flow rate of 15 ml/h. Fractions at approximately 3.5 ml/tube were collected. To monitor the fractionation for the presence of TGF-β_1, 200 μl of each tube was acidified to pH 1.8 with 20 μl of 1 N HCl for 1 h and then neutralized to pH 7.2 with 20 μl 1 N NaOH. TGF-β_1 was then measured by ELISA and mink lung epithelial cell proliferation bioassay as described (7, 8).

ELISA of TGF-β_1. Mouse monoclonal antibody (McAb) 1D11.16 specific for TGF-β was generously provided by Celtrix Pharmaceutical (Santa Clara, CA) and Bristol-Myers Squibb (Seattle, WA). McAb 1D11.16 was used as the capture antibody, and chicken specific anti-TGF-β_1 antibody (R & D, Minneapolis, MN) was used as the indicator antibody in an ELISA described previously (8). Under these experimental conditions, the assay measured only activated TGF-β_1, and did not detect latent TGF-β_1.

Activation of seminal plasma latent TGF-β_1 at various pH. Seminal plasma specimens were adjusted to pH 3.5 and pH 5.0, and pH 7.2. Briefly, 200 μl of specimens were brought to pH 3.5 or pH 5.0 by adding 20 μl of 1 M sodium citrate buffer pH 3.5 or pH 5.0, or by adding PBS pH 7.2. After incubation at room temperature for 30 min, pH of the specimens was neutralized to pH 7.2 by 1 N NaOH. Fifty-microliter aliquots of the specimens were assayed for activated TGF-β_1 by ELISA.

Inhibition of latent TGF-β_1 activation. For measuring activated TGF-β_1 in each protease inhibitor inhibition assay and related experiments, aliquots (200 μl) of pooled chromatographic fractions that had been shown to contain latent TGF-β_1 were incubated with each enzyme inhibitor of various concentrations at pH 5.0 and 37°C for 1 h, and then pH was adjusted to 7.2. The specimens were then analyzed for activated TGF-β_1 by ELISA.

Radioreceptor assay. In experiments with Pefabloc, biologically active TGF-β_1 was also performed by cold competition radioreceptor binding-inhibition assay as described previously (8). Briefly, seminal plasma specimens were adjusted to pH 5.0, mixed with Pefabloc and incubated at 37°C for 1 h. Control was incubated in the absence of Pefabloc. Samples were adjusted to pH 7.2, and analyzed by radioreceptor assay using [^{125}I]-TGF-β_1 (17.5 pM) and TGF-β_1 receptor-rich mink lung epithelia.

Inhibition of latent TGF-β_1 activation by anti-tPA and anti-uPA antibodies. Latent TGF-β_1 preparations (200 μl) were incubated at pH 5.0 in the presence or absence of specific anti-uPA and anti-tPA antibodies at 37°C for 1 h. Activated TGF-β_1 was measured by ELISA and uPA and tPA enzymatic activities were measured by Spectrozyme-PL assay (17). Mouse McAb specific for human uPA, McAb 3689, was purchased from American Diagnostica (Greenwich, CT). Goat PcAb specific for uPA and tPA were described previously (17).

RESULTS AND DISCUSSION

Activation of seminal plasma latent TGF-β_1 at pH 5.0. Our previous data have indicated that the activation or the release of TGF-β_1 from latent TGF-β_1 in human seminal plasma is achieved only at acidic pH (8). To continue the investigation, human seminal plasma was dialyzed and adjusted to pH 3.5 and pH 5.0. After incubation at room temperature for 30 min, the activity of TGF-β_1 released from latent form in seminal plasma was detected by the ELISA specific for TGF-β_1 immunoreactivity. The results showed that the activation of latent TGF-β_1 was most profound at pH 3.5. A moderate activation was achieved at pH 5.0 ($p <$ 0.05 Student's *t* test vs pH 7.2), while a very limited, if any, activation was detected at pH 7.2. Since no *in vivo* physiological condition, except that of gastric juice, will provide such a harsh acidic environment as pH 3.5, pH

5.0, i.e., a pH level commonly found in the contents of the vagina, was used throughout the experiments.

Effect of protease inhibitors on activation of seminal plasma latent TGF-β_1. Human seminal plasma is known to contain acidic proteases (18), and proteases have been implicated in the activation of latent TGF-β_1 in cell culture medium (4). We have used a wide variety of protease inhibitors in order to identify endogenous proteases of seminal plasma that are involved in the activation of latent TGF-β_1.

Human seminal plasma was fractionated and the high molecular weight (100 to 440 kDa and greater) fractions containing acid-activated TGF-β_1 activity as measured by both ELISA and mink lung epithelial cell proliferation bioassay were pooled (7, 8). These pooled fractions were used as the source of latent inactive TGF-β_1 preparation for experiments with the following initial series of protease inhibitors: E64, EDTA, leupeptin, PMSF, pepstatin, plasmin-inhibiting aprotinin, and a fairly new potent serine protease inhibitor Pefabloc (AEBSF), as well as a newly available Sigma's protease inhibitor cocktail that is a mixture of protease inhibitors that contains AEBSF, pepstatin A, E64, bestatin, leupeptin and aprotinin, with broad specificity for the inhibition of serine, cysteine, aspartic proteases and aminopeptidases.

Various protease inhibitors at selected concentrations were incubated with latent TGF-β_1 preparation at pH 5.0 and the physiological temperature of 37°C for 1 h. The mature active TGF-β_1 released from its latent form was detected by ELISA. The results obtained from the experiments revealing the activities, or inactivities, of these protease inhibitors at various concentrations on the activation of latent TGF-β_1 are summarized in Table 1. Using three different concentrations each including the starting concentrations and up to 5 to 50× that were suggested by the manufacturers, only Pefabloc at all three concentrations of 0.2, 1, and 5 mM, and aprotinin at 15 μM; were found to exhibit an inhibition on latent TGF-β_1 activation. The results showing no inhibition at all obtained from the use of the other five protease inhibitors precluded the possibility that such proteases as chymotrypsin, trypsin, thrombin, papain, cathepsins B, D, and L, pepsin, renin, chymosin, and papain would play any meaningful role in the activation of seminal plasma latent TGF-β_1. The use of Sigma's protease inhibitor cocktail alone and in combination with Pefabloc confirmed these results. Further experiments using Pefabloc were thus performed to investigate its inhibitory effect.

Pefabloc dose-dependent inhibition on activation of seminal plasma latent TGF-β_1. Pefabloc at the concentrations of 0.2, 1, and 5 mM was further assessed in additional experiments to determine if the inhibition on the release of biologically active TGF-β_1 from its latent form in seminal plasma was dose-dependent.

TABLE 1

Inhibition of Seminal Plasma Latent TGF-β_1 Activation by Protease Inhibitors[a]

Inhibitor	Concentration[b]	Inhibition[c] (%)
E64	0.14 μM	No
	2.8 μM	No
	28 μM	No
EDTA	0.25 mM	No
	0.5 mM	No
	62.5 mM	No
Leupeptin	0.2 μM	No
	1 μM	No
	5 μM	No
Pepstatin	0.14 μM	No
	1 μM	No
	7 μM	No
PMSF	1 mM	No
	10 mM	No
	50 mM	No
Aprotinin	0.03 μM	No
	0.3 μM	No
	15 μM	28
Pefabloc	0.2 mM	4
	1 mM	24
	5 mM	63
Protease inhibitor cocktail	1:20	9
Pefabloc	2 mM	30
Protease inhibitor cocktail plus Pefabloc		33

[a] Various protease inhibitors at the concentrations shown were incubated with latent TGF-β_1 preparation (200 μl) at pH 5.0 and 37°C for 1 h. The released TGF-β_1 activity was measured by ELISA.

[b] The manufacturers' suggested starting concentrations: E64 (0.14–2.8 μM), EDTA (0.5–1.3 mM), leupeptin (1 μM), pepstatin (1 μM), PMSF (0.1–1 mM), aprotinin (0.01–0.3 μM), Pefabloc (0.4–4 mM), and protease inhibitor cocktail (1:20 dilution).

[c] Average of triplicate assays. The experiments were repeated twice with similar results.

Three more pools of gel filtration fractionated seminal plasma specimens, at protein concentrations of 1.1 mg/ml, 1.0 mg/ml, and 0.9 mg/ml, respectively, were adjusted to pH 5.0 and treated with Pefabloc at each of these concentrations at 37°C for 1 h. Figure 1 (solid circles) depicts the representative results obtained from these specimens, which reveal that the inhibition of latent TGF-β_1 activation by Pefabloc was in a dose-dependent manner.

In addition to ELISA, cold competitive radioreceptor binding-inhibition assay was carried out to ascertain that the released immunoreactive mature TGF-β_1 from latent TGF-β_1 as inhibited by Pefabloc was biologically active mature TGF-β_1. Seminal plasma specimens were again adjusted to pH 5.0 and treated with Pefabloc at the concentrations of 0.2, 1, and 5 mM at 37°C for 1 h, and then subjected to mink lung cell radioreceptor assay. The rationale was that when a lesser amount of seminal plasma latent TGF-β_1 was activated as the result of Pefabloc inhibition, a greater amount of [^{125}I]-TGF-β_1 would be detected to bind to

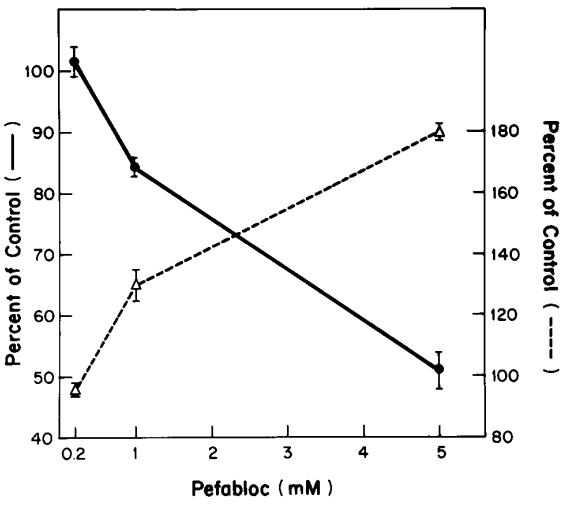

FIG. 1. Effect of Pefabloc on the activation of human seminal plasma latent TGF-β_1 as measured by ELISA and radioreceptor assay. Pefabloc at the final concentrations of 0.2, 1, and 5 mM was added to latent TGF-β_1 preparation that had been adjusted to pH 5.0, and incubated at 37°C for 1 h, and the activity of TGF-β_1 released from latent form was measured by ELISA (solid circles) and by cold competitive radioreceptor binding-inhibition assay (open triangles). The results are shown in mean ± 1 SD from triplicate assays. The experiments were repeated twice with similar results.

TGF-β_1 receptors on the cell surface of mink lung epithelia. As shown in Fig. 1 (open triangles), Pefabloc indeed inhibited the release of biologically active mature TGF-β_1 from latent TGF-β_1. At the concentrations of 0.2, 1, and 5 mM Pefabloc, [^{125}I]-TGF-β_1 radioreceptor assay indicated 96, 135, and 180% bindings, relative to the control (100%) of [^{125}I]-TGF-β_1 binding, to mink lung epithelia. These results obtained from cold competitive radioreceptor binding-inhibition assay confirmed the inhibition of Pefabloc on the activation of latent TGF-β_1 and also validated the ELISA results. The data showing greater than 100% of the control may indicate an inhibition also on the endogenous latent TGF-β_1 of mink lung epithelia origin, which is similar to our previous observation on the inhibition of lymphokine-activated killer cell activity by TGF-β_1 that was synthesized by and secreted from mink lung epithelia (7).

Temperature- and time-dependent manner of inhibition of seminal plasma latent TGF-β_1 activation by Pefabloc. Pefabloc at the concentration of 4 mM was selected for further experiments to determine whether the inhibitory effect of Pefabloc on the proteases responsible for latent TGF-β_1 activation was temperature- and time-dependent. Pefabloc was added to the same series of seminal plasma specimens and incubated at pH 5.0 at 4, 37, and 56°C for 0.5, 1, and 3 h, respectively. As shown in Fig. 2, the released TGF-β_1 activities at 37°C were significantly greater ($p < 0.05$) than those at 56 and at 4°C. Also noted is the result showing that at 37°C a significantly reduced TGF-β_1 activity was always detected in the presence of Pefabloc compared with that in the absence of Pefabloc at all three time points. The released TGF-β_1 activities at 4°C were similar for 0.5, 1, and 3 h of incubation with or without Pefabloc. TGF-β_1 activities detected at 56°C for 0.5, 1, and 3 h of incubation also showed no difference between the presence and absence of Pefabloc. These results indicate that at neither 4 nor 56°C, a measurable Pefabloc-inhibitable protease activity, if any, was involved in the activation of seminal plasma latent TGF-β_1, as the enzymes were either retarded or denatured at these two extreme temperatures.

Figure 2 additionally reveals that the activation of latent TGF-β_1 at 37°C followed a time-dependent course. The longer the incubation time, the greater was the TGF-β_1 activity released from its latent form. Overall, these data further indicate that Pefabloc-in-

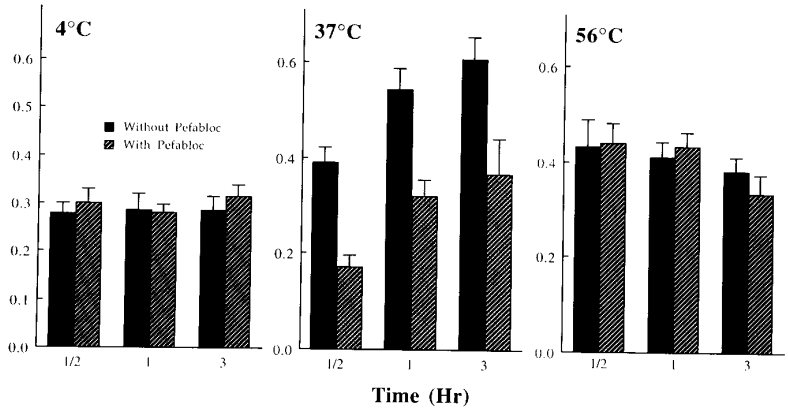

FIG. 2. The temperature- and time-dependent manner of inhibition of human seminal plasma latent TGF-β_1 activation by Pefabloc. Pefabloc at a final concentration of 4 mM was added to latent TGF-β_1 preparation that had been adjusted to pH 5.0, and incubated for 0.5, 1, and 3 h, at 4, 37, and 56°C, respectively. The activity of TGF-β_1 released from latent form was measured by ELISA, and shown in mean ± 1 SD from triplicate assays. The experiments were repeated twice with similar results.

hibitable proteases do participate in the activation of seminal plasma latent TGF-β_1.

Inhibition on activation of seminal plasma latent TGF-β_1 by plasmin, substilisin-like endoproteases, tissue- and urokinase-type plasminogen activators. Pefabloc is a serine protease inhibitor recently available commercially with a moderate range of specificity spectrum. It inhibits trypsin, chymotrypsin, plasmin, kallikrein, substilisin A, thrombin and tPA as listed in the manufacturer's brochure. The inhibitory data as obtained from other protease inhibitors (Table 1) excluded trypsin, chymotrypsin, and thrombin from the proteases that are potential participants in the activation of latent TGF-β_1. Kallikrein, substilisin A, plasmin, and tPA as well as uPA were thus considered the candidate proteases that may play some role in the activation of human seminal plasma latent TGF-β_1.

Two human kallikreins (hk) are present in the prostate and seminal plasma: hk-3 or prostate specific antigen (PSA) of M_r 34 kDa and hk-2 of M_r 30 kDa (19, 20). To determine whether hk-2 and/or hk-3 were the proteases involved in the activation of latent TGF-β_1, Sephacryl S-300 chromatographic fractions, and identified by specific PSA monoclonal antibody (21), containing the M_r range of hk-3 (PSA) and hk-2 were pooled and incubated with latent TGF-β_1 preparation. No release of mature active TGF-β_1 was detected by ELISA. No hk-3 (PSA) immunologic activity was found in the latent TGF-β_1 preparation. Further experiments using purified PSA preparation and recombinant hk-2 preparation also showed negative results on the activation of latent TGF-β_1 (19, 22).

Since substilisins resemble trypsin and chymotrypsin enzymatic activities and human seminal plasma contains a wide variety of proteases (18), substilisins, along with plasmin, tPA and uPA, cannot be ruled out as candidate proteases as a result of the process of elimination as derived from the experiments with proteases inhibitors. Experiments were then performed with additional inhibitors, $ZnCl_2$, $FeSO_4$, ϵ-aminocaproic acid, trasylol, pancreatic trypsin inhibitor, lima bean trypsin inhibitor, and phenol. Table 2 reveals that $ZnCl_2$ and $FeSO_4$, two nonspecific but commonly used inhibitors of plasminogen activators inhibited almost up to 90% of the activation of latent TGF-β_1, whereas substilisin inhibitor and several plasmin inhibitors inhibited only up to 40% of the activation of latent TGF-β_1.

Plasmin is the first protease reported that activates the latent form of TGF-β_1 (4), and the only enzyme so far identified involved in the latent TGF-β_1 activation. Plasmin has been found in human seminal plasma (23). Six substilisin-like endoproteases have been reported in mammalian species (24). Posttranslational endoproteolysis catalyzed by such convertases is essential for the production of biologically active peptides from inactive precursors. Therefore, it is highly likely

TABLE 2

Inhibition of Seminal Plasma Latent TGF-β_1 Activation by Inhibitors of Plasminogen Activators, Plasmin, and Substilisins[a]

Inhibitor	Protease	Concentration[b]	Inhibition[c] (%)
$ZnCl_2$	Plasminogen activators	1.7 mM	30
		5 mM	55
		15 mM	86
$FeSO_4$	Plasminogen activators	0.6 mM	72
		3 mM	87
		15 mM	87
ϵ-Aminocaproic acid	Plasmin	1.5 mM	0
		15 mM	32
Pancreatic trypsin inhibitor	Plasmin	100 μg/ml	36
Lima bean trypsin inhibitor	Plasmin	100 μg/ml	33
Trasylol	Plasmin	15 μM	39
Phenol	Subtilisins	1.5 mM	31
		15 mM	39

[a] Various protease inhibitors at the concentrations shown were incubated with latent TGF-β_1 preparation (200 μl) at pH 5.0 and 37°C for 1 h. The released TGF-β_1 activity was measured by ELISA.

[b] The literature or manufacturers' suggested starting concentrations: $ZnCl_2$ (0.5 mM) (12), $FeSO_4$ (0.5 mM) (12), ϵ-aminocaproic acid (10 μM–5 mM) (13,14), trasylol (15 μM) (15), phenol (50 μM) (16), pancreatic trypsin inhibitor (10–100 μg/ml, per Boehringer-Mannheim), and lima bean trypsin inhibitor (0–100 μg/ml, per Boehringer-Mannheim).

[c] Average of triplicate assays. The experiments were repeated twice with similar results.

that substilisin A, furin, or other substilisin-like endoproteases are also involved in the activation of human seminal plasma latent TGF-β_1. Our data also suggest that either tPA or uPA or both are potentially capable of latent TGF-β_1 activation. Both tPA and uPA are present in human seminal plasma at a mean level of 270 and 6 ng/ml, respectively (25). Approximately 90% of the uPA and 20% of the tPA in seminal plasma are in complexed form with protein C inhibitor, a serine protease inhibitor, which in turn could complex with other serine proteases (26). Also uPA has been found to exhibit a high molecular weight complex at the range similar to that which we have observed in the fractionation of seminal plasma (17), tPA and uPA were the focus of our further experiments.

To test the notion that tPA and uPA are involved in the activation of seminal plasma TGF-β_1, the latent TGF-β_1 preparations were examined for their released TGF-β_1 activity and for their plasminogen activator enzymatic activity followed by incubating with antibodies specific for uPA and tPA, respectively. The results in Table 3 demonstrate that McAb anti-uPA antibody, PcAb anti-uPA and anti-tPA antibodies exhibited inhibitory effect on the activation of seminal plasma latent TGF-β_1. These antibodies exhibited relatively a similar range of inhibition on the activation of

TABLE 3

Inhibition of Seminal Plasma Latent TGF-β_1 Activation by Specific Anti-Urokinase and Anti-Tissue Plasminogen Activator Antibodies

Specimen	Inhibition on TGF-β_1 activation[a] (%)	Inhibition on PA activity[b] (%)
Latent TGF-β_1 preparation[c]	Control	Control
Latent TGF-β_1 preparation + McAb-uPA[d]	15[e]	12
Latent TGF-β_1 preparation + PcAb-uPA[f]	18	15
Latent TGF-β_1 preparation + PcAb-tPA[f]	10	ND[g]
Latent TGF-β_1 preparation + Pefabloc (4 mM)	37	75

[a] By ELISA.
[b] By Spectrozyme-PL assay.
[c] Latent TGF-β_1 preparation (200 μl) was incubated at pH 5.0 without the addition of antibodies or Pefabloc at 37°C for 1 h.
[d] Ten micrograms of McAb 3689 specific for uPA was added to latent TGF-β1 preparation (200 μl) and incubated at pH 5.0 and 37°C for 1 h prior to measurements of TGF-β activity and uPA activity. In a control experiment, 10 μg of McAb 3689 gave greater than 95% inhibition of uPA 0.025 units and 75% of high-molecular-weight uPA complex (27).
[e] Average of triplicate assays. The experiments were repeated twice with similar results.
[f] Goat PcAb specific for uPA (3.4 μg) and 17.8 μg goat PcAb specific for tPA each were added to a latent TGF-β_1 preparation (200 μl) and incubated at pH 5.0 and 37°C for 1 h prior to measurements of TGF-β_1 activity and PA activity. In a control experiment, 3.4 μg of goat PcAb gave greater than 95% inhibition of uPA 0.025 units and 75% of high-molecular-weight uPA complex (27). Goat PcAb tPA also showed similar inhibitory results on tPA enzymatic activity.
[g] Not determined.

latent TGF-β_1 preparation by ELISA (10 to 18%), and on the enzymatic activities of plasminogen activators (12 to 15%). The concentrations of McAb and PcAb are sufficient, of plasminogen activators (12 to 15%). The concentrations of McAb and PcAb are sufficient, since in comparison with purified uPA and tPA controls, these antibodies at the concentrations used inhibited almost quantitatively the plasminogen activator enzymatic activities (27). Pefabloc, used as the positive control in these experiments, was shown as expected to exhibit an inhibition of 37 and 75% on latent TGF-β_1 activation and plasminogen activator enzymatic activity, respectively.

A new finding of the study reported for the first time is on the involvement of substilisin-like endoproteases, tPA and uPA in the activation of seminal plasma latent TGF-β_1. It is noted that after we had finished the experiments and were preparing the manuscript a paper came to our attention. This most recently published report showed that uPA regulates the conversion of latent TGF-β into TGF-β which in turn regulates the activation of basic fibroblast growth factor on smooth muscle cells in culture medium *in vitro* (28). This paper in some way supports the validity of our data demonstrating a role played by uPA on the activation of seminal plasma latent TGF-β_1.

In conclusion, we have shown by simply and systematically using a series of protease inhibitors and specific antibodies for tPA and uPA that plasmin, substilisin-like endoproteases, tPA and uPA are involved in the activation of latent TGF-β_1 in human seminal plasma. The new finding should provide insights into the important role played by this intriguing growth factor in human physiology.

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