Coagulation is initiated by the binding of factor VIIa to tissue factor, with resultant limited factor IX and X activation and thrombin production. Owing to the feedback inhibition of the factor VIIa/tissue factor complex by tissue factor pathway inhibitor (TFPI), additional factor X activation and thrombin generation must proceed through a pathway involving factors VIII, IX, and XI. Experiments designed to elucidate the requirement for amplified factor Xa and thrombin generation in normal hemostasis show that the resistance of plasma clots to tissue plasminogen activator (tPA) and urokinase-induced fibrinolysis is related to the extent of thrombin generation. Inhibition of fibrinolysis is mediated in part by plasma carboxypeptidase-U (CPU), a proenzyme that is proteolytically activated by thrombin in a process enhanced dramatically by the cofactor thrombomodulin. A clot induced in factor IX-deficient plasma with limited amounts of tissue factor in the presence of urokinase (100 U/ml) lyases prematurely, and this defect is corrected by supplementation of the deficient plasma with factor IX (5 μg/ml) or thrombomodulin (20 ng/ml). These additions enhance the rate and extent of CPU activation: in the case of factor IX, presumably by permitting amplified generation of factor Xa and thrombin, and in the case of thrombomodulin, presumably by increasing the degree of CPU activation produced by the low levels of thrombin generated in the absence of factor IX. Pretreatment of the factor IX-deficient plasma with specific anti-CPU antibodies prevents the increased resistance to fibrinolysis produced by addition of factor IX and thrombomodulin. Likewise, when coagulation is induced by thrombin (2 U/ml) in the presence of tPA (60 U/ml), clots formed from plasma deficient in factors VIII, IX, X, or XI lyse prematurely unless the missing factor is replaced or thrombomodulin (20 ng/ml) is added.

From the Division of Hematology, The Jewish Hospital at Washington University Medical Center, St Louis, MO.

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Address reprint requests to George J. Broze, Jr, MD, Division of Hematology, The Jewish Hospital at Washington University Medical Center, 216 S Kingshighway Blvd, St Louis, MO 63110.

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peptidase-N (CPN), that was produced during the coagulation of plasma. Its instability in serum at 37°C led to the designation carboxypeptidase-U (unstable). Shortly thereafter, Campbell et al.1,2 confirmed these studies and referred to the enzyme as carboxypeptidase-R, since it appeared to prefer arginine over lysine residues at the carboxy terminus of substrates. In 1991, Eaton et al.3 isolated a carboxypeptidase proenzyme from plasma based on its ability to bind to a plasminogen affinity column, and called it procarboxypeptidase-B. They noted that the purified carboxypeptidase was proteolytically cleaved by trypsin, thrombin, and plasmin with the apparent release of an amino-terminal activation peptide, and that the activated enzyme was unstable. Later, Wang et al.4 reported that the carboxypeptidase isolated by Eaton et al. and the previously identified CPU were the same, and that the enzyme was present in preparations of plasminogen isolated by lysine-Sepharose affinity chromatography.

As the current study was in progress, Bajzar et al.5 reported that CPU is a thrombin-activatable inhibitor of fibrinolysis and renamed the molecule TAFI. Their investigation was prompted by their previous studies showing that the enhanced fibrinolysis produced by activated protein C was related to its reduction in ultimate thrombin generation,6 and by the observation that this thrombin-mediated inhibition of fibrinolysis required a component that was a contaminant in preparations of purified plasminogen.7,8 In a recent preliminary report, Bajzar and Nesheim9 have shown that thrombomodulin enhances thrombin activation of CPU greater than 1,000-fold. Here, we show that thrombin-dependent inhibition of fibrinolysis mediated through the action of CPU may in part explain the requirement for factors VIII, IX, and XI for sustained hemostasis.

**MATERIALS AND METHODS**

Sodium dodecyl sulfate (SDS), Trizma base, HEPES, EDTA, diisopropylfluorophosphate (DFP), Tween 20, Fast-Flow, phenyl-Sepharose, heparin-agarose, arginine-agarose, ε-aminocaproic acid (EACA), rabbit brain cephalin, and aprotinin were purchased from Sigma Chemical Co (St Louis, MO). The plasminogen affinity column was produced during the coagulation of plasma based on its ability to bind to a plasminogen affinity column, and called it procarboxypeptidase-B. The specific carboxypeptidase-B inhibitor DL-2-mercaptomethyl-3-guanidinethyl-thiopropanoic acid (MERGETPA) and the thrombin inhibitor H-D-phenylalanyl-L-propyl-arginine chloromethyl ketone (PPACK) were from Calbiochem (La Jolla, CA). Affigel-10 was purchased from Bio-Rad Laboratories (Hercules, CA).

**Plasmas.** Barium-absorbed plasma (BAP) was produced from fresh venepuncture blood drawn into 100 mmol/L sodium oxalate (9:1 vol/vol). Following removal of cellular elements by centrifugation (4,000 g for 10 minutes at room temperature), the plasma was absorbed twice with barium sulfate (100 mg/mL at 4°C), dialyzed extensively against 0.15 mol/L NaCl (HBS), pH 7.4, at 4°C, and stored in small aliquots at −70°C. To produce EDTA/BAP, BAP was treated with 0.1 mol/L EDTA for 30 minutes at 37°C and then dialyzed extensively against HBS at 4°C before storage in aliquots at −70°C.

Pooled normal human plasma and plasmas deficient in factors VIII, IX, X, XI, and XII were obtained from George King Biomedical (Overland Park, KS).

**Proteins.** Prothrombin, factor X, and factor IX were purified from fresh frozen plasma, and thrombin (3,500 IU/mg) was produced from purified prothrombin as previously described.10,11 Two-chain tissue plasminogen activator (tPA; 700,000 IU/mg) was obtained from American Diagnostica Inc (Greenwich, CT). Plasminogen was purified from human plasma using lysine-Sepharose and linked to Affigel-10 at a concentration of 5 mg/mL settled gel by the manufacturer’s method. CPN was purified using the method of Hendriks et al.12 Urokinase was the therapeutic product, Abbot kinase, from Abbott Laboratories (Chicago, IL). Following reconstitution of the lyophilized product with water, each milliliter contains 50,000 IU urokinase, 0.5% mannitol, 5% human albumin, and 1% sodium chloride. The factor VIII source was Monoclate-P (Armour, Collegeville, PA). Tissue factor was crude preparation of human brain thromboplastin that had been washed extensively with EDTA.13 A 75-kD partially glycosylated form of soluble human thrombomodulin that lacks the transmembrane and cytoplasmic domains of the native molecule was a kind gift from John Parkinson (Berks Biosciences, Richmond, CA).

**Isolation of CPU.** CPU activity was followed during the purification procedure using a qualitative assay for thrombin-dependent inhibition of fibrinolysis (see below). Fresh blood (1 U) was drawn by venipuncture into sodium oxalate (100 mmol/L, 9:1 vol/vol) and centrifuged at room temperature (4,000 g for 10 minutes) to remove the blood cells. Aprotinin (10 KIU/mL) and DFP (1 mmol/L) were added to the supernatant plasma, and it was stirred with barium sulfate (100 mg/mL) at 4°C for 30 minutes. Following centrifugation (10,000 g for 30 minutes at 4°C), ammonium sulfate (30% saturation) was added to the absorbed plasma, and the mixture was stirred for 60 minutes at 4°C. After centrifugation (10,000 g for 30 minutes at 4°C), the supernatant was dialyzed extensively against 0.01 mol/L HEPES, pH 7.4, at 4°C. The preparation was then applied at room temperature to a 2.5 × 55-cm column of Q Fast-Flow equilibrated in 0.01 mol/L HEPES, pH 7.4, and the column was developed with a 1.6-L gradient from 0 to 0.5 mol/L NaCl. Fractions containing...
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Fig 2. Effect of thrombin generation on fibrinolysis. Lysis of BAP clots formed with factor Xa (500 ng/mL) and variable concentrations of prothrombin or with variable concentrations of thrombin using 100 IU/mL urokinase (A) or tPA (B) in the fibrinolysis assay. Top figure in each panel shows final prothrombin concentrations (µg/mL): 50 (●), 25 (●), 12.5 (●), 6.25 (●), 3.125 (●), and 0.39 (●). Bottom figure in each panel shows final thrombin concentrations (U/mL): 150 (●), 75 (●), 37.5 (●), 15 (●), 4.9 (●), and 1.2 (●).

CPU activity, which eluted between the transferrin and albumin peaks (~0.12 mol/L NaCl), were pooled and applied to a heparin-Sepharose column (50 mL) equilibrated in 0.1 mol/L NaCl/0.01 mol/L HEPES, pH 7.4. After washing with equilibration buffer, the column was eluted with 1.0 mol/L NaCl/0.01 mol/L HEPES, pH 7.4. Fractions containing CPU activity were pooled and applied to a 30-mL column of phenyl-Sepharose equilibrated in 1.0 mol/L NaCl/0.01 mol/L HEPES, pH 7.4. After washing with equilibration buffer, the CPU was eluted with 0.01 mol/L HEPES, pH 7.4. Pooled fractions following phenyl-Sepharose chromatography were applied to a 30-mL column of phenyl-Sepharose equilibrated in 1.0 mol/L NaCl/0.01 mol/L HEPES, pH 7.4. After washing with equilibration buffer, the CPU was eluted with 0.01 mol/L HEPES, pH 7.4. Pooled fractions following phenyl-Sepharose chromatography were applied to a plasminogen-agarose affinity column (4 mL) equilibrated in HBS at a flow rate of 8 mL/h. Following washing with equilibration buffer, the CPU was eluted with a 50-mL gradient from 0 to 0.05 mol/L EACA in HBS with 0.05% Tween 20. Fractions with activity were combined, the pool was concentrated (Amicon YM 10), and aliquots were stored at -70°C. By 12% SDS-PAGE, the isolated CPU appears more than 90% pure and is recognized on Western blot by an antipeptide antibody raised against amino acid residues 156 to 168 of mature CPU (Fig 1). Before testing in fibrinolysis assays, EACA was removed from the CPU preparation by extensive dialysis. The CPU concentration was estimated by absorbance at 280 nm assuming ε280 = 10. Purified CPU was radiolabeled with 125I to a specific activity of 5,000 dpm/ng using Iodo-Beads (Pierce, Rockford, IL).

Qualitative assay for inhibition of fibrinolysis. Immulon-2 flat-bottom microtiter plates (Dynatech Laboratories Inc, Chantilly, VA), which do not support contact activation or factor XI autoactivation (Gailani and Broze, unpublished observations, January 1993), are used for the assay. To a microtiter plate well are added 10 µL thrombin solution, 100 µL of a mixture containing rabbit brain cephalin, calcium chloride, and urokinase or tPA, typically 60 to 125 IU/mL; and calcium chloride, 5 mmol/L for dialyzed BAP and EDTA/BAP, 12.5 mmol/L for citrated plasma samples. In certain experiments, thrombin was omitted and coagulation initiated with tissue factor. Subsequent clot lysis was followed at A405 for 2 to 4 hours using...
thrombin (150 Ul/mL). At various times, EDTA/BAP incubated without metal ions (A), 25 μmol/L cobalt (•), 25 μmol/L manganese (▲), 150 μmol/L manganese (▲), and 150 μmol/L magnesium (▲). EDTA/BAP (A), BAP (▲), EDTA/BAP (▲), and EDTA/BAP regenerated with zinc ions (25 μmol/L, ▲) were tested in the fibrinolysis assay with urokinase (100 IU/mL) and thrombin (150 Ul/mL). At various times, 50 μL S2390 (2 mmol/L) was layered over the clot, and the rate of A405 generation was determined over 10 minutes.

Other. SDS-PAGE and Western blotting were performed as previously described.25 Rabbit polyclonal antibodies against the synthetic peptide CGHAREWISP and purified CPU were produced using standard techniques.25 To measure plasmin activity at the surface of a clot in a microtiter well, at specified times 50 μL S2390 (2.0 mmol/L) is layered on top of the fibrin gel, and the rate of change in A405 is determined over 10 minutes.

RESULTS

Coagulation-dependent inhibition of fibrinolysis. To determine whether the extent of the coagulation process affects subsequent fibrinolysis, factor X–deficient plasma with or without supplementation with purified factor X was induced to clot in microtiter plates with thrombin (4 U/mL), tissue factor (1/1,000 vol/vol), phospholipids, and calcium ions in the presence of 100 IU/mL urokinase or tPA. Fibrinolysis was assessed by the reduction in turbidity (A405) of the plasma clots to a baseline value. Factor X–deficient plasma replenished with purified factor X is resistant to fibrinolysis over the 2-hour time course of the experiment, whereas factor X–deficient plasma lysed prematurely (not shown). To confirm a role for thrombin generation in the inhibition of fibrinolysis, fresh oxalated normal plasma was absorbed with barium sulfate to remove the vitamin K–dependent coagulation factors (including factors VII, IX, X, and prothrombin) and dialyzed into HBS. This BAP was then tested in the fibrinolysis assay using factor Xa (500 ng/mL) with varying concentrations of prothrombin to limit the extent of thrombin generation, or using varying concentrations of thrombin itself to induce coagulation. Increasing the concentrations of prothrombin or thrombin leads to progressive lengthening of fibrinolysis time (Fig 2). The active catalytic site of thrombin is important for thrombin-dependent inhibition of fibrinolysis, since addition of high levels of PPACK-inactivated thrombin (50 μg/mL) to clots formed with 4 U/mL active thrombin does not increase their resistance to fibrinolysis (not shown).

Divalent cation dependence. Preliminary studies showed that the thrombin-dependent inhibition of fibrinolysis is divalent cation–dependent. Pretreatment of BAP with EDTA (0.1 mmol/L) at 37°C for 30 minutes abrogates the anti-fibrinolytic effect of high thrombin concentrations even though calcium ions in excess of the EDTA chelation are provided within the fibrinolysis assay. To investigate the metal dependence of thrombin-dependent inhibition of fibrinolysis, BAP was treated with EDTA (0.1 mmol/L for 30 minutes at 37°C) and dialyzed extensively against HBS. This EDTA-inactivated, dialyzed BAP (EDTNBAP) was then tested in the fibrinolysis assay following incubation with various metal ions at 37°C for 30 minutes. Regeneration of thrombin-dependent inhibition of fibrinolysis in the BAP occurs with nonphysiologic concentrations of cobalt and manganese ions (≥50 μmol/L) and with physiologic concentrations of zinc ions (25 μmol/L) (Fig 3A).

The time course of plasmin production in BAP, EDTA/BAP, and EDTA/BAP regenerated with zinc chloride (25 μmol/L) was assessed by overlaying the clots formed in the fibrinolysis assay at various times with a solution containing...
Fig 4. Effect of purified CPU and CPN on the inhibition of fibrinolysis. EDTA/BAP was supplemented with CPU (5 μg/mL) or CPN (25 μg/mL) before testing in the fibrinolysis assay with urokinase (100 IU/mL) and thrombin (4 or 150 U/mL). EDTA/BAP without supplementation and 4 U/mL (□) or 150 U/mL (●) thrombin; EDTA/BAP with CPU and 4 U/mL (○) or 150 U/mL (●) thrombin; EDTA/BAP with CPN and 4 U/mL (●) or 150 U/mL (△) thrombin.

The generation of plasmin-mediated amidolytic activity at the clot surface is much slower in clots produced from BAP and EDTA/BAP regenerated with 25 μmol/L zinc ions than in EDTA/BAP clots. Thus, thrombin-dependent inhibition of fibrinolysis appears to be due at least in part to the reduced generation of plasmin activity within the clot.

Role of CPU. The metal dependence of the antifibrinolytic activity, its relationship to a reduction in plasmin generation, and its abrogation by the carboxypeptidase-B inhibitor MERGETPA (not shown) are consistent with the role of a carboxypeptidase-B–like enzyme in the process.

Two zinc-containing carboxypeptidases present in plasma have been studied in depth. CPN is a 280,000-MW tetrameric complex of two 83,000-MW noncatalytic heavy chains and two 50,000-MW catalytic light chains. It circulates in plasma (~25 μg/mL) in an active form and is believed to play an important role in modulating the activity of certain mediators of inflammation, for example, bradykinin and kallidin, and complement C3a and C5a. CPU is an approximately 62,000-MWzymogen (Fig 1) that circulates in plasma at an estimated concentration of 5 μg/mL. Its activity is increased following the coagulation of plasma. Addition of purified CPU (5 μg/mL), but not CPN (25 μg/mL),
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Fig 6. Activation of $^{125}$I-CPU by thrombin. A mixture containing $^{125}$I-CPU (2 μg/mL), thrombin (10 μg/mL), phospholipids (1:40 of stock), calcium ions (4 mmol/L), and zinc ions (15 μmol/L) was incubated at room temperature. At the specified times, a portion was removed and the reaction was terminated with an equal volume of 6 mol/L urea, 10% 2-mercaptoethanol, and 10% SDS, and heating at 100°C for 5 minutes. Samples (10 μL) were assayed on SDS-PAGE (15%), and the gel was dried before autoradiography. Lane 1, 1 minute; lane 2, 5 minutes; lane 3, 10 minutes; and lane 4, 20 minutes. MW markers in kilodaltons are shown at right.

to EDTA/BAP reestablishes thrombin-dependent inhibition of fibrinolysis (Fig 4).

Fibrinolysis in factor-deficient plasma. Factor IX–deficient plasma induced to clot with low levels of tissue factor (1:20,000 vol/vol) in the presence of urokinase (100 U/mL) lyses prematurely. Factor IX–deficient plasma supplemented with factor IX (5 μg/mL), thrombomodulin (20 ng/mL), or EACA (1.0 mmol/L), on the other hand, is resistant to fibrinolysis under the same conditions (Fig 5A). The inclusion of rabbit polyclonal anti-CPU IgG, but not preimmune rabbit IgG (not shown), in the mixtures hastens the lysis of clots induced in factor IX–deficient plasma by low levels of tissue factor and abrogates the resistance to fibrinolysis produced by the addition of factor IX and thrombomodulin (Fig 5B).

In similar reactions, radiolabeled CPU was added to the factor IX–deficient plasma, and the time course of $^{125}$I-CPU cleavage was examined by SDS-PAGE and autoradiography. Proteolytic activation of CPU by thrombin or plasmin involves the removal of the amino-terminal portion of the zymogen with the production of a 35,000-MW activated form (CPUa) (Fig 6). However, alternative cleavage at a site within the carboxy-terminal domain of CPU and CPUa leads to degradation products of 53,000 and 25,000 MW, respectively. Addition of factor IX (5 μg/mL) to factor IX–deficient plasma leads to earlier, presumably thrombin-mediated $^{125}$I-CPU activation (Fig 7B vs C). Supplementation of factor IX–deficient plasma with thrombomodulin (20 ng/mL) produces more rapid and more extensive $^{125}$I-CPU activation (Fig 7D). The clot formed in reactions containing factor IX–deficient plasma alone lyses prematurely (Fig 7B), and much of the apparent activation and degradation of $^{125}$I-CPU that occurs late in these reactions may be due to the action of plasmin (Fig 7A and B).

In additional experiments, the fibrinolysis inhibition generated in several factor-deficient plasmas was tested using a simplified system in which thrombin (2 U/mL) rather than tissue factor is used to initiate coagulation, and tPA (60 U/mL) instead of urokinase is used to initiate fibrinolysis. Un-
Fig 8. Fibrinolysis of thrombin-induced clots in factor-deficient plasmas. Plasmas deficient in factor X (•), IX (■), VIII (▲), XI (◆), and XII (△) and normal pooled plasma (○) were tested in the fibrinolysis assay with tPA (60 IU/mL) and thrombin (2 U/mL). (A) No additions; (B) Supplementation of factor X-deficient plasma with factor X (8 μg/mL, ▲), factor IX-deficient plasma with factor IX (5 μg/mL, ■), and factor XI-deficient plasma with factor XI (5 μg/mL, ◆). Normal plasma (○). (C) Addition of thrombomodulin (20 ng/mL) to each of the plasmas tested in (A). The curve representing lysis of the factor X-deficient clot from (A) is reproduced in (B) and (C) for reference.

Under these conditions, the premature lysis of clots from factor X-, IX-, VIII-, and XI-deficient plasmas is also corrected by addition of the missing coagulation factor or addition of thrombomodulin (20 ng/mL), in part confirming the previous results of others. The experiments reported here, initiated in an attempt to understand the hemostatic requirement for the amplified and sustained generation of factor Xa and thrombin produced through the actions of factors VIII, IX, and XI, also support a role for CPU in coagulation-dependent inhibition of fibrinolysis.

Interestingly, thrombomodulin serves as a cofactor for thrombin activation of both protein C and CPU. In the in vitro plasma fibrinolysis system used here, the effect of soluble thrombomodulin on the activation of CPU appears to be predominant. Whether the same is true for cell-associated thrombomodulin or whether other plasma cofactors may modulate the extent or timing of protein C and CPU activation by thrombin/thrombomodulin is not known.

CPU-dependent inhibition of fibrinolysis is associated with a decrease in the generation of plasmin activity within the fibrin clot (Fig 3). Previous studies have shown that

**DISCUSSION**

CPU was discovered several years ago as a carboxypeptidase-B-like activity that appeared following the coagulation of plasma, but it was not until the report by Bajzar et al. that a potential physiologic function of CPU was identified. Prompted by their observation that the apparent profibrinolytic effect of activated protein C was related to its reduction in thrombin generation, the researchers identified CPU as a thrombin-activatable inhibitor of fibrinolysis (TAFI). The experiments reported here, initiated in an attempt to understand the hemostatic requirement for the amplified and sustained generation of factor Xa and thrombin produced through the actions of factors VIII, IX, and XI, also support a role for CPU in coagulation-dependent inhibition of fibrinolysis.

Interestingly, thrombomodulin serves as a cofactor for thrombin activation of both protein C and CPU. In the in vitro plasma fibrinolysis system used here, the effect of soluble thrombomodulin on the activation of CPU appears to be predominant. Whether the same is true for cell-associated thrombomodulin or whether other plasma cofactors may modulate the extent or timing of protein C and CPU activation by thrombin/thrombomodulin is not known.

CPU-dependent inhibition of fibrinolysis is associated with a decrease in the generation of plasmin activity within the fibrin clot (Fig 3). Previous studies have shown that
limited plasmin degradation of fibrin produces polypeptides with carboxy-terminal basic residues, thereby increasing plasminogen binding and the rate of plasminogen activation by both urokinase and tPA.\textsuperscript{27,29} Treatment with pancreatic carboxypeptidase-B abrogates this feedback enhancement of fibrinolysis by removing the carboxy-terminal basic residues from the fibrin polypeptides produced by plasmin.\textsuperscript{29} Whether the action of CPUa involves a similar mechanism, as recently suggested by Redlitz et al.,\textsuperscript{29} and/or is mediated by other antifibrinolytic effects of CPU is not known. Furthermore, the coagulation process may affect subsequent fibrinolysis through means unrelated to CPU.

The in vitro fibrinolysis assay appears to demonstrate an important abnormality of clots produced with hemophilic plasma that may be related to the delayed bleeding seen in individuals with hemophilia, particularly from wounds at sites with high intrinsic fibrinolytic activity. When limiting concentrations of tissue factor are used to initiate coagulation in factor IX–deficient plasma in the presence of a plasminogen activator, the subsequent clot lyses prematurely. This defect can be reversed by supplementation of the deficient plasma with factor IX or by addition of thrombomodulin (Fig 5). Other manipulations, such as decreasing the plasma of TFPI, adding factor VIIa (5 pg/mL), or increasing the plasma that may be related to the delayed bleeding seen in factor IX-deficient plasma in the presence of a plasminogen activator, the subsequent clot lyses prematurely. This defect can be reversed by supplementation of the deficient plasma with factor IX or by addition of thrombomodulin (Fig 5). Other manipulations, such as decreasing the plasma of TFPI, adding factor VIIa (5 pg/mL), or increasing the concentration of tissue factor used to initiate coagulation,\textsuperscript{36-38} also prolong the lysis time of clots formed with factor IX plasma (Broze and Higuchi, unpublished data, May 1995). Most of these interventions lead to increased factor Xa and thrombin production. However, thrombomodulin presumably does not enhance the tissue factor–mediated generation of factor Xa and thrombin in factor IX–deficient plasma, but may instead increase the activation of CPU by the low concentrations of thrombin formed under these conditions.\textsuperscript{17}

REFERENCES


11. Campbell W, Okada H: An arginine specific carboxypeptidase generated in blood during coagulation or inflammation which is unrelated to carboxypeptidase N or its subunits. Biochem Biophys Res Commun 162:933, 1989


33. von dem Borne PAK, Meijers JCM, Bouma BN: Feedback activation of factor XI by thrombin in plasma results in formation of additional thrombin that protects fibrin clots from fibrinolysis. Blood 86:3035, 1995
Coagulation-dependent inhibition of fibrinolysis: role of carboxypeptidase-U and the premature lysis of clots from hemophilic plasma

GJ Jr Broze and DA Higuchi