Conjugation to Antifibrin Fab' Enhances Fibrinolytic Potency of Single-Chain Urokinase Plasminogen Activator

Christoph Bode, MD, Marschall S. Runge, MD, PhD, Sabine Schönermark, MD, Tilman Eberle, John B. Newell, BA, Wolfgang Kübler, MD, and Edgar Haber, MD

Single-chain urokinase plasminogen activator (scu-PA) that had been modified with N-succinimidyl-3-(2-pyridyldithio)propionate was covalently linked by disulfide bonds to the Fab' of a monoclonal antibody specific for the β-chain of fibrin (antibody 59D8). scu-PA–59D8 Fab' conjugate was separated from free scu-PA and two-chain urokinase coupled to 59D8 Fab' by two-step affinity chromatography. First, the reaction mixture was chromatographed on a column containing Sepharose linked to the peptide that had been used as immunogen for antibody 59D8; scu-PA–59D8 Fab' conjugate and unconjugated 59D8 Fab' were retained but not unconjugated scu-PA. Then, the eluate from the peptide-Sepharose column was chromatographed on a column containing Sepharose linked to benzamidine, which acts as a ligand for two-chain urokinase. The molecular weight of the scu-PA–59D8 Fab' conjugate was approximately 100 kDa when electrophoresed on a nonreducing sodium dodecylsulfate-polyacrylamide gel. Enzymatic assay after purification revealed that more than 97% of the scu-PA present in the conjugate retained the single-chain form. The Fab' portion of the conjugate functioned in a manner indistinguishable from that of native antibody 59D8. In an in vitro assay for lysis of fibrin monomer, the fibrinolytic potency of scu-PA–59D8 Fab' was 33-fold more than that of tissue plasminogen activator (p<0.001), 230-fold more than that of unconjugated scu-PA (p<0.0001), and 420-fold more than that of urokinase (p<0.0001). In a human plasma clot assay, scu-PA–59D8 Fab' was significantly more potent than native scu-PA in clot lysis and consumed less fibrinogen at equipotential fibrinolytic concentrations. Potency in vivo, measured in the rabbit jugular vein thrombus model, increased by 29-fold. Thus, conjugation to a fibrin-specific Fab' appears to increase the fibrin affinity and fibrinolytic potency of scu-PA. (Circulation 1990:81;1974–1980)

When plasminogen activator therapy is instituted within 4–6 hours of the onset of symptoms of acute myocardial infarction, there is a significant reduction in morbidity and mortality.1,2 The first generation of plasminogen activators, streptokinase and urokinase, are effective but lack fibrin and hence clot specificity. Second-generation plasminogen activators tissue-type plasminogen activator (t-PA) and single-chain urokinase plasminogen activator (scu-PA) are relatively fibrin specific in vitro3–5 and in vivo.5,6 In patients, however, the administration of t-PA or scu-PA, like that of streptokinase, can also result in hemorrhagic complications.2,7

One approach to improving the specificity of currently available thrombolytic agents is to increase their fibrin affinity by conjugation to an antifibrin antibody. We have studied conjugates between antifibrin monoclonal antibody 59D8 and urokinase8,9 and between 59D8 and t-PA.10,11 Conjugation markedly enhances the thrombolytic potency of the two plasminogen activators, both in vitro and in vivo.

scu-PA is perhaps the most promising plasminogen activator to which direct fibrin specificity could be added. Native scu-PA is fibrin selective, but this selectivity derives from the activation of fibrin-bound plasminogen. Although the mechanism of activation is still not fully understood, it has been established that scu-PA’s direct affinity for fibrin is minimal.12,13 Thus, a conjugate consisting of scu-PA and an anti-
body that contributes high fibrin affinity could combine the fibrin-selective mechanisms of both molecules and thereby constitute a better thrombolytic agent. We report the production and characterization of a scu-PA–59D8 Fab' conjugate and its comparison with urokinase, scu-PA, and t-PA.

**Methods**

Human glycosylated scu-PA that had been highly purified from the conditioned medium of the transformed kidney cell line TCL-598 and stabilized with human albumin was supplied by Sandoz GmbH (Nuremberg, FRG). Low molecular weight two-chain urokinase (urokinase) was a gift from Medac GmbH (Hamburg, FRG). Recombinant t-PA (Actilyse R) was bought from Thomae GmbH (Biberach, FRG). L-Pyroglutamyl-glycyl-l-arginine-p-nitroanilide-trihydrochloride (S-2444), a chromogenic substrate for urokinase, was obtained from KabiVitrum, as was H-D-isoleucyl-l-prolyl-l-arginine-p-nitroanilide-diiodohydrochloride (S-2288). N-Succinimidyl-3-(2-pyridyldithio)propionate (SPDP) was purchased from Pierce Chemical (Rockford, Illinois), and iodine 125-labeled fibrinogen (IBRIN) was purchased from Amersham (Arlington Heights, Illinois). Fresh-frozen plasma was purchased from the University of Heidelberg blood bank. Plasminogen was obtained from the fresh-frozen plasma on lysine-Sepharose according to established procedures. Affinity-purified polyclonal rabbit anti-human albumin immunoglobulin was purchased from Boehringer Mannheim, FRG. p-Aminobenzamidine from Sigma Chemical (St. Louis, Missouri) was coupled to CH-Sepharose 4B from Pharmacia (Piscataway, New Jersey), as described by Holmberg et al. All other chemicals came from Sigma Chemical.

**Preparation and Purification of Monoclonal Antibody 59D8 and Its Fab'**

The identification and characterization of antifibrin monoclonal antibody 59D8 have been described. In short, the antibody was purified from mouse ascites by affinity chromatography on a column of Sepharose-conjugated Gly-His-Arg-Pro-Leu-Asp-Lys-Cys (β-peptide, which corresponds to the seven amino-terminal residues of the β-chain of human fibrin). The antibody was eluted from the affinity matrix with 0.2 M glycine (pH 2.8) and collected into tubes containing a neutralizing amount of 3 M Tris-HCl (pH 8.6). After the antibody had been dialyzed against phosphate-buffered saline azide (PBSA; 0.01 M sodium phosphate, 0.15 M NaCl, 0.02% NaN3, pH 7.4), its purity was assayed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein concentrations were assayed according to Lowry et al with bovine serum albumin used as standard or by measuring optical density at 280 nm.

59D8 Fab' was prepared by digesting the antibody with pepsin. Ten milliliters of antibody solution (2.0 mg/ml in PBSA) was mixed with 1 ml of 1.0 M sodium citrate (pH 2.75) and 1 ml of pepsin solution (0.3 mg/ml in water), the final pH was adjusted to 3.5, and the mixture was incubated for 2 hours at 37°C. The reaction was stopped by the addition of 1.0 ml of 3.0 M Tris/HCl, pH 8.5. (Fab'), was purified from the digest by affinity chromatography on β-peptide-Sepharose as described. The eluate was dialyzed extensively against 0.1 M sodium phosphate (pH 6.8) and reconstituted in an Amicon ultrafiltration chamber to 2 mg/ml. Reduction of the 59D8 (Fab')2 was carried out at room temperature for 3 hours in 1 mM 2-mercaptoethanol, 1 mM EDTA, and 10 mM sodium arsenite, followed by the addition of solid Ellman’s reagent to a concentration of 5 mM. After 30 minutes at room temperature, excess reagent was removed by gel filtration on a Sephadex G-25 column (30×2 cm) equilibrated with 0.1 M sodium phosphate (pH 6.8). In this protected form, 59D8 Fab' has remained structurally and functionally intact at 4°C under sterile conditions for at least 1 year. The thiol form of 59D8 Fab' was easily regenerated by treatment with 10 mM 2-mercaptoethanol for 30 minutes at room temperature, followed by gel filtration.

**Purification of scu-PA**

scu-PA was separated from human serum albumin stabilizer by affinity chromatography on a cyanogen bromide–activated Sepharose 4B column to which polyclonal rabbit anti-human albumin antibody had been coupled. The scu-PA–albumin mixture was repeatedly chromatographed until the scu-PA in the flow-through fraction was electrophoretically pure.

**Electrophoresis**

SDS-PAGE was performed according to the method of Laemmli, and proteins were visualized by staining with Coomassie brilliant blue R.

**Preparation and Purification of scu-PA–59D8 Fab'**

scu-PA (7.5 ml) in NaPi buffer (2.0 mg/ml in 0.14 M NaCl, 3.7 mM sodium phosphate, 1 mM KCl, pH 7.4) was mixed with SPDP (20 mM in absolute ethanol, fivefold molar excess added dropwise), and the reaction was allowed to proceed for 30 minutes at room temperature. The reaction mixture was then dialyzed overnight against several changes of 0.1 M sodium phosphate (pH 6.8). Analysis for 2-pyridyl disulfide content showed 0.8–1.6 residues per scu-PA molecule. The substitution level was kept intentionally low to interfere as little as possible with the specific properties of scu-PA. The thiol form of 59D8 Fab' (8.0 ml at 1.1 mg/ml in 0.1 M sodium phosphate, pH 6.8) was mixed with SPDP-modified scu-PA and allowed to react for 20 hours at room temperature. To minimize the amount of uncoupled 59D8 Fab' in the final preparation, scu-PA was present in the mixture in a twofold molar excess. The reaction was stopped by the addition of a 100-fold molar excess (compared with the protein concentra-
tions) of iodoacetamide in 0.1 M sodium phosphate (pH 8.0).

The scu-PA–59D8 Fab’ conjugate was partially purified from the reaction mixture by sequential affinity chromatography in two steps. The mixture was first applied to β-peptide–Sepharose, which retained conjugate and unconjugated 59D8 Fab’ but not uncoupled scu-PA. After neutralization, the conjugate was dialyzed into 0.1 M sodium phosphate (pH 6.8) and applied to Sepharose containing immobilized benzamidine to remove any two-chain urokinase plasminogen activator (tcu-PA)–59D8 Fab’ conjugate that had been generated during the coupling reaction. The fall-through of this column was concentrated and stored at 4°C. The yield of 59D8 Fab’-conjugated scu-PA was on average about 5% the starting amount of scu-PA. This low yield probably resulted from both the intrinsic limitations of the coupling chemistry and our intentional limitation of the degree of scu-PA substitution (see previous paragraph).

Quantitation of Urokinase, Latent scu-PA, and t-PA Activities

scu-PA (100 μl) was incubated for 45 minutes at 37°C with 100 μl plasmin (2.5 μM in water; final assay concentration, 250 nM) and 800 μl of activation buffer (0.038 M NaCl, 0.05 M Tris, 0.1% bovine serum albumin, pH 7.4). A portion of this mixture (100 μl) was removed and incubated with 800 μl of assay buffer (0.038 M NaCl, 0.05 M Tris, 0.01% bovine serum albumin, 200 IU aprotinin/ml, pH 8.8) and 100 μl of chromogenic substrate S-2444 (final concentration, 0.3 mM). The activity of urokinase, scu-PA (after activation with plasmin), or scu-PA–59D8 Fab’ was measured by continuous monitoring of the absorbance change at 405 nm and expressed in international units (based on the initial rate) by comparing the amidolytic activity of the test substance with that of a freshly resuspended reference vial of urokinase (Medac-Urokinase). The complete structural conversion of scu-PA to tcu-PA under these conditions was confirmed by SDS-PAGE. Quantitation of t-PA activity and comparison with urokinase activity by assay with chromogenic substrate S-2888 has been described in detail.10

Fibrin Monomer–Sepharose Assay

The preparation of 125I-labeled fibrin–Sepharose has been described.22 To assess their fibrinolytic activities, urokinase, t-PA, unconjugated scu-PA, and scu-PA–59D8 Fab’ (units as indicated in 100 μl) were each incubated with 100 μl fibrin–Sepharose for 4 hours. The Sepharose was washed first with 3 ml of a solution containing 0.1 M Tris, 0.1 M NaCl, 0.5% Triton x-100, 0.1% tween 80, and 0.5% bovine serum albumin and then washed three times with 3 ml of 0.1 M Tris, 0.1 M NaCl, and 0.02% NaN3 (pH 7.4) to remove nonspecifically adsorbed plasminogen activator. Thereafter, the affinity matrix was incubated at room temperature with 1 ml of purified plasminogen (0.15 mg/ml in 0.05 M phosphate buffer, pH 7.4). After 15 hours, the mixture was centrifuged, and radioactivity in the supernatant was measured in a gamma counter. The percentage of fibrin lysis was expressed as the ratio of radioactivity present in the supernatant to total initial radioactivity. The results were analyzed by the FIT-FUNCTION program.23

Fibrinogen Assay

The fibrinogen content of samples of citrated human plasma was determined according to Clauss.24

Plasma Clot Assay

The method of Lijnen et al25 was used with some modifications; 5 units of fresh-frozen human plasma obtained from the local blood bank was pooled, aliquoted, and refrozen. Immediately before each experiment, the activities of the various plasminogen activators were calibrated with the S-2444 assay. The amidolytic activities of the activators were determined and related to a standardized concentration of urokinase, and appropriate dilutions were made so that the peptidase activity (units/ml) was identical for each sample. Plasma clots were made by adding to plasma, in the following sequence, 125I-labeled human fibrinogen (100,000 cpm/ml plasma), 0.5 M CaCl2 (final concentration in plasma, 0.05 M), and thrombin (8 NIH units/ml plasma). Immediately after the addition of thrombin, the solution was drawn into Silastic tubing (i.d., 4 mm) and allowed to clot for 30 minutes at 37°C. The tubing was then cut into 2.5-cm sections, yielding clots of approximately 0.2 ml. The clots were removed from the tubing, and each was placed in a plastic vial and washed with 3 ml of 0.15 M NaCl. They were subsequently counted and suspended in 2 ml of fresh-frozen plasma (of the same pool). Experiments were started by the addition of plasminogen activator (or PBSA as control). At various intervals, aliquots of fresh-frozen plasma were removed from each tube for counting. Samples were retained at the end of the experiments for determination of fibrinogen levels.

In Vivo Thrombolysis Model

The rabbit jugular vein model of Collen et al26 was used with the modifications described previously.11 In the experiments reported here, 2 ml PBSA or 2 ml PBSA containing either scu-PA or scu-PA–59D8 was added to 48 ml of 0.9% NaCl in a pediatric infusion container. Five milliliters was administered by bolus infusion, followed by the remainder over 4 hours. Saline (0.9%) was infused for an additional hour, and the rabbit was then killed by infusion of potassium chloride. The amount of radioactivity remaining in the jugular vein segment was determined by gamma counting, and the amount of each activator was determined by its activity in the S-2444 assay (urokina- nase was used as standard). Data were analyzed by the FIT-FUNCTION program,23 as described previously, with the assumption that the maximum lysis was 100%.
Comparison Between scu-PA and scu-PA–59D8 Fab' by FIT-FUNCTION Program

Curves of percent lysis versus plasminogen activator dose (Figures 3 and 6) were compared by first-fitting to each curve a three-parameter function that has been shown to fit such curves well. This function is:

\[
\%\text{Lysis}(x) = A_1 \ast \text{antilogit}(A_2 \ast (\log_{10}(x) - A_3))
\]

where \(x\) is activator dose.

The antilogit function is defined as:

\[
\text{antilogit}(z) = \frac{e^z}{1 + e^z}
\]

The three parameters of the fitted \(\%\text{Lysis}(x)\) function, \(A_1\), \(A_2\), and \(A_3\), can be described as \(A_1\) is the maximum percent lysis achievable at infinite dose in the dose-response curve, \(A_2\) is the rate constant of lysis in units of \(1/(\log_{10} \text{activator dose})\), and \(A_3\) is the rightward shift of the dose-response curve toward less potency in units of \(\log_{10}\) activator dose. If \(A_3\) is negative, then the curve shifts left toward greater potency.

The fit was performed by the nonlinear regression program FIT-FUNCTION in the RS/1 software for the Digital Equipment Corporation VAX computer. In each case, goodness-of-fit was confirmed by an analysis of residuals. FIT-FUNCTION supplies estimates of the standard error of each of the three estimated parameters. The estimated standard errors (SEE) for parameter \(A_3\) were used in an unpaired \(t\) test to compare the estimated values of \(A_3\) for two curves. It is the \(p\) value from this test that is cited in each comparison of potencies. Use of the Bonferroni theorem provided protection against an inadequately small threshold for significant \(p\) values due to multiple comparisons.

Relative potencies were computed for a pair of percent lysis curves whose parameter values were \(A_3\) and \(A_3'\) as:

\[
\text{Relative potency} = 10^{(A_3 - A_3')}
\]

where \(A_3\) is greater than \(A_3'\).

As can be seen from the percent lysis function, this relative potency represents the ratio of the doses of activator required to produce one half of maximum percent lysis. This ratio is well defined even when the percent lysis curves are not "parallel" (i.e., \(A_2\) does not equal \(A_2'\)).

Results

scu-PA–59D8 Fab' Conjugate

The conjugate was separated from unconjugated scu-PA and from tcu-PA–59D8 Fab' conjugate that had been generated during the synthesis and purification procedures by two steps of affinity chromatography. Figure 1A shows the profile when 10 ml (1.5 mg/ml) of the crude reaction mixture was loaded onto a 10-ml \(\beta\)-peptide column. Material that did not bind to the column represented unconjugated scu-PA. The eluate (0.2 M glycine, pH 2.8; see marker) of this column was neutralized by the addition of 3 M Tris-HCl, pH 8.6, and loaded onto a 4-ml benzamidine–Sepharose column (Figure 1B). Here, fall-through represented the desired scu-PA–59D8 Fab' conjugate as well as some uncoupled 59D8 Fab'. The eluate of this column (0.1 M sodium acetate, 0.4 M NaCl, pH 4.0; see marker) contained tcu-PA–58D8 Fab' conjugate.

The parent molecules and products of the synthesis and purification of the scu-PA–59D8 Fab' conjugate were analyzed by SDS-PAGE under reducing and nonreducing conditions (Figure 2). When electrophoresed under nonreducing conditions (Figure 2A), the scu-PA–59D8 Fab' conjugate (lane 2) could be visualized at about 100 kDa as a single band. A 1:1 molar ratio of scu-PA to 59D8 Fab' was to be expected because each molecule contained only one reactive sulfhydryl group. However, the procedure used to purify the scu-PA–59D8 Fab' conjugate did not remove unconjugated 59D8 Fab', which migrates at 50 kDa (compare lane 5). When electrophoresed under reducing conditions (Figure 2B), the disulfide bond linking scu-PA to 59D8 Fab' is broken, and the individual components of the conjugate can be visualized. The scu-PA–59D8 Fab' conjugate (lane 2) is shown to be the sum of its components scu-PA (lane 6) and 59D8 Fab' (lane 5).

The functional characteristics of the scu-PA–59D8 Fab' conjugate were further defined by its purification. Because of the two-step affinity procedure, the conjugate must have the ability to bind to the \(\beta\)-peptide column (i.e., contain a functionally intact
of uncoupled scu-PA was 130,000 units per milligram of protein. On the basis of the molecular weights of scu-PA and 59D8 Fab’, we infer that the final conjugate preparation contained scu-PA–59D8 Fab’ in a molar ratio of 1:2. Given the low substitution of scu-PA (0.8–1.6 residues per molecule; see “Methods”), the conjugate preparation most likely contained 1 free Fab’ per molecule of scu-PA–Fab’ (in a 1:1 ratio).

Fibrin Monomer–Sepharose Assay

scu-PA, urokinase, t-PA, and scu-PA–59D8 Fab’ were compared in the fibrin–Sepharose assay as follows. The concentration of each activator in the absence of fibrin was first determined as described under “Methods.” Samples were diluted so that the activator concentration ranged from 0.01 to 500 units/100 μl (1,000 units/100 μl for urokinase). Figure 3 shows that in the fibrin–Sepharose assay, the fibrinolytic potency of scu-PA–59D8 Fab’ was 33-fold more than that of t-PA (p<0.001), 230-fold more than that of scu-PA (p<0.0001), and 420-fold more than that of urokinase (p<0.0001).

Clot Lysis in Plasma

scu-PA–59D8 Fab’ and its parent molecule scu-PA were compared in an assay for human clot lysis in plasma. Immediately before each plasma clot assay, the peptidase activity of the sample was measured (after plasmin activation) with the S-2444 assay. Appropriate dilutions were made so that the samples

antibody-binding site) and be incapable of binding to benzamidine (i.e., be free of two-chain urokinase activity). This was confirmed by determining latent prourokinase activity (expressed as a percentage of total urokinase activity) at various steps in the conjugate’s synthesis (Table 1). The specific activity of the conjugate was 44,000 units (of latent scu-PA activity) per milligram of protein as assessed by S-2444 assay. In this same assay, the specific activity...
of unconjugated activator and activator–Fab’ conjugate contained identical amidolytic activities. Figure 4 shows that a higher percentage of lysis is achieved with the conjugate when the curves are matched either for concentration of activator or for time. The lag phase characteristic of scu-PA’s action in plasma seems to have been abolished. A comparison between the dose-response curves at matched times for scu-PA–59D8 Fab’ and scu-PA reveals that at 60 minutes, the fibrinolytic potency of the conjugate is 1.8-fold greater ($p<0.0008$). At 120 minutes, the scu-PA–59D8 Fab’ is still 1.8-fold more potent ($p<0.02$) than scu-PA alone; at 180 minutes, the difference is 1.6-fold greater ($p<0.01$). Fibrinogen levels determined from the supernatants of the human plasma clot assay samples (exposed to either scu-PA or scu-PA–59D8 Fab’) are shown in Figure 5. In each instance, the amount of fibrinogenolysis at approximately equal levels of clot lysis was less for scu-PA–59D8 Fab’ than for scu-PA, suggesting a higher degree of fibrin specificity for the conjugate.

**In Vivo Thrombolysis**

We then determined whether the enhanced potency of scu-PA–59D8 Fab’ was evident in vivo (in the rabbit jugular vein model). Figure 6 shows that in these experiments, spontaneous lysis (from infusion of saline only) was 5.6±4.4%. At low concentrations of scu-PA–59D8 Fab’ or scu-PA, there was no statistically significant difference in lysis. However, at high concentrations of scu-PA–59D8 Fab’, there was a significant enhancement in thrombolysis; for example, 300,000 IU scu-PA produced only 25.3±2.3% lysis, whereas 100,000 IU scu-PA–59D8 Fab’ produced 51.7±13.4% lysis. The amount of fibrinogenolysis produced by 100,000 IU scu-PA–59D8 Fab’ was not significantly different from that produced by 300,000 IU scu-PA when examined at 1, 2, 3, 4, or 5 hours. For example, mean fibrinogen concentration for 100,000 IU scu-PA–59D8 Fab’ was 101% of initial fibrinogen concentration at 5 hours, whereas that for 300,000 IU scu-PA was 87%. Combined analysis of all the points in the curve indicates that scu-PA–59D8 Fab’ is 29-fold more potent than scu-PA alone.

**Discussion**

We describe the production, purification, and characterization of a chemical conjugate containing active scu-PA and the Fab’ of antifibrin monoclonal antibody 59D8. The purified conjugate has a molecular weight of approximately 100 kDa and consists of a 1:1 molar ratio of scu-PA to 59D8 Fab’. Compared with urokinase, scu-PA, and t-PA in a fibrin monomer–Sepharose assay, scu-PA–59D8 Fab’ is remarkably more potent in fibrinolysis than any native activator and is more potent than the chemically synthesized urokinase–59D8 and t-PA–59D8 conjugates we reported previously. scu-PA–59D8 Fab’ is also more potent than scu-PA or urokinase in a human plasma clot assay. This enhanced clot lysis...
was accompanied by a sparing of fibrinogen, which signifies increased fibrin specificity.

In the rabbit jugular vein model, we observed a 29-fold increase in the apparent in vivo clot lysing effectiveness of scu-PA–59D8 Fab′. This effect is comparable to what was observed by Collen and coworkers (personal communication) when they coupled scu-PA to an antifibrin antibody specific for the D-dimer. It is curious that such a marked increase in potency is observed in vivo and in the fibrin monomer– Sepharose assay but not in the human plasma clot assay. In the plasma clot model, soluble peptides (products of fibrin degradation) that act as epitopes for the 59D8 antibody are probably released into the supernatant; these soluble peptides may compete with fibrin for antibody binding. On the other hand, in vivo, fibrin-degradation products are cleared rapidly. In the fibrin monomer–Sepharose assay, the plasminogen activator is added first; this allows the antibody to bind to fibrin before the introduction of plasminogen and consequent fibrin degradation. An alternative, less likely explanation for the greater potency of scu-PA–59D8 Fab′ in vivo is that conjugation prolongs the half-life of scu-PA and results in an apparent increase in in vivo potency. This is unlikely because we have previously shown that conjugates between a plasminogen activator and an antibody that does not possess fibrin specificity do not display an increase in apparent in vivo potency.11

Although scu-PA does not have intrinsic fibrin-binding properties, it acts as a fibrin-selective plasminogen activator—presumably because it binds to fibrin-bound plasminogen. By adding a high-affinity fibrin-binding site, we have enhanced the fibrin selectivity of the plasminogen activator. The apparent effectiveness of this scu-PA–59D8 Fab′ chemical conjugate suggests that a recombinant fusion protein containing a fibrin-specific antibody-combining site and the scu-PA catalytic site should be constructed.

References

Key Words • single-chain, urokinase plasminogen activator • fibrinolysis • thrombolysis • antibodies
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