Innovative Approaches to Plasminogen Activator Therapy

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Plasminogen activator therapy for acute myocardial infarction has become standard medical practice. Bleeding complications, however, limit the utility of the currently available agents. This article reviews how the tools of molecular biology and protein engineering are being used to develop safer and more effective plasminogen activators.

THE OCCLUSION OF A CEREBRAL OR CORONARY ARTERY BY a blood clot accounts for the majority of deaths in industrialized countries and is responsible for significant incapacitation and morbidity (*1*). The immediate cause of occlusion is a defect in the vessel wall, usually atherosclerotic, but it is the formation of a thrombus on this defect (*2*) that almost invariably results in the final interruption of blood flow. Perhaps prevention of atherosclerosis will have the most important effect on this process; yet for many in whom prevention is too late, dissolution of the thrombus with plasminogen activator therapy is an attractive method of restoring blood flow.

The activation of plasminogen results in the formation of plasmin, a proteolytic enzyme that degrades fibrin, the principal component of the structural lattice of a thrombus. In the past several years plasminogen activators have been shown to be effective therapeutic agents in coronary artery thrombosis, improving overall survival (*3–7*) while limiting the degree of damage to the heart (*5, 6, 8*). Unfortunately, because plasmin modifies platelet function (*9*) and degrades circulating fibrinogen and clotting factors V and VIII (*10*), as well as thrombus-bound fibrin, plasminogen activator therapy also carries the risk of hemorrhage. When bleeding occurs within the central nervous system, it usually results in grave neurological deficiency and often in death (*11*). Must the risk of bleeding accompany any effort to bring about thrombolysis? Perhaps the ability to selectively rather than systemically generate plasmin would improve the safety of plasminogen activator therapy.

Our review proceeds from the assumption that the ideal thrombolytic agent should be capable of attacking the components of a thrombus while sparing the circulating clotting proteins and platelets. Those components that have been modified by the clotting process offer the most easily defined targets toward which to direct a clot-selective agent. Fibrin, which differs in its covalent structure from its circulating precursor fibrinogen, is one obvious choice. Alternative targets include platelets altered during the clotting process, epitopes comprising the junction between platelet receptors and the proteins that bind to them [such as von Willebrand's factor, fibrinogen, and fibronectin (*12*)], and the covalent link between α_2-antiplasmin and fibrin. Here, we restrict our attention to the fibrin selectivity of plasminogen activators, both as an intrinsic property of certain naturally occurring proteins and as a property that, through the methods of protein engineering, can be enhanced in thrombolytic agents.

Clinical Use of Plasminogen Activators

Although plasminogen activators have been used to treat both arterial and venous thrombosis at many sites within the body, their most dramatic application has been in the therapy of coronary occlusion. Streptokinase, a bacterial protein, and urokinase, a human enzyme initially purified from urine, constitute the first generation of plasminogen activators. When administered within 4 hours of coronary occlusion, streptokinase has been shown to reduce mortality after myocardial infarction by 23% to 81% in a number of randomized trials (*4, 5, 7, 13*). However, the use of this agent is invariably accompanied by a marked depletion of fibrinogen caused by the generation of excess plasmin. Unchecked, the systemic release of plasmin is capable of paralyzing the clotting process, causing what is known as a "lytic state" (*14*). Results obtained with urokinase have been similar in smaller scale clinical trials (*15*).

More recently a second generation of plasminogen activators has become available: tissue-type plasminogen activator (tPA) and single-chain urokinase-like plasminogen activator (scuPA). Unlike streptokinase and urokinase, tPA and scuPA exhibit fibrin-selective plasminogen activation. Although there is now some controversy as to whether the fibrin-selective agents have any clinical advantage over the nonselective agents (*16*), there is little disagreement that a general disruption of the clotting system is to be avoided.

The selectivity of tPA derives from the presence of a fibrin binding site on the molecule. tPA binds fibrin with a K_d of 0.16 μM; when bound, its K_m for plasminogen activation decreases from 83 μM to 0.18 μM and its k_{cat} increases from 0.07 to 0.28 second^{-1}, resulting in an increase in catalytic efficiency of approximately 1000-fold. Although scuPA probably does not bind directly to fibrin (see below), it activates fibrin-bound plasminogen much more readily than plasma plasminogen. Its fibrin selectivity is comparable to that of tPA (*17*).

tPA and scuPA are also considered native plasminogen activators because endothelial (and other) cells secrete them into the circula-

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tion. Initial studies of tPA and scuPA were conducted on proteins purified from cultured cell lines; the Bowes melanoma cell line for tPA, and transformed human kidney cells for scuPA. Both agents have subsequently been produced by recombinant DNA methods (*18*).

The cleavage of native, single-chain tPA by plasmin between amino acids Arg^{275} and Ile^{276} produces two-chain tPA, which is similar in fibrin selectivity and catalytic activity to its single-chain precursor. scuPA is cleaved by plasmin between amino acids Lys^{158} and Ile^{159}. The resulting high molecular weight two-chain urokinase has the catalytic activity of scuPA, but does not have the fibrin selectivity and resistance to plasminogen activator inhibitor I of its single-chain precursor. Low molecular weight two-chain urokinase is the first generation form (Fig. 1). The full-length, high molecular weight form of scuPA is the native plasminogen activator and is the form that has been studied clinically as a second generation plasminogen activator.

tPA has been shown to reduce mortality by 23% to 36% and to result in a depletion of fibrinogen less severe than that caused by streptokinase (*19, 20*). This mitigation of fibrinogen depletion is probably because of the ability of tPA to bind fibrin, which would promote the activation of plasminogen in close proximity to a thrombus. Nevertheless, at doses required for the prompt lysis of coronary thrombi, the administration of tPA can also result in hemorrhage (*19, 20*) accompanied by depletion of both fibrinogen and plasminogen (*19*). Although fibrinogen and plasminogen losses are generally less after tPA administration than after streptokinase administration, it should be noted that measurement of a few selected proteins may not accurately reflect loss of other, more important elements of the clotting system. Clinical experience with scuPA is still limited, although it appears to be an effective thrombolytic agent (*21*); as with tPA the administration of scuPA is also associated with hemorrhage.

Almost as frequently observed in clinical trials has been the tendency for arteries recanalized by tPA to reocclude (*22, 23*), with subsequent loss of the myocardium that had originally been in jeopardy. Not surprisingly, streptokinase, which, as discussed earlier, severely depletes circulating clotting proteins, is associated with a lesser incidence of reocclusion than is tPA (*20, 22*). Streptokinase also reduces blood viscosity, probably because of marked fibrinogen depletion (*24*); whether this effect reduces the incidence of reocclusion is not clear. Preliminary evidence suggests that reocclusion may not be so frequent with scuPA as with tPA (*25*), but a direct comparison has yet to be reported.

Thus despite their fibrin selectivity relative to the first generation of plasminogen activators, the second generation is also beset by significant limitations. Two clinical trials in progress, GISSI-2 and ISIS-3, may determine whether significant differences exist between first and second generation plasminogen activators. The design of a third generation of plasminogen activators composed of more selective molecules will require a detailed understanding of structure-function relationships of plasminogen activators.

Chemical Modification of a Plasminogen-Streptokinase Complex

An acylated plasminogen-streptokinase activator complex (APSAC) has been shown to have enhanced fibrin selectivity (*26*). Acylation reversibly inactivates the catalytic domain of plasminogen, but the inactivated plasminogen is still able to bind to fibrin. When the complex binds to fibrin, the catalytic domain of plasminogen then becomes activated by the streptokinase. Evaluation in animal models showed that APSAC degraded significantly less fibrinogen than did urokinase and streptokinase, but more than did tPA (*26*). The half-life of APSAC in the circulation is also considerably longer than any of the other plasminogen activators (*16*). There are fewer clinical data available for APSAC than for streptokinase and tPA. Although the results from earlier trials were mixed, the investigators of the AIMS trial reported a marked reduction in mortality with APSAC (*27*). If these results are confirmed by other investigations, it would suggest that both fibrin selectivity and a longer half-life are desirable qualities.

Structure and Function of Native Plasminogen Activators

tPA and scuPA are both multidomain proteins. An examination of the function of the individual domains, made either by isolating them after selective proteolytic cleavage or by deleting the DNA segments that encode for their synthesis, may provide the insights necessary to overcome some of the disadvantages inherent in the first and second generation agents.

tPA and scuPA have highly homologous COOH-terminal segments that are responsible for their catalytic activity. In tPA the NH_2-terminal segment effects fibrin binding, whereas in scuPA the function of the NH_2-terminal region has not been determined. In scuPA the NH_2-terminal (or A) chain is lighter than the COOH-terminal (B) chain; in tPA the A chain is the heavier (Fig. 1, A and B).

Structural information is far more complete for tPA than for scuPA. Five domains exist in tPA, each of which is encoded by a single exon or group of exons (*28*) (Fig. 1A). The heavy chain (NH_2-terminus) of tPA contains four domains: a "finger" domain similar to that of fibronectin (*29*), an epidermal growth factor (EGF)–like domain homologous to that of urokinase, protein C and coagulation factors IX and X (*30*), and two "kringle" domains joined by intradomain disulfide bonds similar to those found in plasminogen, scuPA, and prothrombin. The light chain (COOH-terminus) contains the fifth domain, which is homologous to the catalytic regions of other serine proteases.

Gething *et al.* (*28*) have recently described the properties of five deletion mutants from single-chain tPA. The mutants were constructed by modifying intron/exon junctions such that the exon encoding each of the five tPA domains could be precisely deleted. The finger or EGF-like domain (or both) appear to contribute to the high-affinity interaction between tPA and fibrin. Interestingly, mutants lacking only the finger domain display an increased affinity for plasminogen. The absence of the EGF-like domain does not of itself cause a difference in in vitro properties, although the effect of its absence has not been examined in vivo. The two kringle regions appear to be highly homologous and to have equivalent abilities to mediate the stimulation of catalytic activity by fibrin, as evidenced by the fact that deletion mutants lacking both kringles do not bind to fibrin and do not show enhancement of catalytic activity in the presence of fibrin (*31*). Plasminogen activator inhibitor I binds to and inhibits the catalytic segment of tPA. As expected, deletion of all four domains composing the fibrin binding segment does not diminish interaction with plasminogen activator inhibitor I. These results, together with those of van Zonneveld *et al.* (*32*), MacDonald *et al.* (*33*), and Larsen *et al.* (*34*), provide convincing evidence that the five structural domains of tPA have different and autonomous functions.

The light chain (NH_2-terminus) of scuPA contains, in addition to an EGF-like domain, a single kringle region that shows considerable homology with the kringles of tPA (despite the fact that scuPA does not appear to bind fibrin) (*35*) (Fig. 1B). One property that

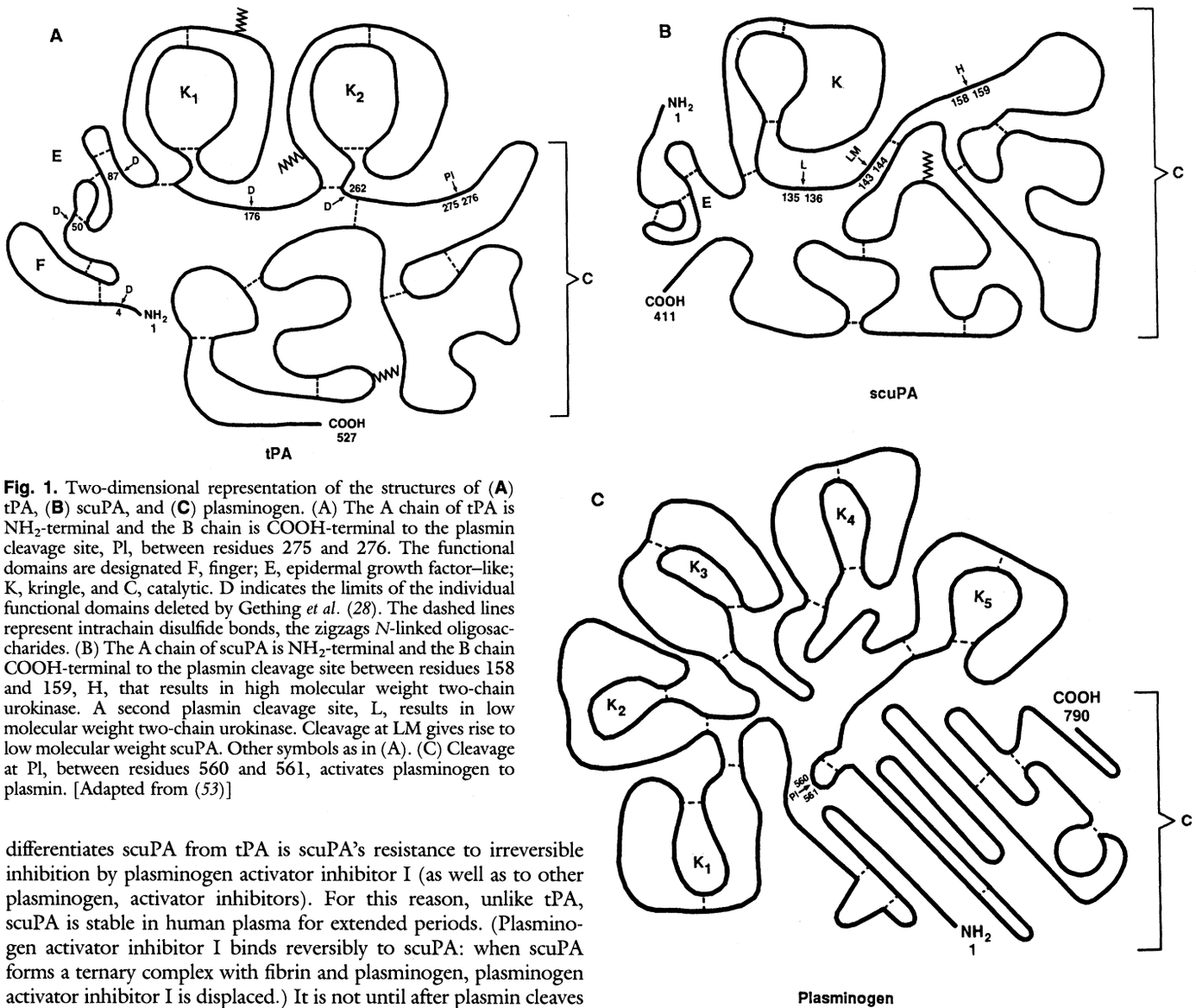

Fig. 1. Two-dimensional representation of the structures of (**A**) tPA, (**B**) scuPA, and (**C**) plasminogen. (A) The A chain of tPA is NH_2-terminal and the B chain is COOH-terminal to the plasmin cleavage site, Pl, between residues 275 and 276. The functional domains are designated F, finger; E, epidermal growth factor–like; K, kringle, and C, catalytic. D indicates the limits of the individual functional domains deleted by Gething *et al.* (*28*). The dashed lines represent intrachain disulfide bonds, the zigzags *N*-linked oligosaccharides. (B) The A chain of scuPA is NH_2-terminal and the B chain COOH-terminal to the plasmin cleavage site between residues 158 and 159, H, that results in high molecular weight two-chain urokinase. A second plasmin cleavage site, L, results in low molecular weight two-chain urokinase. Cleavage at LM gives rise to low molecular weight scuPA. Other symbols as in (A). (C) Cleavage at Pl, between residues 560 and 561, activates plasminogen to plasmin. [Adapted from (*53*)]

differentiates scuPA from tPA is scuPA's resistance to irreversible inhibition by plasminogen activator inhibitor I (as well as to other plasminogen, activator inhibitors). For this reason, unlike tPA, scuPA is stable in human plasma for extended periods. (Plasminogen activator inhibitor I binds reversibly to scuPA: when scuPA forms a ternary complex with fibrin and plasminogen, plasminogen activator inhibitor I is displaced.) It is not until after plasmin cleaves scuPA between residues Lys^{158} and Ile^{159} to form high molecular weight two-chain urokinase that the catalytic site becomes susceptible to irreversible inhibition. Low molecular weight two-chain urokinase derives from subsequent cleavage of the Lys^{135}-Lys^{136} peptide bond. It too is readily inhibited by plasminogen activator inhibitor I.

Domain Deletion and Site-Directed Mutagenesis in the Creation of New Plasminogen Activators

Stump *et al.* (*36*) have described a shortened form of scuPA that results from proteolytic cleavage during purification between residues Glu^{143} and Leu^{144}. scuPA is probably not present in this form in vivo. Low molecular weight scuPA, now expressed by recombinant DNA methods (*37*), does not contain the NH_2-terminal kringle. Surprisingly, although it is only 14 amino acids longer than low molecular weight two-chain urokinase, low molecular weight scuPA (32,000) manifests fibrin selectivity identical to that of native, high molecular weight scuPA (54,000), clearly excluding the kringle from a role in fibrin selectivity. The low molecular weight form also retains another important property of the native form—its resistance to plasminogen activator inhibitor I.

The short half-lives in plasma of both tPA and two-chain urokinase are in part accounted for by their rapid and irreversible inactivation by plasminogen activator inhibitor I. Because plasminogen activator inhibitor I does not irreversibly inactivate scuPA, it should be possible to further prolong scuPA's half-life by preventing cleavage of the Lys^{158}-Ile^{159} peptide bond. Knowing that plasmin specifically cleaves COOH-terminal to a basic amino acid, Nelles *et al.* (*38*) substituted Gly or Glu for Lys^{158} by site-directed mutagenesis. The mutant proteins were not susceptible to plasmin cleavage and could not be activated to form two-chain urokinase. Nor did they lyse plasma clots in vitro. The mutants did, however, activate plasminogen, albeit at a K_m lower than that of native scuPA. These findings give weight to the hypothesis that scuPA, although an active enzyme, must be converted to two-chain urokinase before it can function optimally in fibrinolysis.

Chimeric Molecules

Once it became apparent that the fibrin-binding and catalytic domains of plasminogen activators could function independently,

several laboratories embarked on efforts to design plasminogen activators with properties more desirable than those of the native molecules, by both chemical cross-linking and recombinant DNA methods. With the intent of increasing fibrin selectivity, Robbins and Tanaka (*39*) prepared a hybrid that combines the fibrin-binding domain of plasminogen and the catalytic domain of urokinase (Fig. 2A). This was accomplished by a disulfide cross-linkage between the A chain of plasminogen and the B chain of urokinase. The resulting 92-kD monomer had a lytic activity twofold higher than urokinase in a fibrin-clot assay. This increase derived from an eightfold enhancement in fibrin binding activity and a fivefold stimulation of catalytic activity in the presence of soluble fibrin fragments. Robbins and Boreisha (*40*) next linked the fibrin-binding A chain of plasminogen to the catalytic domain of tPA (B chain) (Fig. 2B). The hybrid showed selective fibrinolytic activity similar to that of tPA. Although the hybrid did not have greater activity than tPA, the experiment did serve to demonstrate that, even though their structures were quite diffreent, the fibrin-binding domains of plasminogen and tPA could be exchanged without resulting in a loss of fibrin selectivity.

To obtain in one molecule both high-affinity fibrin selectivity and resistance to plasminogen activator inhibitor I, Nelles *et al.* (*37*) constructed a recombinant hybrid composed of the A chain (fibrin binding domain) of tPA and the low molecular weight form of scuPA (the catalytic site of urokinase in a form that is not susceptible to plasminogen activator inhibitor I) (Fig. 2C). They spliced the cDNA for tPA, which encodes the 5′ untranslated region and amino acids Ser^{1} through Thr^{263}, to the cDNA for low molecular weight

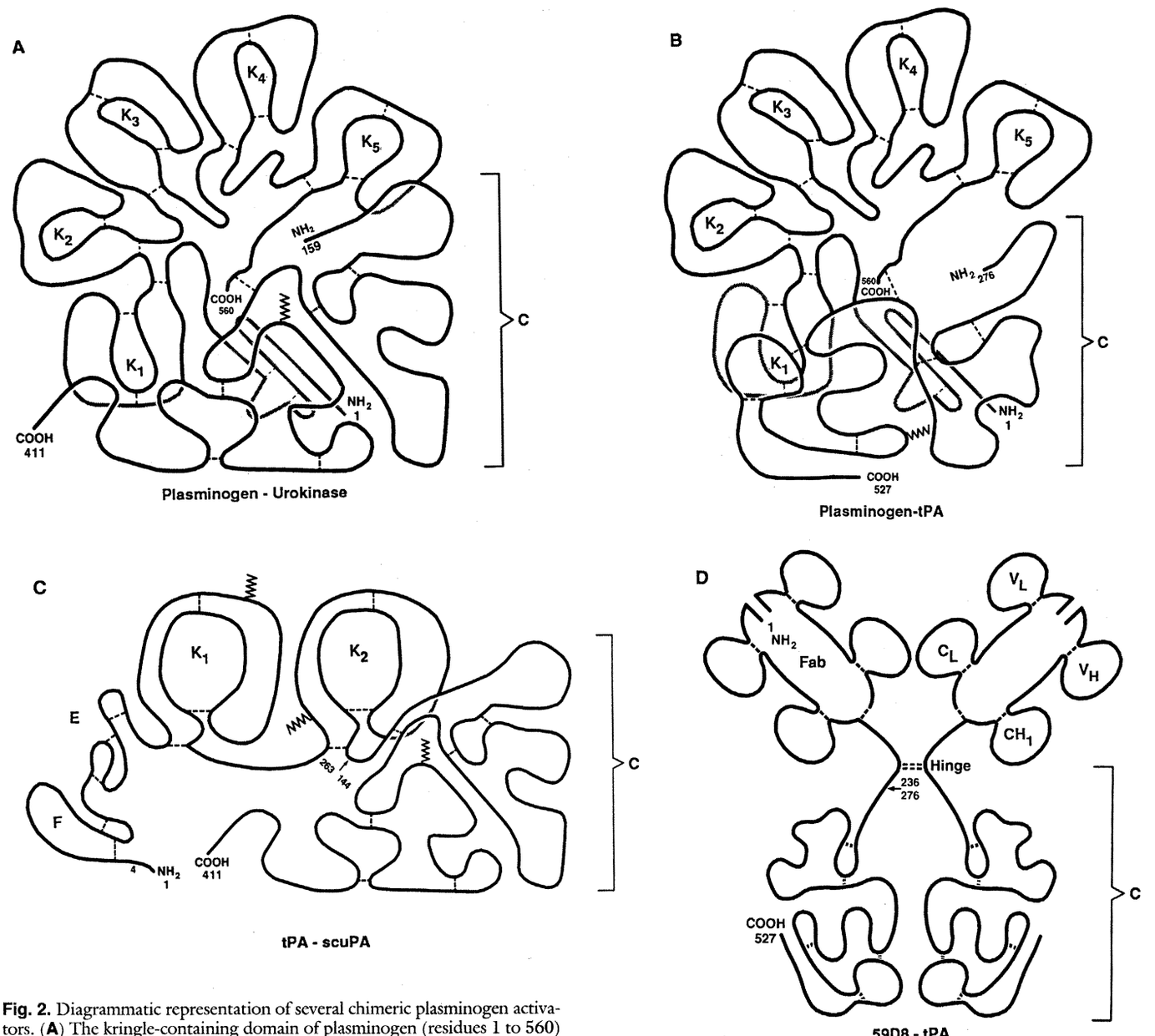

Fig. 2. Diagrammatic representation of several chimeric plasminogen activators. (**A**) The kringle-containing domain of plasminogen (residues 1 to 560) linked to the B chain of urokinase, probably by a disulfide bond between plasminogen Cys^{557} and urokinase Cys^{279}. (**B**) The kringle-containing domain of plasminogen linked to the B chain of tPA, possibly by a disulfide bond between plasminogen Cys^{557} and tPA Cys^{395}. (**C**) The A chain of tPA in contiguous peptide linkage to the B chain of urokinase between tPA Thr^{263} and scuPA Leu^{144} (arrow). (**D**) The Fab region of antifibrin antibody 59D8 in contiguous linkage to the B chain of tPA between residues 236 of the 59D8 heavy chain (Gly in the construct) and Ile^{276} of tPA. VL and VH and CL and CH_1 denote variable and constant regions of the light and heavy chains. The molecule exists as a dimer linked at the immunoglobulin hinge region by a disulfide bond.

scuPA, which codes for Leu^{144} through Leu^{411}, and then expressed the hybrid DNA in Chinese hamster ovary cells. Immunologic epitopes for both tPA and scuPA were present on the expressed protein. Unfortunately, the fibrin-binding activity of the recombinant construct was less than that of native tPA, and the fibrin selectivity of the construct in a human plasma clot assay was less than that of either single- or two-chain tPA.

Antibody-Targeted Fibrinolysis

In light of the experiments discussed thus far, it would seem that the shuffling of domains from existing components of the fibrinolytic system, whether by chemical or recombinant DNA methods, cannot significantly increase fibrin selectivity beyond the point attained by the parent plasminogen activators. An alternative approach to the problem is to use a fibrin-specific antibody in conjunction with a plasminogen activator catalytic site, to achieve an affinity and a selectivity greater than those afforded by the fibrin binding domain of plasminogen or tPA.

The reagent critical to this approach is an antibody that binds to fibrin with high affinity but does not cross-react with fibrinogen. Thrombin acts on fibrinogen to cleave peptides from the NH_2-termini of the fibrinogen Aα and Bβ chains, leading to the noncovalent polymerization of fibrin. The newly revealed six–amino acid sequence at the NH_2-terminus of the fibrin β chain is an epitope that is not available on fibrinogen. We have developed monoclonal antibodies to a synthetic peptide containing this sequence (*41*), which bind to fibrin with high affinity (0.77 n*M*) but do not bind to fibrinogen. In contrast, the binding constant of tPA for fibrin is 0.16 μ*M*. We subsequently harnessed the fibrin selectivity of these antibodies and their fragments to the catalytic activity of plasminogen activators—first as plasminogen activator-antifibrin antibody conjugates, and, more recently, as bifunctional antibodies with specificities for both a plasminogen activator and fibrin.

Plasminogen Activator-Antifibrin Antibody Conjugates

Antifibrin antibody 59D8 [or its antigen-binding fragment (Fab)] was chemically conjugated to urokinase and tPA by means of the disulfide cross-linking reagent *N*-succinimidyl 3-(2-pyridyldithio)propionate (*42, 43*). The resulting urokinase-59D8 and tPA-59D8 molecules were, respectively, 100 times as potent as urokinase and 10 times as potent as tPA in an in vitro fibrinolytic assay (*43*). To determine whether this increased efficacy was physiologically relevant, we compared tPA-59D8 with tPA alone in a human plasma clot assay and in the rabbit jugular vein model (in vivo). tPA-59D8 was 3.2-fold as potent as tPA alone in human plasma (*43*) and 3.0- to 9.6-fold as potent as tPA alone in vivo (over a range of tPA concentrations; the greatest benefit was observed at the lowest tPA concentrations) (*44*). Both in vivo and in vitro, this increased fibrinolytic potency was accompanied by a decrease in the consumption of plasminogen, α_2-antiplasmin, and fibrinogen, indicating that the increase in potency is the result of an increase in selectivity. High molecular weight scuPA chemically cross-linked to antibody 59D8 appears to effect similar increases in fibrinolytic potency (*45*).

Opportunities to chemically link plasminogen activator-antifibrin antibody conjugates are limited to the coupling of available proteolytic fragments such as Fab and the B chain of urokinase or tPA. To permit a detailed search for the optimal structure of a molecule containing both a fibrin-specific antibody binding site and a plasminogen activator catalytic site, we applied recombinant DNA methods. These techniques allow the flexibility of combining a minimal antigen binding structure [the 25-kD variable domain antibody molecule (FV)] with a minimal catalytic domain in a variety of arrangements and at various molecular distances. Two recombinant activator-antibody constructs have been tested thus far, each containing the rearranged 59D8 heavy chain gene. In the first construct the variable exon of the 59D8 heavy chain gene was combined with the CH_1 and hinge exons of mouse immunoglobulin γ2b and the cDNA coding for the B chain of tPA to form the expression vector pSVD8tB (*46*). The plasmid was then transfected into a clone of hybridoma 59D8 that had lost the ability to produce heavy chain. Clones resistant to mycophenolic acid (that is, containing the *Escherichia coli* guanine phosphoribosyltransferase gene of pSVD8tB) secreted a protein of 170 to 180 kD that was recognized by both fibrin- and tPA-specific antibodies. The molecule contained two unmodified antibody light chains of 25 kD each, and two heavy chains of 65 kD each (Fig. 2D). The heavy chains proved to be fusion peptides, extending from residues 1 to 236 of the antibody's heavy chain and from residues 275 to 527 of tPA (the amino acids corresponding to the B chain) (*47*).

The tPA-59D8 recombinant construct bound fibrin with an affinity equal to that of the parent antibody. Its amidolytic activity, measured as the ability to cleave chromogenic substrate S-2288 (H-D-isoleucyl-L-prolyl-L-arginin-*p*-nitroanilide dihydrochloride), was 70% that of single-chain tPA, and its activity as a plasminogen activator, measured in highly purified assays in the absence of fibrin, was equal to that of tPA (when corrected for the loss in amidolytic activity). Surprisingly, however, the chimeric molecule was ineffective in lysing a plasma clot. This was in contrast to results our laboratory had obtained earlier with a chemical conjugate of the B chain of tPA and 59D8 Fab, which had proved as potent as tPA in fibrinolysis. The presence in the recombinant construct of Gly^{275} instead of the expected Arg^{275} would have prevented the plasmin cleavage at this position that normally results in the two-chain form of tPA. We infer that the consequent absence of a free NH_2-terminus on the B chain of tPA prevented the fibrin-mediated enhancement of catalysis that occurs with native tPA.

The second construct shared with the first the 59D8 variable region exon and the mouse immunoglobulin γ2b CH_1 constant region and hinge exons, but also contained a γ2b CH_2 exon and the exons coding for the entire sequence of low molecular weight scuPA (in place of the sequence coding for the B chain of tPA) (*48*). Transfection into 59D8 hybridoma cells that had been selected for the inability to produce heavy chain resulted in a secreted protein that appears to be a dimer containing both immunoglobulin and scuPA epitopes. This scuPA-59D8 chimera differs from the tPA-59D8 protein by the fact that it contains a plasmin-sensitive bond (in this case the Lys^{135}-Lys^{136} bond in scuPA). In contrast to recombinant tPA-59D8, recombinant scuPA-59D8 is an effective fibrinolytic agent. Although a great deal of exploration is still required before the optimal configuration of a plasminogen activator-antifibrin antibody chimera can be determined, it does appear possible to endow plasminogen activators with enhanced fibrin selectivity through the incorporation of a fibrin-specific antibody binding site.

Bispecific Antibodies

A bispecific antibody that recognizes epitopes on both fibrin and a plasminogen activator should be capable of increasing the effective concentration of the plasminogen activator in the proximity of a fibrin deposit. We have synthesized an antiactivator-antifibrin anti-

body by chemically cross-linking fibrin-specific antibody 59D8 to tPA-specific antibody TCL8 (K_d of $5 \times 10^{-9} M$). The resulting conjugate increased the potency of tPA fivefold and consumed less fibrinogen and α_2-antiplasmin in vitro and in vivo (in the rabbit jugular vein model) (*49*). Similar results were obtained in vitro with a bispecific antibody recognizing fibrin and urokinase (*50*).

An alternative and probably more effective method of synthesizing bispecific antibodies is the hybrid-hybridoma technique (*51*). We have subjected clones from a hybridoma producing thymidine kinase–deficient 59D8 and a hybridoma producing hypoxanthine guanine phosphoribosyltransferase–deficient TCL8 to somatic cell fusion in ethylene glycol, and we have selected the surviving clones in hypoxanthine aminopterin thymidine medium. The antibodies secreted by the hybrid hybridomas proved to bind both fibrin and tPA. In an in vitro fibrinolysis assay, the bispecific, hybrid-hybridoma antibodies increased the potency of tPA by 11-fold (*52*). Because of their uniformity, stability, and ease of production, hybrid-hybridoma products are likely to be better candidates for in vivo study than the chemically cross-linked conjugates.

Conclusion

Plasminogen activators are composed of independent functional domains that provide an exceptional opportunity for protein engineering aimed at improving the properties of the natural molecules. Thus far, efforts have focused on the issues of enhanced fibrin selectivity and extended in vivo half-life. Experiments in which functional domains of plasminogen and urokinase, plasminogen and tPA, or tPA and low molecular weight scuPA are joined have provided significant insight into the function of the constituent plasminogen activators, but the hybrids have not had greatly enhanced fibrin selectivity. However, the combination of a high-affinity fibrin-specific antibody binding site and a tPA or scuPA catalytic site appears to increase both the potency and selectivity of the parent plasminogen activator.

A plasminogen activator with enhanced fibrin selectivity cannot differentiate between a thrombus that threatens the heart and one that prevents exsanguination from vascular trauma, for example, from an eroded artery in a peptic ulcer. For this reason, bleeding will be an occasional consequence of even the most fibrin-selective plasminogen activator. Yet because a fibrin-selective plasminogen activator would restrict the action of plasmin to the site of a thrombus, the extent of the activator's effect would be limited to the time it takes for the activator to be eliminated. Thereafter the hemostatic system would return to normal. This effect would be in contrast to that of streptokinase, which necessitates the synthesis and secretion of new clotting proteins before normal hemostasis is restored.

In engineering the ideal plasminogen activator, one must determine the optimal selectivity as well as the precisely appropriate duration of action. The potential exists for enhancing selectivity beyond localization of the fibrinolytic process to the site of a thrombus. It may be possible to differentiate between recently formed and older thrombi on the basis of relative degrees of interchain cross-linking. One might hope that a coronary thrombus that presents as acute ischemic pain would be of more recent origin than a silent fibrin plug that holds back potential bleeding. There is also a growing understanding of the determinants that govern the clearance of a protein from the circulation. By appropriate engineering, it may be possible to impart a circulatory half-life that is consistent with the therapeutic goals of clot dissolution and prevention of reocclusion, yet avoids the risk of an excessively long impairment of the hemostatic system. The work reviewed in this article is but the background and the beginnings of the search for the ideal thrombolytic agent.

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