Molecular Biologic Modification of Plasminogen Activators
An Artful Science

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The application of molecular biology to medical practice has led to the mass production of otherwise limited biological resources for human use. In cardiology, this principle is best exemplified by the development of tissue-type plasminogen activator (t-PA) and its application to a variety of atherothrombotic disorders, principally acute myocardial infarction. The fundamental premise on which the development of t-PA for clinical use was based—namely, that it is more clot selective than other available plasminogen activators—has been shown to be of only modest clinical relevance in that t-PA is, of course, only relatively fibrin selective. At the concentrations required to achieve rapid and efficacious thrombolysis in vivo, significant fibrinolysis occurs. Furthermore, although significantly less fibrinolysis is produced by t-PA than by streptokinase or two-chain urokinases, no significant difference in hemorrhagic complications between t-PA and these fibrin-nonselective plasminogen activators has been demonstrated. In addition to these shortcomings of thrombolytic therapy with t-PA as currently practiced, two other major problems persist: delay in the time to patency noted in many patients (the extreme form of which constitutes resistance of the thrombus to lysis), and reocclusion, occurring in as many as 25% of patients in whom the clot is successfully lysed initially.

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Given these shortcomings and the power of genetic engineering techniques to modify protein structure relatively simply, the cardiology community has anxiously awaited the development of so-called “third-generation” plasminogen activators that have been molecularly tailored to overcome the limitations of standard therapy observed with “second-generation” agents. The article in this issue of Circulation by Jackson and colleagues\(^1\) represents one version of this approach that has yielded interesting results.

The multidomain nature of t-PA suggests that various functional properties of the molecule may be compartmentalized within specific structural regions. Thus, by selective deletion or mutation of specific domains, third-generation molecules may be produced with properties that overcome some of the disadvantages of the “wild-type” form. Each of the five domains of t-PA is encoded by a single exon or group of exons.\(^2\) The aminoterminal or heavy-chain portion of the molecule contains the fibronectin fingerlike domain, an epidermal growth factor-like domain, and two triple-looped “kringle” domains linked by intradomain disulfide bridges that comprise structural regions important for fibrin binding in homologous proteins. The carboxyterminal or light-chain portion of the molecule contains the enzyme active site homologous to that of other serine proteases.

Construction of deletion mutants by several groups led initially to the widely held belief that the five structural domains of t-PA have different and autonomous functions.\(^2-6\) For example, the protease domain contains the enzyme active site and the binding site for plasminogen activator inhibitor type I.

While it has been convenient to view the t-PA molecule as a series of cassettes defined by structural domains with specific and autonomous functional properties, this view is an oversimplification of the well-recognized complexities of protein folding. The identification of the functions of protein domains and their interactions with one another in the protein matrix remains an inexact science. Tertiary structure simply cannot yet be predicted with a high degree of accuracy from primary sequence data. Thus, it should come as no surprise to the thoughtful reader that deletion mutations of molecules like t-PA may produce effects that are not predicted from an assessment of remaining domains that are simplistically assumed not to interact with one another. Consider, for example, the complex relations among domains that determine fibrin binding and stimulation of t-PA activity by fibrin(ogen). van Zonneveld and colleagues\(^5\) first suggested that the stimulating effects of fibrinogen fragments resided in the second (but

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not the first) kringle domain in conjunction with the fibronectin fingerlike and epidermal growth factor–like domains. In addition to this function, the fingerlike domain also determines (rapid) clearance of t-PA and does so with the epidermal growth factor–like domain. Taken together, these data are hardly consonant with the concept of functional domain autonomy; instead, they support the view long known to biochemists that regions remote from one another in the primary sequence of a protein can significantly influence the functional properties of one another.

In the article by Jackson and colleagues, a mutant form of t-PA with a decidedly improved half-life has been engineered and tested in a canine model against wild-type t-PA. The interesting properties of this deletion mutant in vivo again highlight the principle that functional properties of a protein cannot be directly determined on the basis of autonomy of structural domains. This modified form of t-PA is deficient in the epidermal growth factor–like domain, the fibronectin fingerlike domain, and the first kringle domain; as such, it had a markedly prolonged plasma half-life compared with that of wild-type t-PA (58 versus 3 minutes, respectively). In a canine model of platelet-rich coronary thrombosis, this mutant form of t-PA produced more rapid reperfusion and led to a significant reduction in reocclusion rates compared with rates of the wild type. Interestingly, despite the requirement of both the finger and kringle-2 domains for fibrin binding, this mutant t-PA appeared to produce no greater degree of fibrinolysis than wild-type t-PA at doses of mutant that were more efficacious.

The interesting properties of this deletion mutant are not readily predicted from a simple assessment of the purported functions of the remaining domains. For example, equivalent fibrin binding (by implication from the fibrinolysis data) to the wild type is quite unexpected. In addition, the lack of increased hemorrhagic complications and the improvement in reocclusion rates cannot be easily reconciled with the concept of domain autonomy. Rapidly cleared from the circulation, wild-type t-PA may be associated with significant reocclusion rates by virtue of the limited duration of effect of antithrombotic determinants generated by plasmin. In contrast, these effects are also believed to be important determinants of bleeding complications; if this is truly the case, why then were hemorrhagic complications in these animals no different from those treated with the wild-type molecule given that reocclusion rates were significantly better with the mutant?

These initial observations are quite exciting, particularly appearing as they have at a time when many cardiologists are concerned about the scientific and economic validity of continued molecular biologic developments in fibrinolysis. Convincing evidence for truly superior clinical efficacy of t-PA over other thrombolytics has not been forthcoming, and the possibility of realistic improvements in the design of the natural molecule had not until now been realized. The preliminary data presented in this issue support the view that continued research and development in the molecular biology of thrombolytic agents is warranted and may produce unexpected and useful results. There is still an artful side to this powerful science.

References


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