

Letter to the Editor

Recombinant streptokinase: opportunity for an improved agent

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Molecular technology has strongly influenced current approaches in antithrombotic therapy in that proteins with remarkable biological properties, even if present in nature in minuscule amounts and inaccessible locations, can be produced in large quantity and striking purity. Nowhere is this pattern more evident than in the search for the ideal plasminogen activator (PA). Beginning with the successful cloning of the urokinase gene, many sources of PA have been tapped for eventual development as therapeutic agents, including two other native human activators, tissue plasminogen activator (t-PA) and pro-urokinase (pro-UK), two bacterial origin activators from the staphylococcal and streptococcal organisms, and plasminogen activator from the salivary gland of the vampire bat (bat-PA). A relatively late entry into the field is recombinant staphylokinase (SAK), which has been developed quickly from *in vitro* to animal and to pilot studies in patients with acute myocardial infarction.¹ In addition, numerous recombinant forms have been produced and tested; their anticipated superior performance based on the hypothesis that shuffling functional domains from different PAs could improve the activity of those which have evolved in nature. Engineering such new molecules by recombinant technology or by chemical fusion with specific antibodies yields highly interesting biochemical offspring, but relatively few of these invented activators have proved to be functionally superior to the native forms and fewer yet have warranted study in humans.

Striking by its absence has been any attempt to exploit recombinant technology to produce a better SK, even though the gene was successfully cloned in

1984.² Perhaps this is because the manufacture of a sufficient quantity of product has not been a problem using cultured streptococcal organisms. However, a recombinant SK product expressed by a non-streptococcal bacterial organism or by a eukaryotic cell line could have a dramatic effect on a major disadvantageous feature, namely the induction of an antigenic response and of acute nonspecific febrile and/or hypotensive side effects. Compared with the 48-70% incidence of such reactions noted with early, impure preparations, highly purified SK causes fewer side effects, an excess over placebo of 3.5% allergic reactions and 7.9% of hypotension or bradycardia (in patients with acute myocardial infarction).³ This trend towards a lesser incidence of side effects suggests that non-SK antigens or products in the reagent had been responsible for most of these problems, rather than the SK moiety itself. A recombinant SK product possibly could result in a further reduction in infusion associated side effects by eliminating materials with pyrogenic and/or hypotensive effects from the preparation.

Of course, this approach would have little influence on the development of neutralizing anti-SK antibody, noted even in the original description of 'fibrinolysin' by Tillett and Garner in 1933⁴ in patients recovering from recent streptococcal infection. Re-treatment of patients with recombinant or natural SK would risk a therapeutic failure in the face of an anamnestic response. However, there is a potential benefit of recombinant SK in that the antigenicity may be reduced. The rationale would be that certain portions of the molecule that contain antigenic epitopes may not be required for functional activity, and that a recombinant

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protein consisting of the less antigenic portions might be better tolerated by the human host than would the wild-type molecule. Additionally, the risk of an induced immediate anaphylactic reaction, while remote with the chemically-purified products in use today, could be further reduced with recombinant SK, especially if non-SK antigens are responsible for this event.

Evidence does exist that certain portions of SK are less essential for activity and that antigenic potential is not distributed evenly over the protein. SK is comprised of 440 amino acids, of which 26 represent a signal peptide and 414 make up the secreted protein⁵ of molecular size 47000,⁶ and there is striking homology between the SK proteins synthesized by different strains of streptococcus. For example, SK from groups A and C haemolytic streptococcus have an overall amino acid identity of 85%, with 45 conserved substitutions and only 62 non-identical amino acids.⁷ Specific portions (variable regions) of the protein have much lower identity, only 46%, specifically between residues 174–244 and 270–290, where there are 40 and 10 amino acid changes, respectively. Huang *et al.*⁸ postulate that these variable regions may not be required for the functional activity of the protein, in contrast to the nearly identical portions retained by the homologous proteins. These less homologous portions may possess essential properties, perhaps the potential to react with a restricted substrate such as plasminogen. More germane to this issue is the observation that purified recombinant SK molecules lacking the amino terminal 15 amino acids or the carboxy-terminal 41 residues still retain functional (caseinolytic) activity.⁹ Thus, at least two segments of SK could potentially be eliminated without loss of functional activity.

As to the possibility of a patchy distribution of antigenic epitopes, Reed *et al.*⁴ suggest that some segments are more immunogenic than others. For example, anti-SK antibodies isolated from blood bank donors by elution from an SK-agarose column show considerable variation in reactivity with monoclonal antibody-defined epitopes on recombinant SK fragments. Most strikingly, the amino terminal portions (residues 1–13) react with antibodies in 80% of plasma samples, whereas segment 14–127 is devoid of reaction with all of the plasmas. The remaining segments (120–352 and 353–414) showed moderate reactivity, with about 20–35% of plasma samples containing antibody that bind to these portions.

A first approach to reducing the antigenicity of a potential therapeutic SK recombinant protein would be a 14–400 moiety, which would eliminate one highly immunogenic, presumably non-functional portion at the amino terminus as well as a less immunogenic but equally non-functional part of the carboxy terminus. The amino terminal end of such a recombinant SK molecule would now be that portion (14–127) which was virtually free of immunogenicity, at least as indicated by study of a small number of random plasma samples. More dramatic excisions could eliminate the variable portions of SK between 175–244 and 270–290, although the effect of such deletions might well have a tangible impact on the fibrinolytic function of the expressed protein.

It would seem timely to now consider the possibility of decreasing the side effects of SK therapy and of potentially decreasing the immune response leading to neutralizing antibodies against SK by exploiting recombinant technology for this protein, as has been attempted for UK, t-PA, pro-UK, bat-PA and SAK.

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