# STREPTOKINASE-MEDIATED PLASMINOGEN ACTIVATION: MOLECULAR STUDIES USING GENETICALLY ENGINEERED STREPTOKINASE VARIANTS

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## Introduction

Streptokinase triggers a non-proteolytic activation of plasminogen by forming a stoichiometric complex with plasminogen (1), which develops proteolytic activity after complex formation. A molecular approach was applied to address this question by studying the formation of streptokinase-plasminogen complexes on a solid phase and by constructing genetic variants of the streptokinase molecule using recombinant DNA technology (see Figure).

## Materials and Methods

The following streptococcal strains were chosen: NZ 131 (group A *Streptococcus pyogenes*, M-type 49), SP 13013 (group A *S. pyogenes*, M type 1), and H46A (group C *S. equisimilis*). Streptokinase sequences were amplified by polymerase chain reaction (PCR) followed by ligation to pUC18 and subcloning to expression vector pGEX-3X in translational frame with the glutathione-S-transferase gene (2). Transformed colonies were selected and GST-streptokinase was expressed as described by Lizano and Johnston (3).

An internal polymorphic region of the streptokinase molecule implicated in the pathogenesis of glomerulonephritis (4) was deleted and replaced with a double stranded linker constructed by annealing the following oligonucleotides designed to maintain the reading frame upon ligation to the linearized pGEX-3X containing the remainder of the streptokinase gene. Site directed mutagenesis was performed by Unique Site Elimination (U.S.E.) mutagenesis according to Deng and Nickoloff (5). Human Glu-plasminogen was purified according to Deutsch and Mertz (6). The recombinant constructs were assayed according to Kulisek *et al.* (7).

## Results and Discussion

Streptokinase immobilized on affinity matrices via its $NH_2$-terminal fusion to GST or by incorporation of a COOH-terminal poly-histidine "tail" formed active, non-fragmented complexes with plasminogen; this provided an alternative approach to pre-proteolysis plasminogen activation (3).

The N- and the C-terminal conserved domains bind plasminogen independently, yet both must be simultaneously present to achieve a fully active complex with plasminogen. Moreover, mutagenesis studies of glycine 24 of streptokinase previously reported to be indispensable for activity indicated that this residue is rather non-essential for activation. Future efforts to characterize the structure/function relationship of streptokinase may influence the engineering of streptokinase to improve its therapeutic potential and explain its role in streptococcal disease.

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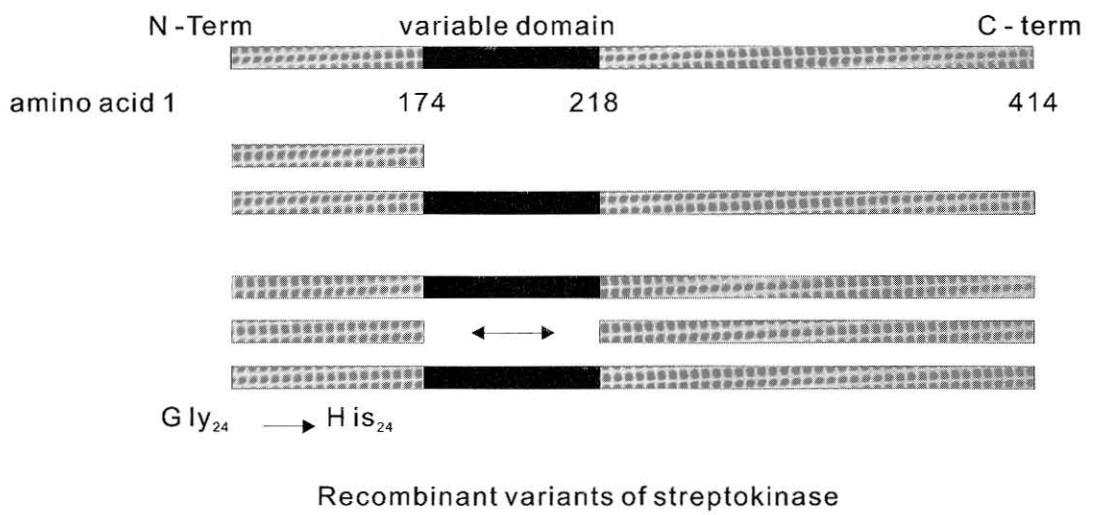


Recombinant variants of streptokinase