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 NH₂-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 proteolytic 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.