

عنوان مقاله:

Construction and expression of streptokinase chimeric molecules with specific activity and enhanced fibrinolytic activity

محل انتشار:

اولین کنگره بین المللی و سیزدهمین کنگره ژنتیک ایران (سال: 1393)

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خلاصه مقاله:

Streptokinase (SK), a bacterial protein produced by streptococci, is one of the main treatments for thromboembolic disorders. However due to lack of fibrin specificity, its application is associated with the risk of hemorrhage. SKs from different strains exhibit different functional activities (plasminogen activation) and specificities which are attributed to the central domains (β -domain) of SK. In this study, chimeric SKs with predicted enhanced activity and improved specificity were constructed by exchange of the central domains of SKs. Two SK molecules with significant different activities and specificities from two distinct streptococci strains were chosen for central domain exchange. Chimeric SKs were constructed by cross-ligation of BstEII/ BsiWI DNA segment encoding the central fragment of sk genes. The assembled sk genes were cloned in pQE30 vector. The constructs were expressed in E.coli M15 host and induced by IPTG. The 6xHis-tagged recombinant SK proteins were purified by NI-NTA chromatography, analyzed by SDS-PAGE and Western blotting. For determination of specific activity, the functional activities were evaluated with chromogenic and clot lysis assay in presence and absence of fibrin. Gel electrophoresis confirmed digestion of the 320nt central domain. The constructs were confirmed by PCR, restriction enzyme analysis and sequencing. The 47-kDa SKs molecules were expressed, purified and the biological activity of chimeric proteins were assayed. Application of SK is limited by shortcomings such as fibrin-non specificity. To address this concern, SK chimeras were constructed by exchange of central domains which seem to play a major role in SK activity and fibrin specificity

کلمات کلیدی:

Streptokinase, thrombolytic agents, chimera

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