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Design, cloning, expression and evaluation of cysteine-substitutes of intact and truncated molecules of streptokinase

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Abstract

Introduction: Thrombosis and the blockage of blood vessels with clots, can lead to acute myocardial infarction and some times even death. Aside from surgical interventions to remove the blockage, the only available treatment is the administration of thrombolytic agents to dissolve the blood clot. Streptokinase (SK) is the most commonly used fibrinolytic drug for this purpose. However, SK has some disadvantages including immunogenicity, short half-life and hemorrhage induction. PEGylation of pharmaceutical proteins by the incorporation of cysteine amino acid is a novel method to decrease their immunogenicity and also to increase their stability and half-life. The ultimate goal of this study was designing and construction of the cysteine analogues of full-length and truncated forms of SK, which posses less hemorrhagic side effects due to fibrin specificity.

Methods: By application of PCR-based site-directed mutagenesis technique, mutants of SK genes, harboring the transversion of AGC codon (serine) to TGC (cysteine), which encoded full-length (amino acids 1-414) and truncated (amino acids 60-386 and 143-386) proteins were established and cloned in pET41a plasmid. Expression of the recombinant SKs was achieved through the induction of *E. coli* transformants. Produced proteins were confirmed by western blotting, purified by affinity chromotography and finally evaluated for their biological activity.

Results: Mutant SK genes were efficiently expressed and due to the fusion of vector-derived His-tag the recombinant full-length and truncated proteins were easily purified. Also despite the replacement of serine to cysteine in the position of 208, the biological activity of the new recombinant protein was still maintained.

Conclusion: The produced mutants of this study provide the possibility of establishing the cysteine specific PEGylation process and improvement of clinical activity of streptokinase protein.

Key words: Streptokinase, PEGylation, Mutagenesis, Cysteine analogue

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