Site-directed mutagenesis of C-terminus of Staphylokinase and its implication on protein function

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INTRODUCTION

Thrombotic complications of cardiovascular diseases are main cause of death and disability and consequently, thrombolysis could favourably influence the outcome of such life-threatening diseases such as myocardial infarction, cerebrovascular thrombosis and venous thromboembolism. Various thrombolytic agents that appeared to have potential for treatment of medical conditions related to blood clotting are plasminogen activators (PAs). Plasminogen activators are serine proteases that catalyze the activation of plasmin (Pli) via proteolytic cleavage of its zymogen form plasminogen(Plg). Plasmin is an important factor in fibrinolysis, the process of breakdown of fibrin polymers formed during blood clotting. Staphylokinase (SAK), a protein produced by Staphylococcus aureus is also known to have profibrinolytic properties and provides a much cheaper therapeutic choice in comparison to mammalian PAs. Various experimental studies had showed that the N and C-terminus regions are obligatory for SAK to achieve activation potential, however their mechanism in catalytic action is missing.

METHODOLOGY

Construction of C-terminus mutants- Four mutants were amplified using specific primers and successfully cloned in *E.coli* using pET9b vector.

Expression and purification- Positive recombinants were expressed in BL21 *E.coli* strain and purified using Ion-exchange chromatography.

Zymographic analysis– An equal concentration of purified proteins were monitored for the formation of a zone of clearance due to caseinolytic activity.

Characterization and kinetic analysis– Plasminogen-coupled chromogenic substrate assay was performed to study activation pattern of each mutant. Further, kinetic studies were performed to determine catalytic efficiency of each mutant.

RESULTS

A) Construction of mutants

SAK C1	Deletion of positively charged residues
SAK C2	Substitution of glutamic acid with alanine
SAK C3	Insertion of three positively charged residues
SAK C4	Insertion of five positively charged residues

Amplification of gene using mutation specific primers

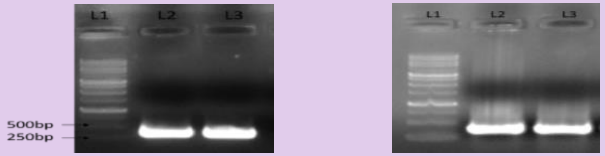

Figure 1: Analysis of amplified product on 1.2% agarose gel (a) L1– DNA ladder, L2– SAKC1 (405bp), L3-SAKC2(411bp) (b) L1– DNA ladder, L2-SAKC3(417bp), L3-SAKC4(426bp)

B) Expression and Purification of proteins

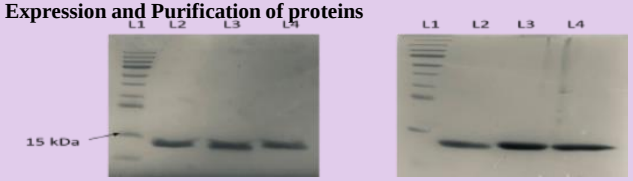

Figure 2: Analysis of purified proteins on 15% SDS PAGE gel (a) L1-Protein ladder,L2- SAK(wild type), L3-SAKC1, L4-SAKC2 (b) L1-Protein ladder,L2-SAK(wild type),L3-SAKC3,L4-SAKC4

C) Zymographic analysis of Plg-specific caseinolytic activity

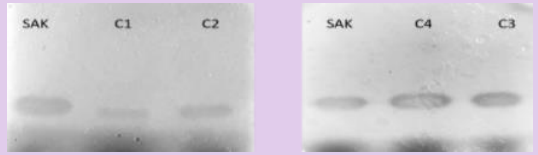

Figure 3: Analysis of caseinolytic activity of proteins on 1% agarose gel containing 10% skimmed milk and 1mg/ml human Plg.

D) Activation pattern studies

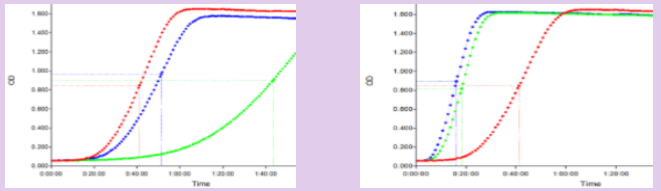

Figure 4: (a) Mutant SAK C1(green)& C2(blue) have longer lag phase of activation as compared to wild type(red) (b) Mutant SAK C3(green)& C4(blue) have shorter lag phase as compared to wild type.

E) Kinetic parameters

Mutant	Vmax (μM/min)	Km (μM)	Kcat (sec^{-1})	Kcat/Km ($\mu M^{-1}s^{-1}$)
SAK	0.05230	1.1	0.313	0.284
SAK C1	0.0348	2.12	0.208	0.098
SAK C2	0.03886	1.680	0.232	0.138
SAK C3	0.09336	0.6845	0.559	0.816
SAK C4	0.1234	0.6431	0.601	0.934

CONCLUSION

The activation pattern studies of these four mutants pointed towards the involvement of C-terminus residues (especially positively charged) in the interaction of SAK with plasminogen. The findings of this study could be utilized to develop SAK with enhanced interaction towards Plg, consequently, with increased fibrinolytic activity.

ACKNOWLEDGEMENT

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