Introduction

The occlusion of coronary arteries due to thrombus formation causes acute myocardial infarction that leads to cardiovascular heart disease (CHD), the most common cause of death and disability for millions of lives every year. To develop a cost effective thrombolytic agent with high efficacy for specific clot lysis has been a desirable goal for the treatment of patients suffering from thrombotic diseases. Physiologically present Pg activators in human body i.e. tPA and urokinase, have been used as recombinant molecules for the treatment of cardiovascular diseases. Both the molecules are being studied to improve their properties as a drug molecule. No doubt, tPA has been used as a preferred choice over urokinase because of its high efficacy in arterial recanalization and high specificity for blood clots. Being highly fibrin specific, tPA does not activate free circulating Pg in plasma and thus causes lesser nonspecific breakdown of plasma proteins. Various improved derivatives of tPA have been approved clinically for the treatment of patients e.g. alteplase, reteplase, tenecteplase, lanolteplase etc. Human tPA and urokinase are immunogenically benign, but are short lived in vivo. Moreover, cost effectiveness consideration due to complex expression and purification systems also poses a major drawback of these Pg activators. Pg activators like SK and SAK have advantage of their cost effective production due to their bacterial origin. Although tPA has a better thrombolytic potential in terms of its immunogenicity, clot dissolving efficacy and high fibrin specificity as compared to SK but due to cost effectiveness, SK is a preferred choice over tPA in developing countries for thrombolytic therapy. Moreover, SK has a better plasma half life as compared to tPA. SAK, another Pg activator of bacterial origin, has shown an immense therapeutic potential because of its high fibrin specificity. Due to its clot specific nature, it causes lesser breakdown of plasma proteins and its thrombolytic potency is equivalent to tPA in terms of arterial recanalization.

The clinical trials have validated the thrombolytic potential of SAK and its advantages over the existing thrombolytics already available in market. However, SAK suffers from two serious disadvantages which need to be addressed and managed properly for use of this molecule in the treatment of thrombotic diseases. SAK is a small protein and gets easily cleared from the blood system when injected into the body. The half life of SAK in plasma is 3-5 min as compared to 30 min of SK. The other disadvantage of SAK as a thrombolytic is its antigenic response. Thus, like the common
drawback of protein therapeutics of bacterial origin, SAK also faces the problems of immunogenicity and short half life. Various strategies have been employed to overcome these disadvantages associated with the therapeutic proteins e.g. chemical conjugation, site directed mutagenesis of antigenic epitopes and cleavage sites of proteolytic enzymes, fusion with proteins or domains, use of drug delivery vehicles etc.

Chemical conjugation of PEG molecules at specific sites in the protein molecule is widely used for improving the half life and reducing the immunogenicity of the therapeutic target protein. There are various methods for attachment of PEG molecule in the protein. But, uncontrolled PEGylation can lead to loss of the function of the protein if functionally important amino acids are masked by PEG and are not exposed to perform their function. There are different types of PEGylation depending upon the strategy employed for the attachment of PEG to the protein. Usually PEG molecules are attached to protein through side chains of lysine residues. SAK contains as many as 20 lysine residues, most of which are essential for its activity, precluding derivatization at lysine residues as an option. Moreover, uncontrolled PEGylation also poses a big disadvantage in its purification to get a homogeneous population. However, site specific PEGylation of SAK can be achieved in a controlled manner by exploiting its amino acid composition. SAK is a small protein (15.5 kDa) with 136 amino acids and most of these residues are involved in interaction with Pg/Pm for making bimolecular enzymatic complex or recruiting substrate Pg which is a multidomain protein (92 kDa). However, SAK molecule does not contain any cysteine, which provides an opportunity to place a cysteine at any non essential position in the molecule by site directed mutagenesis, creating a specific target for site directed PEGylation.

Another approach for improving the therapeutic potential of proteins is the generation of chimeras wherein the proteins or domains with desired features can be linked with the protein under study while maintaining its own potential. Various fusion studies have been applied in SAK to produce chimera to enhance its thrombolytic potential. Fusion of SAK with hirudin to introduce the anticoagulant property in order to prevent reocclusion provides the benefit of thrombolytic and anticoagulant activity in the same protein. Fusion of SAK with kringle 1 of Pg improves the clot lysis efficacy of SAK due to fibrin targeted nature of the molecule. Moreover increase in the clot lysis activity of the chimera can reduce the dose of the drug.
SK is considered a better Pg activator as compared to SAK due to its better catalytic efficiency. Three domains of SK function in a cooperative manner with each other to maximise the activation potential of the molecule. SAK being a single domain molecule, can activate Pg but with relatively lesser efficiency as compared to SK. SAK is structurally similar to a domain of SK and both maintain similar type of contacts with μPm at the interface of their binary activator complexes. However a domain of SK is not fully functional individually and requires the presence of β and γ domains for making extensive contacts with partner Pg/Pm or substrate Pg for better activation of Pg. Fusion of SAK with β and/or γ domain of SK may provide a better understanding of its cofactor activity or may result in a better version of SAK as a Pg activator.