



Designing of Humanized Streptokinase By Domain Engineering

Minor Experiment on Lab in r-DNA Techniques II

Submitted for

Final Year M.Sc., Practical Exam (III Semester)

Mr.G.Thirupugal

II M.Sc., Microbial Gene Technology



**Department Of Microbial Technology
School Of Biological Sciences
Madurai Kamaraj University
India-625 021**

Designing of Humanized Streptokinase By Domain Engineering

Introduction

A blood clot (thrombus) developed in the circulatory system can cause vascular blockage leading to serious consequences including death. A healthy hemostatic system suppresses the development of blood clots in normal circulation, but reacts extensively in the event of vascular injury to prevent blood loss. Outcomes of a failed hemostasis include stroke, pulmonary embolism, deep vein thrombosis and acute myocardial infarction. Pathologies involving a failure of hemostasis and the development of clot require clinical intervention consisting of intravenous administration of thrombolytic agents. Streptokinase is one such agent. Other thrombolytic or fibrinolytic agents include urokinase and the tissue type plasminogen activator (tPA). One clinically important thrombolytic agent, streptokinase.

Thrombolysis or Fibrinolysis

A blood clot or thrombus consists of blood cells occluded in a matrix of the protein fibrin. Enzyme-mediated dissolution of the fibrin clot is known as thrombolysis or fibrinolysis. In mammalian circulation, the enzyme responsible for fibrinolysis is plasmin, a trypsin-like serine protease. The fibrinolytically active plasmin is produced from the inactive protein plasminogen, which is present in circulation. Conversion of the inactive plasminogen to fibrinolytic plasmin involves a limited proteolytic cleavage, which is mediated by the various plasminogen activators. Two plasminogen activators that occur naturally in blood are the tissue type (tPA) and the urokinase type (uPA). The fibrinolytic activity in circulation is modulated by inhibitors of plasminogen activators (e.g., plasminogen activator inhibitor-1, PAI-1, a fast-acting inhibitor of tPA and uPA) and plasmin (e.g., α 1-antiplasmin, α 2 macroglobulin).

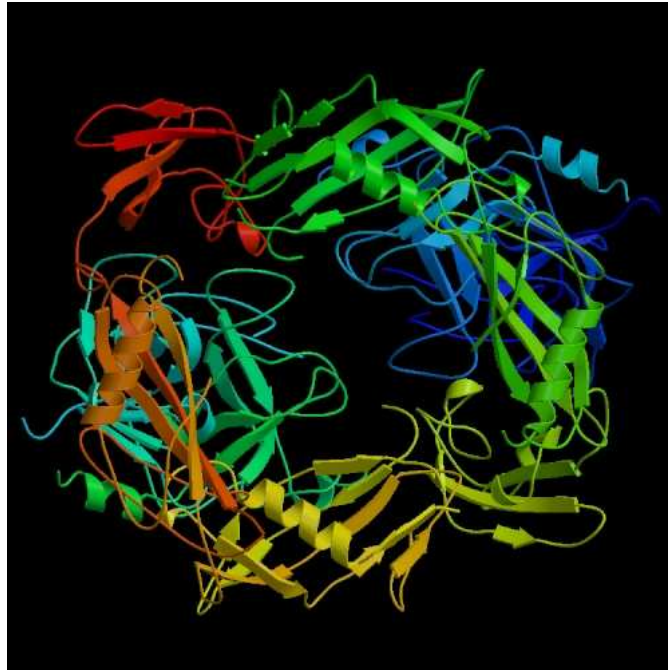
Streptokinase

Three major available thrombolytic agents (i.e., tPA, uPA, sPA), the microbial sourced streptokinase is the least expensive, but immunogenic. Human tPA and uPA are immunogenically benign, but are short-lived in vivo and therefore of a limited therapeutic effectiveness. Comparative clinical trials and cost-effectiveness considerations suggest that streptokinase is the drug of choice for thrombolytic therapy but this is debatable. Streptokinase is a nonhuman protein, and its introduction into the circulatory systems can illicit severe anaphylactic response, including death. The risk of this immune response is dependent on the level of the anti-streptokinase antibodies present in circulation. This immunogenicity restricts multiple treatments with streptokinase. The plasmin produced through the streptokinase-mediated activation of plasminogen breaks down streptokinase. This limits the in vivo half-life of streptokinase to about 30 min. Although streptokinase survives in circulation significantly longer than does tPA (a half-life of about 5 min), this is still short for efficient therapy. Unlike microbial streptokinase, human urokinase is not antigenic or pyrogenic, but its recovery from urine is expensive and supply is limited for any extensive use in therapy. Like streptokinase, urokinase activates both the circulating plasminogen and the clot-bound plasminogen. Because plasminogen activation by uPA and streptokinase is not specific to the clot-bound plasminogen, the use of these activators is associated with a serious risk of hemorrhage.

Streptokinase structure & Function

The extracellular enzyme streptokinase (EC 3.4.99.22) is produced by various strains of h-hemolytic streptococci. The enzyme is a single-chain polypeptide that exerts its fibrinolytic action indirectly by activating the circulatory plasminogen. Streptokinase has a molar mass of 47 kDa and is made up of 414 amino acid residues. The protein exhibits its maximum activity at a pH of approximately 7.5 and its isoelectric pH is 4.7. The protein does not contain cystine, cysteine, phosphorous, conjugated carbohydrates and lipids.

3D structure of The Catalytic Domain Of Human Plasmin and Streptokinase

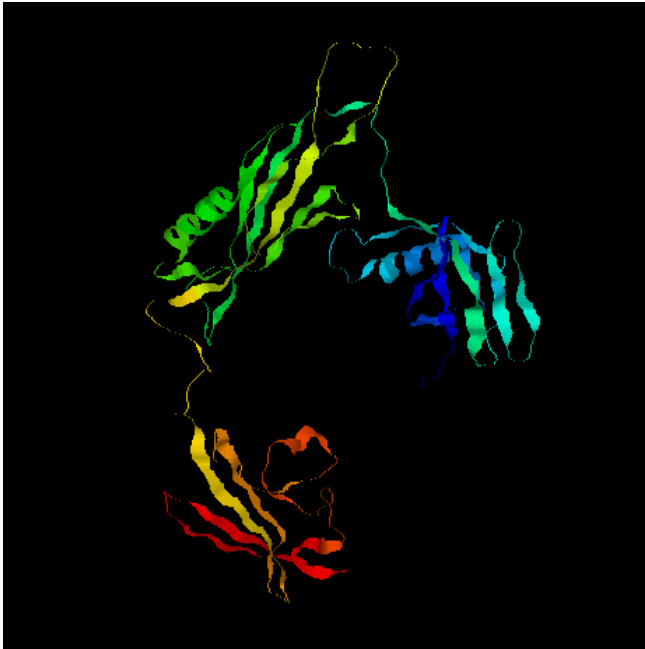


Streptokinase consists of multiple structural domains (i.e., , h- and g-domains) with different associated functional properties. Scanning calorimetric analysis suggests that the protein is composed of two distinct domains. The N-terminal domain (i.e., residues 1–59) has been found to complement the low plasminogen activation ability of the 60–414 amino acid residue domain of the protein.

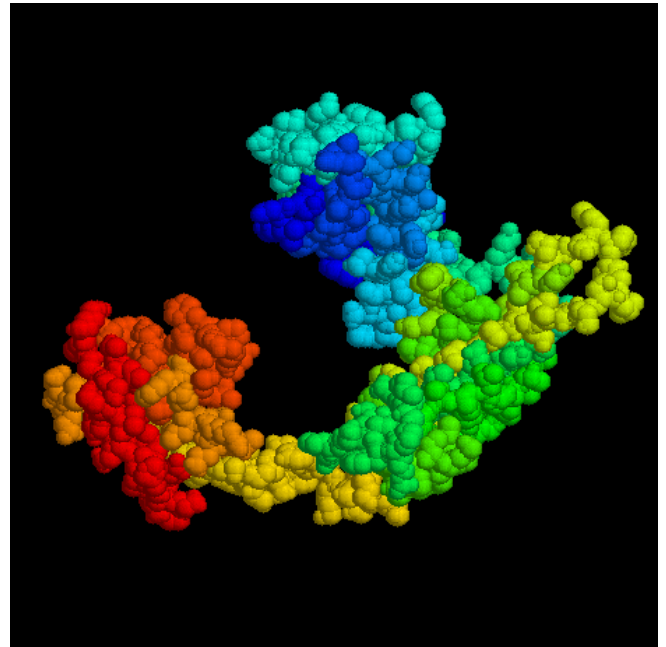
Streptokinase is known to activate plasminogen both by fibrin-dependent and fibrin independent mechanisms. Streptokinase interacts with plasminogen through multiple domains. The C-terminal domain of streptokinase is involved in plasminogen substrate recognition and activation. Similarly, the Asp41–His48 region of streptokinase is important in binding to the substrate plasminogen. The coiled region of the streptokinase g-domain is said to be essential for plasminogen activation. Similarly, the streptokinase h domain is involved in forming the streptokinase–plasminogen complex that is responsible for activating the plasminogen. Streptokinase binds preferentially to the extended conformation of plasminogen through the lysine binding site to trigger conformational activation of plasminogen. Streptokinase–plasminogen activator complex interacts with plasminogen through long range protein–protein interactions to maximize

catalytic turnover The first 59 amino acid residues seem to have multiple functional roles in streptokinase. Without these N-terminal residues, streptokinase has an unstable secondary structure. Loss of residues 1–59 greatly reduces the activity of the remaining streptokinase fragment (i.e., residues 60–414)

3D Structure of Streptokinase



Ribbon Model

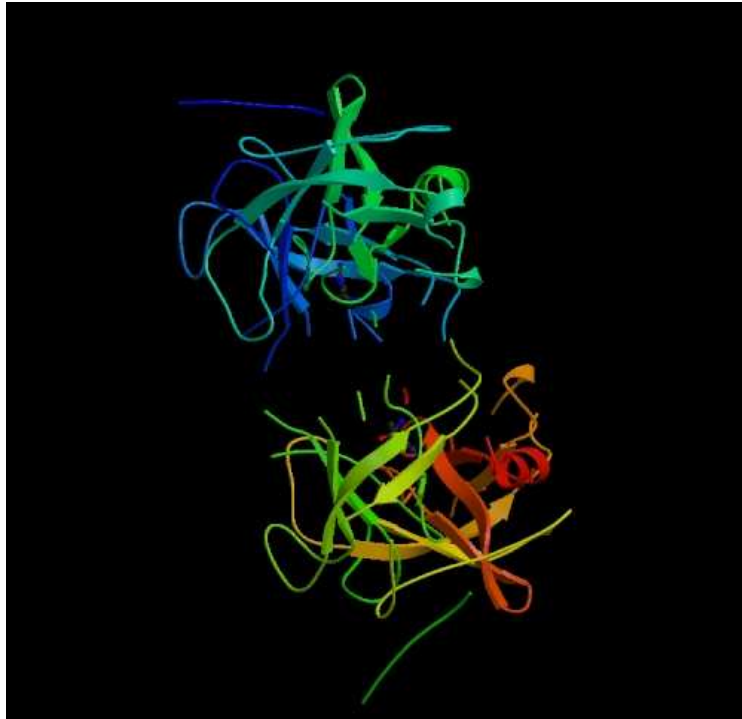


Space-Fill Model

Urokinase

Urokinase-type plasminogen activator (u-PA) promotes fibrinolysis by catalyzing the conversion of plasminogen to the active protease plasmin via the cleavage of a peptide bond. When localized to the external cell surface it contributes to tissue remodelling and cellular migration; inhibition of its activity impedes the spread of cancer. u-PA has three domains: an N-terminal receptor-binding growth factor domain, a central kringle domain and a C-terminal catalytic protease domain. The biological roles of the fibrinolytic enzymes render them therapeutic targets.

3D Structure of Urokinase

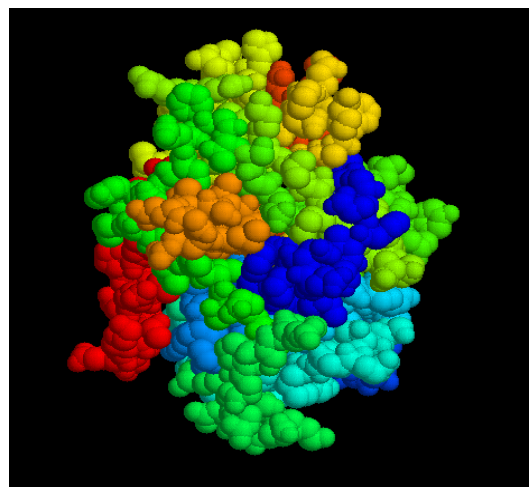


Urokinase Domain D

Residues length: 128 amino acids



Ribbon Model



Space-Fill Model

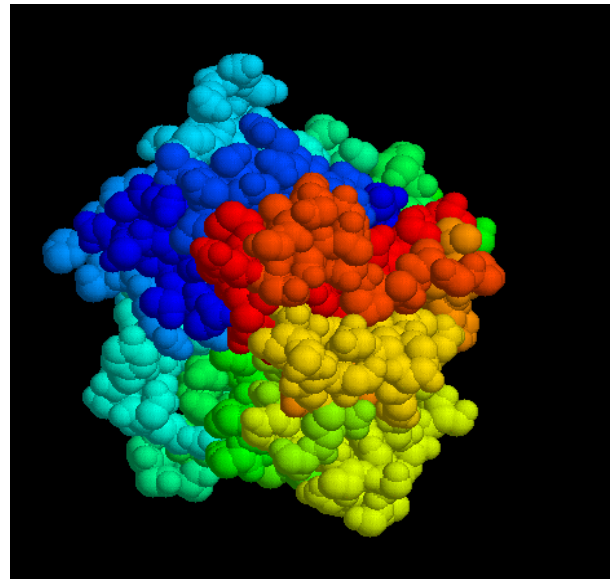
Enhancing Activity of Streptokinase

Immunogenicity of streptokinase and its relatively short half-life in circulation limit therapeutic potential of this protein. Streptokinase in circulation is proteolytically degraded by plasmin. Consequently, research has focused on structurally modifying streptokinase to extend half-life, reduce or eliminate immunogenicity, and improve plasminogen activation. Any structural change needs to be informed by a thorough structure–function analysis of streptokinase domains and this has been the subject of extensive investigation. Structurally modified streptokinases have been produced in several ways including genetic mutation, recombinant DNA technology and chemical or enzymatic modification of the native streptokinase.

Humanization of Streptokinase

Domain shuffling

Urokinase Domain D and Streptokinase Plasminogen activator Domain



Conclusion

Native streptokinase is useful for cost-effective thrombolytic therapy in clinical practice, but its use is not risk free. Domain engineering helpful modifications of streptokinase has been used for extending its half-life in circulation, improving plasminogen activation, and reducing or eliminating immunogenicity.

Software's used

- ❖ **Cn3D 4.1**
- ❖ **Chimera**
- ❖ **Chemera**
- ❖ **Rasmol**
- ❖ **Spdbv**
- ❖ **ESyPred 3D**
- ❖ **Geno 3D**
- ❖ **3D PSSM**
- ❖ **Protein Data Bank**