

Production and partial purification of streptokinase from *Streptococcus pyogenes*

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Received: 19 January 2011 / Received in revised form: 13 March 2010, Accepted: 19 March 2011, Published online: 01 July 2011,
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Abstract

Streptokinase is as effective as recombinant tissue plasminogen activator (tPA) in treating acute myocardial infarction and it is certainly more cost-effective. In view of the relatively recent availability of the competing recombinant tPA, skepticism is being expressed about the continued viability of streptokinase therapy. Despite this research on streptokinase continues, and it remains a vital and affordable therapy especially in the world's poorer healthcare systems. Our present study focuses on the production of streptokinase from *Streptococcus pyogenes* species and partial purification of streptokinase by ammonium sulfate precipitation, dialysis and column chromatography. The enzyme is quantified by Lowry's method and its electrophoretic mobility and molecular weight were determined by SDS-PAGE.

Key words: Streptokinase, *Streptococcus pyogenes*, tissue plasminogen activator, SDS-PAGE.

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 (Collen et al. 1988). Streptokinase is one such

agent. Other thrombolytic or fibrinolytic agents include urokinase and tissue type plasminogen activator (tPA).

The extracellular enzyme streptokinase (EC 3.4.99.22) is produced by various strains of β -hemolytic *Streptococci*. The enzyme is a single polypeptide that exerts its fibrinolytic action indirectly by activating the circulatory plasminogen. The complete amino acid sequence of streptokinase was first established by Jackson and Tang (1982). Streptokinase has a molar mass of 47 kDa and is made up of 414 amino acid residues (Malke and Ferretti 1984). The protein exhibits its maximum activity at a pH of approximately 7.5 and its isoelectric pH is 4.7 (Castellino 1976). The protein does not contain cystine, cysteine, phosphorous, conjugated carbohydrates and lipids. Other physical and chemical data on streptokinase have been reported by De Renzo et al (1967). Streptokinase produced by different groups of streptococci differs considerably in structure (Malke 1993).

Commercial production of streptokinase requires special attention to biosafety considerations because the protein is potentially immunogenic to process workers. In addition, care is necessary if streptokinase is being produced using natural strains of *Streptococci* because all streptokinase producing streptococci are potentially pathogenic. The present study is to produce and partially purify the streptokinase enzyme from *Streptococcus pyogenes*.

Materials and methods

Sample collection

The culture was obtained from microbiology department, Aravind Eye hospital, Tirunelveli, India. It was subculture on blood agar medium and Todd Hewitt Broth (THB) medium and stored at 4 °C. Confirmation of microorganism was done with blood agar medium, Todd Hewitt broth medium, gram staining and biochemical tests.

Production of streptokinase

The bacteria were grown in 25 ml of THB at 37 °C. By increasing the turbidity to the level of OD-0.6 at 600 nm, it was subcultured in 250 ml of broth. It was observed that the optimum pH for cell growth

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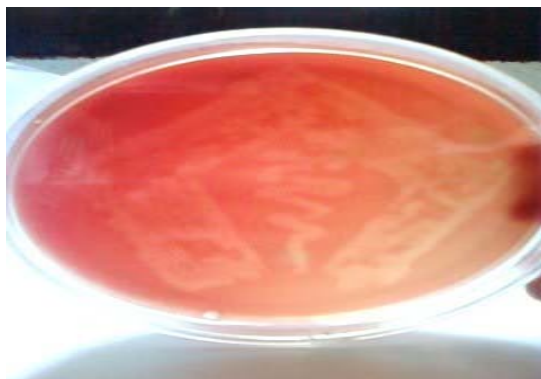


Figure 1. *Streptococcus pyogenes* on blood agar plates

and streptokinase activity was at the neutral condition (pH-7). To improve the growth condition, the pH of the culture was maintained at 7.0 during incubation at 37 °C for 8 hours by adding sterile 4% (w/v) glucose and 5N NaOH. The culture was centrifuged for 25 minutes at 10,000 g. The supernatant was filtered through a 0.45µm cellulose acetate filter. The supernatant which was filtered was collected and labelled as crude enzyme. (Mohammad Babashamsi et al. 2009). Then the streptokinase was purified by ammonium sulfate precipitation, dialysis and using column chromatography.

Biological activity of streptokinase

Radial caseinolysis assay

The skim milk agar medium was prepared and wells were punctured in agar plate. 25 µl of streptokinase enzyme was loaded into the wells and kept for 12 hours incubation at 37 °C (Yanjun duan et al. 1998).

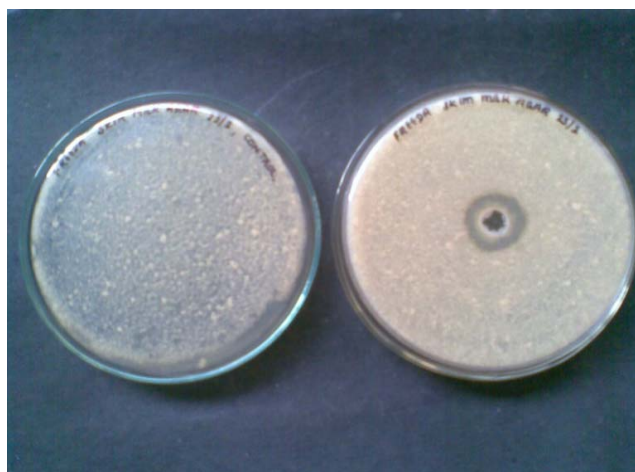


Figure 2. Biological activity of streptokinase- Radial Caseinolysis assay.

Assay of streptokinase activity

The streptokinase activity was determined by the method used by Silverstein (1975) and Park et al. (1991). It is based on the observation that streptokinase-human plasma complex can hydrolyze the artificial substrate, CLN (Park et al. 1991).

Table 1. Result for biochemical tests

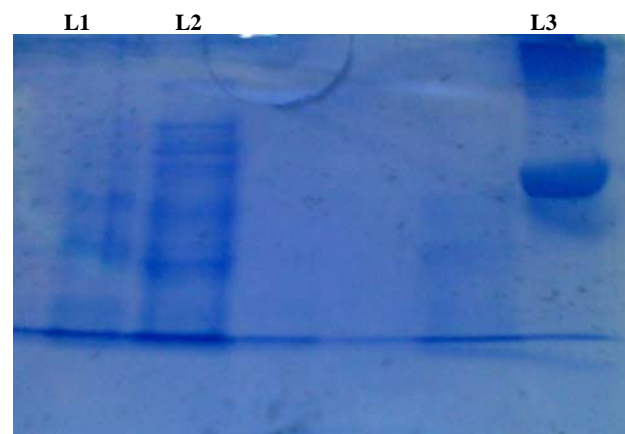
TEST	<i>Streptococcus pyogenes</i>
Indole	Negative
Methyl red	Positive
Voges proskauer	Negative
Citrate utilization	Negative

Protein estimation

The concentration of protein present in crude enzyme, partially purified and purified enzyme were estimated by Lowry's method (Lowry 1951).

SDS-Polyacrylamide gel electrophoresis (SDS PAGE)

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 25 Mm Tris/192 Mm glycerin buffer (pH 8.3) that contained 0.1% (w/v) SDS as the running buffer, as described by Laemmli (1970).



**LANE 1 : Purified enzyme,
LANE 2 : Crude Enzyme,
LANE 3 : Standard Marker (BSA)**

Figure 3. SDS PAGE analysis of streptokinase expression

Results and discussion

The β hemolysis zone was found on blood agar plate (Fig. 1) showed the conformation of microorganism. After 24 hours incubation, turbidity was found in the THB medium. The organism was confirmed by Gram's staining and it was shown to be gram positive cocci. The organism was confirmed by IMViC test and it was confirmed as *Streptococcus pyogenes* (Table 1). Streptokinase enzyme was produced in Todd Hewitt Broth (THB) of pH 7.8 from the *Streptococcus pyogenes*.

The streptokinase enzymes were purified using ammonium sulphate precipitation; dialysis and column chromatography and the result were mentioned in Table 2. The total proteins were precipitated with

Table 2. Results for purification of streptokinase by Ammonium sulfate precipitation, dialysis and column chromatography.

Purification Steps	Enzyme Activity	Total Activity	Total protein (mg)	Specific Activity	Purification Fold	Recovery (%)
Crude Extract	8066.7	806.7	1.21	666.7	1	100%
Dialysis	7600	760	0.77	987	1.48	94.2%
Column Chromatography	6600	660	0.45	1466.7	2.20	81.8%

65% saturation of ammonium sulfate. Purification of proteins by ammonium sulfate precipitation is one of the most widely used preliminary purification procedures. It is based on the differential solubility of different proteins in salt solutions. This is followed by increased protein-protein interactions, leading to the aggregation or precipitation of the proteins. The precipitated proteins were dialyzed against 1 Mm Tris-buffer and the proteins were purified.

The streptokinase enzymes were eluted in DEAE cellulose column by using 10mM Tris buffer, pH 7.0 with linear gradient NaCl (25-30 mM). The fractions were collected at time intervals. The column chromatographic procedure employed here was purely based on the anionic binding of DEAE cellulose column with a streptokinase enzyme.

The crude extract, precipitated protein and purified protein were estimated by Lowry method. From this the concentration of the crude enzyme was found to be 1.21 mg/ml, the concentration of the partial purified enzyme is 0.77 mg/ml and the concentration of the purified enzyme was 0.45 mg/ml. In Radial Caseinolysis assay, the clear zones were found around the loaded wells on skim milk agar plate was indicated caseinolytic activity of streptokinase. (Fig. 2)

In SDS-PAGE, Crude enzyme, precipitated enzyme and purified enzyme were electrophoresised on SDS-PAGE. The purified protein was seen as a band approximately 47 kDa. Appearance of SK as single band at 47kDa size confirmed the presence of single protein in the preparation. The molecular weight of Streptokinase was determined to be approximately 47 kDa.

Acknowledgement

We sincerely thank to the department of Biotechnology, SNMV Arts and Science College, Malumichampatti, Coimbatore for providing the laboratory facilities to carry out this research work.

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