Sci. J. Fac. Sci. Minufia Univ., 2009, Vol. XXIII, 105-121

PURIFICATION AND CHARACTERIZATION OF STREPTOKINASE PRODUCED BY S. PYOGENS AND S. EQUISIMILIS

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ABSTRACT

In an attempt to purify streptokinase produced by S. pyogenes and S. equisimilis, it was first purified by 45% and 50% ammonium sulfate precipitation respectively followed by dialysis over night. Partially purified enzyme samples were applied to Sephadex G-100 column. Gel filtration step resulted in 30 and 32 fold increase in specific activity of streptokinase produced by S. pyogenes and S. equisimilis respectively. Further purification was obtained by Sephacryl S-200 column which increased S. pyogenes and S. equisimilis enzymes specific activity to more than 60%and 47 respectively. Purification of both enzymes with the ion-exchange DEAE Sephadex A-50 column resulted in more than 160%and 164% increase in the specific activity of the purified enzyme. The highest residual activity of both S. pyogenes and S. equisimilis purified streptokinase was obtained at pH 8. However, purified enzymes retained almost all of their activity at pH 7.The specific activity was significantly decreased at alkaline pH values. Both S. pyogenes and S. equisimilis purified enzymes showed a maximum activity at 37oC. Moreover, S. pyogenes streptokinase kept more than 90% of its activity at 45oC. Residual activity decreased significantly when temperature was elevated to 60oC. S. equisimilis streptokinase lost almost 60% of its activity at 60oC, but the residual activity remained over 80% at 45oC. S. pyogenes purified enzyme kept more than 86% of its activity after incubation at 50oC for 60 minutes. Half the enzymatic activity was obtained after exposure to 60oC for 30 minutes. In case of S. equisimilis purified streptokinase, almost all the enzyme activity survived incubation at 50oC for the entire 60 minutes. However, the enzyme kept only 40% of its activity at ٦0oC for 50 minutes. Higher degrees of temperature significantly

decreased the enzyme activity. The degree of thermostability for both purified enzymes decreased directly with either the increase of temperature or the increase of the exposure time. Kinetics study for substrate activation with purified S. pyogenes streptokinase showed a maximum velocity (Vmax) of the reaction was 1.183 □M/ min, and a km value of 0.285 □M/ml when N-p-tosyl-glycyl-prolyl-lysine-p-nitroanilide acetate was used as the substrate for liberated plasmin. Similar results for plasminogen activation were obtained after incubating human plasminogen with purified S. equisimilis streptokinase. Reaction rate for substrate hydrolysis by liberated plasmin has a maximum velocity of 1.176 □M/min. Km of the reaction was 0.287 □M/ml.

KEYWORDS: STREPTOKINASE, GEL FILTRATION, ION EXCHANGE DEAE SEPHADEX A-50, ENZYME KINETICS

INTRODUCTION

The most common circulatory disorders that affect the human health nowadays are strokes and myocardial infarction (Baruah *et al.*, 2006). These diseases are consequently developed after formation of blood clot (thrombus), which consists of blood cells trapped in a matrix of the protein fibrin. Fibrinolytic agents that are produced by bacteria – especially streptokinase and staphylokinase- were found to be the most effective and inexpensive drugs that can be used as a treatment for these kinds of disorders (Sikri and Bardia, 2007). For a safe and convenient application of these pharmaceutical drugs, it must be obtained in a very pure state since it is produced from potentially pathogenic microbes. Several methods have been reported for streptokinase purification. There are many differences between them according to the source of enzyme, and the method of fermentation.

The enzyme was purified using DEAE-cellulose column chromatography followed by column electrophoresis in sucrose density gradient. The DEAE-cellulose column step was repeated to remove impurities found in the purified enzyme solution. (De Renzo, 1967). The enzyme was purified by a similar procedure but it was modified by combination of ion exchange DEAE Sephadex A-50 and gel filtration Sephadex G-100 chromatography columns. (Taylor and Botts, 1968). Tomar (1968) purified streptokinase from Varidase using a different procedure (Banerjee, *et al.*, 2004). The enzyme was fractionated either by hydroxyapatite chromatography or ammonium sulfate fractionation. Precipitation of streptokinase with 40–50% ammonium sulfate resulted in a two- to threefold increase in specific activity. The precipitate was recovered by centrifugation and

dialyzed against 0.09 M sodium chloride. The dialyzed solution was further purified by gradient elution from a DEAE-cellulose chromatography column followed by ultra filtration (Tomar, 1968). A highly pure streptokinase was recovered from the relatively crude commercial Kabikinase (Kabi Vitram, Sweden) by Einarsson *et al.* (1979). Similarly, ammonium sulfate fractionation was first used to obtain a crude precipitate of streptokinase. The precipitate was redissolved and subjected to gel filtration chromatography (Leigh, 1994). The eluted enzyme fractions were further purified using column chromatography on DEAE-cellulose or DEAE-Sepharose (Einarsson *et al.*, 1979; Ohkuni *et al.*, 1992; Avilan *et al.*, 1997). Several affinity chromatography methods have been discussed for purifying streptokinase (Castellino *et al.*, 1976; Rodriguez *et al.*, 1992, 1994). Insolubilized di-isopropyl fluorophosphates (DIP) plasmin was used as the affinity ligand (Castellino *et al.*, 1976). This ligand was produced by the conversion of plasminogen to plasmin with urokinase and inhibition of the proteolytic activity of plasmin by DIP. Purification through the affinity column caused a 30% decrease in the enzyme activity, suggesting incomplete inhibition of the plasmin affinity ligand bound to the chromatography matrix.

Affinity purification methods with monoclonal antibody as a ligand have been used to purify streptokinase (Banerjee, *et al.*, 2004), acylated plasminogen or plasmin as the affinity ligand (Rodriguez *et al.*, 1992). The acylation of plasminogen or plasmin was carried out with p-nitrophenyl-p-guanidino benzoate (NPGB). Plasminogen acylation with NPGB allowed it to be used as an affinity ligand without requiring activation to plasmin. This probably reduced the plasmin associated proteolysis of streptokinase. Rodriguez *et al.* (1994) used a combination of human plasminogen and monoclonal antibody against streptokinase for chromatographic purification of streptokinase. Both ligands were bound to Sepharose as the chromatographic matrix. Immobilized NPGB acylated plasminogen for affinity purification was also used for the enzyme purification using a solution of urea as the eluent. Streptokinase has been purified from the filtrate of a streptococcal fermentation broth using hydrophobic interaction chromatography on phenyl- or octyl-Sepharose column (Pupo *et al.*, 1999). A gradient elution with 21% ammonium sulfate was used to recover the enzyme. Further purification involved gel permeation and ion exchange chromatography steps (Banerjee, *et al.*, 2004).

Separation of streptokinase by reverse-phase HPLC has been used for purifying a bovine plasminogen activator from culture supernatants of the bovine pathogen *S. uberis*. A single protein with a molecular mass of 32 kDa was detected in the eluted active fraction. This plasminogen activator lacked the C-

terminal domain that is characteristic of the *S. equisimilis* streptokinase (Johnsen *et al.*, 1999).

MATERIALS AND METHODS

Bacterial growth and streptokinase production

S. pyogenes and *S. equisimilis* were used for streptokinase production, the two strains are known as hyperproducers of the enzyme. Cells were activated by growing them overnight on Strep-base medium containing 10 g tryptone, 5 g yeast extract, 2 g K_2HPO_4, 5 g NaCl, and 5 g glucose per liter. The pH was adjusted to 7.4 by the addition of diluted NaOH. Cells were allowed to grow at 37°C with shaking at 100 rpm using a shaker incubator. One ml of the previously grown cultures was used to inoculate 100 ml of Brain Heart Infusion (BHI) medium for enzymatic production. Cells were allowed to grow at 37°C with shaking at 100 rpm. After incubation for 24 hours cultures were taken and centrifuged in a microcentrifuge at 8,000 rpm for 2 minutes. Supernatants were kept on ice for further purification steps.

Streptokinase activity

Streptokinase activity was determined by the colorimetric method with N-p-tosyl-glycyl-prolyl-lysine-p-nitroanilide acetate (Chromozym PL; Boehringer) as the substrate for plasmin (Wong *et al.*, 1994; Yazdani and Mukherjee, 2002). Samples were mixed with plasminogen and the mixture was incubated at 37°C for about 5 minutes. The substrates mixture containing the chromozym dissolved in 50 mM Tris-HCl buffer pH 8.0 was then added to the enzyme-substrate mixture. The reaction was incubated for 20 minutes at 37°C and the change in absorbance at 405 nm was monitored at 37°C by using a spectrophotometer. Units of enzyme activity were calculated with a standard streptokinase curve.

Protein determination

Protein concentrations in different fractions were determined as described by (Bradford, 1976). Protein content was measured at 595 nm using a spectrophotometer. A standard curve was made using Bovine serum albumin as a standard protein. Blank reagent was 100 µl of the propitiate buffer and 5 ml of protein reagent.

Purification of Streptokinase

Precipitation of streptokinase by $(NH_4)_2SO_4$

Fifty ml crude enzyme sample was precipitated with solid ammonium sulfate (45% saturation) in an ice-bath then it was allowed to stand for 1 hour at 4°C. The mixture was centrifuged at 12,000 rpm for 30 minutes. Pellets were dissolved in a minimal volume of 50 mM Tris-HCl buffer, pH 8.0 then dialyzed

overnight against the same buffer. Samples from dialyzed enzyme samples were taken for determination of protein content and the streptokinase activity.

Sephadex G-100 column

Three ml of the dialyzed streptokinase was applied to the Sephadex G-100 (2 x 30 cm) column. The enzyme was eluted with 50 mM Tris-HCl, pH 8.0 containing 10 mM $CaCl_2$ at a flow rate of 3 ml/minute. Fractions (3 ml) were collected at 4°C after which the absorbance at 280 nm, and enzyme activity were determined. Active fractions were collected and reprecipitated on ice with solid $(NH_4)_2SO_4$. After standing overnight at 4°C, the precipitate was removed by centrifugation at 12,000 rpm 30 minutes at 4°C. The precipitate was re-dissolved in a small volume of the above buffer and stored at –20°C to be used in further purification and characterization experiments.

Sephacryl S-200 column

One ml of enzyme sample was applied to the Sephacryl S-200 column (1 x 20 cm). Enzyme was eluted with buffer at a flow rate of 0.7 ml/min Fractions (1 ml) were collected at 4°C after which the absorbance at 280 nm and enzyme activity were determined. Active fractions were collected and reprecipitated on ice with solid $(NH_4)_2SO_4$. After standing overnight at 4°C, the precipitate was removed by centrifugation at 12,000 rpm 30 minutes at 4°C. The precipitate was re-dissolved in a small volume of the above buffer and stored at –20°C to be used in further characterization experiments.

DEAE Sephadex A-50 column

Streptokinase sample was further purified by dissolving in 3 ml of 0.10 M NaCl-0.01 M Tris buffer (pH 8.0) and dialyzed for 24 hr at 4°C against the same buffer. Enzyme sample was then adsorbed and fractionated on DEAE Sephadex A-50 (Pharmacia Fine Co., Uppsala, Sweden) at 4°C with a linear (0.1 M-0.5 M) NaCl-Tris gradient. DEAE Sephadex A-50 column (1 x 20 cm) was washed with 500 ml of 0.1 M NaCI, 0.01 M Tris buffer (pH 8.0) and allowed to equilibrate with this buffer over 24 hr after a final adjustment of the pH of the slurry to pH 8.0 with 0.1 N HCI. Flow rate was adjusted to be 20 ml per hr. Sample fractions were collected in 1 ml. The protein concentration was determined by absorbance measurements at 280 nm with a spectrophotometer. The effluent containing streptokinase was pooled and dialyzed for 24 hr at 4°C against 0.001 M phosphate and 0.3 M NaCl at pH 7.4.

Protein electrophoresis of purified enzymes

Purified streptokinase samples eluted from DEAE Sephadex A-50 column were applied to Sodium dodecyl sulphate polyacrylamide gel electrophoresis using a discontinuous buffer system described by Laemmli (1970). After assembly of the gel apparatus, 10 ml of each enzyme sample was applied in each

well. Protein marker as well as streptokinase standard (Hermentin *et al.,* 2005) were applied and electrophoresis was carried out at 100 volts in Tris-Glycine-SDS running buffer containing 3 g Tris base, 14.4 g Glycine, and 1 g SDS per liter of distilled water. Electrophoresis was carried out until the tracking dye (Bromophenol blue) reached the bottom of the gel. Gel was then stained in 50 ml of staining solution containing 0.125 % Comassie blue for 45 minutes with shaking at room temperature. Finally, stained gel was de-stained in de-staining solution that contained 10% glacial acetic acid and 50% methanol.

Characterization of purified streptokinases

Determination of optimum pH

The optimum pH of the purified extracellular streptokinase was determined over a pH range (4-12). The buffers used in this test were 0.1 M acetate buffer for pH 4 and pH 6, 0.1 M Tris-HCl buffer for pH 7.0 and pH 8.0, and 0.1 M carbonate buffer for pH 9.0, 10.0, 11.0 and pH 12.0.

Determination of optimum temperature

The optimum temperature of the purified enzyme was determined over a temperature range of 20°C-80°C. The pHs of the reactions were adjusted to be 7.0 for all temperatures (Derechin, 1961).

Thermostability of the purified streptokinases

Purified streptokinase was tested for its thermal stability. Aliquots of purified enzyme and 0.1 M Tris-HCl buffer, pH 7.0 were heated for different time intervals (10-60 minutes) at temperatures from 50°C to 80°C. Similarly, mixtures of equal volumes of purified enzyme and plasminogen were preheated at temperatures from 50°C to 80°C for the same time intervals. Substrate was added to each reaction mixture after the exposure time at the temperatures above. The remaining activity of streptokinase was determined as mentioned before.

Kinetics of purified enzymes

Both purified *S. pyogenes* and *S. equisimilis* streptokinases were tested for their plasminogen activation kinetics. Ten nM samples of human Plasminogen were mixed with 10 µl of the purified streptokinase sample under investigation for 5-min at 37°C. The substrate mixture containing serial dilutions of N-p-tosyl-glycyl-prolyl-lysine-p-nitroanilide acetate dissolved in 50 mM Tris-HCl buffer pH 8.0 was then added to the enzyme-plasminogen mixture. The reaction was incubated for 10 minutes at 37°C and the change in absorbance at 405 nm was monitored at 37°C by using a spectrophotometer. Units of enzyme activity were calculated with a standard streptokinase curve (Sazonova *et al.*, 2001).

RESULTS AND DISCUSSION

Crude enzyme samples obtained after centrifugation of both *S. pyogenes* and *S. equisimilis* cultures were further purified with salting out using ammonium sulfate (Clark and Switzer, 1977). It is also known that, in addition to ammonium sulfate, proteins can be precipitated by organic solvents such as acetone and isopropyl alcohol on the principle of isoelectric precipitation (Simpson, 2004). This method can be used as alternative method of salting out the enzyme by ammonium sulfate precipitation. Samples of crude enzyme produced by *S. pyogenes* and *S. equisimilis* were precipitated separately by 45% and 50% solid ammonium sulfate respectively and increased volumes of some organic solvents (results are not shown). Precipitation of streptokinase with ammonium sulfate resulted in more than 13 fold purification of streptokinase enzyme produced by both strains. Precipitated samples were dialyzed against Tris-HCl buffer pH 7.4. This step resulted in 16-fold purification of the enzyme in case of *S. pyogenes* (Table 1) and 18-fold purification in case of *S. equisimilis* (Table 2). Dialyzed enzyme samples were further purified with Sephadex G-100. For further purification, streptokinase eluted from Sephadex G-100 column was divided into two samples, the first sample was applied to Sephacryl S-200 column. The second sample was applied to DEAE Sephadex A-50 column. Figure (1) illustrates the fractionation profile of the streptokinase produced by *S. pyogenes* when the enzyme sample was eluted at a flow rate of 36 ml/hr. Active fractionations started form fraction 7 and ended with fraction 17. A similar pattern of enzyme fractionation was obtained when streptokinase produced by *S. equisimilis* was applied to Sephadex G-100 column (Figure 2). Sephadex G-100 purification step resulted in 30 and 32 fold increase in specific activity of streptokinase produced by *S. pyogenes* and *S. equisimilis* respectively (Table 1 and Table 2).

Table (1): Purification table of streptokinase produced by *S. pyogenes* after application of Sephadex G-100 and Sephacryl S-200 columns.

Purification Step	Units/ml	mg protein/ml	Specific Activity	Fold Purification
Cell free supernatant	83.64	4.91	17.03	1.0
Pellet after $(NH_4)_2SO_4$ Precipitation	912.09	3.95	230.73	13.5
Pellets after dialysis	870.29	3.11	279.43	16.4
After Sephadex G-100	737.02	1.44	512.72	30.1
After Sephacryl S-200	602.55	0.72	838.34	49.2

Table (2): Purification table of streptokinase produced by *S. equisimilis* after application of Sephadex G-100 and Sephacryl S-200 columns.

Purification Step	Units/ml	mg protein/ml	Specific Activity	Fold Purification
Cell free supernatant	77.52	4.67	16.59	1.0
Pellet after $(NH_4)_2SO_4$ Precipitation	755.47	3.47	217.47	13.1
Pellets after dialysis	726.30	2.40	303.16	18.3
After Sephadex G-100	702.58	1.32	533.19	32.1
After Sephacryl S-200	584.11	0.74	786.47	47.4

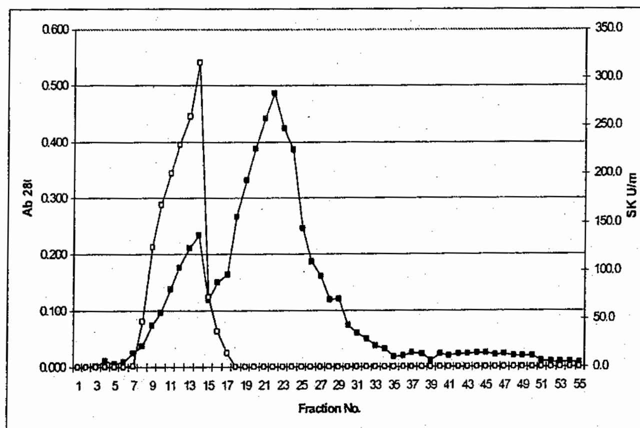


Figure (1): Fractionation pattern of *S. pyogenes* streptokinase after purification with Sephadex G-100 column. ■ and □ represent absorbance at 280 nm and streptokinase activity (Units/ml) respectively.

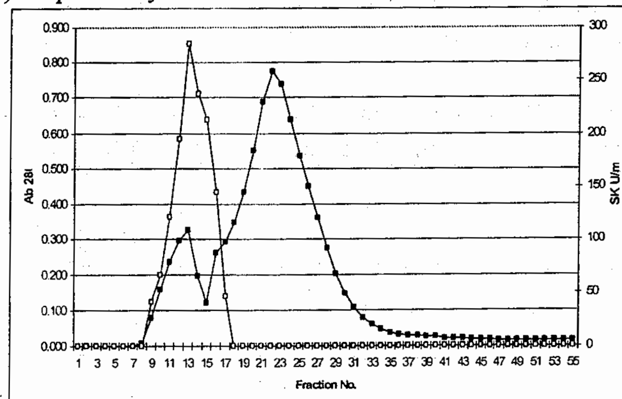


Figure (2): Fractionation pattern of *S. equisimilis* streptokinase after purification with Sephadex G-100 column. ■ and □ represent absorbance at 280 nm and streptokinase activity (Units/ml) respectively.

Purification of enzymes by a combined method of more than one chromatographic column significantly increases the purification fold of the purified enzyme (Banerjee *et al.*, 2004). To increase the purification degree of streptokinase produced by *S. pyogenes* and *S. equisimilis*, eluted samples from Sephadex G-100 were further applied to Sephacryl S-200 column. Figure (3) show the fractionation pattern of *S. pyogenes* purified enzyme. Sephacryl S-200 column purification increased the enzyme specific activity more than 60% increase when compared to that obtained from Sephadex G-100 column. Purification fold was also increased significantly to more than 49 purification fold (Table 1). *S. equisimilis* streptokinase eluted from Sephadex G-100 column was further purified with Sephacryl S-200

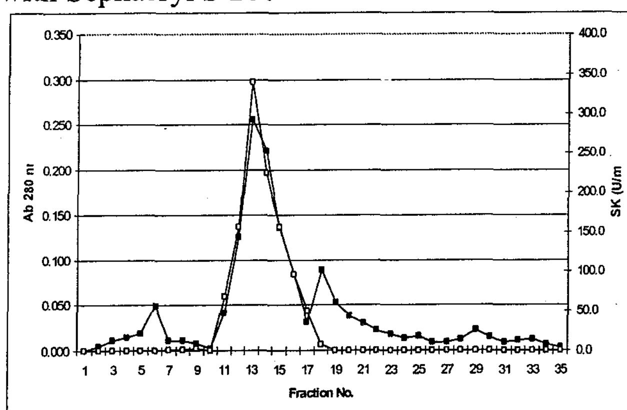


Figure (3): Fractionation pattern of *S. pyogenes* streptokinase after purification with Sephacryl S-200 column. ■and □represent absorbance at 280 nm and streptokinase activity (Units/ml) respectively.

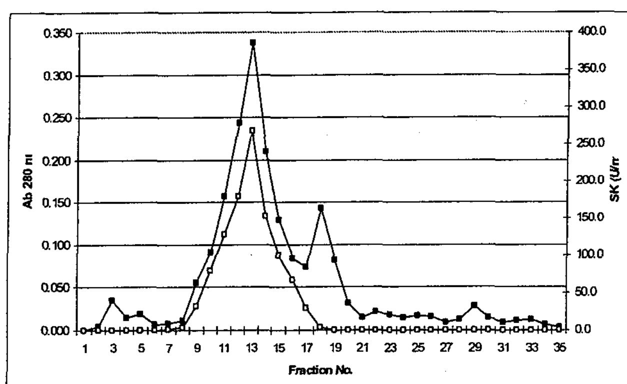


Figure (4): Fractionation pattern of *S. equisimilis* streptokinase after purification with Sephacryl S-200 column. ■and □represent absorbance at 280 nm and streptokinase activity (Units/ml) respectively.

column (Figure 4). This second purification step resulted in more than 47% increase in the specific activity of the purified enzyme. Sephacryl S-200 column also increased the purification to more than 47 fold (Table 2). In a parallel purification step, streptokinase samples eluted from Sephadex G-100 column were applied to the ion-exchange chromatography column, DEAE Sephadex A-50 in an attempt to compare the degree of enzyme purification with the previously obtained results from the combined (Sephadex G-100, Sephacryl S-200) purification method. Figure (5) illustrates the fractionation pattern of *S. pyogenes* enzyme - previously purified with Sephadex G-100 column - after application to the ion-exchange column. The ion-exchange chromatography column resulted in more than 160% increase in the specific activity of the purified enzyme. This purification step resulted also in more than 78 fold purification of the enzyme. In a similar way, DEAE Sephadex A-50 column increased the specific activity of the purified *S. equisimilis* streptokinase (Figure 6) to more than 164%. This purification step resulted in 85 fold purification of the enzyme. Figure (7) shows SDS-PAGE analysis of both purified enzymes, the analysis shows a single band for both enzymes that matches with the streptokinase standard band.

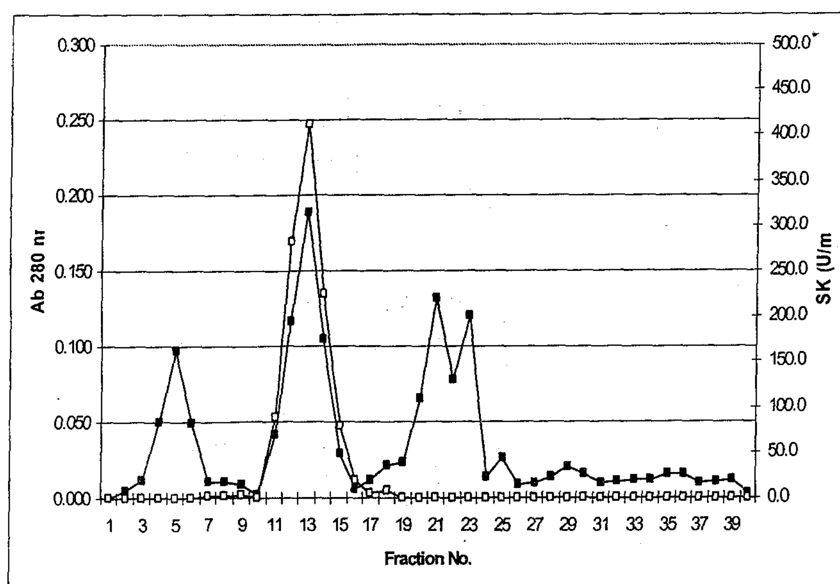


Figure (5): Fractionation pattern of *S. pyogenes* streptokinase after purification with DEAE Sephadex A-50 column. ■ and □ represent absorbance at 280 nm and streptokinase activity (Units/ml) respectively.

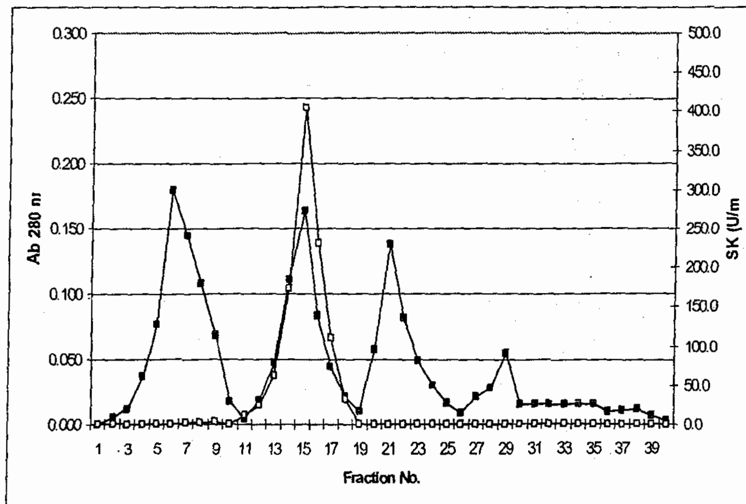


Figure (6): Fractionation pattern of *S. equisimilis* streptokinase after purification with DEAE Sephadex A-50 column. ■ and □ represent absorbance at 280 nm and streptokinase activity (Units/ml) respectively.

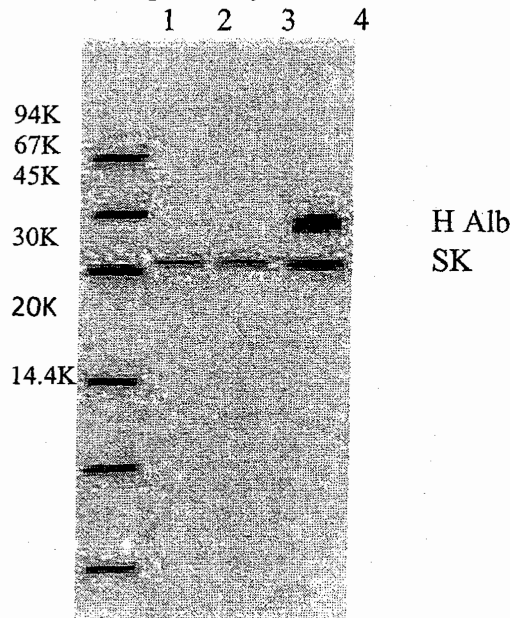
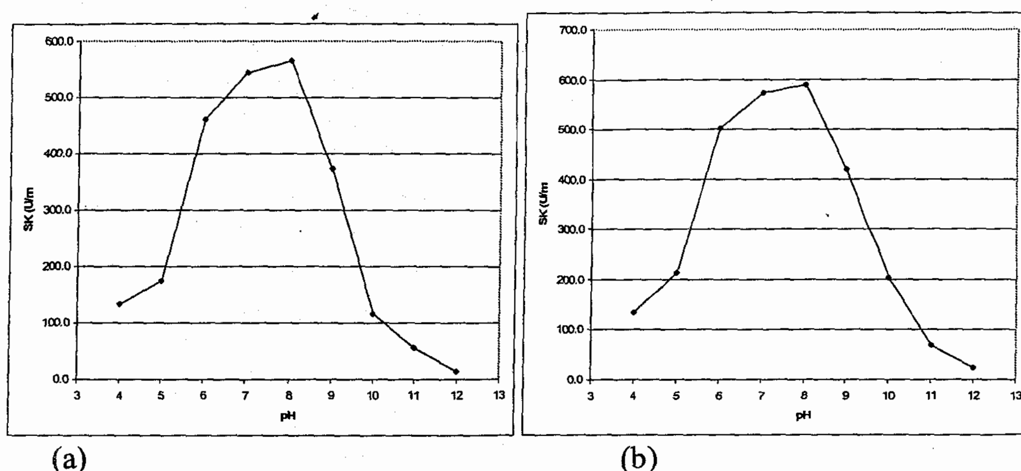


Figure (7): SDS-PAGE analysis of purified streptokinases after elution from DEAE Sephadex A-50 column. Lane 1 contains the molecular weight standards. Lane 2 and lane 3 contain S. pyogenes and S. equisimilis purified streptokinases respectively. Lane 4 contains human serum albumen and streptokinase standards.

Characterization of purified streptokinases

Optimum pH for purified streptokinases activity

The highest residual activity of both *S. pyogenes* and *S. equisimilis* purified streptokinase was obtained at pH 8 (Figures 8). However, purified enzymes retained almost all of their activity at pH 7. The specific activity was significantly decreased at alkaline pH values.

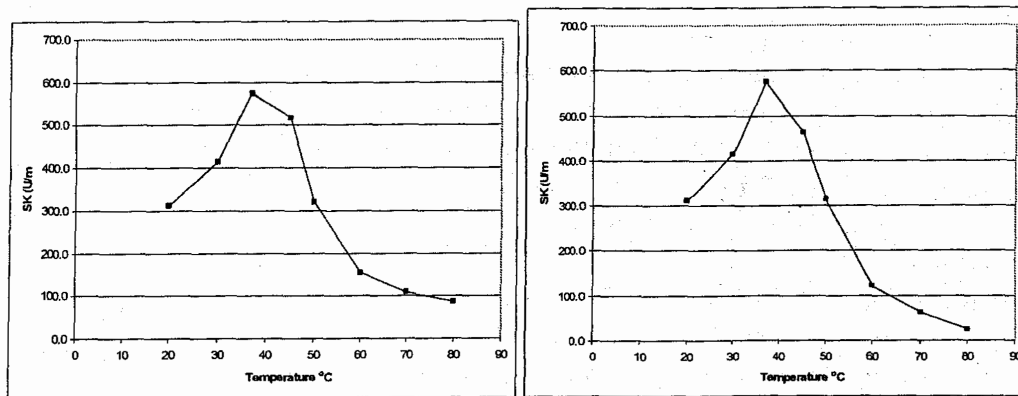


(a) (b)

Figure (8): Effect of different pH values on the activity of (a) *S. pyogenes* , and (b) *S. equisimilis* purified streptokinases.

Optimum temperature for purified streptokinases activity

The optimum temperature of the purified enzyme was determined as before. The optimum temperature for *S. pyogenes* purified streptokinase was 37°C. Moreover, the enzyme kept more than 90% of its activity at 45°C. Residual activity decreased significantly when temperature was elevated to 60°C (Figure 9). Optimum temperature for *S. equisimilis* purified streptokinase activity was 37°C. The enzyme lost almost 60% of its activity at 60°C, but the residual activity remained over 80% at 45°C (Figure 9).

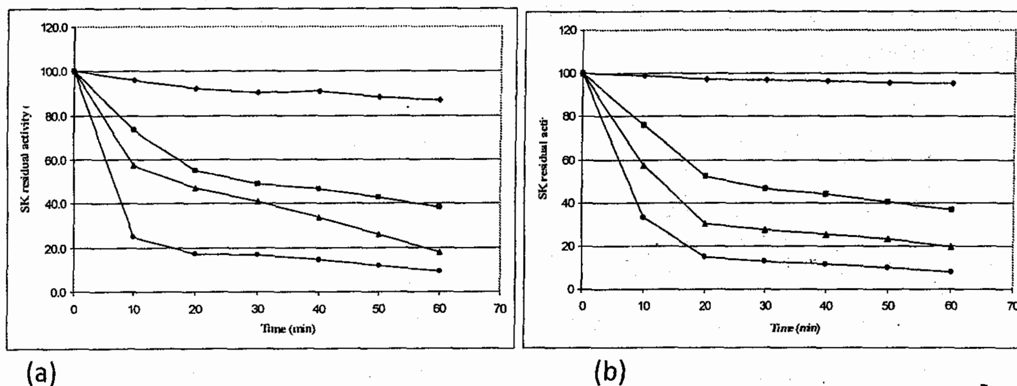


(a) (b)

Figure (9): Effect of different temperature degrees on the activity of (a) *S. pyogenes* and (b) *S. equisimilis* purified streptokinases.

Thermostability of purified streptokinases

S. pyogenes purified enzyme kept more than 86% of its activity after incubation at 50°C for 60 minutes. Half the enzymatic activity was obtained after exposure to 60°C for 30 minutes (Figure 10). The degree of thermostability decreased directly with either the increase of temperature or the increase of the exposure time. In case of *S. equisimilis* purified streptokinase, almost all the enzyme activity survived incubation at 50°C for the entire 60 minutes. However, the enzyme kept only 40% of its activity at 60°C for 50 minutes (Figure 10). Higher degrees of temperature significantly decreased the enzyme activity.

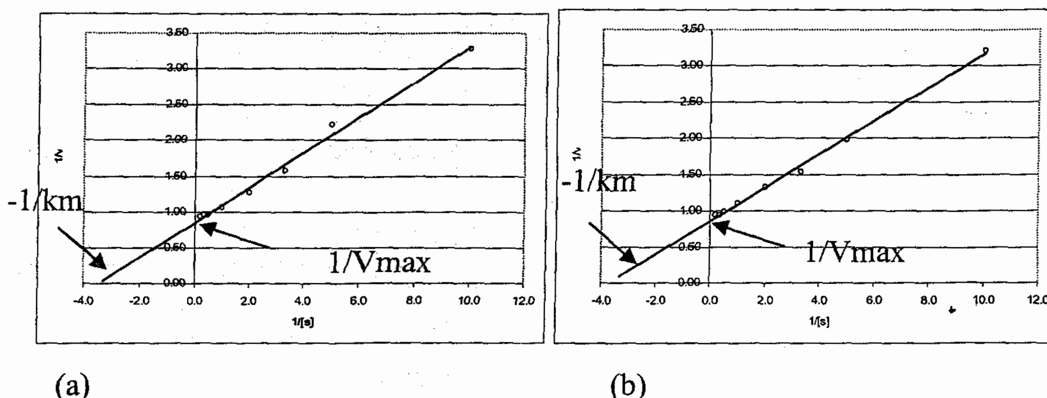


(a) (b)

Figure (10): Thermostability of (a) *S. pyogenes*, *and (b) S. equisimilis* purified streptokinases. Symbols ♦, ■, ▲, and ● represent streptokinase residual activity percentage at 50°C, 60°C, 70°C, and 80°C respectively.

Kinetics of purified enzymes

Kinetics of substrate activation with purified enzyme was investigated to determine the rate of enzymatic reaction for the produced plasmin. Figure (11) shows Lineweaver-Burk plot of human plasminogen activation after incubating plasminogen with purified *S. pyogenes* streptokinase for 5 minutes at 37°C. N-p-tosyl-glycyl-prolyl-lysine-p-nitroanilide acetate was used as the substrate for liberated plasmin. Maximum velocity (Vmax) of the reaction was 1.183 μM/ min, with a km value of 0.285 μM/ml. Similar results were obtained for plasminogen activation with purified *S. equisimilis* streptokinase, N-p-tosyl-glycyl-prolyl-lysine-p-nitroanilide acetate hydrolysis by liberated plasmin has a maximum velocity of 1.176 μM/min. Km of the reaction was 0.287 μM/ml (Figure 12).



(a) (b)

Figure (10): Lineweaver-Burk plot of human plasminogen activation after incubating plasminogen with (a) purified *S. pyogenes* streptokinase, and (b) purified *S. equisimilis* streptokinase.

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تنقية و دراسة خصائص إنزيم الإستربتوكينيز المنتج بواسطة الإستربتوكوكاس بيوجينيز و الإستربتوكوكاس إكويزيميليس

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في محاولة لتنقية إنزيم الإستربتوكينيز المنتج بواسطة السلالات *S. pyogenes* و *S. equisimilis* تم ترسيب الإنزيم بواسطة ملح كبريتات الأمونيوم بتركيز ٤٥% و ٥٠% متبوعاً بالغسيل بواسطة الأنبوب الغشائي. و أخذت العينات المنقاه جزئياً لكى تنقى أكثر بإستخدام عامود الفصل الكروماتوجرافى *Sephadex G-100*. و قد أدت خطوة التحليل الكروماتوجرافى إلى زيادة درجة نقاء الإنزيم ٣٠ و ٣٢ مرة لكل من إنزيم الإستربتوكينيز المنتج بواسطة السلالات *S. pyogenes* و *S. equisimilis* على الترتيب. و عند إستخدام عامود الفصل الكروماتوجرافى *Sephacryl S-200* للحصول على درجة نقاء أعلى، تم زيادة درجة النقاء لكلا الإنزيمين المنتجين بواسطة السلالاتين *S. pyogenes* و *S. equisimilis* إلى ٦٠% و ٤٧% على الترتيب. و عند تنقية كلا الإنزيمين بإستخدام عامود التبادل الأيونى *DEAE Sephadex A-50* زادت درجة النقاء إلى ١٦٠% و ١٦٤% على الترتيب. و تم الحصول أعلى درجة فعالية متبقية من نشاط الإستربتوكيتيز المنقي المنتج بواسطة كل من *S. pyogenes* و *S. equisimilis* عند درجة الحموضه ٨. ومع ذلك ، فإن الإنزيمات المنقاه احتفظت تقريبا بكل نشاطها في درجة الحموضه ٧. و قد إنخفضت درجة الفاعلية انخفاضا كبيرا في قيم درجة الحموضه العالية. و قد أظهرت الإنزيمات المنقاه من كلا من *S. pyogenes* و *S. equisimilis* أقصى نشاط عند ٣٧ درجة مئوية, علاوة على ذلك ، فإن إنزيم الإستربتوكيتيز المنتج بواسطة *S. pyogenes* احتفظ بأكثر من ٩٠ ٪ من نشاطه في ٤٥ درجة مئوية. و إنخفضت درجة الفعالية المتبقية بدرجه كبيرة عندما رفع درجة الحرارة إلي ٦٠ درجة مئوية. وفقد إنزيم الإستربتوكيتيز المنتج بواسطة *S. equisimilis* ما يقرب من ٦٠ ٪ من نشاطه في ٦٠ درجة مئوية، ولكن تبقى أكثر من ٨٠ ٪ من نشاط الإنزيم عند ٤٥ درجة مئوية. وإحتفظ الإنزيم المنقى من *S. pyogenes* بأكثر من ٨٦% من نشاطه وذلك بعد تحضينه عند ٥٠ درجة مئوية لمدة ساعة كاملة, ولكن النشاط الإنزيمي ظل محتفظاً بنصف كفاءته بعد التعرض لدرجة ٦٠ مئوية لمدة ٣٠ دقيقة. أما في حالة الإنزيم المنقى من *S. equisimilis* , فقد إحتفظ الإنزيم بكامل نشاطه تقريباً بعد تحضينه عند درجة ٥٠ مئوية لمدة ساعة كاملة. ولكن الإنزيم إحتفظ بحوالى ٤٠% من نشاطه بعد تحضينه عند ٦٠ درجة مئوية لمدة ٥٠ دقيقة, و قد تبين أن درجات الحرارة العالية تؤثر بشكل ملحوظ على نشاط الإنزيم , فإن درجة الثبات الحراري لكل من الإنزيمين المنقيين تقل مع زيادة درجة الحرارة أو زيادة زمن التعرض للحرارة العالية. و قد أظهرت دراسة النشاطات التفاعلية لإنزيم الإستربتوكيتيز المنقى و المنتج بواسطة *S. pyogenes* سرعة قصوى لتنشيط المتفاعل تصل إلى ١،١٨٣ ميكرومول/ دقيقة, و km تصل إلى ٠،٢٨٥ ميكرومول/ مل و ذلك عند إستخدام المركب *N-p-tosyl-glycyl-prolyl-lysine-p-nitroanilide acetate* كمتفاعل لإنزيم البلازمين المتحرر. و قد تم التحصل على نتائج مشابهه عند تنشيط البلازمينوجين بإنزيم الإستربتوكيتيز المنقى و المنتج بواسطة *S. equisimilis* , فقد وصلت سرعة التفاعل القصوى لتحليل المتفاعل بواسطة إنزيم البلازمين المتحرر إلى ١،١٧٦ ميكرومول/ دقيقة, و km تصل إلى ٠،٢٨٧ ميكرومول/ مل.