

Preparation of Plasminogen by Affinity Chromatography

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Abstract

Background: Plasminogen is one of the compounds derived from human plasma. Activation of plasminogen produces plasmin. Plasmin is able to lyse fibrinogen, fibrin, and some other human plasma proteins. The aim of the present work was to study the separation of human plasminogen by affinity chromatography using gel lysine Sepharose.

Materials and Methods: Normal human plasma was used as the starting material. By increasing its concentration of ethanol from 0% to 8% in pH 7.2 at -3°C the centrifugation was carried out and the supernatant I was separated from precipitate I. Then supernatant fraction I was used for separation of human plasminogen from plasma and further purification was carried out using affinity chromatography using affinity gel lysine Sepharose.

Results: In our study we could obtain plasminogen with the concentration of 0.5%. In normal human plasma the concentration of plasminogen is about 200mg/l. Comparing the result with the concentration of plasminogen in normal human plasma shows about 25 folds higher concentration.

Conclusion: Using affinity chromatography method a 25-fold increase in plasminogen concentration was achieved which shows this method is a promising method for the preparation of plasminogen from human plasma.

Keywords: Plasminogen, plasmin, plasma, fractionation.

Introduction

Plasminogen is one of the compounds derived from human plasma by fractionation. When plasminogen is activated to plasmin, it has the property of lysing fibrinogen, fibrin, and some other proteins¹. In another words, plasmin converts fibrin into soluble products and also hydrolyses some other proteins². Congenital plasminogen deficiency is an illness that results in inflamed growth on the mucous membranes such as the eyelids and the inside of the mouth³. Propagation of the growths is usually triggered by infections or injury, but they may also occur spontaneously in the absence of known triggers⁴. The prevalence of congenital plasminogen deficiency is about 1.6 per one million people. A reduction in functional plasminogen results in less produced plasmin to break down fibrin, leading to a buildup of fibrin⁵. The excess fibrin and the resulting inflammation of the tissue result in the inflamed growths characteristic of congenital plasminogen deficiency.

Formerly plasmin was given parentally for the treatment of thrombotic disorders. Plasmin can be used in combination with deoxyribonucleic for the debridement of wounds^{6,7}. Also plasminogen has been investigated as a thrombolytic agent and has been used in conjunction with other blood products in wound sealant preparations^{8,9}.

Usually, isolation of plasminogen needs complex methods^{10,11}. Using chromatographic techniques have great impact in separation and purification of human plasma derived compounds¹². Among different chromatographic techniques for isolation of human plasma derived products, anion exchange chromatography and affinity chromatography are more applied^{13,14}.

Ito et al. have separated Glu-plasminogen, Lys-plasminogen and plasmin using high-performance affinity chromatography on a micro particulate polyvinyl alcohol gel as the supporting material and p-aminobenzamidine as the specific ligand¹⁵.

In another work Cole and Mertz have prepared crude bovine plasminogen from bovine serum by 30% saturation with ammonium sulphate and they reached a 28-fold purification of crude bovine plasminogen¹⁶.

The aim of the present study was the separation of human plasminogen by affinity chromatography using gel lysine Sepharose. The starting material which we used for chromatography was fraction I supernatant which we prepared using human plasma fractionation.

Materials and Methods

In the current work fraction I was obtained by adjustment of parameters such as ethanol concentration, pH, ionic strength, temperature, and protein concentration as described before¹⁷. We used normal human plasma as the starting material. By increasing its concentration of ethanol from 0% to 8% in pH 7.2 at -3°C the centrifugation was carried out and the supernatant I was separated from precipitate I. Preparation of plasminogen with fraction I supernatant as raw material was followed by the affinity chromatography technique. First the gel of Heparin-Sepharose was suspended in the buffer and applied to the column. Then the column was washed by the solution of sodium chloride (1 M) to remove all proteins bound to the gel due to ion exchange effects. After removal of all impurities the elution of plasminogen was carried out by applying the solution of ϵ -aminocaproic acid (0.1 M).

Results

In normal human plasma the concentration of plasminogen is about 200mg/l. In our study we could obtain plasminogen with the concentration of 0.5%, which in comparison with the concentration of plasminogen in normal plasma shows about 25 folds higher concentration.

Figure 1 shows the SDS-PAGE electrophoresis of plasminogen with different concentrations at $\frac{1}{4}$, $\frac{1}{2}$, and non diluted concentration. The SDS-PAGE electrophoresis showed a strong band at 92 kDa related to plasminogen, which normally has a molecular weight of 90 to 92 kDa. The strongest band marked with 4 is related to non-diluted plasminogen and weaker bands marked with 3 and 2 are related to $\frac{1}{2}$ and $\frac{1}{4}$ diluted plasminogen respectively.

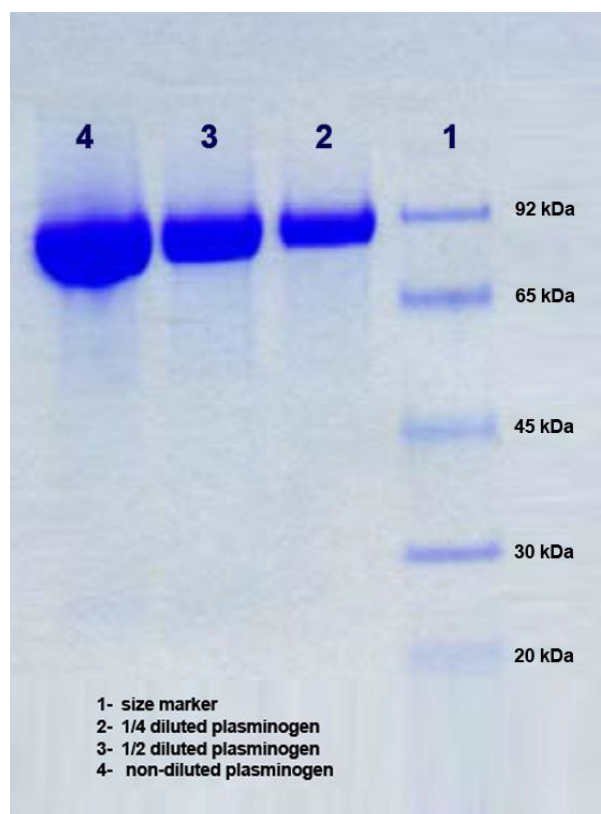


Figure 1: SDS-PAGE electrophoresis of plasminogen with different concentrations.

Discussion

Blood macromolecules usually can be separated from human plasma by plasma fractionation and in combination with chromatographic method^{18,19-23}. Plasminogen has been prepared using different methods²⁴⁻²⁶. Benfeldt et al. studied a procedure for the isolation of plasminogen and plasmin from bovine milk. Pure undegraded plasminogen with N-terminal amino acid sequence and mobility in SDS-page similar to plasminogen isolated from bovine blood was obtained²⁷.

In another work Cole and Mertz prepared crude bovine plasminogen from bovine serum by 30% saturation with ammonium sulphate, and it was partially purified by isoelectric precipitation at pH^{5,3}, and then it was purified further by chromatography on a column of diethylaminoethyl-cellulose¹⁶. Additional purification of the column product was obtained by precipitation of plasminogen from solution with potassium phosphate and they reached 28-folds purification of crude bovine plasminogen¹⁶.

Ito et al. separated Glu-plasminogen, Lys-plasminogen and plasmin by high-performance affinity chromatography on a micro particulate polyvinyl alcohol gel as the supporting material and p-aminobenzamidine as the specific ligand¹⁵.

In the present study, plasminogen with a concentration of 0.5% was achieved which in comparison with the concentration of plasminogen in normal plasma (200 mg/L) shows about 25-fold higher concentration. Finding of our study was in line with Cole and Mertz who purified bovine plasminogen 28-folds from crude plasminogen¹⁶.

Conclusion

Using affinity chromatography method a 25-fold increase in plasminogen concentration was achieved which shows this method is a promising method for the preparation of plasminogen from human plasma.

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