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Affinity purification of plasminogen by radial-flow affinity chromatography

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ABSTRACT

A method for the purification of plasminogen using immobilized L-lysine on a membrane, the whole system being constructed in a radial flow cartridge, is described. Human plasma was applied to the cartridge at 20 ml/min. The results showed that under the chromatographic conditions chosen, in a single pass, >85% recovery of plasminogen was attained with a 110-fold increase in specific activity.

INTRODUCTION

With the recent advances in biotechnology, there is a large demand for purification methods that can achieve high product purity in a few simple steps. Affinity chromatography has become a method of choice as high purity can be obtained in a single step [1,2].

Plasminogen is the inactive precursor of the proteolytic enzyme plasmin, which is responsible for the dissolution of fibrin clots in the blood. The purification of plasminogen using L-lysine bound on Sepharose has been reported [3]. Although, the method proved to be satisfactory, the flow-rates were very low (75 ml/h) and it was time consuming. Bound L-lysine has been used in many other applications, and is a very useful ligand for the purification of plasmin and plasminogen of various species [4,5], plasminogen activator [6] and ribonucleic acids [7].

This paper describes a method for purifying plasminogen from human plasma with L-lysine immobilized on a radial-flow membrane cartridge system. The method provides a new means of obtaining large amounts of plasminogen within a few hours and could be easily extended owing to the linear scale-up capability of this system, which has been demonstrated previously using *p*-aminobenzamidine as a ligand [8].

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EXPERIMENTAL

L-Lysine coupling

A Zetaffinity 250 cartridge (Cuno, Cergy-Pontoise, France) containing modified cellulose was used as chromatographic medium. The hydroxyl derivative was used for ligand coupling. The activation of hydroxyl functions was accomplished with a 1.5% metaperiodate solution. Lysine was dissolved in 0.05 *M* sodium phosphate containing 0.25 *M* NaCl and recycled overnight through the cartridge. After reduction with $NaBH_4$, the cartridge was drained and the unbound reactive sites were blocked with 2% glycine ethyl ester hydrochloride solution.

Plasminogen purification from human plasma

The cartridge containing bound lysine was equilibrated with 0.1 *M* sodium phosphate (pH 7.4) containing 0.05 *M* NaCl and 0.01% Tween 80. After centrifugation and filtration, the plasma was diluted with equilibration buffer (0.5 v/v) and applied to the cartridge at 20 ml/min. The cartridge was washed with equilibration buffer until the absorbance at 280 nm reached the baseline, then elution was carried out with 0.2 *M* 6-aminocaproic acid in equilibration buffer. Before analysis, bound fractions were dialysed overnight at 4°C against 0.01 *M* 6-aminocaproic acid in equilibration buffer. The protein content was measured using the Bio-Rad protein assay and the plasminogen activity with the Kabi substrate S-2251. Purity was evaluated by 7.5% sodium dodecyl sulphate polyacrylamide gel electrophoresis.

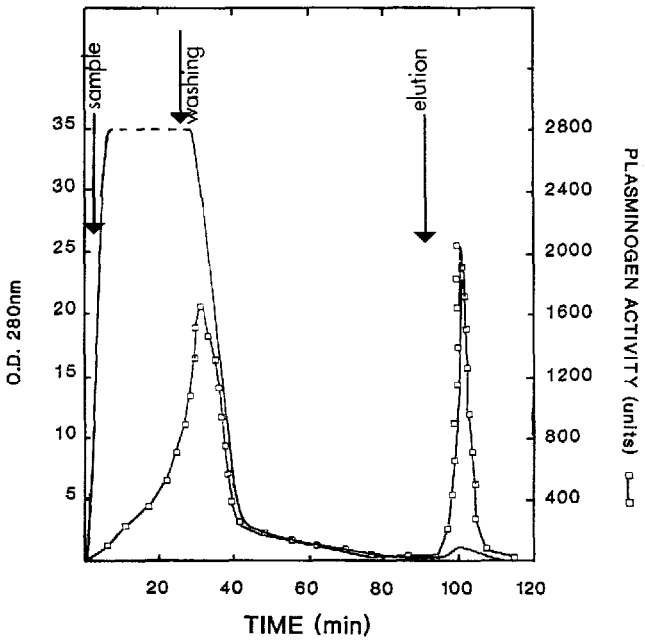

Fig. 1. Chromatogram of the purification of plasminogen from human plasma using lysine bound on a radial-flow cartridge. Chromatography was performed as described under Experimental.

TABLE I

RESULTS OF PLASMINOGEN PURIFICATION FROM HUMAN PLASMA USING LYSINE BOUND ON A RADIAL-FLOW CARTRIDGE

Device	Plasma volume (ml)	Plasminogen activity eluted (units)	Specific activity eluted	Purification	Total recovery (%)
No. 60 disc[a]	50	2452	406	100 ×	88
No. 250 capsule[b]	400	8948	358	144 ×	57
No. 250 capsule[c]	250	6045	410	124 ×	74

[a] Entire fractionation done at 4°C.
[b] 400 ml of plasma diluted to 600 ml in equilibration buffer.
[c] 250 ml of plasma diluted to 500 ml in equilibration buffer.

RESULTS AND DISCUSSION

The chromatograms (Fig. 1) shows that plasminogen was retained by the bound lysine. Electrophoretic analysis demonstrated that the eluted fractions were devoid of any visible protein contamination. The results in Table I show that in a single pass a very good increase of purity was obtained with satisfactory recoveries. This purification procedure is a very convenient method for fast and easy recovery of plasminogen, as a whole chromatographic cycle could be performed within 2 h.

This radial-flow chromatographic method has been used in many applications involving ion exchange and has proved to be extremely useful, particularly when the product of interest is present at low concentration in a very large sample, *e.g.*, monoclonal antibodies from hybridoma cell culture supernatant [9] or recombinant proteins [10]. Moreover, the linear scale-up capability of these radial-flow cartridges has already been demonstrated with ion exchangers [11] and affinity chromatography [8]. Hence the method described here could easily be scaled up to very large amounts within a short period of time.

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