

Optical rotations were measured with a Rudolph photoelectric spectropolarimeter using a tube of 1 dm length. For the details, see Ref.<sup>12</sup>. The experimental error is probably  $\pm 20^\circ$  for molecular rotations (*cf.* Ref.<sup>12</sup>). In some cases it was impossible to make measurements down to 300  $\mu\mu$  because of intense light absorption by the compound under study. The course of a typical curve for  $\text{CH}_3\text{SO}(\text{CH}_2)_5\text{NHCSNH}_2$  between 350 and 300  $\mu\mu$  is indicated by the following values; [M], and  $\lambda$  in parentheses; -625 (350), -755 (330), -840 (320), -930 (310), -1 030 (300  $\mu\mu$ ).

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## Purification of Human Plasminogen on DEAE-cellulose

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In a recent communication a method for the purification of human plasminogen was outlined<sup>1</sup>. The most important step of this method was an adsorption of a partially purified plasminogen preparation on columns of DEAE-cellulose equilibrated with an ammonium acetate buffer, pH 9.0, containing lysine to a concentration of 0.01 M. Under these conditions about 80 % of the protein was adsorbed, whereas 60–80 % of the plasminogen passed through the column in the frontal fraction together with about 20 % of the protein. We were prompted to use lysine in this connection by the observation of Alkjaersig, Fletcher and Sherry<sup>2</sup>, who found that  $\epsilon$ -aminocaproic acid and lysine increased the solubility of plasminogen and plasmin preparations at neutral reaction, probably due to a dissolution of a protein complex.

As starting material we used a plasminogen preparation obtained from Cohn's fraction III by dialysis against a phosphate buffer of pH 7.0 and low ionic strength. The specific activity ranged from 8 to 20 times that of fraction III. This variability was shown to be caused by the poor reproducibility of the first step. Therefore we now have excluded this step.

Plasminogen is easily adsorbed on DEAE-cellulose, previously equilibrated with ammonium acetate buffer, pH 9.0, but if lysine is added to the buffer it is not adsorbed. Thus it was near at hand first to adsorb plasminogen to DEAE-cellulose from a weakly alkaline buffer without lysine and then to try to elute the activity with the same buffer to which lysine had been added. In the following a method based on this principle is outlined.

A preparation obtained from Cohn's fraction II + III by precipitating the plasminogen at low ionic strength and pH 5.3 was used as starting material. Preparations of this type have almost the same specific activity as fraction III and the yields are higher. About 35 g DEAE-cellulose (Brown Comp. New Hampshire, U.S.A.) were suspended in 0.04 M ammonium acetate buffer pH 9.0. The slurry was poured in a column with a diameter of 4 cm and packed with

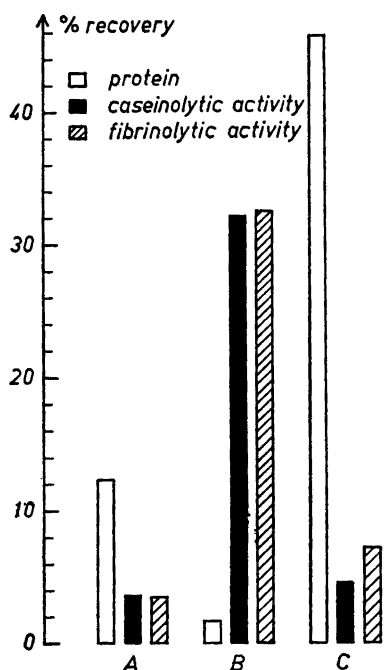


Fig. 1. Adsorption and step-wise elution of human plasminogen on DEAE-cellulose. The buffers are:

- A) 0.04 M ammonium acetate, pH 9.0.
- B) 0.04 M ammonium acetate, pH 9.0 containing lysine to a concentration of 0.01 M.
- C) 0.04 M ammonium acetate, pH 4.0 containing lysine to a concentration of 0.01 M.

an excess air pressure of about 50 mm Hg. The column was equilibrated with the same buffer. 10 g of the starting material was suspended in 500 ml of the equilibration buffer and dialysed against the same buffer during 6–8 h. Some of the material did not dissolve and was removed by centrifugation. The opalescent supernatant was applied to the column, which was then

developed with about 1 000 ml of the equilibration buffer. The elution was then performed stepwise with 0.04 M ammonium acetate buffer, pH 9.0 and 4.0, both buffers containing lysine to a concentration of 0.01 M. The protein concentration in the effluent was followed by measuring the optical density at 280  $\mu$  in a Beckman DU photometer.

The fractions were acidified with hydrochloric acid to pH 3.5, dialysed against distilled water and precipitated with cold acetone at a concentration of 40 % at  $-15^{\circ}\text{C}$ . The precipitates were lyophilized and the fractions tested with the caseinolytic method of Müllertz<sup>3</sup> and the fibrinolytic method of Christensen<sup>4</sup>.

The result of such an experiment is shown in Fig. 1, where the amount of lyophilized protein, caseinolytic and fibrinolytic activity in the fractions are expressed as per cent of the starting material. In the frontal peak appears about 12 % of the protein but only 4 % of the plasminogen. The main part of the plasminogen thus has been adsorbed to the ion exchanger. When the column is eluted with the same buffer, to which lysine has been added, a small peak appears containing only about 1–2 % of the protein but about 30–35 % of the activity, which means a purification of about 20 times as compared to the starting material. With the acidic buffer, finally, 40 % of the protein material and 5 % of the plasminogen is eluted. Five preparations have been made according to the method outlined. The increase in specific activity in the second fraction (B in Fig. 1) has ranged from 20 to 30 times as compared to the starting material and the yield has ranged from 30 to 35 %.

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