Purification and Biochemical Properties of Human Plasminogen

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Plasminogen is the plasma precursor of the fibrinolytic enzyme, plasmin. The activation of plasminogen isolated from various animal species by streptokinase, a protein of bacterial origin, has received considerable attention in recent years. A two-step reaction has been postulated wherein streptokinase reacts with a substance termed proactivator (1), which is present in highest concentration in human plasma and human plasminogen preparations and apparently is absent from the plasma of certain animal species such as the cow, to form an activator of human plasminogen (2) and the plasminogens of other animal species (3). The existence of proactivator as an entity distinct from human plasminogen is still hypothetical, since it has not been isolated in pure form. In fact, evidence has been presented (4, 5) which indicates that proactivator is human plasminogen.

The purpose of this study was to attempt to isolate proactivator in pure form. Column chromatography of human plasminogen, prepared by the method of Kline (6), on carboxymethyl cellulose, has resulted in a 2- to 4-fold purification. Although a high degree of homogeneity is apparent from physical-chemical (7) and serological data, chromatographed plasminogen still retains essentially the same ratio of proactivator activity to plasminogen activity as that in crude preparations. The results of these studies are therefore interpreted as further evidence for the identity of proactivator and human plasminogen.

EXPERIMENTAL

Preparation of Plasminogen—Human plasminogen was partially purified by the method of Kline (6) or by the following modification. Seven grams of Fraction III1 were suspended in 100 ml water, adjusted to pH 3.0 with N H2SO4, and stirred for 1 hour. To avoid foaming, stirring was carried out at a fairly slow but constant rate in this step and in all subsequent steps. The suspension was centrifuged at 4000 r.p.m. for one-half hour and the precipitate discarded. The supernatant fluid was adjusted to pH 8.5 with N NaOH. After storage at 5°C for 1 day, the precipitate was collected by centrifugation at 4000 r.p.m. for one-half hour and dissolved in 0.1 M sodium formate-formic acid buffer, pH 3.0. In agreement with the more recent studies of Kline (8), precipitation at pH 8.5 yielded a purer fraction than precipitation at a lower pH.

Preparation of Carboxymethyl Cellulose Column—CM-cellulose was prepared from 325 mesh Solka-Floc cellulose (9), or type 20 was purchased from the Brown Paper Company. Approximately 10 g of CM-cellulose were suspended in 100 ml of 0.1 M HCl, stirred for several minutes, and filtered with the aid of suction. After several acid washes, the CM-cellulose was washed several times with water and then with the buffer employed in the adsorption step. All substances which absorb at 280 μM were removed by this procedure. Washed CM-cellulose was finally suspended in enough buffer to give a thin suspension and packed in a column under a pressure of 10 p.s.i.

Adsorption and Elution of Plasminogen—Chromatography was carried out at 5°C. A concentrated solution of plasminogen was dialyzed against the appropriate buffer and applied to the column slowly to permit adsorption. Two equal volume cylinders (10) were used to provide a gradient elution system. Flow rates, which were predetermined for each column size, did not exceed 15 ml per cm2 per hour.

Examination of Effluent. (1) Protein Measurement—Eluate fractions were examined for relative protein concentration in a Beckman DU spectrophotometer by determining the extinction at 280 μM. One optical density unit was found to be equivalent to 0.1 mg of N or 625 μg of protein. Specific activity was defined as proactivator units per optical density unit or per mg of N. One optical density unit was designated as the amount present in one ml of a solution which gives an optical density of unity.

(2) Determination of Proactivator Activity—The assay, which has been described (5), is based on the activation of bovine plasminogen by mixtures of streptokinase and proactivator, the latter substance being present in human plasminogen preparations. The amount of bovine plasmin formed is measured by the extent of hydrolysis of LMe.2 The method of diluting human plasminogen was modified in order to obtain soluble and stable plasminogen. At pH 6.4, μg quantities of human plasminogen are completely soluble in solutions containing lysine.3 Therefore plasminogen was first diluted in 10−3 M phosphate buffer, pH 6.4, containing 2 × 10−2 M lysine. This solution was further

1 Fraction III was obtained by Dr. Henry Pieriera of these laboratories through the courtesy of Dr. T. Gerlough of E. R. Squibb and Sons, and Dr. J. N. Ashworth of the American Red Cross.

2 The abbreviations used are: LMe, L-lysine methyl ester; TAME, tosyl L-arginine methyl ester.

3 J. J. Hagan, F. B. Ablondi, and E. C. De Renzo, to be published.
diluted in $10^{-1}$ M phosphate buffer, pH 6.4, in which the plasminogen remains soluble, in order to reduce the lysine concentration which would otherwise produce high blank values. The standard deviation of five assay values of a human plasminogen fraction was found to be $\pm 15\%$ of the mean value. One unit of proactivator is that quantity which under the assay condition brings about the hydrolysis of 0.84 $\mu$ mole of LMe in 60 minutes.

Assay for Plasminogen and Plasmin—Certain effluent cuts were assayed for plasminogen and plasmin by the procedures described below.

(1) Determination of Plasminogen in Fibrinolytic Assay—The method of Christensen (11) served as a measure of fibrinolytic activity which could be derived from a given plasminogen preparation. Two thousand units of streptokinase per clot were sufficient for activation. Phosphate buffer containing lysine was used as a diluent for plasminogen. One fibrinolytic unit was defined as that amount of plasminogen which lysed a clot in 20 minutes.

(2) Determination of Plasminogen and Plasmin Activities with Synthetic Substrates—Streptokinase-activatable plasminogen and spontaneous plasmin activities were measured by the hydrolysis of TAMe (5, 12). One unit of activity was defined as that quantity which hydrolyzes 1 $\mu$ mole of TAMe in 30 minutes under the conditions of the assay. LMe was also employed as a substrate for measuring streptokinase-activatable plasminogen as well as plasmin activities (5). Phosphate buffer containing lysine was again used as a diluent for plasminogen. One unit of activity was defined as that amount which under the conditions of assay hydrolyzes 0.84 $\mu$ mole of LMe in 90 minutes.

(3) Determination of Plasminogen by Digestion of Casein—A modification of the procedure described by Kunitz (13) was followed. The reaction mixture was composed of 2 ml. of a 5% Hammersten casein solution, 0.5 ml (50 to 250 units) of streptokinase and 0.5 ml of human plasminogen, all dissolved in 0.1 M phosphate buffer, pH 7.2. After incubation at 37° for 30 minutes, the reaction mixtures and blanks were precipitated by the addition of an equal volume of 10-l trichloroacetic acid and the supernatant fluid was determined by measuring their absorbancy at 280 mp in a Beckman DU spectrophotometer. One unit of caseinolytic activity was defined as that quantity of human plasminogen which under the conditions of assay gave an increase in trichloroacetic acid-soluble products of 10$^{-3}$ optical density units.

(4) Determination of Plasmin Activity by Fibrin Plate Method—The fibrin plate method of Astrup and Mullertz (14) as modified by Von Kaulla (15) was also employed as a measure of plasmin activity. Plasminogen preparations were diluted in lysine-phosphate buffer before assay. A standard reference curve was prepared by plotting on log-log paper plasmin concentration in terms of LMe-esterase units against size of lysed zones (diameter in mm). It was found that 100 LMe-esterase units of plasmin produced a 30-mm$^2$ zone of lysis on heated fibrin plates. The size of lysed zones resulting from four dilutions of an unknown were plotted in the same manner. Plasmin activity of the unknown was then calculated in terms of LMe-esterase units. That concentration of plasmin which lysed a 30 mm$^2$ zone of lysis was taken to be equivalent to 100 LMe-esterase units.

Immunochemical Analysis—An acid extract of Fraction III containing $10^{-4}$ M lysine was adjusted to pH 7.5 and sterilized by passage through a Seitz filter. Six New Zealand white rabbits, weighing 24 to 3 kg, were given intravenous injections with 1 ml of antigen (2.8 mg of protein) five times over a period of 13 days. On the 1st and 3rd day, a 1-ml subcutaneous injection was included in the schedule. At the 32nd day, a second series of five intravenous injections was started and continued over a period of 12 days. Antibodies were detected on the 48th day by the agar diffusion technique of Ouchterlony (16). At 61 days, the rabbits were bled by heart puncture and the serum collected and dried in the frozen state.

Agar plates were prepared from a 1% solution of Noble agar in $10^{-1}$ M phosphate buffer containing $10^{-1}$ M lysine and sodium ethyl mercurethiosalicylate (1 part per 5000) as a preservative. The warm solution was poured into flat bottom Petri dishes to a depth of $\frac{1}{4}$ inch. After cooling, stainless steel antibiotic assay cups were placed on the agar and sealed by having an additional $\frac{1}{4}$ inch layer of warm agar poured on the plate. Antibody and antigen, both containing sodium ethyl mercurethiosalicylate as a preservative, were added to the appropriate cups and left to diffuse at room temperature.

RESULTS

Chromatography of Plasminogen on CM-cellulose Columns—Attempts to purify plasminogen, which had been purified by the method of Kline, by chromatography on anion exchange columns, were complicated because of its low solubility in the operative pH range of the exchangers. However, at pH 3.0, the region of maximal stability and solubility of plasminogen, CM-cellulose was found to be a good adsorbant of proactivator activity.

Shown in Fig. 1 is an elution diagram of plasminogen chromatographed on CM-cellulose. A decreasing pH gradient was set up by the gradual addition of $10^{-1}$ M HCl to $10^{-1}$ M formate-formic acid buffer, pH 2.75. About one-half of the protein did not adsorb and passed through after 1 column volume. This cut contained a small portion of proactivator activity. The bulk of the proactivator activity was eluted between pH 2.4 and 1.8. Proactivator and plasminogen assay values of pooled effluent fractions are shown in Table I. Within the standard deviation of the pH gradient (250 ml 10$^{-1}$ M HCI). Recovery of protein and proactivator was essentially quantitative.

![Fig. 1. Effluent diagram of human plasminogen prepared by the method of Kline. Conditions: CM-cellulose column, size 43 X 0.92 cm, volume 28 ml; protein charge = 68.5 mg N; decreasing pH gradient (230 ml 10$^{-1}$ M Na Formate-formic acid, pH 2.75 to 260 ml 10$^{-1}$ M HCl). Recovery of protein and proactivator was essentially quantitative.](http://www.jbc.org/)
of the assay methods, the ratios of proactivator to plasminogen for the starting material and for the purest eluate fractions were the same.

Rechromatography of Plasminogen on CM-cellulose by Decreasing pH Gradient Elution—After concentration and equilibration by dialysis, the center fraction (Pool 2) of the activity peak shown in Fig. 1 was rechromatographed under the same conditions. The results are shown in Fig. 2. The specific activity of proactivator was found to be constant throughout most of the main peak. The pooled eluate was found to contain about 10% plasmin by TAME-esterase assay in the absence of streptokinase, indicating that plasmin was not completely separated from plasminogen or that plasmin was formed during the chromatographic and/or the assay procedure.

Rechromatography of Plasminogen by Increasing Salt and pH Gradient—Since the solubility of plasminogen is increased by lysine through the middle pH range, an attempt was made to elute plasminogen from CM-cellulose by increasing the pH with buffer containing lysine. The purest fraction from a once chromatographed plasminogen preparation was absorbed on CM-cellulose at pH 2.9 in \(10^{-2} \text{ M} \) sodium formate-formic acid buffer. An increasing pH gradient was established by the gradual addition to the formate buffer of a buffer composed of \(2.5 \times 10^{-1} \text{ M} \) citric acid and \(10^{-3} \text{ M} \) lysine-HCl adjusted to pH 6.5 with NaOH. The effluent diagram is shown in Fig. 3.

Minor components were eluted between pH 2.9 and pH 5.0, whereas proactivator was eluted above pH 5.0. Again the specific activity of proactivator was greatest at the center of the peak. Fibrin plate assays showed that pooled fractions from the main peak all contained plasmin activity and the purest fractions contained the least amount of plasmin relative to proactivator (Table II). Although this variable plasmin content contributed to the lower specific activity of proactivator on both sides of the peak, incomplete resolution of other minor components at the left of the peak also contributed to the lower specific activity of proactivator in this region.

Chromatography of Plasminogen by Other Elution Schedules—Although the nature of the adsorption and elution phenomena which occur under the conditions described above is not clearly understood, there can be no doubt that purification of proactivator activity is achieved. Ionic strength gradients at constant pH or stepwise decreasing pH elution schedules did not resolve the components present in partially purified plasminogen preparations.

Stability of Chromatographed Plasminogen—Chromatographed plasminogen maintained at pH 2.0 in elution buffers or dialyzed against \(10^{-3} \text{ M} \) HCl could be stored at 5°C for 1 month with no loss in activity. Preparations kept in the frozen state were stable

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**Table I**

Comparison of proactivator and plasminogen activities of chromatographed plasminogen

<table>
<thead>
<tr>
<th>Fraction analyzed</th>
<th>Proactivator specific activity &lt;sup&gt;a&lt;/sup&gt;</th>
<th>Proactivator specific activity &lt;sup&gt;b&lt;/sup&gt;</th>
<th>Plasminogen specific activity &lt;sup&gt;c&lt;/sup&gt;</th>
<th>Ratio of plasminogen to proactivator &lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting material</td>
<td>unit/mg N</td>
<td>unit/ml</td>
<td>unit/ml</td>
<td>TAME-esterase units/ml</td>
</tr>
<tr>
<td>Eluate</td>
<td>35,000</td>
<td>40,000</td>
<td>1140</td>
<td>1:35</td>
</tr>
<tr>
<td>Pool (1)</td>
<td>20,000</td>
<td>3,600</td>
<td>120</td>
<td>1:30</td>
</tr>
<tr>
<td>Pool (2)</td>
<td>82,000</td>
<td>27,000</td>
<td>660</td>
<td>1:41</td>
</tr>
<tr>
<td>Pool (3)</td>
<td>50,000</td>
<td>6,300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Proactivator determined by the method of Ablondi and Hagan (5); see "Experimental" section for details.

<sup>b</sup> Streptokinase-activatable TAME-esterase activity (5). See "Experimental" section for details.

<sup>c</sup> Prepared by the method of Kline (6). See "Experimental" section for details.

<sup>d</sup> The numbers correspond to the eluate pools shown in Fig. 1.

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**Fig. 2.** Effluent diagram of once chromatographed human plasminogen. Conditions: CM-cellulose column, size 43 X 0.92 cm, volume 28 ml; plasminogen charge = 7.8 mg N; decreasing pH gradient (230 ml \(10^{-3} \text{ M} \) sodium formate-formic acid buffer pH 2.75 to 280 ml \(10^{-1} \text{ M} \) HCl). Recovery of protein, 98%; recovery of proactivator, 86%. Specific activity of proactivator: before chromatography, 82,000 units per mg N; after chromatography, 84,000 units per mg N. X—X, pH; •—•, OD units at 280 nm; O—O, proactivator units per ml; Δ—Δ, proactivator units per OD unit (280 nm).

**Fig. 3.** Effluent diagram of once chromatographed plasminogen. Conditions: CM-cellulose column, size 43 X 0.92 cm, volume 28 ml; plasminogen charge, 23.4 mg N; pH gradient, 0.01 M Na formate-formic acid, pH 3.0 to 0.25 M citric acid-0.1 M lysine pH 6.5. Recovery of protein, 98%; recovery of proactivator, 86%. X—X, pH; •—•, OD units at 280 nm; O—O, proactivator units per ml; V—V, plasmin (LMEase units per ml); Δ—Δ, proactivator units per optical density unit.
TABLE II
Activities of pooled fractions from increasing salt and pH gradient elution

<table>
<thead>
<tr>
<th>Fraction number</th>
<th>Proactivator activity</th>
<th>Proactivator specific activity</th>
<th>Plasmin activity</th>
<th>Ratio of plasmin units to proactivator units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>units/ml</td>
<td>units/O.D.50</td>
<td>units/ml</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>266</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>700</td>
<td>935</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11,000</td>
<td>6,500</td>
<td>390</td>
<td>1:28</td>
</tr>
<tr>
<td>5</td>
<td>39,000</td>
<td>14,500</td>
<td>125</td>
<td>1:312</td>
</tr>
<tr>
<td>6</td>
<td>24,000</td>
<td>10,700</td>
<td>210</td>
<td>1:115</td>
</tr>
<tr>
<td>7</td>
<td>3,200</td>
<td>5,200</td>
<td>84</td>
<td>1:38</td>
</tr>
</tbody>
</table>

* The fraction number corresponds to the number shown in Fig. 3.

† Proactivator determined by the method of Ablondi and Hagan (5); see “Experimental” section for details.

‡ Determined on heated fibrin plates (15). See “Experimental” for details.

TABLE III
Biochemical activities of chromatographed and crude plasminogen

<table>
<thead>
<tr>
<th>Plasminogen fraction assayed</th>
<th>Proactivator activity</th>
<th>Proactivator specific activity</th>
<th>Streptokinase-activatable caseinolytic activity</th>
<th>Streptokinase-activatable LMe-esterase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>units/ml</td>
<td>units/O.D.50</td>
<td>units/ml</td>
<td>units/ml</td>
</tr>
<tr>
<td>Fraction III</td>
<td>160</td>
<td>160</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>Chromatographed (decreasing pH gradient)</td>
<td>160</td>
<td>8,000</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>Fraction III</td>
<td>15,000</td>
<td>160</td>
<td>1,800</td>
<td>1,800</td>
</tr>
<tr>
<td>Chromatographed (decreasing pH gradient)</td>
<td>17,000</td>
<td>10,700</td>
<td>2,200</td>
<td>2,200</td>
</tr>
<tr>
<td>Fraction III</td>
<td>2,500</td>
<td>160</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>Chromatographed (increasing pH and salt gradient)</td>
<td>2,500</td>
<td>14,500</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Plasma</td>
<td>2,000</td>
<td>28</td>
<td>68</td>
<td>68</td>
</tr>
<tr>
<td>Chromatographed (decreasing pH gradient)</td>
<td>2,200</td>
<td>9,000</td>
<td>66</td>
<td>66</td>
</tr>
</tbody>
</table>

* Averages of two determinations on two different chromatographed plasminogen preparations.

for a few days, but prolonged storage resulted in a loss of proactivator activity. Salt-free preparations that had been lyophilized at pH 3.0 were initially stable but lost 90% of their proactivator and plasmin activities after 2 weeks in the dry state.

Enzyme Activities of Chromatographed Plasminogen—A summary of these results is presented in Tables III and IV. The ratios of (a) proactivator units to fibrinolytic units, (b) proactivator units to streptokinase-activatable caseinolytic units, and (c) proactivator units to streptokinase-activatable TAME-esterase units remain reasonably constant through a 50 to 90-fold purification of proactivator over Fraction III and a 300-fold purification of proactivator over plasma.

Activation of Chromatographed Plasminogen by Trypsin—Twenty milliliters of chromatographed plasminogen (14 mg N) were mixed with 20 ml of glycerol (17) and lysine was added to a concentration of 10⁻⁶ M. The pH was adjusted to 7.6 with NaOH. Then 1 mg of crystalline trypsin was added and the reaction mixture was preserved by the addition of sodium ethyl mercurithiosalicylate (1 part per 10,000). The mixture was incubated at 37°C for several days. Aliquots were assayed periodically for proactivator, plasminogen, and plasmin activities by methods described above, and for trichloroacetic acid-soluble products (17). The results (Fig. 4) show a direct relationship between the disappearance of proactivator activity and the formation of plasmin and trichloroacetic acid-soluble substance which absorbs at 280 μm. They indicate that purified proactivator is converted to plasmin by the liberation of at least one trichloroacetic acid-soluble peptide.

Also of interest is the observation that total streptokinase-activatable LMe-esterase activity increases during the formation of plasmin and the disappearance of proactivator activity. Because of the complexities inherent in the assay system, the reason for this increase remains obscure.

Immunochemical Analysis of Chromatographed Plasminogen—The photographs shown in Fig. 5 were taken after 27 and 47 hours of incubation at 37°C. The results of these experiments are presented in Tables III and IV. The ratios of (a) proactivator units to fibrinolytic units, (b) proactivator units to streptokinase-activatable caseinolytic units, and (c) proactivator units to streptokinase-activatable TAME-esterase units remain reasonably constant through a 50 to 90-fold purification of proactivator over Fraction III and a 300-fold purification of proactivator over plasma.

TABLE IV
Ratios of biochemical activities present in chromatographed and crude plasminogen

<table>
<thead>
<tr>
<th>Plasminogen fraction assayed</th>
<th>Ratio of proactivator units to fibrinolytic units</th>
<th>Ratio of proactivator units to streptokinase activatable caseinolytic units</th>
<th>Ratio of proactivator units to streptokinase activatable TAME esterase units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction III</td>
<td>1.8:1</td>
<td>9.15:1</td>
<td></td>
</tr>
<tr>
<td>Chromatographed</td>
<td>2.4:1</td>
<td>10.1:1</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td>30.3:1</td>
<td></td>
</tr>
<tr>
<td>Chromatographed</td>
<td></td>
<td>33.8:1</td>
<td></td>
</tr>
</tbody>
</table>

* Averages of two determinations on two different chromatographed plasminogen preparations.

FIG. 4. Activation of chromatographed plasminogen by trypsin. O—O, streptokinase, activatable LMe-esterase (plasminogen and plasmin); Δ—Δ, LMe-esterase (plasmin); X—X, trichloroacetic acid soluble products; ●—●, proactivator units per ml. See text for details.
DISCUSSION

Kline (6) has reported the purification and crystallization of plasminogen, and the physicochemical properties of plasminogen prepared by this procedure have been examined by Shulman et al. (18). Electrophoretic and ultracentrifuge studies by the latter investigators indicated that such plasminogen preparations contain about 30% impurity. The results of the present experiments show that major impurities in this type of preparation can be removed by chromatography on CM-cellulose. The physicochemical characteristics of chromatographed plasminogen, which show a high degree of homogeneity, are presented in the following paper (7).

Considerable controversy concerning the existence of proactivator as an entity distinct from plasminogen still exists (19). By the methods described in this paper “proactivator activity” was not separated from “plasminogen activity.” Furthermore, although the purity of chromatographed plasminogen was increased up to 4 times that previously described in the literature, which represents a 300- to 400-fold purification over plasma, the ratio of proactivator activity to streptokinase-activatable plasminogen activity remained essentially the same. These findings are interpreted as supporting the concept, previously proposed (5), that proactivator and plasminogen are identical.

It should be stated that the evidence presented herein for the identity of proactivator with plasminogen can only be considered suggestive. In the light of the present enzymatic and immunological studies and the physical studies reported in the next paper (7), more than one molecular species is present, even in the most purified preparations. It remains a real possibility that proactivator and plasminogen are not identical. This, however, is contradicted by the maintenance of a constant ratio of proactivator activity to streptokinase-activatable plasminogen activity through a several-hundred-fold purification and the high degree of homogeneity of the purified preparation (7).

Chromatographed plasminogen still displays plasmin activity. That plasmin can account for the proactivator activity, however, is highly unlikely. It has been previously shown that a mixture of streptokinase and plasminogen containing a large concentration of plasmin (5) or trypsin-activated plasmin is, at best, a poor activator of bovine plasminogen. The data of the experiments on trypsin activation of plasminogen, which demonstrate a decrease in proactivator activity as plasmin activity increases, further support this contention; in fact such data would be expected if plasmin were formed from proactivator.

SUMMARY

1. Human plasminogen, prepared by the Kline procedure, was chromatographed on carboxymethyl cellulose to yield fractions up to 4 times purer in proactivator content.

2. Highly purified plasminogen displayed the same ratio of proactivator activity to streptokinase-activatable plasminogen as crude plasminogen.

3. Chromatographed plasminogen was shown, by immunological analysis, to consist of at least two antigenic components.

4. The most highly purified plasminogen preparations still contain a small amount of plasmin activity.

5. The problem of the identity of human plasminogen and proactivator is briefly discussed.

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