Plasminogen, the Serum Proenzyme Activated by Factors from Cells Transformed by Oncogenic Viruses

JAMES P. QUIGLEY, LILIANA OSSOWSKI, and E. REICH
From The Rockefeller University, New York, New York 10021

SUMMARY

The serum factor that participates in the fibrinolytic activity associated with tumor and transformed cells has been purified by two independent methods. The chromatographic, electrophoretic, and biochemical properties of this molecule are consistent with the properties of the known fibrinolytic precursor, plasminogen. Purified plasminogen was isolated by affinity chromatography from fetal bovine, chicken, dog, and human sera and its activation by factors released from a variety of transformed cell cultures, including human tumor cell lines, was examined. The specificity of activation is absolute in the avian system, i.e. chicken plasminogen is activated only by the factor from cultures of chick embryo fibroblasts transformed by the Rous sarcoma virus. The mammalian plasminogens are activated to varying extents by the activators from all transformed cells tested.

It has been shown (1, 2) that avian and mammalian fibroblasts, transformed to malignancy by oncogenic viruses or chemical carcinogens, produce a potent fibrinolytic activity when cultured under appropriate conditions; in contrast, little or no such activity appears in parallel control cultures of normal cells. More recently, enzymatic activity has been detected in primary cultures of chemically induced mouse and rat tumors, and in a variety of human tumor cell lines. These observations confirm the original description of his phenomenon by Fischer (3).

Because there are indications that the fibrinolytic activity of a transformed culture may in part determine several phenotypic parameters associated with transformation (1, 2), it appears desirable to isolate the active principles and characterize their catalytic properties. Previous analysis of the induction of fibrinolysis has already established that the fibrinolytic enzyme can be generated in vitro by the interaction of two protein factors: one of these is present in the sera of all avian and mammalian species, and the second factor is produced by transformed cells (1, 2). Since the active enzyme is formed and can be assayed under cell-free conditions in vitro, the purification and characterization of both cell and serum factors can be undertaken successfully. In this paper we describe the isolation of the serum factor and its identification as the known fibrinolytic precursor, plasminogen. The two accompanying reports describe, respectively, the role of the enzyme in mediating a sequence of morphological changes that accompany transformation, and the characterization of the cell factor as a plasminogen activator.

EXPERIMENTAL PROCEDURE

Cell Cultures—The materials and procedures used in the preparation and maintenance of chicken, hamster, and mouse embryo fibroblasts were exactly as described previously (1, 2). Infection of mouse embryo fibroblasts with Rous sarcoma virus, infection of mouse embryo fibroblasts with murine sarcoma virus, and the isolation of a transformed clone of simian virus 40 (SV40)-infected hamster embryo fibroblasts were as already reported (1, 2). The human transformed cell lines were RPMI 41, an osteosarcoma, and RPMI 8342, a malignant melanoma, kindly supplied by Dr. Daniel B. Rifkin; these were cultured in plastic Petri dishes using medium RPMI 1640 (Grand Island Biological Co.) supplemented with fetal bovine serum (final concentration 10%).

Preparation of Serum Free Culture Fluids Containing Cell Factor—Serum-free culture fluids were obtained by washing the cells twice with TD buffer (0.024 M Tris-HCl, pH 7.4, 0.14 M NaCl, 0.000 M KCl, and 0.000 M Na2HPO4) and then incubating them in Eagle’s medium for 16 to 18 hours (1, 2). The serum-free supernatant was collected and centrifuged at low speed to remove intact cells and cell debris. The supernatant, which contains the active cell factor, was dialyzed exhaustively against 0.1 M Tris buffer, pH 8.1, and kept frozen (-20°C) until used in the assay system. The cell factor preparations used in this paper, unless otherwise stated, were from cultures of mouse embryo cells transformed by murine sarcoma virus.

11F-Fibrin-coated Petri Dishes—The 35-mm Petri dishes used in the assay system were coated with 11F-fibrin at a final specific activity of 300 to 500 cpm per µg of fibrin as described previously (1, 2). To determine the total radioactivity on the Petri dishes in each experiment, the insoluble 11F-fibrin was exhaustively digested with trypsin and the radioactivity released was measured. Dishes were prepared as follows: plastic Petri dishes, 35-mm, were coated with 11F-fibrinogen (500 cpm per µg) at a concentration of 10 µg per cm2. After drying for at least 24 hours at 45°C, the plates were incubated for 2 hours with medium supplemented with fetal bovine serum (2.5% concentration), and then washed twice with TD buffer.

Assay of Fibrinolytic Activity—For assay of cell-free fibrinolysis, an appropriate aliquot of a standard preparation of crude cell factor (equivalent to approximately 1 ml of serum-free culture fluid) was mixed with the fractions to be tested for serum factor activity. The assay was performed in a final volume of 1 ml using

RECEIVED FOR PUBLICATION, SEPTEMBER 20, 1973

4306
plastic Petri dishes (35-mm diameter) coated with 125I-fibrin. Incubation was at 37° and release of radioactivity into the medium was followed by removing known aliquots of the total volume at selected time intervals and measuring the radioactivity in a gamma counter (Packard Instrument Co., Downers Grove, Ill.). Following a short lag period, the release of radioactivity was linear with time of incubation until 70% of the fibrin had been solubilized; the release of radioactivity was also linear with the concentration of serum factor. That the solubilization of radioactivity is due to proteolytic digestion of the insoluble fibrin has already been documented (1, 2).

Ammonium Sulfate Fractionation of Dog Serum—Dog serum was diluted by the addition of 4 volumes of cold TD buffer. Solid ammonium sulfate was added, with constant stirring, to 20% saturation, the mixture stirred for 30 min, and the precipitate was harvested by centrifugation in a refrigerated centrifuge (Sorvall RC-2B) at 10,000 X g for 20 min. The resulting pellet was dissolved in one-quarter of the volume of the original serum and dialyzed exhaustively against TD buffer. This procedure was repeated to obtain the proteins precipitating, respectively, between 20 and 40% and 40 and 60% of saturation with ammonium sulfate. The supernatant from 60%, saturation, and all of the other fractions, were equilibrated with TD buffer by exhaustive dialysis. The entire procedure was performed at 4°.

SP-Sephadex Ion Exchange Chromatography—Sephrose 4B was activated with cyanogen bromide according to the procedure of Cuatrecasas et al. (4). Lysine was then coupled to the activated Sepharose and an affinity column (1.0 x 11 cm) prepared according to the method of Deutsch and Mertz (5). The unbound lysine was removed by washing the column in 0.3 M potassium phosphate buffer, pH 7.4, containing 0.001 M EDTA.

Polyacrylamide Gel Electrophoresis—Sodium dodecyl sulfate polyacrylamide gels and the buffer systems were prepared according to the method of Laemmli (6). A vertical slab gel electrophoresis apparatus was employed. The upper stacking gel was 3% acrylamide and the lower separating gel 10% acrylamide. Polyacrylamide gels and the buffer systems were prepared according to the method of Laemmli (6). A vertical slab gel electrophoresis apparatus was employed. The upper stacking gel was 3% acrylamide and the lower separating gel 10% acrylamide. The samples were denatured and reduced by boiling for 2 min in sodium dodecyl sulfate (final concentration 2%) and β-mercaptoethanol. The samples were then applied, with 0.25 ml of sample, to the gel. The electrophoresis was run at 4°.

RESULTS

Purification of Fibrinolytic Factor from Dog Serum—Dog serum (20 ml) was fractionated using ammonium sulfate precipitation as described under "Experimental Procedure." A 10- and 20-μl aliquot of each fraction was assayed for fibrinolytic activity. Essentially identical chromatograms were obtained and a standard curve was constructed using the column effluent as a standard. The specific activity of the column effluent was 3.5 units/mg.

Ammonium Sulfate Fractionation of Dog Serum—Dog serum was diluted by the addition of 4 volumes of cold TD buffer. Solid ammonium sulfate was added, with constant stirring, to 20% saturation, the mixture stirred for 30 min, and the precipitate was harvested by centrifugation in a refrigerated centrifuge (Sorvall RC-2B) at 10,000 X g for 20 min. The resulting pellet was dissolved in one-quarter of the volume of the original serum and dialyzed exhaustively against TD buffer. This procedure was repeated to obtain the proteins precipitating, respectively, between 20 and 40% and 40 and 60% of saturation with ammonium sulfate. The supernatant from 60%, saturation, and all of the other fractions, were equilibrated with TD buffer by exhaustive dialysis. The entire procedure was performed at 4°.

SP-Sephadex Ion Exchange Chromatography—Sephrose 4B was activated with cyanogen bromide according to the procedure of Cuatrecasas et al. (4). Lysine was then coupled to the activated Sepharose and an affinity column (1.0 x 11 cm) prepared according to the method of Deutsch and Mertz (5). The unbound lysine was removed by washing the column in 0.3 M potassium phosphate buffer, pH 7.4, containing 0.001 M EDTA.

Polyacrylamide Gel Electrophoresis—Sodium dodecyl sulfate polyacrylamide gels and the buffer systems were prepared according to the method of Laemmli (6). A vertical slab gel electrophoresis apparatus was employed. The upper stacking gel was 3% acrylamide and the lower separating gel 10% acrylamide. The samples were denatured and reduced by boiling for 2 min in sodium dodecyl sulfate (final concentration 2%) and β-mercaptoethanol (0.5 M) immediately before application to the surface of the stacking gel.

Materials

The sera and powdered culture medium were purchased from Grand Island Biological Co. (Grand Island, N.Y.). Human serum was prepared from blood drawn from two normal healthy adult males. All Petri dishes were disposable Falconware. The chromatographic materials were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Streptokinase (Varidase) was purchased from Lederle Laboratories (Pearl River, N.Y.). Ammonium sulfate ultrapure special enzyme grade, was obtained from Schwarz-Mann (Orangeburg, N.Y.). All other materials and reagents were the best commercially available grades.

Table I

<table>
<thead>
<tr>
<th>Ammonium sulfate fraction (mg)</th>
<th>Protein Recovery</th>
<th>Specific activity (mg)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. 0-20%</td>
<td>6</td>
<td>0.9</td>
<td>&lt;1</td>
</tr>
<tr>
<td>II. 20-40%</td>
<td>480</td>
<td>2.9</td>
<td>91</td>
</tr>
<tr>
<td>III. 40-60%</td>
<td>300</td>
<td>0.04</td>
<td>&lt;1</td>
</tr>
<tr>
<td>IV. 60% supernatant 0.1</td>
<td>420</td>
<td>0.1</td>
<td>3</td>
</tr>
<tr>
<td>Original whole serum 1400</td>
<td>100</td>
<td>1.1</td>
<td>100</td>
</tr>
</tbody>
</table>

* Specific activity is expressed as counts per min released per hour per mg of protein × 10^-4" in a fibrin plate assay.

Since acid-labile inhibitor(s) of fibrinolytic activity is present in whole serum, the serum is incubated at pH 5 for 2 hours at 24° to inactivate it. The serum is then neutralized to pH 7.4 and assayed for fibrinolytic activity, and the specific activity is determined. This value is used to calculate total recovery of fibrinolytic activity in the various fractions.

The active ammonium sulfate fraction (Fraction II, 4 ml, 290 mg of protein) was dialyzed exhaustively against 0.5 M sodium acetate buffer, pH 5, diluted with distilled water to a concentration of 0.1 M sodium acetate, and then adsorbed to a column of SP-Sephadex (1.4 × 40 cm), previously equilibrated with 0.1 M sodium acetate buffer. The column was washed successively with 0.1 M and 0.25 M sodium acetate (pH 5) until the absorbance of the eluate at 280 nm had returned to base-line (Fig. 1); more than 60% of the adsorbed protein was eluted under these conditions. A linear gradient of sodium acetate buffer (pH 5) was then applied, with 0.25 M and 1.0 M as limiting concentrations. Following the elution of two major peaks of inactive protein, two smaller peaks were eluted from the columns (1 and 1I); both peaks contained material that was active in fibrinolysis when combined with cell factor. These two active peaks together accounted for 3 to 4% of the total protein and 80% of the fibrinolytic activity applied to the column. The recovery of protein and fibrinolytic activity in this step was routinely 80 to 85% and 70 to 85%, respectively, and the over-all purification was about 100-fold with respect to the starting serum. Essentially identical results were obtained when the factors from chicken, human, and fetal bovine sera were purified by the same procedure.

When the proteins in the peaks were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and β-mercaptoethanol, each active peak was found to consist of a major component (apparent mol wt 85,000) and a number of other minor components. Analysis of the same fractions on columns of Sephadex G-200 indicated that the molecular weight of the fibrinolytically active components was in the range of 80,000 to 90,000.
lytic activity by the standard W-fibrin plate method employing mouse cell factor. No fibrinolytic activity was observed in the peak fractions obtained on SP-Sephadex chromatography (Fig. 1) was assayed for fibrinolytic activity by the standard '25I-fibrin plate assay system, containing either cell factor or 0.1 ml of Tris buffer containing 100 units of streptokinase (100 units). MSV HF, conditioned medium from mouse fibroblast cultures transformed by murine sarcoma virus.

Streptokinase Activation of Partially Purified Serum Factor—The preceding studies of the chromatographic and electrophoretic properties of the partially purified serum factor preparations suggested that the active component might be plasminogen, the precursor of the known fibrinolytic enzyme, plasmin. Several series of experiments were therefore performed to test this possibility.

One characteristic property of dog plasminogen is its conversion to plasmin by streptokinase (7). Consequently, each of the peak fractions obtained on SP-Sephadex chromatography (Fig. 1) was assayed for fibrinolytic activity when activated either by streptokinase or by cell factor. Fractions 78 to 82 and 88 to 93 were pooled and designated Peak I and II, respectively. 125I-Fibrin-coated Petri dishes were incubated with 1.0 ml of 1 m Tris buffer, pH 8.1, 20 µl of Peak I or II, and either 0.1 m of cell factor or 0.1 ml of Tris buffer containing 100 units of streptokinase. The radioactivity released from the plates after various periods of incubation at 37° was determined, and the specific activity of each peak was calculated for both cell factor stimulated and streptokinase stimulated activity.

There was no fibrinolysis in the absence of either activator. The specific fibrinolytic activity of Peak I relative to that of Peak II was 0.60 when activated by cell factor, and 0.62 when activated by streptokinase. These results suggest that the serum factor is plasminogen. Additional experiments were undertaken to verify this conclusion.

Affinity Chromatography—Deutsch and Mertz (5) have shown that human plasminogen can be purified by affinity chromatography on lysine-substituted Sepharose 4B, and we have used this procedure to identify the active component of dog serum. Crude dog serum was passed through an affinity column and the fractions were tested for (a) plasminogen, by assaying for fibrinolysis in the presence of streptokinase, and (b) serum factor activity, by assaying in the presence of cell factor. The results are presented in Fig. 2 and Table II. The bulk of the serum protein was not adsorbed to the column and emerged free of detectable fibrinolytic activity with either activator. A minor protein fraction that comprised less than 1% of the total serum protein

**Table II**

<table>
<thead>
<tr>
<th>Column fraction</th>
<th>Protein</th>
<th>Cell factor-stimulated</th>
<th>Streptokinase-stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>Recovery%</td>
<td>Specific activity*</td>
</tr>
<tr>
<td>Fractions 3 to 17</td>
<td>1390</td>
<td>99</td>
<td>0</td>
</tr>
<tr>
<td>Fractions 31 to 33</td>
<td>8.4</td>
<td>0.6</td>
<td>410</td>
</tr>
<tr>
<td>Original whole serum</td>
<td>1400</td>
<td>100b</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* Specific activity is expressed as counts per min released per hour per mg of protein × 10⁻⁴.

**Fig. 1.** Cation exchange chromatography on SP-Sephadex of the fibrinolytic factor isolated from a 20 to 40% ammonium sulfate fraction of dog serum. A 20 to 40% ammonium sulfate fraction (Fraction II, 290 mg of protein) was dialyzed against 0.5 m sodium acetate, pH 5, for 24 hours at 4°. The dialyzed sample was diluted with distilled water to bring the concentration of acetate to 0.1 m and loaded onto a column (1.4 × 40 cm) of SP-Sephadex, previously equilibrated with 0.1 m sodium acetate, pH 5. All procedures were carried out at 4°. After the sample (20 ml) was applied, the column was eluted successively with 0.1 m sodium acetate, pH 5, 0.25 m sodium acetate, pH 5, and a linear ionic gradient of 0.25 m to 1.0 m sodium acetate, pH 5. Fractions (2 ml) were collected at a flow rate of 15 ml per hour. Absorbancy at 280 nm was recorded and a 20-µl aliquot of each fraction was assayed for fibrinolytic activity by the standard '25I-fibrin plate method employing mouse cell factor. No fibrinolytic activity was observed in the absence of cell factor. The slope of the ionic gradient was determined by conductivity measurements on the individual fractions.

**Fig. 2.** Purification of fibrinolytic serum factor by affinity chromatography. Dog serum (20 ml) was passed over a column (1.0 × 11 cm) of lysine-substituted Sepharose 4B, equilibrated with 0.3 m potassium phosphate buffer, pH 7.4, containing 0.001 m EDTA at 4°. Absorbance at 280 nm was recorded. The serum was filtered through the column at 20 ml per hour and fractions of 2 ml were collected. The column was then washed with 0.3 m potassium phosphate containing 0.001 m EDTA, pH 7.4, until the absorbance at 280 nm returned to base-line. The fibrinolytic activity was eluted with 0.2 m ε-aminocaproic acid (εACA) in 0.1 m potassium phosphate-0.001 m EDTA, pH 7.4. Fractions 28 to 36 were dialyzed against 0.3 m phosphate-0.001 m EDTA, pH 7.4, buffer at 4° to remove ε-aminocaproic acid. A 10-µl aliquot of each fraction was analyzed for fibrinolytic activity in a standard '25I-fibrin plate assay (see "Experimental Procedure") containing either mouse cell factor or streptokinase (100 units).
that the product obtained from a single cycle of affinity chromatography may be uncertain. It is of interest to note that the product contains some carbohydrate, the molecular weight determination of which was performed as described under "Experimental Procedure." The gels show A, 20 to 25 μg of whole dog serum; B, 4 to 6 μg of active serum protein isolated by affinity column (Fractions 31 to 33, Fig. 2); and C, 4 to 6 μg of active serum protein that had been recycled through a second affinity column which reduces the trace amounts of contaminating serum protein.

was eluted by ɛ-aminocaproic acid, a lysine analog and a known inhibitor of plasmin; this peak coincided with the peaks of fibrinolytic activity stimulated by cell factor and by streptokinase. As seen in Table II, all of the protein was recovered from the column, and the recovery of the cell factor-stimulated and streptokinase-stimulated activity was also quantitative. No fibrinolysis occurred in the absence of an activator. The degree of purification achieved was approximately 200-fold for both streptokinase and cell factor-stimulated enzyme, in agreement with the previous report (5).

The profile of purified dog plasminogen on sodium dodecyl sulfate-β-mercaptoethanol polyacrylamide gel electrophoresis is shown in Fig. 3. Plasminogen is visible as a faint band in the profile of native dog serum (A). The molecular weight of the isolated plasminogen (B) is calculated to be 90,000; however, in view of the report by Robbins and co-workers (8) that plasminogen contains some carbohydrate, the molecular weight determined by sodium dodecyl sulfate-β-mercaptoethanol polyacrylamide gel electrophoresis may be uncertain. It is of interest that the product obtained from a single cycle of affinity chromatography was not entirely pure, since several faint contaminating bands are seen; these may be removed by a second cycle of affinity chromatography. The plasminogen obtained in this way usually appears as a triplet (C). This indicates a heterogeneity in molecular weight of the isolated plasminogen preparations and is consistent with the work of Wiman and Wallén (9).

As a final test for the identity of the serum factor and plasminogen, the plasminogen fractions which were isolated from two cycles of affinity chromatography were pooled and analyzed by chromatography on SP-Sephadex to compare their properties with those of the partially purified serum factor (Fig. 1). Aliquots of the individual fractions were assayed for fibrinolytic activity in the standard 125I-fibrin plates system containing either cell factor from mouse cells transformed by mouse sarcoma virus or streptokinase. Conductivity measurements were performed on Fractions 40 to 80 to determine the slope of the ionic gradient. All assays contained excess cell factor or streptokinase, respectively. MSV HF-conditioned medium from mouse fibroblast cultures transformed by murine sarcoma virus.

![Fig. 3. Polyacrylamide gel electrophoresis of dog serum and the active fraction from dog serum isolated by affinity chromatography. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed as described under "Experimental Procedure." The gels show A, 20 to 25 μg of whole dog serum and the calibrated molecular weights of individual serum proteins, determined in a separate experiment; B, 4 to 6 μg of active serum protein isolated by affinity column (Fractions 31 to 33, Fig. 2); C, 4 to 6 μg of active serum protein that had been recycled through a second affinity column which reduces the trace amounts of contaminating serum protein.](http://www.jbc.org/content/245/21/4309/F1.large.jpg)

![Fig. 4. Cation exchange chromatography on SP-Sephadex of plasminogen isolated and purified by affinity chromatography. Plasminogen (10 mg in 20 ml of phosphate-buffered saline), previously purified through two cycles of affinity chromatography, was dialysed against 0.5 M sodium acetate, pH 5, diluted with distilled water to a final concentration of 0.1 M sodium acetate, and loaded onto a SP-Sephadex column (1.4 × 35 cm) equilibrated with 0.1 M sodium acetate buffer, pH 5. Stepwise and gradient elution with sodium acetate buffers were carried out as described in Fig. 1. Fractions (3 ml) were collected at a flow rate of 15 ml per hour. Absorbance at 280 nm was recorded and a 20-μl aliquot of each fraction was assayed for fibrinolytic activity in the standard 125I-fibrin plates system containing either cell factor from mouse cells transformed by mouse sarcoma virus or streptokinase. Conductivity measurements were performed on Fractions 40 to 80 to determine the slope of the ionic gradient. All assays contained excess cell factor or streptokinase, respectively. MSV HF-conditioned medium from mouse fibroblast cultures transformed by murine sarcoma virus.](http://www.jbc.org/content/245/21/4309/F2.large.jpg)
its purity verified by sodium dodecyl sulfate /mercaptoethanol each of these. The final product was always characterized and represented approximately 0.4% of the total protein in fetal bovine serum, and close to 0.8% of the protein in chicken, dog, and human plasminogens were slightly larger, being close to 90,000.

Activation of Various Plasminogens by Cell Factors from Different Cultures of Transformed Cells—It has been found (1, 2) that the level of fibrinolysis generated by transformed cultures depends on the nature of the serum supplement, and there is a characteristic species-specific pattern of activating and non-activating sera for each type of transformed cells. The mechanisms that determine these diverse patterns of serum effects have not been defined; they could be due to differing concentrations or stability of various serum inhibitors, or to other variables. The availability of purified plasminogens from different species allows one possible mechanism to be tested, namely, whether the pattern of activating sera is determined primarily by the interaction of different cell factors and the respective plasminogen molecules. We have therefore tested four purified plasminogens for activation by cell factors from several transformed cultures and human tumor cell lines. The formation of plasmin was measured by the release of $^{125}$I-fibrin from the surface of Petri dishes. Since a limiting concentration of cell factor is used in each assay and since the plasmid molecules derived from different plasminogens have the same specific activity, the release of the $^{125}$I is an indirect measure of the relative activation of the different plasminogens by the various cell factors. The results are presented in Table III. The background release of radioactivity in the assay in the absence of any plasminogen accounts for solubilization of 3 to 4% of the total radioactivity on the dish. This background level was not exceeded by any of the plasminogen preparations alone, showing that these were free of plasmin (Line 1). The same was true when cell factor preparations from normal cultures were mixed with the different plasminogens, indicating that normal chick embryo fibroblasts did not produce significant amounts of plasminogen activators (Line 2). Finally, the cell factor preparations from different transformed cultures did not catalyze fibrinolysis in the absence of plasminogen, and were therefore not contaminated with either plasminogen or plasmin.

Chicken plasminogen showed the most exacting specificity for activation since it was activated only by the cell factor from Rous sarcoma virus-transformed chick fibroblast cultures. With the possible exception of vampire bat saliva (13), this is the first known activator of chicken plasminogen. The mammalian plasminogens were all activated, although to different extents, by the various cell factors, including that from transformed chick cells, although the latter appeared to have a preference for chicken plasminogen. The cell factors derived from mammalian cells resembled each other closely in specificity of activation, since they all activated dog plasminogen most effectively, followed by human and fetal bovine plasminogens; all of the mammalian cell factors failed to activate chicken plasminogen. The known activator of bacterial origin, streptokinase, acted only on two of the plasminogens tested, dog and human.

**DISCUSSION**

The results described in this paper establish that the serum factor required for fibrinolysis in transformed fibroblast cultures is plasminogen. This identity is based on the following characteristics. (a) The molecular weight and catalytic properties of the serum factor are the same as those previously determined for plasminogen in other laboratories (8, 9, 12). (b) The properties of plasminogen and the serum factor are identical during chromatography on affinity columns and on cation exchange resins. (c) The factor in dog and human sera that is activated by factors from transformed cells is identical with that activated by the bacterial activator streptokinase. (d) As shown in an accompanying contribution (14), activation of plasminogen by cell factor is associated with the conversion of the single polypeptide of plasminogen to the two polypeptide chains of plasmin, just as in the case of activation by streptokinase or urokinase (15).

All of the above properties demonstrate that the serum factor is plasminogen, the precursor of the known fibrinolytic protease, plasmin. Plasminogen can be activated by a protein that is released by transformed and by tumor cells. Therefore, the formation of elevated levels of plasminogen activator provides a fundamental enzymatic distinction between a variety of solid tumors and their normal tissue counterparts, and this enzymatic difference may account for many of the cellular phenomena associated with transformation in culture (16). Since pathological modifications of clotting and fibrinolysis are commonly associated with malignant and other diseases in man, the implications of the present findings are the subject of continuing investigations.

The failure of mammalian cell factors to activate purified chicken plasminogen provides a satisfying explanation for the limitation of fibrinolysis and morphological transformation that occurs when SV40-transformed hamster cultures are incubated in media supplemented with chicken serum. However there is

**Table III**

<table>
<thead>
<tr>
<th>Source of cell factor</th>
<th>Fibrinolytic activity (radioactivity released, % of total) with plasminogen purified from serum of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasminogen from chicken, dog, human (two normal adult males), and fetal bovine serum was isolated and purified on separate lysine-Sepharose affinity columns. A crude cell factor preparation was obtained from each of the indicated cultures as described under &quot;Methods.&quot; The assay system consisted of 1.0 ml of cell factor preparation and 5 µg of the respective plasminogen, incubated on a $^{125}$I-fibrin-coated Petri dish. After 90 min at 37°C the medium on the Petri dish was removed and the radioactivity determined in a gamma counter. The radioactivity released into the medium is expressed as the per cent of the total radioactivity present on the Petri dish.</td>
</tr>
<tr>
<td>Chicken</td>
<td>1. No addition 3 4 3 3 4 4</td>
</tr>
<tr>
<td>Dog</td>
<td>2. Chicken, normal 4 4 4 4 4 4</td>
</tr>
<tr>
<td>Human-1</td>
<td>3. Chicken, transformed by Rous sarcoma virus 40 13 23 21 37 4</td>
</tr>
<tr>
<td>Human-2</td>
<td>4. Mouse, transformed by MSV 6 6 4 1 3 1</td>
</tr>
<tr>
<td>Fetal bovine</td>
<td>5. Hamster, transformed by SV40 4 3 5 20 13 18 4</td>
</tr>
<tr>
<td></td>
<td>6. Human, osteosarcoma 6 7 5 5 49 43 4</td>
</tr>
<tr>
<td></td>
<td>7. Human, melanoma 4 4 4 4 41 20 3</td>
</tr>
<tr>
<td></td>
<td>8. Streptokinase 3 7 0 5 4 50 3 3</td>
</tr>
</tbody>
</table>

*4 K. C. Robbins, personal communication.*
no comparable specificity in the activation of mammalian plasminogens by cell factors from transformed mammalian cells. For example, purified fetal bovine plasminogen is effectively activated by the factors from all of the tested transformed mammalian cultures. Thus the restriction of fibrinolysis and morphological change produced by fetal bovine serum (2, 17) is more likely due to the content of macromolecular protease inhibitors, the presence of these probably accounts for most of the patterns of activating and nonactivating sera observed to date (1, 2).

Acknowledgments—We thank L. Steiner, M. Safaiepour, and A. Chu for expert technical assistance, Dr. Robert Klett for helpful advice and discussions, and Dr. David J. Loskutoff for his criticism of the manuscript.

REFERENCES


* D. Loskutoff and E. Reich, unpublished results.
Plasminogen, the Serum Proenzyme Activated by Factors from Cells Transformed by Oncogenic Viruses
James P. Quigley, Liliana Ossowski and E. Reich


Access the most updated version of this article at http://www.jbc.org/content/249/13/4306

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/249/13/4306.full.html#ref-list-1