Streptokinase-activatable Proactivator of Human and Bovine Plasminogen

AKIKAZU TAKADA, YUMIKO TAKADA, AND JULIAN L. AMBRUS

From the Springville Laboratories, Roswell Park Memorial Institute, Springville, New York 14141, and the State University of New York at Buffalo, Buffalo, New York 14203

SUMMARY

A proactivator for both human and bovine plasminogen was fractionated from human plasma or euglobulin by gel filtration. The proactivator, which was free from plasminogen, was purified by three passages through Sephadex G-200, two passages through Bio-Gel A-150m, and stepwise elution through DEAE-cellulose. The ionic strength of medium containing purified proactivator was decreased by dialysis, resulting in precipitation of proteins with little proactivator activity. The supernatant, approximately 5 times as pure as before dialysis, was concentrated and was gel-filtered through Sephadex G-200. The fractions of highest proactivator activity were shown to be in the fractionation range of Sephadex G-200, smaller than the exclusion limit of the gel. There was no cross reactivity with a highly purified preparation of human plasminogen in immunodiffusion tests. A mixture of the proactivator with streptokinase could not digest casein or the methyl ester of tosylarginine in the absence of plasminogen. Activator prepared from the proactivator had a stronger activator activity for bovine plasminogen than did complexes of streptokinase with plasmin and plasminogen. Small amounts of streptokinase converted the proactivator into activator, which in turn converted human plasminogen into plasmin; but the same amounts of streptokinase could scarcely activate plasminogen in the absence of added proactivator. Very large amounts of streptokinase, however, activated plasminogen equally well in the presence or absence of proactivator. In sharp contrast to caseinolysis, esterolysis of tosylarginine with plasminogen was unaffected by the presence or absence of the proactivator, regardless of how much streptokinase was added.

The proactivator lost 60% of its activity by heating at 85° for 10 min, and almost 100% by heating for 20 min. It lost 50% of its activity in 30 min at pH 2, but was relatively stable at pH 11.

Various investigators proposed (1-3) that the activation of bovine plasminogen by streptokinase in the presence of a trace amount of human globulin takes place in two steps. A human plasminogen proactivator first reacts with streptokinase to form a plasminogen activator, which then transforms bovine plasminogen into plasmin. In contrast to human plasma, bovine plasma apparently lacks the proactivator. This may be the reason for the inability of streptokinase to activate bovine plasminogen alone.

In a study involving hydrolysis of the methyl ester of lysine, Kline and Fishman (4) demonstrated that human plasmin itself could serve as a proactivator. Markus and Werkheiser (5, 6) proposed a scheme to the effect that there are two proactivators in human plasma, one becoming an activator (Activator I) on the addition of streptokinase, and the other being a complex (Activator II) of streptokinase with plasminogen (or plasmin). These authors hypothesized that Activator I could activate both human and bovine plasminogen. Ling, Summaria, and Robbins (7, 8) found that a complex of streptokinase with human plasmin serves as an activator for bovine plasminogen. Summaria et al. (9) have reported that streptokinase, free or complexed, acts directly on human plasminogen.

The present authors (10-13) have demonstrated that there are proactivators in human plasma, and that at least one such proactivator (termed Proactivator A) is not dependent on the presence of plasminogen or plasmin. In the present study, such a proactivator, after activation by streptokinase, did not digest casein or tosylarginine methyl ester, but exhibited a high activator activity in relation to bovine clots. This proactivator served as a proactivator not only for bovine plasminogen, but also for human plasminogen. Physical and immunochemical characterization of this proactivator, particularly in comparison with plasminogen is also described in this paper.

MATERIALS AND METHODS

Fresh human plasma was obtained from the Red Cross. Commercial preparations of bovine fibrinogen (Cohn's Fraction I, Armour), bovine thrombin (Parke-Davis), streptokinase (Lederle Laboratories), Hammersten casein (Nutritional Biochemicals), and the methyl ester of tosylarginine (tosyl-AME', Mann) were used.

Human plasminogen was prepared according to the method of Kline and Fishman (14). Highly purified human plasminogen (15.4 mg per ml, 26.3 casein units per ml, lot 332A) was kindly given to us by Dr. K. C. Robbins, of the Michael Reese Research Foundation, Chicago.

1 The abbreviation used is: tosyl-AME, tosylarginine methyl ester.
Proactivator of Human and Bovine Plasminogen

**Fig. 1.** Relationship of reciprocal of lysis time to proactivator concentration. *Abscissa* represents volume of proactivator solution (milliliters), and hence relative proactivator concentration. After 0.9 ml of each dilution was activated with 0.1 ml of 500 units per ml of streptokinase, clot lysis time was measured in minutes (see text).

**Fig. 2.** *A,* elution pattern for an euglobulin preparation (30 ml) from human plasma (50 ml), eluted with 0.1 M Tris-HCl buffer, pH 8.0, through a Sephadex G-200 column (3 × 35 cm); 15 ml fractions. O---O, optical densities (protein) of effluents of individual fractions, measured at 280 nm; ■—■, caseinolysis; Δ—Δ, tosyl-AME esterolysis (e.g., that of Fraction 12 was 6 pmol of acid liberated by 0.2 ml of the effluents). *B,* elution pattern for Fractions 6 to 8 of the sample represented in *A,* eluted through a Sephadex G-200 column (3 × 45 cm), 15-ml fractions. O---O, optical densities protein, as in *A;* ●—●, reciprocals of lysis times (× 10³), and hence relative clot lysis activities; X, lysis areas (mm²) on heated bovine fibrin plates in the presence of streptokinase, thus expressing relative plasminogen concentrations. *C,* elution pattern for Fractions 8 to 10 of the sample represented in *A,* eluted through a Sephadex G-200 column (3 × 45 cm); 15-ml fractions. O---O, optical densities, as in *A;* ●—●, reciprocals of lysis times (× 10³) as in *B.* No fractions exhibited lysis on heated fibrin plates.

Gel filtration was performed by the method of Flodin and Killander (15). Sephadex G-200 (Pharmacia) and Bio-Gel A-150m (Bio-Rad, Richmond, California) were washed first with 0.1 N HCl, then with 0.1 N NaOH, and finally with 0.1 M Tris-HCl buffer, pH 8.0. For each experiment, a column was packed with gel. After adsorption of a sample placed on the gel bed, the column was eluted with Tris-HCl buffer.

DEAE-cellulose (Bio-Rad), was prepared in the same manner as the filter gels, but fractionation was performed by means of stepwise elution. Protein was determined by measuring absorbance at 280 nm. Lysis time for fibrin clots was determined according to the method described previously (13). An 0.9-ml sample of the proactivator solution, prepared as described later, was mixed with 0.1 ml of a solution containing 500 Christensen units of streptokinase per ml, and the mixture was incubated for 10 min at 37°. After incubation, 0.2 ml of the mixture was combined with 0.4 ml of 0.067 M phosphate buffer, pH 7.4 (containing 0.008% CaCl₂ and 0.9% NaCl), 0.3 ml of 0.33% bovine fibrinogen solution, and 0.1 ml of 100 units per ml of thrombin solution. This reaction mixture was incubated at 37° and the time for complete lysis of the clot that formed was measured in minutes.

**Fig. 3.** *A,* elution pattern of Fractions 8 to 10 of sample represented in Fig. 2B, eluted with 0.1 M Tris-HCl buffer, pH 8.0, through a Bio-Gel A-150m column (3 × 33 cm); 7-ml fractions. *B,* elution pattern of Fractions 27 to 33 of sample represented in *A;* conditions exactly as in *A.

More concentrated solutions of the proactivator exhibited a plateau in the lysis time, because the quantity of bovine plasminogen in the fibrin clot was a limiting factor. Within the concentration range represented in Fig. 1, however, the assay system was presumably adequate.

Bovine fibrin plates were prepared as described before (13, 16). The heated fibrin plates (17) were used to detect the presence of plasminogen in samples applied.

Caseinolytic activity was performed according to a slightly modified method of Norman (18). A mixture of 1 ml of 4% casein solution, 1 ml of effluent, and 0.1 ml of 10,000 units per ml of streptokinase solution was incubated at 37° for 30 min. Three milliliters of 10% trichloracetic acid were added, and the mixture was kept at 4° overnight. The mixture was centrifuged for 20 min at 3,000 rpm and the optical density of the supernatant was determined by measuring absorbance at 280 nm. Caseinolytic activity was calculated by the method of Remmert and Cohen (19). The casein unit was defined as the amount of enzyme that liberated 450 μg of tyrosine soluble in trichloracetic acid in 1 hour (20).

Tosyl-AME esterolysis was determined by the method of Troll and Sherry (3), and described in detail previously (13). The results were expressed as the number of micromoles of acid liberated during the 45-min incubation period.

Guinea pig and bovine euglobulin preparations obtained from fresh guinea pig and bovine serum were used as sources of plasminogen.

Immunodiffusion was performed with Gelman immunodiffusion sets. Antibodies to purified plasminogen (40.5 casein units per ml) and to the proactivator under study were obtained.
by injecting the antigens together with complete Freund adjuvant into rabbits. Antigens were injected weekly for 4 weeks, and blood was taken from the carotid artery. Serum was prepared. After the antigens and antibodies were placed in respective wells, slides were left in a humidity chamber at room temperature for about 20 hours, and then were placed in fresh 1% NaCl solution for 20 hours, the NaCl solution being changed twice. They were stained with buffalo black.

Disc electrophoresis was performed with the Canalco apparatus according to the method of Davis (21). Acrylamide was used as a gel matrix.

RESULTS

Isolation and Purification of Proactivator of Plasminogen

In order to obtain a highly purified sample of proactivator, 30 ml of euglobulin solution were prepared from 50 ml of human plasma and were fractionated by means of a Sephadex G-200 column (3 x 35 cm) (Fig. 2, A to C). Fractions 6 to 8 exhibited no caseinolysis or tosyl-AME esterolysis in the presence of any quantity of streptokinase, but induced a trace of lysis on heated fibrin plates (Fig. 2A), which are much more sensitive than casein or tosyl-AME. The pooled solutions of Fractions 6 to 8 (45 ml) were passed twice through a Sephadex G-200 column (3 x 45 cm) (Fig. 2, B and C). Fractions 8 to 10 from the second passage through the Bio-Gel A-150m column, was dialyzed against 0.1 M Tris-HCl, pH 8.0, and then against various volumes of distilled water at 4°C, giving media of several different concentrations. Fig. 5A shows the results. Decreasing the ionic strength at low temperature caused precipitation. Each medium was centrifuged for 30 min at 4°C, and the residue was restored to the original volume by the addition of Tris-HCl of the same ionic strength as the supernatant. The fibrinolytic activities of both the supernatants and the residues were determined by a clot lysis method. B, supernatant a in A was concentrated 10 times, and 1 ml was eluted through a column (2 x 45 cm) of Sephadex G-200. Fractions were 3 ml each.

Figures 26 to 30 from the second passage through the Bio-Gel column were pooled and 30 ml were passed through a DEAE-cellulose column (3 x 10 cm). After saturation with 0.02 M phosphate buffer, pH 8.0, the DEAE-cellulose column was subjected to stepwise elution with 60 ml of 0.2 M phosphate buffer, pH 8.0, 60 ml of 0.3 M phosphate buffer, pH 8.0, and 150 ml of 0.3 M phosphate buffer, pH 8.0, containing 1 M NaCl (Fig. 4). To avoid aggregation of protein components, the samples were not concentrated in most of the steps of gel filtration or chromatography. The effluents were pooled and applied as they were to the next column, except for the second passage through Bio-Gel A-150m. This proactivator, obtained from DEAE-cellulose column, was dialyzed against 0.1 M Tris-HCl, pH 8.0, and then against various volumes of distilled water at 4°C, giving media of several different concentrations. Fig. 5A shows the results. Decreasing the ionic strength at low temperature caused precipitation. Each medium was centrifuged for 30 min at 4°C, and the residue was restored to the original volume by the addition of Tris-HCl of the same ionic strength as the supernatant.

Decreasing the ionic strength to one-tenth of its initial value precipitated 80% of the proteins, but most of the proactivator activity remained in the supernatant. Further decreases in the ionic strength did not cause any further loss in the proactivator activity of the supernatant.

After dialysis against 30 times its volume of water, the supernatant was concentrated to 1 ml (10 times its initial concentration), and was gel-filtered through a column (2 x 45 cm) of Sephadex G-200. The peak for blue dextran (for measuring the void volume) was at Fraction 13, and that for proactivator activity was at Fraction 15 (Fig. 5B). These results indicate that the molecular weight of the proactivator falls in the fractionation range of Sephadex G-200.

It is not possible to give the yield in each step of the purification.
tion procedures, because a very small amount of human plasminogen or plasmin, if present as a contaminant, would result in activator activity, because it is claimed that streptokinase-plasmin or plasminogen complex are strong activators of plasminogen. Thus, the yield would not be calculated until it is absolutely sure that the present proactivator solution is not contaminated with plasmin or plasminogen.

**Immunodiffusion**—The effluents of Fractions 14 and 15 were each concentrated so as to contain 4 mg of protein per ml. The 1.5 ml of each concentrate were mixed with an equal volume of Freund’s adjuvant and injected weekly into rabbits. Fig. 6, A and B, shows the results. The proactivator in Fig. 6A was from Fraction 14, and that in Fig. 6B was from Fraction 15. In Fig. 6 there was no cross reaction between proactivator and antiplasminogen or between plasminogen and antiproactivator.

**Disc Electrophoresis**—Fig. 7 shows the results of disc electrophoresis. A represents the proactivator (Fraction 14 in Fig. 5B) and B represents human euglobulin. In this experiment, the proactivator gave only two lines (one strong and the other weak), whereas human euglobulin (the starting material) gave many dark-colored lines. Further evaluation of homogeneity of the present proactivator is carried out (a) by making a slice of the gel and determining the site with proactivator activity, and (b) by the use of immunoelectrophoresis.

**Physical and Biological Properties of Proactivator**

The proactivator solution subjected to further characterization had the following properties. The protein content was 3.71 mg per ml. When 0.9 ml of the solution was activated by 0.1 ml of 500 units per ml of streptokinase for 10 min at 37° and 0.2 ml of the activated solution was mixed with 0.3 ml of 0.33% bovine fibrinogen solution, 0.4 ml of phosphate buffer, and 0.1 ml of 100 units per ml of thrombin solution, the bovine fibrin clot that formed was lysed in 9 min. In the absence of either proactivator or streptokinase, no lysis occurred within a period of more than 24 hours.

**Activation of Highly Purified Human Plasminogen by Proactivator in Presence of Streptokinase**—To 0.5-ml portions of the highly purified plasminogen preparation were added 0.5-ml portions of proactivator solution, and to 0.5-ml portions of either plasminogen preparation or proactivator solution were added 0.5-ml or 0.6-ml portions of Tris buffer. To the 1.0-ml mixtures were added 0.1-ml portions of 10 or 100 units per ml of streptokinase solution. Table I shows the results. Plasminogen in the presence of either 1 or 10 units of streptokinase gave much more caseinolysis in the presence of the proactivator than in its absence; no caseinolysis was produced by plasminogen in the absence of streptokinase, or by proactivator in the presence of streptokinase but in the absence of plasminogen.

**Heat Stability of Proactivator**—Samples of the stock solution of the proactivator were heated at 85° for various lengths of time ranging from 5 to 30 min. After cooling in an ice-cold bath, each sample was subjected to a clot lysis determination. Results indicate that 60% of the activity was lost in 10 min of heating at 85°, and that practically no activity was left after 20 min at 85°.

**pH Stability**—The pH of one sample of the stock solution of the proactivator was adjusted to 2 with 1 N HCl, and that of another sample was adjusted to 11 with 1 N NaOH. These two
samples, together with a control sample whose pH was left at 7.4, were allowed to stand at room temperature for 30 min. The results of clot lysis indicate that the proactivator lost 50% of its activity at pH 2, but was stable at pH 11.

Effects of Various Amounts of Streptokinase on Lysis Time in Presence of Constant Amount of Proactivator—Fig. 8 indicates that the activator activity of the proactivator increased with increased amounts of streptokinase up to about 200 units of the latter, where it reached a plateau, probably because the amount of plasminogen in the bovine clot was a limiting factor.

Effect of Amount of Streptokinase on Activation of Nonhuman Plasminogen by Proactivator—Fig. 9, A and B, shows the relationships of the quantity of streptokinase to caseinolysis by guinea pig and bovine plasminogen. With less than 100 units of streptokinase, the proactivator could not activate either type of nonhuman plasminogen; but when the quantity of streptokinase was greater than 1000 units, activation increased very rapidly.

Bovine and guinea pig plasminogen were not activated by streptokinase in the absence of the proactivator. Thus, it is the proactivator that could convert nonhuman type of plasminogen into plasmin after the addition of streptokinase. It is, however, not known whether the proactivator preparation contained a single proactivator for plasminogens of all animal species or one each for human and nonhuman types of plasminogens, because the activation curves for these two types of plasminogens are quite different (Figs. 9 and 10).

Effect of Amount of Streptokinase on Activation of Human Plasminogen by Proactivator—Fig. 10 indicates the results of experiments in which the relationship of the quantity of streptokinase to caseinolysis was determined by using human plasminogen in the presence or absence of the proactivator. In contrast to the
Proactivator of Human and Bovine Plasminogen

Vol. 245, No. 23

6394

Relationship of activation of nonhuman plasminogen to concentration of streptokinase (SK). Each reaction mixture contained 0.5 ml of either proactivator solution (shaded symbols) or Tris buffer (opened symbols), together with 0.5 ml of euglobulin solution (prepared from 5 ml of serum) as a source of plasminogen, and was activated with 0.1 ml of streptokinase solution at one or another of several different concentrations. Abscissa represents units of streptokinase, and ordinate represents optical density in caseinolysis.

Increasing the streptokinase concentration in the system described in Fig. 10 beyond 100 units resulted in a plateau in the activator activity curve. One possible explanation is that 100 units of streptokinase were enough to activate the proactivator needed for activating all of the plasminogen present (0.075 casein units), and hence increasing streptokinase beyond 100 units had no effect on the rate of caseinolysis. To study this point, larger amounts of plasminogen were used. The amount of plasminogen used in the experiment represented in Fig. 11 was 0.75 casein unit, 10 times as much as was used in the preceding experiment, and yet the results obtained in the two experiments were essen-

tially the same. This is especially clear when Curve A', obtained in the second experiment, is compared, as in Fig. 11, with a normalized version of Curve A, the curve obtained in the first experiment. (The normalization reflects the fact that the optical density is necessarily much greater in the presence of 10 times as much plasminogen.) This result means that the plateau is not due to any limitation imposed by the amount of plasminogen.

The only question that remains concerning the inadequacy of the proposed explanation is whether or not the amount of proactivator used is indeed still in excess when 10 times as much plasminogen is used. However, the amount of caseinolysis produced by 0.75 casein unit of plasminogen with 100 units of streptokinase in the presence of various amounts of proactivator was shown not to deviate significantly from a constant.

Effect of Proactivator on tosyl-AME Esterase Activity of Human Plasminogen in Presence of Streptokinase—An experiment similar to that represented in Fig. 10 was performed, but involving tosyl-AME esterolysis rather than caseinolysis. To mixtures each consisting of 0.2 ml of 0.1 M tosyl-AME and 0.3 ml of 0.5 M Tris buffer, pH 9.0, were added various concentrations of streptokinase in 0.1 ml volumes, and the various samples were in-
cubated at 37° for 5 min. Then a mixture of 0.2 ml of plasminogen and 0.2 ml of either proactivator or Tris buffer was added to each previously incubated sample, and the resulting mixtures were kept in a water bath at 37° for 40 min. If the same activation mechanism is involved in tosyl-AME esterolysis as in caseinolysis, esterolysis in the presence of small amounts of streptokinase should be more extensive in the presence of proactivator than in its absence. Tosyl-AME esterolysis was found to proceed similarly in the presence of proactivator as in its absence, regardless of the concentration of streptokinase (Fig. 12), thus indicating that the activation mechanisms for caseinolysis and tosyl-AME esterolysis are different.

**DISCUSSION**

Kline and Fishman (22) proposed that proactivator is not a separate substance, but that streptokinase forms with plasminogen a complex that serves as an activator for both human and bovine plasminogen. Later they showed that a complex of streptokinase with human plasmin is an activator for bovine plasminogen (4). Kline (23) has also reported the existence of a strong covariance between plasminogen and proactivator concentrations.

Ling et al. (7, 8) have asserted that the activator of bovine plasminogen is only a complex of streptokinase with plasmin. These authors, however, tried to demonstrate proactivator activity in a fairly purified plasminogen preparation. It is also possible that a proactivator, different from plasminogen or plasmin, is present in crude human plasma.

Astrup and Müllertz (16) and Lassen (17) used the fibrin plate method to differentiate activator from plasmin. If plasmin is present, it lyases both the standard and heated fibrin plates. If only activator is present, however, it lyases only the standard plate. Activator converts to plasmin the plasminogen in the fibrin network in the standard plate (the heated plate contains no undenatured plasminogen), and the plasmin in turn digests the fibrin.

In previous studies, the present authors (10-13) also used this method. Because streptokinase converts proactivator into activator, this method will distinguish between plasminogen and proactivator in the presence of streptokinase. The proactivator studied by this group does not contain plasmin or plasminogen, because it does not lyse a heated fibrin plate, even after activation by streptokinase (Fig. 2B). Accordingly, it is not a complex of streptokinase with plasmin or plasminogen. This conclusion is supported by the results of physical and immunological assays.

In the sample represented in Fig. 2B, activator activity was shown in Fractions 7 to 14 which had no caseinolytic or tosyl-AME esterolytic activity. If those fractions contained small amounts of plasmin or plasminogen, and if plasmin or plasminogen was essential to the proactivator activity, Fractions 15 to 17 should have exhibited stronger activator activity than Fractions 10 and 11, because the amounts of plasminogen or plasmin are larger in Fractions 15 to 17 than in Fractions 10 and 11.

Ling et al. (7, 8) reported that a complex of plasmin with streptokinase did not have proteolytic activity. Accordingly, the earlier fractions in Fig. 2, B or C, might contain plasminogen, which forms a complex with streptokinase, and the resulting complex might not have proteolytic activity strong enough to lyse the heated fibrin plate or to exhibit caseinolytic activity, even though it might have a strong activator activity, resulting in intense fibrinolysis of bovine clots. Since the same amount of streptokinase was added to each fraction, in the experiments represented by Fig. 2B, the amounts of complexes of streptokinase with plasmin (or plasminogen) should be at least as large in Fractions 10 and 11 as in Fractions 15 to 17.

Fig. 2B shows that Fractions 10 and 11 exhibited far stronger fibrinolytic activity than did Fractions 15 to 17. Fraction 17 exhibited a very low activator activity, although it lysed heated plates, indicating that plasmin was present. The fact that the fractions that contain plasmin do not exhibit a high activator activity suggests that the activator produced from proactivator by streptokinase may have a much stronger activator activity than the complex of streptokinase with plasmin which occurs in the later fractions.

Experiments represented in Fig. 10 indicate that low streptokinase contents (less than 50 units) resulted in greater caseinolytic activity in a mixture of plasminogen with proactivator than in a mixture of plasminogen with buffer. At high concentrations of streptokinase, the rate of caseinolysis was not higher in the mixture of the proactivator and plasminogen than plasminogen alone throughout the incubation period. In other words, the addition of the proactivator solution to fractions containing plasminogen did not influence the velocity at which plasmin appeared. This result is radically different from that obtained at low concentrations of streptokinase. If the activation of plasminogen does not involve activator derived from proactivator, then an increase in the concentration of activator should bring about an increase of plasmin formed within a specified reaction time, regardless of the concentration of streptokinase. If another kind of activator is formed at high concentrations of streptokinase, however, and if this other kind of activator is potent enough to mask the action of activator derived from proactivator, little difference might be observed on the addition of a solution of a proactivator to a fraction containing plasminogen.

Some authors have calculated the ratio of streptokinase to plasminogen in the complex, and have concluded that the two substances are united in a mole to mole ratio (8, 24, 25). A ratio of 1 mole of streptokinase to 1 mole of plasminogen is equal to a ratio of 2273 units of streptokinase to 1 casein unit of plasminogen (8). In the experiments represented in Fig. 10 plasminogen contained 0.15 casein unit per ml, and 0.5 ml of plasminogen was mixed with 0.1 ml of streptokinase solution. When more than 500 units of streptokinase were added, the addition of proactivator did not make any difference; here the ratio of streptokinase to plasminogen was more than 50:0.075 (667:1). If all of the streptokinase (500 units) reacted with plasminogen (0.15 casein unit), one third of the plasminogen (0.22 casein unit) should be in the form of the streptokinase-plasminogen complex. When 200 units of streptokinase were added to plasminogen, all of the plasminogen should have been in the form of the complex. Nevertheless, the mixture still showed extensive caseinolytic activity. This phenomenon is under intensive study in our laboratories. The addition of the proactivator solution to plasminogen in the presence of various amounts of streptokinase did not make any difference with respect to tosyl-AME esterolysis (Fig. 12), thus suggesting that the proactivator did not participate in the activation of plasminogen to plasmin when tosyl-AME was used as a substrate. It is not known whether there are two kinds of plasminogen, one requiring proactivator for its activation and the
other not, or two kinds of plasmin, one formed through the interaction of plasminogen with activator derived from pro-
activator, and the other resulting from a direct attack of strep-
tokinase on plasminogen. We summarized some of the dif-
fferences of the properties between the present proactivator and
human plasminogen.

1. The proactivator is acid-labile, plasminogen being stable
shortly.

2. The proactivator loses activity even at 56°C whereas
plasminogen is only denatured over 85°C (17).

3. The proactivator is eluted in the macroglobulin fractions by
Sephadex G-200 gel filtration. Human plasmin or plasminogen
was reported to have a molecular weight of approximately
89,000 (26).

Summario et al. (9) found that plasminogen contaminated with
plasmin but treated with a plasmin inhibitor could be activated
by streptokinase alone and hence that activation can occur in
the absence of any complex of streptokinase with plasmin. In
the present series of studies, we have repeatedly failed to find
signs indicating that our proactivator preparation is contami-
nated with plasmin or plasminogen.

Even if our preparation contained very small amounts of
plasmin undetectable with the present methods, the amounts
could not have been much larger than those in the plasminogen
preparation used by Summario et al. (9). Consequently, the
further addition of undetectable amounts of plasmin in our
proactivator, if any, to the plasminogen preparation, which was
heavily contaminated with plasmin (9), could not have ap-
preciably influenced the amount of streptokinase-plasmin com-
plex formed between streptokinase and plasmin in the plasmino-
gen preparation, thus the rate of caseinolysis.

As Table I shows, addition of our proactivator to the highly
purified plasminogen preparation resulted in a significant in-
crease in the rate of the caseinolysis caused by the plasminogen
in the presence of 1 or 10 units of streptokinase. This finding
is of course not in the least incompatible with any observation
that streptokinase forms a complex with human plasmin or
plasminogen, or even that it attacks human plasminogen
directly. All that our finding implies in this regard is that the
proactivator is not merely a complex of streptokinase with plas-
min or plasminogen.

In other words, the present series of studies indicates that the
euglobulin fraction of human plasma contains an entity that
exhibits proactivator activity, but is not dependent on the
presence of plasmin or plasminogen. Efforts to determine other
properties of this entity, particularly its molecular weight, are
under way.

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