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Studies on Plasminogen Activators

I. Sucrose Density Gradient Distribution of Activators from Normal and Lytic Human Plasma

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Molecular sieve fractionation and density gradient distribution of fibrinolytically active human plasma fractions showed that a plasminogen activator isolated from spontaneously lytic human plasma obtained *post mortem*, and the SK-activator are different molecular forms. While the natural fibrinolytic activator has an approximately similar size as human plasmin (>albumin) the SK-activator forms a >200 000 molecular weight complex in native and lytic plasma. When plasma proteolytic inhibitors were investigated in the corresponding sieve fractions, comparable to normal anti-plasmin capacity was obtained in the normal plasma albumin and the lytic sieve fraction containing the natural plasminogen activator. It was concluded that the SK-activator estimated to be a 1:1 complex between SK and a plasma protein (proactivator, plasminogen, plasmin) is a larger protein complex in native plasma under present conditions, either by association of SK with a macroglobulin or by the formation of an enzyme-substrate complex larger than provided through the stoichiometric reaction between 1 mole of plasminogen and 1 mole of SK. The complex formation took place at pH 8 in the presence of 1 M NaCl in normal as well as strongly lytic plasma, conditions which should not favour an association complex between plasma proteins. The proteins participating in the complex formation were not completely digested through the proteolytic effects of thrombin or plasmin under the conditions of post mortem fibrinolysis in blood, since the SK-complex was formed also in plasma containing the preformed plasminogen activator. The lytic plasma used was fibrinogen free or did not clot with thrombin, under conditions where added human fibrinogen could be clotted.

Different sieve patterns on Sephadex G-200 were recently reported [1] with natural and artificial plasminogen activator from human plasma. In accordance with the plasma protein fractionation by Gelotte *et al.*[2] the activator complex formed in normal plasma with streptokinase (SK), a protein of bacterial origin,[3] was sieved together with the 19 S macroglobulins, while a natural fibrinolytic activator from *post mortem* plasma appeared to have a

molecular size between the 7 S globulins and albumins. This spontaneously formed activator compound appearing in blood after sudden death is presumably similar to the plasminogen activator described by Müllertz [4,5] from healthy humans after anoxaemic death. It may also be identical with the tissue activator found by Astrup and Permin,[6] and Astrup.[7] The origin and nature of the natural blood activator causing physiological thrombolysis in the presence of fibrin deposits, are still comparatively unknown.[8–12] It is formed from a proenzyme (the proactivator protein) present in human plasma,[9] or it may originate in the tissues and arrive into the blood through an increased permeability of the endothelium. Both origins are therefore possible as concerns the fibrinolytic activator in *post mortem* blood.[1] In the present work evidence is presented to sustain different molecular sizes between the SK-activator and an active globulin in *post mortem* spontaneously lytic human plasma.

MATERIALS AND METHODS

Human normal mixed blood bank plasma * and *post mortem* plasma ** collected at different time periods following sudden death were used in this investigation. *Normal plasma* contained citrate dextrose anticoagulant solution according to the USP formula 65 ml per 400 ml blood separated by pressure and stored at $-20°$. *Post mortem* blood was drawn from the femoral vein with siliconized equipment into polythene tubes kept in ice and centrifuged immediately at 900 *g* at 5° for 20 min. Plasma samples were subsequently analyzed for free fibrinolytic activity and clottability.[1] Two types referred to as *lytic plasma* were used: 1. nonclottable (5 – 10 units of thrombin),[1] with predominantly free plasminogen activator activity [3-5] and none or traces of proteolytic activity, 56 – 100 % antiplasmin, 76.5 % antitrypsin capacity, 2. with low fibrinogen (0.015 – 0.018 %) or defective clotting ability, no free fibrinolytic activity (activator or protease), 28 % antiplasmin, 88 % antitryspin capacity. No fibrinolytic activity in sieve fractions.

Plasma fibrinogen content was determined according to Bergström *et al.*[13] as described in detail before.[1] A recovery of approximately 80 % of the mean value for mixed normal human plasma [13] was obtained after the addition of a purified human fibrinogen (Kabi, Grade L) to lytic plasma. Nonclottable refers to plasma that did not coagulate with 5 – 10 N.I.H. units of thrombin (Topostasine, Hoffman LaRoche, Basel, Switzerland) in 2 h at room temperature.

Gel filtration on Sephadex G-200 (40 – 120 μ, Pharmacia, Uppsala, Sweden) was performed according to Gelotte *et al.*[1,2] using about 500 ml bed volumes (column diameter 3.8 cm). Elutions were performed with 0.05 M Tris-HCl buffer pH 8 containing 1 M NaCl at flow rates 6 – 8 ml per half hour. The total elution volume for the plasma proteins excluded in three peaks (I – III) was about 43 % of the total bed volumes (average of six runs). In all cases 8 ml of plasma was applied to the molecular sieving. All the operations were performed in a cold room at $+ 5°$. The elution profiles of plasma proteins were measured by the absorbancy at 280 mμ (Beckman DU spectrophotometer) from all the eluate fractions (1:20 diluted) (LKB 3400 B RadiRac fraction collector).

Concentration of pooled plasma protein fractions was made by *ultrafiltration* according to Everall and Wright,[14] using cellulose tubing (Arthur H. Thomas Co., Philadelphia, USA) and a negative pressure by waterpump of about 56 cm mercury. Ultrafiltrated fraction pools of normal human plasma (see Fig. 5) had an ionic strength $\mu = 6 \times 0.182$ (peak I), 6×0.186 (peak II), 6×0.195 (peak III) and were brought to $\mu = 0.15$ by dialysis against 0.15 M NaCl at $+5°$ over night (UF-fractions). Ionic strength measure-

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ments were made with a Philips conductivity bridge PR 9500 and interpolated from a standard curve with 0.003 M NaCl.

Electrophoresis of UF-fractions was performed in a Spinco Durrum electrophoresis apparatus for 18 h at 2.5 mA, room temperature, using the Beckman Model R Paper electrophoresis system [15] (Spinco Division, Beckman Instruments Inc., Palo Alto, California, USA) and dyeing method (Spinco procedure B).

Protein determinations were performed by the Folin-Cu method according to Lowry *et al.*[16] and Eggstein and Kreutz.[17]

Fibrinolytic activity was measured by the fibrin plate method [18,19] using human and bovine fibrinogen (Kabi, Grade B 1, lyophilized purity 85—95 % of total protein content) [20] described in detail before.[1] Activator assay was performed with the plasminogen-rich bovine fibrinogen,[18] while protease activity was obtained using the plate assay after destruction by heat [19] of the plasminogen.

Furthermore the *clot lysis test* was used essentially according to Fletcher [21] and Ploug and Kjeldgaard.[22] Test tubes 7.5 by 10 mm were placed in ice. Ice cold 0.05 M Tris-HCl buffer pH 7.8 containing NaCl to $\mu = 0.15$, 0.2 ml was pipetted into the tubes together with 0.2 ml plasma or plasma equivalent amounts of UF-fractions. Ice cold 0.2 % fibrinogen solution, 1 ml was rapidly added followed by 0.1 ml thrombin solution (100 units per ml in saline). The tube was rapidly inverted and placed in a water bath at + 37°. At the moment of clotting (after 40—60 sec) a glass bead (450 mg diameter 7 mm) was placed on the surface. Time was recorded for the bead to reach the bottom of the tube. The reproducibility of the method was tested with purified urokinase (Leo Pharmaceutical Products, Denmark). A linear reference curve was obtained with 2.5—100 units [22] of urokinase. Samples to be tested were checked for ionic strength.[23] If solutions of $\mu \geqq 0.15$ were applied to the clot lysis test substitution was made by dissolving the fibrinogen in distilled water instead of the buffer.

The streptokinase (SK) used was a highly purified preparation (Kabikinas) obtained with β-haemolytic streptococci. The reported molecular weight for the fibrinolytically active component was about 50 000, the protein content 0.015 μg per unit. SK had no effect on heated or unheated bovine fibrin plates, but produced activation of unheated human fibrinogen. The *SK-activator complex* was prepared with 1000 units of SK per ml of normal or lytic human plasma, as described before.[1]

Density gradient ultracentrifugation was performed with the Spinco Model L ultracentrifuge using the swinging out bucket rotor SW 39. Runs were performed with a linear sucrose gradient of 5 to 20 % in 0.05 M Tris-HCl buffer pH 8 containing 1 M NaCl. The gradient-making device designed by Salo and Kouns [24] was used. Ice cooled samples of 0.25—0.35 ml of UF-fractions of the respective enzymes were layered on top of 4.5 ml gradient solution in cellulose nitrate tubes (5 ml capacity) and placed into the cooled (+5°) rotor. Runs were performed for 20—21.5 h at 38 000 rpm according to Kunkel,[25] in the cold (0—5°). Fractionation was made according to the drop method counting 20 drops per fraction after perforating the tubes with a syringe. Fraction tubes were kept in ice during the fractionation procedure. Tests for fibrinolytic activity were made subsequently. Human serum albumin (mol.weight 69 000) (Kabi, protein standard 20 %) was spun as a standard for reference to molecular weight, applying 0.35 ml 4 % solution diluted with 1 M NaCl. The preparation was 88 % monomer albumin with 12 % dimer by Sephadex G-200 gel filtration, and contained 99 % homogeneous protein by paper electrophoresis. Freshly prepared cold sucrose solutions were used for making the gradient. Controls with sucrose were run on fibrin plates.

Trypsin inhibitor was determined according to the method by Erlanger *et al.*[26] using benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPA) (Mann Research Laboratories, New York, USA) as substrate with an excess of trypsin (2 × crystallized, Worthington, Freehold, New Jersey, USA), following the procedures as described by Ganrot.[27] BAPA was dissolved in dimethylsulphoxide [26] using 0.5 ml in a final volume of 25 ml with 0.05 M Tris-HCl pH 8.2 containing 0.02 M $CaCl_2$ and 0.002 M substrate. Trypsin was stored frozen in a stock solution, 1 mg per ml 0.0025 N HCl, and diluted with buffer for the experiments to 30 μg per 2 ml.[26] Incubations were performed for 10 min at +25° with 50 and 100 μl plasma or plasma equivalent samples from UF-fractions, running controls with trypsin. Absorbancy was read at 410 mμ from acetic acid solutions.[26]

Antiplasmin distribution was determined by proteolysis on heated bovine fibrin plates by the incubation of 0.114 casein units of purified plasmin (Kabi, Grade A, 310

units in 50 % glycerol, diluted 1:10 with buffer) together with an approximately equal volume (0.03 ml) of plasma equivalent UF-fractions. Inhibitory effect was estimated relative to the activity (mm^2) of a control with plasmin alone (20 h at +37°).

RESULTS AND DISCUSSION

The result of an experiment with molecular sieving of the natural plasminogen activator in lytic plasma (type 1) is illustrated in Fig. 1. The activator activity in the sample tested prior to gel filtration was 310 mm^2 on the unheated bovine fibrin plate, and traces (±) of proteolytic activity (heated fibrin). Precipitated material due to storage at −20° was removed by centrifugation for 10 min at 900 *g*, +5°. The eluates corresponding to the three protein peaks obtained by absorbancy measurements were pooled. The elution profile obtained with a similar sample of lytic plasma was presented before.[1] The main peak of fibrinolytic activity was obtained in the plasma albumin range,[28] as found with 0.06 ml aliquots on unheated fibrin plates. Traces of activity occurred on heated fibrin. The fibrinolytic activity obtained with the protein sieve fractions after concentration by ultrafiltration is shown in Fig. 1. Approximately equal activities were obtained on unheated human fibrin plates. Comparable activities were obtained in a second run with the same plasma, yielding in UF-III fraction 400 m^2 (unheated) and 56 mm^2 (heated) activity on fibrin plates. With an euglobulin precipitate from the plasma used in these experiments, performed by 1:20 dilution with 0.014 % acetic acid and redissolved to the original plasma volume in Tris-buffer pH 7.3, comparable activities were obtained with bovine (499 mm^2) and human (400 mm^2) unheated fibrin,

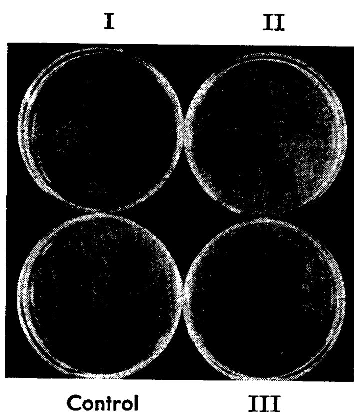

Fig. 1. Activity obtained on unheated fibrin plates (16 h, +37°) in protein sieve fractions from lytic plasma (Type 1), 0.03 ml plasma equivalent volumes of corresponding UF-fractions (undialyzed). I, (0.064 ml) no activity (±); II, (0.05 ml) 203 mm^2; III, (0.046 ml) 446 mm^2, and a control of the original plasma 364 mm^2.

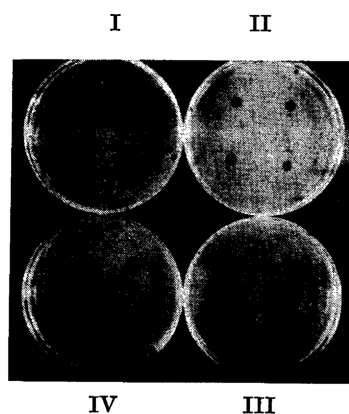

Fig. 2. Fibrinolytic SK-activator traced (unheated, bovine fibrin plates) with 0.03 ml directly from the pooled sieve fractions of 8 ml lytic plasma (Type 2) incubated with SK 1000 units per ml. Total pool volumes: I, 45.4 ml (500 mm^2); II, 71 ml; III, 55 ml; IV, 77 ml (compared to dialyzed UF-fractions, Table 1).

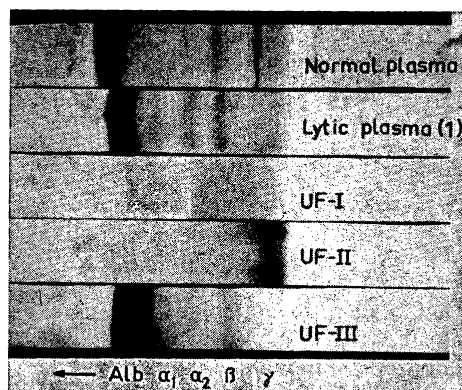

Fig. 3. Protein distribution by paper electrophoresis in sieve fractions from lytic plasma (see Fig. 1). Plasma equivalent volumes of UF-fractions I, II, III compared with 6 μl of unfractionated lytic and normal plasma.

and 54 mm^2 on heated fibrin plates. The low fibrinolytic activity obtained with this plasma was inhibited by 0.001 M ε-aminocaproic acid, and therefore apparently due to a low content of free plasmin.

The electrophoretic distribution of plasma proteins corresponding to the molecular sieve pattern of lytic plasma is shown in Fig. 3. As found by these analyses (Figs. 1, 3) the natural plasminogen activator was eluted predominantly in the albumin range, with some activity in the protein peak II range. The dominating protein in the sieve fraction containing most of the fibrinolytic activity was albumin. However, an association with plasma albumin [29] may be ruled out due to the following considerations: 1) a high salt concentration was used to minimize protein-protein interactions,[2] 2) by the molecular sieve method the activator appeared between the albumin (4.6 S) and the 7 S sieve range, and 3) all the activity could be precipitated together with the euglobin fraction from the unfractionated lytic plasma. This is in agreement with results obtained below with the density gradient ultracentrifugation. A calculation according to the method by Martin and Ames [30] based upon the sedimentation rate compared with human serum albumin (Fig. 6 b) indicated similarity with 5.5—6 S material.

The elution profiles obtained with lytic plasma show the similar three protein peaks characteristic for normal human plasma (Figs. 4,5). Protein determination of the peak UF-fractions gave the following percentage distribution between normal/lytic sieve fractions: I, 15.2/18.5, II, 27.6/28.2; III, 57.2/53.3 with totals of 540 mg/498 mg protein respectively (uncorrected for losses during the UF-procedures). A comparable distribution was also obtained with the respective electrophoretic protein patterns (Fig. 3). The changes occurring during the process of spontaneous lysis were detected mainly by a lack of clottable fibrinogen and the appearance of the plasminogen activator component in plasma. Normal plasma protein sieve fractions (UF) had no activity on fibrin plates.

Artificial fibrinolytic activator was prepared[1] by activation of lytic plasma with SK. The activated samples were submitted to the similar molecular

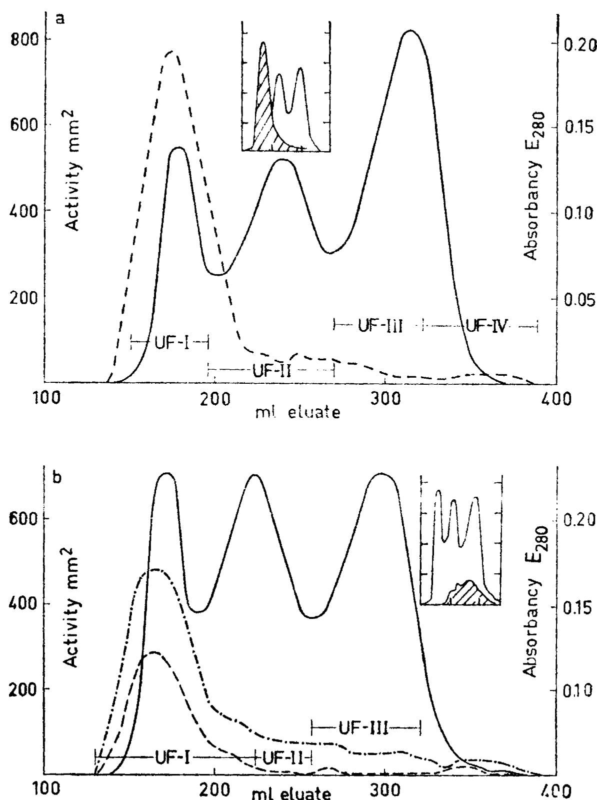

Fig. 4. Elution of fibrinolytic activity (- - -) by gel filtration on Sephadex G-200 of SK activated lytic plasma a) type 2, b) type 1. Plasma protein profile (—) measured by absorbancy at 280 mμ. Plasminogen activation was determined on unheated bovine fibrin plates using 0.03 ml (- - -) and 0.06 ml (—•—) from all the single eluate fractions. No activity (traces) on heated fibrin. Inset figures (shaded areas) a) elution of SK-activator from activated normal human plasma b) natural plasminogen activator from *post mortem* plasma (for details see previous publication [1]).

sieve procedures. Fibrinolytic activity eluted from the columns was traced in the single fractions (Fig. 4 a, b) by plate analyses.

The SK-activator was eluted together with the first protein peak (Fig. 2, 4 a,b), following the sieve pattern of plasma macroglobulins.[2] Regardless of whether normal [1] or lytic plasma were used as substrates for SK, the active component was obtained in the sieve fraction immediately following V_0. With lytic plasma type 2, containing no free fibrinolytic activity, the activity elution profile (Fig. 4 a) was comparable to that obtained with SK from normal human plasma,[1] while a lower activity level was attained in lytic plasma containing preformed plasminogen activator (Fig. 4 b).

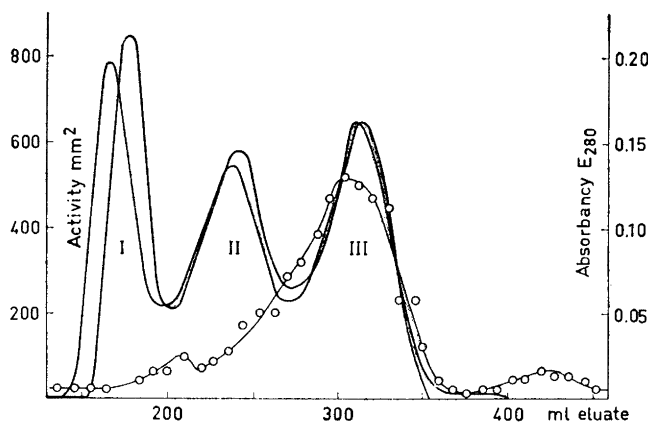

Fig. 5. Molecular sieve elution of 8000 units of SK, by activity measurement on human fibrin plates (○) with 0.03 ml eluate. Comparison with the normal plasma protein sieve pattern (—) obtained from two separate columns with 8 ml human plasma, measured by absorbancy at 280 mμ.

Plate analyses (Table 1) and clot lysis tests (Table 2) were made with UF-fractions corresponding to the sieve fractions in Fig. 4 a and b. It was found that while the main part of activity was located with the macroglobulin fraction plasminogen activating effect was also found in other UF-fractions after the SK-treatment Attempts to inactivate SK at pH 9 to 10 proved unsuccessful.[10] Tests on human fibrin plates (control value 544 mm² with 0.03 ml) yielded 93 % activity after 2 h standing at room temperature pH 9 (250 units per ml), 84 % after 24 h, and 88 % after a week at + 5°, pH 10. It was concluded that SK would not become inactivated within the time limit for a complete gel filtration experiment. Due to the small molecular size SK would be expected to interfere in the elution range of albumin (UF—III, UF—IV, Fig. 4 a) if not all was used for activator complex formation. Gel filtration of 8000 units of SK dissolved in 0.15 M NaCl showed that uncoupled SK eluted over a broad range compared with the sieve pattern of normal

Table 1. Comparative activity (mm²) on human and bovine fibrin with sieve fractions of SK-activated lytic plasma (type 2) by plate analyses (16 h, +37°).

	UF-fractions			
Fibrin plate	I	II	III	IV
Bovine unheated	504	172	105	90
Bovine heated	44	±	±	0
Human unheated	375	186	105	103

Means of four determinations.

human plasma proteins (Fig. 5). This may explain the lysis reactions obtained with UF-fractions II—IV from SK-activated plasma (Table 2). The comparatively high lysis effect obtained with the normal human UF—II fraction is apparently due to the higher plasminogen content in this sieve fraction,[31,32] compared to spontaneously lytic plasma, as was indicated by the corresponding lysis times (Fig. 4 a and b, UF—II).

Table 2. Clot lysis times (sec) with human *post mortem* plasma sieve fractions from Sephadex G-200.

Plasma	UF-fractions			
	I	II	III	IV
type 1 *	> 14 400	> 14 400	4 605	—
type 1 SK-activated **	277	3 060	4 145	—
type 2 SK-activated	373	2 159	4 120	3 363
normal plasma SK-activated	973	669	4 055	—

* with direct plasma 8880 sec, ** with SK 1000 units per ml > 10 800 sec, $\mu = 0.133-0.155$. From left to right measured with same preparations of bovine fibrinogen.

The presence of the natural plasminogen activator in the UF—I and SK-activator in the UF—III sieve fractions was verified by an increased clot lysis in accordance with the fibrinolytic sieve pattern from plasma type 1 (Fig. 4 b, inset figure).

Shorter lysis times were recorded with SK-activated lytic and normal plasma in the sieve fractions III and IV (Table 2). Although the presence of activator could be verified in plasma type 1 the existence side by side of the two plasminogen activators was therefore difficult to demonstrate after SK-activation of plasma containing the preformed activator. Furthermore the proportion of SK-activator after artificial activation under optimal conditions was much higher than the natural plasminogen activator isolated from spontaneously lytic plasma. Several causes to the increased lysis times may, however, be found (see Kline [33] and Ling *et al.*[34]), by the presence of human globulins in these sieve fractions.[2,28]

The eventual relationship in these plasmas between antiplasmin activity and spontaneous fibrinolytic activity was investigated. As shown by Lewis [32] and Ganrot,[27] antiplasmin and antitrypsin [27] are eluated together with albumin. Close to normal plasmin inhibiting capacity was found in the UF—III sieve fractions (Table 3). The interference of free plasmin (mol.weight 89 000 [34]) in these fractions would therefore not be expected. A higher plasmin effect (activation) obtained in this assay with UF—II fractions is apparently due to unaltered plasminogen left in the lytic plasma samples. Some alterations compared to normal antitrypsin capacity were obtained with the lytic fractions (Table 3).

Table 3. Antitrypsin and antiplasmin capacity in sieve fractions as % inhibitory effect.

Assay	UF-fraction No.	Plasma Normal	Plasma type 1	Plasma SK-activated type 2
Antiplasmin	I	47	63	—
	II	(53)	(58)	(53)
	III	69	66	63
Antitrypsin	I	18	6	—
	II	24	13	20
	III	98	96	92

In parentheses measured activation. The antitrypsin capacity in normal SK-activated plasma was 8 (I), 24 (II) and 100 (III) %, respectively. The table lists antitrypsin effect obtained with 100 μl.

Sucrose density gradient ultracentrifugation was run with concentrated preparations of the natural and SK-activator. Assuming that the SK-activator obtained from type 1 and 2 plasma were identical protein complexes (Fig. 4 a and b), the corresponding UF—I sieve fractions were pooled and further concentrated by ultrafiltration. A preparation was obtained with the fibrinolytic activity of 483 mm^2 (unheated) and 95 mm^2 (heated) per 0.03 ml (27 % antiplasmin and 28—57 % antitrypsin capacity). The UF—III sieve fraction containing the natural plasminogen activator [1] (see Fig. 3 b, inset)

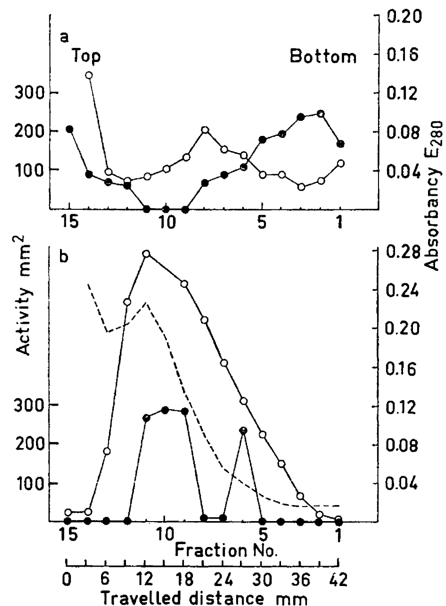

Fig. 6. Sucrose gradient distribution by ultracentrifugation of fibrinolytic activity (●) with concentrated molecular sieve fractions a) SK-activator complex, UF-I, 0.25 ml, b) natural activator compound UF-III, 0.35 ml. Sieved plasma protein materials (○) as measured by absorbancy at 280 mμ. Projected on diagram b (- - -) a run with a human serum albumin preparation (protein standard). Second (top) peak caused by the content of sodium-*o*-ethylmercurithiobenzoate, 0.8 mg %. The gradient (from left to right) of 5 to 20 % sucrose was made up in 0.05 M Tris-HCl buffer pH 8 with 1 M NaCl. Fractions were collected from the bottom by the drop counting method.

was treated similarly (240 mm^2 per 0.03 ml, 95 mm^2 per 0.03 ml 1:10 diluted sample; activity unheated). As shown in Fig. 6 the SK-activator activity (a) was obtained in the region corresponding to higher molecular weight materials (bottom), while the natural compound sedimented slower and near the albumin peak (b).

Comparatively large quantities of protein were required with the natural activator preparation because of difficulties to locate the fibrinolytic activity. The plasminogen activating effect was measured on unheated bovine fibrin plates with 0.06 ml from the drop fractions and incubated for 16 h at 37° (Fig. 6 a). Incubation was continued for 52 h at 25° with the drop fractions in Fig. 6 b. Alternatively activation was obtained after 23 h at 37° and a continued 20 h at 25° with the pooled drop fractions. The large protein peak corresponds to the native human plasma albumin. As shown in Fig. 6 a, a fibrinolytically partly inert protein was separated from the active SK-complex in the gradient run. Similar results were obtained in two subsequent runs with each material. The second peak of activity (Fig. 6 b) was verified by repeating the activity measurements with the ultrafiltrated, pooled drop fractions Nos 5—8 and 9—11, respectively. In an experiment employing 0.25 ml to the gradient run activity was obtained only towards the top region.

Some approximate estimations [30] of the sedimentation rates were made based on the gradient distributions obtained with the respective sieve fractions. As shown in Fig. 6 a the major part of fibrinolytic activity sedimented towards the bottom of the tube, while a major protein component (8.6 S) of fibrinolytically inert material separated from it. Flodin and Killander [28] found by sucrose density gradient ultracentrifugation antibody activities in the two first peaks, 19 S towards the bottom and 7 S near the middle of the tube. Part of the material used for ultracentrifugation to locate the SK-activator contains a considerable portion of material from the second sieve fraction (Fig. 4 b, UF—I), explaining the comparatively large protein peak obtained in the middle (Fig. 6 a), which contains more protein than could be expected by the overlapping of the sieve fractions I and II.[28] The fibrinolytically active material sedimented somewhat slower than 19 S macroglobulin (14 S). Further experimentation is needed for a closer identification, as well as the purification this way of the SK-activator complex, as indicated by the positions of the activity and protein peaks. The increase of both protein and activity in the top layers (Fig. 6 a) may be connected to lipoprotein sedimentation [35] (or may in part be due to an accumulation of bottom material around the syringe and obtained in the top fraction [24]). The peak of SK-activator activity corresponded to about 337 000 molecular weight material.

As shown in Fig. 6 b, the peak of native plasma albumin coincided with the peak of Cohn precipitated human serum albumin (4.6 S). The estimated molecular weight for the fibrinolytically active component in the third sieve fraction (inset Fig. 4 b) as calculated from fractions Nos. 9—10 (Fig. 6 b) was about 106 000 to 81 000 corresponding approximately to the suggested size of plasmin.[34] In this region the maxima of β_1-globulin and an α_2-globulin occur somewhat before that of albumin.[28] This coincides with the region found for the active globulin in present studies, sustaining Müllertz' original findings [4,5] that the euglobulin precipitate contains the plasminogen activator

from spontaneously active human blood, confirmed also by the shorter lysis time obtained with material from the third sieve fraction (Table 2) compared with sieve fractions I and II.

Due to the use of different batches of fibrinogen preparations (see Methods) with slight differences in the plasminogen content, comparison of the lysis times in Table 2 should be made within each series only. The general lytic appearance in all sieve fractions obtained after incubation of *post mortem* plasma with high SK concentration, did also occur with the third sieve fraction of SK-activated normal human plasma.

This general lytic appearance may be connected with unassociated SK obtained in the third and second sieve ranges (Fig. 5), or is a consequence of the plasmin producing effect of the SK-activator.

The complex formation involving large molecules in native plasma may be a primary phase in the mechanism of fibrinolytic activation by SK connected to the SK-antibody reactions. According to Fletcher *et al.*[36] the specific biochemical effect of SK on plasminogen does not occur until varying amounts of circulating antibody has been neutralized. As shown in the present experiments the maximal fibrinolytic effect with SK was always found in the first sieve fraction. This binding of SK to a complex, however, does not exclude a subsequent or simultaneous 1:1 reaction with plasminogen at available active sites.

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