# EFFECT OF ENDOGENOUS ACTIVATION OF FIBRINOLYSIS ON HUMAN PLASMONOGEN

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Plasminogen (PG) is one of the main components of the fibrinolytic system of human blood, which is converted into the active enzyme plasmin on activation of the latter [1]. There is evidence in the literature that changes may take place in PG under the influence of activation induced by streptokinase or urokinase [14, 16]. However, the effect of endogenous activation of fibrinolysis on human PG has been inadequately studied. We know that the strongest activation of the fibrinolytic system is observed in sudden death [3, 5], and for that reason blood plasma from persons who have died suddenly can be used as the source of activated PG.

The aim of this investigation was to study the effect of natural endogenous activation of fibrinolysis on human PG.

## EXPERIMENTAL METHOD

PG was isolated from freshly frozen donor's plasma and fibrinolytically active plasma (FAP) from blood obtained from persons dying suddenly [5], by affinity chromatography [9]: 75 ml of filtered plasma was passed (at the rate of 75 ml/h) through a column (20 × 100 mm) containing 13 ml of CNBr-lysine-sepharose 4B, equilibrated with 0.05 M phosphate buffer, pH 7.4. The sepharose was washed to remove nonspecifically bound protein, first with the starting buffer and later with the same buffer, containing 0.5 M NaCl. PG was eluted with a 0.2 M solution of ε-aminocaproic acid. The optical density ($E_{280}$) and protein concentration [13] were determined in the resulting fractions. PG and plasmin activity were studied by the caseinolytic method and expressed in caseinolytic units (CU) in 1 ml of sample [7]. PG activator activity was determined by the method of lysis of fibrin disks and expressed in millimeters per square [12]. The content of PG + plasmin, of fibrinolysis inhibitors $\alpha_2$-macroglobulin ($\alpha_2$-MG), and $\alpha_1$-antitrypsin ($\alpha_1$-AT) and the fibrin/fibrinogen ratio were investigated immunochemically [8]. The relative molecular weight of PG was determined by gel-filtration on Sephadex G-200 [2]. The donor's plasma was passed initially through Sephadex,

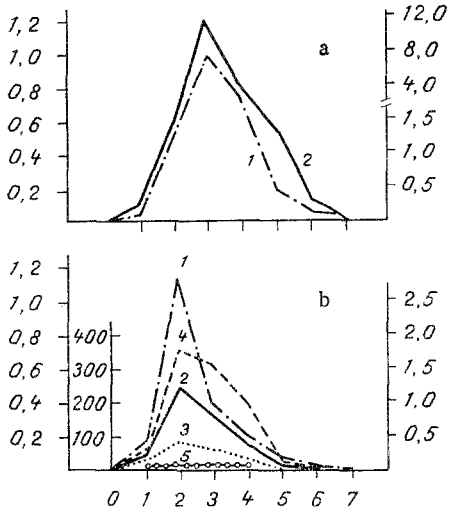


Fig. 1. Analysis of eluates obtained by affinity chromatography of PG from donors' plasma (a) and from FAP (b). Abscissa, No. of fractions; ordinate: on left — $E_{280}$, on right — CU/ml. 1) Protein (in $U_{OD}$/ml); 2) PG (in CU/ml); 3) plasmin (in CU/ml); 4) PG activator (in $mm^2$); 5) $\alpha_2$-MG, $\alpha_1$-AT, fibrin/fibrinogen (immunochemical analysis).

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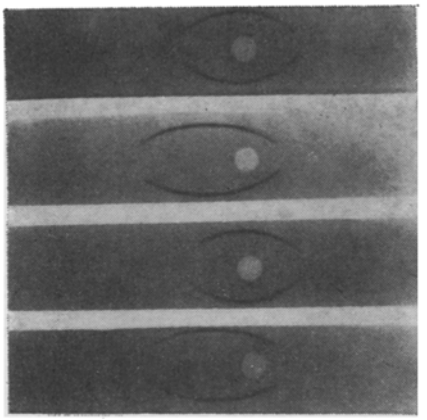


Fig. 2

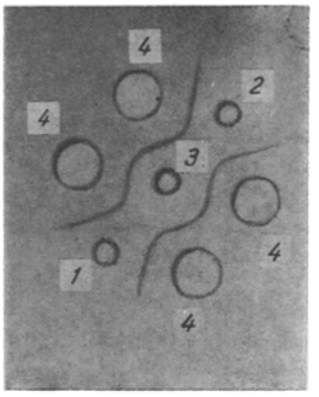


Fig. 3

Fig. 2. Immunoelectrophoretic analysis of PG preparations and antisera to human PG. 1, 3) PG preparation from FAP; 2, 4) PG preparations from donors' plasma. a) Antiserum to human PG from donors' plasma; b) antiserum to human PG from Behringwerke; c) antiserum to human PG from FAP.

Fig. 3. Immunodiffusion analysis of antisera to human PG. 1) Antiserum to human PG from donors' plasma; 2) antiserum to human PG from FAP; 3) antiserum to human PG from Behringwerke; 4) standard human PG antigen.

followed by the PG preparations. Marker proteins were identified in the resulting fractions with the aid of specific antisera (Behringwerke, West Germany): $\alpha_2$-MG (mol. wt. 725 kilodaltons — kD), IgG (150 kD), PG (91 kD), transferrin (80 kD), and antithrombin-III (65 kD). The isoelectric point of PG was determined by the method of isochromatic focusing on a column with cellulose DEAE-52. The pH gradient was created by means of a 2% solution of ampholines. To estimate the immunochemical purity of the PG preparations, immunoelectrophoresis was used [4].

The PG preparations were used to immunize Chinchilla rabbits in order to obtain specific antisera. The immunization cycles consisted of four injections, each of 0.5-1 mg protein, mixed with Freund's complete adjuvant, every 9 days, with alternation of the sites of injection (subcutaneously, intramuscularly, into the popliteal lymph nodes, and subcutaneously). Blood was taken 7 days after the last injection of the preparation. Exhaustion and purification of the antisera were carried out with an immunosorbent prepared on the basis of glutaraldehyde [10] and the PG-free protein fraction obtained after passage of the plasma through lysine-sepharose. The resulting antisera were compared with a commercial antiserum to PG (Behringwerke).

## EXPERIMENTAL RESULTS

The only component of the fibrinolytic system of the blood which was found in the samples of donors' plasma studied was PG, the average concentration of which was 4.0 ± 0.2 CU/ml. The PG level in samples of FAP was reduced by 4-7 times and, besides PG, plasmin (0.15-0.28 CU/ml) and plasminogen activator (area of lysis 144-225 $mm^2$) also were found in them.

By affinity chromatography a protein pool containing PG only was obtained from the donors' plasma (Fig. 1a). The yield of PG as protein was 0.29-0.35%, and as specific activity 90.4-95.7%. The specific caseinolytic activity of PG was on average to 294 times higher than in the original plasma. During affinity chromatography of FAP one protein pool (Fig. 1b), also was obtained; the content of native PG in it was 10-14 times less than in the preparation from donors' plasma. The yield of the PG preparation from FAP, as protein, was reduced to 0.19-0.22%, and the loss of PG/plasmin during the isolation procedure was 51.2-59.0%. Besides PG, plasmin and PG activator also were found in the preparation (Fig. 1b). Inhibitors of fibrinolysis ($\alpha_2$-MG and $\alpha_1$-AT) and also trace amounts of fibrin/fibrinogen were discovered immunochemically in the PG preparation from FAP. The physicochemical properties of the PG preparations also differed. The relative mol. wt. of PG from donors' plasma was 90-95 kD,

and the isoelectric point was 6.61-6.72, whereas the PG preparation from FAP had mol. wt. 100-120 kD and an isoelectric point of 6.96-7.27. Immunoelectrophoresis of the preparations with monospecific antiserum to human PG revealed differences in electrophoretic mobility; the preparation from FAP exhibited more marked cathodal mobility (Fig. 2).

Purified antisera obtained after immunization of rabbits with PG preparations from donors' plasma and FAP were monospecific, and on immunoelectrophoresis with PG preparations they gave a picture similar to that when commercial antiserum was used (Fig. 2). Antisera to PG from FAP were identical to antisera to PG from donors' plasma and antisera to PG from "Behringwerke" (Fig. 3).

These investigations thus showed that the PG preparation from FAP, obtained by affinity chromatography, differs considerably from the PG preparation from donors' plasma. The PG preparation from donors' plasma, so far as its physicochemical properties are concerned, is highly purified Glu-PG (the native form of PG with glutamic acid as the N-terminal amino acid [6]. Many other proteins were found in the PG preparation from FAP, evidence of the complex interactions between the components of the fibrinolytic system in response to natural endogenous activation. Under the influence of activators (plasma or vascular) appearing in the blood stream in the case of sudden death [1], the concentration of native PG in the plasma falls sharply due to conversion into Lys-PG (the activated form of PG with lysine as the N-terminal amino acid) or into plasmin. This can be deduced from a change in the physicochemical properties of PG: a decrease in relative electrophoretic mobility, a shift of the isoelectric point [11]. Lys-PG and plasmin interact with fibrin/fibrinogen and form complexes with fibrinolysis inhibitors [6]. This explains the appearance of components with no biological specificity for lysine-sepharose in the PG preparation from FAP: $\alpha_2$-MG, $\alpha_1$-AT, fibrin/fibrinogen. Binding of activated PG with fibrinolysis inhibitors leads to considerable losses during its isolation from FAP, since the inhibitors come out in the effluent. This multiple interaction of Lys-PG and plasmin with other proteins, and also adsorption on lysine-sepharose are effected through the presence of several lysine-binding sites on the PG/plasmin molecule [6]. This suggests that during PG activation, the functional activity of the lysine-binding sites is preserved.

Manifestation of plasminogen activator activity in the PG preparation from FAP is evidence that it can be adsorbed on lysine-sepharose.

The molecular weight of the activated PG/PM was found to be much higher than that known for Lys-PG and plasmin (81-90 kD). We know that this increase in molecular weight, determined by gel chromatography, may be connected with the conformation of the PG molecule. However, the effect of complex formation by activated PG with other proteins can also be postulated.

The fact that identical monospecific antisera to human PG can be obtained from donors' plasma and FAP is evidence that the native and activated PG and plasmin possess common immunochemical determinants.

These experiments also showed that the method of affinity chromatography on lysine-sepharose can be used to study complex interaction between components of the fibrinolytic system during activation of fibrinolysis.

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