DNA, a Controlling Factor in the Conversion of Plasminogen to Plasmin

Very little information is available concerning the relation of nucleic acids to plasminogen activation. Plasminogen is one of the key zymogens to produce plasmin. Because of plasmin's vital role in blood coagulation, the conversion of plasminogen to plasmin has been studied extensively[1–4].

During the preparation of plasminogen activator from porcine heart, following the procedure of BACHMAN et al.[5], a fast nucleoprotein peak was eluted from the Sephadex G 200–G 100. The peak contained a nucleoprotein which was labile to the action of deoxyribonuclease activity. This nucleic acid, when abtained after deproteinization of the nucleoprotein, inhibited the activation of human plasminogen by the heart plasminogen activator[6].

The present report describes the formation of porcine heart DNA-human plasminogen complex, and the ability of the DNA to inhibit the conversion of human plasminogen to active plasmin.

Materials and methods. Human plasminogen (Merck lot 81607) was purified by gel filtration[4] and used in the present studies. The procine heart plasminogen activator[5] employed showed a single non-symmetrical non-homogeneous peak with a sedimentation constant of $S_{20,w}$ equivalent to 2.7 at protein concentration of 3 mg/ml. Porcine heart DNA was prepared[7] from porcine heart, then treated with trypsin, re-isolated by 3 times extraction with equal volume of water saturated phenol. The aqueous phase was then extracted with ether and the DNA was precipitated by 6 volumes of isopropanol, and dissolved in 0.01 *M Tris* buffer (pH 7.4) containing 0.01 *M* NaCl. The sodium salt of this DNA in 10^{-2} *M* buffer pH 7.4 has E(P) at 260 nm of 6150, corresponding to an absorbance of 18.9% of 1 mg/ml solution.

The conversion of plasminogen to plasmin was followed by the caseinolytic[8], and esterolytic (LMe)[9] activi-

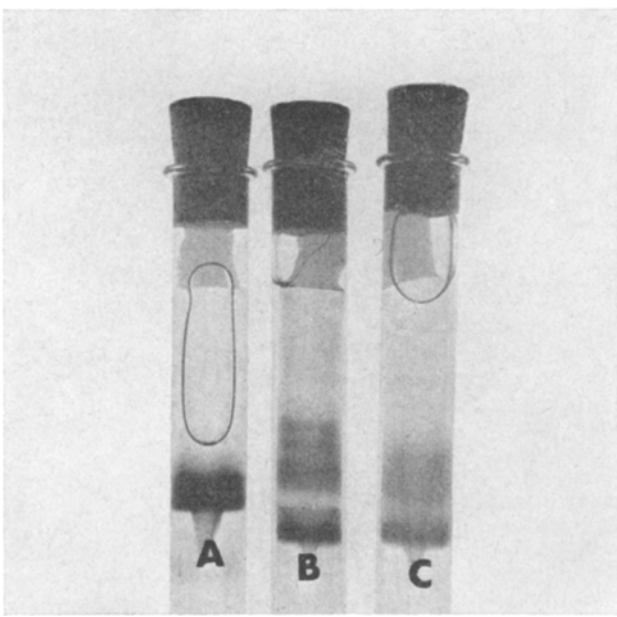

Fig. 1. Disc electrophoretic resolution on polyacrylamide gel containing 0.15 *M* ε-aminocaproic acid, of human plasminogen (A), and in presence of porcine heart plasminogen activator (B). (C) Human plasminogen-porcine heart DNA treated with the plasminogen activator. All these solutions were in 0.01 *M tris*-HCl buffer of pH 6.5, incubated at 25 °C for 30 min.

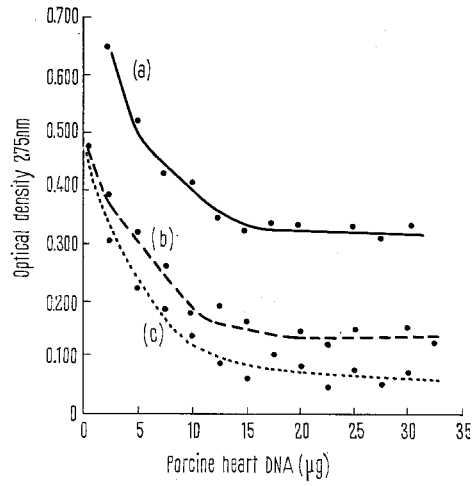

Fig. 2. Inhibition of porcine heart activator-activation of human plasminogen by increasing amounts of porcine heart DNA. Human plasminogen was incubated at pH 6.2 and 25 °C for 30 min first with increasing quantities of the porcine heart DNA, then with 3, 5 and 50 µg of the porcine heart activator per milligram of plasminogen. The solutions were incubated for additional 30 min. The caseinolytic activities[8] of each was then determined.

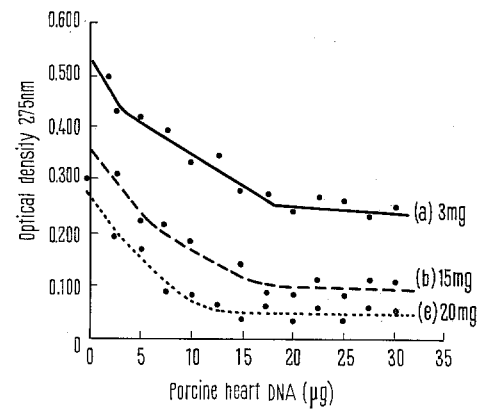

Fig. 3. Inhibition of porcine heart activator-activation of human plasminogen by porcine heart DNA. Human plasminogen was first incubated at pH 6.2 and 25 °C with increasing quantities of the porcine heart DNA, then with 3, 15 and 20 µg of the porcine heart plasminogen activator per milligram of the human plasminogen. Incubation was continued for additional 30 min then the esterolytic (LMe) hydrolytic activity of each solution was determined[9].

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ties. Disc electrophoretic resolution was carried out on columns of polyacrylamide gel[10] containing 0.15 *M* e-aminocaproic acid.

Results and discussion. The electrophoretic profile in Figure 1 shows that purified human plasminogen (1 mg/ml) at pH 6.5, and after incubation at 25 °C for 30 min migrated as a single polydisperse major zone (profile A). When incubated with porcine heart plasminogen activator (0.01 mg/ml) at pH 6.5, the human plasminogen migrated in at least 4 zones (profile B). When porcine heart DNA-human plasminogen complex was incubated with porcine heart plasminogen activator, the mixture migrated in at least 2 zones. Profile C of Figure 1, shows also reduced number of migrating zones, indicating limited activator activity. Under these experimental conditions, with the quantities used, the porcine heart activator was not detected on the polyacrylamide gel.

Progressive decrease in the release of caseinolytic activity is seen in Figure 2, curve a, where 3 μg of porcine heart plasminogen activator was added to incubation solutions of human plasminogen and increasing amounts of porcine heart DNA. Stronger inhibition of the release of caseinolytic activity is shown by curves b and c, where 5 μg and 30 μg of the plasminogen activator were added.

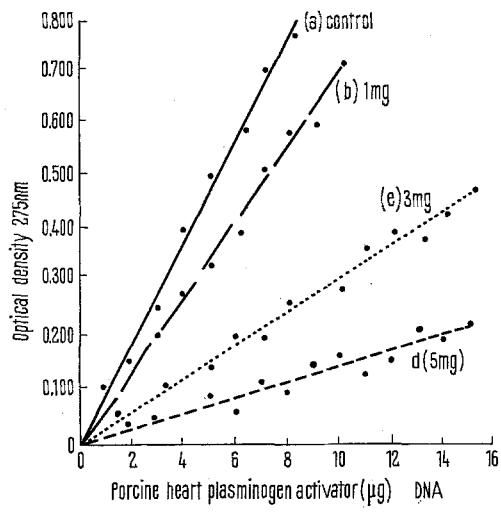

Fig. 4. Inhibition of porcine heart activator-activation of human plasminogen by porcine heart DNA. Human plasminogen was first incubated with 1, 3, and 5 μg of porcine heart DNA to 1 mg of human plasminogen, at pH 6.2 and 25 °C for 30 min. Increasing quantities of the porcine heart plasminogen activator was then added. Incubation was continued for additional 30 min. The esterolytic (LMe) hydrolytic activity of each was finally determined[9].

The inhibitory effect of increasing amounts of porcine heart DNA on the catalytic release of esterolytic (LMe) activity from human plasminogen is shown in Figure 3. Progressive inhibition of plasminogen activation followed by the assay of the activated enzyme, plasmin activity released from the action of 3, 15 and 20 μg of porcine heart plasminogen activator indicate possible complex DNA-plasminogen formation. The esterolytic activity of plasminogen-activator-activated human plasminogen in 30 min incubated solutions is a function of the concentration of the activator. However, the esterolytic activity decreases by the addition to the plasminogen solution of porcine heart DNA. The degree of inhibition depends on the ratio of plasminogen to DNA. The inhibitory effect of DNA on the release of esterolytic activity from human plasminogen is shown in Figure 4.

The results described could be interpreted as follows: Human plasminogen combined with porcine heart DNA forming a complex. The formation of this new class of DNA complexes was first suggested by the work of HOFSTEE[11] in which he described the ability of chymotrypsinogen to form soluble stoichimetric complexes with DNA. The present preliminary observations demonstrated the formation of plasminogen-DNA complexes, and suggested the ability of the DNA to protect the zymogen from the action of a tissue plasminogen activator. Studies are in progress aimed at the ability of the DNA in the complex to protect the zymogen from the action of various plasminogen activators.

Résumé. Les résultats de ces études démontrent la formation de «complexes» entre l'acide désoxyribonucléique et la plasminogène. L'acide désoxyribonucléique inhibe l'action catalytique de la plasminogène humaine sur la plasmine.

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Fibrinolytic System, Fibrinogen Degradation Products and Soluble Fibrin Monomer Complexes of Renal Venous and Aortal Blood

Renal venous blood obtained from rabbit, dog or man during surgery by direct puncture under general anaesthesia shows an elevated fibrinolytic activity[1–3]. High values of fibrinogen degradation products (FDP) were found[3] in dog renal blood obtained by venous puncture. These experiments suggested an enhanced continuous fibrinolysis originating in renal vessels. A continuous secretion of plasminogen activator into the systemic circulation was postulated.

Recently several investigators found that renal venous blood obtained by selective catheterisation had a slightly higher fibrinolytic activity than arterial and peripheral venous blood[4,5]. The comparison of these literature data indicates that renal venous blood obtained by direct puncture always showed much higher fibrinolytic activity than the renal venous blood obtained by catheterization.

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