NOTE

Isolation of Feline Plasma Plasminogen

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ABSTRACT. Plasminogen (Pmg), the precursur protein of proteolytic enzyme plasmin was isolated from cat plasma according with the modification of Deutsch and Mertz's method. Purified protein had approximately 96,000 dalton at SDS-PAGE and PI was 6.0 and 6.5. Pmg values in plasma collected from 28 individuals of male and female adult cats were measured using anti feline Pmg rabbit serum. In this experiment, 0.34±0.07 g/l (mean ± standard deviation) (max. 0.67-min. 0.24) were obtained.——KEY WORDS: feline; plasminogen.

Plasminogen is the precursur protein of the proteolytic enzyme plasmin which is a dissolvent of fibrin clot in blood. Recently, the function of activating complement has been reported of this protein plasmin [9, 12]. In case of disseminated intravascular coagulation (DIC), measuring the plasminogen is also an important test in human.

Although the protein has been purified from humans [2, 10], rabbits [3, 4, 6, 10], dogs [7, 10], sheep [8], ducks [3], bovine [10] and cats plasma [3, 10] by different methods, purification method and the characteristic of feline plasminogen has been hitherto unavailable. In this report, the authors have described our isolation procedure of plasminogen from feline plasma by affinity chromatography, gel filtration and preparative zone electrophoresis. Furthermore, the normal concentration of the protein in plasma has been measured under the condition of using antiseraum. The procedure is a modification of Deutsch and Mertz's method [5].

The experiments in the present study were carried out with healthy young adult cat blood that collected into anticoagulant composing of 0.8 g citric acid, 2.2 g sodium citrate and 2.45 g glucose in 100 ml of distilled water (6 volumes of blood: 1 volume of anticoagulant). After centrifugation at 1,500 G for 15 minutes, the plasma was decanted. And then, 200 ml of plasma was diluted by 170 ml of 0.1 M sodium phosphate buffer (PB) at pH 7.4 containing 0.003 M ethylenediamine tetraacetic acid disodium (EDTA-2Na) and aprotinin (20 KIU/ml), and was applied directly to a column of lysine-Sepharose 4B (1.5 by 35 cm, Pharmacia Fine Chemicals AB) pre-washed with the buffer at the flow rate of 42 ml per hour. The column was then washed with the previous solution until the absorbance of the eluent at 280 nm at the same flow rate was fallowed below 0.025. At this point, the bound protein to the gel was eluted with 0.2 M e-aminocaproic acid (EACA) containing 0.003 M EDTA-2Na at pH 7.4 at the flow rate of 18 ml per hour. Fractions of approximately 5 ml were collected and read for absorption at 280 nm in a spectrophotometer. This profile obtained with the elution of plasminogen bounding to lysine-Sepharose 4B column is shown in Fig. 1. These fractions containing plasminogen were determined by immunoelectrophoresis (IEP) against anti feline whole plasma from rabbit and 7.5% polyacrylamide gel electrophoresis.
(PAGE), and were pooled. In electropherogram, two precipitates were observed; probably one of which was plasminogen and another was immunoglobulin G (IgG). A similar result was gained by 7.5% PAGE. With a quantitative immunochemical determination of IgG, 0.002 g IgG/l (about 3% of the preparation) was demonstrated.

The EACA was removed from the elutes by gel filtration on Sephadex G-25 column (PD-10, Pharmacia) with 0.1 M phosphate buffer saline (PBS) at pH 7.4.

The sample was concentrated to 7 ml by ultrafiltration through a PM-10 membrane (Diaflo apparatus; Amicon Corporation), loaded to Sephacryl S-200 column (2.6 by 90 cm, Pharmacia), and developed with the same buffer at 15 ml per hour flow rate. The fraction collected was approximately 5 ml. The elution pattern obtained S-200 chromatography is shown in Fig. 2. The step served to separate a small impurity on the frontal and a moderate impurities on the rear side of the main plasminogen fraction. This rear sidal peak was probably a modified plasminogen reported by Sodetz et al. [11].

The fractions containing the highest concentration of plasminogen from 53 to 57 were selected, concentrated to 7 ml by ultrafiltration finally, and dialyzed against veronal-veronal-Na buffer; pH 8.6. \(\mu=0.05\). The concentrated solution was next applied to a preparative zone electrophoresis of Pevicon bufferized with the same veronal buffer (21 by 21.5 by 0.5 cm).

This electrophoresis was carried out following the horizontal technique using a continuous buffer system. The migration was pursued for 12 hours with a voltage gradient of about 20 V/cm.

After migration, the block was divided out at 5 mm intervals in a pararell direction of electrophoresis. The protein was extracted from the slice with 0.1 M PBS at pH 7.4, and the preparations containing plasminogen were dialyzed against 0.05 M Tris (Tris (hydroxymethyl) aminomethane)-HCl-0.012 M NaCl solution at pH 7.4, prior to future use. All the steps in the operation were performed at a cold temperature (5°C).
Finally, the isolated material was examined by 7.5% PAGE and 4 to 20% gradient PAGE, and molecular weight estimations were performed on 12.5% gel in sodium dodecyl sulfate (SDS, Laemmli method) using molecular weight markers. Feline plasminogen had three major and two minor bands in PAGE, and molecular weights determined approximately 96,000 at SDS-PAGE. This molecular weight was larger than of human (93,000) or rabbit cases (87,000–88,000) [11]. Profiles of these experiments are shown in Fig. 3.

The PI of this protein was 6.0 and 6.5 by isoelectric focusing on polyacrylamide gel using PI markers. Furthermore, the specific activity of isolated protein had 7.8 unit per mg assayed by Abiko et al. [1] using streptokinase, casein and N-Tosyl-L-Arginine methyl estel-HCl (TAME).
The New Zealand-White rabbit was immunized by the protein in emulsion using Freund's complete adjuvant (protein 3.4 mg/ml), subcutaneously. After three weeks, a single precipitate was observed on IEP, in Fig. 4.

Fresh plasma were collected from 28 individuals of male and female adult cats, and plasminogen values were measured using the rabbit anti-feline plasminogen serum by Laurell's electroimmunoassay in Fig. 5. This procedure was discharged under the condition of 1% agarose gel with 0.075 M barbital buffer at pH 8.5. An uniform field strength of 4 V/cm was applied for 12 hours at 10°C.

In this experiment, 0.34±0.07 g/l (mean±standard deviation) (max. 0.67−min. 0.24) [in male 0.33±0.08 g/l (max. 0.67−min. 0.25) and in female 0.34±0.07 g/l (max. 0.52−min. 0.24)] were obtained.

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REFERENCES

要 約
ネコ血漿プラスミノゲンの分離と血漿中含有量について（短報）: 古川敏紀・杉山文博1（筑波大学基礎医学系，
1動物実験センター）——線溶系蛋白プラスミンの前駆体であるプラスミノゲン（Pmg）をネコ血漿から、Deutsch
& Mertz 法に準じ、分離した。ネコ Pmg は分子量 96,000（SDS-PAGE）を有し、数本のバンド（4〜20% gra-
dient-PAGE）を確認し得た。精製物をウサギに免疫して得た抗体を用いて、レケット免疫電気泳動により、
ネコ成体56頭（雄 3.0 kg, 雌 2.5 kg 以上）の血漿 Pmg 量を測定し、最大 0.67 g/l, 最小 0.24 g/l, 0.34±
0.07 g/l（平均±標準偏差）を得た。