Isolation of Feline Plasma Hemopexin

Toshinori FURUKAWA, and Fumihiro SUGIYAMA

Institute of Basic Medical Sciences, and Laboratory Animal Research Center, University of Tsukuba. 1-1-1 Tennodai, Sakura-Mura, Nishari-Gun, Ibaraki, 305, Japan

(Received 13 February 1984/ Accepted 16 July 1984)

ABSTRACT. Feline hemopexin (Hx) was isolated from serum with the procedure on the modification of Vretblad et al. Purified Hx had 71,000 and 74,000 dalton at 4–30% PAGE, and the solution immunized to rabbit foot pad. After 3 weeks, only one precipitate was observed between feline plasma and antiserum with IEP and Ouchthalony method. Hx level in 51 healthy adult cat plasma was 1.11 ± 0.27 g/l (mean ± standard deviation) by RIE. — KEY WORDS: feline, hemopexin.

Hemopexin (Hx), synthetized in the liver, is a glycoprotein of β fraction on electrophoresis of forming an equimolar complex with heme or other porphyrins and that is a member of the class of plasma proteins with specific transport functions. On human articular rheumatism, infections, cancer and diabetes, the Hx level increase in plasma, whereas hemolytic anemia decrease its level. When hemolytic anemia occurs, Hx seems to be important similar to haptoglobin, functionally. Determination of plasma Hx might therefore be a valuable aid in judging the severity of hemolytic disorders, since the Hx level is a reliable indicator of plasma heme accumulation [1]. The present paper describes first the purification procedure of feline Hx with the modification of Vretblad et al. [2] and secondly the value of 51 feline serum Hx by Rockelet electroimmunoassay (RIE). Although the Hx concentration is readily determined immunochemically by the single radial immunodiffusion method (SRID), we used Rocket method because RIE was more sensitive than SRID method.

One hundred ml serum was adjusted to pH 8.0 with 1 M NaOH, and mixed with 43 ml of 50% polyethylene glycol (PEG 4000) solution. After stirring for 1 hr, the precipitate was removed by centrifugation at 3,000 rpm for 15 min.

The supernatant was adjusted to pH 4.0 with 1 M HCl, added 14.6 g solid PEG 4000 (final concentration of PEG was 25%) and stirred for 1 hr. After centrifugation at 3,000 rpm for 15 min, the solution was decanted and the precipitate was dissolved in distilled water, and the pH was adjusted to 7.5 with 1 M NaOH. These procedures were carried out at 4°C. The pH was furthermore adjusted to 6.0 by 1 M HCl, the solution was centrifuged at 5,000 rpm for 10 min and the supernatant was dialyzed against 0.05 M sodium acetate buffer at pH 5.2 and loaded to a DEAE-Sepharose CL-6B column (2.5 by 30.6 cm) equilibrated with above buffer at the flow rate of 85 ml per hr. Fractions of approximately 5 ml each were collected. The fractions from No. 26 to No. 36 were pooled (this elution profile is shown in Fig. 1) and then the pH was adjusted to 7.2 with saturated Na2HPO4 solution.

This solution containing discharging protein was applied to wheat germ lectin-Sepharose 6MB affinity column (1.5 by 7 cm) equilibrated with 0.05 M sodium
Fig. 1. Elution profile of 15–25% PEG precipitate on a DEAE-Sepharose CL-6B column.

The column (2.5 by 30.6 cm) was equilibrated with 0.05 M sodium acetate buffer at pH 5.2 at the flow rate of 85 ml/hr. The precipitate from 15 to 25% polyethylene glycol 4000 was dialyzed against the above buffer. After application, the column was washed with the same buffer at the same flow rate and collected discharging protein (tube number from 26 to 36).

Fig. 2. Wheat germ lectin-Sepharose 6MB affinity chromatogram.

The sample containing hemopexin was loaded to the affinity column (1.5 by 7 cm) equilibrated with 0.05 M sodium phosphate buffer at pH 7.0 containing 0.2 M NaCl and 0.02% (W/V) NaN₃ at room temperature. Elution profile was measured by the absorbance at 280 nm. The absorbed protein was developed with the buffer containing N-acetyl-D-glucosamine (100 mg/ml) at the flow rate of 30 ml/hr.

Fig. 3. Anion exchange chromatographic pattern on Mono-Q column.

The column of Mono-Q (FPLC system, Pharmacia) was washed and equilibrated with 0.05 M Tris-HCl buffer (pH 8.6) at the flow rate of 0.5 ml/min. The sample was applied and eluted with a gradient between the equilibration buffer and 0.5 M Tris-HCl buffer at pH 4.0. The second to third peak contained hemopexin.

phosphate buffer containing 0.2 M NaCl and 0.02% (W/V) NaN₃ at pH 7.0. Once the sample was loaded, the column was washed with the buffer solution at a flow rate of 30 ml/hr. The solution containing Hx was then eluted with 20 ml of the buffer containing 100 mg N-acetyl-D-glucosamine per ml. The elution pattern is shown in Fig. 2. All the eluates from this column were pooled, and checked with immunoelectrophoresis (IEP) and 7.5% polyacrylamide gel electrophoresis (PAGE). These results revealed that the transferrin was contaminated.

The sample was then dialyzed against 0.05 M Tris-HCl buffer at pH 8.6, and loaded onto an anion exchange chromatocolumn (Mono-Q, HR5/5) by FPLC system (Pharmacia Fine Chemicals AB) equilibrated with the same buffer, then developed with a gradient between 0.05 M Tris-HCl buffer at pH 8.6 and 0.5 M Tris-HCl buffer at pH 4.0. The flow rate was maintained at 1 ml per min, 0.5 ml fraction in each tube were collected, and the absorbance at 280 nm was monitored. The elution profile revealed resolution of four major peaks (Fig. 3). The first peak, frac-
Fig. 4. Electrophoretogram of feline hemopexin.

a) 4–30% gradient polyacrylamide gel electrophoretogram.

Two major bands which reveal 71000 and 74000 dalton respectively were observed.

b) Immunoelectrophoretogram of feline plasma against anti feline hemopexin.

After electrophoresis of 1 μl feline plasma, only one line was observed against anti feline hemopexin after placing in a moistened chamber for 24 hr at room temperature.

tions from No. 10 to No. 20, contained transferrin and the second to third peak Hx.

The second and the third peaks had 71,000 and 74,000 daltons by 4–30% gradient PAGE (Fig. 4) using MWMs, respectively. Each sample was observed to have the same band with 4–20% gradient and 7.5% regular PAGE. The solutions were concentrated to 66 μg per ml, emulsified with the same volume of Freund’s complete adjuvant and immunized 1 ml to rabbits foot pad. After 3 weeks, the boosters were given. Only one precipitate was observed with IEP (Fig. 4b) and Ouchtalony methods.
Hemopexin values in 51 feline plasma were measured by RIE using anti feline Hx serum (Fig. 5). RIE was performed with 1% agarose containing 1% antiserum in 0.01 M sodium barbital buffer at pH 8.6 at 7 mS of specific conductivity. Blood was collected from the jugular vein and put in EDTA-2Na. These plasma were carbbamilated by 2 M potassium cyanate, and diluted with gel buffer to 16, 64 and 256. Five μl of the buffer-diluted samples were applied in the wells. Electrophoresis was continued with a voltage of 300 V constantly on the power supply corresponding to 8 to 10 V/cm in the gel for 4 hr at 10°C. After electrophoresis, the gel was washed in phosphate buffer saline for periods of at least 72 hr with stirring, and stained by Coomassie brilliant blue G-250.

Feline Hx level in plasma was 1.11±0.27 g/l (mean±standard deviation). Specifically, 1.16±0.29 g/l, 1.06±0.24 g/l were gained from 26 males and 25 females, respectively.

ACKNOWLEDGEMENTS. The authors should like to thank Prof. Yasuoka for kindly reading the manuscript and for his useful suggestions. A part of this investigation was supported by grant No. 57771558 from the Scientific Research Fund of the Japanese Ministry of Education, Science and Culture.

REFERENCES