Journal of Chromatography, 137 (1977) 471–474

CHROM. 9975

Note

Purification of α-fetoprotein from rat amniotic fluid by gel filtration

CARLOS LAMAR, Jr. and J. ROBERT CANTEY

Veterans Administration Hospital, and Department of Medicine, Medical University of South Carolina, Charleston, S.C. 29401 (U.S.A)

(First received January 31th, 1977; revised manuscript received March 4th, 1977)

Rat α-fetoprotein (AFP) and albumin are present in amniotic fluid but it has been difficult to separate them because of similarities in their size. Partial purification of AFP has been achieved by the use of block electrophoresis[1], iso-electric focusing[2], and immunoabsorption[3]. Recently, several workers[4,5] have reported satisfactory purification by absorbing the α-fetoprotein on an estradiol–Sepharose column. Absolute purification has required a combination of two or more of the available techniques. This report describes a straight-forward method of obtaining pure rat α-fetoprotein from amniotic fluid requiring only one chromatographic column. Our procedure has proved successful after several of the methods mentioned above had been used and yielded disappointing results. The procedure described in this report is useful for isolating small quantities of AFP sufficient for certain studies and to raise specific antibodies. However, the yield is low and the method is therefore not applicable when large quantities are required.

EXPERIMENTAL

Glass columns (150 × 2.5 cm) were obtained from Glenco. These were filled with Ultragel AcA-44 (LKB), which is a preswollen gel with a polyacrylamide and agarose gel matrix that separates molecules according to size and permits high resolution because of its rigid uniform beads that do not pack under pressure. AcA-44 separates species with molecular weights of 12,000–130,000 and is used at room temperature. The effluent buffer of 0.01 *M* phosphate (pH 7.4) was pumped upward through the column at the rate of 0.36 ml/min by a Buchler Peristaltic Pump.

Electrophoresis was performed for 16 h at 125 mA in a Pharmacia apparatus GE-4 using a gradient gel slab in which the concentration of polyacrylamide was 4–30%. The buffer used was tris-boric acid at pH 8.35 and Coomassie Blue was used for staining the slab. Destaining of the slab with 7% acetic acid was accomplished in a Pharmacia GD-4 apparatus.

Ouchterlony plates for double immunodiffusion were prepared on glass slides using 1% agarose in borate saline (pH 8.4).

Rabbit anti-rat transferrin, γ-globulin, and albumin were obtained from Cappel Laboratories. Rat α-fetoprotein and goat anti-rat α-fetoprotein used in the initial studies were generously supplied by Dr. Samuel Sell (San Diego, Calif., U.S.A.).

Protein solutions were concentrated by applying a positive pressure at 4° in an Amicon stirred cell fitted with a PM30 membrane. Proteins were determined by the method of Lowry *et al.*[6].

Three rats between the 12th and 17th day of gestation were anesthetized and the uterus was removed *in toto*, washed and stored overnight in distilled water in the refrigerator. 20 Fetuses with intact amniotic sacs were removed and approximately 0.5 ml of amniotic fluid was withdrawn from each by using a 26-gauge needle. The amniotic fluid was pooled and after centrifugation and without concentration, 5 ml of the amniotic fluid with a total protein content of 2.4 mg/ml was placed on a 150-cm Ultragel column (a 100-cm column can be used with similar results) and 6-ml portions of the effluent were collected.

RESULTS

Fig. 1 is a representation of a typical gel filtration run. Small quantities of γ-globulin appear in the void volume of 324 ml and α-fetoprotein, albumin and transferrin appear in 456–480 ml of effluent volume. Each individual 6-ml fraction comprising this main peak was assessed separately for the presence of albumin, transferrin and α-fetoprotein by gradient gel electrophoresis (Fig. 2) and double immunodiffusion on Ouchterlony plates (Fig. 3). As can be noted in Fig. 3, the top well, which contained the first portion of the peak, contained α-fetoprotein (total protein approximately 150–350 μg) but no albumin or transferrin.

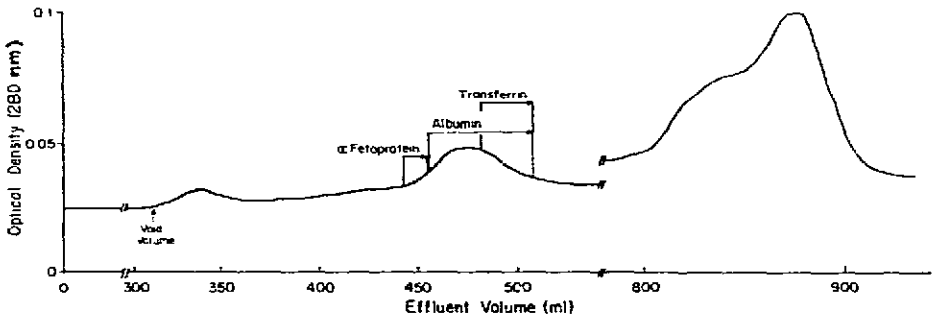

Fig. 1. Elution pattern of rat amniotic fluid on a 150 × 2.5 cm Ultragel AcA-44 column with 0.01 *M* phosphate buffer (pH 7.4). Portions of 6 ml were collected. The void volume was 324 ml. The small initial peak (eluted between 330 and 350 ml) represents globulin. α-fetoprotein, albumin and transferrin constituted different portions of the second, main, peak (see text for explanation). The final large peaks between 800 and 900 ml elution volume were not identified.

After five similar separations of additional aliquots of amniotic fluid, the initial portions of each peak from each run were pooled and concentrated to a volume of 5 ml. The total protein of this solution was 1.7 mg. This material was subjected to chromatography on the same Ultragel column and the initial portion (6 ml) of the peak was collected. The yield of α-fetoprotein was approximately 800 μg or approximately 7% of the original 12 mg.

Approximately 300 μg of pure α-fetoprotein in 2 ml of 0.05 *M* phosphate buf-

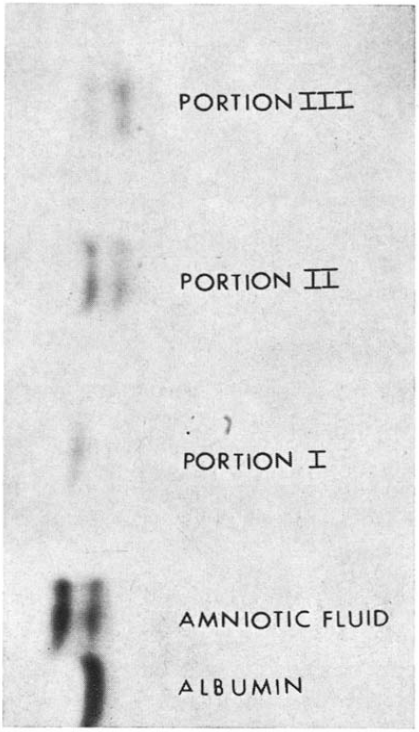

Fig. 2. Polyacrylamide gradient (4–30%) gel electrophoresis of albumin, amniotic fluid, and different portions of the effluent peak described in Fig. 1. Portion I contains α-fetoprotein, portion II contains nearly equal concentrations of albumin and α-fetoprotein, and portion III contains albumin and a small amount of α-fetoprotein.

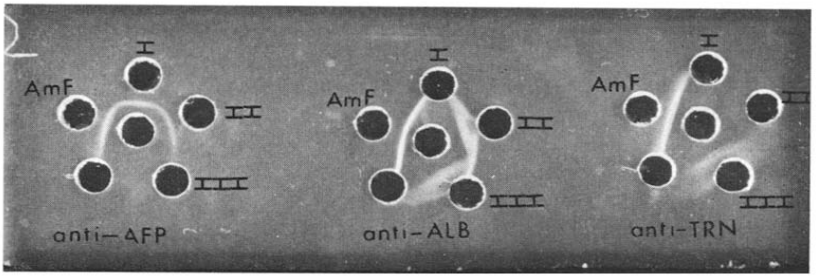

Fig. 3. Double immunodiffusion on Ouchterlony plates of rat amniotic fluid (protein, 2.4 mg/ml) (AmF), portion I contains 6 ml (protein, 0.2 mg/ml); portion II contains 18 ml (protein, 0.4 mg/ml); and portion III contains 18 ml (protein, 0.5 mg/ml) of the effluent peak described in Fig. 1. The center well of the first set contained anti-α-fetoprotein, the second set contained anti-albumin, and the third set contained anti-transferrin antibodies.

fer pH 7.4 was mixed with an equal volume of Freund's adjuvant and injected intramuscularly into a guinea pig on two occasions 10 days apart. On the 16th day following the first injection, 5 ml of blood were removed from the guinea pig by heart puncture and the serum was assessed for antibodies to albumin and α-fetoprotein. As can be seen in Fig. 4, only antibodies to α-fetoprotein were present.

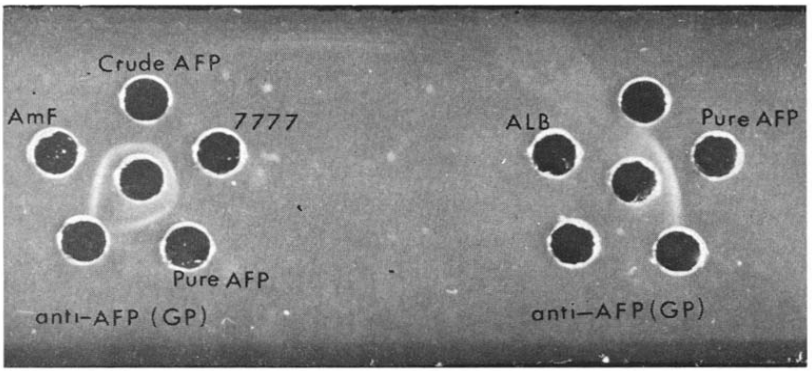

Fig. 4. Double immunodiffusion of the guinea pig anti-α-fetoprotein serum and several proteins on Ouchterlony plates. The center wells contain the anti-α-fetoprotein serum from a guinea pig injected with purified AFP prepared as described in the text. The side wells of the first set contain amniotic fluid (protein, 2.4 mg/ml) (AmF), partially purified α-fetoprotein (protein, 23.7 mg/ml) (crude AFP), serum from a rat bearing a Morris hepatoma 7777 (protein, 70 mg/ml) (7777), and purified α-fetoprotein (protein, 0.26 mg/ml) (pure AFP). The side wells of the second set contained albumin 5 mg/ml, and pure α-fetoprotein 0.26 mg/ml.

DISCUSSION

It has been demonstrated that α-fetoprotein can be effectively separated from contaminating proteins by Ultragel AcA 44 chromatography when individual components of the resulting elution "peak" are taken and studied separately. A second chromatographic separation of several α-fetoprotein fractions yielded a product that was pure as determined by gradient gel electrophoresis, double immunodiffusion, and its ability to elicit monospecific antisera in guinea pigs. Although small quantities of AFP are obtained, sufficient quantities are easily purified to raise antibodies in animals and for electrophoretic and immunological studies. The procedure is simple enough to allow continuous on-going purification if milligram quantities of AFP are needed.

REFERENCES

1 J. A. W. Kirsch, R. W. Wise and I. T. Oliver, *Biochem. J.*, 102 (1967) 763.
2 S. Sell, I. Jalowayski, C. Bellone and H. T. Wepsic, *Cancer Res.*, 32 (1972) 1184.
3 S. Nishi and H. Hirai, *Biochim. Biophys. Acta*, 278 (1972) 293.
4 R. Arnon, E. Teicher, M. Bustin and M. Sela, *FEBS Lett.*, 32 (no. 2) (1973) 335.
5 J. Uriel, D. Bouillon and M. Dupiers, *FEBS Lett.*, 53 (no. 3) (1975) 305.
6 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.