# AFFINITY CHROMATOGRAPHY PURIFICATION OF RAT $\alpha_1$-FOETOPROTEIN

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## 1. Introduction

During the last ten years, the foetal and carcinoma antigens, particularly the $\alpha_1$-foetoprotein (AFP) have been much studied [1].

The hypothesis of the involvement of the $\alpha_1$-foetoprotein in cellular differentiation, during foetal or tumor development, has been the starting point of many researches in immunology and oncology [2, 3].

One of the necessary steps in these studies is the preparation of ponderal amounts of native AFP. Several purification methods have already been described [4–6].

We present here a simplified method of preparation of rat AFP by affinity chromatography.

## 2. Material and methods

### 2.1. *Animals*

19 ± 2 Day-old rat embryos, Charles Rivers C.D. strain, were collected and washed in physiological saline. The blood was obtained from mixed male and female embryo pools, after decapitation and cardiac puncture. The serum prepared after retraction and centrifugation was stored at −20°C.

### 2.2. *Antisera*

They were prepared in our laboratory as already described [7]. The 'foetal' antibodies, directed against the $\alpha_1$-foetoprotein, the $\alpha_2$M and the lipoprotein esterase, were prepared by applying a total anti-embryo immunoserum to a Sepharose-4B column coupled with adult rat serum. The method is the same as that which will be described for the purification of AFP.

An antiserum rich in antibodies directed against $\alpha_2$M, obtained from rabbits immunized with serum from adult rats presenting an inflammatory reaction, after a turpentine subcutaneous injection, was a kind gift from R. Engler.

The protein assay was performed according to the method of Lowry and al. [8].

### 2.3. *Polyacrylamide gel electrophoresis and immunoelectrophoresis* were performed as already described [7], and stained with Amido Black.

### 2.4. *Immunodiffusion experiments* were done by Ouchterlony's method.

### 2.5. *Purification method*

#### 2.5.1. Principle

In order to purify ponderal amounts of AFP, we have prepared a column with Sepharose-4B coupled with a rabbit antiserum raised against normal adult rat serum. The passage through this gel of an embryo serum should leave all adult antigens fixed on the coupled Sepharose, while the specific foetal protein(s) should flow freely from the column.

#### 2.5.2. Detailed procedure

It involved four steps:

(i) *Preparation of the anti-adult rat serum γ globulin fraction*

25 ml of antiserum diluted 10 times in distilled water were precipitated with 40% ammonium sulfate. The precipitate was washed 3 times with 40% ammonium sulfate and dissolved again in 30 ml dis-

tilled water. This solution was dialysed 48 hr at 4°C against a 0.1 M borate buffer, pH 8.6, containing 0.5 M NaCl (Buffer T).

(ii) *Preparation of the Sepharose-γ globulin column*

15 g of CNBr-Sepharose-4B (Pharmacia) were suspended in 100 ml of distilled water. After swelling (16 min) the gel was washed during 20 min on a Buchner filter with 3 l of a HCl $10^{-3}$ M solution. It was then washed once with 100 ml of borate buffer T and incubated 12 hr with the γ globulin solution.

When this coupling step was achieved, the gel was applied to a Whatman column (1.5 cm diameter, 40 cm height), and washed successively with 100 ml 0.1 M borate buffer with 1 M ethanolamine (pH 10), 100 ml acetate buffer containing 1 M NaCl, and finally 100 ml 0.1 M borate buffer pH 8.6 containing 1 M NaCl.

(iii) *Preparation of the total foetal fraction* (TF Fraction)

Whole foetal serum was applied to a carboxy-methyl-cellulose column (Whatman CM 52), and eluted with 0.01 M phosphate buffer pH 6, in order to remove hemoglobin. The hemoglobin was retained, while the serum proteins with an isoelectric pH lower than 6 were rapidly eluted. This eluate constituted the 'TF' fraction.

(iv) *Affinity chromatography step*

The 'TF' fraction is applied on the Sepharose-γ globulin column and eluted with the borate buffer T.

The flow was controlled by a polystaltic BUCHLER pump (20 ml/hr). After elution of the foetal antigen(s), the adult antigens fixed on the column are removed with the aid of 0.1 M acetate–3 M NaSCN buffer pH 4.

The column is regenerated and used once more.

## 3. Results

Fig. 1 shows the elution pattern of the column; a single symmetrical protein peak is seen. The corresponding eluate has a concentration of 15 mg/28 ml which represents a yield of 34%.

The protein preparation is analysed by electrophoretic, immunodiffusion and immunoelectrophoretic techniques.

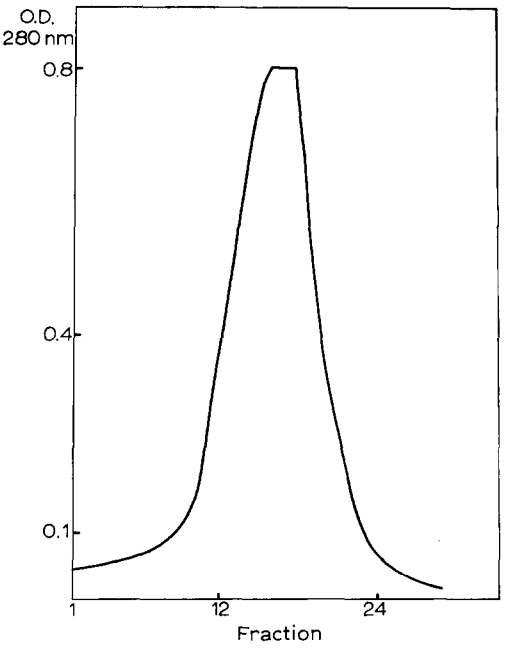


Fig. 1. Elution by a 0.1 M borate buffer pH 8.6, 0.5 M NaCl of a serum fraction (obtained after hemoglobin removal) applied to a Sepharose anti-adult γ globulin column.

The electrophoresis (fig. 2) in 12% polyacrylamide gels shows that the purified AFP is composed of 2 bands of different staining intensities; their relative percentage is established by registering at 610 nm the optical density of the electrophoregrams stained with amido-black on a Gilford gel scanner.

The cathodic fraction represents 67%, while the anodic fraction is 33% of the purified protein.

Specific staining techniques demonstrate the absence of transferrin [9] and hemoglobin [10] contamination.

Electrophoresis in 7% polyacrylamide gels containing only 1% of bis-acrylamide, performed in conditions that allow migration of high molecular weight protein molecules, shows that there is no contamination with high molecular weight proteins like $\alpha_2$M and β lipoprotein.

In some of our preparations, we have found a more cathodic protein (10%) similar to that which has already been described in several cases [7–11].

The immunodiffusion of the purified protein against several anti rat immune sera has demonstrated the absence of other contaminants: indeed,

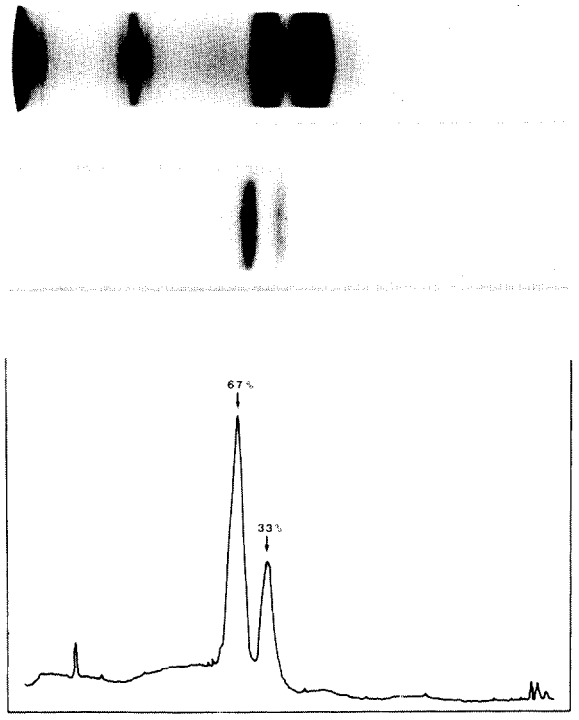


Fig. 2. Polyacrylamide (12%) gel electrophoresis at 4°C of the purified AFP, compared to the total rat embryo serum. The relative percentage of the two stained bands is established by registering the optical density at 610 nm.

the immunodiffusion experiments of the purified preparation against anti-albumin, anti whole adult serum, anti-inflammatory adult serum (rich in $\alpha_2$M) have been entirely negative.

The fig. 3 shows an immunoelectrophoresis of the purified protein comparatively with the whole embryo serum. A single precipitation arc is seen in the case

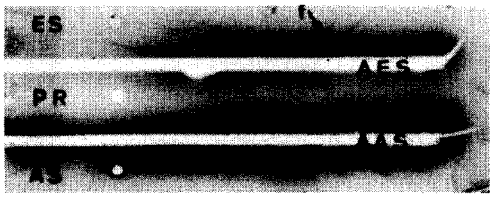


Fig. 3. Immunoproteinogram of the purified protein (PR) obtained after diffusion of an anti rat embryo immune serum (AES) and an anti adult rat immune serum (AAS). An embryo serum (ES) and an adult serum (AS) are used as reference. f: $\alpha_1$-foetoprotein.

of the purified fraction after diffusion against the immune anti-embryo serum. It corresponds to the $\alpha_1$-foetoprotein. No adult contaminant can be demonstrated.

When an antiserum containing the three 'foetal' antibodies (5) (i.e. $\alpha_2$M, $\beta$ lipoprotein esterase and $\alpha_1$-foetoprotein) is used, there is again a single precipitation arc, at the $\alpha_1$-foetoprotein level.

## 4. Conclusion and discussion

The purification method described here is simple, rapid and gives high yields of protein. It allows the preparation of relatively important amounts of highly pure $\alpha_1$-foetoprotein: no contamination with albumin, hemoglobin and transferrin can be demonstrated.

The antibodies directed against the serum proteins of adult rat, fixed on the Sepharose 4B, will retain the adult antigens present in the foetal serum, while the hemoglobin has been removed on a carboxymethyl cellulose column. We should consequently obtain a mixture of $\alpha_1$-foetoprotein, $\alpha_2$M and lipoprotein esterase. In fact, these two latter antigens, have been eliminated during the purification procedure, as demonstrated with immunological and electrophoretic techniques. This is not surprising, as it is known that the lipoprotein esterase [5] as well as the $\alpha_2$M [12] are to be found in the adult serum in lower than the foetal but non negligible concentrations.

Our results indicate, consequently, that the amount of antibodies against these two antigens in the immune sera used for the affinity chromatography are sufficient to remove the corresponding foetal antigens.

The purified $\alpha_1$-foetoprotein prepared by us shows a molecular heterogeneity already described by other authors [13, 14]. The two forms can be detected only by electrophoresis in gels with a high acrylamide content (12%). Their relative proportions are 67% for the more cathodic and 33% for the more anodic form. When subjected to immunoelectrophoresis, however, the two forms give a single precipitation arc in the presence of an anti-foetal immune serum.

We have studied the physico-chemical characteristics of the binding of 17 $\beta$-estradiol by this purified

protein and also its affinity for corticosterone [15]. This study has demonstrated the absence of transcortine contamination. We are now trying to separate on a preparative scale the two molecular forms of the $\alpha_1$-foetoprotein in order to study their properties comparatively.

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