AFFINITY CHROMATOGRAPHY OF HUMAN, RAT AND MOUSE α-FETOPROTEIN ON ESTRADIOL-SEPHAROSE ADSORBENTS

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Received 13 March 1975

1. Introduction

Rat and mouse serum α-fetoprotein (αFP) possesses high binding affinity for estrogens as demonstrated by biochemical [1–4] and immunological [5] methods. The results concerning human serum αFP are controversial. By either equilibrium or sucrose gradient centrifugation no significant estrogen-binding capacity could be demonstrated [3,4]. Gel diffusion immunoprecipitates of serum αFP from a patient bearing primary liver cancer, showed, however, estrogen-binding activity [5] and, more recently, the specific binding of human serum αFP to estradiol–Sepharose columns was reported, although attempts to eluate the αFP from the adsorbent were unsuccessful [6].

In the present paper we describe a single procedure to isolate rat and mouse αFP from amniotic fluids after adsorption on estradiol-Sepharose beads and elution with saturated solutions of estrone in 15% aqueous dioxane. We also report preliminary results on the chromatographic behaviour of rat and mouse, as well as human αFP upon insolubilized estradiol adsorbents. The results obtained provide further evidence that all three αFP possess estrogen-binding properties, although the proportion of active versus inactive αFP molecules present in a biological fluid varies greatly from one species to another.

2. Materials and methods

Rat and mouse amniotic fluids were obtained by puncturing the amniotic sacs of 15 to 20 day-old embryos. The fluids from each species were pooled separately, centrifuged at 5000 rev/min and stored at −20°C. Human serum from a patient bearing a primary liver cancer and a high level of αFP (> 2 mg/ml) was fractionated by precipitation with 40% saturated ammonium sulfate. The supernatant after centrifugation at 18 000 rev/min in a Sorvall refrigerated centrifuge was decanted, dialyzed against phosphate-buffered saline (PBS), concentrated to initial volume by ultrafiltration and stored at −20°C.

2.1. *Biochemicals*

Estradiol-Sepharose adsorbent (0.45 μmol estradiol/ml) prepared by coupling estradiol-17β-monohemisuccinate to diaminononane-Sepharose [6] was kindly provided by Dr Arnon (The Weizman Institute, Israel). Estrone was purchased from Roussel-UCLAF (France). Agarose was obtained from the Industrie Biologique Française (Gennevilliers, France).

2.2. *Immunochemical and immunologic techniques*

Specific rabbit antisera to rat, mouse and human αFP were prepared as previously described [7]. Polyvalent antisera to rat, mouse and human serum proteins were obtained following similar immunization procedures. Glucose oxidase-labelled goat antibody to rabbit immunoglobulines was a gift of Dr Avrameas (Institut Pasteur, Paris).

Quantitation of αFP was carried out by immunoelectrodiffusion [8] using the double-antibody technique of Guesdon and Avrameas [9]. The smallest concentration of αFP measurable was 40 ng/ml. Qualitative tests for αFP, serum albumin and other serum proteins were done either in Ouchterlony plates [10] or by immunoelectrophoresis [11].

2.3. *Electrophoresis*

Electrophoretic runs were performed in acrylamide-agarose composite gels (5% acrylamide, 0.8% agarose) according to the method of Uriel [2]. After staining with Coomassie Blue, densitometric diagrams of the resolved proteins were obtained in a Vernon densitometer equipped with a digital integrator.

3. Results

3.1. *Affinity chromatography of αFP*

All the operations were carried out at 4–6°C in the following manner: 0.2 g of wet estradiol-Sepharose beads were first washed by repeated suspension and centrifugation (5000 rev/min, 10 min) in 5 ml aliquots of PBS. After the last centrifugation, 1 ml of αFP solution (amniotic fluid or serum fraction) was added to the pelleted beads (0.3 ml) and the suspension was gently stirred over-night. The suspension was centrifuged again, the supernatant removed and the beads washed as above until the optical density (280 nm) of the supernatant dropped to zero. The packed beads were then suspended for 2 hr in 1 ml of a saturated solution of estrone in PBS containing 15% dioxane. After centrifugation, the desorbed proteins were recovered in the supernatant and stored at +4°C.

3.2. *Chromatographic behavior of mouse, rat and human αFP*

Electrophoregrams of rat and mouse amniotic fluids, before and after adsorption of estradiol-Sepharose, are shown in fig. 1 and 2, respectively. It can be seen that the proteins most significantly retained by the

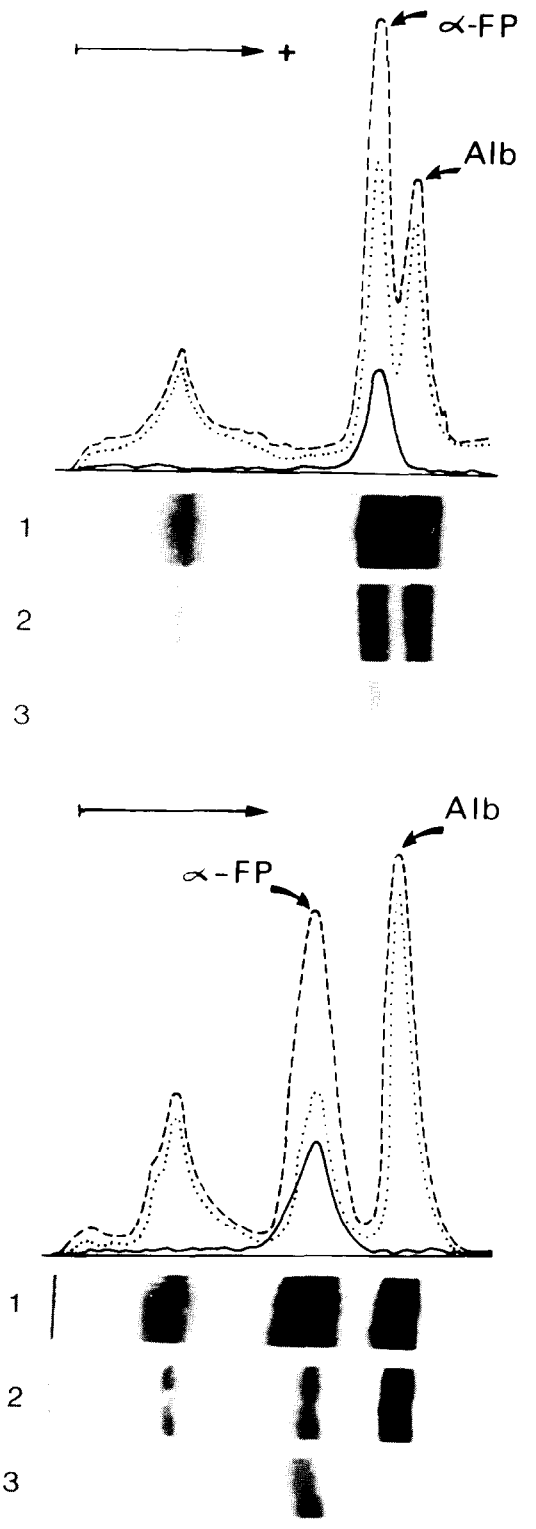

Fig.1. Electrophoretic patterns in acrylamide-agarose gels and densitometric diagrams of 1) rat amniotic fluid (– – –), 2) rat amniotic fluid after adsorption on estradiol-Sepharose beads (·····), 3) eluate after desorption with a saturated solution of estrone in 15% aqueous dioxane (——). αFP, α-fetoprotein; Alb. serum albumin.

Fig.2. Electrophoretic patterns in acrylamide-agarose gels and densitometric diagrams of 1) mouse amniotic fluid (– – –), 2) mouse amniotic fluid after adsorption on estradiol-Sepharose beads (·····), 3) eluate after desorption with a saturated solution of estrone in 15% aqueous dioxane (——). αFP, α-fetoprotein; Alb. serum albumin.

adsorbent correspond to the electrophoretic area of αFP. In the same figures are represented the electrophoretic patterns of the proteins eluted with estrone solutions. In each case, one single band with the electrophoretic mobility of αFP can be observed. The identity of the eluted protein with αFP was confirmed by immunodiffusion tests using specific antisera to rat and mouse αFP. No other serum proteins were detected in the eluted material.

When the αFP enriched fraction of human serum was allowed to react with the same adsorbent, the amount of retained proteins was much less than in the case of rat and mouse amniotic fluids. Nevertheless, some proteins were recovered either after elution with 1 M acetic acid or with estrone solution. Serum albumin and αFP were present in the eluate following acetic acid desorption, but only αFP was detected after the subsequent treatment with estrone in 15% aqueous dioxane. The whole quantity of αFP recovered was 0.46 μg, less than 0.1% of the total αFP contained in the sample (2.50 mg). This low estradiol-binding capacity of human αFP explains previous failure to demonstrate such an estrophylic property by less sensitive methods [3,4].

Quantitative data of the affinity chromatography assays carried out with mouse and rat αFP are compiled in table 1. The specificity of αFP binding appears clearly in both cases, although some differences can be seen. Less rat αFP (0.40 mg) is bound by the same volume (0.3 ml of estradiol adsorbent) than mouse αFP (0.70 mg). Also the yield of rat αFP – per cent of fetoprotein recovered relative to the whole αFP in the sample – is half that of mouse αFP. On the other hand, similar yields – 25% of rat αFP and 42% of mouse αFP

Table 1
Affinity chromatography on estradiol-Sepharose

Sample (1 ml)	Proteins (mg)			
	Total*	αFP**	Albumin***	Other***
Mouse amniotic fluid (MAF)	3.4	1.30	1.05	1.10
MAF after chromatographic assay	2.45	0.60	0.90	0.95
Mouse αFP eluted in 15% dioxane	–	0.55	none†	none†
αFP yield†		42%		
Rat amniotic fluid (RAF)	2.60	1.05	0.75	0.80
RAF after chromatographic assay	1.95	0.65	0.60	0.70
Rat αFP eluted in 15% dioxane	–	0.26	none†	none†
αFP yield††		25%		

* Estimated by the biuret method.
** Estimated by immunoenzymatic method.
*** Estimated from densitometric diagrams after gel electrophoresis and protein staining with Coomassie Blue.
† By immunodiffusion tests with homologous antisera.
†† % αFP recovered relative to total αFP in the sample.

– were obtained when 0.5 ml of amniotic fluids, instead of 1 ml, were chromatographed on 0.3 ml of adsorbent.

These observations suggest the presence of two populations of αFP molecules in either rat or mouse amniotic fluid, the proportion of αFP molecules without estradiol-binding ability being higher in rats than in mice. Recent data obtained by equilibrium dialysis have led to a similar conclusion relative to the functional heterogeneity of rat serum αFP [13].

4. Discussion

The use of saturated solutions of estrone in 15% aqueous dioxane has enabled us to develop a single procedure for the isolation of rat and mouse αFP specifically adsorbed on estradiol-agarose derivatives. The isolated αFP are indistinguishable from the native proteins as far as the electrophoretic mobility and the immunological properties are concerned. The method can be extended to a preparative scale since yields of about 2 and 4 mg of rat and mouse αFP, respectively, per gram of adsorbent should be expected in a single run.

The results presented here also provide further evidence of the estrogen-binding properties of rat, mouse and human αFP already demonstrated by immunological methods [5]. These results confirm the specificity of the binding of human αFP on estradiol-Sepharose adsorbents previously reported by Arnon et al. [6].

On the other hand, studies on the localization of liver αFP by cell affinity labelling with tritiated estrogens and catecholamines [13,14] had led to the hypothesis that αFP may occur in two distinct molecular variants, one of which possesses only estrogen binding activity. The hypothesis seems to be supported by the analysis of the chromatographic data reported here which strongly suggests the existence in rat and mouse amniotic fluid as well as in sera patients with primary liver cancer of both type of αFP molecular variants. Much work will be necessary to investigate the nature and the physiological significance of such heterogeneity.

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