AFFINITY CHROMATOGRAPHY OF MOUSE AND RAT ALPHA-FETOPROTEIN ON IMMOBILIZED DIETHYLSTILBESTROL

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Alpha-fetoprotein (AFP) is the principal embryo-specific mammalian protein, and intensive study of its properties has led to the discovery of its biosynthesis in a chemically induced hepatoma in mice [1] and in a human hepatocellular carcinoma [3]. Mouse and rat AFP possess high affinity and specificity for estrogens, and this property has been used to isolate and purify the above-mentioned proteins by affinity chromatography on immobilized estrogens, such as estrone and estradiol [6, 7, 10, 11]. In experiments of this kind we showed [4] that sorbents containing immobilized diethylstilbestrol bind human AFP with high selectivity and reversibly, and can be used to isolate it.

The aim of this investigation was to study, by affinity chromatography, the ability of mouse and rat AFP to bind with the immobilized synthetic estrogen analog diethylstilbestrol (DES), and to obtain AFP preparations on this basis.

EXPERIMENTAL METHOD

As the source of mouse and rat AFP we used amniotic fluid, obtained by puncture of the amniotic cavity of female rats on the 14th-18th day of pregnancy, or from female mice on the 17th-19th day of pregnancy. The amniotic fluid thus collected was centrifuged at 8000 rpm for 20 min, then dialyzed against a 50 mM solution of sodium chloride, buffered with 20 mM triethanolamine buffer (pH 7.8) for 2 days. For affinity chromatography of mouse and rat AFP we used a sorbent containing DES, immobilized on sepharose, activated by 4-β-hydroxyethylsulfonyl-2-aminoanisole sulfate [2]. The method of immobilization of diethylstilbestrol was described by the writers previously [4]. A column with the sorbent (volume 30 ml) was equilibrated with a 50 mM solution of sodium chloride, buffered with 20 mM triethanolamine buffer (pH 6.8). The same solution was used for washing to remove unbound proteins. Elution of the material bound with the sorbent was carried out with 10% butanol solution in 10 mM veronal-medinal buffer (pH 8.6) at the rate of 30 ml/h. Optical density of the unbound and eluted fractions was monitored with a "Uvicord LKB" densitometer (Sweden). Experiments with amniotic fluid of rats and mice, incubated beforehand with free estrogens, were carried out separately. For this purpose, 50 ml of amniotic fluid was incubated with 0.02% estradiol solution overnight at 4°C with constant mixing. Next, the amniotic fluid was centrifuged at 6000 rpm for 20 min, and then subjected to affinity chromatography on DES-sepharose. AFP in the amniotic fluid of the rats and mice, and also in the fractions obtained, was determined by immunodiffusion analysis in agar, by titration with a standard test system [5]. Antisera against mouse and rat AFP were obtained by immunizing rabbits with the corresponding amniotic fluid in a volume of 0.2 ml subconjunctivally, and 0.8 ml subcutaneously, in an equal volume of Freund's adjuvant. Immunization was carried out for 1 month with an interval of 1 week, and subsequent reimmunization after 2 months with the same dose of antigen. On the 7th day after the last injection blood was taken and the antiserum obtained was subjected to specific absorption with the corresponding blood serum of adult mice and rats. Analytical disk-electrophoresis of the AFP preparations was carried out in 7% polyacrylamide gel [8].

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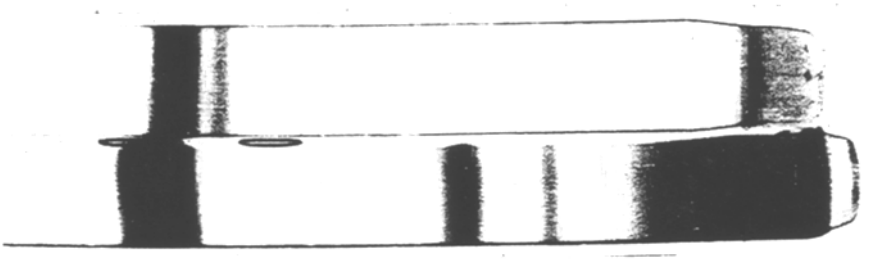

Fig. 1. Affinity chromatography of mouse (a) and rat (b) AFP on sorbent with immobilized diethylstilbestrol. Optical density recorded in continuous-flow cuvette of "Uvicord" densitometer at 280 nm. Continuous line indicates optical density, broken line AFP content. Peak A corresponds to placement profile, peak B to elution profile.

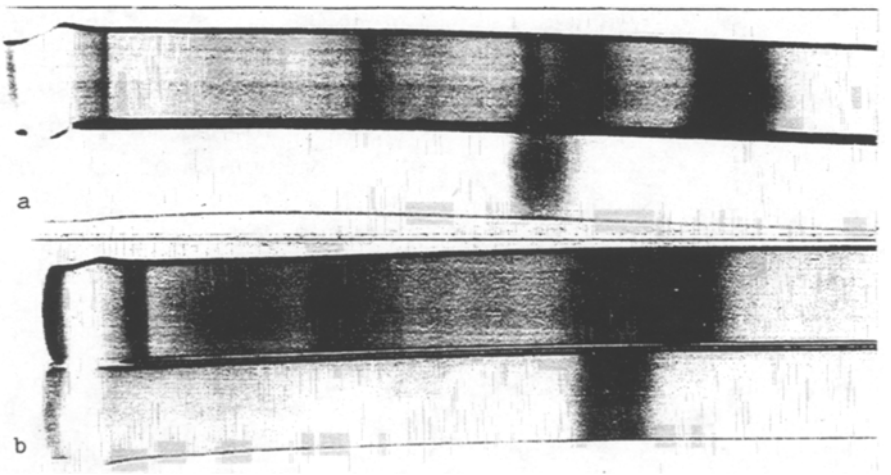

Fig. 2. Disk electrophoresis of mouse (a) and rat (b) AFP preparations obtained by affinity chromatography on sorbent with immobilized diethylstilbestrol. Control – amniotic fluid of corresponding animal. 1) AFP preparation, 2) amniotic fluid. Arrows indicate bands for AFP (↑) and albumin (↓).

EXPERIMENTAL RESULTS

On passing 50 ml of mouse amniotic fluid containing AFP in a concentration of 1.28 g/liter through the column with the sorbent, the AFP bound with the sorbent and came out on elution with a maximal concentration of 2.8 g/liter. Under these circumstances 27 mg of AFP was obtained, corresponding to 42% of the original content in 50 ml of amniotic fluid (Fig. 1a). Affinity chromatography of the rat amniotic fluid, containing about 6 mg of AFP in 50 ml, showed that rat AFP binds with the sorbent by a much greater degree than mouse AFP. On elution of the bound protein, an AFP preparation with maximal concentration of 5.2 g/liter was obtained. The total quantity of the AFP preparation obtained was 48 mg, and the yield was 75% of the original content of AFP in the test amniotic fluid, correspondingly (Fig. 1b). Electrophoresis of the mouse and rat AFP preparations thus obtained showed a high degree of purity (Fig. 2).

The experiments thus showed that mouse and rat AFP possess high specificity of binding with immobilized diethylstilbestrol. This property enables pure preparations of mouse and rat AFP to be obtained in one stage by affinity chromatography on DES-sepharose, directly from the primary biological material, namely amniotic fluid. The sorbent has good capacity, so that 50 ml of amniotic fluid can be applied at once to a 30-ml column and AFP preparations obtained in considerable amounts. For instance, from 64 mg of AFP, contained in the original material,

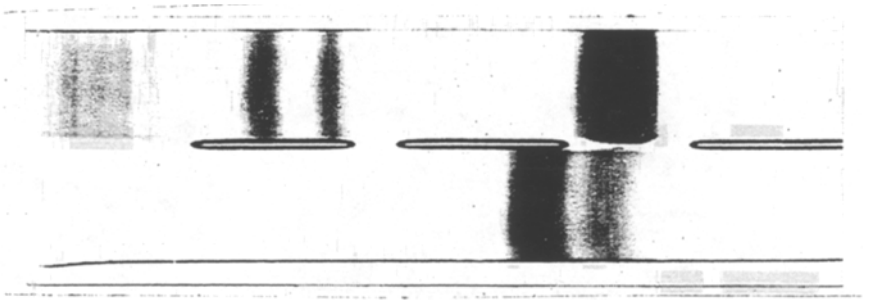

Fig. 3. Affinity chromatography of rat AFP on sorbent with immobilized diethylstilbestrol, after preliminary incubation of rat amniotic fluid with 0.02% estradiol. Optical density recorded at 280 nm in continuous-flow cuvette of "Uvicord" densitometer. Continuous line indicates optical density, broken line AFP content. Peak A corresponds to placement profile, Peak B to elution profile.

27 mg of mouse AFP and 42 mg of rat AFP respectively were obtained. The yields of AFP preparations obtained on DES-sepharose are not inferior to those obtained by other workers on estradiol-sepharose and 2-amino-succinyl-estrone-sepharose [6, 10, 11]. It is interesting to note that during affinity chromatography on estradiol-sepharose [10] the yield of mouse AFP, which was 42%, exceeded the yield of rat AFP (25%). During affinity chromatography on DES-sepharose the yield of mouse AFP also was 42%, whereas for rat AFP it was three times greater (75%), possible evidence of the relatively higher affinity of rat AFP toward immobilized DES than estradiolsepharose. Attention must also be paid to the relative mildness of the conditions of elution of AFP from DES-sepharose compared with those known previously [6, 10].

Considering that mouse and rat AFP possess high affinity for free estradiol [9, 10], experiments were carried out to study affinity chromatography of AFP incubated beforehand with free estradiol. Preliminary incubation of 50 ml of rat amniotic fluid with 0.02% estradiol followed by affinity chromatography on DES-sepharose showed that AFP in this case is only slightly adsorbed, and 640 μg of it was eluted, or 1% of the initial quantity of AFP (Fig. 3). Mouse AFP, after preliminary incubation with estradiol under analogous conditions, behaved in the same way during affinity chromatography, even less of it being eluted (160 μg), about 0.25% of the original amount of AFP.

Preliminary incubation of mouse and rat amniotic fluid with free estradiol thus leads to a sharp decrease in binding capacity of AFP relative to immobilized DES. It can be postulated that estradiol and diethylstilbestrol compete for the same binding sites on the mouse and rat AFP molecule. "Covering" of the estrogen-binding sites by free estradiol prevents interaction of AFP with immobilized diethylstilbestrol. It can be tentatively suggested that the conformation of DES, formed after its immobilization by the method we have described, is sufficiently favorable for binding estrogen-receptor sites on the surface of the AFP molecule.

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