AFFINITY CHROMATOGRAPHY OF HUMAN ALPHA-FETOPROTEIN ON IMMOBILIZED ESTROGENS

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α-Fetoprotein (AFP), which has attracted the attention of investigators as a protein associated with hepatoma and hepatocellular carcinoma [1, 3], is widely used to produce immunodiagnostic sera, and the problem of its isolation is accordingly interesting. Rat and mouse AFP can be isolated with high yields by the method of affinity chromatography on immobilized estrogens [7], whereas the question of the estrogen-binding activity of human AFP remains open.

The aim of this investigation was to determine the estrogen-binding capacity of human AFP by the method of affinity chromatography on specific sorbents containing immobilized estrogens.

EXPERIMENTAL METHODS

AFP was isolated by affinity chromatography on immobilized estrogens from a butanol extract of abortion material (AFP concentration about 50 μg/ml, or 0.1% of total protein) [4]. The butanol extract was prepared as follows. Butyl alcohol was added to the abortion material in the ratio of 1:5, the product was thoroughly mixed and allowed to stand overnight at 4°C, after which it was centrifuged at 6000 rpm for 45 min. The aqueous-protein phase containing AFP was separated and dialyzed for 24 h against running tap water, and then for 24 h against a 0.05 M solution of NaCl buffered with triethanolamine (pH 6.8) or bicarbonate (pH 7.8) buffer. The presence of AFP in the butanol extract of the abortion material and in the fractions isolated by affinity chromatography was tested by the immunodiffusion method by titration with a standard test system [5]. Antisera against AFP were obtained by immunization of rabbits with the AFP preparation, followed by adsorption with the donors' serum protein. To isolate AFP from butanol extract of abortion material affinity chromatography was carried out on a column containing immobilized estrone or diethylstilbestrol. The column was equilibrated with 0.05 M NaCl solution, buffered with triethanolamine (pH 6.8) or bicarbonate (pH 7.8) buffer. The same solution was used to wash off the unbound proteins. Elution was carried out with 10% butanol solution in 0.01 M Veronal–Medinal buffer (pH 8.6). The optical density of the eluted fractions was determined by means of a "Uvicord" continuous-flow densitometer (LKB, Sweden). The resulting fractions were analyzed by polyacrylamide gel disk-electrophoresis [6]. Estrone was immobilized by the following method. To 100 ml of sepharose Cl 4B were added 100 ml of distilled water and sodium periodate up to a concentration of 0.1 M; the sample was mixed on a magnetic mixer for about 2 h, washed with distilled water, transferred into 0.1 M solution of hexamethylenediamine, degassed under vacuum, and replaced on the magnetic mixer for 2 h. After this 2 g of sodium borhydride was added and the product was kept for a further 1 h with mixing, then washed with distilled water and transferred into dioxan, to which was added 0.5 g of estrone-0,3-hemisuccinyl, 4 g of dicyclohexylcarbodi-imide, and 1 g N-hydroxysuccinimide in 100 ml of dioxan. The mixture was left on the magnetic mixer for 24 h. The prepared sorbent was washed to remove unreacted substances and catalysts with 2 liters of ethanol, 2 liters of dioxane, 2 liters of a 50% aqueous solution of dioxan, 2 liters of a 50% aqueous solution of ethanol, and 2 liters of distilled water. The diethylstilbestrol was immobilized on sepharose, activated by the sulfuric acid ester of 4-β-hydroxyethylsulfonyl-2-aminoanisole [2]. For this purpose, 100 ml of sepharose Cl 4B was treated with 1 g of the sulfuric acid ester of 4-β-hydroxyethylsulfonyl-2-

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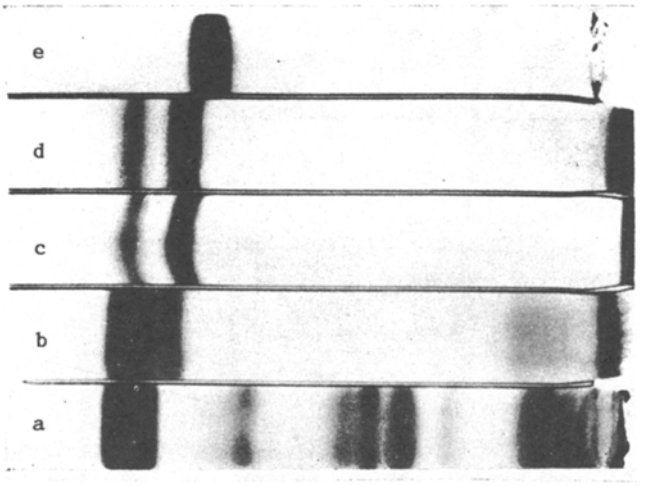

Fig. 1. Disk electrophoresis of AFP preparations: a) extract of abortion material; b) AFP preparation isolated on immobilized diethylstilbestrol; c) AFP preparation isolated on immobilized estrone (extract of abortion material was incubated first with estrone; d) AFP preparation isolated on immobilized estrone; e) AFP preparation after rechromatography on immobilized estrone.

aminoanisole and 2 g sodium carbonate, thoroughly mixed, and heated for 1 h at 100°C. The product was then washed with hot distilled water and cooled, and subsequent procedures were carried out at 0-4°C. To 100 ml of sepharose 100 ml of 10% HCl solution was added, the sample was carefully mixed, and 2 g $NaNO_2$ was added gradually in the cold with constant mixing, to carry out the diazotization reaction. The product was then washed with cold distilled water, transferred into cold acetone, after which 100 ml of a 1% solution of diethylstilbestrol in cold acetone and 2 g of sodium bicarbonate were added to 100 ml of activated sepharose. The mixture was stirred overnight at 4°C. The prepared sorbent was washed with 2 liters of acetone, 2 liters of ethanol, 2 liters of a 50% aqueous solution of ethanol, 2 liters of a 50% aqueous solution of acetone, and 2 liters of distilled water.

EXPERIMENTAL RESULTS

As a result of affinity chromatography of 80 ml of abortion material treated with butanol, on sorbent containing immobilized estrone, an AFP preparation was obtained with a concentration of 80-100 μg AFP/ml and with a yield of 6-10% of the initial quantity. On polyacrylamide gel electrophoresis two zones of migration of proteins with mobility of albumin and AFP were observed in the isolated preparation; their relative proportions were about the same (Fig. 1d).

As a result of affinity chromatography of 80 ml of butanol extract of abortion material on sorbent containing immobilized diethylstilbestrol an AFP preparation was obtained with a concentration of 320-350 μg AFP/ml and with a yield of up to 95% of the initial quantity. Electrophoretic investigation of the preparation revealed several proteins, of which albumin accounted for about 80% and AFP for about 15% (Fig. 1b).

Rechromatography of the preparation obtained with immobilized diethylstilbestrol on sorbent containing immobilized estrone removed much of the contaminating albumin and yielded an AFP preparation with a purity of up to 95% and with a total yield of about 70% (Fig. 1e).

During affinity chromatography of cell-free extract of abortion material obtained without butanol treatment the AFP was not adsorbed on sorbents containing immobilized estrogens. Preincubation of the butanol extract of abortion material with estrone, estradiol, estriol, and estrone-O,3-hemisuccinyl followed by affinity chromatography on immobilized estrogens did not reduce the yield of the AFP preparation, but reduced its albumin content to some extent (Fig. 1c). Meanwhile preliminary incubation of the butanol extract of abortion material with diethylstilbestrol led to a reduction of the AFP yield in the resulting preparation by about half.

The results are evidence that estrogens can bind with human AFP under certain conditions. Human AFP in biological fluids is evidently bound with estrogens; the binding constant, moreover, is so high that exchange of hormone molecules is virtually impossible and AFP untreated with butanol therefore does not bind the hormone immobilized on sepharose. After treatment of abortion material with butanol, as a result of which dissociation of the estrogen–AFP complex takes place, human AFP becomes capable of binding the immobilized hormone. The binding is very strong in character and the complex does not dissociate during attempts at elution with 1 M and 2 M NaCl solutions and also by the use of a whole range of detergents. A very good eluting agent under these circumstances is a 10% solution of butanol in isotonic sodium chloride or in 0.01 N Veronal–Medinal buffer, pH 8.6. The orientation of the hormone immobilized on sepharose also is important. In the present experiments on sorbents containing estradiol or estrone, immobilized through C-17, the yield of AFP on affinity chromatography was very small (about 0.5-1%) and this was accompanied by a high concentration of ballast proteins, mainly albumin, although these data also are evidence in support of binding of human AFP with estrogenic hormones.

Meanwhile the results of chromatography both of the butanol extract of abortion material and of AFP preparations incubated beforehand with free estrone and estradiol, are evidence that binding of AP with free estrogens does not take place, confirming prevous observations [7]. It can be tentatively suggested that binding of estrogens with human AFP is specific in character, through the participation of unknown protein intermediaries, the discovery of which is an interesting problem, and the microenvironment of the immobilized estrogen thus as it were models the conditions of binding of human AFP in vivo.

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EFFECT OF LOW DOSES OF HYDRA PEPTIDE MORPHOGEN ON PROTEIN SYNTHESIS IN THE INTACT AND REGENERATING RAT LIVER

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Hydra peptide morphogen (HPM), described in 1981 by Schaller and Bodenmüller, accelerates regeneration of injured organs of the Coelenterata and Polychaeta [4, 9]. This oligopeptide has been found by immunochemical methods in the gastrointestinal tract and brain of mammals and also in human blood plasma [4, 8, 9]. Data on the biological effect of this

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