COMPETITIVE AFFINITY CHROMATOGRAPHY OF HUMAN ALPHA-FETOPROTEIN ON IMMOBILIZED DIETHYLSTILBESTROL

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By the use of affinity chromatography on immobilized estrogens it is possible to obtain animal [5, 6, 8] and human [3] alpha-fetoprotein (AFP) in high yields in one or two steps. It has also been noted that when aqueous-butanol extracts of abortive material, in which the AFP concentration is very low by comparison with albumin (200-500 times lower), are used in order to isolate human AFP, the principal protein impurity which must be removed from AFP preparations is albumin, which binds immobilized estrogens highly effectively [3]. Meanwhile human AFP is known not to bind free estrogens [6, 8], and this has been confirmed also in experiments with affinity chromatography of butanol-treated abortive material [3].

The aim of this investigation was to study the effect of competition between free steroid hormones and an immobilized ligand (diethylstilbestrol; DES) on the yield of human AFP during affinity chromatography.

EXPERIMENTAL METHOD

Affinity chromatography of AFP was carried out with an aqueous-butanol extract [3] of human fetal-placental complex, obtained at medical abortion. AFP and albumin were determined by titration with a standard test system [4] by the immunodiffusion method. Affinity chromatography was performed on a column with immobilized DES, linked to sepharose by diazo-coupling [3] through hydroxyethylsulfanyl-2-aminoanisole [1]. To study the effect of competition between free steroid hormones and immobilized DES on the yield of AFP, experiments were carried out with aqueous-butanol extracts of abortive material, incubated beforehand with free steroids: estrone, estradiol, estriol, testosterone, hydrocortisone, and deoxycorticosterone, and the synthetic estrogen analog — DES. These hormones were introduced in the form of an alcoholic solution into aqueous-butanol extracts of abortive material until the concentration reached 0.015-0.025%. The material was then placed on a magnetic mixer and incubated with continuous mixing for 8-12 h at 4°C, after which it was centrifuged to remove unbound and undissolved hormone. The material was then subjected to affinity chromatography on a column with DES-sepharose (40 ml), equilibrated with 0.05 M sodium chloride solution, buffered with triethanolamine buffer, pH 6.8. Unbound proteins were then removed by washing with the same buffer. Elution was carried out with a 10% solution of butanol in 0.01 M veronal-medinal buffer, pH 8.6. To standardize the conditions, 150 ml of aqueous-butanol extract of abortive material with an AFP concentration of 80 mg/liter was applied to the column. The rate of flow of the material during application, rinsing, and elution was 30 ml/h. To standardize the conditions on the column, 150 ml of test material, equalized for AFP concentration (80 mg/liter), was usually applied to the column. The optical density of the eluted fractions was monitored by means of a continuous-flow Uvicord densitometer (LKB, Sweden). The fractions were analyzed by polyacrylamide gel (PAG) disk electrophoresis [7].

EXPERIMENTAL RESULTS

During affinity chromatography of 150 ml of aqueous-butanol extract of abortive material on immobilized DES an AFP preparation containing 6.7 mg of AFP was obtained. Considering that the initial material contained 12 mg of AFP, the yield was

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TABLE 1. Effect of Preincubation of Butanol Extract of Abortive Material with Free Steroid Hormones on Binding of AFP with Immobilized DES

Hormone	Concentration, %	Amount of AFP applied, mg	Amount of AFP eluted, mg	Yield of AFP, %	AFP/albumin
Control	—	4,0	3,8	95	1/10
		7,2	5,9	82	1/10
		9,6	6,1	63	1/10
		12,0	6,7	56	1/10
Estrone	0,015	12,0	10,3	86	1/2
	0,025	12,0	10,5	88	1/2
Estradiol	0,015	12,0	10,6	88	2/1
	0,025	12,0	10,9	91	2/1
Estriol	0,015	12,0	11,0	92	1/1
	0,025	12,0	11,2	93	1/1
Testosterone	0,015	12,0	11,9	99	1/2
	0,025	12,0	11,1	92	1/2
Hydrocortisone	0,015	12,0	9,1	76	1/2
Deoxycorticosterone	0,015	12,0	9,3	77	1/1,5
DES	0,015	12,0	4,9	41	1/2
		7,2	4,2	58	1/2
Initial material		—	—	—	1/100

56%. Addition of ethanol to the initial material up to 1-3% had no effect on the yield of AFP. Incidentally, passage of a smaller amount of abortive material was accompanied by a relatively higher percentage yield of AFP (Table 1). The ratio of albumin to AFP in the preparations obtained was virtually independent of the amount of material passed through, and was about 10:1.

Adsorption of proteins on immobilized DES is evidently accompanied not simply by a decrease in the number of protein-free sites of the immobilized DES, but also by possible steric hindrances, created by screening of nearby molecules of the immobilized ligand by adsorbed macromolecules from albumin and AFP molecules capable of binding with them, and this is reflected in the fairly rapid decrease in the percentage yield of AFP with an increase in the volume of aqueous-butanol extract of abortive material passed through. Preliminary incubation of the test material with free steroid hormones led not only to an increase in the percentage yield of AFP during affinity chromatography on immobilized DES (Fig. 1), but also led to an increase in the relative content of AFP in the preparation by comparison with albumin (Fig. 2). The results of a study of the effect of competition of free steroids with immobilized DES on the yield of AFP are given in Table 1.

Table 1 shows that the most significant effect on the AFP yield was that of preincubation with testosterone, in the presence of which virtually all the AFP bound with the sorbent, and the ratio of AFP to albumin in the preparation was 1:2, which is considerably better than that in preparations obtained from material without incubation with the hormone (1:10). Least contamination with albumin was observed in preparations obtained after preincubation with estradiol, where the ratio of AFP to albumin was 2:1. A hormone concentration of between 0.015 and 0.025% had no effect on the AFP; albumin ratio and was constant for each hormone. As a rule an increase in hormone concentration led to an increase in the yield of AFP; the exception was testosterone, with which, when used in a concentration of 0.015%, the maximal yield of AFP up to 99% was observed, and DES, addition of which in a concentration of 0.015% led to a decrease in the AFP yield compared with the control.

Free steroid hormones, it can be concluded, compete during affinity chromatography with immobilized DES for receptor binding sites on steroid-binding proteins, occupy these sites, and thereby prevent binding of the given proteins with the immobilized ligand. Meanwhile steroid hormones present in the free state do not bind with AFP, which, during weakening of competition for immobilized DES by albumin, binds in a greater amount with the sorbent. Free DES can evidently bind with AFP, leading to a decrease in the yield of AFP during chromatography in its presence. AFP possesses much greater affinity for immobilized DES than albumin, for in native abortive material and in aqueous-butanol extract of abortive material the AFP/albumin ratio was about 1:100, whereas in the preparation obtained by affinity chromatography on immobilized DES, this ratio was about 1:10.

It follows from the results of this investigation that DES can be regarded as a group ligand for albumin and AFP, and the results can be regarded as an example of affinity chromatography on group biospecific ligand-sorbents [2] under conditions of competition both of free hormones with immobilized DES for protein receptor sites, and of sorbent-binding proteins with

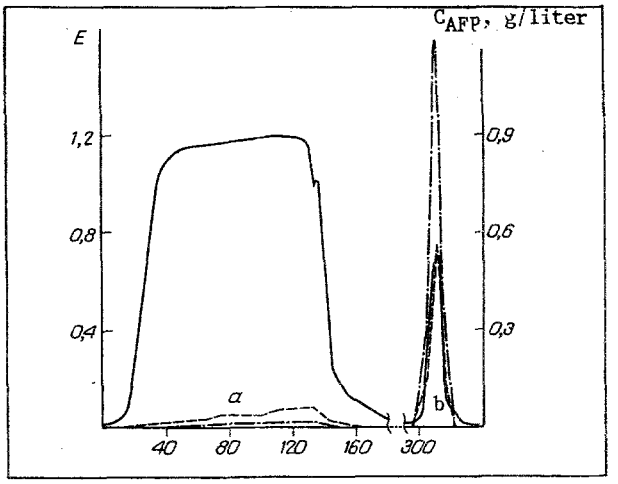

Fig. 1. Affinity chromatography of human AFP on DES-sepharose. Abscissa, volume (in ml); continuous line, optical density; broken line, AFP concentration in experiment without preincubation with hormones, line of dots and dashes, with preincubation with 0.015% estrone, a) peak corresponds to profile of passage of material through column, b) to profile of elution of protein.

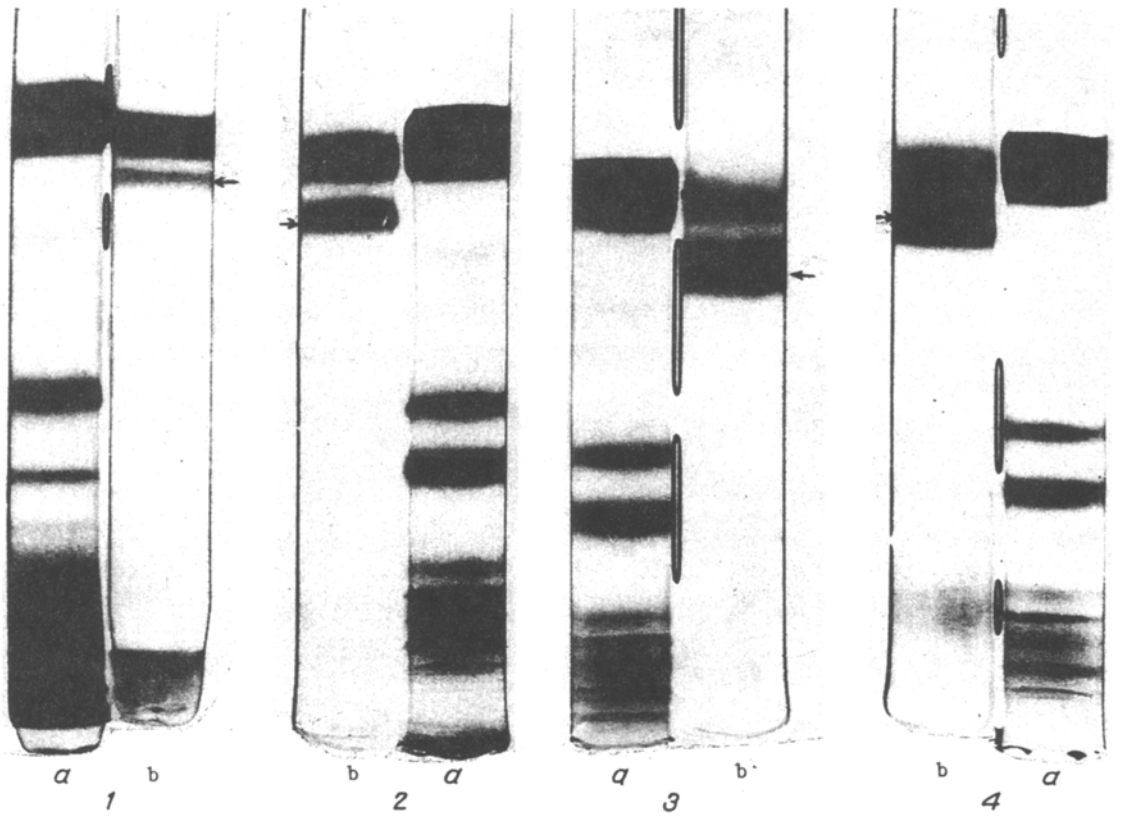

Fig. 2. PAG electrophoresis of human AFP preparations obtained by affinity chromatography on DES-sepharose. a) Control, butanol extract of abortive material, b) preparation of human AFP, 1) AFP preparation obtained without preliminary incubation with hormones, 2) obtained with preincubation with 0.015% estrone, 3) after preincubation with 0.015% estradiol, 4) after preincubation with 0.015% estriol.

AFP for immobilized DES. The approach demonstrated in this investigation may perhaps find application in affinity chromatography of highly specific ligands on group biospecific sorbents as competitors with the aim of increasing the yield of one of the proteins binding this sorbent.

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CHANGES IN ELECTRICAL PARAMETERS OF THE BLOOD IN THE EARLY PERIOD AFTER BURNS

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Key words: burns; rat erythrocytes; blood.

Homeostatic, biological, and biochemical constants and processes are based on interaction of electric charges. Disturbances of these constants and processes are at the same time changes in the electric charge of cells, and also of other electrical characteristics of the blood. The diagnosis of these disturbances by means of instruments recording electrical parameters of the blood and its cells and the discovery of their correlation with the features of various disorders are of great interest, because they may prove to be simpler, more accessible, and cheaper than methods of clinical and biochemical analysis currently in use.

Blood cells, if the acid-base balance has the normal value, have a negative charge on their surface, which plays an important role in all physiological processes: gas exchange, adsorption of amino acids, proteins, and their breakdown products, antigens and antibodies, enzymes, foreign substances entering the blood. In pathological states the electric charge of the cells may change substantially as a result both of changes in the physicochemical structure of the cell surface and in the composition of the external medium — with the appearance of antibodies, abnormal proteins, and cell breakdown products in the blood [4, 6].

Normal erythrocytes possess dielectric properties. The dielectric constant of erythrocytes depends on their polarization and their relaxation time, which, in turn, is determined by the state, number, and character of the cells, molecules, and particles present in the liquid medium with erythrocytes. The dielectric constant, and also the geometric dimensions and shape of the sample of erythrocytes determine the electrical capacity of the latter which, if the electrical potential is the same, may vary depending on the changes in magnitude of the charge. Our investigations [1] showed that severe burn trauma causes a rapid and marked decrease in the specific surface resistance of the blood, confirming data in the literature on an increase in the specific electrical conductance of the cells as a result of their injury [3].

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