BINDING OF 5S ESTRADIOL RECEPTOR TO POLY-DEOXYNUCLEOTIDES

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SUMMARY

Calf uterus cytosol was incubated with (^{3}H)estradiol and fractionated on Sephadex G-200. Two (^{3}H)estradiol-binding protein fractions were obtained with sedimentation coefficients of 5.1 S and 3.5 S, respectively. The 5.1 S fraction bound to poly dT, poly dA:dT and poly dG:dC to a higher extent than to calf thymus DNA. The 3.5 S fraction did not bind to DNA.

Steroid hormones bind to specific receptor proteins in the cytoplasm of responsive cells(1). The interaction takes place with receptors which sediment in sucrose gradients at approximately 4S in the presence of 0.4 M KCl (2). The complexes undergo a temperature dependent transition into a 5S form which binds to nuclear chromatin (3,4). At present it is uncertain what the role is of various chromosomal components in the latter binding process. Certain nuclear acidic proteins have been proposed to act as acceptor sites (5-7), but some data indicate that histones may also be involved (8,9). Since retention of the receptor-hormone complex in nuclei is abolished by treatment with DNase, the DNA may also play an important role (4,10). Steroid receptors bind to DNA-cellulose (11,12), and this method has been applied to purify these receptors (13). It is therefore of interest to determine to what degree steroid receptors bind to various synthetic polynucleotides as compared to DNA. In the present

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paper we present a simple in vitro method for studying the interaction of estradiol receptors with DNA in solution. We have used this system to investigate the binding of these receptors with various commercially obtained poly-deoxynucleotides.

MATERIALS and METHODS

6,7-(^{3}H)Estradiol (41 Ci/mmole) was obtained from The Radiochemical Centre,Amersham,England. Sephadex G-200 and Sepharose 2B were products of Pharmacia, Uppsala, Sweden. Dithiothreitol (Clelands Reagent) was obtained from Sigma, St.Louis,Mo., and Aquacide II from Calbiochem, San Diego, Calif. Calf thymus DNA was prepared according to the method of Kay et al.(14).Yeast RNA was obtained from BDH, Poole,England. The biopolymers dG:dC (8 OD/ml),dA (7.8 OD/ml),dT (5 OD/ml),dAT (7 OD/ml),dA:dT(8 OD /ml) and dG (8 OD/ml) were obtained from General Biochemicals, Chagrin Falls, Ohio. Protein content was assayed according to the method of Lowry et al.(15).Radioactivity was assayed in the Packard Tri-Carb Liquid Scintillation Spectrometer, making corrections for quenching. Preparations of estradiol receptors were stored at -70°C.

RESULTS

Freshly obtained calf uterus (10 g) was homogenized in the cold in 20 ml buffer containing 10 mM Tris-HCl (pH 7.4), 1.5 mM EDTA and 1 mM dithiothreitol (TED buffer). The homogenate was submitted to centrifugation at 0°- 4°C for 30 min at 45,000 x g in the Sorvall RC-2 ultracentrifuge. The sediment was discarded and (^{3}H)estradiol was added to the supernatant (cytosol) to make a final concentration of 2 nmolar. The mixture was incubated for 30 min at 20°C and then dialyzed for about 4 h in the cold against solid Aquacide II in order to reduce the volume to approximately 2 ml. The concentrate was submitted to gel filtration on a column of Sephadex G-200 in the cold. As is shown in Fig.1, two peaks

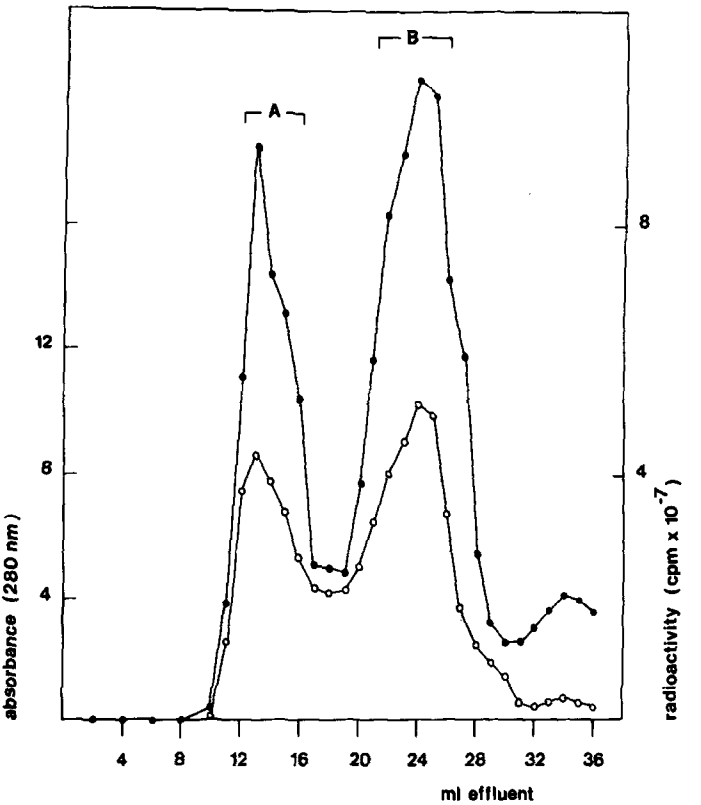

Fig.1. Sephadex G-200 chromatography of calf uterus cytosol after incubation with (^{3}H)estradiol. Column size 1 cm x 78 cm. Elution was carried out in the cold with TED buffer and fractions of 1 ml were collected. (o),Absorbance at 280 nm; (•), Radioactivity.

of protein-bound radioactivity were obtained after elution volumes of 13 ml (Fraction A) and 24 ml (Fraction B),respectively. The relative amounts of radioactivity in these peaks varied in different experiments. A small peak of unbound (^{3}H)estradiol was eluted after 34 ml effluent volume. When Fraction A and Fraction B were studied in the analytical ultracentrifuge equipped with absorption optics (17), these fractions sedimented as single symmetrical peaks. Fractions A and B were found to have sedimentation coefficients of 5.1 S and 3.5 S, respectively. A portion of Fraction A was incubated for 5 min in the cold with

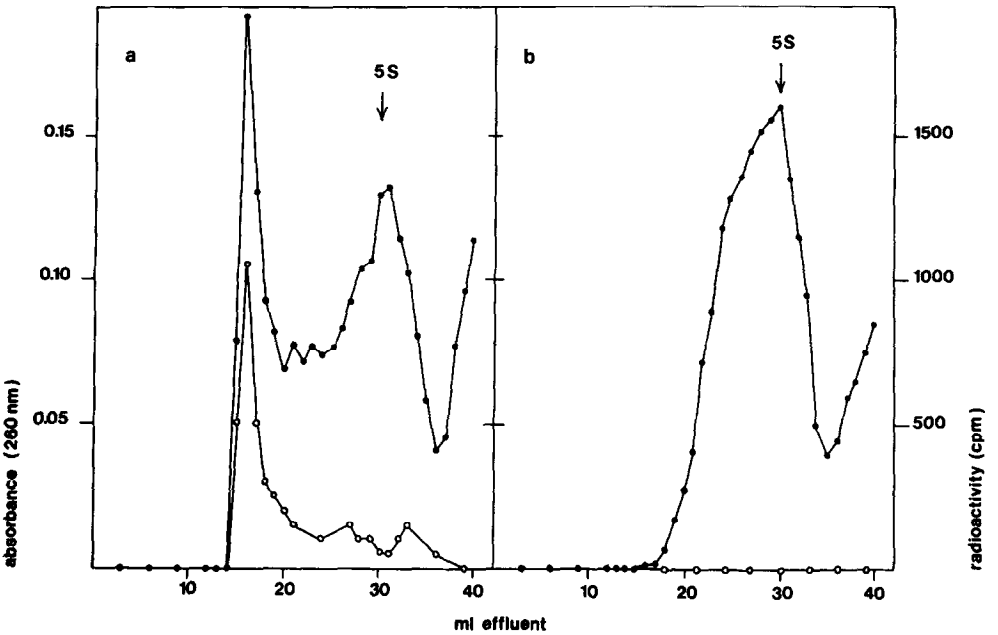

Fig.2. Binding of 5S estradiol receptor to DNA. Sepharose 2B chromatography of (a)Fraction A + 21 µg calf thymus DNA;(b) Fraction A. Each sample had a total volume of 0.7 ml in TED buffer and contained 0.7 mg Fraction A (65,600 cpm/mg protein). The mixtures were incubated at 4°C for 5 min and were then applied to a column of Sepharose 2B (column size 1 cm x 78 cm). The column was eluted with TED buffer in the cold, and fractions of 1 ml were collected. (o),Absorbance at 260 nm; (•), Radioactivity.

calf thymus DNA, and the mixture was fractionated on a column of Sepharose 2B. Part of the radioactivity was bound to the DNA and emerged from the column in the void volume (Fig.2a). In a control experiment (Fig.2b) when Fraction A alone was incubated and submitted to gel filtration, no radioactivity was eluted in the void volume. In both cases, a small amount of unbound (^{3}H) estradiol was removed from the column after 40 ml elution volume. Incubation of DNA with (^{3}H)estradiol in the absence of Fraction A, followed by gel filtration did not result in binding of radi-

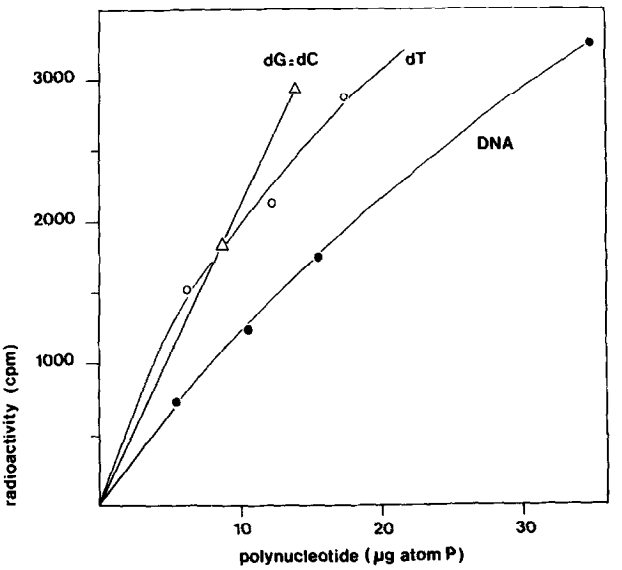

Fig.3. Binding of 5S estradiol receptor to biopolymers. Portions of Fraction A (0.7 mg; 65,600 cpm/mg protein) were incubated at 4°C for 5 min with various amounts of calf thymus DNA (●), poly dT (o) and poly dG:dC (Δ). The mixtures were submitted to gel filtration on Sepharose 2B columns, and the u.v.absorption and radioactivity of the polynucleotide peaks emerging in the void volumes were assayed.

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oactivity to the DNA peak eluted in the void volume (not shown in fig.)

Fig.3 shows the results obtained when portions of Fraction A were incubated with different amounts of calf thymus DNA, poly dT or poly dG:dC. There was an increase in amount of radioactivity bound with increased polynucleotide concentration in the incubation mixture. Poly dT and poly dG:dC bound significantly more 5 S receptor than did calf thymus DNA. The receptor-binding capacities of several other biopolymers were investigated in a similar manner. Poly dA:dT bound approximately the same amounts of 5 S receptor as did poly dT within the concentration range studied (0 - 20 µg atom P of poly dA:dT).The receptor-binding

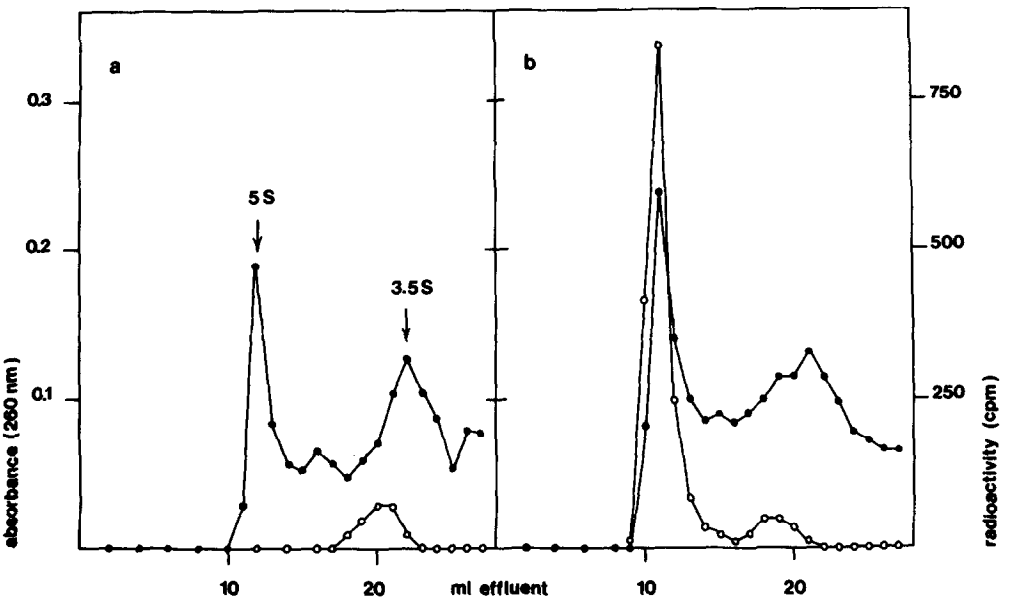

Fig.4. Sephadex G-200 chromatography of (a) Fraction B; (b) Fraction B + 53 µg calf thymus DNA. Each sample had a total volume of 0.7 ml in TED buffer and contained 0.7 mg Fraction B (245,000 cpm/mg protein). (o),Absorbance at 260 nm;(●),Radioactivity.

capacities of poly dG, poly dA, poly dAT, yeast RNA and heat-denatured (20 min at 100°C) calf thymus DNA could not be determined since these materials were retarded on Sepharose 2B columns and practically no material was eluted in the void volume. Fraction B was also studied. When this fraction was resubmitted to gel filtration on a column of Sephadex G-200, part of the radioactivity was eluted as 5 S receptor (Fig.4a) indicating a partial conversion of 3.5 S to 5 S receptor during rechromatography. A partial dissociation of (^{3}H)estradiol also occurred since part of the radioactivity was removed from the column after about 40 ml elution volume. When Fraction B was incubated with calf thymus DNA and the mixture was studied by gel filtration (Fig.4b) the elution profile of radioactivity was essentially the same as in Fig.4a. The finding that there was practically no

increase in radioactivity in the DNA peak, indicated that there was practically no binding of 3.5 receptor to DNA under these experimental conditions.

DISCUSSION

The experiments were carried out with buffer containing dithiothreitol, since this substance has been found to protect estradiol receptors during prolonged storage (16). We found that receptors could be stored for several weeks at -70°C in dithiothreitol-containing buffers without significant loss of binding activity.

Fractionation of uterine cytosol on Sephadex G-200 revealed the presence of two estradiol-binding protein fractions with sedimentation coefficients of 5.1 S and 3.5 S, respectively. These probably represent the 5S and 4S receptors generally referred to in the literature; there are reports that the sedimentation coefficients of estradiol receptors in salt-containing sucrose gradients are close to 5.2 S and 3.8 S, respectively (18). For simplicity, the approximate value of 5S for the 5.1 S receptor has sometimes been used in this article. Our studies indicate that this protein binds to the single-stranded homopolymer dT and the double-stranded homopolymer pairs dG:dC and dA:dT to a higher extent than to calf thymus DNA. On the basis of this finding it seems possible that long chain deoxyhomopolymers linked to cellulose or to other inert carriers may be useful tools for the isolation and purification of 5S receptors from mammalian tissues. The finding that 5S receptors have a high affinity for homopolymers *in vitro* may also indicate that homopolymer stretches on DNA may be binding sites for 5S receptors. Deoxyadenylate-rich and deoxyguanylate-rich regions are present in mammalian DNA in much higher concentration than in bacterial and viral

DNA (19). The attachment of 5 S receptors to such loci on DNA may lead to gene activation, perhaps by inducing the weakening of the constraint certain arginine-rich histones impose on the supercoiling of DNA (20).

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