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PROGESTERONE RECEPTOR COMPONENTS: IDENTIFICATION OF SUBUNITS BINDING TO THE TARGET-CELL GENOME*

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SUMMARY

The chick oviduct cytoplasmic progesterone receptor has been shown to consist of two 4S components, A and B, by chromatography on DEAE-cellulose. Both A and B are high-affinity, specific progesterone-binding proteins which cannot be interconverted or recombined. Both appear bound in isolated oviduct nuclei *in vitro*. The two components differ in their affinity for DNA and chromatin. Component A binds only to pure DNA whereas component B binds only to oviduct chromatin.

INTRODUCTION

IT IS now a generally accepted concept that an early step in the mechanism of action of steroid hormones is the association of the hormone with a specific receptor protein in the cytoplasm of the target cell[1]. Subsequently, this hormone-receptor complex is transported into the cell nucleus[2] and can be found associated with chromatin[3–5]. In previous studies, we have described a tissue-specific, high-affinity cytoplasmic progesterone receptor protein[6, 7] of the chick oviduct[8–10] which appears to function by accumulating in nuclei [11, 12] and by binding specifically to oviduct chromatin[13, 14]. The tissue specificity of this effect, together with the early hormone effects on nuclear RNA synthesis[15, 16] indicated to us that this intranuclear localization of cytoplasmic receptor-hormone complexes represented initial steps in the oviduct response to progesterone. If the interaction of the receptor-hormone complex with chromatin acceptor sites was indeed a functional event *in vivo*, then it was most important to understand the molecular events of this interaction *in vitro*.

EXPERIMENTAL

Chemicals. All chemicals were reagent grade and obtained from Fisher except ammonium sulfate, sucrose, and Tris (Mann Research, ultrapure) or as noted.

Temperatures. All steps were performed at 0°C except as noted.

Steroids. [1,2-^{3}H]-progesterone (33·8 Ci/mmol) was obtained from New England Nuclear. Crude cytosol progesterone receptor was labeled by adding this 1:5 progesterone solution (in 20% ethanol) dropwise to the cytosol (5 μl progesterone/ml cytosol).

Scintillation counting. Radioactivity was determined in 0·6 ml aqueous samples

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by adding 5 ml of scintillation fluid containing toluene: Triton X-100 (Beckman) and Spectrafluor (Amersham Searle), 1000:521:42 (by vol.). Counting efficiency for ^3H was 33%.

Sucrose gradient centrifugation

Linear gradients of 5 to 20% sucrose in TESH buffer (0·01M Tris-HCl, pH 7·4, 0·001M Na_2EDTA, 0·012M 1-thioglycerol) (no KCl) or in TESH buffer containing 0·3M KCl (high KCl) were prepared in 5 ml polyallomer tubes (Beckman). The tubes were centrifuged for 16 h at 45,000 rpm in a Spinco SW-50·1 rotor at 0–2°C and handled as described previously [11, 12].

Preparation of plasma and cytoplasmic supernatant fractions

Female Rhode Island Red chicks were used for the preparation of the progesterone receptor. The animals were injected daily for 15 days with 5 mg of diethylstilbestrol (DES) in sesame oil administered subcutaneously. The animals were killed, the oviducts removed and washed in 0·9% NaCl. The pooled oviducts were minced in four volumes of TESH buffer. The mince was homogenized with a polytron PT-10 (Brinkmann Inst.). This homogenate was used to prepare cytosol (S_1) by centrifugation at 120,000 *g* for 1 h.

Hen plasma or serum were prepared from Rhode Island Red laying hens by centrifuging blood for 15 min at 27,000 *g*. The supernatant fraction was centrifuged for one hour at 120,000 *g* in a Spinco SW-40 rotor. This high speed [^3H]-progesterone at 4°C. Label bound to receptor was detected by the charcoal

Ammonium sulfate precipitation

A saturated ammonium sulfate-TESH buffer (pH 7·4) was added dropwise to a labeled cytosol solution to give an ammonium sulfate saturation of 30%. The precipitated material was collected by centrifugation and the pellet redissolved in a small volume of TESH to give a preparation designated P_1.

Agarose gel filtration

A 2·5 cm × 100 cm agarose A-0·5m (Bio-Rad) column was equilibrated with TESH, 0·3M KCl as described previously [5]. Samples (P_1) were applied in 1–2 ml volumes; 3 or 6 ml fractions were collected at 0° and small aliquots were counted for ^3H. The peak fractions of bound radioactivity were pooled and concentrated by ultrafiltration.

DEAE-cellulose ion exchange chromatography

Microgranular DEAE-cellulose (Whatman DE-52) in TESH was used in a 2·0 cm × 5 cm jacketed column. Samples were applied in TESH and the column was then washed with 30 ml TESH. The elution was carried out using either a 150 ml KCl gradient from 0 to 0·3M KCl in TESH or stepwise elution with 30 ml of TESH, 0·15M KCl, followed by 30 ml of TESH, 0·3M KCl.

Assay of receptor components in crude fractions

To measure the presence of components A and B in crude cytosol or nuclear extracts, a shortened isolation procedure was used. Samples with a KCl concentration less than 0·03M were applied directly to a 5 ml DEAE-cellulose column and eluted stepwise with KCl.

Preparation of receptor without bound steroid

Oviduct cytosol was prepared as described above but no radioactive steroid was added. The receptor was precipitated at 30% saturation of ammonium sulfate and then chromatographed on DEAE-cellulose by stepwise elution. An aliquot (100 μl) of each fraction was added to 10 μl of buffer containing 1 ng of [^{3}H]-progesterone at 4°C. Label bound to receptor was detected by the charcoal assay as described below. Fractions from the column having the most binding activity in the A and B peak regions were pooled for kinetic studies.

Binding assay using charcoal–dextran–bovine plasma albumin

Measurement of hormone binding to receptor component was done using a modification of the charcoal-dextran method of Korenman[22]. Labeled samples (100 μl) to be assayed were combined with 0·9 ml of charcoal assay mixture containing 0·5% acid and base-washed charcoal (Norit), 0·05% dextran, 0·2% bovine plasma albumin (BPA, Sigma), and 0·02% NaN_3 in TESH, 0·3M KCl pH 7·4, mixed and allowed to stand in ice for 30 min. The solution was centrifuged at 1500 *g* and the supernatant fraction was decanted and counted for ^{3}H.

Receptor specificity–hormone competition studies

Crude cytosol preparation and purified components A and B were assayed for their hormone binding specificity by the method of Korenman[22]. Receptor preparations (0·5 ml) were incubated at 0° with 10,000 cpm of [^{3}H]progesterone together with varying amounts of unlabeled progesterone or other steroids over a range of 0·25–100 ng. After 16 h, unbound hormone was removed by adding 0·5 ml charcoal–dextran–BPA as described above. The competition ability of each steroid was then computed relative to unlabeled progesterone.

Binding kinetics–rate of association

To start the binding reaction, 1·0 ml aliquots of an unlabeled receptor fraction received 10 μl of various dilutions of stock [^{3}H]-progesterone in H_2O. The binding reaction was stopped at specific times by adding 100μl samples to tubes containing 1 μg of unlabeled progesterone (10 μl). These samples were then assayed for receptor-bound radioactivity by the charcoal adsorption assay. The data were plotted by the method of Best–Belpomme *et al.*[22] to determine the second-order association rate constants for each receptor preparation tested.

Binding kinetics–rate of dissociation

To 1·0 ml samples of labeled A, B or cytosol, unlabeled progesterone (1 μg) was added to start the dissociation experiment. At various times, 100 μl aliquots were removed and assayed by the charcoal binding assay. Semi-logarithmic plots of bound radioactivity vs. time gave straight lines for the pseudo first-order dissociation reaction. The first-order dissociation constant was determined from the slope of each curve. Equilibrium constants for the receptor-hormone binding reactions were calculated as the ratio k_d/k_a, assuming one independent class of binding sites per receptor molecule.

Extraction of binding proteins labeled with [^{3}H]*-progesterone in vivo*

Chicks stimulated with DES for 15 days received 200 μCi (0·3 ml) of [^{3}H]-progesterone in 90% saline − 10% ethanol by intravenous injection. After 30 min

the animals were killed and the oviducts were removed and processed as described above to prepare binding proteins.

Nuclear binding protein components from oviduct slices incubated with [1,2-^{3}H]-*progesterone in vitro*

Oviduct slices from DES-primed chicks (2·4 g were incubated for 5 min at 0° in 6 ml of Basal Eagle's Medium (BEM) containing 10^{-8} M [^{3}H]-progesterone and then transferred to fresh BEM without progesterone and incubated 20 min at 0°, 23° or 37°. The slices were then transferred to 10 ml of TESH. Cytosol and nuclear binding proteins were prepared from these tissues as described previously [6, 12]. Both preparations were chromatographed on DEAE-cellulose to prepare receptor components A and B.

Uptake of cytoplasmic binding proteins by isolated oviduct nuclei

For each binding protein uptake assay, nuclei were prepared from 0·5 g of DES-treated chick oviducts as described previously [11, 12]. Each nuclear pellet was resuspended in a 2·0 ml sample of labeled receptor preparation or resuspended in a blank of TESH, 0·15M KCl containing [^{3}H]-progesterone (10 ng). After a 20-min incubation at 0°, 30 μg of unlabeled progesterone were added. The nuclei were centrifuged at 5000 *g*, washed twice with 3 ml TESH, and then extracted with 1·0 ml TESH, 0·4M KCl for 40 min. The preparation was centrifuged at 26,000 *g*, and the supernatant fraction was saved for sucrose-gradient analysis.

Interaction of binding proteins with DNA

The techniques of Toft (this symposium) were used to study the interaction between binding proteins and DNA. The interaction was detected by measuring the disappearance of the normal 4S receptor peak on sucrose gradients when DNA (> 14S) was present. Either crude cytosol or binding component A or B containing 20–40,000 c.p.m. of bound [^{3}H]-progesterone was incubated for 1 h at 4°C with 100 μg of purified native calf thymus DNA (Sigma) or native chick DNA in TESH containing 0·1M KCl. The final reaction volume was 0·35 ml. After incubation, 0·2 ml samples were layered on 5–20% sucrose gradients in TESH, 0·1M KCl.

Interaction of binding proteins with chromatin

Binding component preparations or crude cytosol were assayed for chromatin binding activity by a method which has been described in detail elsewhere [13, 14]. Binding protein preparations containing up to 100,000 c.p.m. [^{3}H]-progesterone bound/ml were added (0–200 μl) to 50 μg of oviduct or spleen chromatin in TESH, 0·15M KCl, pH 7·0, containing 0·5 mg bovine serum albumin, mixed, and allowed to stand at 0° for 1 h. The final reaction volume was 0·5 ml. Then the chromatin was pelleted, washed, and resuspended in 0·005M Tris, 0·15M NaCl, 0·01M $MgCl_2$, pH 7·0. The chromatin was then collected on Millipore filters (HAWP). The filters were dried and counted for ^{3}H.

RESULTS

Our earlier studies of receptor biochemistry used crude soluble cytoplasmic oviduct preparations. In order to study receptor-mediated hormone effects more

directly, it was desirable to isolate receptors relatively free of cytoplasmic contaminants. The starting material for this purification was soluble oviduct cytoplasmic fraction from chicks treated for 15 days with diethylstilbestrol. This cytosol was prelabeled *in vitro* with [^{3}H]-progesterone.

Initial purification was done by adding ammonium sulfate to 30% saturation Table 1). The pelleted proteins contained 67% of the binding activity of the cytosol. This step produced a 25-fold increase in specific activity expressed as bound radioactivity per mg protein. The procedure precipitated less than 1% of the transcortin in a labeled plasma preparation. This small amount had much lower specific activity than the crude plasma. Thus, this step eliminated plasma contaminants.

The ammonium sulfate precipitate was next chromatographed by agarose gel filtration, and a single binding peak eluted. The macromolecular binding material was then concentrated by ultrafiltration and chromatographed on a DEAE-cellulose column as shown in Fig. 1.

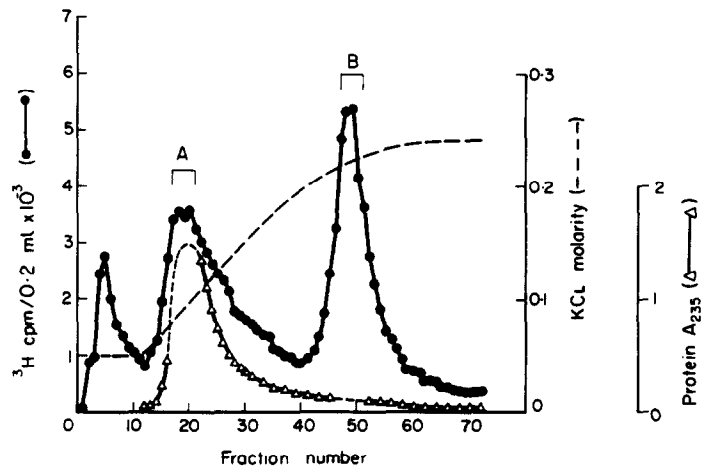

Fig. 1. DEAE-cellulose chromatography of binding proteins after ammonium sulfate precipitation, Whatman DE-52 ion exchanges equilibrated in 0·01M Tris-HCl, pH 7·4 0·001M Na_2EDTA, 0·012M thioglycerol (TESH) used in column 2·0 cm × 5·0 cm at 0°. The applied proteins in TESH were washed through the column with TESH; the column was then eluted with a KCl gradient (----) in TESH. Aliquots (0·1 ml) of 3·0 ml fractions were counted for radioactivity (●--●). Protein concentration determined by absorbance at 235 nm compared to bovine serum albumin standards (△--△).

The first peak was not retarded by the resin, co-chromatographed with free progesterone, and had no binding activity on sucrose gradients. When the column was then eluted with a KCl gradient, two peaks of radioactivity, A and B, eluted in equal amounts. The first, fraction A, eluted with the major protein peak while the more acidic second peak, fraction B, eluted at higher KCl molarity. Fractions A and B prepared in this way were about 1000 and 3000-fold purified compared to the original homogenate.

As shown in Fig. 2, both components A and B sedimented as 4S macromolecules on sucrose gradients containing KCl just as native cytosol receptor did. However, under low-salt conditions, in which native receptor sedimented as an 8S species, the binding activity of peak A was lost and the radioactivity was distributed throughout the tube.

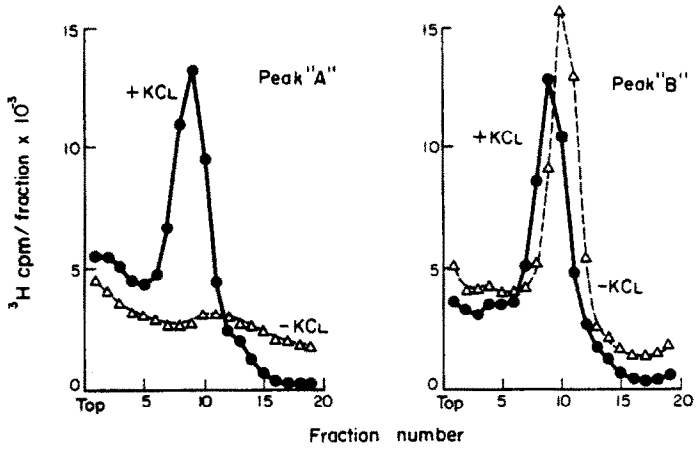

Fig. 2. Sucrose gradient analysis of chick oviduct progesterone receptor components obtained by DEAE-cellulose chromatography. Samples (0·2 ml) of receptor layered over 5–20% sucrose in TESH buffer with 0·3M KCl as indicated. Centrifugation in Spinco SW-50·1 rotor at 45,000 rev/min for 16 h at 2°C. Fractions (0·25 ml) collected and diluted with 0·5 ml H_2O for radioactivity determination. Left panel: analysis of peak A in gradient with 0·3M KCl (●--●) and in TESH only (△--△). Right panel: analysis of peak B in gradient with 0·3M KCl (●--●) and in TESH only (△--△).

Peak B remained a stable 4S molecule. No 8S material has been found, and A and B have not been interconverted or recombined. Thus A and B are physically distinct from each other and from native cytosol receptor in solutions without KCl. The purified materials were stable for at least 100 h at 0°, but were still sensitive to heat and freezing.

To test whether both A and B were specific progesterone receptors we tested their relative binding affinity for several other steroids in a competition assay. Table 2 shows that the progesterone binding specificity of crude cytosol has been retained by the ammonium sulfate pellet P_1 and by purified components A and B from DEAE-cellulose. All four receptor fractions tested had the same pattern of hormone specificity, binding progesterone and its biologically active metabolite 5α-pregnane-3,20-dione much more tightly than testosterone, estradiol, or cortisol.

The similarity between the purified and crude material was then studied kinetically. Table 3 shows that cytosol receptor, fraction A, and fraction B all had similar equilibrium constants. The low equilibrium constant shows that A and B both exhibit the typical tenacious binding behavior reported for tissue hormone receptors. A and B have also been shown not to be metabolic enzymes since neither A nor B metabolizes progesterone *in vitro*.

Thus, the highly purified receptor components represent the same type of binding activity seen in native cytosol, and are stable enough to warrant further investigation of their biological effects.

Since the receptor components A and B were purified from cytosol labeled *in vitro*, we then questioned whether both components also bound progesterone *in vivo*. To study this, chicks were injected with tritiated progesterone, and oviduct cytosol was prepared 30 min later. The cytosol was resolved into receptor components A and B on DEAE-cellulose by a stepwise KCl elution. Figure 3

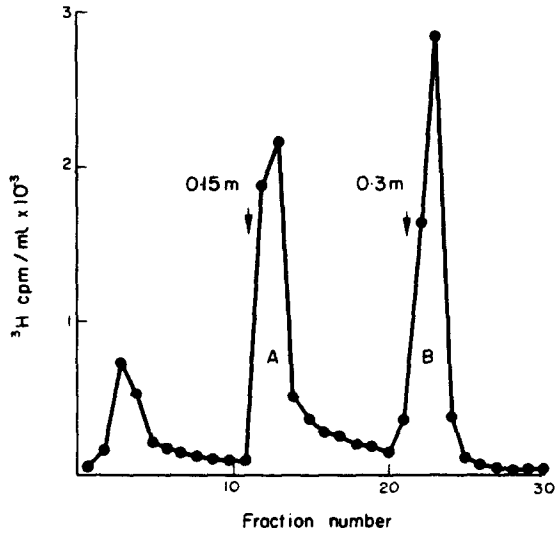

Fig. 3. DEAE-cellulose stepwise KCl elution profile of receptor components A and B from chick oviduct cytosol prepared 30 min after injection of 200 μCi [1,2-^{3}H]-progesterone via left wing vein. Cytosol (1·0 ml) applied in TESH to 5 ml column of DEAE-cellulose. Aliquots (0·1 ml) from KCl eluted fractions (3·0 ml) counted for ^{3}H.

shows that the crude cytosol labeled *in vivo* contained both receptor components, again in equal amounts.

A variety of cytologic, radioautographic and biochemical studies [17–19] have demonstrated that steroid hormones appear in target-cell nuclei soon after hormone administration. Our early studies had shown a requirement for receptors to transport progesterone to nuclei [11, 12].

Since receptors isolated from nuclei behaved as a single 4S species on sucrose gradients, it was of interest to ask which 4S cytoplasmic component, A or B, appeared in nuclei. To study this, oviduct slices were incubated *in vitro* with tritiated progesterone. Nuclei purified from these oviducts contained salt-extractable receptors which chromatographed on DEAE-cellulose as shown in Fig. 4. Significantly, both cytoplasmic components A and B appeared in the nuclei in equal amounts.

To study this nuclear uptake process more carefully, prelabeled A or B, crude cytosol, or buffer alone were incubated with purified oviduct nuclei. After incubation the washed nuclei were extracted with salt, and the receptors sedimented on sucrose gradients in KCl.

Figure 5 shows the results of that experiment. The two higher peaks show the 4S receptor activity extracted from nuclei incubated with components A or B. The lower dotted line represents the nuclear 4S component found when crude cytosol was incubated with nuclei. The lowest curve shows the lack of 4S binding when tritiated progesterone was incubated with nuclei in buffer alone. Thus, both A and B are taken up by isolated nuclei *in vitro*.

Due to the appearance of both components in nuclei, we asked the following question: do receptors have specific roles in target-cell nuclei, and do the two receptor components have separate functions?

Their possible roles in the nucleus were studied in the following experiments.

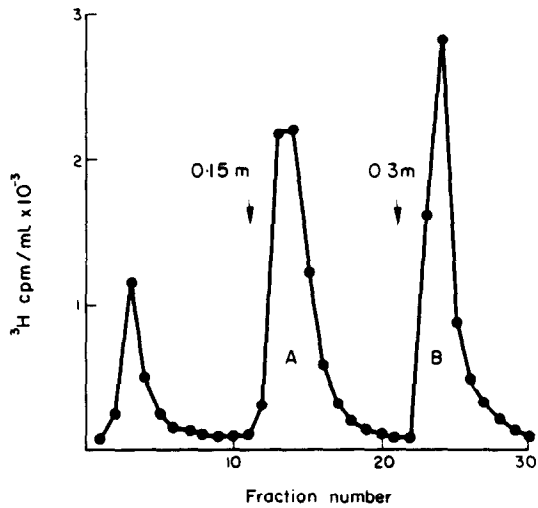

Fig. 4. DEAE-cellulose stepwise KCl elution of nuclear receptors as described in Fig. 3. Oviduct slice incubated at 0° for 5 min in Basal Eagle's Medium with 10^{-7}M [1,2-^{3}H]-progesterone. Slices then transferred to fresh Eagle's, and incubated 30 min at 37°C. The tissues were then rinsed, homogenized in TESH, and the nuclear pellets obtained. After several buffer-sucrose washes with 0·2% Triton X-100, nuclei were collected by centrifugation and receptors extracted with 0·3M KCl in TESH for 40 min at 0°C. Extracts were diluted 1 : 10 with TESH and chromatographed as shown to detect ^{3}H in A and B fractions (●--●).

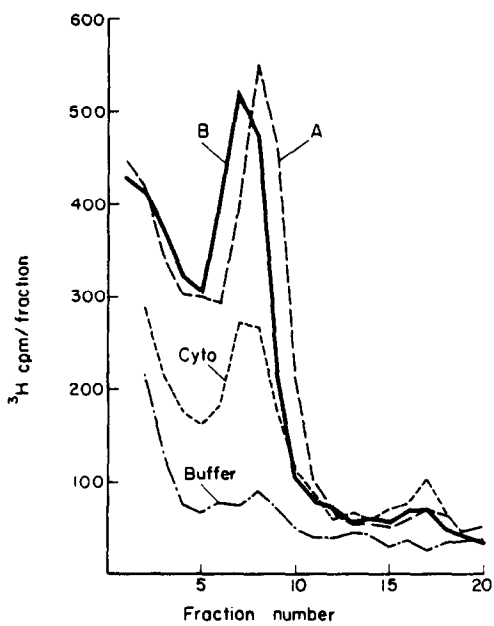

Fig. 5. Sucrose-gradient analysis of receptors extracted from isolated nuclei. Receptor preparations labeled with [^{3}H]-progesterone were incubated at 0°C with purified oviduct nuclei for 30 min. Then the nuclei were collected by centrifugation, washed, and extracted with 0·3M KCl in TESH for 40 min. This extract was applied to sucrose gradients containing 0·3M KCl. The 4S receptor peaks shown were from nuclei incubated with either cytosol (· · ·), Fraction A (---), Fraction B (———) or [^{3}H]-progesterone in buffer only (-·-·).

When A or B was incubated with DNA and then sedimented on sucrose gradients in 0·1M KCl[20], the results in Fig. 6 were obtained. The left panel shows that the normal 4S binding peak of component A was bound to the DNA and carried down the centrifuge tube. This effect is not species specific; it occurs with either calf or chick DNA.

The right panel, however, demonstrates that component B did not interact with the DNA since the 4S peak remained intact. Thus, the two components differ markedly in this operational way.

Other work in this laboratory has shown that many cytoplasmic steroid receptors will interact only with their respective target cell chromatins[21].

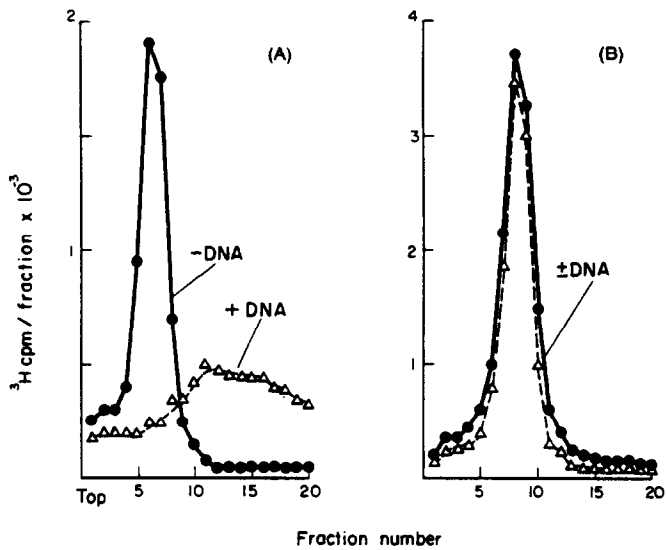

Fig. 6. Sucrose-gradient analysis for receptor interaction with DNA. Gradients in 0·1M KCl were used. Receptor preparations were combined with 100 μg DNA for 2 h at 0°C. Then the preparations were centrifuged to pellet the DNA (14S). Panel A: Receptor component A without DNA (●–●) and after DNA treatment (△--△). Panel B: Receptor component B without (●–●) and with DNA (△--△).

As an *in vitro* assay for chromatin binding, receptor components A and B were incubated with oviduct or spleen chromatin[13, 14]. Chromatin-bound receptor was separated by centrifugation from unbound receptor. When increasing amounts of A or B were incubated in this way, the results of Fig. 7 were obtained. Fraction B demonstrated enhanced binding to oviduct chromatin compared to a non-target tissue such as spleen. On the other hand, fraction A, although stable under these conditions, showed no binding to either chromatin preparation. Thus, another difference between the two components was detected by this procedure.

DISCUSSION

We have thus purified and characterized two 4S components of the chick oviduct progesterone receptor system. The similarity between the crude and purified materials lends further support to previous work done using crude preparations. Furthermore, the components account for the functional activity of crude

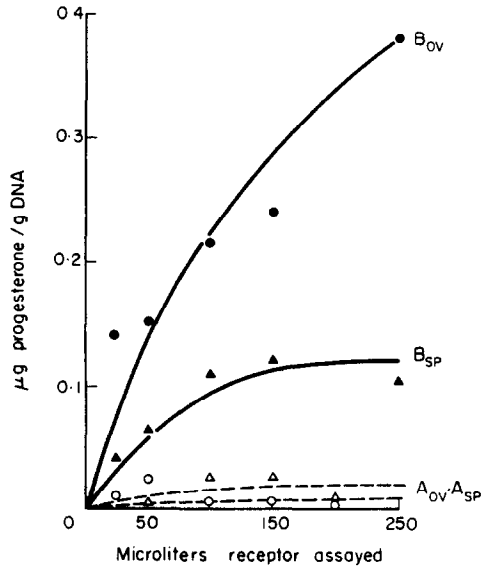

Fig. 7. Binding of purified receptor components to chromatin. Labeled receptor preparations in TESH, 0·15M KCl combined with 60 μg purified chick oviduct or chick spleen chromatin. Final volume was 0·5 ml. After 1·0 h chromatin was pelleted by centrifugation, washed twice with buffer, aggregated with $MgCl_2$, and collected on Millipore filters for counting. Plots show component B binding to chromatin of oviduct (●–●) or spleen (▲–▲), and component A binding to chromatin of oviduct (○--○) or spleen (△--△).

cytosol receptor, suggesting that the crude material consists of equimolar amounts of components A and B.

The possibility is therefore suggested that these two receptor components could play separate roles during the initial interaction of the receptor-hormone complex with chromatin in the target cell. Component B may specifically bind to the chromatin protein while component A simultaneously interacts with the DNA itself. If the hormone-receptor complex is indeed the inducer unit for steroid hormone modulation of nuclear RNA transcription, then this differential binding to the genome may prove to be of major importance to steroid hormone action.

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DISCUSSION

Munck: Bert, is there a temperature-dependent step in the interaction of the complete receptor complex with the chromatin? And can you say anything about whether such a step is necessary for the separated components of the receptor for binding to DNA or to the acidic protein?

O'Malley: It is not a temperature-dependent step in that it binds quite well at 0°C. Our impression is that the transfer into the nucleus and the delivery to the acceptor site may be accelerated slightly by heat. We are presently in the midst of removing off these acidic proteins and trying to determine whether they are interacting with the receptor while they are separated free from chromatin.

Crabbé: First may I express my appreciation for this beautiful presentation. I would like to know whether the acidic protein coating DNA and which supposedly plays a critical role in the expression of hormonal action was present from the outset. I gather that the data about progesterone are all obtained with DES-treated oviducts. Is the acidic protein somehow "induced" by this DES treatment? Secondly, I would like to have your views on how (provided the acidic protein is also inside the nucleus) the nuclear progesterone-receptor complex finds its way around on the DNA chain and "hits" the gene that would be critical for transcription eventually. In other words, is the acidic protein evenly distributed along the DNA chain, or does this acidic protein form a heterogenous population?

O'Malley: That is an interesting question, and one we did look at (Spelsberg, Steggles, O'Malley, *Biochim. biophys. Acta.*, in press). Fortunately, we could do this with the oviduct in that we had an unstimulated tissue. The undifferentiated oviduct, before estrogen treatment, has some capacity for progesterone to induce a small amount of avidin. As you treat with estrogen, you increase the capacity of this tissue to respond to progesterone by making avidin. We first looked at the total quantity of cytoplasmic receptor during this treatment and we found that the quantity of cytoplasmic receptor went up with estrogen treatment, and we induced the total specific activity of receptor relative to other proteins. So before we even knew about the acceptor proteins, we figured that because of the influence of estrogens the oviduct had more total receptor and could therefore respond better to progesterone. When we examined the DNA acidic proteins, there was very little change in the total amount of acidic proteins, but an increase in the amount of acceptor capacity for the receptor per quantity of chromatin was noted. So it seems that estrogen increases both the amount of receptor and of acceptors. The cell is then geared to produce a maximum response.

The acidic protein fraction in general is a very heterogeneous fraction: there may be 18–22 polyacrylamide bands in the AP3 fraction alone. In reference to the DNA chain, there are probably many acidic protein acceptor sites. We are coming to the realization that there is not simply one gene site to which the receptor binds. There are probably a number of reaction sites within the nucleus. It appears most likely that the cell also delivers more steroid-receptor complex into the nucleus than it needs. This would thus create a saturation condition within the nucleus. The steroid-receptor complex may pick out the acceptor sites by a conformational protein–protein recognition which has a high affinity in favor of the bound state. We know nothing yet about the mechanism for release of the receptor from nuclear chromatin.

Van der Molen: As I understand, Bert, you have been monitoring most of your binding on the basis of the tritiated progesterone. Would it be possible, once you have the receptors, to shed the progesterone as a marker and to demonstrate that the receptor itself, rather than the steroid, will give the interactions with the genome? I was thinking about a proof of the interaction between the acidic protein and the receptor without the use of a labelled steroid. Is that a possibility?

O'Malley: It should be possible eventually, except that there is very little receptor in the cell, it is difficult to label by amino acid tritium incorporation.

Jensen: I might say that the problem at the moment is that you need the radioactive steroid as a marker. So this question of being able to label the receptor protein with some other isotope is a very important one. We believe we have now labelled the estrogen receptor with ^{32}P *in vivo*, and it appears that the ^{32}P of the receptor and the tritium of the steroid move together into the nucleus on *in vitro* incubation. Once one has a way of looking at the protein without the steroid, there are lots of things you can do with it. Whether there is phosphorus in the progesterone receptor too, one does not know, but I have the feeling that phosphorus may be a component of all these receptor proteins.

Siiteri: Dr. O'Malley, I gather that you feel that the role of histones is minor in the processes involved in expression of genome activity, contrary to Dr. Bonner and some others who have shown some rather specific effects of hormones in terms of increasing template activity. Since you have a receptor which recognizes acidic proteins—specific acidic proteins, presumably—and also DNA, how does the receptor ignore, if you will, the histones which are on the outer portions of the chromatin?

O'Malley: That is an interesting question, especially in view of the fact that the receptor is an acidic protein with an "isoelectric point." of 4–4·5. The histones are very basic, and one would think there could be a natural charge interaction. With respect to the receptor interaction of chromatin, one could consider that there is probably a specific conformational interaction of protein to protein because it is an acidic protein and an acidic protein interacting. But in fact, at least on an affinity basis, they do tend to ignore the histones.

We have a series of projects going on to understand what makes a cell differentiate from one cell into another, and what maintains the restriction of a particular cell. Why does a given cell make only certain proteins and RNA's, and are the levels of RNA's in the cells regulated by simple molecular inductions? Our evidence indicates that the major regulatory molecules in the chromatin are the acidic proteins. Not only does this so-called "inducer complex" interact with the

acidic proteins, but the acidic proteins also seem to be important in maintaining chromatin restriction, i.e. they direct chromatin to make RNA's of a certain type, e.g., erythrocyte chromatin to make erythrocyte RNA's, liver chromatin to make liver RNA's. When one makes hybrid chromatins by substituting acidic proteins, one changes the type of RNA's made on the basis of the source of acidic proteins. Dr. Spelsberg has done that in our lab, and by similar methods Drs. Paul and Gilmore in Scotland have come to the same conclusions.

I do not know exactly how chromatin is structured. One could conceive that within chromatin there are areas of naked DNA, areas of DNA covered by acidic protein which interact with inducers and thus modulate the quality of RNA being made in the cell. Finally, there may be areas of DNA covered by both acidic protein and histone which perhaps are permanently restricted. The histones may thus be important in permanent restriction of genes but not in fine modulation by inducers to change the amounts of RNA's transcribed from certain genes. Interestingly enough Dr. Allfrey has shown that you take naked DNA, add increasing amounts of histones, and shut down all transcription of the DNA by RNA polymerase. But recent evidence generated by Dr. Spelsberg reveals that if you add acidic proteins from that tissue to the DNA first, then histones can no longer completely shut down transcription of the DNA. The acidic proteins maintain certain open areas on the DNA, even though the two macromolecules have opposite charges. My money at this point lies with the acidic proteins as the most important regulators of cell function and the important determinants in cell specific restriction of transcription.

Wira: You may have already answered this question, but I am wondering if progesterone is necessary for your A fraction to bind to DNA.

O'Malley: I do not have a clear answer yet.