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Progesterone Receptors of Chick Oviduct: Identification of 6S Receptor Dimers

WILLIAM T. SCHRADER,* SHARON S. HEUER, AND BERT W. O'MALLEY

Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77025

A 6S progesterone receptor form has been identified in chick oviduct cytosol fractions. It has been shown to consist of equal amounts of two 4S receptor subunits, A and B, previously shown to bind in nuclei to DNA and chromatin sites respectively. The 6S form is tentatively defined as a dimer of both A and B subunits. It is present in cytosol under conditions in which large *in vitro* aggregate formation (8S) is prevented. The 6S dimer co-chromatographs with authentic receptor B on DEAE-cellulose, but does not bind to phosphocellulose. Dissociation of the dimer with salt or temperature liberates the receptor subunits in their monomeric 4S forms.

Earlier work in our laboratory has identified progesterone receptor proteins of the chick oviduct (Sherman *et al.*, 1970; Schrader and O'Malley, 1972). These proteins have been shown to be specific for progesterone and its biologically active metabolite, 5α-pregnane-3,20 dione (Smith *et al.*, 1974; Strott, 1974). The receptors appear to function by complexing with progesterone in the cytoplasm and then moving into the nucleus, where the complexes associate with specific sites of the oviduct chromatin (Spelsberg *et al.*, 1971; Spelsberg *et al.*, 1972). These interactions are considered likely intermediates in the progesterone induction of messenger RNA synthesis of the egg-white protein, avidin, and for all other gene regulatory events associated with the hormone's biologic activity (O'Malley *et al.*, 1969; O'Malley and Means, 1974). Although the pathway for this process is not completely clear, it seems to involve binding to a limited number of nuclear sites which are present in greater numbers in target cell nuclei than in nontarget nuclei (O'Malley *et al.*, 1971; Buller *et al.*, 1974a). Whatever the tissue source, however, the sites bind receptors strongly by a process requiring hormone on the protein (Buller *et al.*, 1974b).

Our approach to the study of these interesting macromolecules has been to isolate and purify receptors for progesterone and to characterize their interaction with nuclear constituents. Such an analysis has shown that there are at least two progesterone receptors differing in both physico-chemical properties and nuclear function (Schrader *et al.*, 1972; O'Malley and Schrader, 1972). Two 4S forms of the receptors, of molecular weights 110,000 and 117,000 daltons, have been purified nearly to homogeneity (Schrader *et al.*, 1975a; Kuhn *et al.*, 1975; Schrader *et al.*, 1975b). Significantly, separation of the two forms, termed receptors A and B respectively, also separated the two known types of nuclear interaction, namely, binding to DNA (receptor A) and binding to chromatin (receptor B) (Schrader *et al.*, 1972; O'Malley *et al.*, 1972).

As originally detected, the receptor A and B components were present in about equal amounts in cytoplasm. When receptors were taken up by nuclei, the nuclear extracts also showed nearly equal amounts of both forms (Schrader *et al.*, 1972). This suggested the possibility that the two forms might exist in the cell as a dimer or higher aggregate. Such a coupling of two receptors, possibly with much different intranuclear binding activities, led us to suggest that they might function as a dimer *in vivo*. The B subunit was envisaged as acting as a "specifier" unit, leading the dimer to proper loci in the chromatin where the A subunit's DNA-binding activity would somehow then function as a regulator of gene

* To whom reprint requests should be sent.

activity (O'Malley *et al.*, 1972; O'Malley *et al.*, 1973).

We were unable to prove the existence of dimers between receptors A and B, nor to recombine the isolated purified forms. Hence, this idea had no experimental evidence to support it. In this communication we report the use of phosphocellulose chromatography (Schrader *et al.*, 1975a) and sucrose gradient ultracentrifugation to analyze receptor forms in oviduct cytosol. A distinct receptor form, heavier than the monomers, has been detected. This heavier 6S form is shown to be dissociated by either ionic strength or brief warming into equal amounts of the A and B receptor subunits. Furthermore, the partially purified A receptor subunit has been recombined with B subunits from cytosol to reconstitute the 6S receptor dimer *in vitro*.

These observations lead to some speculation regarding the biologically active form of receptors *in vivo*.

MATERIALS AND METHODS

Chemicals and Reagents

Sources for all chemicals and reagents have been outlined in previous publications (Schrader *et al.*, 1972). Ion-exchange resins (DEAE-cellulose and phosphocellulose) were from Reeve-Angel. [1,2,$^3H_2$] progesterone (50 Ci/mmole) was from New England Nuclear. Nonradioactive progesterone was from Steraloids. Both were used without further purification.

Buffers

One standard buffer was used for all experiments. The ionic strength was varied appropriately by the addition of potassium chloride as indicated. The buffer, Buffer A, contained 10 mM Tris-HCl, pH 7.4-1 mM Na_2EDTA-12 mM 1-thioglycerol.

All steps of the procedures were performed at 0°-2° except as noted in the text and figure captions.

Scintillation Counting

Radioactive samples were counted in a toluene-POPOP-PPO fluor containing 33 percent Triton X-100 with a counting efficiency of 33 percent as described in earlier publications (Schrader *et al.*, 1972).

Sucrose Gradient Ultracentrifugation

This procedure was done as described previously (Schrader *et al.*, 1972) in 5 percent to 20 percent sucrose in Buffer A. Where required, 0.3 M KCl was added as listed in the text and figure captions. Gradients were run in Beckman SW-50.1 rotors at 0° for 16 hours at 45,000 rpm. The tubes were pierced and fractionated (0.2 ml fractions) in an ISCO Model D gradient fractionator.

Animals

White Leghorn chicks 30 days of age were used in all experiments. The animals were stimulated daily before use for two weeks with 5 mg/day of diethyl-stilbestrol in sesame oil (subcutaneous route of injection).

Preparation of Cytosol

Chicks were killed by cervical dislocation and the oviducts were removed, rinsed in 0.9 percent NaCl and homogenized at 0° in 4 ml Buffer A/g tissue as described previously (Schrader *et al.*, 1972; Schrader, 1974; Schrader *et al.*, 1974). Cytosol (cytoplasmic soluble fraction) was prepared by ultracentrifugation at 0° for 1 hour at 140,000 × g. Floating fat was aspirated from this cytosol and it was labeled with ^{3}H-progesterone by addition of the isotope to a final concentration of 10^{-8} M.

Ion-exchange Chromatography

Small columns (5 ml) of either DEAE-cellulose or phosphocellulose (PC) were prepared and equilibrated in Buffer A as described previously (Schrader and O'Malley, 1972; O'Malley and Schrader, 1972; Schrader, 1974). Receptors were applied to the columns in Buffer A and washed with 50 ml Buffer A.

Linear KCl gradients in Buffer A were used to elute the receptors from the columns. Samples were assayed from each fraction for radioactivity and conductivity. Free progesterone does not bind or elute from either column.

RESULTS

When labeled cytosol was analyzed on sucrose gradients the results of Figure 1 were obtained. When 0.3 M KCl was included in the gradient, a radioactive peak at about 4.2S was observed as has been shown previously (Sherman *et al.*, 1970; Schrader and O'Malley, 1972; Toft and O'Malley, 1972). Earlier studies had shown this peak to contain both A and B receptor forms. When gradients containing only Buffer A without KCl were analyzed, however, a more complex profile was seen. The receptors showed only a slight shoulder at the 4S region and a double peak at 6S and 8S. The two higher molecular weight forms normally contained about equal amounts of radioactivity. Our past experiments had failed to show this doublet adequately (Schrader and O'Malley, 1972; Toft

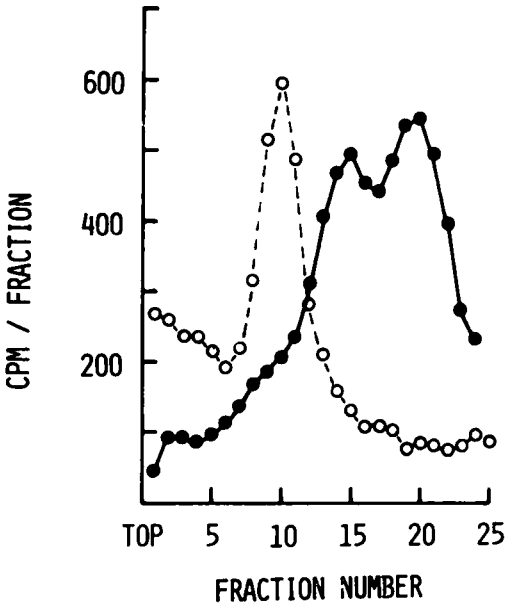

FIG. 1. Sucrose gradient ultracentrifugation of oviduct progesterone receptors from estrogen-primed chicks. Oviducts were removed from chicks stimulated for 14 days with diethylstilbestrol, homogenized in Buffer A (4 ml/g) and cytosol was prepared and labeled with [^{3}H] progesterone as described in Methods. Samples (200 μl) were layered over 5% to 20% sucrose gradients in Buffer A (●——●) or in Buffer A containing 0.3 M KCl (O- - -O). The tubes were centrifuged in a Beckman SW-50.1 rotor at 2° for 16 hours at 45,000 rpm. Tubes were pierced, 0.2 ml fractions were collected, and each fraction was counted for ^{3}H as described in Methods. Standard proteins run under identical conditions were determined in separate experiments by the absorbence of each fraction at 280 nm. Hemoglobin (4.2S) and aldolase (7.9S) ran in fractions 11 and 19 respectively.

and O'Malley, 1972). Generally, either one of them was seen, or at best, a broad peak encompassing the entire lower half of the gradient had been observed. Due to the problems other laboratories had experienced in understanding the complex sedimentation behavior of estrogen receptors in uterus (Chamness and McGuire, 1972; Jensen *et al.*, 1969), no concerted effort had been made to analyze the low-salt forms of the progesterone receptor complexes. As will be apparent below, however, an important observation had already been made earlier: Toft and O'Malley (1972) had reported that chicks which had not been stimulated by estrogen contained progesterone receptor which did not aggregate to an 8S form. In other words, the ability of the receptor to form this 8S complex was itself estrogen-dependent.

In similar experiments, shown in Figure 2, oviducts from unstimulated chicks showed a normal 4S receptor peak on high salt sucrose gradients, but no 8S form in gradients without KCl. Significantly, the no-KCl profile did show the existence of a 6S species. This complex, then, was also salt-dissociable but occurred regardless of the estrogen history of the tissue.

It was therefore of interest to analyze the receptors in cytosol more closely in order to study this 6S entity.

Our laboratory has developed several ion-exchange methods useful for differentiating among various receptor forms (Schrader, 1974). The first of these applied to the cytosol

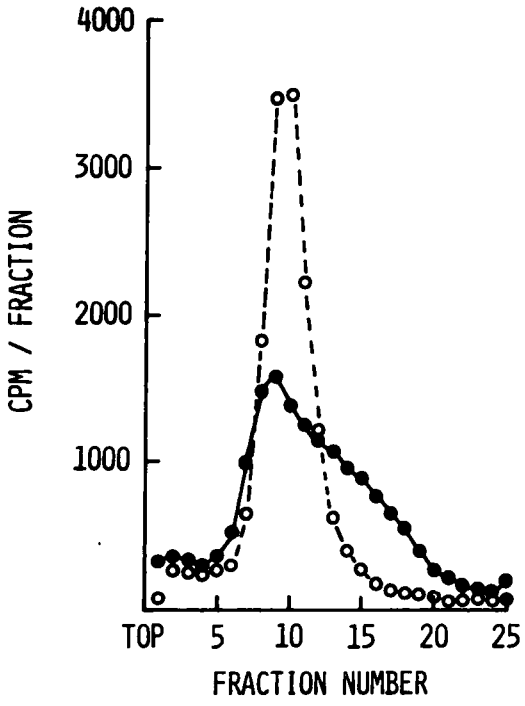

FIG. 2. Sucrose-gradient ultracentrifugation of oviduct progesterone receptors from immature, untreated chicks. Oviducts were removed from 7 day old chicks, processed into cytosol, labeled and analyzed on sucrose gradients as described in Methods and Figure 1. Gradients contained either Buffer A alone (●——●) or Buffer A containing 0.3 M KCl (O- - -O).

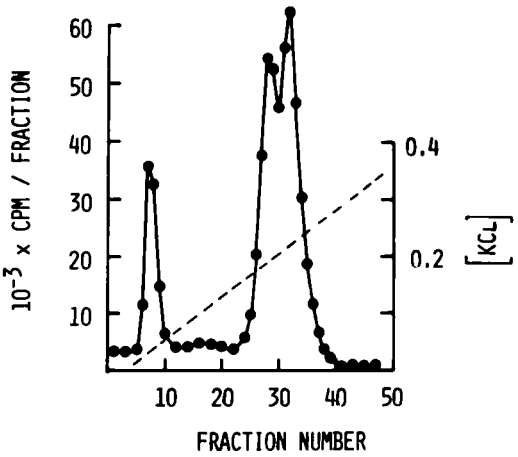

FIG. 3. DEAE-cellulose column chromatography of labeled oviduct cytosol. Cytosol from estrogen-primed chicks was labeled with [^{3}H]progesterone in Buffer A. It was applied to a 5 ml DEAE-cellulose column and washed with 50 ml Buffer A. The column was eluted with a 100 ml gradient of KCl in Buffer A from 0 to 0.4 M KCl. Fractions (2 ml) were collected and counted for ^{3}H (●——●). Conductivity of each fraction was determined and converted to KCl molarity (- - -).

directly was DEAE-cellulose as shown in Figure 3. It can be seen in this figure that the pattern is markedly different from that previously reported (Schrader and O'Malley, 1972) in which an ammonium sulfate precipitate was chromatographed. First, corticosteroid binding globulin is now seen as the first peak off the column at about 0.05 M KCl. The second peak at 0.1 M KCl represents the small amount of receptor A protein free in the cytosol.

Most of the radioactivity chromatographed as a pair of peaks at 0.2 M and 0.24 M KCl respectively. Since authentic receptor B elutes at 0.2 M KCl, we first analyzed the 0.24 M KCl peak by sucrose-gradient ultracentrifugation. In experiments not shown here, we found that this peak represented the 8S receptor form. The peak at 0.2 M KCl was then examined by the same procedure and found to sediment as a pair of peaks at 4S and 6S. This suggested that the 0.2 M KCl cytosol peak contained both the receptor B form and also the 6S form.

To establish this more clearly, it was necessary to prepare 6S receptor free of 8S contaminants. This was done by sucrose gradient ultracentrifugation as shown in Figure 4. Stancel and co-workers (1973) had shown that the 8S receptor aggregate involved complexing of the receptors with some other molecules present in the cytosol. These molecules were even present in nontarget tissues. These workers noted that the association of receptors with these factors began only after homogenization of the tissue and could be prevented or greatly retarded by preparing cytosol as a more dilute homogenate than was normally done. This technique was used in the experiments shown in Figure 4.

As the cytosol was progressively diluted, less 8S receptor was seen but more 4S remained in the preparation. Significantly, there was very little change in the amount of 6S complex, again pointing to the notion that this form was present in the tissue *in vivo*.

EFFECT OF DILUTION ON RECEPTORS

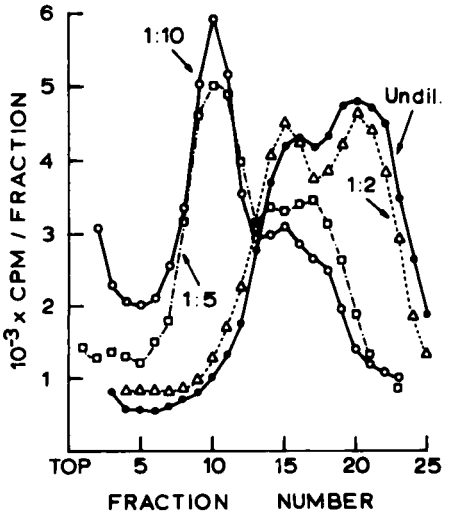

FIG. 4. Sucrose-gradient ultracentrifugation of labeled cytosols following dilution. Oviduct cytosol from estrogen-primed chicks was prepared as described in Methods. Aliquots of labeled cytosol were diluted immediately after preparation by addition of cold Buffer A. Final dilutions of cytosol were: undiluted, (●——●); diluted 1:2 (Δ- - -Δ); diluted 1:5 (□- - -□); and diluted 1:10 (○——○). These preparations had protein concentrations of about 20, 10, 4 and 2 mg/ml respectively. After dilution the samples were analyzed by sucrose-gradient ultracentrifugation in Buffer A as described in Methods and Figure 1. After counting, the uncorrected counts in each fraction were adjusted to indicate a constant amount of radioactivity on each gradient.

Sucrose gradients of 1:10 diluted cytosol were run, pierced at 0° and the 6S regions were collected. When these pooled fractions were chromatographed on a DEAE-cellulose column, the results of Figure 5 were obtained. The 6S receptor chromatographed at 0.2 M KCl, and thus was indistinguishable from authentic receptor B by this technique. Further proof of their similarity was obtained by mixing equal amounts of labeled B receptor and the 6S complexes, and rechromatographing on DEAE-cellulose. This procedure showed a single peak at 0.2 M KCl.

The 6S form was then analyzed by phosphocellulose chromatography. On this resin, receptor B elutes at 0.26 M KCl (Schrader *et al.*, 1975a; Schrader *et al.*, 1975b). Interestingly, the 6S receptor form did not bind to PC at all in Buffer A but washed through the column intact.

This chromatographic method was used to determine whether the 6S receptor form consisted of both A and B receptor components. Such a possibility was intriguing, since the existence of AB dimers *in vivo* might account for the appearance of equal amounts of both monomers in nuclei following hormone administration (Schrader *et al.*, 1972). The results of this study are shown in Figure 6. When 6S receptors were treated with either 0.15 M KCl or heated to room temperature for 30 minutes, the complexes were converted into two forms in equal amounts which bound to the column and eluted at 0.26 and 0.3 M KCl respectively. These receptors were shown to be receptors B and A by rechromatography with authentic receptor standards.

This experiment strongly suggested that the 6S receptor complex is a dimer of receptors A and B, which has distinctive charge properties different from the monomers.

A definitive proof that the 6S is an A-B complex would require reassociation of A and B monomers *in vitro* to form this complex. Conversion of labeled ^{3}H-A receptor to a 6S complex by incubation with unlabeled oviduct

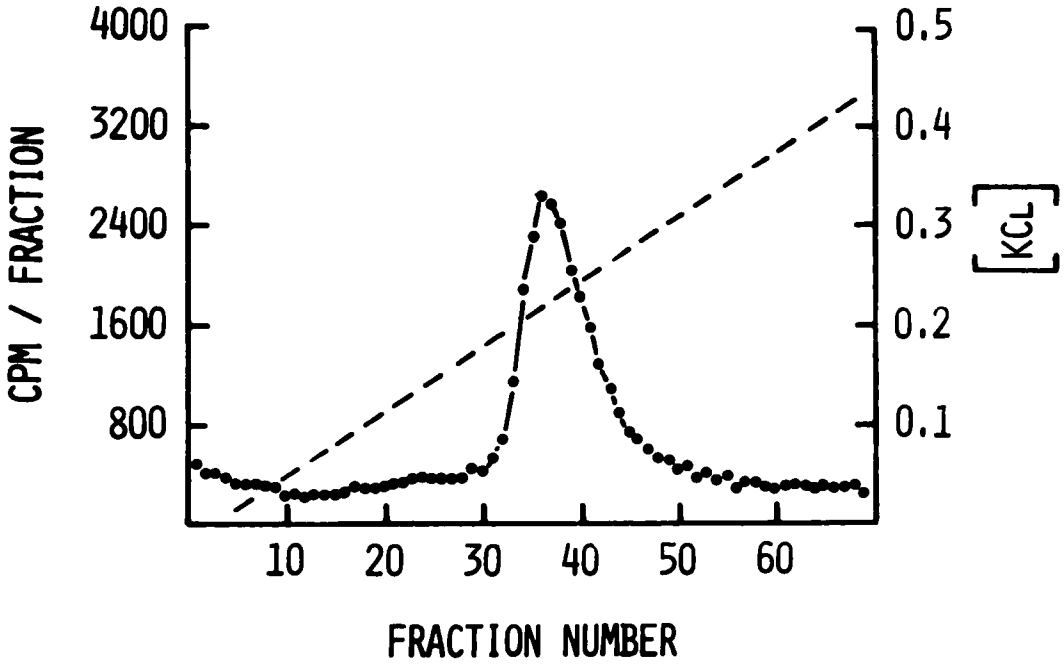

FIG. 5. DEAE-cellulose column chromatography of 6S receptor complexes. Oviduct cytosol was labeled and diluted 10-fold as described in Figure 4. Six identical sucrose gradients in Buffer A were run using this material as described in Figures 1 and 4. The gradients were tapped at 0°, and fractions 14–16 were pooled. The pooled fractions were applied to a 5 ml DEAE column, washed in Buffer A and eluted with a KCl gradient. Radioactivity (●——●) and conductivity (- - -) were determined as described in Methods and Figure 3.

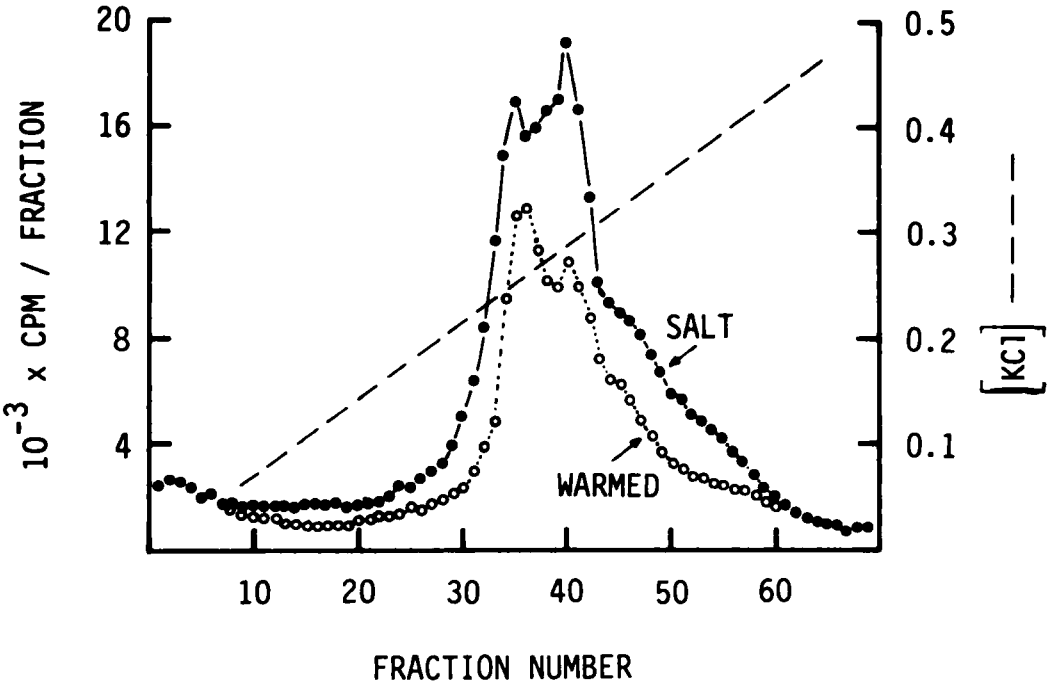

FIG. 6. Phosphocellulose column chromatography of dissociated 6S receptors. Labeled 6S receptors in Buffer A were collected in the drop-through fraction of a 5 ml PC column. They were treated either by warming to room temperature for 30 min (O- - -O) or by adding an equal volume of Buffer A containing 0.3 M KCl to bring the sample to 0.15 M in KCl (●——●). The KCl-treated sample was re-diluted with an equal volume of Buffer A after 30 min to give a final KCl concentration of 0.075 M. Both samples were then analyzed by column chromatography on a 5 ml phosphocellulose column in Buffer A by the same procedure described for DEAE columns in Methods and Figures 3 and 5. Conductivity was used to determine KCl concentrations of each fraction (- - -).

cytosol is shown in Figure 7. No 8S receptor was formed, but virtually all of the A components were converted to a species sedimenting at 6S, apparently by complexing with B subunits in the cytosol. Since endogenous receptor hormone binding sites in the cytosol were blocked with nonradioactive progesterone, and since no label sedimenting at 8S was observed, this conversion cannot be explained simply by acquisition of label by 6S dimers already present in the cytosol.

The 6S receptor dimer thus can exist in cytosol prepared in Buffer A. Since this ionic strength is well below that of the cell, it was important to determine whether this species was stable at physiologic salt concentrations. To attempt to answer this question, the experiment shown in Figure 8 was performed. Labeled 6S receptors from cytosol were collected in the drop-through fraction of a phosphocellulose column and subjected to sucrose gradient ultracentrifugation in gradients containing various levels of KCl. As shown in the figure, conversion from 6S to 4S was about half complete at 0.1 M KCl, and was 100 percent at 0.2 M KCl. This information suggests that the 6S dimer can exist as a metastable species at physiologic (*in vivo*) conditions.

DISCUSSION

The experiments described above give evidence for a 6S progesterone receptor dimer which might be stable *in vivo* and contain both receptor monomers. Such a molecule would be able to interact with either DNA or chromatin binding sites.

Other laboratories have reported estrogen receptors of about 6S in size (Giannopoulos and Gorski, 1971; Erdos *et al.*, 1971). One of

these reports was an attempt to determine the nature of the receptors as they exist *in vivo* (Erdos *et al.*, 1971). It is interesting that their conclusion was that 4S, 6S and 8S receptors could be found. We are presently studying the inter-conversion among these various forms more completely, using both size and charge characteristics. Since the 8S receptor is essentially absent in diluted preparations and appears in a time-related fashion *in vitro*, we have viewed this form as being artifactual, although of considerable analytical interest. The monomers A and B and their nuclear binding sites have drawn most of our attention.

Purification of both monomers by two different procedures (Kuhn *et al.*, 1975; Schrader *et al.*, 1975b) has shown that receptors A and B are 4.2S molecules with molecular weights of 110,000 and 117,000 daltons respectively. Neither SDS nor acid-urea gel electrophoresis has given any indication that these monomers are themselves composed of subunits. Our thinking, therefore, is that the hormone and nuclear site of each monomer are both on the same polypeptide chain.

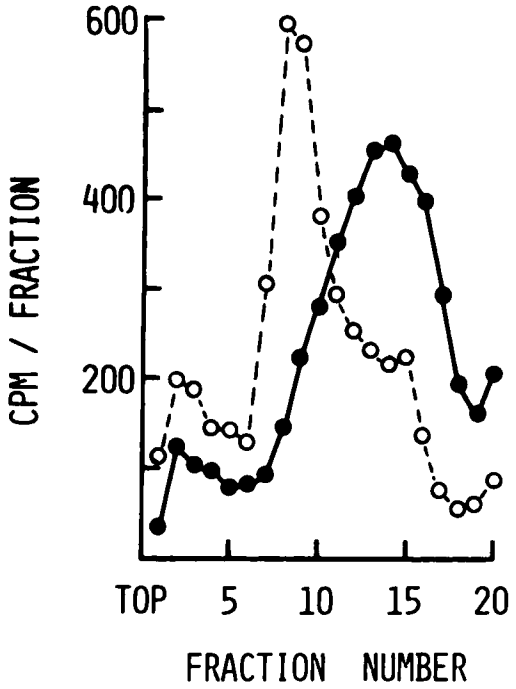

FIG. 7. Recombination of monomers to make 6S receptor form. Labeled receptor subunit A was prepared by stepwise KCl elution of a 5 ml DEAE column to which receptors precipitated with ammonium sulfate had been applied as described in earlier publications (Schrader and O'Malley, 1972; Schrader *et al.*, 1972). This sample was analyzed on a sucrose gradient containing Buffer A (O- - -O) as described in Methods and Figure 1. The sample was also combined with an equal volume of unlabeled oviduct cytosol in which endogenous receptor hormone-binding sites were blocked by addition of nonradioactive progesterone (●——●).

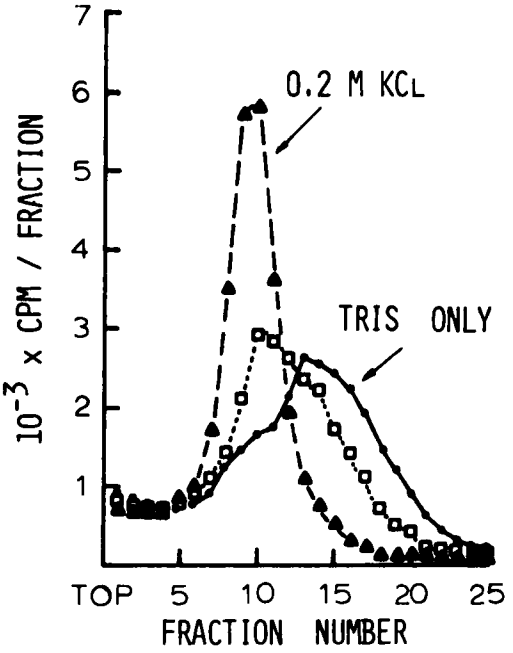

FIG. 8. Salt stability of 6S receptor form. Labeled 6S receptors were prepared as described in Figure 6. Aliquots (200 μl) were layered over sucrose gradients in Buffer A containing either no KCl (●——●), 0.1 M KCl (□···□), or 0.2 M KCl (▲——▲). The gradients were run as described in Methods and Figure 1.

Our present study, however, reestablishes our interest in the association of receptors into functional aggregates. It is interesting to speculate at this point as to the possible role such a dimer might play in the cell. First of all, both types of nuclear binding (DNA and chromatin sites) apparently occur *in vivo*. Both subunits A and B appear in nuclei. Thus, the controversy in the literature over the nature of nuclear "acceptor" sites may in fact be shown to be due to the presence of receptors like the ones described here which have both DNA and chromatin binding sites, but on separate molecules.

If receptor A binding to DNA is in fact the event which modulates gene activity, then receptor B in the dimer may guide the dimer to specific genes destined for control. These genes may be defined by their acidic proteins. Receptor B would then be fulfilling a "specifier" role. Such a bi-functional receptor model was hinted at by us in an earlier communication (O'Malley *et al.*, 1972). At that time, however, no coupling of receptors A and B had been described. There was thus no mechanism to account for a concerted (A + B) gene regulatory process other than independent localization of both receptors coincidentally in equal amounts in the nucleus.

If our initial observations about the nature of the 6S dimer prove correct, this molecule may in fact account for many of these observations. In companion studies of estrogen-mediated gene regulation (Schwartz *et al.*, 1975; Tsai *et al.*, 1975), a model for gene regulation emerges which also features a bi-functional or multi-step induction process. Receptors are thought to interact with acidic proteins adjacent to the polymerase initiation site and then migrate or "search" along the genome until they recognize a specific regulatory site. By our future studies of specific gene regulation in the chick oviduct, we hope to understand more rigorously the mechanism of action of these receptor-hormone complexes.

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REFERENCES

BULLER, R. E., SCHRADER, W. T., AND O'MALLEY, B. W. (1974a). Progesterone-binding components of chick oviduct: The kinetics of nuclear binding. *J. Biol. Chem.* (in press).

BULLER, R. E., TOFT, D. O., SCHRADER, W. T., AND O'MALLEY, B. W. (1974b). Progesterone-binding components of chick oviduct. Receptor activation and hormone dependent binding to purified nuclei. *J. Biol. Chem.* (in press).

CHAMNESS, G. C., AND MCGUIRE, W. L. (1972). Estrogen receptor in the uterus: Physiological forms and artifacts. *Biochemistry* **11**, 2466-2472.

ERDOS, T., *et al.* (1971). Studies on the uterine cytoplasmic estradiol receptor. *In:* Advances in the Biosciences VII (G. Raspe, ed.), Pergamon Press, New York, pp. 119-135.

GIANNOPOULOS, G., AND GORSKI, J. (1971). Estrogen-binding protein of the rat uterus. Different molecular forms associated with nuclear uptake of estradiol. *J. Biol. Chem.* **246**, 2530-2536.

JENSEN, E. V., NUMATA, M., SMITH, S., SUZUKI, T., BRECHER, P. I., AND DESOMBRE, E. R. (1969). Estrogen-receptor interaction in target tissues. *Dev. Biol. Suppl.* **3**, 151-171.

KUHN, R. W., SCHRADER, W. T., SMITH, R. G., AND O'MALLEY, B. W. (1975). Progesterone-binding proteins of chick oviduct. Purification by steroid affinity chromatography. *J. Biol. Chem.* (submitted).

O'MALLEY, B. W., MCGUIRE, W. L., KOHLER, P. O., AND KORENMAN, S. G. (1969). Studies on the mechanism of steroid hormone regulation of synthesis of specific proteins. *Recent Progr. Hormone Res.* **25**, 105-160.

O'MALLEY, B. W., TOFT, D. O., AND SHERMAN, M. R. (1971). Progesterone-binding components of chick oviduct. II. Nuclear components. *J. Biol. Chem.* **246**, 1117-1122.

O'MALLEY, B. W., AND SCHRADER, W. T. (1972). Progesterone receptor components: Identification of subunits binding to the target-cell genome. *J. Steroid Biochem.* **3**, 617-629.

O'MALLEY, B. W., SPELSBERG, T. C., SCHRADER, W. T., CHYTIL, F., AND STEGGLES, A. W. (1972). Mechanisms of interaction of a hormone receptor complex with the genome of a eukaryotic target cell. *Nature* **235**, 141-144.

O'MALLEY, B. W., SCHRADER, W. T., AND SPELSBERG, T. C. (1973). Hormone-receptor interactions with the genome of eukaryotic target cells. *In:* Receptors for Reproductive Hormones, (B. W. O'Malley and A. R. Means, eds.), Plenum Press, New York, pp. 174-196.

O'MALLEY, B. W., AND MEANS, A. R. (1974). Female steroid hormones and target cell nuclei. *Science* **183**, 610-620.

SCHRADER, W. T., AND O'MALLEY, B. W. (1972). Progesterone-binding components of chick oviduct. IV. Characterization of purified subunits. *J. Biol. Chem.* **247**, 51-59.

SCHRADER, W. T., TOFT, D. O., AND O'MALLEY, B. W. (1972). Progesterone-binding protein of chick oviduct. VI. Interaction of purified progesterone receptor components with nuclear constituents. *J. Biol. Chem.* **247**, 2401-2407.

SCHRADER, W. T. (1974). Methods for extraction and quantification of receptors. *Methods in Enzymology* **36**, 187-211.

SCHRADER, W. T., SOCHER, S. H., AND BULLER, R. E. (1974). Steroid hormone-receptor interactions with

nuclear constituents. Methods in Enzymology **36,** 292–313.

SCHRADER, W. T., BULLER, R. E., KUHN, R. W., AND O'MALLEY, B. W. (1975a). Molecular mechanisms of steroid hormone action. J. Steroid Biochem. (in press).

SCHRADER, W. T., KUHN, R. W., AND O'MALLEY, B. W. (1975b). Progesterone-binding proteins of chick oviduct. Purification from oviduct of laying hens. J. Biol. Chem. (submitted).

SCHWARTZ, R. J., TSAI, M. J., TSAI, S. Y., AND O'MALLEY, B. W. (1975). Effect of estrogen on gene expression in the chick oviduct: Changes in the number of RNA polymerase binding and initiation sites in chromatin. J. Biol. Chem. (in press).

SHERMAN, M. R., CORVOL, P. L., AND O'MALLEY, B. W. (1970). Progesterone-binding components of chick oviduct: Preliminary characterization of cytoplasmic components. J. Biol. Chem. **245,** 6085–6096.

SMITH, H. E., SMITH, R. G., TOFT, D. O., NEERGAARD, J. R., BURROWS, E. P., AND O'MALLEY, B. W. (1974). Binding of steroids to progesterone receptor proteins in chick oviduct and human uterus. J. Biol. Chem. (in press).

SPELSBERG, T. C., STEGGLES, A. W., AND O'MALLEY, B. W. (1971). Progesterone-binding components of chick oviduct. III. Chromatin acceptor sites. J. Biol. Chem. **246,** 4188–4197.

SPELSBERG, T. C., STEGGLES, A. W., CHYTIL, F., AND O'MALLEY, B. W. (1972). Progesterone-binding components of chick oviduct. V. Exchange of progesterone-binding capacity from target to nontarget tissue chromatin. J. Biol. Chem. **247,** 1368–1374.

STANCEL, G. M., LEUNG, K. M. T., AND GORSKI, J. (1973). Estrogen receptors in the rat uterus. Relationship between cytoplasmic and nuclear forms of the estrogen binding protein. Biochemistry **12,** 2137–2141.

STROTT, C. A. (1974). Metabolism of progesterone in the chick oviduct: Relation to the progesterone receptor and biological activity. Endocrinology **96,** 826–837.

TOFT, D. O., AND O'MALLEY, B. W. (1972). Target tissue receptors for progesterone: The influence of estrogen treatment. Endocrinology **90,** 1041–1045.

TSAI, M. J., SCHWARTZ, R. J., TSAI, S. Y., AND O'MALLEY, B. W. (1975). Effect of estrogen on gene expression in the chick oviduct: Rifampicin-resistant initiation sites. J. Biol. Chem. (in press).