TISSUE SPECIFIC BINDING IN VITRO OF PROGESTERONE-RECEPTOR TO THE CHROMATINS OF CHICK TISSUES

A. W. Steggles, T. C. Spelsberg and B. W. O'Malley

Departments of Obstetrics and Gynecology, Medicine and Biochemistry

Vanderbilt University School of Medicine
Nashville, Tennessee

Received February 9, 1971

SUMMARY

When H^3-progesterone is incubated with chick oviduct cytosol, it binds to a specific receptor protein. The subsequent binding of this complex to oviduct chromatin is greater than that of free progesterone alone. This specific affinity for the progesterone-receptor complex is not observed with spleen, heart, liver or erythrocyte chromatin. Progesterone incubated with the cytosols of non-target tissues such as liver or spleen shows no specific binding to chromatin from any source. Artificial complexes of chick histone and DNA display a low degree of non-specific binding. The results suggest that the chromatin of target tissue (oviduct) may contain "acceptor" sites for the hormone-receptor complex contained in the cytoplasm of the cell. The genome may thus be preprogrammed to receive the hormone receptor complex as it enters the nucleus of the target cell.

INTRODUCTION

Recent studies have shown that steroid hormones initially combine with specific receptor proteins in the cytosol of target tissue cells, and that these complexes are then transported into the nucleus and become closely associated with the chromatin (1-3). When steroid hormones are administered in vivo, a steroid hormone-receptor complex can subsequently be extracted from the target tissue chromatin (4-6). Subsequent to the association of hormones with chromatin, an increase in template activity of the chromatin as well as the synthesis of new species of RNA has been noted (5,7,8). The exact nature or biological meaning of the interactions of hormones with chromatin of target tissues,

however, remains obscure. If such an association is the cause of gene activation, then it may represent a fundamental event in the hormonal regulation of gene activity.

We undertook investigations into the nature of the interaction of progesterone with the chromatin of target (oviduct) and non-target (spleen, heart and erythrocyte) tissues of chickens. Progesterone is known to cause specific changes in the gene expression (3,8) as well as alterations in the template capacity of oviduct chromatin (3). Recent studies in our laboratory (9) have identified a cytosol receptor protein specific for progesterone in the oviducts of estrogen-treated chicks. The progesterone initially binds to this cytosol receptor and is transported into the nucleus where it becomes associated with chromatin (10). Studies in vitro have demonstrated that: 1) the oviduct cytosol receptor is essential for the nuclear retention of progesterone; 2) oviduct nuclei but not lung or spleen nuclei, can bind progesterone; and 3) nuclear-bound progesterone, extractable with high salt, is associated with an acidic protein which is indistinguishable from the cytosol receptor (10).

METHODS AND MATERIALS

The liver, spleen, and heart were removed from untreated 14-28 day old chicks (Rhode Island Reds), rinsed in cold .01M Tris-HCl, pH 7.5 buffer, and stored at -20°C. Oviducts from treated chicks (subcutaneous injections of 5mg estrogen daily for 15 days) and mature erythrocytes from 2 year old hens were also used.

The frozen tissue was finely minced with scissors in 5-10 vol. of 0.5M sucrose in 0.002M $CaCl_2$, 0.25M KCl, 0.05M Tris-HCl pH 7.5 buffer (buffer A). The suspension was homogenized with 10 strokes in a teflon-glass homogenizer, poured through 4

layers of cheesecloth, and centrifuged 10 minutes at 6000 x g. The pellet was rehomogenized in cold 2.0M sucrose in buffer A and the final concentration of sucrose adjusted to 1.75M with buffer A. This homogenate was centrifuged 20 minutes at 25,000 x g, the pellet of nuclei saved, and the supernate with floating cell debris rehomogenized and recentrifuged. The combined pellets were rehomogenized in 1.75M sucrose in buffer A and the solution centrifuged as above. The pellets of partially purified nuclei were gently resuspended in 0.5M sucrose + buffer A with 0.2% triton X-100, filtered through wire or gauze (100 mesh), and centrifuged 10 minutes at 10,000 x g. The purified nuclei were used as a source for chromatin isolation and purification as described previously (11).

It has been shown that kinetic studies, using saturating enzyme and nucleotide levels, can be used to estimate the extent of DNA in chromatin available for transcription; these values correlate well with values obtained by DNA-RNA hybridization experiments (18). In these experiments, the reaction mixture, reaction conditions, and E. Coli RNA polymerase enzyme were essentially those described by Burgess (12). Measurement of C^{14}-UMP incorporation in the presence of rate-limiting amounts of chromatin has been described previously (11). The percent of DNA in each chromatin available for transcription was calculated by using the rate of incorporation of C^{14}-UMP in reactions with pure DNA as 100%.

The chemical analysis of chromatin has previously been described (11) and is based on previous studies by other investigators (13). All proteins were analyzed by the method of Lowry *et al.* (14) and DNA by the method of Burton (15). Each preparation of chromatin was initially analyzed for histone and

nonhistone protein, RNA, DNA, and for the integrity of the species of histones by acrylamide gel electrophoresis (16), as well as the ability of each to serve as a template in the in vitro DNA-dependent RNA synthesis. Periodically, the chromatin preparations were reanalyzed for protease degradation.

RESULTS AND DISCUSSION

Incubations of H^3-progesterone with oviduct cytosol followed by density gradient sedimentation show that the majority of the label is bound to a protein which sediments as the progesterone cytosol receptor (9). Incubations in 0.15M NaCl at 4°C for 1 hour constituted optimal binding conditions of the H^3-progesterone-receptor complex to oviduct chromatin. As shown in Figure 1, the extent of binding of H^3-progesterone-receptor complex to oviduct chromatin was much greater than to the chromatins of spleen and erythrocytes. Similar differences were observed with heart chromatin and to a lesser extent with liver chromatin. It seems, therefore, that the deoxyribonucleoprotein of a progesterone target tissue either contains specific macromolecules or is structurally oriented (preprogrammed) in some manner so as to specifically bind the progesterone-receptor complex; the deoxyribonucleoprotein of other (non-target) tissues does not possess this capacity.

To check the requirement of the cytosol receptor for this binding, free progesterone alone and progesterone preincubated with the cytosols of other tissues (Fig. 2) was substituted for oviduct cytosol. No specific binding of H^3-progesterone was observed, i.e., the extensive association of the hormone with oviduct chromatin was lost. A similar lack of specificity was observed when progesterone bound to serum proteins such as chick plasma transcortin was incubated with various chromatins.

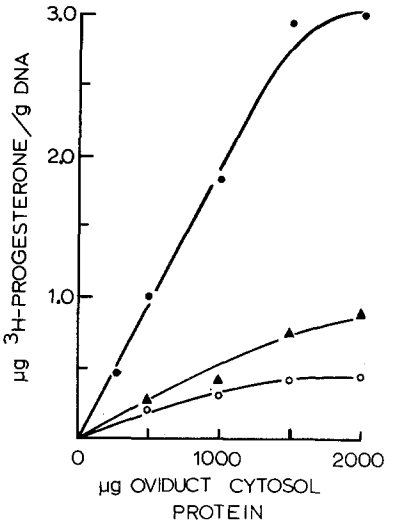

Figure 1. Binding of H^3-progesterone-receptor complex to 30-50 μg of oviduct chromatin (●) from 15 day estrogen-treated chicks, erythrocyte chromatin (▲) from 2 yr old hens, and spleen chromatin (o) from untreated chicks. Cytosol containing the progesterone receptor was isolated from 10-20 day estrogen-treated chick oviducts and labeled with H^3-progesterone (1.5×10^{-8}M) at 4° for 1 hr. Increasing amounts of the H^3-progesterone-oviduct receptor complex were incubated for 1 hr at 4°C with 30-50 μg of chromatin in a final colume of 0.5ml containing 0.15M NaCl. The solution was centrifuged 10 minutes at 1200 x g to sediment the chromatin. The chromatin was resuspended in 2.0ml of cold .15M NaCl and recentrifuged. The pellet was then resuspended in 1.0ml of cold 0.15M NaCl + 0.01M $MgCl_2$ and filtered with vacuum through Millipore filters (0.45μ pore size, 24mm dia. from the Millipore Corp., Bedford, Mass.). Each reaction vessel was washed twice with the same cold buffer and each filter was washed with 20ml of cold buffer. After drying at room temperature, the filters were counted in a Beckman Scintillation spectrometer using 5ml of the standard Toluene-based POP-POPOP solution. After counting, the filters were dried and incubated in 0.3M $HClO_4$ for 30 min at 90°C. The cooled solutions were analyzed for DNA (29). Specified amounts of standard DNA were dried on filters and treated similarly each time to serve as the standard in the quantitative procedures. The hydrolysis procedure quantitatively extracts all DNA from the filters. In each experiment reactions containing H^3-progesterone and cytosol, but no chromatin, were included. The radioactivity on the filters of these solutions was subtracted as background from those solutions which included chromatin. The radioactivity per filter was then correlated with the μg DNA per filter and the results expressed as μg H^3-progesterone bound per g DNA (as chromatin).

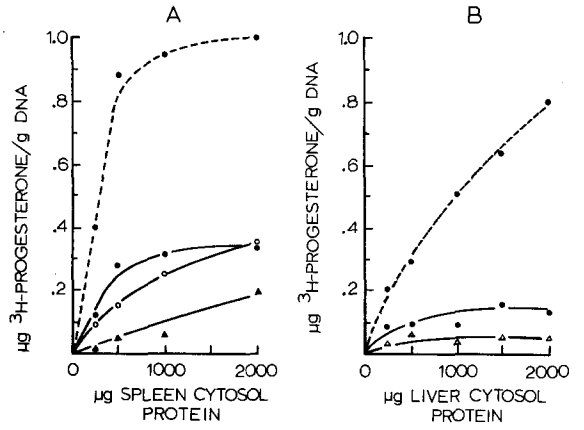

Figure 2. Binding of H^3-progesterone, preincubated with various cytosols, to 30-50 µg of different chromatins. The solid lines represent (A) the binding of H^3-progesterone-spleen cytosol complex and (B) the binding of H^3-progesterone-liver cytosol complex. The chromatins were isolated from (●) oviducts from 15 day estrogen-treated chicks, (o) spleen, and (Δ) liver from untreated chicks, and (▲) mature erythrocytes from 2 year old hens. The dotted lines represent the binding of H^3-progesterone-oviduct cytosol complex to oviduct chromatin for comparative purposes. See the legend of Figure 1 for procedures.

Therefore, the cytosol receptor protein for progesterone, previously shown to occur in oviduct but not in spleen or other tissues (3,9,10), appears to be essential for the extensive binding of progesterone to the oviduct chromatin.

Since steroid hormones have been found associated in vivo with histones (17), two complexes of pure DNA and histone were made and their association with the progesterone-receptor complex measured. Figure 3 shows that the binding of H^3-progesterone to these artificial DNA-histone complexes was lower than that to native oviduct chromatin. Since the extent of open template in the complexes is greater than that in native oviduct chromatin, the presence of open DNA does not appear to be a factor involved in the extensive binding to oviduct chromatin. Al-

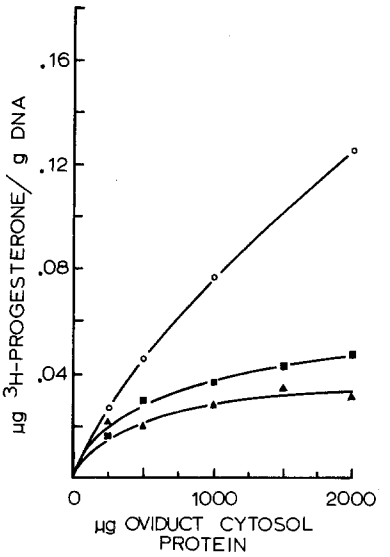

Figure 3. Binding of H^3-progesterone, preincubated with oviduct cytosol, to 30-50 µg of (o) native oviduct chromatin (histone/DNA = .95; nonhistone/DNA = .54; % open template = 3.8), (■) DNA-histone complex A (histone/DNA = .90; % open template 9.0), and (▲) DNA-histone complex B (histone/DNA = 1.3; % open template = 5.0). Purified chick oviduct histone was added slowly with mixing to purified chick oviduct DNA in (A) 3 mg histone to 3 mg DNA and in (B) 6 mg histone to 3 mg DNA. After standing at 4°C for 30 minutes, the insoluble complex was collected by centrifugation, purified and analyzed as described for native chromatin (11).

though the histone/DNA ratio in the complexes was similar to that found for native oviduct chromatin, the binding was still minimal, suggesting that the histones do not singularly participate in the binding of progesterone to oviduct chromatin.

It is noteworthy to mention that the chromatins isolated by various procedures have been shown to synthesize RNA in vitro and the species of RNA transcribed are similar to those synthesized in vivo (18). Consequently, isolated chromatin retains the native-like properties assigned to the functioning chromatin in cells. Since oviduct cytosol is required for specific binding,

the interesting possibility is raised that the hormone binds to oviduct chromatin together with the cytosol receptor and that the nonhistone "chromosomal" protein, reported to be associated with many chromatin bound steroids (4,6,18,19), was simply the cytosol receptor protein.

The specific binding of the progesterone-receptor complex to oviduct chromatin suggests that a hormone target tissue may require not only the presence of a receptor protein to accumulate and transport the hormone to the nucleus, but also the genetic material must be preprogrammed to receive the hormone-receptor complex. The exact chemical composition of these chromatin "acceptor" sites is presently under investigation.

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