Acceptor sites for the oestrogen receptor in hen oviduct chromatin

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Partially purified hen oviduct oestrogen receptors, charged with [^3H]oestradiol, were shown to specifically bind *in vitro* to purified hen oviduct chromatin. Maximal binding occurred within 60 min at 0°C in a Tris buffer containing 0.1 M-KCl and 0.5 mM-phenylmethanesulphonyl fluoride. The binding of the [^3H]oestradiol–receptor complexes to intact purified chromatin was saturable, whereas the receptor binding to hen DNA remained linear. Saturation was further demonstrated by the minimal acceptor binding of receptor charged with [^3H]oestradiol plus 200-fold oestradiol compared with [^3H]oestradiol receptors at equal [^3H]oestradiol concentrations. Scatchard analysis of [^3H]oestradiol–receptor binding to chromatin above DNA levels gave indications of high-affinity binding with a low capacity. Further, the nuclear binding was tissue-specific since the binding to hen spleen chromatin was negligible. To further uncover the specific acceptor sites, proteins were removed from hen oviduct chromatin by increasing concentrations of guanidine hydrochloride (1–7 M). Those residual fractions extracted with 3–7 M-guanidine hydrochloride had the highest acceptor activity (above DNA levels) with the peak activity uncovered by 5 M-guanidine hydrochloride. To further characterize the oestrogen-receptor acceptor sites, oviduct chromatin was bound to hydroxyapatite in the presence of 3 M-NaCl and then protein fractions were extracted sequentially with 1–7 M-guanidine hydrochloride. Each fraction was then reconstituted to pure hen DNA by reverse gradient dialysis. [^3H]Oestradiol receptors were found to bind to the greatest degree to the fraction reconstituted from the 5 M-guanidine hydrochloride protein extract. Reconstituted nucleosidic proteins (NAP) from combined 4–7 M-guanidine hydrochloride protein extracts showed saturable binding by [^3H]oestradiol receptors, whereas binding to hen DNA did not saturate. The high affinity, low capacity, and specificity of binding of oestrogen receptors to NAP was similar to that found in intact chromatin. Thus, chromatin acceptor proteins for the oestrogen receptor have been partially isolated and characterized in the hen oviduct and display properties similar to that reported for the acceptor proteins of the progesterone receptor.

The non-histone proteins of chromatin have been suggested as a component of chromatin acceptor sites for steroid-hormone–receptor complexes (Spelsberg *et al.*, 1971; O'Malley *et al.*, 1972). Binding of steroid-hormone–receptor complexes to acceptor sites is one event of a series of steps involved in the mechanism of action of steroid hormones. Steroid hormones first enter the target cell and bind to specific receptor molecules. The steroid-hormone–receptor complexes are activated and then translocate to the nucleus, where im-

mediate alterations in gene transcription occur. The events in the nucleus that cause the initiation of gene regulation by steroid hormones involve the binding of steroid–receptor complexes to chromatin 'acceptor' sites (Thrall *et al.*, 1978). The non-histone protein–DNA complexes, which are believed to constitute the acceptor sites, have been extensively studied with regard to avian progesterone–receptor interactions (Spelsberg *et al.*, 1976, 1979; Spelsberg, 1982). Recently, these types of studies have been extended to other steroid-hormone receptors, target tissues and animal species (Klyzsejko-Stefanowicz *et al.*, 1976; Perry & Lopez, 1978; Taylor *et al.*, 1980; Tsai *et al.*, 1980; Ruh *et al.*, 1981a,b, 1982).

Abbreviations used: PMSF, phenylmethanesulphonyl fluoride; GuHCl, guanidine hydrochloride.

Kon *et al.* (1980) have recently described the characteristics of the hen oviduct oestrogen receptor and its interaction with nuclei. The chick and hen oviduct oestrogen-receptor molecule possessed the characteristics of other oestrogen receptors; namely, high affinity, low capacity and a specificity for oestrogenic compounds. Oestrogen-receptor complexes bound to nuclei in a cell-free system and demonstrated saturability and specificity. We have extended these studies and report the characterization of the binding of hen oviduct oestrogen-receptor complex to chromatin, the isolation of non-histone (acceptor) proteins bound to the DNA that are responsible for the oestrogen receptor binding and the reconstitution of the non-histone protein-DNA complexes to regenerate the 'acceptor' sites. We compared these data with results obtained with progesterone-receptor complexes. This is the first report describing the binding of two steroid-hormone-receptor complexes to reconstituted chromatin acceptor sites in the same target tissue.

Methods and materials

Source of tissue

Oviducts and spleens from normal laying hens were obtained from a local poultry company, immediately trimmed of fat and connective tissue, frozen on dry ice and stored at -80°C .

Specific chemicals

Steroids and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Fisher Scientific Co. (Pittsburgh, PA, U.S.A.). 17β -[2,4,6,7(n)- ^3H]Oestradiol (sp. radioactivity 115 Ci/mmol) and [6,7(n)- ^3H]progesterone (sp. radioactivity 50 Ci/mmol) were from New England Nuclear Corp. (Boston, MA, U.S.A.). The purities of the labelled and unlabelled steroids were monitored by high-pressure liquid chromatography using C_{18} μ Bondapak columns (Waters Associates, Milford, MA, U.S.A.) under a reverse phase with a programmed linear gradient of increasing acetonitrile and decreasing water concentrations.

Preparations of hen oviduct oestrogen receptor

All receptor preparations were performed at 4°C and used the general methods of Kon *et al.* (1980) unless otherwise stated. Frozen hen oviducts were thawed in 3 vol. (w/v) of buffer [300 mM-KCl/100 mM-Tris/10 mM-EDTA/5% (v/v) glycerol/12 mM-monothioglycerol (added fresh) (pH 8.0)] for 30 min at 4°C and then gently homogenized in a Waring blender. The oviducts were further homogenized in a Teflon/glass homogenizer and centrifuged at 21600g for 10 min in an HB-4 rotor (Sorvall). The supernatant was passed through four single layers of cheesecloth, to remove floating debris, and centrifuged at 250000g for 60 min in a

60Ti rotor (Beckman). The floating lipid layer was aspirated away and only the clear cytosol was used. The protein content of the cytosol was determined by using the Bio-Rad Protein Assay (Bradford, 1976). The cytosol was charged with 10 nM-[^3H]oestradiol, mixed for 15 min, then the solution was made 0.5 mM with respect to PMSF. The cytosol was incubated further at 10°C for 16–20 h. The [^3H]oestradiol-receptor complexes were partially purified by 30% $(\text{NH}_4)_2\text{SO}_4$ precipitation for 1 h at 4°C . The solution was centrifuged at 21600g for 10 min in an HB-4 rotor, the supernatant decanted and the receptor pellets stored in the tubes at -80°C until used. Under these conditions the oestrogen receptor could be stored for at least 2 weeks. In certain experiments we used [^3H]progesterone-labelled progesterone receptors. These were prepared from chick oviducts as described previously (Thrall & Spelsberg, 1980; Spelsberg & Halberg, 1980).

Preparation of partially deproteinized hen oviduct chromatin

Hen oviduct chromatin was prepared from purified oviduct nuclei as described previously (Pikler *et al.*, 1976), with modifications as described by Boyd & Spelsberg (1979).

Hen oviduct chromatin was gently homogenized with a Teflon/glass homogenizer at 4°C and divided into portions. Chromatin portions were precipitated with 0.2 M-KCl for 10 min and recovered as pellets by centrifuging at 21600g for 10 min in an HB-4 rotor. The chromatin pellets were homogenized in 0.7 M-GuHCl/50 M- NaH_2PO_4 /Na $_2\text{HPO}_4$ (pH 6) and the proteins were extracted for 1 h at 4 ml of extraction buffer per mg of chromatin as DNA. The mixtures were centrifuged at 106400g for 60 h in 45Ti rotors (Beckman). After decanting the supernatant, the residual chromatin pellets were resuspended in 2 mM-Tris/0.1 mM-EDTA (pH 7.5) ('TE buffer') at 1 ml/mg of DNA and dialysed against 100 vol. of TE buffer for 16 h with four buffer changes. These residual chromatin fractions were freeze-dried and resuspended in a minimal amount of TE buffer for acceptor activity assays.

Preparation of reconstituted nucleosidic proteins

The chromosomal proteins containing the acceptor sites were isolated and reconstituted to hen DNA as described previously for the progesterone-receptor acceptor proteins (Spelsberg *et al.*, 1976, 1979, 1982; Thrall *et al.*, 1978). Hydroxyapatite (HTP; BioRad) was hydrated (25 g dry/50 mg of DNA) overnight in 5 vol. (v/w) of 3 M-NaCl/100 mM- NaH_2PO_4 /Na $_2\text{HPO}_4$ (pH 6.0) ('NaCl/ PO_4 buffer'). This hydroxyapatite slurry was filtered and 5 vol. (v/v) of NaCl/ PO_4 buffer was added per dry weight of original hydroxyapatite. The hen oviduct chromatin in TE buffer was added to

this slurry and bound to the hydroxyapatite with occasional gentle mixing for 2h. The slurry was filtered and 4 vol. of the NaCl/PO₄ buffer added for 10min followed by filtration to remove the histones. Most of the non-histone proteins were sequentially extracted from the hydroxyapatite-bound dehistonized chromatin by using a series of solutions containing 1–7M-GuHCl in 100mM-NaH₂PO₄/Na₂HPO₄, pH 6.0. Each solution was used at 5 vol. (v/w) for 1h at 4°C with one repeat extraction. The GuHCl filtrates were dialysed thoroughly against 100vol. of deionized water at 4°C and freeze-dried and saved for reconstitution to hen DNA.

The non-histone protein fractions and the hen DNA were rehydrated separately for 4h in 6M-GuHCl in 1mM-mercaptoethanol/10mM-EDTA/0.2mM-PMSF/50mM-sodium acetate, pH 6.0 ('MEPA buffer') and then mixed together at a protein/DNA equivalent of 2:5 for 1h. The samples were then placed in dialysis bags and equilibrated against 6M-GuHCl in MEPA buffer for 4h and the protein reconstituted to DNA using a 16h reverse gradient dialysis reducing the GuHCl concentration from 6M to 0M. Samples were centrifuged at 82400g for 24h (70.1Ti rotor) to pellet the reconstituted protein-DNA complexes. The reconstituted nucleocacidic proteins were resuspended in minimal TE buffer and used for binding experiments.

Acceptor-site assay for the hen oviduct oestrogen receptor

Receptor pellets were redissolved in (10mM-Tris/1mM-EDTA/12mM-monothioglycerol/0.5mM-PMSF (pH 7.5) ('TESHP buffer') at one-third the original cytosol volume, gently hand-homogenized in a Teflon/glass homogenizer (Glenco) and incubated at 4°C for 1h. The [³H]oestradiol-receptor-complex solution was then dialysed for 1h against 100vol. of TESHP buffer and centrifuged at 90500g for 10min in a 60Ti rotor (Beckman). The [³H]-oestradiol-receptor complex solution was recharged with 5nM-[³H]oestradiol for 1h and then used directly for the acceptor binding assays.

Chromatin or reconstituted nucleocacidic proteins (at 50µg of DNA per assay) were added to silicon-treated disposable culture tubes. The [³H]-oestradiol-receptor-complex solution (200µl) was added to start binding. The final 500µl assay also contained TESHP buffer and 0.1M-KCl. After a 1h incubation at 4°C, the chromatin-receptor complexes were precipitated with 100µl of a 1% solution of streptomycin sulphate by the method of Webster *et al.* (1976). Briefly, the assays were incubated for 30min and then centrifuged at 9000g for 10min using a JS 7.5 rotor (Beckman). The pellets were washed with 1ml of 0.02% streptomycin sulphate and centrifuged twice more. The pellets were collected on Millipore filters and rinsed four times

with 1ml of 0.02% streptomycin sulphate. Dry filters were counted in 5ml of Spectrofluor scintillation fluid at 50% efficiency and then the filters were removed, dried and assayed for DNA (Burton, 1956).

Results

Initial studies were concerned with isolation, partial purification, labelling and stabilization of the hen oviduct oestrogen receptor. It was determined that 'charging' hen oviduct oestrogen receptors with 10nM-[³H]oestradiol for 16h was most efficient and specific if the temperature was 10°C. At the lower temperatures of 4°C and 7°C, specific binding was lower and at 15°C most of the binding was non-specific as determined by the difference between total binding ([³H]oestradiol) and non-specific binding ([³H]oestradiol + 200-fold unlabelled oestradiol concentration). To reduce potential proteinase activity, PMSF was used. It was found that the presence of 0.5mM-PMSF during the [³H]oestradiol charging incubation gave improved results without affecting the final acceptor assay. After dialysis of the partially purified [(NH₄)₂SO₄-precipitated] [³H]oestradiol-receptor complex, it was beneficial to recharge the hen oviduct oestrogen receptor with 5nM-[³H]oestradiol. The sex steroid-binding protein reported by Smith & Taylor (1982) and Ruh & Toft (1982) to exist in chicken oviduct and to bind large excesses of oestradiol was not present in our partially purified oestrogen-receptor preparation, since progesterone would not compete with [³H]oestradiol for binding to receptors, whereas diethylstilboestrol or oestradiol were effective competitors.

Fig. 1 shows the optimal time for binding the partially purified hen oviduct [³H]oestradiol-receptor complex to hen oviduct chromatin by using the binding assay as described in the Methods and materials section. The zero-time binding blank was determined by immediately adding the streptomycin sulphate after adding the [³H]oestradiol-receptor complex to the assay. This binding blank was subtracted from all other values. The binding of [³H]oestradiol-receptor complex to purified chromatin continued to increase during the first hour of incubation but remained constant during the next hour. Other periodic checks never detected a binding difference between 60min and 90min of incubation. Therefore, all subsequent acceptor assay experiments were performed at 60min of incubation.

The ability of [³H]oestradiol-receptor complex to saturate binding sites on chromatin was next investigated (Fig. 2). The acceptor-site assay was performed as usual, except that the [³H]oestradiol-receptor complex was titrated over a 10-fold range from 25µl to 250µl of [³H]oestradiol-receptor complex solution per 500µl of total assay.

Fig. 1. The effect of time on the binding of [^3H]-oestradiol-receptor complexes to hen oviduct chromatin

Oestrogen receptors from hen oviduct cytosol were charged with [^3H]oestradiol for 16 h at 10°C and then partially purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation. Receptor pellets were redissolved in buffer, given an additional charge of [^3H]oestradiol for 1 h, dialysed, and $200\mu\text{l}$ portions were incubated with $50\mu\text{g}$ of hen oviduct chromatin (as DNA) for various times up to 2 h. Receptor-chromatin complexes were precipitated by streptomycin sulphate, collected on Millipore filters, counted and DNA determinations were made. A zero-time binding blank was subtracted from all values. For full details see the Methods and materials section.

Fig. 2. Effect of increasing the concentration of [^3H]-oestradiol-receptor complex on the binding to hen oviduct chromatin

Receptor preparations, chromatin preparations and acceptor-site assays were as described in the legend to Fig. 1. Binding to chromatin or pure hen DNA was for 1 h. (a) shows the binding curves of [^3H]oestradiol-receptor complex to chromatin and DNA; (b) shows the calculated binding to chromatin minus DNA binding.

The chromatin binding saturated somewhere between 150 and $200\mu\text{l}$ of [^3H]oestradiol-receptor complex, whereas the binding to hen oviduct DNA remained linear throughout (Fig. 2a) and never displayed a tendency toward saturable binding, even at much higher concentrations of [^3H]oestradiol-receptor complex (results not shown). The binding of [^3H]oestradiol-receptor complex to chromatin was generally more than double that of DNA. When the DNA binding values were subtracted from the respective chromatin binding values, a saturable binding curve was evident (Fig. 2b). From this binding curve a Scatchard plot (Scatchard, 1949) gave an estimate of the quantity of binding (40 fmol of [^3H]oestradiol-receptor complex/mg of chromatin DNA) and of the binding affinity (K_d approx. 30 pM), which indicated a very-high-affinity binding of the [^3H]oestradiol-receptor complex for some limited component on chromatin.

Next the binding of [^3H]oestradiol-receptor complex to chromatin, after it was partially deproteinized by GuHCl, was examined. Hen oviduct chromatin was extracted with buffer or $1\text{--}7\text{ M}$ -

GuHCl and the residual chromatin used in acceptor-site assays. The binding of [^3H]oestradiol-receptor complex to intact chromatin (buffer only) was subtracted from the values obtained with the binding of [^3H]oestradiol-receptor complex to chromatin extracted with $1\text{--}7\text{ M}$ -GuHCl. As shown in Fig. 3, there was a slight but consistent increase in binding to chromatin previously extracted with 1 M -GuHCl. This binding was lost with a 2 M -GuHCl extraction. However, $3\text{--}5\text{ M}$ -GuHCl extraction increasingly uncovered oestrogen-receptor acceptor sites in hen oviduct chromatin. Some of this acceptor activity was removed by $6\text{--}7\text{ M}$ -GuHCl. These experiments demonstrated that increased acceptor activity could be made available by GuHCl extraction, possibly by removing masking proteins as reported previously for both the progesterone-receptor binding to oviduct chromatin (Webster *et al.*, 1976; Spelsberg *et al.*, 1976, 1979) and the oestrogen receptor binding to uterine chromatin (Ruh *et al.*, 1981b). At the higher GuHCl con-

Fig. 3. The effects of binding [³H]oestradiol-receptor complexes to hen oviduct chromatin previously extracted with increasing concentrations of GuHCl

The residual (partially deproteinized) chromatin was collected by ultracentrifugation, dialysed, freeze-dried and resuspended in buffer before binding 200 μ l of [³H]oestradiol-receptor complex to 50 μ g of chromatin (as DNA) for 1 h. Results are expressed as fmol/mg of DNA (corrected for whole-chromatin binding) and are means $\pm$ S.E.M. (represented by the bars). For further details see the Methods and materials section.

Fig. 4. The effect of binding [³H]oestradiol-receptor complexes or [³H]progesterone-receptor complexes to various reconstituted nucleoacidic protein fractions from hen oviduct chromatin

Non-histone proteins were sequentially extracted from purified hen oviduct chromatin with 1–7 M-GuHCl, dialysed, freeze-dried and reconstituted to pure hen DNA during a 16 h reverse gradient dialysis from 7 to 0 M-GuHCl. Acceptor-site binding assays were as described in the legend to Fig. 1 and expressed as fmol of [³H]oestradiol-receptor complex or [³H]progesterone-receptor/mg of DNA. For further details see the Methods and materials section. Results are means $\pm$ S.E.M. (represented by the bars) and are corrected for DNA binding.

centrations, the acceptor sites in turn also dissociated from the DNA.

The non-histone proteins were then reconstituted to pure hen DNA to regenerate the acceptor sites for the oestrogen-receptor complex. As shown in Fig. 4, [³H]oestradiol-receptor complex binding to the reconstituted nucleoacidic protein fractions began to increase above DNA binding levels with the 3 M-GuHCl-extract reconstitutions. The highest acceptor activity for the oestrogen receptor was with reconstitutions from the 5 M- and 7 M-GuHCl extracts, with a distinct loss of acceptor sites with the 6 M-GuHCl extraction. On the same reconstituted nucleoacidic proteins, the [³H]progesterone-receptor complex had a similar pattern of binding to the progesterone-receptor acceptor sites; it was subsequently found that the exact molarity of GuHCl that dissociates most of the acceptor activity for oestrogen or progesterone receptor depends on the length of time the GuHCl is exposed to the chromatin. These results indicate that (1) the acceptor activity for both oestrogen receptor and progesterone receptor can be dissociated from, and reconstituted back to, the DNA, (2) these activities for both receptors are tightly bound to the DNA and

(3) the major acceptor activity for both receptors is roughly extracted with a similar concentration of GuHCl.

Additional experiments determined, in general, the specificity of [³H]oestradiol-receptor complex binding to reconstituted nucleoacidic proteins. We combined the proteins from the 4–7 M-GuHCl extracts to reconstitute a pooled nucleoacidic proteins. Increasing concentrations of [³H]oestradiol-receptor complex were then bound to this pooled nucleoacidic protein fraction to determine the saturation and affinity of binding (Fig. 5). Fig. 5(a) shows that similar to native nucleoacidic proteins, the [³H]oestradiol-receptor complex binding to reconstituted nucleoacidic protein was about twice that of DNA, with saturation beginning to occur at 200–250 μ l of [³H]oestradiol-receptor complex, whereas the binding to DNA remained linear. Identical results were obtained when the protein concentration in the assay was kept constant. When DNA binding was subtracted from nucleoacidic protein binding and Scatchard analysis performed (Fig. 5b), the binding quantity of [³H]oestradiol-receptor complex was about 80 fmol/mg of chromatin DNA with a K_d of about 0.15 nM. Although

Fig. 5. Effect of increasing concentrations of $[^3\text{H}]$ -oestradiol-receptor complex to a pooled nucleocacidic protein fraction

Non-histone proteins from 4–7 M-GuHCl extracts of hen oviduct chromatin were combined and reconstituted as described in the legend to Fig. 4. Various concentrations of $[^3\text{H}]$ oestradiol-receptor complex were incubated with the reconstituted nucleocacidic proteins as described in the legend to Fig. 2. (a) shows $[^3\text{H}]$ oestradiol-receptor complex binding to nucleocacidic proteins (O) on hen DNA (Δ). (b) shows binding to nucleocacidic proteins minus DNA binding.

the reconstituted nucleocacidic protein fraction appears to have more acceptor sites but a lesser affinity for binding oestrogen receptors than the native chromatin, the binding to both types of acceptor sites is still saturable and of high affinity.

The binding of specific $[^3\text{H}]$ oestradiol-receptor complex to various chromatin, DNA or nucleocacidic protein fractions was also determined (Fig. 6). Hen oviduct oestrogen receptors were labelled with either 10 nM- $[^3\text{H}]$ oestradiol (for total binding) or $[^3\text{H}]$ oestradiol plus a 200-fold excess of unlabelled oestradiol (for non-specific binding). After precipitation, dialysis and recharging with either $[^3\text{H}]$ oestradiol or $[^3\text{H}]$ oestradiol plus a 200-fold excess of oestradiol, the oestrogen-receptor preparations were adjusted, by adding some free $[^3\text{H}]$ oestradiol, to equivalent levels of $[^3\text{H}]$ oestradiol concentrations

Fig. 6. Specificity of $[^3\text{H}]$ oestradiol-receptor complex or free $[^3\text{H}]$ oestradiol binding to various chromatin fractions

Hen oviduct oestrogen receptor charged with $[^3\text{H}]$ -oestradiol plus ($\square$) or minus ($\blacksquare$) a 200-fold excess of oestradiol was incubated with oviduct or spleen chromatin, pure hen DNA or reconstituted nucleocacidic proteins (Recon. NAP). Free $[^3\text{H}]$ oestradiol plus ($\blacksquare$) or minus ($\square$) a 200-fold excess of oestradiol was also incubated with reconstituted nucleocacidic proteins. Filter blank binding was subtracted from all values. Results are expressed as c.p.m./mg of DNA and are means $\pm$ s.e.m. (indicated by the half-bars). For further details, see the Methods and materials section.

before acceptor-site assay. As shown in Fig. 6, the binding of specific $[^3\text{H}]$ oestradiol-receptor complex occurred in the order of reconstituted nucleocacidic proteins > oviduct chromatin > oviduct DNA > spleen chromatin, with the latter (a non-target tissue chromatin) showing almost no binding. In addition, whereas $[^3\text{H}]$ oestradiol-receptor complex displayed high binding, free $[^3\text{H}]$ oestradiol shows a very low binding to the reconstituted nucleocacidic proteins, indicating that the oestrogen receptor is required for binding to this nuclear fraction as it is for chromatin binding. It should be noted that using total $[^3\text{H}]$ -oestradiol-bound receptor minus non-specific $[^3\text{H}]$ -oestradiol bound receptor in acceptor-site assays only indicates that specific $[^3\text{H}]$ oestradiol-receptor complex is binding to chromatin or nucleocacidic proteins and not that $[^3\text{H}]$ oestradiol-receptor complex is binding to specific acceptor sites.

Discussion

These studies show for the first time that the nuclear acceptor sites involved in the cell-free binding of hen oviduct [^3H]oestradiol-receptor complex involve tightly bound non-histone proteins. The acceptor site assays were shown to require a receptor, and to involve a time-dependent, saturable and high-affinity binding and to be tissue-specific. That simple absorption of the [^3H]oestradiol-receptor complex to the chromatin played only a minor role in this binding is supported by the saturable binding of high levels of [^3H]oestradiol-receptor complex to the oviduct chromatin and to the reconstituted oviduct nucleic acid protein fraction, but not to the pure hen DNA, which only displayed a linear binding for [^3H]oestradiol-receptor complex. Also, the low level of specific receptor binding to chromatin from spleen, a non-target tissue, negates non-specific absorption of the [^3H]oestradiol-receptor complex to chromatin in general and indicates that the [^3H]oestradiol-receptor complex binding to the target tissue (oviduct) chromatin is specific.

It is interesting that the binding of [^3H]oestradiol-receptor complex to the GuHCl-extracted oviduct chromatins displayed a marked increase at the higher GuHCl concentrations, indicating an unmasking of acceptor sites. Comparable results were found by Ruh *et al.* (1981a) in various partially deproteinized calf uterine chromatin preparations. In that case acceptor was seemingly unmasked by 1 M-, 4 M- and 7 M-GuHCl, whereas we have found in the hen oviduct chromatin that 1 M-GuHCl unmasked only a small but consistent amount of acceptor activity and that 3–5 M-GuHCl unmasked greatly increased numbers of binding sites for oestrogen receptor (Fig. 3). These results are similar to those found for chick oviduct progesterone receptor, which has enhanced binding to chromatin after removal of certain non-histone proteins that mask many of the acceptor sites. As estimated by Fig. 6, over half of the [^3H]oestradiol-receptor complex binding sites in nucleic acid proteins seem to be 'masked', a finding similar to the [^3H]progesterone-receptor complex binding reported previously (Webster *et al.*, 1976; Spelsberg *et al.*, 1976, 1979; Thrall *et al.*, 1978; Spelsberg, 1982). Therefore, the most interesting general analogies appear in the 4–5 M-GuHCl extraction region for both the oviduct oestrogen receptor and progesterone receptor (Fig. 4) and for the calf uterine oestrogen receptor (Ruh *et al.*, 1981a).

Also of interest are the indications that the acceptor activity for oestrogen receptors appears to be dissociated from the DNA at high concentrations of GuHCl and can be 'reconstituted' to the pure hen DNA. These properties are very similar to

the 'acceptor activity' for the hen oviduct progesterone receptor, which has been shown to be an acidic low-molecular-weight protein, tightly bound to the DNA (Webster *et al.*, 1976; Spelsberg *et al.*, 1976, 1979; Thrall *et al.*, 1978; Spelsberg, 1982). In addition, there are indications that there may be two or more classes of [^3H]oestradiol-receptor complex acceptor sites, as demonstrated by the increased binding activity of nucleic acid proteins reconstituted from both 5 M- and 7 M-GuHCl extracts of oviduct chromatin. This observation of more than one region of acceptor activity has also been reported for reconstituted nucleic acid proteins from calf uterine chromatin extracts (Ruh *et al.*, 1982) separated by flat-bed isoelectrofocusing. Spelsberg *et al.* (1979) have shown a similar phenomenon of 2–3 acceptor species for the progesterone receptor separated by isoelectrofocusing.

We would suspect that the hen oviduct chromatin acceptor sites for the oestrogen receptor and progesterone receptor are not identical, which is suggested by the studies of Kon & Spelsberg (1982) on receptor binding to oviduct nuclei. However, additional purification of the acceptor proteins would be needed to answer this question. The acceptor proteins for the avian oviduct progesterone receptor have also recently been shown to require certain 'specific' DNA sequences in order to generate [^3H]progesterone-receptor binding. Whether or not the acceptor sites for the avian oviduct [^3H]progesterone-receptor involve the same or a similar class of acidic proteins as for the hen oviduct [^3H]progesterone-receptor, as well as specific DNA sequences, remains to be determined.

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