Characteristics of Different Cytoplasmic and Nuclear Estrogen Receptors Appearing with Continuous Hormonal Exposure*

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Biochemical properties of cytosol estrogen receptor (ERc) and nuclear estrogen receptor (ERN) from rat uteri continuously exposed in vivo to 17β-[2,4,6,7-3H]estradiol ([3H]E2) for 6 h have been studied on the basis of immunological recognition and chromatographic elution patterns. Overall concentrations of ERc and ERN did not change during this time period when receptor-saturating concentrations of [3H]E2 were maintained (Jakesz, R., Kasid, A., and Lippman, M. E. (1983) J. Biol. Chem. 258, 11798–11806); however, biochemical characteristics were different in ERc and ERN after short or long term hormonal exposure. When ERc from rats treated with estradiol for 30 min was applied to HAP or DEAE columns, two different ER binding components were seen. DNA binding in a cell-free system revealed that these binding components represented an activated and a nonactivated ERc population. After long term hormonal exposure (6 h), only one component of ERc with low DNA binding could be shown despite the preservation of an equivalent quantity of cytoplasmic binding activity. This binder does not react with a monoclonal antibody directed against extranuclear estrogen receptor species. These data suggest disappearance of the activated ERc population, with appearance of a new, immunologically nonrecognizable ERc species with 6 h of continuous hormonal exposure. Elution profiles of ERN on HAP chromatography reveal 2 different binding components at 30 min and at 6 h of continuous [3H]E2 exposure. There is an increase of the population eluted at higher molarity after 6 h of in vivo treatment. This later eluting binding component is the major DNA binder in vitro. ERN from both time points are recognized immunologically by monoclonal antibody. After reaction with the antibody, the sedimentation coefficient shifted to 8–9 S on sucrose gradients, but the previously described faster sedimentation of ERN extracted 6 h after injection persisted. We conclude that ER in both cellular compartments undergoes time-dependent alterations, which may be involved in the initiation of hormone action.

Our previous work has shown that it is possible to maintain rat uterine ER1 levels over a 6-h period when [3H]E2 serum concentrations are maintained which are sufficient to saturate cytosol binding sites (1). We have found that with this continuous hormonal exposure in vivo, which may more clearly resemble physiologic conditions, cytosol and nuclear ER binding sites undergo changes in sucrose gradient behavior, dissociation kinetics, and in binding to DNA-cellulose in vitro without major quantitative alteration. Most current knowledge about characterization of ER is derived from in vitro experiments, dealing with potentially artificial changes such as exposure of cytosol preparations to heat (2), to salt (3), to DNA (4), or ammonium sulfate (5). Under these conditions, the cytosol receptor sedimenting at 4 S changes its sedimentation coefficient to 6 S and the dissociation pattern becomes slow and monophasic (6). This receptor is called activated or transformed. An open question in this repertoire of changes remains the biologic significance of these findings.

In our previous work, we found that a relatively large proportion of estrogen binding activity remained cytoplasmic in localization throughout a continuous [3H]E2 exposure for 6 h, a situation which we expected would lead to a complete translocation of ERc to the nucleus (7). Sucrose gradient behavior and DNA binding suggested that the cytoplasmic hormone binding activity present after 6 h of estrogen treatment was still ER. A puzzling observation was that this competitive binding moiety did not show any dissociation of in vivo bound ligand (1). We therefore studied the nature of this ER population 6 h after in vitro treatment.

Our previous investigations also showed that nuclear receptor levels did not decrease over the 6-h time period studied. Since the sucrose gradient patterns of nuclear extracts obtained after 30 min and 6 h of hormone exposure were different, it seemed important to characterize the nature of the changes in these nuclear binding moieties.

We characterized the different ER populations 1) by their immunological recognition by a monoclonal antibody (D547 Spy) raised against extranuclear estrogen receptor protein of MCF-7 human breast cancer cells (8); 2) by DEAE-cellulose chromatography, which has shown to be a useful method in separating activated and nonactivated ERc from calf uterus (9) and glucocorticoid receptor in liver cytosol (10, 11); and 3) by hydroxylapatite chromatography (12). It has been shown that hydroxylapatite does not adsorb steroids (13), and further, using HAP columns, it is possible to chromatographically characterize ERc which is salt-extracted from the nucleus. Using these techniques, we found consistently that both ERc and ERN change in their biochemical characteristics with 6 h of continuous hormonal exposure. Thus, our data support

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1 The abbreviations used are: ER, estrogen receptor; ERc, cytosol estrogen receptor; ERN, nuclear estrogen receptor; [3H]E2, 17β-[2,4,6,7-3H]estradiol; HAP, hydroxylapatite; DCC, dextran-coated charcoal; UTT, 1,1,1,3,3,3-hexachloro-2,2-bis-(p-chlorophenyl)ethane.
important time-dependent qualitative changes in uterine hormone binding without the previously described quantitative loss of receptor.

**EXPERIMENTAL PROCEDURES**

**Animals**—Immature female 17-21 day-old Osborne-Mendel rats were obtained from the National Institutes of Health, Small Animal Section (Bethesda, MD).

**Chemicals**—17β-2α, 6α, 7-3H estradiol (115 Ci/mmol) diluted prior to use to 25 Ci/mmol. γ-[3H] Globulin and [3H]ovalbumin was purchased from New England Nuclear (Boston, MA); unlabeled 17β-estradiol was obtained from Steraloids (Pawling, NY) and prepared in absolute ethanol. All reagents were analytical grade and were purchased from the following companies: Dextran T-40 from New England Nuclear, Sigma (St. Louis, MO), hydroxyapatite Bio-Gel HTP from Bio-Rad (Richmond, CA), DNA-cellulose from PL-Biochemicals (Milwaukee, WI), sucrose from Schwarz-Mann (Spring Valley, NY), DEAE-cellulose, DE-52 from Whatman, Ltd (Maidstone, Kent, England), DTT, ammonium sulfate, and Tris from ERL (Rockville, MD), and Sephadex G-25 from Pharmacia (Piscataway, NJ).

**Buffers**—Buffers and their compositions are as follows: TED, 10 mM Tris, 1 mM EDTA, 0.7 mM DTT; TEDG containing 10% glycerol (v/v); TEDGK, TEDG containing 600 mM KC1 (pH 8.5); PD, 10 mM potassium phosphate, 0.7 mM DTT; PDG, PD containing 10% glycerol (v/v); and PDGK, PDG containing 600 mM KC1. Unless otherwise stated, buffers were adjusted to pH 7.4 (4 °C).

**Preparation of ERc and ERN**—Preparation of uterine cytosol and nuclear fractions treated with simultaneous 100-fold excess of unlabeled 17β-estradiol for 30 min at 0 °C was achieved by treatment of cytosol charged with 5 nM [3H] estradiol for binding to DNA-cellulose as described. (1). For chromatography experiments, 5-6 uterine eq/ml of cytosol were processed which resulted in a protein concentration of 5-6 mg/ml of cytosol. ERc were extracted by sonication of 5-6 uterine eq/ml in TEDGK or DEAE buffer. ERN were extracted by sonication of 5-6 uterine eq/ml in TEDGK or DEAE buffer. ERN were extracted by sonication of 5-6 uterine eq/ml in PDGK buffer. ERc and ERN were separated by HAP chromatography. HAP was initially washed 3 times with TEDG and then incubated with 600 mM KC1. Column of HAP (1 × 5 cm) was equilibrated in TED buffer, pH 7.4. Following application of ER preparations, the columns were first washed with TED buffer until radioactivity was reduced to background values and then eluted with 50 ml of a linear 10-50 mM potassium phosphate gradient prepared in PDG. 1.5-ml fractions were collected and aliquots were counted for radioactivity. After establishing linearity of the gradients, 10 cm of the column were washed with 10 volumes of TED buffer. 2-ml fractions were collected and aliquots were measured for radioactivity and [3H]radioactivity. 1-ml peak fractions were assayed for binding to DNA-cellulose for 60 min at 0 °C with constant shaking as described previously (1).

**Results**

**Antireceptor Monoclonal Antibody Reactivity with ERc and ERN**—In studies described in companion paper (1), we found differences in the sedimentation coefficients of ERc and ERN 30 min and 6 h after in vivo treatment. We questioned whether the conformational or other modifications which were responsible for time-dependent alterations in receptor sedimentation properties would affect monoclonal antibody reactivity with estrogen receptor. We, therefore, used a monoclonal antibody (DS47 Sp7) prepared against ER in MCF-7 human breast cancer cells for binding studies. Previous work had demonstrated that this monoclonal antibody recognized rat uterine ER (8). Using optimal reaction conditions as described previously (8), 40% of the ERc obtained 30 min after hormone injection was recognized by the antibody (Fig. 1a), and the immunoreceptor complexes shifted to the 7 S region in 5-20% sucrose gradients, which is in agreement with earlier results (20). Control experiments with native ERc reacted with monoclonal antibody showed complete recognition of receptor with shift of all specific binding down the gradient which is also consistent with previously published results (8, 20) (data not shown). These data suggest that ERc preparations isolated 30 min after hormone injection contain two ER populations with different immunoreactivity. Surprisingly, the ERc obtained 6 h after [3H]estradiol injection was not recognized by the antibody (Fig. 1b). We conclude that during 6 h of continuous hormonal stimulation, a significant structural change occurs, which prevents antibody recognition of the putative receptors. ERc from both time points are recognized equally well (Fig. 1c). Monoclonal antibodies against ER recognize different structural features of the receptor. DS47 Sp7 used in these studies is known to recognize the DNA binding portion of the receptor. Thus, loss of antibody reactivity and DNA binding might be expected to occur simultaneously. Other data (1) also strongly suggest that this binding moiety is estrogen receptor and not some alternative binding moiety. The ERc obtained 30 min after hormonal stimulation had a sedimentation coefficient of 5 S which shifted to 8.3 S after reactivity with the monoclonal antibody. The remaining ER sedimented at 4.5 S which is significantly different from the entire population of ERc obtained prior to reaction with the monoclonal antibody (1). The ERc obtained from uteri 6 h after in vivo treatment (which had a sedimentation coefficient of 5.3 S) after reaction with the antibody showed...
a different sedimentation coefficient (8.6 S) from that of ERN, antibody complexes prepared after 30 min of hormone treatment. The receptor protein remaining after reaction with the monoclonal antibody sedimented at about 4.8 S. From the results with ERN, we conclude that two different receptor populations exist at both time points. These are differentially precipitated by DCC pellets. 200-μl aliquots of the cytosol were incubated for 15 min at 0 °C with or without a 200-fold excess of unlabeled competitor. Aliquots of the labeled cytosol were then incubated at either 0 °C (α, nonactivated receptor) or 28 °C for 30 min (b, activated receptor). Cytosol preparations were applied to HAP columns and eluted with a linear 10–500 mM potassium phosphate (KP) gradient. The figure shows a typical pattern representative of 3 different experiments.

**Table 1**

**Binding of partially purified ERc to DNA-cellulose**

<table>
<thead>
<tr>
<th>Protein concentration/incubation</th>
<th>Binding to DNA-cellulose</th>
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<tr>
<td>8 mg</td>
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<td>Peak I</td>
<td>120</td>
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<td>Peak II</td>
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<td>Peak I after (NH₄)₂SO₄ purification</td>
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<tr>
<td>Peak II after (NH₄)₂SO₄ purification</td>
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Characteristics of Cytosol and Nuclear ER

**Fig. 1.** Sucrose density gradient sedimentation behavior of ERc and ERN reacted with monoclonal antibody. Cytosol was prepared in TEDG from rats injected for 30 min or 6 h with 2 injections of 0.5 μg of [3H]E2 prepared in saline and in oil in the absence or presence of 200-fold molar excess of unlabeled E2 (C, total binding; ○, nonspecific binding). After removal of the unbound steroid by DCC pellets, 200-μl aliquots of the cytosol were incubated for 60 min at 0 °C with an antireceptor monoclonal antibody (D547 Spy) (8). Nuclei were prepared from the uteri and ERc were released by sonication in TEDGK. Aliquots of the DCC-treated high speed supernatant were incubated with the monoclonal antibody for 60 min at 0 °C (○, ERN, 30 min; ●, ERN, 6 h). 200-μl aliquots were layered onto 5–20% sucrose gradient prepared in TEDG, pH 7.4, containing 0.4 M KCl.

**Fig. 2.** Elution of nonactivated and activated ERc on HAP chromatography. Cytosol (protein concentration 5–6 mg/ml) was prepared in PDG buffer from uteri of untreated immature rats and charged for 90 min at 0 °C with 5 nM [3H]E2 with or without a 200-fold excess of unlabeled competitor. Aliquots of the labeled cytosol were then incubated at either 0 °C (α, nonactivated receptor) or 28 °C for 30 min (b, activated receptor). Cytosol preparations were applied to HAP columns and eluted with a linear 10–500 mM potassium phosphate (KP) gradient. The figure shows a typical pattern representative of 3 different experiments.
Fig. 3. Elution profile and DNA-cellulose binding of ERc from in vivo treated rats. Cytosol (5–6 mg of protein/ml) was prepared from uteri of rats treated for 30 min or 6 h in vivo with 0.5 μg of [3H]E2 in the absence or presence of 200-fold excess of unlabeled competitor. Samples were loaded on pre-equilibrated HAP columns and eluted with a linear 10–500 mM potassium phosphate (KP) gradient (L). Aliquots were bound in duplicate to DNA-cellulose (E) as described under “Experimental Procedures.” This figure shows a representative pattern of 3 different experiments. c, Mixing experiments in which cytosol preparations from rats injected with either [3H]E2 for 30 min or unlabeled E2 for 6 h were incubated together for 1 h at 4 °C prior to coelution through HAP column.

We next tried to verify our results showing different characteristics of ERc from uteri treated for 30 min or 6 h in vivo by use of a different chromatographic system. Ion exchange chromatography has previously been shown to be useful for ER characterization in calf uterus (5, 9) for both progesterone receptor in chick oviduct (22) and glucocorticoid receptor (10, 11). With this technique, ERc obtained 30 min after in vivo treatment with estradiol (Fig. 4a) revealed 2 major peaks eluting at 100 and 170 mM KCl. The small peak eluting at 30 mM KCl may represent adsorbed, previously dissociated [3H]E2. The binding of the peak fractions to DNA-cellulose showed results comparable to the HAP chromatography data. The earlier eluting peak bound to a lesser extent (12 to 16%) compared to the later eluting peak (28 to 36%). The ERc obtained 6 h after in vivo treatment (Fig. 4b) showed only one peak eluting at 60 mM KCl. Binding studies with DNA-cellulose revealed only a weak affinity for DNA binding of ERc obtained after 6 h of continuous hormonal exposure (10%). These data are consistent with our results using HAP chromatography. The ERc obtained 30 min after hormone treatment contained 2 separable components which eluted at different salt concentrations and showed different affinities for DNA. The ERc obtained after 6 h of continuous hormonal treatment showed only one predominant receptor population. Interestingly, 30-min in vivo labeled ERc eluted at a low KCl

150 mM before starting the binding reaction. As indicated in Table I, peak II showed 3 times higher binding capacity than that of peak I and probably represents activated ERc. The peak fractions recovered from HAP chromatography were also precipitated with 30% saturated ammonium sulfate. This purification step increased the binding ability to DNA-cellulose nearly 3-fold. The partially purified peak II (activated ERc) still showed a much higher binding ability than the purified peak I. We conclude that in vitro activation leads to an increase of an ERc form eluting at a higher potassium phosphate molarity on HAP chromatography. Apparently, HAP chromatography is a useful method for discriminating between activated and nonactivated ERc populations.

We then investigated the chromatographic patterns of cytosol fractions prepared from uteri of animals treated in vivo for 30 min (Fig. 3a) or for 6 h (Fig. 3b) with a simultaneous double injection of 0.5 μg of [3H]E2 in saline and oil with or without 200-fold excess of unlabeled estradiol. The chromatographic profile of ERc obtained 30 min after injection, very closely resembled the pattern of the native ERc (Fig. 2a). Two sharp peaks eluting at a similar potassium phosphate molarity as the in vitro native ERc were detected. Binding of peak fractions to DNA-cellulose again revealed preferential binding of peak II to DNA. The ERc obtained 6 h after estradiol exposure (Fig. 3b) showed a different elution pattern. The majority of the total competitive ER binding eluted at 30 mM potassium phosphate. No ERc eluting at higher salt concentrations was observed. The DNA binding ability of ERc, peak I, from uteri of rats treated with hormone for 6 h was similar to the binding of peak I from ERc of rats treated for 30 min or peak I from in vitro labeled cytosol (Table I). These results show that the activated component in ERc present at 30 min after in vivo [3H]E2 treatment, which binds DNA-cellulose preferentially, disappears with continuous in vivo 6-h hormonal exposure of animals. Thus, despite the apparent preservation of total ERc binding activity, profound changes in elution pattern and binding ability to DNA-cellulose occur in ERc obtained after 6 h of continuous hormonal exposure in vivo.

To address the question of whether the alterations in ER at 6 h were due to modifications introduced during cell disruption and receptor extraction, we performed a mixing experiment, the results of which are shown in Fig. 3c. We find that in such a mixing experiment, no changes in the HAP column elution profiles of the 30 min in vivo labeled ERc were observed when coeluted with 6-h in vivo labeled ERc. Therefore, we conclude that certain time-dependent changes in receptor characteristics, that we report here, are not due to various nonreceptor cytosolic factors or proteolytic enzymes which would alter the chromatographic pattern and also the efficiency of DNA binding, but rather are due to in vivo modifications in ER appearing within 6 h of continuous hormonal administration. Furthermore, we have also prepared ERc obtained from animals treated for 30 min or 6 h with estradiol in the presence of 15 mM molybdate or 50 mM leupeptin. When compared by chromatography on HAP column, we did not find any change in the elution patterns previously described (data not shown). While the exact function of the multiple receptor forms seen here must obviously await functional assays of receptor, the present data do suggest substantial in vivo modification of ER.
molarity compared with the 6-h labeled ERc. This together with the previously described methods of analyses suggested that these receptors were not identical (1). Furthermore, we emphasize that our previous experiments rule out the possibility that the binding moiety in the cytosol fraction was α-fetoprotein (1).

Elution Pattern of ERN on HAP Chromatography and Binding to DNA-cellulose—We next examined the HAP elution profiles of ERN obtained 30 min or 6 h post-[3H]E2 injections. The 30-min labeled ERN showed 2 sharp peaks eluting at 60 and 130 mM potassium phosphate (Fig. 5a). ERN extracted 6 h after in vivo hormone treatment showed a similar pattern, with one peak eluting at 50 mM and the second at 120 mM potassium phosphate. However, following 6 h of continuous treatment, quantitatively more binding activity eluted in the second peak, which is in contrast to the behavior of the ERc from rats injected with E2 for 6 h.

Since we were able to show that the ERN labeled at both time points contained 2 separable binding components, we wanted to investigate the binding capacities of these components to DNA-cellulose. Since preparations of specific nuclear acceptor binding sites are not available and since it has been proposed that the receptor site may contain both a protein and a DNA binding site (27), we compared the DNA binding ability of crude, desalted, and partially purified ERN preparations (Fig. 6). We found a 50% increase in DNA-cellulose binding of 6-h in vivo labeled ERN when compared with the ERN obtained from 30-min treated rats (Fig. 6a). Furthermore, using desalted nuclear extracts prepared from 6-h hormone-treated animals, we found similar increases in the binding of ERN to DNA-cellulose (Fig. 6b). The preferential binding of 30-min or 6-h in vivo labeled ERN which eluted in peak II was reproducibly detected when stepwise eluted ERN (Fig. 6c) or ammonium sulfate precipitated receptor fractions (Fig. 6d) were tested for binding to DNA-cellulose. We therefore conclude that, as in the cytosol preparations (Figs. 2 and 3),

**Fig. 4.** DEAE-cellulose chromatography of ERc. Cytosol was prepared in TEDG from uteri of rats treated for a, 30 min or b, 6 h with 0.5 μg of [3H]E2 in the absence or presence of 200-fold excess of nonlabeled E2. Columns were eluted with 100 ml of a 10–500 mM linear KCl gradient in TEDG buffer. Peak fractions were incubated with DNA-cellulose (II).

**Fig. 5.** HAP chromatography pattern of ERN. ERN was extracted in PDGK by sonication from uteri of rats treated either with [3H]E2 for a, 30 min or b, 6 h and layered on pre-equilibrated HAP columns. Since the nonspecific binding was less than 10% in all nuclear fractions, competed samples were not used in these experiments. Elution was performed with 60 ml of a linear 10–500 mM potassium phosphate gradient (KP, III). 1.5 ml of fraction were collected and measured for radioactivity and conductivity.

**Fig. 6.** DNA-cellulose binding of different ERN preparations. Nuclear extracts were prepared in PDGK from the uteri of rats injected with [3H]E2 in absence or presence of 200-fold excess of unlabeled E2 for 30 min or 6 h. Aliquots of nuclear KCl extracts were passed through a pre-equilibrated Sephadex G-25 column to remove salt. Other aliquots were layered on a pre-equilibrated HAP column, washed, and stepwise eluted with 10 ml of 100 and 200 mM potassium phosphate. Aliquots of peak fractions were precipitated with ammonium sulfate (30% saturation), redissolved, and analyzed for binding to DNA-cellulose. a, Crude nuclear extract (600–700 μg of protein/ml); b, desalted ERN; c, peak I and peak II eluted from HAP columns (160–200 μg of protein/ml); d, peak I and II after ammonium sulfate precipitation (250–300 μg of protein/ml). Data shown are averages of 3 experiments.
The ER$_C$ eluting at higher KCl molarity contains the major DNA binding moiety. The reason for the increased DNA binding in crude ER$_C$ extracts obtained 6 h after [3H]E$_2$ treatment is the increased percentage of ER$_C$, peak II, which is directly responsible for the DNA binding.

**DISCUSSION**

Our results indicate that 30 min after injection of receptor-saturating amounts of [3H]E$_2$, both cytosol and nuclear compartments contain two different ER species. The cytosol contains an activated and a nonactivated receptor population indicated from DNA binding studies of ER components derived from HAP and DEAE-cellulose chromatography (Figs. 3e and 4a). This result is consistent with an earlier observation, that the dissociation kinetics of ER$_C$ from uteri of rats treated for 30 min behaves in a biphasic pattern (1). One might have assumed that under physiologic temperatures in the presence of saturating concentrations of ligand the total receptor population would become activated. There is no obvious explanation for our observation that only part of the ER$_C$ becomes transformed. One possibility may be that under maximal hormonal stimulation the cytosol lacks a “translocation” or “activation factor” described for different receptor systems by several groups (23-26). Another explanation may be that this nonactivated receptor becomes translocated together with the activated ER$_C$, but has functions different than becoming bound to DNA. There are 3 arguments in favor of this theory. 1) ER$_C$ obtained 30 min after in vivo treatment shows two different ER species, which elute on HAP chromatography at comparable molarities as ER$_C$ obtained 30 min after injection (Figs. 3 and 5). 2) ER$_N$ prepared from uteri of rats treated with estradiol for 30 min reacts incompletely with monoclonal antibody directed against ER. This unrecognized population has the same sedimentation constant as the ER$_C$ obtained from rats treated with hormone for 30 min (Fig. 1, a and c). 3) The two nuclear receptor populations released by HAP chromatography show a similar DNA binding pattern as the ER$_C$ from rats treated with estradiol for 30 min (Figs. 3, 6, and Table I). The peak eluting at a higher potassium phosphate molarity has a preferential DNA binding ability. These results may also be seen in the light of a series of studies on the progesterone receptor system of the chick oviduct by Schrader et al. (22) and Buller et al. (27). It has been postulated that upon entrance of the A-B receptor dimer into the nucleus, the B subunit binds to the protein portion of the acceptor site, followed by release of the A subunit which is then able to interact with the DNA (27). There are several indications that this could also be true for the rat uterine ER system in vivo. 1) ER$_C$ obtained 30 min after in vivo treatment contains an activated and a nonactivated population. Two different subunits have been reported to exist in the progesterone receptor from the chick oviduct as demonstrated by DEAE-chromatography (22). 2) The nuclear fraction also contains 2 different binding components which change over time. 3) One of these components shows preferential DNA binding.

The ER$_C$ obtained after 6-h in vivo treatment seems to be a different species from the ER$_C$ obtained after short term hormonal stimulation. It has important characteristics which differ from either in vitro labeled or short term in vivo treated ER$_C$. 1) We previously reported that ER$_C$ obtained after 6 h of in vivo hormonal treatment shows no dissociation of ligand at different temperatures (1). 2) It has minimal immunological reaction with a specific monoclonal antibody directed against ER (Fig. 1b). 3) It has a very low affinity for DNA-cellulose. 4) It has a different elution profile on DEAE showing only one early eluting peak. The biological significance of the ER$_C$ derived from uteri under chronic hormonal stimulation is uncertain. It seems to be a nonactivated receptor form. But whether it is a recycled ER form coming from the nucleus which had undergone major changes, or if it is an ER species which did not become translocated because of the saturation of nuclear binding sites or because of its poor DNA binding ability remains unknown. A similar nontranslocating cytoplasmic steroid binding moiety, called the Z protein, has been recently described in the hormone-stimulated chick oviduct (28). It may also be possible that in our system the cytosol receptor 6 h after hormonal stimulation may represent a receptor precursor protein as has been suggested by Taylor and Smith (28) for the Z protein.

The chromatographic elution profiles of activated and nonactivated states of different receptors from various sources behave differently. Glucocorticoid receptors either in thymocytes or in hepatocytes show an earlier elution of the activated receptor, whereas the nonactivated population elutes at a higher molarity (10, 11, 16, 29). Progesterone receptor from chick oviduct shows a similar phenomenon (30). In contrast, ER$_C$ in the nonactivated state derived from rat or calf uterus (9) elutes at a lower molarity than activated ER$_C$. Therefore, it is uncertain whether the activation step in different receptor systems is based on similar biochemical alterations.

There are several reports dealing with different ER$_N$ populations discriminated in vitro. One is the presence of KCl-extractable and KCl-nonextractable ER$_N$ (31). It seems unlikely that one of the described ER$_N$ fractions generated by HAP chromatography (Fig. 5) is similar to these KCl-resistant ER$_N$ primarily because the amount of this ER$_N$ population does not exceed 10% of the total ER$_N$ amount (32). For the same reason, it seems to be unlikely that one of these receptor components is similar to unfilled nuclear binding sites discovered by Carlson and Gorski (33). Another study describes type I and type II nuclear binding sites (34). These type II binding sites can only be detected by exchange assays with higher amounts of [3H]E$_2$ in the absence of DTT. Since they have not been directly identified in vivo and since their biochemical properties are not entirely clear, we cannot rule out the possibility that these sites bear some relation to our observed ER$_N$ binding components.

We conclude that certain biochemical properties of ER$_C$ and ER$_N$ change during a 6-h period of continuous hormonal exposure. We find that cytosol prepared 30 min after [3H]E$_2$ injection contains an activated and a nonactivated ER$_C$ species. The nuclear compartment contains 2 different receptor forms with either short or long term hormonal exposure and it remains to further investigations to elucidate the relationship between these observations and coupling of receptor to specific gene activation.

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