# SPECIFIC EFFECT OF ESTROGENS ON AN INTERACTION BETWEEN THE UTERINE ESTRADIOL RECEPTOR AND DNA

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## 1. Introduction

Many experimental data indicate that estradiol ($E_2$) binds to its specific cytosol receptor (Rc) in uterine cells and that the $RcE_2$ complex thus formed is then transferred to the nucleus where it interacts with chromatin "acceptor" sites [1–3]. This translocation is induced *in vitro* by estradiol and by higher concentration of esterone ($E_1$) and estriol [4–5] and it is also seems to be favored by high concentrations of androgens [4]. We have looked for a possible *in vitro* interaction between Rc and DNA in order to understand the mechanism by which Rc is translocated and bound to nuclei, to define the nature of the "acceptor sites" localised in uterine chromatin, and eventually to approach the mechanism of stimulation of transcription by estradiol.

The present paper reports that the uterine Rc binds strongly with DNA, and that DNAase and KCl (0.5 M) treatment applied to the already formed complex liberates the whole Rc. Although some Rc binds in the absence of ligand, this interaction is markedly increased by estradiol and estrone but is not modified by 3-deoxy estradiol*.

## 2. Methods

[6,7-$^3$H]estradiol (specific activity = 45 Ci/mM), [6,7-$^3$H]estrone (specific activity = 45 Ci/mM) were obtained from CEA and their purity were checked by thin-layer chromatography on Silica gel F 254 Merck using benzene–ethylacetate, 3:2, v/v. Non radioactive steroids were from Roussel-Uclaf. Calf thymus DNA type I, containing 1–2% protein, was from Sigma. Single stranded DNA was obtained by heating DNA at 100° for 10 min and checked to give about 30% hyperchromicity at 260 nm. The uterine high speed supernatant (cytosol) was prepared from immature calf or rats (22 days) as previously described [6] in a 10 mM Tris-HCl pH 7.4–1.5 mM EDTA buffer; it contained approx. 2 mg protein/ml. Usually the cytosol was incubated with [$^3$H]estradiol (6 nM) at 0–2° up to binding equilibrium. Then DNA (200 μg/ml) was combined with the cytosol at 0–2° for 3 hr. The complex formed was then separated from the unbound Rc, using sucrose gradient (5–20%) ultracentrifugation at 2–4°. In some experiments, in order to test the effect of ligand on the DNA–Rc interaction, radioactive estradiol, absent during both incubation of cytosol with DNA and sucrose gradient centrifugation was finally added to the collected fraction. The number of estradiol specific binding sites of Rc was evaluated either in counting directly the fractions obtained after sucrose gradient centrifugation or using the dextran coated charcoal assay [6–7]. Radioactivity was counted with a 60% efficiency using a toluene scintillation mixture. Protein and DNA concentrations were determined by the

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Folin [8] and Burton [9] techniques or by UV absorption at 280 nm and 260 nm, respectively. After incubation, no metabolism or alteration of estrogens were shown using thin-layer chromatography.

## 3. Results

### 3.1. *The $RcE_2$ complex co-sediments with DNA in a hypoionic buffer*

When uterine cytosol previously incubated with estradiol and DNA was layered on a sucrose gradient buffered with Tris-EDTA, the radioactive estradiol migrated with DNA, which peaked between 14 and 30 S according to the DNA preparation used. In addition, there was a displacement of the 260 nm absorbing material which was dependent on the protein concentration used. When DNA and [$^3$H]estradiol had been incubated in the absence of Rc, either with Tris buffer, containing or not BSA, or with a 60° treated uterine cytosol, or with liver cytosol, estradiol (2 nM) did not migrate with DNA (fig. 1). In the cytosol, in the absence of added DNA, estradiol was sometimes bound to aggregates present in the 14–30 S area (fig. 3a). Whatever the significance of these aggregates might have been, their evaluation was needed in order to quantify more precisely the Rc–DNA interaction (see sect. 3.3).

### 3.2. *The $RcE_2$ complex can be liberated from DNA after DNAase or KCl treatment*

When DNAase was added to the already formed DNA–Rc complex, the 8 S cytosol receptor seemed to be liberated. This result indicated that the peak seen in the 14 S to 30 S area was not the consequence of an irreversible aggregation of Rc frequently observed after different treatments [10]. The absorption curve at 260 nm indicated that DNA was mainly digested

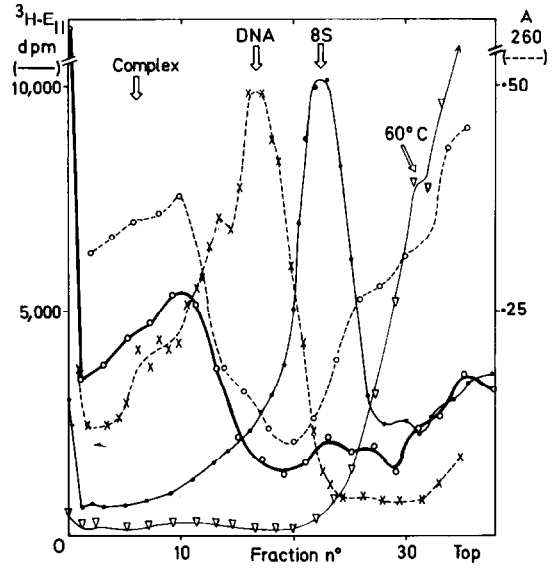


Fig. 1. Interaction in a salt free-medium. Calf uterine cytosol (2 mg protein/ml) was incubated with [$^3$H]estradiol (2 nM) for 90 min at 0–2°. One part was then agitated with calf thymus DNA 200 μg/ml for 120 min at 0–2° and subsequently centrifuged through a 5–20% sucrose gradient at 2–4° (○—○—○). The collected fractions were measured for radioactivity content (——) and for UV absorption at 260 nm (– – – –). Another part has been heated 10 min at 60° before mixing with DNA (▽—▽—▽). The cytosol containing estradiol with DNA (●—●—●) and the DNA without cytosol (x—x—x) were also centrifuged separately.

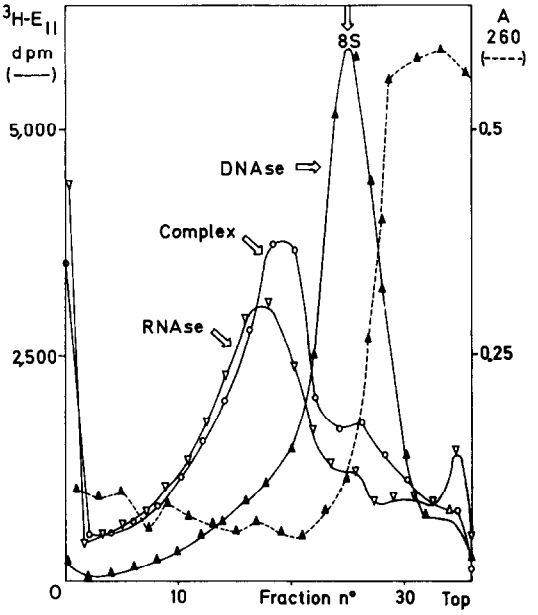


Fig. 2. Effect of DNAase on the DNA–$RcE_2$ complex. Calf thymus DNA, uterine cytosol and [$^3$H]estradiol were incubated as indicated in fig. 1 and separated into 3 lots: a) One was heated for 20 min at 25° (○—○—○), b) the second was treated by DNAase, Worthington, 40 μg/ml, 20 min at 25°, in the presence of 7 mM $MgCl_2$ (▲—▲—▲), and c) the third part was treated by RNAase, Worthington, 75 μg/ml, for 60 min at 2° (▽—▽—▽). The 3 lots were then cooled, centrifuged through a sucrose gradient centrifugation at 2–4° and the fractions were assayed for radioactivity content (——) or UV absorption (– – – –).

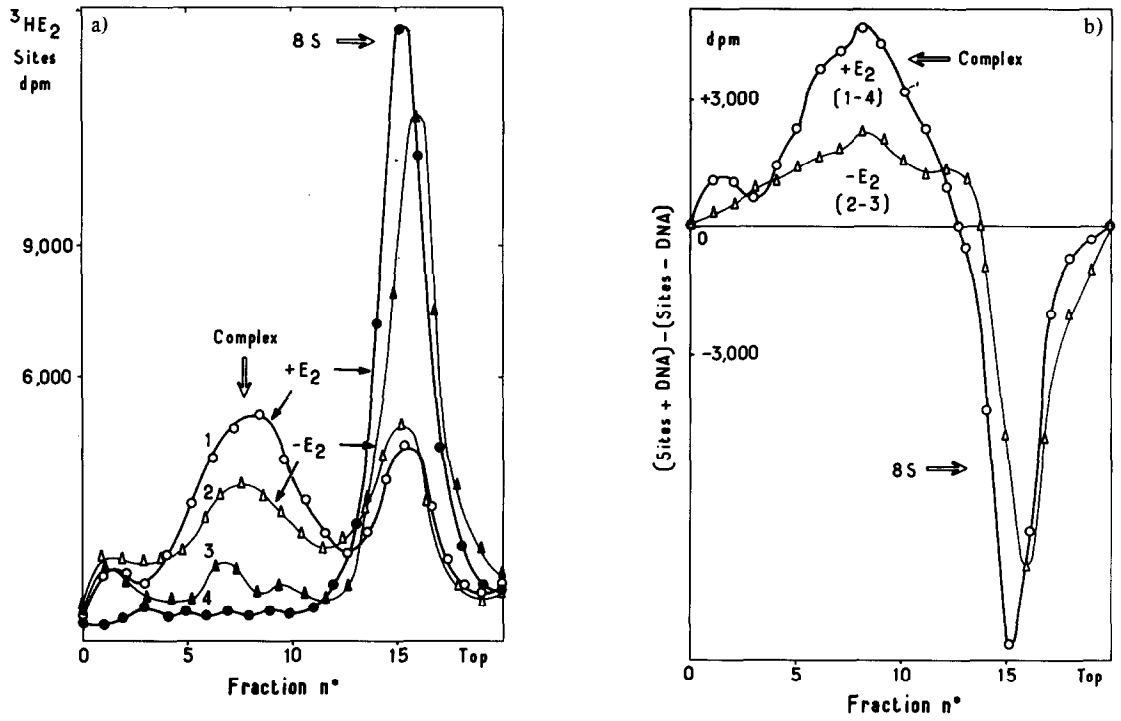


Fig. 3. Effect of estradiol and DNA on the migration of cytosol $E_2$ binding sites in a sucrose gradient. a) Direct representation: DNA and uterine cytosol were kept in contact at 0–2° during 2 hr without (△—△—△) or with (○—○—○) 6 nM [$^3$H]$E_2$, then the DNA bound Rc was separated from the unbound Rc by sucrose gradient centrifugation. The collected fractions (≃ 0.6 ml) were further incubated at 0–2° for 90 min with saturating concentration of [$^3$H]estradiol, and the maximum number of specific binding sites for estradiol were assayed in each fraction using the charcoal technique. The estradiol sites in the control cytosol without (▲—▲—▲) or with (●—●—●) estradiol were assayed parallely. The specific estradiol binding sites were corrected for the same degree of inactivation. b) Differential representation: The $E_2$ binding sites finally measured in the fractions collected after centrifugation of cytosol (curves 3 or 4 of fig. 3a) were subtracted from the $E_2$ binding sites recovered in the corresponding fractions obtained when cytosol had been incubated with DNA (curves 1 or 2). Two curves were obtained, which represented the actual effect of DNA on the repartition of estradiol binding sites in the presence (○—○—○) or absence (△—△—△) of estradiol.

after this DNAase treatment. The presence of RNAase did not liberate Rc, suggesting that DNA was not implied in the process. These results indicate that DNA–protein interaction was involved in the formation of the 14 S–30 S complex, and that Rc interacted with DNA. Whether this interaction is direct or via another component of the cytosol awaits further experimentation.

The dissociation of Rc from DNA was also observed by increasing ionic strength. The receptor migrated as a 5 to 8 S polydisperse peak in a 0.15 M KCl medium. It was totally liberated from DNA as a 4 to 5 S peak in the presence of 0.50 M KCl, conditions under which the majority of the nuclear receptor was extracted from the uterine nuclei [11].

### 3.3. *Estrogens favor specifically the interaction of Rc with DNA*

The effect of several steroids on the Rc–DNA interaction was evaluated according to the difference observed between the DNA–Rc complex formed in the presence of the steroid and that formed in its absence. The amount of Rc, bound to DNA or unbound, was indirectly measured according to its maximum binding capacity for estradiol. These sites were determined by the charcoal dextran assay, on fractions which were collected after sucrose gradient centrifugation. It has been verified that the presence of DNA did not modify the validity of the charcoal assay, since the plateau level of the saturation curve of the Rc–$E_2$ complex was similar in cytosol, with or

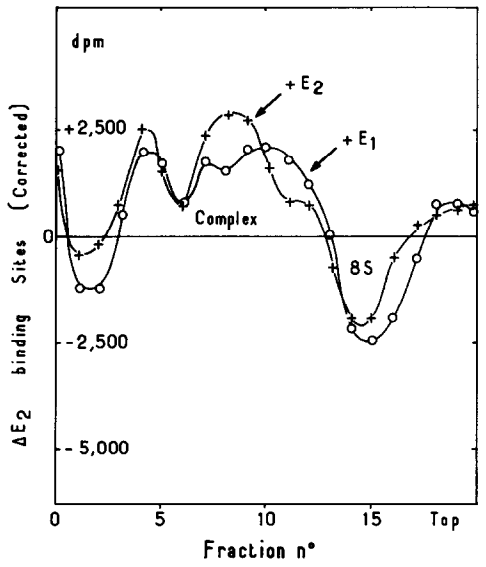


Fig. 4. Effect of estradiol and estrone on a DNA–Rc interaction. Using the differential representation described under fig. 3b, two curves were drawn for each of the steroid which had been tested during the interaction. The curve obtained in the absence of steroid was subtracted from that obtained in its presence, in order to obtain the actual effect of [$^3$H]-estrone (22.5 nM) (○—○—○) or [$^3$H]estradiol (6 nM) (+—+—+) on the DNA–Rc interaction.

Table 1

| Steroids tested | % Increase of $E_2$ binding sites in the DNA region | |
|---|---|---|
| | Aggregates included | Aggregates excluded |
| Estradiol* (6 nM) | 25 ± 6 | 80 ± 40 |
| Estrone° (22.5 nM) | 12 | 65 |
| 3-Deoxy estradiol° (50 nM) | 5 | 0 |
| Testosterone† (250 nM) | 10 ± 6 | 15 ± 25 |

The effect of the indicated steroids on the DNA–Rc interaction have been tested, as described in the text and under figs. 3 and 4. Results were in percent increase of $E_2$ binding sites present in the DNA region (≥ 14 S), they were obtained from the representations used in figs. 3a and 4. * Mean of 11 experiments ± S.E. † Mean of 5 experiments ± S.E. ° Mean of 3 experiments.

without DNA (not shown). The possible increase of the specific $E_2$ binding sites present in the DNA region after $E_2$ treatment could be a priori the consequence of at least 3 different processes: first, protection of $E_2$ binding sites against inactivation; second, increase of the aggregation of Rc in the DNA area and finally increase of the actual DNA–Rc interaction. Consequently, in order to evaluate more precisely the actual effect of steroids on the Rc–DNA interaction, their effect on the inactivation and aggregation of Rc were taken into account: i) The protective effect of $E_2$ (or other steroids) on the $E_2$ receptor sites was systematically corrected, in increasing the $E_2$ sites recovered in the absence of $E_2$, up to the level of those found in its presence. In this respect, it was verified that DNA did not modify the rate of Rc inactivation in the presence or absence of $E_2$. ii) The eventual effect of steroids on the amount of aggregates present in the cytosol, containing no exogenous DNA, was parallely evaluated in control experiments and corrected as follows: the $E_2$ binding sites present in cytosol control were subtracted from those found after incubation of cytosol with DNA (fig. 3).

Estradiol (6 nM) was shown to increase the amount of the Rc–DNA complex whereas it seemed to protect Rc against the formation of aggregates (fig. 3). In addition to the increase of the 14 S–30 S complex after $E_2$ treatment, the parallel decrease of the unbound 8 S receptor was also an indication of the degree of the interaction (fig. 3). In the absence of any ligand, some Rc–DNA interaction occurred, suggesting that a non specific binding of Rc to DNA was not totally removed under our experimental conditions. The actual effect of the steroids was obtained in subtracting the sites found in the absence of steroids during the interaction from those measured in their presence (fig. 4). This representation emphasized the fact that estradiol (6 nM) and estrone (22.5 nM), which are both biologically active estrogens were efficient on this interaction. The estradiol induced increase of the DNA–Rc interaction was also shown when using single stranded DNA (not shown).

Non estrogenic steroids, which did not compete with $E_2$ on its receptor site, such as progesterone or cortisol were practically inactive on the DNA–Rc

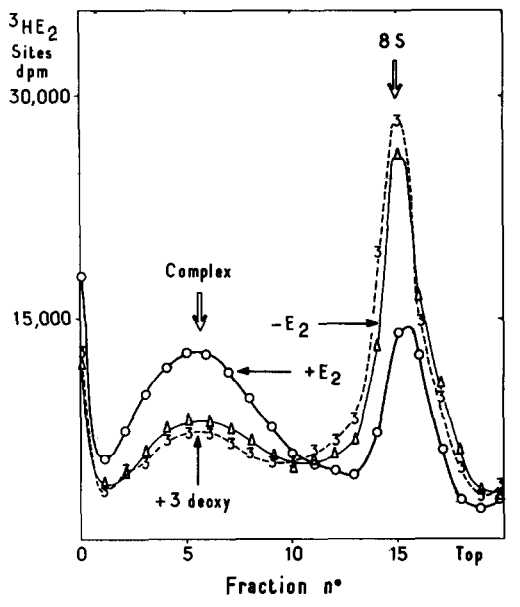


Fig. 5. Absence of effect of 3-deoxy estradiol on a DNA–Rc interaction. DNA was mixed with 3 lots of uterine cytosol previously incubated with [$^3$H]estradiol 6 nM (o—o—o), 3-deoxy estradiol 50 nM (3–3–3) and no steroid (△—△—△). The estradiol binding sites were then measured, after sucrose gradient ultracentrifugation, as described under fig. 3a. The control cytosols were not represented since they did not markedly modify the results.

interaction. In addition, the phenol function seemed to be needed to increase the affinity of Rc to DNA since the 3 deoxy estradiol was inefficient when used at concentrations for which no competition on $E_2$ sites of Rc was observed (fig. 5, table 1). In the case of testosterone (250 nM), a slight increase of the Rc–DNA interaction was found which was more often inferior to that obtained by using estradiol. The significance of this effect and its relationship with that observed when testosterone was applied *in vitro* on the whole uteri [4] are under study.

The whole results (summarized in table 1) indicate the importance of the correction dealing with the aggregates and emphasize the fact that the activity of steroids on the DNA–Rc interaction seems to be roughly parallel to their binding affinity for Rc on one hand, and to their uterotrophic activity on the other hand.

## 4. Discussion

A similar interaction between the estradiol receptor complex and DNA has been recently independently described by others using sucrose gradient centrifugation [12, 13] or DNA cellulose chromatography [14, 15]. The present paper emphasizes the fact that the 8 S receptor can be recovered after its binding to DNA using DNAase treatment, and that estradiol and estrone, but not 3-deoxy estradiol favor specifically this DNA–Rc interaction performed at 0° in a salt free medium.

It is unlikely that estrogens modify DNA itself since they did not bind to it at the low concentrations used as shown in sect. 3.1. More probably, the *in vitro* effect of these hormones on the binding of Rc to DNA reveals some modification of Rc induced by its specific ligands. In addition, these results suggest that Rc contains at least two different binding sites, one for estrogens and the second for DNA, the affinity of the second site being increased by estrogen binding. $E_2$ in this respect seems to behave as an allosteric effector, although no cooperativity of binding has been demonstrated. Whether or not such an interaction occurs *in vivo* is questioned, since we find no marked DNA specificity (unpublished) under the reported experimental conditions. In addition, the molecular form (4 S, 5 S, 8 S or aggregates) under which the receptor enters the nucleus is a subject of controversy. The relationships of the effect of estradiol on the Rc–DNA interaction described above at 0–2° in a salt-free medium, with another effect of estradiol on the transformation of Rc at 25° from 4 S to 5 S [16] is not yet clear, although it has been recently reported that DNA could also favor this kind of transformation [15]. However, the fact that the effect of steroids on the DNA–Rc interaction were approximately parallel to their affinity for Rc on one hand, and to their uterotrophic activity on the other hand, strongly favors a biological significance of such an interaction. Moreover, using a more physiological buffer, we have recently shown that this DNA–Rc complex is also stable and saturable when increasing the concentration of Rc (J. André and H. Rochefort, in preparation). The mechanism of formation of the nuclear receptor from Rc and that of the stimulation of RNA tran-

scription, which are both induced by the presence of estrogens in the uterus could be enlightened by this DNA–protein interaction. The translocation of Rc to the nucleus might be totally explained by its binding to DNA, the fact that the nuclear membrane contains DNA [17] favors this possibility. Alternatively the reported DNA–Rc interaction might be one of the last steps implied in the translocation of the receptor estradiol complex into the nucleus. In this case, estrogens could also favor previous steps such as activation of Rc in the cytoplasm [15] and/or translocation of the receptor through nuclear membranes. The role of non histone protein(s) might be involved in modifying the Rc–DNA interaction, by increasing its specificity and/or its affinity [18]. The stimulation of RNA transcription by estradiol which is one of the first metabolic responses of the cell to the hormone could also be the consequence of this binding to DNA, as it seems to be the case for 3′5′-cyclic AMP in *E. coli* [19]. Quantitative studies performed under more specific and physiological conditions are now in progress to test these possibilities.

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