Estradiol-binding Kinetics of the Activated and Nonactivated Estrogen Receptor*

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The dissociation of \( [\text{H}] \)estradiol from the estrogen receptor of calf uteri followed a two-component exponential function. The first component rate constant, \( k_{-1} \), was 0.12 \( \pm \) 0.01 \text{min}^{-1}, while the second component, \( k_{-2} \), had a rate constant of 4.0 \( \pm \) 0.3 \times 10^{-3} \text{min}^{-1} \) at 25°C. Increasing the fractional activation of the receptor before initiating the \( [\text{H}] \)estradiol dissociation measurement subsequently produced a corresponding decrease in the fraction of the first component without a change in either rate constant.

Sucrose gradient centrifugation analysis indicated that incubation of the estrogen receptor at 25°C in 0.4 M sodium thiocyanate inhibited the 4 to 5 S receptor transformation; only the 4 S monomer was observed. The release of \( [\text{H}] \)estradiol from the 4 S receptor at 25°C in 0.4 M sodium thiocyanate follows a single exponential function with a rate constant of 0.17 min^{-1}, similar to the first component of the biphasic dissociation curve in the absence of sodium thiocyanate. Sucrose gradient centrifugation analysis of the 5 S receptor dimer showed that the \( [\text{H}] \)estradiol dissociation at 25°C in 0.4 M KCl was a single exponential function with a rate constant of 4.4 \times 10^{-3} \text{min}^{-1}. The \( [\text{H}] \)estradiol association rate constants, \( k_{+1} \), were approximately 1 \times 10^8 \text{M}^{-1} \text{min}^{-1} \) at 25°C, whether measured in the presence of 0.4 M KCl, in 0.4 M sodium thiocyanate, or without salt. The association constants, \( k_{+} \), calculated from the rate constants (\( k_{+} / k_{-1} \) and \( k_{+} / k_{-2} \)) indicated that the estrogen receptor has two affinity states. The inactive 4 S monomer has a lower affinity for estradiol (1 \times 10^8 \text{M}^{-1}) than the activated 5 S receptor dimer (2.8 \times 10^{10} \text{M}^{-1}). Consequently, estradiol-binding drives the monomer-dimer equilibrium toward the higher affinity 5 S dimer state of the receptor. These data demonstrate that the 4 to 5 S receptor transformation is a biochemical regulatory mechanism modulated by estradiol.

Two forms of the estrogen receptor have been described, a cytoplasmic form with a sedimentation coefficient of 4 S and a nuclear 5 S species. The nuclear 5 S form is generated from the cytoplasmic 4 S receptor by an estradiol- and temperature-dependent reaction \((1-3)\). The activation or transformation of the receptor from the 4 S to the 5 S state has also been investigated in cell-free systems; activation of the receptor is contingent upon brief incubation of the estradiol receptor complex at 20°C-37°C \((4, 5)\). The analyses of the receptor transformation process indicate that the 4 S state of the receptor is a monomer with a molecular weight of 7 to 8 \times 10^4, which by an estradiol-dependent dimerization reaction associates with a second monomer to form the 5 S receptor, with a molecular weight of 13 to 14 \times 10^4 \((6, 7)\).

Numerous attempts have been made to characterize the \( [\text{H}] \)estradiol-binding properties of the receptor as they relate to the receptor's regulatory function, but without success. Equilibrium binding analysis of \( [\text{H}] \)estradiol, usually performed at 4°C, shows a single class of binding sites, without evidence reflecting a molecular transition from a nonactive to an active state of the receptor, and limited evidence of ligand-binding cooperativity \((8-10)\). The interesting findings of Best-Belpomme et al. \((10)\) and Sanborn et al. \((11)\) indicate that the dissociation of \( [\text{H}] \)estradiol from the receptor shows a complex biphasic curve that is inconsistent with the simple binding process observed by the equilibrium measurements and the Scatchard analysis.

We report in this paper that the two-component exponential dissociation of \( [\text{H}] \)estradiol from the receptor is generated by the nonactive and active states of the receptor. The first, rapid \( [\text{H}] \)estradiol dissociating component is a property of the nonactive 4 S receptor, while the second, slower dissociating component is a property of the active 5 S receptor; estradiol shifts the receptor from a lower to a higher affinity state.

**EXPERIMENTAL PROCEDURES**

**Materials** The \( 17\beta \)([2,4,6,7-\text{H}]estradiol (98.5 or 109 Ci/mmol) was obtained from Amersham/Searle. Its radiochemical purity was verified by thin layer chromatography in benzene:ethanol (9:1) or chloroform:methyl acetate (3:1) solvent systems. Estradiol \( 17\beta \), estriol, diethylstilbestrol, and testosterone were obtained from Steraloids, and progesterone from Schwarz/Mann. Trypsin (204 units/mg) was purchased from Worthington, and soybean trypsin inhibitor (type I-S) from Sigma. The Tria and sucrose were ultrapure grades from Schwarz/Mann. All other reagents used were analytical grade.

**Preparation of Calf Uterine Cytosol**-Calf uteri weighing less than 15 g were stripped of connective tissue, placed in liquid nitrogen, and stored frozen at -80°C until used. The uteri were reduced to a powder with a liquid nitrogen-cooled steel mortar and pestle, and homogenized in 3 to 5 volumes of cold 40 mM Tris, 1 mM...
RESULTS

Dissociation of [3H]Estradiol from Estrogen Receptor – The dissociation of [3H]estradiol from the estrogen receptor, measured by the replacement of labeled with unlabeled estradiol at 29°, showed a biphasic two-component first order dissociation curve (Fig. 1). The [3H]estradiol dissociation rate constant of the first, more rapid component, \( k_{-1} \), after subtraction of the second, slower dissociating component, was linear, with a \( k_{-1} \) of 0.16 ± 0.02 min\(^{-1}\) (Table I). The dissociation rate constants of the second component, \( k_{-2} \), was 8.8 ± 0.7 \times 10^{-3} \text{ min}^{-1}, or only 6% of \( k_{-1} \) (Table I).

The biphasic dissociation curve was corrected for receptor lability or inactivation by measuring the specifically bound [3H]estradiol in the absence of unlabeled estradiol during the 29° incubation; there was no receptor loss after a 2-h incubation, 5% inactivation after 4 h, and significant inactivation after 4 to 6 h (Fig. 1). Experiments were usually performed over a 2- to 3-h period; correction for receptor loss, when necessary, allowed the isolation of the receptor inactivation process from the [3H]estradiol dissociation kinetics. The non-specific [3H]estradiol binding of the uterine cytosol was approximately 5 to 8% of the total binding measured and did not change with time of incubation at 29°. An identical biphasic dissociation curve was seen whether unlabeled estradiol, diethylstilbestrol, or estrone was used to replace the [3H]estradiol that had been dissociated from the receptor at 29°.

The addition of testosterone or progesterone did not replace the specifically bound [3H]estradiol during the 29° incubation. The kinetic parameters of the biphasic [3H]estradiol dissociation were identical whether the fraction of the receptor occupied initially by [3H]estradiol was 14, 36, or 81% of the total estrogen receptor concentration (Table II).

The replacement of [3H]estradiol with unlabeled estradiol...
during the 29° incubation is reversible, since the bound, unlabeled estradiol was replaced by [3H]estradiol. Cytosol preincubated with 5 nM [3H]estradiol at 0° was assayed for dissociation and inactivation at 29°; after 1 h 43% of the initial [3H]estradiol remained specifically bound. The unbound steroid was removed by charcoal/dextran adsorption, 5 μM unlabeled estradiol was added, and the dissociation was initiated by incubation at 29°. Each point was corrected for nonspecific binding; receptor inactivation was insignificant. The k-, component for the 25 (6) and 40 min (8) preincubations at 29° were: 0 min (8), 0.23 min-1; 5 min (0), 0.17 min-1; 10 min (m), 0.18 min-1; and 15 min (C), 0.21 min-1. The k-, component for the 25 (A) and 40 min (C) preincubation at 29° was absent. The k-, was similar for all dissociation curves, 9.5 ± 0.1 × 10^-3 min-1. Estradiol preincubated at 29° for 30 min was cooled to 0° and equilibrated with [3H]estradiol; 5 μM estradiol was added and the [3H]estradiol dissociation kinetics measured (V). Inset, the fraction of the first component of the biphasic dissociation curve versus time of receptor activation at 29°.

**Fig. 2.** Dissociation of [3H]estradiol (E,) from the activated estrogen receptor. Uterine cytosol in Buffer TD was equilibrated with 5 nM [3H]estradiol at 0° for 1 h. Aliquots were incubated for 0, 5, 10, 15, 25, and 40 min at 29° to activate the receptor, then cooled. The free [3H]estradiol was removed by charcoal/dextran adsorption, 5 μM unlabeled estradiol was added, and the dissociation was initiated by incubation at 29°. Each point was corrected for nonspecific binding; receptor inactivation was insignificant. The k-, component for the 25 (A) and 40 min (C) preincubation at 29° was absent. The k-, was similar for all dissociation curves, 9.5 ± 0.1 × 10^-3 min-1. Estradiol preincubated at 29° for 30 min was cooled to 0° and equilibrated with [3H]estradiol; 5 μM estradiol was added and the [3H]estradiol dissociation kinetics measured (V). Inset, the fraction of the first component of the biphasic dissociation curve versus time of receptor activation at 29°.

**Activation of Estrogen Receptor—Warming the [3H]estradiol-receptor complex from 0 to 29° induces receptor activation, as indicated by either an increase in the receptor's capacity to bind to nuclei or an increase in the sedimentation coefficient of the receptor from 4 S to 5 S (4, 5, 14). The [3H]estradiol-receptor complex was incubated at 29° for 0, 5, 10, 15, 25, and 40 min; the dissociation of the [3H]estradiol from the receptor was measured after the addition of an excess of unlabeled estradiol (Fig. 2). The fraction of [3H]estradiol dissociating with the kinetics of the first component decreased with increasing time of the initial 29° incubation (without the excess unlabeled steroid). The decrease in the fraction of the first component is not due to receptor inactivation, since the initial 29° incubation did not reduce the amount of specifically bound [3H]estradiol. Neither was the rate constant k,- (0.20 ± 0.01 min^-1) changed when the receptor was preincubated for 0, 5, 10, or 15 min at 29°. However, preincubation at 29° for 25 or 40 min, followed by receptor dissociation, showed the slower [3H]estradiol dissociation (k-,) kinetics without the first component. The decrease in the fraction of the first component was not linear with the time the receptor was preincubated at 29° (Fig. 2, inset), which suggests the involvement of complex kinetics in the transformation of the receptor from a state showing k,- kinetics to a state showing k- kinetics. A plot of the inverse of the fraction of the first component versus the time of incubation (receptor activation) at 29° indicates that the transition from the more rapid to the slower estradiol-dissociating component is a second order reaction (data not shown). This is consistent with our previous observations of the 4 S to 5 S receptor transformation (5).

Incubating the estrogen receptor at 29° for 30 min before equilibration with [3H]estradiol at 0°, a process which does not induce receptor activation (4, 5), resulted in a biphasic [3H]estradiol dissociation curve identical with the curve of the nonactivated receptor (Fig. 2). The disappearance of the first component was dependent upon the presence of estrogen during the 29° preincubation. The second component did not show a change in k,- with preincubation at 29°. Instead, a parallel shift upward was observed as the fraction of the first component diminished (Fig. 2). The data suggest that the fraction of the first component (k,-) present is dependent upon the nonactive estrogen receptor.

**Dissociation of [3H] Estradiol from Receptor Analyzed by Sucrose Gradient Centrifugation—**The nonactivated 4 S estrogen receptor complex had a rapid [3H]estradiol dissociation similar to k-, while the activated 5 S estrogen receptor

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**Table II**

| Initial bound [3H]estradiol (nM) | Fraction of total [3H]estradiol binding | k-1 (min^-1) | k-2 (×10^7 min^-1) |
|-------------------------------|----------------------------------------|--------------|------------------|
| 0.41                          | 0.14                                   | 0.15         | 5.3              |
| 1.04                          | 0.06                                   | 0.15         | 5.1              |
| 2.32                          | 0.81                                   | 0.12         | 5.1              |

**Fig. 2.** Dissociation of [3H]estradiol (E,) from the activated estrogen receptor. Uterine cytosol in Buffer TD was equilibrated with 5 nM [3H]estradiol at 0° for 1 h. Aliquots were incubated for 0, 5, 10, 15, 25, and 40 min at 29° to activate the receptor, then cooled. The free [3H]estradiol was removed by charcoal/dextran adsorption, 5 μM unlabeled estradiol was added, and the dissociation was initiated by incubation at 29°. Each point was corrected for nonspecific binding; receptor inactivation was insignificant. The k- component for the 25 (A) and 40 min (C) preincubation at 29° was absent. The k- was similar for all dissociation curves, 9.5 ± 0.1 × 10^-3 min-1. Estradiol preincubated at 29° for 30 min was cooled to 0° and equilibrated with [3H]estradiol; 5 μM estradiol was added and the [3H]estradiol dissociation kinetics measured (V). Inset, the fraction of the first component of the biphasic dissociation curve versus time of receptor activation at 29°.
showed a slower \(^{3}H\)estradiol dissociation, similar to \(k_{-1}\) of the two-component dissociation curve. Uterine cytosol was equilibrated with 10 nM \(^{3}H\)estradiol, then made 0.4 M with respect to sodium thiocyanate (NaSCN) to retard the hydrophobic interactions between the 4 S estrogen receptor and its complementary monomeric unit, thereby preventing the formation of the 5 S dimer during incubation at 25°. The \(^{3}H\)estradiol receptor was incubated at 25° with or without an excess of unlabeled estradiol for 0, 2, 5, 10, and 15 min. The dissociation of the radioactive steroid was stopped by cooling the aliquots to 0° and adding charcoal/dextran. Sucrose gradient centrifugation analysis showed that the predominant form of the receptor was 4 S and that the bound \(^{3}H\)estradiol was exchanged for unlabeled estradiol during incubation at 25°. A single first order dissociation curve was seen when the replacement of radioactivity of the 4 S peak was plotted (Fig. 3B). The rate constant was 0.17 min⁻¹, in good agreement with the 0.12 min⁻¹ value observed for \(k_{1}\) of the two-component \(^{3}H\)estradiol dissociation kinetics at 25° (Table I). When the 5 S estrogen receptor was subjected to sucrose gradient centrifugation for 16 h at 50,000 rpm, the receptor showed a slower \(^{3}H\)estradiol dissociation, similar to \(k_{-1}\) of the two-component \(^{3}H\)estradiol dissociation kinetics at 25° (Fig. 4A). The labeled estrogen dissociated from the receptor as a single component with a rate constant of \(4.4 \times 10^{-3}\) min⁻¹ at 25° (Fig. 4B), identical with the \(k_{-1}\) of the \(^{3}H\)estradiol dissociation curve (Table I). Thus, the increase in the \(^{3}H\)estradiol dissociation rate in the presence of 0.4 M NaSCN was not caused by the ionic strength of the buffer but by the ability of the 0.4 M NaSCN to retain the estrogen receptor in the nonactive 4 S form.

The influence of 0.4 M KC1 or 0.4 M NaSCN on the dissociation of \(^{3}H\)estradiol from the receptor at 25°, measured by the charcoal/dextran adsorption method, showed results similar to those from sucrose gradient centrifugation analysis. In the presence of 0.4 M KC1 the activated estrogen receptor showed a single dissociating component with a rate constant of \(4.3 \approx 0.3 \times 10^{-3}\) min⁻¹, similar to the \(k_{-1}\) of the two-component dissociation curve observed in Buffer TD. In the presence of 0.4 M NaSCN the estrogen receptor showed a single dissociating component with a rate constant \((0.11 \pm 0.01)\) min⁻¹ comparable to the \(k_{-1}\) of the biphasic dissociation curve (Fig. 5).

The 0.4 M KC1 or 0.4 M NaSCN did not influence the \(^{3}H\)estradiol association reactions at 0° or 25°. The association reactions were measured under second order conditions. The rate constants were independent of both receptor and \(^{3}H\)estradiol concentrations. The association rate constants in Buffer TD, Buffer TD with 0.4 M KC1, or 0.4 M NaSCN were not significantly different (Table III), which indicates that the NaSCN only affected the dissociation rate of \(^{3}H\)estradiol. Using either the fast \((k_{-1})\) or the slower \((k_{-2})\) dissociation rate constant, an estimation of the association constants \((K_{a})\) from the ratios \(k_{-1}/k_{+1}\) and \(k_{-2}/k_{+2}\) indicates a 25- to 30-fold difference in the affinity between the two states of the receptor at 25°.

**Influence of Trypsin on Kinetics of \(^{3}H\)Estradiol Dissociation**—Limited proteolysis of the estrogen receptor by trypsin or endogenous proteases at 0° produces an estrogen-binding fragment of the receptor which sediments at approximately 4 S and has a molecular weight of \(6 \times 10^{4}\), which is lower than that of the native 4 S estrogen receptor (7 to \(8 \times 10^{4}\)) (15, 16). The trypsin-treated estrogen receptor cannot undergo either the temperature-induced 4 S to 5 S receptor transformation or the temperature-enhanced nuclear-binding activity (17).

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**Fig. 3.** Sucrose gradient analysis of \(^{3}H\)estradiol (E₁) dissociation from the 4 S estrogen receptor. Uterine cytosol was equilibrated with 10 nM \(^{3}H\)estradiol for 1 h at 0°, then made 0.4 M with respect to NaSCN for 4 h at 0°. Aliquots were incubated at 25° for the times noted, with or without the addition of 1 \(\mu\)M estradiol. The unbound estradiol was adsorbed by charcoal/dextran and the aliquots were subjected to sucrose gradient centrifugation for 16 h at 50,000 rpm at 1° (A). The percentage of \(^{3}H\)estradiol remaining bound to the 4 S receptor with time at 25°; the dissociation rate constant was 0.17 min⁻¹ (B).

**Fig. 4.** Sucrose gradient analysis of \(^{3}H\)estradiol (E₂) dissociation from the 5 S estrogen receptor. Uterine cytosol was equilibrated with 10 nM \(^{3}H\)estradiol for 1 h at 0°, then incubated at 28° for 30 min, cooled to 0°, and made 0.4 M with respect to NaCl for 4 h at 0°. The remaining conditions are identical with those in Fig. 3A (A). The percentage of \(^{3}H\)estradiol remaining bound to the 5 S receptor with time at 25°; the dissociation rate constant was \(4.4 \times 10^{-3}\) min⁻¹ (B).
Table III

| Cytosol in | k₁ | k₂ | k₄/k₃ | k₄/k₂ |
|-----------|----|----|-------|-------|
| Buffer TD | 0.12 ± 0.01 (6) | 4.0 ± 0.5 (6) | 1.1 ± 0.1 (5) | 0.9 ± 0.3 (5) |
| +0.4 M KCl | 0.13 ± 0.01 (4) | 4.3 ± 0.3 (4) | 0.7 ± 0.01 (2) | 0.5 ± 0.2 (3) |
| +0.4 M NaSCN | 0.11 ± 0.01 (4) | 1.1 ± 0.2 (3) | 1.0 ± 0.3 (5) | 0.9 ± 0.3 (5) |

**DISCUSSION**

The first component of the biphasic [³H]estradiol dissociation curve is the lower affinity state of the receptor, the nonactive 4 S monomer, with a rate constant of k₁. This conclusion is based upon a number of experimentally consistent observations. The disappearance of the amount of the first component is inversely related to the time that the [³H]estradiol-receptor complex is preincubated at 25° before the [³H]estradiol dissociation from the receptor is measured. Preincubation of the receptor without estradiol fails to activate the receptor and consequently does not induce the disappearance of the k₁ component (Fig. 2). Sucrose gradient centrifugation analysis demonstrates that sodium thiocyanate was effective in inhibiting the 4 S to 5 S receptor transformation and in maintaining the receptor in the 4 S state that shows a single rapidly dissociating component. The rate constants were 7 × 10⁻³ min⁻¹ and 12 × 10⁻³ min⁻¹, respectively (Fig. 6B). Trypsin had an identical effect on the dissociation kinetics of the estrogen receptor whether the receptor was in the activated or nonactivated state. The receptor inactivation curves (Fig. 6) indicate that no significant specifically bound [³H]estradiol was lost during the 25° incubation; the soybean inhibitor effectively inhibited the trypsin activity. In comparison with the native receptor, trypsin-modified estrogen receptor showed a loss of biphasic [³H]estradiol dissociation kinetics suggesting a loss of the capacity to transform from a nonactive to an active state of the receptor.
rate constant identical with the $k_-$ of the biphasic dissociation curve (Fig. 3). Sucrose gradient centrifugation analysis demonstrates that the 5 S form of the receptor has a slower rate of estradiol dissociation, equal to the $k_-$ of the biphasic $[^3H]$estradiol dissociation curve (Fig. 4).

The association rate constant $k_{+,1}$ of estradiol binding by the receptor is not significantly influenced by the absence or presence of 0.4 M KCl or 0.4 M sodium thiocyanate (Table III).

Assuming that the association rate constant $k_{+,1}$ is an appropriate value for both states of the receptor, the association constants ($K_+$) of the 4 S and 5 S receptor were calculated from the ratios of the rate constants $k_{+,1}/k_-$ and $k_{+,4}/k_-$, respectively. This is a reasonable assumption since the formation of the 5 S dimer requires that the 4 S monomer be complexed with estradiol (4, 5) and that the existence of a second association rate constant of estradiol associating with an "estradiol-free activated 5 S dimer" is an unlikely pathway.

The two-component exponential dissociation of estradiol from its receptor shows that the inactive state of the receptor has a faster estradiol dissociation rate and a lower affinity for estradiol (1 x $10^6 \text{ M}^{-1}$) than the active receptor (2.8 x $10^6 \text{ M}^{-1}$). Since the affinity of the 5 S receptor dimer for estradiol is higher than that of the 4 S monomer, estradiol shifts the monomer-dimer equilibrium from the inactive to the activated state of the receptor, demonstrating that estradiol binding is a modulator of the 4 S to 5 S receptor transformation. The biphasic curve of estradiol dissociating from the receptor depends on three parameters: the two dissociation rate constants $k_-$ and $k_-$ and the fraction of the estradiol dissociating with the $k_-$ parameter. The fraction of the estradiol dissociating from the receptor with the faster rate constant $k_-$ is dependent upon the competition between the rate at which estradiol dissociates from the 4 S receptor and the rate of the 4 S monomer dimerizing to form the 5 S receptor. The 4 S receptor dimerization reaction is characterized by the second order rate constant, $k_{act}$ (Fig. 7).

These data indicate that a complex interplay exists between estradiol-binding and the state of the receptor. The $[^3H]$estradiol equilibrium binding to the receptor at 0-4°C has generally been accepted to indicate the presence of a single class of binding sites (9) and does not detect the transition of the receptor from the nonactivated to the activated state. The receptor's complex estradiol dissociation kinetics reveals the presence of two states of the receptor. The assumption based upon the Scatchard analysis of a single class of binding sites may be incorrect. The receptor's regulatory characteristics and properties that are influenced by estradiol would be very different, depending upon the structure of the receptor. At the present time there is no compelling evidence to indicate whether the 5 S receptor dimer is composed of two estrogen-binding 4 S monomers, or a 4 S estrogen binding monomer and a second, non-estrogen binding monomer (5, 6, 18). Wyman (19) and Levitzki and Schlessinger (20) have described the theoretical basis for the kinetic behavior of oligomerizing protein systems and their relationships to the Hill coefficient, a measure of cooperativity. The dimerization of identical monomers can show positive cooperativity even in the absence of subunit-subunit interactions, provided a sufficient difference in the affinity for the ligands exists in the two states of the monomer-dimer equilibrium.

If we assume that the 5 S receptor is composed of two estrogen-binding 4 S monomers, while considering the higher affinity of the 5 S dimer for estradiol, the positive cooperativity of estradiol-binding should be present. Although most studies of estradiol binding by the receptor indicate that the Scatchard or Hill plots show limited or no evidence of cooperativity (9), several investigators have reported Scatchard plots with "vaulted ceilings," which suggest positive cooperativity (10, 11). We also observed Scatchard plots with vaulted ceilings and Hill coefficients of 1.3 to 1.7. Because of the possibility of differential receptor inactivation at lower, as compared with higher, steroid concentrations, and of small errors in separating free and bound steroids, we cannot be certain that the deviation from linearity of the Scatchard plots at low estradiol concentrations is not due to experimental artifacts rather than to positive cooperativity. In addition, the equilibrium binding analysis of the estrogen receptor is usually carried out at 0-4°C. Under these conditions the 4 S to 5 S receptor transformation is very slow (5), consequently limiting the equilibrium between the lower and higher estradiol affinity states of the receptor. The diffusion in affinity between the lower and higher affinity states is approximately 25-30-fold, as estimated from the kinetic data (Table III), which is well within the range of the values reported and the limits of accuracy of $[^3H]$estradiol equilibrium measurements (9).

If we assume that the 5 S receptor is a heterodimer composed of an estrogen-binding monomer and a second, non-estrogen-binding monomer, then positive cooperativity of estradiol binding may not necessarily be observed. The non-estrogen-binding monomer would associate with the estrogen-binding monomer during receptor transformation, increasing the affinity for estradiol by the maintenance of the 5 S receptor. This phenomenon which is similar to the heliotropic effect (21) is distinct from the positive cooperative model of the homodimer.

A disparity between the affinities of the cytoplasmic and nuclear forms of the estrogen receptor has been previously reported. Some reports (22-24) suggest that the cytoplasmic form of the estrogen receptor has an estradiol affinity approximately 10-fold higher than the nuclear estrogen receptor. (The estradiol affinity of the nuclear estrogen receptor was indirectly measured by the $[^3H]$estradiol exchange method at 37°C (25) and compared with an equilibrium binding assay of the cytosol at 0°C). This is contrary to our observation that the activated 5 S receptor or nuclear form has a higher affinity

\begin{itemize}
  \item A. C. Notides and D. E. Hamilton, unpublished observations.
\end{itemize}
than the 4 S receptor. The basis for this apparent disagreement may be related to use of the nuclear [3H]estradiol exchange or to the molecular interactions of the estrogen receptor with the chromatin. The activated 5 S receptor has a greater propensity than the 4 S receptor to associate with some component(s) of the nucleus, "acceptor sites," thereby initiating the synthesis of mRNAs (23). The details of the molecular interactions of the receptor with the chromatin are not known, although a number of models have been proposed (26–28). The molecular action or termination of the receptor-chromatin interaction may involve a factor(s) or condition(s) that induces the receptor to revert to a lower affinity state favoring estradiol dissociation from the receptor dimer, followed by dimer dissociation. Possibly, during the nuclear receptor [3H]estradiol exchange assay only the lower affinity state of the receptor is measured.

The action of trypsin at O-4" on the estrogen receptor results in limited proteolysis of the receptor and a reduction of its molecular weight but does not destroy the estrogen-binding site (17). As a consequence of the limited proteolysis, the receptor loses its biphasic estradiol dissociation characteristics and shows an increase in the rate of estradiol dissociation from the receptor, dependent upon the amount of trypsin added and, presumably, the extent of proteolysis of the receptor.

The complex dissociation kinetics suggests the need for a reassessment of the assumption based upon equilibrium binding data, that receptor interaction is a simple equilibrium involving estradiol and a single class of receptor binding sites.

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