DNA Allosterically Modulates the Steroid Binding Domain of the Estrogen Receptor*

Michael Fritsch, Roy D. Welch, Fern E. Murdoch, Iain Anderson, and Jack Gorski†

From the Department of Biochemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706

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The ability of DNA to allosterically alter the conformation of the estrogen receptor's (ER) steroid binding domain was investigated. Using dissociation kinetics we observed that when DNA was bound to the DNA binding domain of the rat uterine ER the rate of estrogen dissociation from the steroid binding domain increased almost 2-fold. This change in the rate of estrogen dissociation depended on DNA concentration. DNA used and correlated with the thermodynamic binding affinities (Kd) of the ER for two different DNA sequences. We were unable to detect a DNA-induced change in the trypsin cleavage pattern of the amino terminal end of the ER. Using a whole cell dissociation kinetics assay with MCF-7 breast cancer cells we observed a 7-fold slower rate of estrogen dissociation from the ER within the cell than from the ER in vitro. This suggests that additional factors, other than DNA binding, may modify the steroid binding domain within the cell. We conclude that DNA can allosterically modulate the structure of the steroid binding domain of the ER, and we hypothesize that this conformational change may be necessary for the full transcriptional activity of the ER.

The ER* protein is a member of the steroid receptor superfamily of DNA binding, nuclear transcription factors (1, 2) and can be divided into distinct functional domains (Fig. 1). The steroid binding domain is responsible for high affinity binding of estrogen (3) but has also been implicated in transcriptional activation and protein dimerization (Refs. 4, and references therein, and 5). The DNA binding domain mediates ER binding to specific DNA enhancer sequences called estrogen response elements (ERE), proposed to localize the ER to a sequence. We demonstrate that when DNA is bound to the DNA binding domain of the ER, the ability of the steroid binding domain to bind estrogen is altered, as measured by a change in the dissociation rate of estrogen. These data support a model in which DNA can act as an allosteric modulator of the steroid binding domain of the ER. We observe no change in the proteolytic cleavage pattern of the amino terminus with or without DNA bound to the ER, indicating that the allosteric effect of DNA may be specific to the steroid binding domain. Finally, we show that within the cell the dissociation rate of estrogen from the ER is prolonged over that observed in vitro, suggesting that additional factors within the cell may modulate the functional activity of the steroid binding domain of the ER.

EXPERIMENTAL PROCEDURES

Materials—The sources of materials used in these studies are as indicated: [2,4,6,7-3H]estradiol (85-115 Ci/mmol) and [2,4,6,7-3H]estradiol (90-115 Ci/mmol) in ethanol were obtained from Du Pont-New England Nuclear; Tris buffer, β-mercaptoethanol, insulin, EDTA, HEPES, bovine pancreas trypsin (Type III-S), bovine lung aprotinin, and phenylmethylsulfonyl fluoride were from Sigma; SDS-PAGE reagents including low range SDS-PAGE standards were obtained from Bio-Rad; sodium bicarbonate came from Mallinckrodt; methylcellulose was from Fisher Scientific Co.; antibiotics for cell culture were from Gibco; bovine calf serum was from HyClone; Dulbecco's modified Eagle's medium (without phenol red and sodium bicarbonate) and HBSS were from Sigma; CMP-EDTA was made from reagents without calcium and magnesium and with 1 mM EDTA.

Estrogen Receptor Preparation—Uterine cytosols were prepared from 19-day-old Sprague-Dawley female rats at three uteri/ml as described previously (10) except in TM buffer (10 mM Tris, pH 7.5, at 25 °C, 10 mM β-mercaptoethanol). The cytosol was made to 5 mM in tritiated estradiol ([3H]E2) or 30 mM in tritiated estradiol ([3H]E2). A 200-fold excess of diethylstilbestrol (DES), an unaltered estrogen agonist with high affinity for ER, was added to replicate samples to determine nonspecific binding. Samples were incubated for 45 min at 4 °C to occupy the ER, followed by 45 min at 28 °C to transform the ER to its DNA binding form (11, 12). All in vitro occupied ER in these studies were heat transformed. The cytosol samples were returned to ice and made to 150 mM KCl (TM buffer). The nonspecific binding was the same in the presence and absence of DNA.

Synthetic Oligonucleotides—The sequences were previously described (13); vitERE (5'-CATCCAGATCAGTGTCTCGATC3') and mutERE (5'-GATCCAGATCAGTGTCTCGATC3'). In transient transfection assays the vitERE mediates an estrogen response, whereas the mutERE does not. Both strands of the vitERE and mutERE oligonucleotides were synthesized in the Department of
Biochemistry at the University of Wisconsin-Madison. Single-stranded oligonucleotides were dissolved in 10 mM Tris, 1 mM EDTA, 200 mM NaCl, annealed with the complementary strand, and the concentration of double-stranded DNA determined spectrophotometrically (10). Each 25-base pair double-stranded oligonucleotide was considered a single binding site for the determination of the DNA concentration.

**Dissociation Kinetics—**[^1] [H]E₂ occupied ER in TKM buffer was incubated with no DNA, mutER, or vitER for 1 h at 4 °C. An equal volume of TKM with 3 μM DES was added to compete with dissociated [H]E₂ for binding to ER and incubation continued at 28 °C. An equal volume of TKM with ethanol vehicle was added to control reactions to demonstrate any loss of ER during the course of the assay. At the indicated times the tubes were placed at 4 °C and ice-cold buffer added to stop the dissociation of [H]E₂. The amount of specific [H]E₂ remaining at each time was determined using the hydroxylapatite assay (14). The ratio of the amount of specific [H]E₂ bound at each time ([ER]) to the amount of specific [H]E₂ bound at time 0 ([ER]₀) represents the fraction of [H]E₂ that remained bound to ER. For E₂ the experiment was performed as above except ER was occupied with 30 nM [H]E₂ and dissociation performed for 4 °C for the indicated times. The equation ln([ER]₀/[ER]ₜ) = -kt describes the dissociation of ligand (15); therefore, the slopes of the lines in plots of ln([ER]₀/[ER]ₜ) versus time represent the dissociation rate constants k. The rate constants were estimated by use of the computer program KINETICS (16), and the half-lives (t₁/₂) were calculated from the equation t₁/₂ = -ln(0.5)/k.

The dose-response curves of DNA's effect on estrogen dissociation were obtained by incubating the [H]E₂ occupied ER with varying concentrations of either vitER or mutER for 1 h at 4 °C in TKM buffer. The highest concentration of each oligonucleotide was assumed to saturate ER as estimated from binding studies at 4 °C. An equal volume of 3 μM DES in TKM was added and the reactions were incubated at 28 °C for 75 min. Specific [H]E₂ remaining at each concentration of DNA was determined as described above. The fraction of ER in the slow dissociation state at each DNA concentration was calculated as follows:

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\text{Specific dpm at each } [\text{DNA}] = \frac{\text{Specific dpm at saturating } [\text{DNA}]}{\text{Specific dpm at zero } [\text{DNA}]} \times \text{specific dpm at saturating } [\text{DNA}]
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The specific dpm at saturating [DNA] represent the amount of [H]E₂ bound to the ER when all the ER is bound to DNA (i.e. in the fast dissociation state). The specific dpm at zero [DNA] represents the amount of [H]E₂ bound to the ER when no DNA is bound to the ER (i.e. in the slow dissociation state). The denominator represents the maximum change in the specific dpm caused by DNA binding. The numerator represents the change in specific dpm at each [DNA]. As more ER becomes bound to DNA, the specific dpm at each [DNA] should approach the specific dpm at saturating [DNA]. The numerator reflects the amount of ER not bound to DNA, which therefore remains in a slow dissociation state.

**Immunoblotting—** Samples were treated with SDS-PAGE sample buffer and separated by SDS-PAGE (10% acrylamide), transferred to nitrocellulose, and immunostained using the procedure described previously (18). The primary antibody was a 1:1000 dilution of affinity purified ER715, a polyclonal antibody raised against a 15-amino acid peptide from the D region of the rat ER.

**Trypsinization of ER—** The ER was trypsinized using 2.5 μg/ml final concentration of trypsin for various amounts of time up to 1 h at 4 °C. Trypsin stock (0.5 mg/ml) was made fresh from powder of the morning of each experiment and diluted into the sample several hours later. Trypsinization was stopped using a mixture of aprotinin at a final concentration of 50 μg/ml and PMPS at 15 μM. This mixture of protease inhibitors completely inhibited trypsin action at these concentrations. Control tubes were treated with trypsin that had been preincubated with these protease inhibitors.

**Cell Culture—** MCF-7 breast cancer cells were passaged 85–90 times in phenol red-free Dulbecco's modified Eagle's medium with 5 mM HEPES, 44 mM sodium bicarbonate, 10% bovine calf serum (estrogen-free), 1 μg/ml insulin, and antibiotics (100 units/ml penicillin G, 100 μg/ml streptomycin sulfate, and 0.25 μg/ml amphotericin B as fungizone). The cells were split 1:10 on each passage, which was every 7 days. For individual experiments the cells were cultured for 6–7 days in 100-mm plates, fed every 2–3 days, and harvested 24 h before harvesting. This cell line has about 150,000 ER/cell.[^2]

**Whole Cell Dissociation Kinetics—** The medium was removed and each plate of cells rinsed once with 5 ml of CMF-EDTA and 5 ml of fresh CMF-EDTA was added and incubated at 37 °C for 10 min to release the cells from the plates. Cells were pelleted at 150 × g for 4 min at room temperature, and resuspended in 3 ml of DMEM/plate. The cells were divided into two equal samples and an equal volume of 10 nM [H]E₂ in HBSS, or 10 nM [H]E₂ plus 2 μM DES in HBSS was added. The cells were incubated at 37 °C for 15 min with occasional mixing to occupy the ER. The cells were released on ice with a 1:6 dilution to a concentration of about 800,000 cells/ml. One milliliter of the cell suspension was aliquoted into each tube, and 0.5 ml of HBSS with 3 μM DES was added at 37 °C at time 0 to initiate the dissociation kinetics assay. To determine the loss of ER during the dissociation assay, 0.5 ml of HBSS without DES was added to control tubes. At the appropriate times the tubes were placed at 4 °C, and 1 ml at room temperature, and resuspended in 3 ml of DMEM/plate. The tubes were spun, and 0.5 ml of the supernatant was added to 3.5 ml of ice-cold HBSS added to stop the dissociation of [H]E₂. Cells were spun at 150 × g for 4 min at 4 °C, washed twice with 2 ml of HBSS, 0.1% methylcellulose, 0.1% bovine serum albumin and twice with 1.5 ml of HBSS, 0.1% methylcellulose. The cell pellet was resuspended in 0.1 ml of TE (10 mM Tris-CI plus 1.5 mM EDTA), and 0.5 ml of 0.6 ml 200 mM NaCl containing 0.1 mg/ml ethidium bromide from temperature with occasional mixing. The tubes were spun, and 0.5 ml of the supernatant was added to 3.5 ml of Ready-Set scintillation fluid (Beckman) and subjected to scintillation counting. The amount of specific [H]E₂ remaining at each point was calculated as described above.

**ER Extraction from MCF-7 Cells—** The ER was occupied in situ as described above. The cells were spun at 150 × g for 5 min at 4 °C, resuspended in 0.6 ml/plate of TEM (TM plus 1.5 mM EDTA) at 4 °C, homogenized by 30 strokes in a Dounce homogenizer with a B pestle, allowed to sit 5–10 min, and homogenized 15 more strokes. A nuclear pellet was prepared by centrifuging the homogenized cell suspension at 3000 × g for 10 min at 4 °C. The nuclear pellet was resuspended in 0.6 ml/plate of 0.6 M KCl in TEM and allowed to sit 90 min at 4 °C with occasional mixing. The extract was centrifuged in a fixed angle rotor (TLA-100.2) at 436,000 × g for 40 min in a Beckman table top TLA 100 ultracentrifuge at 4 °C. The supernatant containing occupied nuclear ER was diluted 1:8 so that the final salt concentration was 100 mM. Dissociation kinetics were performed on aliquots as described for rat uterine cytosolic ER except at 37 °C.

Unoccupied MCF-7 cytosolic ER was prepared by resuspending nonestrogen-treated cells in 0.67 ml/plate of TEM at 4 °C and homogenizing as above. The extract was centrifuged in a fixed angle rotor (TLA-100.2) at 436,000 × g for 10 min in a Beckman table top TLA 100 ultracentrifuge at 4 °C. The supernatant containing unoccupied cytosolic ER was collected. Occupation of ER and dissociation kinetics were performed as for the rat uterine cytosolic ER except in TEM without KCl and at 37 °C. It has been shown previously that [KCl] has no effect on [H]E₂ dissociation from the ER (19, 20).

**RESULTS**

**DNA Binding to the ER Alters the Steroid Binding Domain's Ability to Bind Estrogen—** We employed kinetic analysis to investigate whether DNA binding to the ER could modulate the steroid binding domain's ability to bind estrogen. Since the steroid binding domain alone is needed for estrogen binding (21), any DNA-induced change in the half-life of estrogen dissociation would necessarily reflect a change in the conformation of the steroid binding domain. Fig. 2a shows the dissociation kinetics assay of [H]E₂ in the absence of DNA was 40.3 ± 1.6 min. A 25-base pair oligonucleotide containing the ERE from the Xenopus vitellogenin A2 gene (vitER) or a 2-base pair mutation (mutER) (13) was added to the reaction at a final oligonucleotide concentration of 20 nM. Based on thermodynamic

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[^1]: M. Fritsch and J. Gorski, unpublished data.

[^2]: Fig. 1. The functional domains of the rat ER (41). The amino acids are numbered from amino terminus to carboxyl terminus (1-600).
FIG. 2. Dissociation kinetics of estrogen from the ER in the absence and presence of DNA. A, rat uterine cytosolic ER occupied with [3H]E2 in TM buffer was placed at 28 °C for varying amounts of time either in the presence of 3 μM DES (solid lines) or in the absence of DES (dashed lines) with no DNA (closed circles), 20 nM mutERE (open squares), or 20 nM vitERE (closed squares). The natural logarithm of the ratio of the amount of specific [3H]E2 bound at time zero ([ER]o) to the amount of specific [3H]E2 bound at time t ([ER]t) was plotted against the time at 28 °C. The data shown are representative of four independent experiments. B, the experiment was performed as in A except ER was occupied with [3H]E2 and dissociation performed at 4 °C for the indicated times.

equilibrium binding constants obtained at 4 °C, this concentration of vitERE saturates the ER, whereas the mutERE occupies less than 6% of the ER (17). The dissociation half-life of [3H]E2 from the ER in the presence of 20 nM mutERE was 39.2 ± 1.1 min, which was equivalent to the control value in the absence of DNA. The dissociation half-life of [3H]E2 from ER in the presence of 20 nM vitERE was significantly shorter, 26.8 ± 0.9 min.

Estriol is an estrogen with a lower binding affinity for the ER than estradiol (22). This allows the dissociation rate to be measured at 4 °C. Fig. 2B shows the dissociation kinetics of [3H]E2 from the ER at 4 °C ± DNA. The dissociation half-life of [3H]E2 from the ER in the absence of DNA was 15.4 ± 1.1 h and 13.4 ± 0.8 h in the presence of 20 nM mutERE. In the presence of 20 nM vitERE, the dissociation half-life was significantly shorter, 7.8 ± 0.4 h.

We wanted to ensure that the loss of estrogen measured in these assays was due strictly to dissociation and not to denaturation or loss of ER. The total amount of measured ER did not change in the absence of DES as competitor during the assay (dashed lines in Fig. 2). Fig. 3 shows an immunoblot of the cytosolic ER treated with or without vitERE and incubated at 0 °C or at 28 °C for 75 min. The amount of full-length (66 kDa) ER detected did not change under the assay conditions used. Thus, the presence of DNA and heating to 28 °C had no effect on the total amount of intact ER present during the dissociation kinetics assay.

The DNA-induced Change in the Steroid Binding Domain Is Mediated by the DNA Binding Domain—The equivalent dissociation rates of estrogen from the ER observed in the absence of DNA or in the presence of mutERE eliminated the possibility of a general DNA polyanion effect on the steroid binding domain. The data in Fig. 2 suggest that the ER can exist in one of two states with regard to the rate of estrogen dissociation; a slow state when DNA is not bound and a fast state when DNA is bound to the ER. This model predicts that the fraction of ER in the fast state of estrogen dissociation will correlate with the fraction of ER bound to DNA. To test this, we added increasing concentrations of either vitERE or mutERE to cytosolic ER and measured the amount of [3H]E2 that remained bound to ER at a single time point under conditions described for Fig. 2A. Fig. 4 illustrates the conversion of the ER from the slow state of estrogen dissociation in the absence of DNA to the fast state of estrogen dissociation as the concentration of DNA is increased. The data in Fig. 4 were calculated and plotted as described under “Experimental Procedures.” A much higher concentration of mutERE than vitERE was needed to saturate the response. These results were consistent with a binding phenomenon, because the curves were sigmoidal and the responses to DNA were saturable. We estimated the concentration of DNA at which 50% of the ER was in the slow state of dissociation (50%slow) by fitting the data in Fig. 4 to the model of a rectangular hyperbola, which is mathematically equivalent to the Michaelis-Menten equation, with nonlinear least squares regression using the SAS PROC NLIN computer program (23). In this study the concentration of vitERE at 50%slow was 0.5 ± 0.2 nM and for mutERE the concentration was 100 ± 40 nM, which gives a relative 50%slow value of 200, indicating

![Fig. 3. Immunoblot of rat uterine cytosol ER under various conditions encountered during the course of dissociation kinetics. Rat uterine cytosol ER was prepared as described under “Experimental Procedures.” Following transformation at 28 °C, the cytosol was either incubated with 60 nM vitERE or TM buffer (no DNA) for 4 h at 4 °C. The samples were then left at 0 °C or placed at 28 °C for 75 min and then treated as described under “Experimental Procedures.” The positions at which the molecular weight markers migrated are indicated on the left.](image-url)
that 200 times more mutERE was needed to produce the same effect as vitERE. The 50%slow values are within 2–8-fold of the binding affinities (Kd) for each of these oligonucleotides binding to ER as determined at 4 °C (17).

Considering the differences under which the DNA binding assays and these dissociation kinetic assays were performed, especially temperature, these 50%slow values are close to the expected range for the oligonucleotides binding to ER. Clearly the conversion of the ER from the slow to the fast state of binding to ER as determined at 4 °C (17).

The dissociation half-life of estrogen longer than that observed in vitro (18). Therefore, most of the steroid binding domain must be intact in each fragment to be detected by both methods and a stepwise cleavage must be occurring from the amino terminal end of the ER. The dissociation half-life of estrogen is a function of the ER's environment. The half-life of dissociation of [3H]E2 from the unoccupied, rat uterine cytosolic ER was 59.4 min (data not shown). Thus, the DNA binding domain of ER is necessary to observe the DNA-induced change in the amino terminus, at least we cannot detect such a change by the method of partial trypsinization.

**Additional Factors Contribute to the Dissociation Rate of Estrogen from the ER within the Cell**—We have shown that DNA binding to the ER can alter the dissociation kinetics of estrogen as measured in vitro. When DNA is bound to the ER, estrogen dissociates faster from the steroid binding domain. Since it is assumed that the occupied ER is bound to DNA within the cell (2, 25), does the dissociation rate of estrogen from the ER within the cell differ from the dissociation rate of estrogen from the ER in an in vitro system? Using a whole cell assay, we rapidly occupied the ER using [3H]E2 within MCF-7 breast cancer cells at 37 °C. Fig. 6A shows the dissociation kinetics of [3H]E2 from the ER within the cell at 37 °C with DES as competitor. For direct comparison the dissociation rate of [3H]E2 from MCF-7 ER was also measured in vitro at 37 °C. Fig. 6B shows the dissociation of [3H]E2 from ER measured in vitro, when ERs were occupied in situ (triangles) or in vitro (squares). As can be seen in Fig. 6, during the course of the dissociation kinetic studies at 37 °C we observed a loss of ER within the cells (open circles, Fig. 6A) and in vitro (open triangles and squares, Fig. 6B). To determine the corrected [3H]E2 dissociation rate, the slope of the line with no DES was subtracted from the slope of the line with DES.

The dissociation of [3H]E2 was strikingly affected by the ER’s environment. The half-life of dissociation of [3H]E2 from the ER within the cell was 59.4 ± 1.7 min (Fig. 6A). A dramatically shorter half-life of 8.6 ± 0.7 min was obtained when the unoccupied MCF-7 ER was extracted into a cytosol, occupied with [3H]E2, and dissociation kinetics performed in vitro at 37 °C (squares, Fig. 6B). Thus, factors within the cell other than DNA binding must contribute to making the dissociation half-life of estrogen longer than that observed in vitro. Interestingly, if the ER was occupied in situ, extracted from the nucleus, and dissociation kinetics performed on this nuclear ER in vitro at 37 °C, we observed an intermediate half-life of dissociation of 24.4 ± 1.2 min (triangles, Fig. 6B). These data suggest that the DNA-induced increase in the rate of estrogen dissociation simply represents an in vitro assay of a conformational change in the steroid binding domain mediated by DNA binding to the ER. However, within the cell additional factors, possibly including other proteins or cova-
stereoid hormone receptor family induces an allosteric conformational change in the receptor's steroid binding domain. When DNA is bound to the ER, there is an approximate 2-fold increase in the rate of estrogen dissociation. This change in the rate of estrogen dissociation reflects a DNA-induced change in the conformation of the steroid binding domain. The quantitative relationship between DNA binding and steroid dissociation (Fig. 4) confirms that DNA is acting through the DNA binding domain of the ER. Thus, the steroid binding and DNA binding domains are not completely independent of each other. Steroid receptor-steroid interactions have demonstrated alterations in the protein's structure (Ref. 26 and references therein). Estrogen, however, does not appear to have a significant effect on ER binding to DNA (10, 27, 28). Few studies have looked at changes in the protein induced by DNA binding. An early study of the effects of polynucleotides on steroid receptor's structure/function using calf uterine progesterone receptor showed that nonspecific nucleic acids, in particular polyguanylate (poly(G)), were able to prevent and reverse the heat-induced loss of progesterone binding ability (29). However, the site of interaction of these polynucleotides with the progesterone receptor was not demonstrated. We report here evidence that ER binding to specific DNA sequences, through the DNA binding domain, can alter the structure of the steroid binding domain as measured by a change in the rate of estrogen dissociation.

DNA-induced conformational changes have been reported for other proteins, but the changes have not been localized to a different domain as shown here. Proton nuclear magnetic resonance was used to show a conformational change in Xcro upon binding the 17-mer operator O0-3 (30) and fos and jun (32) have been shown to undergo a change in the amount of α-helix upon binding to their respective DNA elements as determined by circular dichroism. Recently, two other yeast transcription factors, transcription factor IID (33) and pheromone receptor transcription factor (PRTF) (34) have been reported to undergo alterations in protease sensitivity induced by DNA binding. The nature of these changes and how they relate to the function of these proteins remain under study. Interestingly, PRTF was found to undergo a conformational change only upon binding a specific DNA sequence. We show in our studies that the steroid binding domain undergoes a conformational change upon binding either the vitERE or the mutERE, although 200 times more mutERE was needed because the binding affinity of the ER for the mutERE is much lower.

The significance of the DNA-induced change in the structure of the steroid binding domain is unknown but may be a necessary component for establishing a properly functioning transcriptional complex (35, 36). The steroid binding domain has been shown to be necessary for the complete hormone-induced transcriptional activation of some genes (21), whereas a hormone-independent transcriptional activation function, at least for some genes, has been proposed for the amino terminus (4, 37, 38). We hypothesize that the importance of the DNA-induced conformational change in the steroid binding domain involves the regulation of receptor interactions with other proteins needed for transcriptional activation of a target gene. This control could restrict nuclear proteins from interacting with the ER unless it was bound to DNA. We propose that DNA and estrogen may act together to transform the receptor to the proper conformation for transcriptional activation.

Whether DNA binding can alter the conformation of the amino-terminal end of the ER is less clear. When the amino-terminal domain of the glucocorticoid receptor was removed...
using α-chymotrypsin, there was a decrease in the DNA binding specificity of the resulting 39-kDa fragment (39). The authors hypothesized that interaction between these two domains might occur. Using proteolytic cleavage mapping of the rat cytosolic ER, we observed no DNA-induced changes in the amino-terminal end of the protein, suggesting that DNA may specifically modulate the conformation of the steroid binding domain of the ER and not the amino terminus. Thus, DNA may be able to regulate only one of the two proposed transcriptional activation domains of the ER.

The DNA-induced change in the structure of the ER’s steroid binding domain is measurable as an almost 2-fold faster rate of estrogen dissociation in vitro. Within the cell the dissociation rate of estrogen could be affected by a variety of factors, including binding of ER to DNA. In Fig. 6 we show that within the cell the dissociation rate of estrogen is much slower than it is in vitro. We propose that under the influence of estrogen and DNA the ER changes conformation, which within the cell allows the ER to make the proper contacts with other mediating transcription factors or the basal transcriptional machinery (40). This interaction of ER with other protein factors may also modify the steroid binding domain’s conformation, resulting in the slower rate of estrogen dissociation measured by the whole cell assay. The correct cascade of conformational changes in the ER might be necessary to build the proper protein complex capable of initiating transcription. The in situ occupied nuclear ER allowed to undergo estrogen dissociation in vitro also showed a slower rate than the in vitro occupied cytosolic ER. Perhaps the ER undergoes a covalent modification within the cell after estrogen binding or additional extracted nuclear proteins may interact with the ER, even in vitro, and thereby modify the steroid binding domain and slow the rate of estrogen dissociation.

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