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DIFFERENCES IN THE FORM OF THE SALT-TRANSFORMED ESTROGEN RECEPTOR WHEN BOUND BY ESTROGEN VERSUS ANTIESTROGEN

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Summary—Our laboratory has previously reported that antiestrogen binding to molybdate-stabilized non-transformed estrogen receptor results in a larger form of the receptor in 0.3 M KCl when compared with estrogen bound receptor. Estradiol promoted the formation of monomers in the presence of 0.3 M KCl whereas antiestrogen appeared to promote dimer formation. We have extended these studies examining the rabbit uterine salt-transformed estrogen receptor partially purified by DEAE–cellulose chromatography. We previously demonstrated that estrogen receptor prepared in this way bound to different sites on partially deproteinized chromatin subfractions or reconstituted chromosomal protein/DNA fractions when the receptor was complexed with estrogen vs antiestrogen. Analysis of these receptor preparations indicated that DEAE–cellulose step-elution resulted in a peak fraction which sedimented as a single 5.9S peak in 5–20% sucrose density gradients containing 0.3 M KCl for receptor bound by the antiestrogens H1285 and *trans*-hydroxytamoxifen. However, receptor bound by estradiol sedimented as 4.5S. These receptor complexes bound DNA–cellulose indicating that these partially purified receptors were transformed. DEAE rechromatography or agarose gel filtration of the partially purified antiestrogen–receptor complexes resulted in significant dissociation of the larger complex into monomers. Incubations of 5.9S antiestrogen-receptor complexes with antibodies against nontransformed steroid receptor-associated proteins (the 59 and 90 kDa proteins) did not result in the interaction of this larger antiestrogen–receptor complex with these antibodies (obtained from L. E. Faber and D. O. Toft, respectively). Our results support the concept that antiestrogen binding induces a different receptor conformation which could affect monomer–dimer equilibrium, thus rendering the antiestrogen–receptor complex incapable of inducing complete estrogenic responses in target tissues.

INTRODUCTION

Our laboratory has demonstrated multiple acceptor sites in mammalian chromatin which display different degrees of estrogen receptor binding capacity and affinity for estrogen– vs antiestrogen–receptor complexes (reviewed in Refs [1, 2]). We [3–5] and others [6–9] have also demonstrated that antiestrogen interaction with the estrogen receptor alters the receptor such that its physicochemical characteristics differ from that of the estradiol–receptor complex. Thus, the biological responses of antiestrogens may be the end result of such altered ligand–receptor interaction. Therefore, the distinction between estrogen- and antiestrogen-receptor binding to multiple sets of different chromatin acceptor sites may be a result of more fundamental differences in initial ligand-receptor interaction, i.e. proper transformation of receptors may be permitted when the proper ligand binds to the receptor [10, 11].

We previously determined that there is a difference in physicochemical properties between estrogen– and antiestrogen–receptor complexes prior to transformation [3–5]. In experiments using calf uterus and rat uterus and pituitary we examined partially purified estrogen receptor from ion-exchange chromatography and found that the nontransformed molybdate-stabilized receptor, bound by estradiol, eluted as two forms which sedimented as a 4S and 6S species. However, the receptor bound by the triphenylethylene antiestrogen, H1285, eluted primarily as one peak and sedimented as a 6S species. These data were consistent with the possibility that antiestrogen binding to the estrogen receptor stabilized the receptor in the dimeric form affecting a monomer–dimer equilibrium.

In the present study, we characterized the partially purified salt-transformed estrogen receptor bound by estradiol vs high affinity triphenylethylene antiestrogens. Since our prevous studies suggested that antiestrogens promote dimer formation, the question arose as to whether this larger form was, in fact, a receptor dimer, or whether antiestrogens promoted monomer binding to another protein, such as a heat

shock protein. Therefore, we investigated the possibility of the interaction of antiestrogen-bound receptor with the 59 or 90 kDa receptor associated proteins [12, 13].

EXPERIMENTAL

Chemicals

The high affinity antiestrogen [^{3}H]H1285 (H1285 = 4 (*N,N*-diethylaminoethoxy)-4′-methoxy-α-(*p*-hydroxyphenyl)-α′-ethylstilbene) (sp. act. 20 Ci/mmol), was prepared in our laboratory from H1285 [14]. [^{3}H]*trans*-Hydroxytamoxifen (sp. act. 16 Ci/mmol) was a gift from Dr Benita Katzenellenbogen. 17-β[6,7-^{3}H]Estradiol (60 Ci/mmol), and [^{14}C]ovalbumin and [^{14}C]gamma-globulin standards were obtained from New England Nuclear Corporation, Boston, Mass. DEAE–cellulose (DE-52) was purchased from Whatman (Clifton, N.J.). Agarose (Bio-Gel A-1.5 m) was obtained from Bio-Rad Laboratories (Richmond, Calif.). Bovine pancreas RNase was obtained from Calbiochem (La Jolla, Calif.). MOPC-21 mouse IgG1 was obtained from Sigma (St Louis, Mo.).

The AC-88 antibody prepared against the avian 90 kDa heat shock protein [13] was obtained from Dr D. O. Toft. The immune IgG (EC1) prepared against the rabbit uterine 59 kDa protein [15] was a gift from Dr L. E. Faber.

Cytosol preparation

Mature rabbit uteri were obtained frozen from Pel-Freez (Rogers, Ark.). Uteri were partially thawed, minced and homogenized in 3 vol TED buffer (10 mM Tris–HCl, 1.5 mM EDTA, 1 mM dithiothreitol, pH 7.5) containing 0.5 mM PMSF. Sodium molybdate was added where indicated from a concentrated stock. All procedures were performed at 4°C. The homogenate was centrifuged at 170,000 *g* for 30 min to obtain cytosol. The cytosol was incubated with 10–20 nM radiolabeled ligand for 90 min, treated with a pellet from an equal vol of 1% Dextran-coated charcoal (1% w/v) Norit A activated charcoal and 0.1% (w/v) Dextran in buffer for 10 min, centrifuged, and the supernatant used for DEAE–cellulose chromatography or sucrose density gradient analysis. In one set of experiments cytosol was incubated with 5 mg RNase for 30 min at 4°C after the incubation with [^{3}H]H1285. The RNase was prepared with 150 mM NaCl, pH 5.0, and boiled for 10 min at 90°C in a water bath. The concentration of the RNase was 4 mg/ml.

DEAE–cellulose column chromatography

DEAE-step elution was performed as previously described [14]. DE-52 columns (10 ml, 17 × 35 mm) were washed with 50 ml TED buffer. In some experiments TED buffer contained 1 mM molybdate. The cytosol samples were loaded onto the columns. The bulk of the protein was eluted with 40 ml TED buffer containing 0.1 M KCl and the receptor was then eluted with 20 ml TED buffer containing 0.4 M KCl in 3 ml fractions. Aliquots (50 μl) were counted in 4 ml scintillation fluid (0.4% Omnifluor, 25% Triton X-114 in xylene) at 46% efficiency to determine the peak receptor fraction.

Receptor interaction with receptor-associated proteins

In order to investigate the possibility that antiestrogen–receptor complexes interact with 59 or 90 kDa receptor-associated proteins, aliquots of DEAE-peak fractions were incubated for 1 h at 4°C with Faber's immune IgG (EC1) prepared against the rabbit uterus 59 kDa protein or Toft's AC-88 antibody prepared against the avian 90 kDa heat shock protein prior to sucrose density gradient analysis.

Sucrose density gradients

Linear 5–20% sucrose gradients (3.6 ml) containing 300 mM KCl in TESH buffer (10 mM Tris, 1.5 mM EDTA, 12 mM monothioglycerol) were prepared and chilled at 4°C. DEAE–cellulose peak fractions (200 μl) were layered on the sucrose gradient and centrifuged for 15 h in a SW56 rotor at 225,000 *g*. Fractions (4 drops) were collected from the top and counted in 4 ml scintillation fluid. [^{14}C]Ovalbumin (3.7S) and [^{14}C]gamma globulin (6.6S) were added either as internal standards or in parallel gradient tubes.

Gel filtration column chromatography

Aliquots of receptor peak fractions from the ion-exchange colulmn were loaded onto Agarose A-1.5 m columns (100–200 mesh; 1.5 cm i.d. × 90 cm) and 60 drop fractions were collected in 10 mM Tris, 1.5 mM EDTA, 10 mM monothioglycerol, 10% glycerol buffer, pH 7.5, containing 300 mM KCl. Radioactivity was determined in aliquots of gel filtration column fractions. The Stoke's radii (R_s) of the standards used to calibrate the columns were thyroglobulin (8.6 nm), catalase (5.2 nm), bovine serum albumin (3.5 nm), ovalbumin (3.05 nm), and cytochrome c (1.7 nm).

RESULTS

We previously demonstrated that 20–30-fold purified rabbit uterine transformed estrogen– and antiestrogen–receptor complexes can be prepared by DEAE–cellulose column chromatography [16]. Using step-elution with 0.1 M KCl followed by 0.4 M KCl partial purification of the salt-transformed receptor can be obtained in the absence or presence of 1 mM molybdate, which stabilizes the receptor but permits transformation. As reported previously the elution profile for both [^{3}H]estradiol– and [^{3}H]H1285–receptor complexes was similar and binding of ligand was specific for the estrogen receptor since 200-fold excess non-radiolabeled ligand virtually eliminated the elution of radioactively labeled receptor complexes [16]. In contrast to several other antiestrogens,

H1285 displays very little nonspecific binding. [^{3}H]*trans*-Hydroxytamoxifen–receptor complexes also eluted as a single peak with DEAE-step elution and the non-specific binding was less than 15%. The KCl concentration in the peak fractions was approximately 0.3 M.

Sucrose density gradient analysis

We examined the sedimentation characteristics of the receptor in cytosol or in the DEAE peak fraction when bound to [^{3}H]estradiol or to [^{3}H]H1285. Cytosolic receptors bound by [^{3}H]estradiol sedimented as 4.8S whereas [^{3}H]H1285–receptor complexes sedimented as 6.6S (Fig. 1). Similar results were obtained with the DEAE peak fraction. [^{3}H]Estradiol receptors sedimented as a 4.5S form whereas [^{3}H]H1285 receptors sedimented as a single 5.9S peak in 5–20% sucrose density gradients (Fig. 2A). Similar results were obtained whether or not the receptors were prepared in 1 mM molybdate. [^{3}H]*trans*-Hydroxytamoxifen–receptor complexes also sedimented as a larger form (Fig. 2B) suggesting that receptor-dimer stabilization was not limited to H1285. Both the partially purified [^{3}H]estradiol– and [^{3}H]H1285–receptor complexes were transformed as demonstrated by binding to DNA–cellulose (data not shown). If the peak fraction containing [^{3}H]H1285 receptors was diluted 10-fold with buffer without KCl to reduce the KCl concentration to 30 mM, loaded onto another DEAE–cellulose column, and again eluted with 0.4 m KCl, the receptors eluted as a single 4.8S peak (Fig. 3).

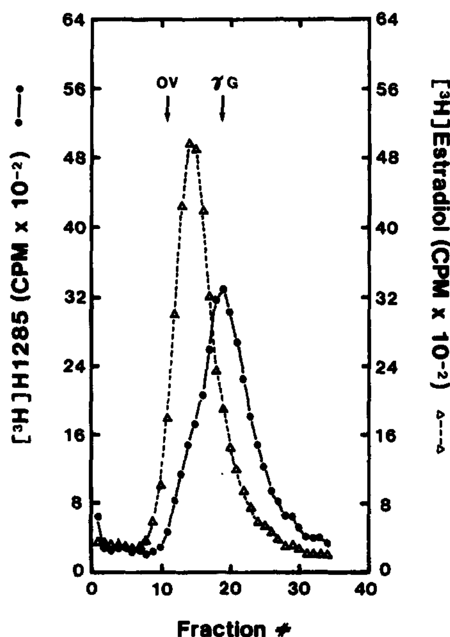

Fig. 1. Sucrose density gradient analysis of the cytosolic estrogen receptor. Cytosol was prepared in 10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 0.5 mM PMSF and was labeled with 20 nm [^{3}H]H1285 or [^{3}H]estradiol. After dextran-coated charcoal treatment 200 μl was layered on 5–20% sucrose density gradients containing 0.3 M KCl. [^{14}C]Ovalbumin (3.7S) and [^{14}C]gamma globulin (6.6S) were used as sedimentation markers. Tubes were centrifuged at 225,000 *g* (SW 56 rotor) in a Beckman L5-50 ultracentrifuge for 15 h. Gradient fractions were collected, counted, and plotted as total bound cpm.

Gel filtration analysis

DEAE-purified [^{3}H]estradiol– and [^{3}H]H1285–receptor complexes were further analyzed by gel filtration chromatography to ascertain whether the observed sedimentation differences between estrogen– and antiestrogen–receptor complexes would also be reflected in differences in Stokes' radii. [^{3}H]Estradiol–receptor complexes had one major form with a Stokes' radius of 4.3 nm. However, [^{3}H]H1285–receptor complexes were resolved into two forms with Stokes' radii of 4.3 and 7.4 nm (Fig. 4).

It appears that gel chromatography caused dissociation of some of the larger receptor form into a smaller form as seen with DEAE-rechromatography, indicating that the larger [^{3}H]H1285 receptor form can be dissociated into smaller components. These data are similar to the data obtained for molybdate-stabilized non-transformed estrogen receptor bound by estrogen vs antiestrogen [3–5] and support the concept of a monomer–dimer relationship, where antiestrogen stabilizes the dimeric form.

Receptor-associated macromolecules

Although our previous studies and reports from other laboratories (reviewed in Ref. [10]), as well as this current study suggest that antiestrogens promote receptor homodimer formation, we tested for other macromolecules that could result in a heterodimer. Cytosolic estrogen receptor labeled with [^{3}H]H1285 was incubated with RNase for 30 min at 4°C and DEAE-purified receptors obtained as described above in order to determine if receptor–RNA complexes could account for the larger forms observed with antiestrogens. However, no shift in sedimentation was seen with RNase treatment (data not shown).

We also questioned whether antiestrogens promoted monomer binding to another protein, such as heat shock protein or other non-transformed receptor associated proteins. Therefore, we investigated the possibility of antiestrogen receptor interaction with the 59 kDa rabbit uterine receptor-associated protein described by Faber[12] as well as the 90 kDa heat shock protein [13] by using antibodies directed against these proteins. Again, no shift in sedimentation was seen when EC1 antibody (Fig. 5A) or AC-88 antibody (not shown) were incubated with aliquots of DEAE-purified antiestrogen–receptor complexes. These experiments were performed with several different antibody concentrations. However, EC1 did recognize the estrogen receptor in the non-transformed state when bound by either antiestrogen (Fig. 5B) or estrogen (not shown) and caused a shift in sedimentation.

DISCUSSION

Since partially purified estrogen receptors bound by estrogen vs antiestrogen display differing interactions with chromatin acceptor sites [1, 2, 10, 11, 16], it can be hypothesized that antiestrogens cause a subtle but significant alteration in the conformation of the estrogen receptor. The mixed agonist/antagonist responses elicited by antiestrogens may be the end result of such altered ligand–receptor interactions.

H1285 is a potent and very effective antiestrogen in a variety of species and tissues. The relative binding affinity of H1285 is approximately 1100% (with estradiol as 100%) in rat uterus and pituitary [5], 300% in rabbit uterus [16], and 100% in MCF-7 cells [17]. Its binding affinity is similar to *trans*-hydroxytamoxifen, although H1285 is more potent in some species. H1285 is an effective antiestrogen at low doses; however, H1285 displays both agonistic and antagonistic properties in all tissue layers of the rat uterus [18]. The lower affinity antiestrogens such as tamoxifen, nafoxidine and clomiphene have less than 10% relative binding affinity. Thus, large doses are required to achieve antiestrogenicity in uterine tissue [18]. In MCF-7 cells [17] H1285 is a 100-fold more potent inhibitor of cell proliferation than tamoxifen but equivalent with *trans*-hydroxytamoxifen. Thus, the biological effectiveness of triphenylethylene antiestrogens correlates with binding affinity for the estrogen receptor. This, in turn, may effect the stability of receptor dimer formation.

Previous studies from our laboratory had suggested that antiestrogens promote a larger form of the estrogen receptor. This was shown for the non-transformed molybdate-stabilized calf uterine [3, 4] and rat uterine and pituitary [5] estrogen receptors. Based on our studies as well as reports from other laboratories [6, 7] we proposed that antiestrogens stabilize the receptor as a homodimer. This could result from an antiestrogen-induced conformational change in the receptor which retards the dissociation of the 5-6S dimer into 4S monomers or protects the antiestrogen receptor complex from factors/enzymes which might cause the estrogen bound receptor to readily dissociate into monomers. In either event, the data indicated that antiestrogen binding resulted in a larger form of the receptor as measured by sucrose density gradient analysis in 0.3 M KCl, as well as gel filtration analysis. Since only transformed receptors interact with nuclear components leading to altered gene expression, we directed our current studies to the

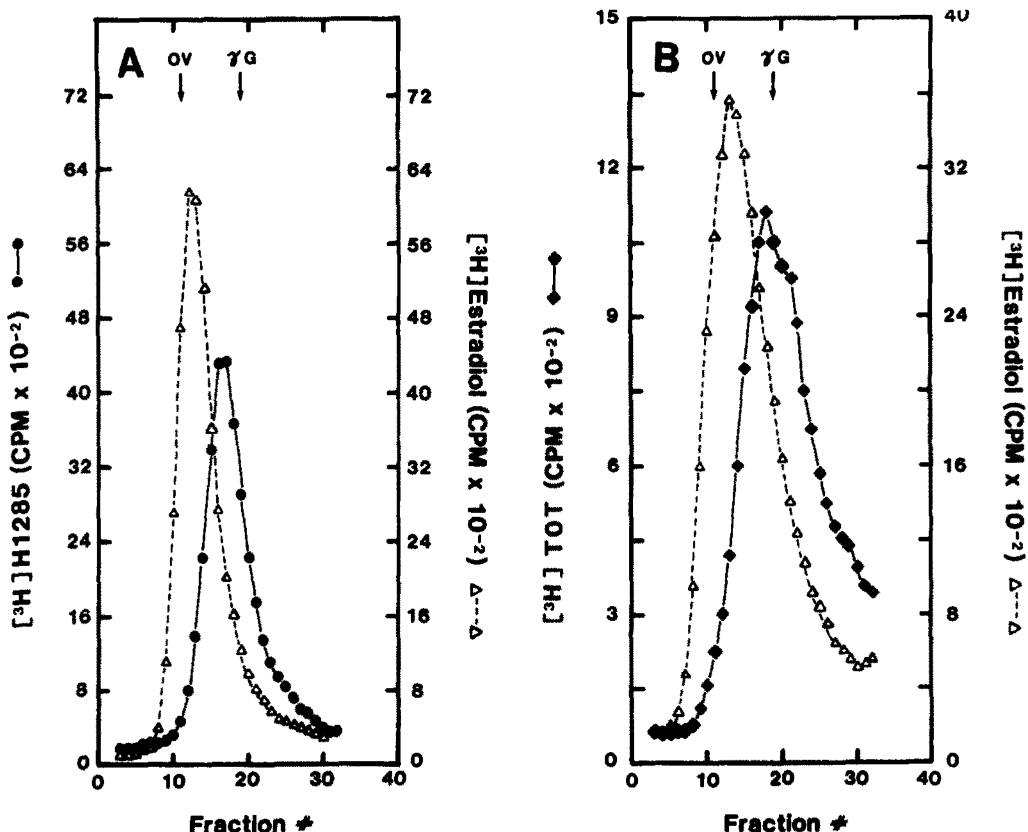

Fig. 2. Sucrose density gradient analysis of [^{3}H]H1285—(A) and [^{3}H]*trans*-hydroxytamoxifen—(B) receptor complexes from DEAE-cellulose. Cytosol was prepared as described in Fig. 1 and partially purified with DEAE-cellulose column chromatography using step-elution. Aliquots (200 μl) of the peak fraction were layered on 5–20% sucrose density gradients containing 0.3 M KCl. Conditions of the sucrose density gradient analysis were as described in Fig. 1.

salt-transformed partially purified receptor. The results of these studies again indicate that the high affinity triphenylethylene antiestrogen, H1285, promotes a larger form of the receptor (~6S). However, this receptor form can dissociate to the 4S form by dilution. These data are consistent with other reports which suggest that antiestrogens promote the homodimerization of the lighter monomeric form of the nuclear estrogen receptor [6, 7].

The 6S form of the antiestrogen–receptor complex appears to be composed of two identical monomeric subunits; it does not appear to be a receptor monomer associated with RNA or the 59 kDa receptor-associated protein. The rabbit 59 kDa protein was first reported to be associated with the mammalian 8.5S progestin receptor, but has since been shown to be associated with the non-transformed estrogen, androgen and glucocorticoid receptors [19]. Since in our studies H1285 impeded monomer formation, it was postulated that the larger 6S form might be the result of continued association of the 4S receptor monomer with the 59 kDa protein, even though the receptor was salt-transformed. However, our data clearly indicate that this is not the case.

The studies addressing the 90 kDa heat shock protein are not as conclusive. AC-88 antibody has been shown to interact with the free 90 kDa mammalian heat shock protein but not the 9S non-transformed receptor complex [20]. We postulated that if the estrogen receptor bound by H1285 continued to be associated with the 90 kDa protein but was dissociated from other components of the 9S complex, then AC-88 might interact with the 6S form. Since we saw no shift in the size of the receptor after incubation with AC-88, it is still possible that the 6S form of the [^{3}H]H1285–receptor complex is a result of receptor subunit association with heat shock protein. However, we consider this unlikely. A recent report suggests that a member of the 70-kDa family of stress proteins is associated with the human and avian progesterone receptors, as determined by immunoblot assay of purified progesterone receptor with a monoclonal antibody to the 72–73 kDa stress proteins [21]. Although this is the only known antibody to bind to nonsteroid binding proteins associated with the transformed receptor, in our system this same monoclonal antibody did not recognize the nontransformed rabbit uterine estrogen receptor as determined by density shift (unpublished observations).

It has been suggested that the transformed estrogen receptor is a dimer *in vivo* and that the agonist-bound homodimer binds to DNA *in vitro* [22, 23]. Recently, it was reported that estrogen receptor binds as dimers to its response element (ERE), and that estrogen plays an important role in the formation of these stable, DNA binding estrogen receptor dimers. The

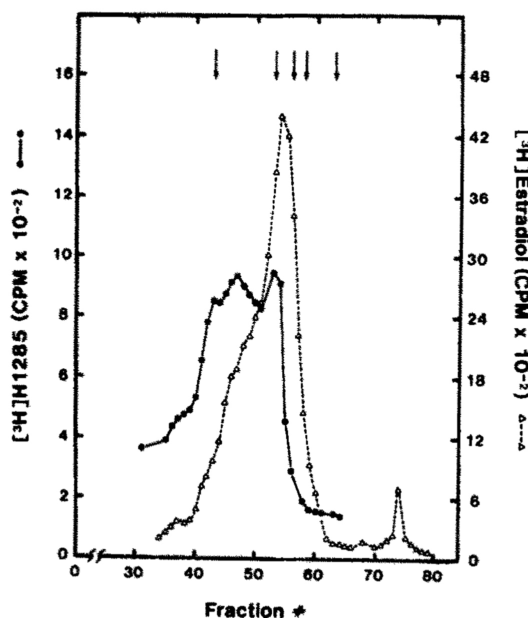

Fig. 4. Gel filtration analysis of estrogen– and antiestrogen–receptor complexes. Cytosol was incubated with 10 nM [^{3}H]estradiol or [^{3}H]H1285, and fractionated on DEAE–cellulose. Peak fractions were loaded onto Agarose 1.5 m columns, eluted with 10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 5% glycerol, 0.3 M KCl and fractions collected. Calculations were according to the formula $K_{av} = V_e - V_0/V_t - V_0$ where V_e represents the elution volume, V_0 the void volume, and V_t the total volume of packed bed. The arrows indicate the following Stokes' radius markers (left to right): thyroglobulin (8.6 nm), catalase (5.2 nm), bovine serum albumin (3.5 nm), ovalbumin (3.05 nm) and cytochrome c (1.7 nm).

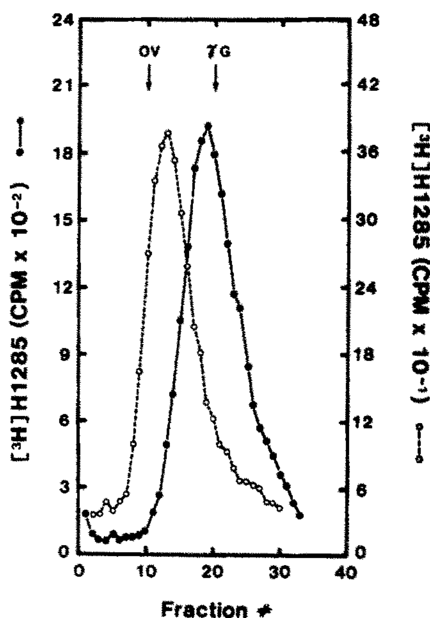

Fig. 3. Sucrose density gradient analysis of [^{3}H]H1285–receptor complexes after rechromatography on DEAE–cellulose. The peak fraction from step-elution (●——●) was diluted 10-fold with buffer without KCl and applied to a new DEAE–cellulose column (○——○). Elution from the DEAE column and sucrose density gradient analysis were as described in text.

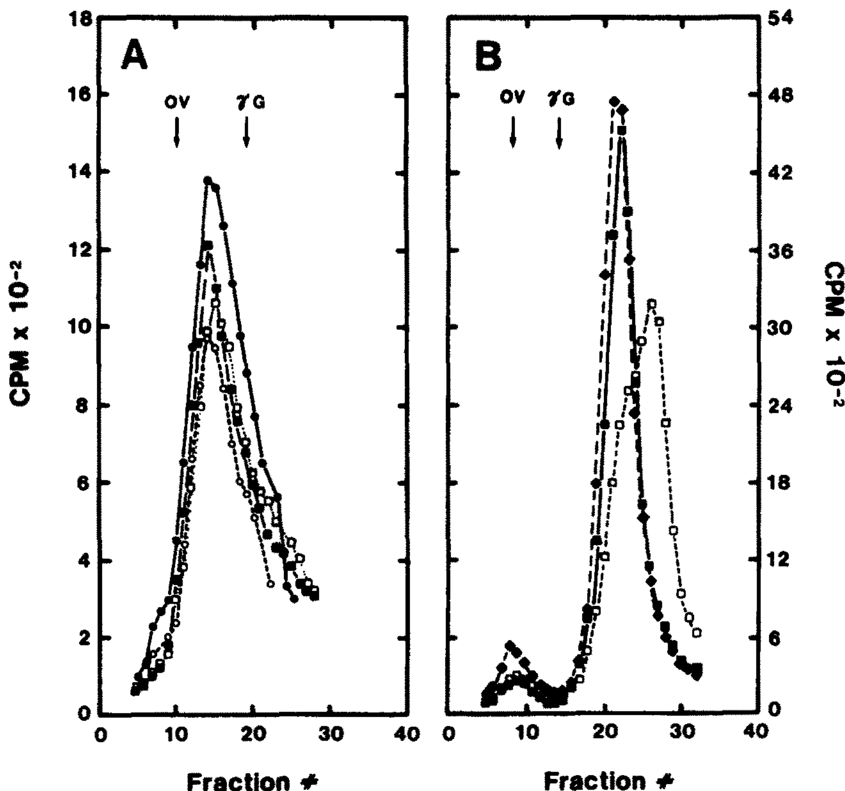

Fig. 5. Sucrose density gradient sedimentation analysis of receptor complexes with the EC1 antibody. (A) Aliquots of the peak fraction (200 μl) from the DEAE purification of [^{3}H]H1285- and [^{3}H]*trans*-hydroxytamoxifen-receptor complexes were incubated for 1 h at 4°C with 21 μg EC1 immune IgG (provided by L. E. Faber). Conditions of sucrose density gradient analysis were as described in Fig. 1. Open symbols represent incubation with antibody. [^{3}H]H1285-receptor (●——●, ○——○); [^{3}H]*trans*-hydroxytamoxifen-receptor (■——■, □——□). (B) Aliquots of cytosol (200 μl) radiolabeled with [^{3}H]*trans*-hydroxytamoxifen in the presence of 10 mM molybdate were incubated for 1 h at 4°C with 21 μg EC1 immune IgG or MOPC-21 non-immune IgG and loaded onto sucrose density gradients containing 10 mM molybdate minus KCl. [^{3}H]*trans*-Hydroxytamoxifen-receptor minus EC1 (■——■); + EC1 (□——□); + MOPC-21 IgG (◆——◆).

symmetry of the palindromic ERE is essential for high-affinity binding by estrogen receptor, supporting all the more the likelihood that the receptor binds to ERE as a dimer. The regulatory implications of dimer formation have been previously examined [22] and a monomer–dimer equilibrium model was proposed. This model allows for the regulation of transformed receptor dimers by the concentration of both estrogen and receptor in a given cell.

These reports are not necessarily in conflict with our findings here. Rather, our previous research and these current studies suggest a model of hormone/anti-hormone action in which dissociation of the receptor from chromatin acceptor sites has a regulatory role. We previously reported that antiestrogens, including the triphenylethylene H1285, caused a prolonged nuclear retention of the occupied estrogen receptor [24]. This is in contrast to the rapid and immediate processing of the estrogen-bound receptor. We propose that the mechanism of antiestrogen antagonism may not be simply a matter of dimer formation, but rather the relative stability of that dimerization. It may be that the estrogen receptor binds efficiently to the palindromic ERE as a ligand-induced dimer and in doing so initiates transcription. It is possible that in order to attain a full estrogenic response, a sequence of receptor recycling and reactivation must follow the ERE binding; i.e. the estrogen receptor must be dissociated from DNA and associated chromosomal proteins so that later phases of the estrogenic response may occur. The monomer–dimer equilibrium may be such that the estradiol–receptor dimer, after having bound to the ERE, then dissociates to monomers. Past observations that antiestrogens cause prolonged nuclear retention and our present finding which suggests that antiestrogens stabilize the dimer form of the transformed estrogen receptor together point to the possibility that antiestrogens act by inhibiting dimer dissociation into monomers at the ERE, thereby preventing estrogen receptor processing [25, 26] and further responses to estrogen. This model may also explain why it is possible that some antiestrogens have partial agonist activity since the initial binding of the dimer to the ERE is not inhibited.

There are also reports that other hormone antagonists cause stabilization of the steroid receptor complexes in a larger form. The glucocorticoid antagonist 17α-methyltestosterone appears to prevent the cytosolic 10S receptor from dissociating into the 4S DNA-binding subunit [27]. The calf uterine progesterone receptor bound by the antagonist RU486 vs the agonist R5020 also shows retardation of the conversion of the 8S RU486 form to the 4S form [28]. Similar results were also reported for the rabbit uterine salt-transformed progesterone receptor [29]. RU486 slowed down the dissociation of the 90 kDa protein from the receptor protein impairing DNA binding. The cumulative results of these studies suggest another mechanism whereby certain ligands that bind receptor promote antagonist responses instead of agonist responses, i.e. impaired dissociation of components that comprise the native or non-transformed steroid hormone receptor.

In summary, transformed estradiol–receptor complexes *in vivo* may exist as dimers which can readily dissociate into monomers. However, antiestrogen binding to the receptor may inhibit full dissociation as a result of a receptor conformation that is different than that induced by estrogen binding. This conformational change could affect the monomer–dimer equilibrium which may be one mechanism responsible for the mixed agonist/antagonist responses elicited by antiestrogens.

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REFERENCES

1. Ruh T. S., Ruh M. F., Singh R. K. and Butler W. B.: Antiestrogen action in MCF-7 cells. In *Receptor Mediated Hormone Antagonism* (Edited by M. K. Agarwal). Walter de Gruyter, Berlin (1987) pp. 307–328.
2. Ruh T. S., Ruh M. F. and Singh R. K.: Nuclear acceptor sites: interaction with estrogen- versus antiestrogen-receptor complexes. In *Steroid Receptors in Health and Disease* (Edited by V. K. Moudgil). Plenum Press, New York (1988) pp. 233–250.
3. Ruh M. F., Brzyski R. G., Strange L. and Ruh T. S.: Estrogen and antiestrogen binding to different forms of the molybdate-stabilized estrogen receptor. *Endocrinology* **112** (1983) 2203–2205.
4. Keene J. L., Ruh M. F. and Ruh T. S.: Interaction of the antiestrogen [^{3}H]H1285 with the two forms the molybdate-stabilized calf uterine estrogen receptor. *J. Steroid Biochem.* **21** (1984) 625–631.
5. Jasper T. W., Ruh M. F. and Ruh T. S.: Estrogen and antiestrogen binding to rat uterine and pituitary estrogen receptor: evidence for at least two physicochemical forms of the estrogen receptor. *J. Steroid Biochem.* **23** (1985) 537–545.
6. Katzenellenbogen B. S., Miller M. A., Mullick A. and Sheen Y. Y.: Antiestrogen action in breast cancer cells: modulation of proliferation and protein synthesis, and interaction with estrogen receptors and additional antiestrogen binding sites. *Breast Cancer Res. Treat.* **5** (1985) 231–243.
7. Sasson S. and Notides A. C.: The inhibition of the estrogen receptor's positive cooperative [^{3}H]estradiol binding by the antagonist, clomiphene. *J. Biol. Chem.* **257** (1982) 11540–11545.
8. Pavlik E. J., Nelson K., van Nagell J. R., Donaldson E. S., Walden M. L., Hanson M. B., Gallion H., Flanigan R. C. and Kenady D. E.: Hydrodynamic characterizations of estrogen receptors complexed with [^{3}H]-4-hydroxytamoxifen: evidence in support of contrasting receptor transitions mediated by different ligands. *Biochemistry* **24** (1985) 8101–8106.
9. Evans E., Baskevitch P. P. and Rochefort H.: Estrogen-receptor-DNA interaction. *Eur. J. Biochem.* **128** (1982) 185–191.
10. Ruh T. S. and Ruh M. F.: Antiestrogen action: Properties of the estrogen receptor and chromatin acceptor sites. In *Recent Advances in Steroid Hormone Action* (Edited by V. K. Moudgil). Walter de Gruyter, Berlin (1987) pp. 102–132.
11. Singh R. K., Ruh M. F., Butler W. B. and Ruh T. S.: Acceptor sites in chromatin for receptor bound by estrogen versus antiestrogen in antiestrogen-sensitive and resistant MCF-7 cells. *Endocrinology* **118** (1986) 1087–1095.
12. Tai P. K. and Faber L. E.: Isolation of dissimilar components of the 8.5S nonactivated uterine progestin receptor. *Can. J. Biochem. Cell Biol.* **63** (1985) 41–49.
13. Toft D. O., Sullivan W. P., McCormick D. J. and Riehl R. M.: Heat shock proteins and steroid hormone receptors. *Biochem. Actions Horm.* **14** (1987) 293–316.
14. Keene J. L., Sweet F., Ruh M. F. and Ruh T. S.: Interaction of the radiolabeled high-affinity antiestrogen [^{3}H]H1285 with the cytoplasmic estrogen receptor. *Biochem. J.* **217** (1984) 819–826.
15. Nakao K. N., Meyers J. E. and Faber L. E.: Development of a monoclonal antibody to the rabbit 8.5S uterine progestin receptor. *Can. J. Biochem. Cell Biol.* **63** (1985) 33–40.
16. Singh R. K., Ruh M. F. and Ruh T. S.: Binding of [^{3}H]estradiol- and [^{3}H]H1285-receptor complexes to rabbit uterine chromatin. *Biochim. Biophys. Acta.* **800** (1984) 33–40.
17. Sheen Y. Y., Ruh T. S., Mangel W. F. and Katzenellenbogen B. S.: Antiestrogenic potency and binding characteristics of the triphenylethylene H1285 in MCF-7 human breast cancer cells. *Cancer Res.* **45** (1985) 4192–4199.
18. Ruh M. F., Connors N. A. and Ruh T. S.: The effects of the high affinity antiestrogen, H1285, on uterine growth and morphology. *Comp. Biochem. Physiol.* **77C** (1984) 89–93.
19. Tai P. K., Maeda Y., Nakao K., Wakim N. G., Duhring J. L. and Faber L. E.: A 59-kilodalton protein associated with progestin, estrogen, androgen, and glucocorticoid receptors. *Biochemistry* **25** (1986) 5269–5275.
20. Kost S. L., Smith D. F., Sullivan W. P., Welch W. J. and Toft D. O.: Binding of heat shock proteins to the avian progesterone receptor. *Molec. Cell. Biol.* **9** (1989) 3829–3833.
21. Estes P. A., Suba E. J., Lawler-Heavner J., Elashry-Stowers D., Wei L. L., Toft D. O., Sullivan W. P., Horwitz K. B. and Edwards D. P.: Immunologic analysis of human breast cancer progesterone receptors. 1. Immunoaffinity purification of transformed receptors and production of monoclonal antibodies. *Biochemistry* **26** (1987) 6250–6262.
22. Gordon M. S. and Notides A. C.: Computer modeling of estradiol interactions with the estrogen receptor. *J. Steroid Biochem.* **25** (1986) 177–181.

23. Kumar V. and Chambon P.: The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. *Cell* **55** (1988) 145–156.
24. Ruh T. S., Baudendistel L. J., Nicholson W. F. and Ruh M. F.: The effects of antioestrogens on the oestrogen receptor. *J. Steroid Biochem.* **11** (1979) 315–322.
25. Horwitz K. B. and McGuire W. L.: Nuclear mechanisms of estrogen action: effects of estradiol and anti-estrogens on estrogen receptors and nuclear processing. *J. Biol. Chem.* **253** (1978) 8185–8191.
26. Horwitz K. B. and McGuire W. L.: Nuclear estrogen receptors, effect of inhibitors on processing and steady-state levels. *J. Biol. Chem.* **255** (1980) 9699–9705.
27. Raaka B. M., Finnerty M. and Samuels H. H.: The glucocorticoid antagonist 17α-methylestroterone binds to the 10S glucocorticoid receptors and blocks agonist-mediated dissociation of the 10S oligomer to the 4S deoxyribonucleic acid-binding subunit. *Molec. Endocr.* **3** (1989) 332–341.
28. Moudgil V. K. and Hurd C.: Transformation of calf uterine progesterone receptor: analysis of the process when receptor is bound to progesterone and RU38486. *Biochemistry* **26** (1987) 4993–5001.
29. Renoir J.-M., Radanyi C. and Baulieu E. E.: The antiprogesterone RU486 stabilizes the heteroligomeric, non-DNA binding, 8S-form of the rabbit uterus cytosol progesterone receptor. *Steroids* **53** (1989) 1–20.