Physical Properties of Estrogen Receptor Complexes in MCF-7 Human Breast Cancer Cells

DIFFERENCES WITH ANTI-ESTROGEN AND ESTROGEN

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We have examined the binding of two high affinity radiolabeled anti-estrogens, 2-[4-(2-pyrrolidin-1-yl-ethoxy)phenyl]-2-(3-[3H]-4-hydroxyphenyl)-1-phenyl-1-nitroethene ([3H]Cl628M) and 1-[4-(2-dimethylaminoethoxy)phenyl]-1-(4-hydroxyphenyl)-2-(4-[3H]phenyl)but-1(2)-ene ([3H]trans-hydroxytamoxifen) to the estrogen receptor from MCF-7 human breast cancer cells and have used hydrodynamic methods to determine the molecular properties of estrogen and anti-estrogen receptor complexes from these cells. Saturation binding analysis indicates that each compound binds predominately to a single class of high affinity binding sites with \( K_d \) of 1.3 \( \times \) 10\(^{-10}\) M for [3H]Cl628M and 1.4 \( \times \) 10\(^{-10}\) M for [3H]trans-hydroxytamoxifen, and 2.2 \( \times \) 10\(^{-10}\) M for [3H]C1628M. Marked differences are seen in the sedimentation rate and chromatographic properties of the nuclear estrogen receptor when complexed with anti-estrogen as opposed to the estrogen, estradiol (E\(_2\)). The nuclear E\(_2\) receptor sediments at 4.1 \( \pm \) 0.03 S on high salt (0.4 M KCl) sucrose gradients; by contrast, receptor complexes with C1628M or trans-hydroxytamoxifen sediments as a 5.5 \( \pm \) 0.08 S peak. Upon chromatography on Sephadex G-200 columns equilibrated with buffer containing 0.4 M KCl, the E\(_2\) nuclear receptor complexes appear to have a Stokes radius of 4.8 \( \pm \) 0.20 nm, while the nuclear C1628M and trans-hydroxytamoxifen complexes have a Stokes radius of 5.93 \( \pm \) 0.20 nm. These sedimentation coefficients and Stokes radii correspond to calculated molecular weights of 83,000 for the nuclear E\(_2\) receptor complex and 137,000 for the nuclear C1628M and trans-hydroxytamoxifen receptor complexes. By contrast, cytoplasmic estrogen receptors labeled with C1628M, trans-hydroxytamoxifen, or E\(_2\) have similar sedimentation coefficients of 4.1 \( \pm \) 0.03 S and Stokes radii of 4.39 \( \pm \) 0.30 nm, corresponding to a molecular weight of 76,000.

The differences in physical properties of nuclear estrogen and anti-estrogen receptor complexes are obliterated in the presence of 3 M urea where estrogen and anti-estrogen complexes of 81,000 molecular weight (3.9 S, 4.94-nm Stokes radius) are obtained. These data are consistent with the association of the nuclear anti-estrogen receptor complex with an additional protein of \( \approx \) 55,000 molecular weight, an association that can be reversed by 3 M urea. This anti-estrogen-promoted change in receptor association with another cellular component may be an important aspect of the estrogen-antagonist and growth-inhibiting properties of these compounds.

The classical scheme of estrogen action, wherein estrogen binds to the cytoplasmic receptor to form an activated complex which then translocates to the nucleus and interacts with chromatin sites to modulate gene expression, still appears to be a suitable model for the interaction of estrogen in target cells, including the mammary gland (1-3). Anti-estrogens, which are effective inhibitors of the growth of hormone-dependent breast cancers (4-6), compete with estrogen for binding to cytoplasmic receptor sites and translocate the receptor complex to the nucleus (5, 7). The nuclear anti-estrogen receptor complex, however, appears to be only partially active in promoting specific biological responses and is effective in blocking the action of estrogens (7-10). This suggests that the receptor anti-estrogen complex might be physically different from the receptor estrogen complex; receptor conformation or size could be altered, or its interaction with other cellular components might be modified.

Most efforts to characterize the interaction of anti-estrogens with receptor have utilized indirect exchange or competition assays (5, 7, 11). Moreover, previous efforts to characterize directly anti-estrogen receptor complexes using radiolabeled anti-estrogens have been hampered by the low affinity of these compounds for estrogen receptor, which results in the dissociation of the complex during characterization (7). The recent availability of radiolabeled anti-estrogens, having affinities for estrogen receptor comparable to that of the natural estrogen estradiol, now make possible the direct characterization of these complexes (12, 13).

The MCF-7 cell line, derived from a human metastatic breast carcinoma, is a model system for study of the regulation of breast cancer cell growth by estrogens and anti-estrogens. These cells contain high levels of estrogen receptor and estrogen-responsive progestin binding activity, and growth of these

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† The trivial names and abbreviations used are: estradiol (E\(_2\)), estradiol-1,3,5(10)-triene-3,17β-diol; Cl628M, 2-[4-(2-pyrrolidin-1-yl-ethoxy)phenyl]-2-(3-[3H]-4-hydroxyphenyl)-1-phenyl-1-nitroethene; [3H]Cl628M, 2-[4-(2-pyrrolidin-1-yl-ethoxy)phenyl]-2-(3-[3H]-4-hydroxyphenyl)-1-phenyl-1-nitroethene; trans-hydroxytamoxifen (trans-OH-Tam), 1-[4-(2-dimethylaminoethoxy)phenyl]-1-(4-hydroxyphenyl)-2-phenylbut-1(2)-ene; R\(_n\), Stokes radius.
cells is inhibited by anti-estrogens (8-11). In this paper, we compare the receptor interactions of two radiolabeled high affinity nonsteroidal anti-estrogens, [\(^{3}H\)]Cl282M and [\(^{3}H\)]trans-hydroxytamoxifen, with those of the potent natural estrogen [\(^{3}H\)]estradiol and examine the physicochemical properties of receptor following anti-estrogen or estrogen binding.

Our findings demonstrate a substantial alteration in sedimentation rate and chromatographic properties of the nuclear estrogen receptor from MCF-7 cells when complexed with either of the high affinity anti-estrogens, Cl282M or trans-hydroxytamoxifen, as opposed to the estrogen estradiol. These changes in receptor properties may be an important aspect underlying the estrogen-antagonist antitumor properties of these compounds.

MATeRIALS AND METHODS

MCF-7 cells obtained from Dr. Charles McGrath of the Michigan Cancer Foundation (Detroit, MI) were maintained in closed 150-cm\(^2\) Corning T-flasks incubated at 37 °C and were passaged in a logarithmic growth phase (10). Growth medium was Eagle’s minimal essential medium containing Hank’s salts supplemented with 1% nonessential amino acids (Gibco, Grand Island, NY), 2 mM L-glutamine (Gibco), 0.006 μg/ml of insulin (Sigma), 5% calf serum (Gibco), 0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (Gibco), 50 μg/ml of gentamicin (Scherer, Kenilworth, NJ), 100 units/ml of penicillin (Gibco), and 50 μg/ml of streptomycin (Gibco).

Four days prior to use, cells were transferred to harvest medium, which was the same as growth medium except that calf serum was replaced by charcoal-dextran stripped calf serum prepared by incubating 100 ml of serum with 5 ml of charcoal-dextran solution (0.50% Norit A, 0.05% dextran in 0.14 NaCl) for 30 min at 55 °C. This procedure removed 96-98% of a trace amount of [\(^{3}H\)]estradiol. The charcoal was removed by centrifugation-filtration, and the serum was filter-sterilized and stored at -20 °C. Harvest medium was also supplemented with 10 mM hydrocortisone. Cells were fluid-changed with fresh harvest medium on 4 consecutive days prior to each experiment to enable the removal of endogenous steroids.

[\(^{2}H\),\(^{6}H\)] Estradiol (108 Ci/mmol) was obtained from Amersham. [\(^{3}H\)]Cl282M (22 Ci/mmol) and [\(^{3}H\)]trans-hydroxytamoxifen (27 Ci/mmol) were prepared and kindly provided by Drs. John Katzenellenbogen and David Robertson, Department of Chemistry, University of Illinois (14, 15). Radioinert estradiol was obtained from Sigma.

**Binding Analysis—**Cells from 10 near-confluent T-150 flasks were harvested as previously described (10), suspended in 2.0 ml of 5 mM sodium phosphate, pH 7.4 at 4 °C, 10 mM thiglycerol, and 10% glycerol (PTG buffer), and homogenized in a Dounce homogenizer using the B pestle. The homogenate was centrifuged (10 min, 800 x g), and the supernatant was collected. The crude nuclear pellet was washed twice more at 0-4 °C with buffer and the supernatants combined. The supernatant fraction (cytosol) was centrifuged at 180,000 x g (30 min) and then diluted to 12 ml with PTG buffer. Aliquots of cytosol (200 μl) were incubated at 0-4 °C for 20 h with [\(^{3}H\)]estradiol, [\(^{3}H\)]Cl282M, or [\(^{3}H\)]trans-hydroxytamoxifen at concentrations ranging from 1 x 10\(^{-10}\) M to 6 x 10\(^{-8}\) M. An aliquot was withdrawn for determination of total radioactivity, and unbound ligand was then removed by incubating 1 part of charcoal-dextran slurry with 9 parts of extract for 8 min at 0-4 °C. The charcoal was pelleted by a 3-min centrifugation at 15,600 x g, and an aliquot of the supernatant was withdrawn for counting. Parallel tubes contained the radioactive ligand plus a 100-fold excess of radioinert estradiol to assess nonspecific binding as described in detail in Ref 13.

Sucrose Gradient Analyses—MCF-7 cells from two near-confluent T-150 flasks were incubated for 1 h with 20 nM [\(^{3}H\)]Cl282M or [\(^{3}H\)]trans-hydroxytamoxifen or for 0.5 h with 10 nM [\(^{3}H\)]estradiol at 37 °C. These times gave maximal localization of anti-estrogen and estradiol receptor complexes in the nucleus (10). The cells were harvested, washed, as described above, and homogenized in 1 ml of PTG buffer. The homogenate was centrifuged at 800 x g for 10 min, and the resulting nuclear pellet was washed twice with 1 ml of ice-cold PTG buffer. The washed pellet was resuspended in 0.125 ml of PTG buffer to which 0.375 ml of 10 mM Tris-HCl, pH 8.5, 1.5 mM EDTA, 10 mM thiglycerol, 10% glycerol, 0.8 M KCl was added and incubated at 4 °C for 1 h with resuspension every 15 min. This procedure extracted reproducibly over 90% of the [\(^{3}H\)]E\(_2\), or [\(^{3}H\)]anti-estrogen receptor complexes as determined by ethanol extraction. The suspension was centrifuged for 30 min at 180,000 x g, and the supernatant was treated for 8 min at 0-4 °C with charcoal-dextran slurry (1 part of slurry, 9 parts of extract). A 900-ml aliquot was layered on linear 5-20% sucrose gradients (3.6 ml) formed in PTG buffer containing 0.4 mM KCl. The gradients were centrifuged at 357,000 x g in an SW 60 rotor for 15 h, and two-drop fractions were collected for counting. Cells were also treated with radioactive ligand plus 100-fold excess of radioinert estradiol to assess the level of nonspecific binding. Recovery on all gradients was greater than 90%. Sedimentation coefficients were determined according to Martin and Ames (16) relative to the internal markers [\(^{14}C\)ovalbumin (3.5 S) and [\(^{14}C\]γ globulin 6.6 S) which were included in each gradient (13).

**Binding to cytoplasmic sites was assessed in the cell-free cytosol (supernatant (180,000 x g x 30 min)) prepared from control previously untreated cells or following incubation of cells with tritiated estradiol or anti-estrogen for 1.5 h at 0-4 °C. In the latter case, the cells were**
then harvested, washed, and homogenized in 0.5 ml of PTG buffer, as above. The homogenate was centrifuged at 150,000 × g for 30 min, treated with charcoal-dextran, and then sedimented for 15 h at 0-20% sucrose gradients formed in PTG buffer containing 0.4 M KCl. Sucrose gradients (5-20% sucrose) containing 30.0 M urea and 0.4 M KCl were prepared in PTG buffer. Centrifugation was for 17 h at 357,000 × g at 4 °C.

**RESULTS**

**Analysis of the Binding of Anti-estrogens to the MCF-7 Cytosol Receptor**—Saturation binding analyses for estradiol and the anti-estrogens are shown in Fig. 1. These data indicate the presence of one class of high affinity cytosol binding sites with equilibrium dissociation constants ($K_d$) of $1.3 \times 10^{-9}$ M for E$_2$, $2.2 \times 10^{-10}$ M for CI628M, and $1.4 \times 10^{-10}$ M for trans-hydroxytamoxifen binding to the estrogen receptor. Scatchard binding analysis (17), therefore, indicates that the affinity of these anti-estrogens for receptor is very close to that of estradiol and that each compound binds to a similar number of sites. Moreover, competitive binding analysis (10) and the full displacement of [3H]anti-estrogen binding by unlabeled estradiol (see below) indicate that these compounds bind in a mutually competitive manner to the cytosol estrogen receptor.

**Sedimentation Behavior of Tritiated Anti-estrogen and Estrogen Receptor Complexes**—To determine whether the physicochemical properties of the receptor anti-estrogen complex differ from those of the receptor estrogen complex, MCF-7 cells were incubated for 0.5-1 h at 37 °C with a receptor-saturating concentration of radiolabeled E$_2$, CI628M, or trans-hydroxytamoxifen. The nuclear receptor sites were then extracted with 0.6 M salt and analyzed on sucrose gradients. (This extraction procedure solubilized greater than 90% of the tritiated estradiol or anti-estrogen receptor complexes.) Fig. 2 shows that the receptor estradiol complex sediments at 4.1 S while CI628M and trans-hydroxytamoxifen complexes sediment more rapidly, at 5.5 S. By contrast, the cytoplasmic receptor, when complexed with any of these estrogen or anti-estrogen ligands, following in vitro cytosol or whole cell labeling at 0–4 °C, showed similar sedimentation rates (4.1 S) on high salt sucrose gradients (Fig. 3).

**Gel Chromatography of Nuclear and Cytosolic Anti-estrogen and Estrogen Receptor Complexes**—Nuclear and cytoplasmic receptor complexes with radiolabeled anti-estrogen or estradiol were chromatographed on a Sephadex G-200 column that had been standardized previously with a series of proteins of known Stokes radii (18–20). The elution profiles of the receptor complexes are shown in Fig. 4 (top), and the elution positions of the complexes ($K_w$) are indicated on the column calibration line (Fig. 4, bottom). The nuclear CI628M and trans-hydroxytamoxifen complexes eluted ahead of the nuclear estradiol complex, indicating that the nuclear anti-estrogen receptor complexes have a larger Stokes radius (5.93 and nonspecific binding (open circles). The protein concentration of the extracts was 0.5 μg of protein/0.3 ml of extract applied to each gradient.

**FIG. 2.** High salt sucrose gradient analysis of salt-extracted nuclear receptor after incubation of cells at 37 °C with radiolabeled E$_2$, CI628M, or trans-hydroxytamoxifen. MCF-7 cells were incubated for 0.5 h with 10 nM $[^3H]E_2$ or for 1 h with 20 nM $[^3H]CI628M$ or $[^3H]trans$-hydroxytamoxifen at 37 °C. Parallel T-150 flasks were incubated with the indicated concentration of radiolabeled compound plus 10-fold excess of radioioint E$_2$. The cells were harvested and washed, and the nuclear salt extract was prepared and charcoal-dextran treated as described in "Materials and Methods." A 300-μl aliquot was sedimented in 5-20% sucrose gradients containing 0.4 M KCl (15 h at 4 °C at 357,000 × g). $[^3H]Ovumulin (OV, 3.5 S) and $[^14C]γ$-globulin ($γ G, 6.5 S$) were included in all gradients as markers, and their positions are designated by arrows. The boldface curve represents the difference between total binding (closed circles) and nonspecific binding (open circles). The protein concentration of the extracts was 0.5 μg of protein/0.3 ml of extract applied to each gradient.
and their positions are designated by arrows. The extracts was 0.4 mg of protein/0.3 ml applied to each gradient.

nonspecific binding (open circles). The protein concentration represents the difference between total binding (closed circles) and the cytoplasmic receptor, whether complexed with estradiol or anti-estrogen, has a calculated molecular weight of 75,600.

Utilizing the Stokes radii estimated from Sephadex G-200 column chromatography and the sedimentation coefficients determined from sucrose gradient analyses, the molecular weights of the complexes were calculated by the equation of Siegel and Monty (21) and are summarized in Table I. The cytoplasmic receptor was isolated for characterization as indicated in the legend to Fig. 3. The nuclear receptor complexes were isolated from cells incubated at 37 °C for 0.5 h with radiolabeled E2, for 1 h with radiolabeled CI628M or trans-hydroxytamoxifen. All receptor complexes were treated with charcoal-dextran prior to column application as described under "Materials and Methods." Parallel incubations containing radioactive ligand plus a 100-fold excess of radioinert E2 were also analyzed to assess nonspecific binding. Catalase (CA, 5.13 nm), bovine serum albumin (BSA, 3.63 nm), ovalbumin (OV, 2.80 nm), soybean trypsin inhibitor (STZ, 2.26 nm), and myoglobin (MYO, 2.02 nm) were chromatographed as standards. R is the Stokes radius in nanometers, and Kc is the distribution coefficient where Kc = V_v/V_t, V_v is the void volume measured with [3H]leucine, V_t is the total volume of the gel bed measured with [3H]leucine, V_v is the void volume measured with blue dextran 20000, and V_t is the elution volume of the proteins. The dimensions of the column were 1.5 × 24 cm. Top, the elution profiles of the cytosol (—) and nuclear (——) receptor complexes. Bottom, the relationship between R and Kc for the protein standards and receptor complexes. In the top, the E2 nuclear receptor values are plotted at one-tenth of the values actually seen on the column, while the CI628M cytosol and trans-hydroxytamoxifen cytosol receptor values are plotted at 3 times the values obtained so that all receptor profiles could be compared more readily on one scale.

**Fig. 3.** High salt sucrose gradient analysis of cytoplasmic receptor after incubation of cells at 0–4 °C with radiolabeled E2, CI628M, or trans-hydroxytamoxifen. MCF-7 cells were incubated for 1.5 h at 0–4 °C with radiolabeled E2, CI628M, or trans-hydroxytamoxifen. Parallel T-150 flasks were incubated with the indicated concentration of each ligand plus a 100-fold excess of radioinert E2. The cells were harvested and washed, and the cytoplasmic extract was prepared and charcoal-dextran treated as described under "Materials and Methods." A 300-μl aliquot was layered on 5–20% sucrose gradients containing 0.4 M KCl and centrifuged for 15 h at 357,000 × g. Marker proteins, [3H]ovalbumin (OV, 4.15 S) and [14C]globohulin (γG, 6.6 S), were included in each gradient, and their positions are designated by arrows. The boldface curve represents the difference between total binding (closed circles) and nonspecific binding (open circles). The protein concentration of the extracts was 0.4 mg of protein/0.3 ml applied to each gradient.

**Fig. 4.** Estimation of Stokes radii of estrogen and anti-estrogen receptor complexes by Sephadex G-200 column chromatography. MCF-7 cells were incubated for 1.5 h at 0–4 °C with radiolabeled E2, CI628M, or trans-hydroxytamoxifen, and the cytoplasmic receptor was isolated for characterization as indicated in the legend to Fig. 3. The nuclear receptor complexes were isolated from cells incubated at 37 °C for 0.5 h with radiolabeled E2, for 1 h with radiolabeled CI628M or trans-hydroxytamoxifen. All receptor complexes were treated with charcoal-dextran prior to column application as described under "Materials and Methods." Parallel incubations containing radioactive ligand plus a 100-fold excess of radioinert E2 were also analyzed to assess nonspecific binding. Catalase (CA, 5.13 nm), bovine serum albumin (BSA, 3.63 nm), ovalbumin (OV, 2.80 nm), soybean trypsin inhibitor (STZ, 2.26 nm), and myoglobin (MYO, 2.02 nm) were chromatographed as standards. R is the Stokes radius in nanometers, and Kc is the distribution coefficient where Kc = V_v/V_t, V_v is the void volume measured with [3H]leucine, V_t is the total volume of the gel bed measured with [3H]leucine, V_v is the void volume measured with blue dextran 20000, and V_t is the elution volume of the proteins. The dimensions of the column were 1.5 × 24 cm. Top, the elution profiles of the cytosol (—) and nuclear (——) receptor complexes. Bottom, the relationship between R and Kc for the protein standards and receptor complexes. In the top, the E2 nuclear receptor values are plotted at one-tenth of the values actually seen on the column, while the CI628M cytosol and trans-hydroxytamoxifen cytosol receptor values are plotted at 3 times the values obtained so that all receptor profiles could be compared more readily on one scale.
The nuclear estradiol receptor complex has a slightly greater molecular weight (83,400) due to a larger Stokes radius. This small change may simply reflect a change in conformation. The nuclear anti-estrogen receptor complexes, by contrast, have a far greater molecular weight (137,000). Such a large increase in calculated molecular weight is unlikely to be ascribable to conformational change or to a change in $\bar{v}$ and most likely represents an increase in mass as a result of association with another component (see “Discussion”).

Characterization of Estradiol and Anti-estrogen Receptor Complexes in the Presence of Urea—Urea is known to dissociate protein molecules held together by weak interactions (22, 23). Since the binding of estradiol and anti-estrogen to receptor was found to be very stable in concentrations of urea, characterization was carried out in 3.0 M urea. The results obtained in 3.0 M urea are depicted in Figs. 5 and 6.

**FIG. 5.** Effect of urea upon sedimentation of nuclear (left) and cytosol (right) receptor complexes. Nuclear and cytosol estradiol and anti-estrogen receptor complexes were prepared exactly as described in the legends to Figs. 2 and 3 and were sedimented on 5-20% sucrose gradients containing 0.4 M KCl and 3.0 M urea with [14C]ovalbumin (OV) and [14C]γ globulin (γG) as internal markers. The boldface curve represents the difference between total binding (closed circles) and nonspecific binding (open circles). The protein concentration of the extracts ranged from 0.41 to 0.46 mg of protein/300-μl aliquot applied to each gradient.
TABLE I

Physical parameters of estrogen and anti-estrogen receptor complexes from gel chromatography and sucrose gradient analysis

Experimental values are given as mean ± standard error of the mean with number of determinations indicated in parentheses. Sedimentation coefficients were obtained relative to protein standards by density gradient sedimentation (Figs. 2 and 3). R, was calculated from the linear regression line of log R, versus K, for five protein standards (Fig. 4) using the mean K, from two determinations of each standard. Molecular weights were calculated from the mean Stokes radius and sedimentation coefficient by the equation of Siegel and Monty (21). The frictional ratio (f/f,) due to shape was calculated from the equation f/f, = R, (4πμN)/3 (y + ρ)1/2 = 13.96 R,/(M/8) where ρ is assumed to be 0.73 cm2 g−1 and y (degree of solvation) is assumed to be 0.2 g/g of protein, and R, is in nanometers.

| Receptor complex | Sedimentation coefficient | Stokes radius (nm) | Frictional ratio | Molecular weight |
|------------------|--------------------------|--------------------|-----------------|-----------------|
| Estradiol nuclear | 4.1 ± 0.03 (10)          | 4.84 ± 0.20 (3)    | 1.55            | 83,400          |
| CI628M nuclear   | 5.5 ± 0.06 (6)           | 5.22 ± 0.20 (3)    | 1.60            | 137,000         |
| Trans-hydroxytamoxifen nuclear | 5.5 ± 0.06 (4) | 4.95 ± 0.18 (3) | 1.60 | 137,000 |
| Estradiol cytosol | 4.1 ± 0.02 (10)          | 4.39 ± 0.23 (4)    | 1.45            | 75,600          |
| CI628M cytosol   | 4.1 ± 0.03 (6)           | 4.39 ± 0.20 (3)    | 1.45            | 75,600          |
| Trans-hydroxytamoxifen cytosol | 4.1 ± 0.03 (3) | 4.30 ± 0.30 (3) | 1.45 | 75,600 |

Fig. 6. Stokes radii of the cytoplasmic and nuclear estradiol and anti-estrogen receptor complexes calculated by Sephadex G-200 chromatography with 3 M urea. The Sephadex G-200 column (1.5 × 24 cm) was equilibrated with PTG buffer containing 0.4 M KCl and 3 M urea at pH 7.4 and standardized with proteins of known Stokes radii. The receptor extracts were prepared exactly as described in the legends to Figs. 2 and 3. The value for each protein standard or receptor preparation is the mean of two determinations. CA, catalase; BSA, bovine serum albumin; OV, ovalbumin; ST, soybean trypsin inhibitor; MYO, myoglobin.

up to 4 M, we examined the behavior of the estradiol and anti-estrogen receptor complexes on sucrose gradients containing urea. (Over a 24-h period at 0–4 °C, the [3H]estradiol or [3H]anti-estradiol binding capacity of the nuclear or cytosol estrogen receptor preparations decreased by less than 10% in 1–3 M urea, as reported previously for uterine estrogen receptor. See Ref. 23.) Cells were incubated with radiolabeled E2, CI628M, or trans-hydroxytamoxifen at 37 °C, and the nuclear receptor was then salt-extracted and sedimented in gradients containing 3 M urea and 0.4 M salt (Fig. 5). Each nuclear receptor complex sedimented as a single peak at 3.9 S; this is in contrast to the profiles observed in the absence of urea (Fig. 2). Cytoplasmic receptor sites also sedimented at 3.9 S, regardless of ligand (Fig. 5). Analysis of estradiol and anti-estrogen receptor complexes on Sephadex G-200 columns equilibrated with 3 M urea and 0.4 M KCl and calibrated with a series of protein standards showed the cytoplasmic or nuclear receptors complexed with E2 or anti-estradiol to elute similarly, corresponding to a Stokes radius of 4.94 nm (Fig. 6). The molecular weights calculated from the Stokes radii and sedimentation coefficients are 81,000 for all the receptor species.

**DISCUSSION**

In the past, studies of receptor anti-estrogen interaction have utilized indirect exchange assays to detect receptor (4, 5, 7). This was necessary because of the lack of radiolabeled anti-estrogens with high affinities for estrogen receptor. In the present study, two high affinity radiolabeled anti-estrogens were utilized (7, 12, 13) to study the physical properties of receptor anti-estrogen complexes in MCF-7 human breast carcinoma cells.

Our findings indicate that anti-estrogens promote a change in receptor form that may be the result of a change in its association with other cellular components such that the MCF-7 nuclear estrogen receptor migrates as a dimeric form when complexed with anti-estrogen as compared with estrogen. The fact that the cytoplasmic receptor shows similar properties when complexed with estrogen or anti-estrogen ligands and that this heavier form is not seen upon incubation of the cytosol anti-estrogen receptor complex at 30 or 37 °C suggests that the shift in sedimentation rate and size of the receptor appears to be associated with exposure to the nuclear compartment.

The nuclear anti-estrogen complexes sedimented at 5.5 S and eluted with a Stokes radius of 5.93 nm, corresponding to a calculated molecular weight of 137,000. This represents an increase in molecular weight over that of the nuclear estradiol receptor complex too great to be explained by conformational change and suggests that the receptor is associating with other components (23). To determine whether the anti-estrogen receptor complexes were associated with other cellular components, properties of the estradiol and anti-estrogen complexes were studied on sucrose gradients and on Sephadex columns equilibrated with 3 M urea. Under these conditions, cytoplasmic and nuclear receptors, whether complexed with estradiol or anti-estradiol, sedimented at 3.9 S, and they all eluted from Sephadex columns with a Stokes radius of 4.94 nm. These values correspond to a calculated molecular weight of 81,000.

Urea alters the hydrodynamic properties of the cytoplasmic receptor complexes and nuclear estradiol complex, with the Stokes radius increasing and the sedimentation coefficient decreasing, in a manner expected for moderate concentrations of urea. However, the molecular weights of the cytoplasmic receptor complexes and the nuclear estradiol receptor complex calculated from the Stokes radius and sedimentation coefficient remain unchanged. The presence of 3 M urea has a more profound effect on the nuclear anti-estrogen receptor complex. The changes in hydrodynamic properties observed in the presence of urea are consistent with dissociation of a component of approximately 55,000 molecular weight, resulting in a form of the nuclear anti-estrogen receptor complex that is now indistinguishable from that of the nuclear estradiol receptor complex.

In some estrogen target tissues, including the uterus and pituitary, activation of estrogen receptor (a process by which the cytoplasmic estradiol receptor complex is converted to a form with enhanced affinity for nuclear acceptor sites) is believed to involve a dimerization of receptor or its interaction with a dissimilar protein and is manifest by a change in sedimentation velocity (the 4 S to 5 S transformation) on high salt sucrose gradients (24–26). It is important to stress that
the alteration in sedimentation rate and size of the anti-
estrogen nuclear receptor complexes probably does not rep-
sent activation of the receptor in the classical sense, as the
estradiol receptor complex from MCF-7 cells is not changed
in these properties upon activation. Both cytoplasmic and
nuclear estradiol receptor complexes from MCF-7 cells sedi-
ment at 4.1 S (as shown previously, Ref. 27) and have similar
Stokes radii.

Our findings suggest that anti-estrogens may antagonize
estrogen action by promoting a change in receptor that results
in its association with another cellular component. It is tempt-
ing to speculate that this anti-estrogen-promoted interaction
may be biologically nonproductive and would prevent or block
receptor from associating with biologically important chro-
matin-binding or “acceptor” sites (28). Such a model could
explain the deficient activation of estrogen-dependent re-
sponses by anti-estrogens and the growth inhibition observed
in MCF-7 cells treated with anti-estrogens (5, 8, 10). Further
studies should allow a continuing examination of this hypoth-
thesis.

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