Interactions of synthetic estrogens with human estrogen receptors

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Abstract

Synthetic estrogens have diverse chemical structures and may either positively or negatively affect the estrogenic signaling pathways through interactions with the estrogen receptors (ERs). Modeling studies suggest that 4-(1-adamantyl)phenol (AdP) and 4,4’-(1,3-adamantanediyldiphenol (AdDP) can bind in the ligand binding site of ERα. We used fluorescence polarization (FP) to compare the binding affinities of AdP, AdDP and 2-(1-adamantyl)-4-methylphenol (AdMP) for human ERα and ERβ with the binding affinities of the known ER ligands, diethylstilbestrol (DES) and 4-hydroxytamoxifen (4OHT). Competition binding experiments show that AdDP has greater affinity for both ERs than does AdP, while AdMP does not bind the receptor proteins. The relative binding affinities of AdDP and AdP are weaker than the affinity of DES or 4OHT for both ERs with the exception of AdDP, which binds ERβ with higher affinity than does 4OHT. We also found that AdDP and AdP cause differential conformational changes in ERα and ERβ, which result in altered affinities of the ERs for fluorescein-labeled estrogen response elements (EREs) using a direct binding FP assay. The results show that ERβ liganded with either AdDP or AdP has greater affinity for human pS2 ERE than the ERβ–4OHT complex. The data suggest that synthetic molecules like adamantanes may function as biologically active ligands for human ERs. This demonstrates the importance of considering the potential of novel classes of synthetic compounds as selective ER modulators.

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Introduction

The estrogen receptor (ER) is a ligand-dependent transcriptional factor with domain structure (Kumar et al. 1987) and belongs to a supergene family that includes receptors for steroid and thyroid hormones, vitamin D₃ and retinoic acid (Evans 1988). In the absence of a ligand the ER resides in the cell nucleus associated with heat-shock proteins (Joab et al. 1984, Sanchez et al. 1990). Binding of the hormone estrogen causes the ER to undergo conformational changes leading to dissociation of the heat-shock proteins and formation of stable receptor dimers (Kumar & Chambon 1988). The ligand-occupied receptor dimers interact with estrogen response elements (EREs) located within the regulatory region of target genes, and then are able to interact with other cellular components to either activate or suppress transcription of a target gene in a promoter- and cell-specific manner (Tora et al. 1989).

Recently it has been discovered that a second ER (ERβ) exists, in addition to the traditional ER, now called ERα. ERα and ERβ share common physical and functional properties; they also have high degrees of homology in their ligand binding domains (LBDs) and DNA binding domains (Kuiper et al. 1996, Mosselman et al. 1996). Both ERs have similar affinities for 17β-estradiol (E₂), recognize the same EREs and are expressed in distinct and overlapping tissues (Couse et al. 1997).

In addition to the endogenous estrogens, a group of exogenous chemicals called xenoestrogens can display estrogen-like functions in estrogen responsive tissues. The source of xenoestrogens can be dietary in nature including phytoestrogens (Kurzer & Xu 1997) or industrial chemicals (pharmaceuticals, pesticides, pollutants) (Korach et al. 1997). The xenoestrogens interact with the ERs and can either induce a response that mimics endogenous estrogen stimulation or produce an inactive receptor–ligand complex that inhibits the transcription of ER regulated genes.

The synthetic xenoestrogens have diverse chemical structures but there are some common structural motifs among these compounds. The most essential structural motif that elicits estrogenic activity is a phenol that is relatively unhindered, attached to a rather bulky hydrophobic structure (Katzenellenbogen 1995). Adlercreutz & Mazur (1997) have noted that although the length and the width of the E₂ molecule fit compactly within the LBD of the ER protein, binding of E₂ within this domain leaves large unoccupied regions opposite the B- and C-rings. Endo et al. (1999) have recently described binding of
Materials and Methods

Materials

The steroids E₂, DES and 4OHT were obtained from Sigma Chemical Co. (St Louis, MO, USA). AdP, AdDP and AdMP were from Aldrich (Milwaukee, WI, USA).

Human recombinant ERα and ERβ, and fluorescein-labeled E₂ (ES2) were purchased from Pan Vera Corporation (Madison, WI, USA).

Fluorescein end-labeled Xenopus vit A2, human pS2 ERs and glucocorticoid response element (GRE) were custom synthesized by Oligos Etc. (Wilsonville, OR, USA).

Molecular modeling

Molecular modeling studies were done using SYBYL molecular modeling software (SYBYL release 6·6; Tripos, St Louis, MO, USA). All molecules were drawn using SYBYL sketcher and minimized with the Powell method using simplex initial optimization and Tripos force field.

ES2–ER direct binding experiments

Recombinant human ERα and ERβ were serially diluted from 256 nM to 0·5 nM in screening buffer (100 mM potassium phosphate, pH 7·5; 100 µg/ml bovine gamma globulin; 0·02% sodium azide) to a final volume of 100 µl in borosilicate test tubes. ES2 was added to each test tube to a final concentration of 1 nM and incubated for 60 min at room temperature. The FP of each tube was measured on a Beacon 2000 Fluorescence Polarization Instrument (Pan Vera Corporation) with a 490 nm excitation filter and 530 nm emission filter (Jameson & Sawyer 1995). FP values were plotted vs ER concentration.

Kₐ was calculated using a nonlinear least-square curve fitting program (Prizm; Graphpad Inc., San Diego, CA, USA) as the concentration of ER at which half of the ligand is bound.

Competitive binding experiments

DES, 4OHT, AdP, AdDP and AdMP were tested for their ability to displace the ES2 molecule from ERα–ES2 and ERβ–ES2 complexes.

Serial dilutions of each competing compound were prepared from an 8 mM ethanol stock solution in screening buffer. Preincubated ERα or ERβ (13 nM) and ES2 (1 nM) were added to produce a final volume of 100 µl. After 60 min incubation at room temperature, the polarization values at each competitor’s concentration were measured using the Beacon 2000 FP system with a 490 nm excitation filter and 530 nm emission filter. The polarization values were converted to percent inhibition using the equation Iₜₐₜ = (P₀ − P)/(P₀ − P₁₀₀) × 100, where P₀ is the
polarization value at 0% inhibition, $P_{100}$ is the polarization value at 100% inhibition, and $P$ is the observed FP at each concentration point. Free ES2 (100% inhibition) was used as a positive control and ER–ES2 complex (0% inhibition) was used as a negative control. Polarization values were transformed into percent inhibition in order to normalize the differences at 0% inhibition for each run. The percent inhibition vs competitor concentration curves were analyzed by nonlinear least-squares curve fitting and yielded $IC_{50}$ values (the concentration of competitor needed to displace half of the bound ligand). To compare the binding affinities of the tested compounds, $IC_{50}$ values were converted to relative binding affinities (RBAs) using $E_2$ as a standard. The $E_2$ RBA was set equal to 100, and the RBA value for each of the phytoestrogens was calculated using the formula $RBA = (IC_{50} E_2 / IC_{50}$ competitor) \times 100.

**Preparation of EREs**

EREs from *Xenopus vit* A2 gene, ERE from human pS2 gene and consensus GRE were tested to bind ER\(\alpha\) and ER\(\beta\) (Table 1). The sense DNA strands (Oligos Etc.) containing EREs and GRE were labeled with fluorescein attached via a six-carbon spacer at the 5’ terminus. The 35 bp double stranded oligonucleotides were prepared by annealing equimolar concentrations of the sense and antisense strands in 10 mM Tris–HCl, pH 7.8 and 150 mM NaCl. This mixture was heated to 95 °C for 10 min and slowly cooled (30 min) to room temperature. To remove any hairpin formations the double stranded DNA was purified by 12% polyacrylamide (1:19 bisacrylamide:acrylamide) gel electrophoresis containing 89 mM Tris–borate, 2.5 mM EDTA, pH 8.3 and 10% ammonium persulfate.

### Table 1  Double stranded DNA sequences containing EREs and GRE. The underlined sequences represent the limits of the 13 bp reverse repeat of the EREs and the GRE, containing 5 bp arms and a 3 bp spacer region

<table>
<thead>
<tr>
<th>Sequence</th>
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<tbody>
<tr>
<td><strong>Xenopus vit</strong> A2 ERE</td>
</tr>
<tr>
<td>5’ XGT CCA AAG CTG TTC CCAGT GTC ACTGG ACT AGT TTC AA 3’</td>
</tr>
<tr>
<td>3’ CA GGT TCT ACG AGC ACCGG AC TAG TTT CAA AA 5’</td>
</tr>
<tr>
<td><strong>Human pS2 ERE</strong></td>
</tr>
<tr>
<td>5’ XGT CCA AAG TCA GGTCA CGG TGGCC TG ATC AAA GTT 3’</td>
</tr>
<tr>
<td>3’ CA GGT TTC ACG ACCGG AC TAG TTT CAA AA 5’</td>
</tr>
<tr>
<td><strong>Consensus GRE</strong></td>
</tr>
<tr>
<td>5’ XGT CCA AAG TCA GAACA CAG TG TCTG TGA TTC AAA GTT 3’</td>
</tr>
<tr>
<td>3’ CA GGT TTC AGT CT TGTG ACT AGC ACCGG AC TAG TTT CAA AA 5’</td>
</tr>
</tbody>
</table>

ER–ERE direct binding studies

To further investigate the estrogenic properties of the xenoestrogens we performed direct binding experiments and measured the abilities of ER\(\alpha\) and ER\(\beta\) to associate with *Xenopus vit* A2 ERE and human pS2 ERE in the presence of synthetic ligands. ER\(\alpha\) and ER\(\beta\) were serially diluted from 450 nM to 0.8 nM in DNA binding buffer (10 mM potassium phosphate, pH 7.8; 0.1 mM EDTA; 50 μM magnesium chloride; 10% glycerol). The ERs were incubated for 30 min with concentrations of each of the xenoestrogens required to saturate ER\(\alpha\) and ER\(\beta\) as determined by competitive binding experiments, and then for 10 min with poly(dI–dC) (1 μg/5 μg protein) at room temperature. The binding, initiated by adding fluorescein-labeled synthetic oligonucleotide EREs (final concentration 0.5 nM), was allowed to proceed at room temperature for 60 min. The polarization values of each ER concentration were then measured on the Beacon 2000 instrument with 490 nm excitation and 530 nm emission maximums. The binding isotherm was constructed by plotting percent saturation vs ER concentration using the formula $S_{s} = (P - P_{0}) / (P_{100} - P_{0}) \times 100$, where $P_{0}$ is the polarization value of $E_2$ at 0% saturation, $P_{100}$ is the polarization value of $E_2$ at 100% saturation, and $P$ is the observed FP at each concentration point. $K_d$ was calculated from the binding curves using a nonlinear least-squares curve fitting program. The binding affinities of ER\(\alpha\) and ER\(\beta\) (liganded with synthetic estrogens) for EREs were also calculated in terms of RBA (RBA = ($K_d$ $E_2$ / $K_d$ competitor) \times 100).

In order to prove the reliability and specificity of the method we compared the binding affinities of ER\(\alpha\) and ER\(\beta\) (liganded with $E_2$) for fluorescein-labeled *Xenopus vit* A2 ERE and fluorescein-labeled GRE. At the concentration range tested no ER–GRE complexes were formed as opposed to the high affinity binding of both ERs to the consensus ERE (Nikov et al. 2000).

**Results**

**Modeling studies**

Figure 2 illustrates how AdDP may be accommodated within the LBD of ER\(\alpha\) with the two \textit{para} phenol groups of the AdDP molecule positioned similarly to the C-3
phenolic hydroxyl group and the 17β-hydroxyl group of an E2 ligand. We have also modeled the polar interactions between ERα and AdDP (Fig. 3).

**Ligand binding specificity of ERα and ERβ**

To determine the affinity of the adamantyl phenols for ERα and ERβ we first measured the ability of ES2 to associate with the receptor proteins (Fig. 4). One nanomolar of the labeled ligand was titrated with increasing concentrations of the ERs to produce the binding isotherms. Kd values for both receptors were calculated from the saturation curves; for ERα the Kd was 25 nM and for ERβ it was 10 nM. The affinity of the labeled ES2 ligand was approximately 2-fold higher for ERβ than for ERα.

We then performed competition binding of DES, 4OHT, AdP, AdDP and AdMP with the ER–ES2 complex. The binding affinities (IC50 values) of the tested compounds were determined from the competition curves (Fig. 5, Table 2).
The planar aromatic compound DES is a potent synthetic estrogen agonist and binds with high affinity to both ERs. The binding affinity of DES for ERα and ERβ we measured is about 1.3-fold higher than the affinity of E2 (Table 2), which is in agreement with the previously reported affinity of DES for ER (Bolger et al. 1998).

We found that 4OHT has a 7-fold lower affinity for ERα than does E2 and a 10-fold lower affinity for ERβ. The binding affinity of 4OHT for ERα is approximately 1.5-fold higher than for ERβ (Table 2).

AdDP binds with 15-fold and 7-fold lower affinity to ERα and ERβ than does E2, respectively. AdP binds with lower affinity to both ERs than does AdDP. The affinity of AdP for ERα is 77-fold lower than that of E2, and the affinity of AdP for ERβ is 17-fold lower. Both AdP and AdDP have higher affinities for ERβ than for ERα (Table 2). AdMP does not displace the labeled ES2 ligand from either ERα or ERβ in the concentration range tested (Fig. 5).

**ER–xenoestrogen complex binding to human pS2 and Xenopus vit A2 EREs**

The ERβ–DES complex binds to Xenopus vit A2 ERE with affinity similar to that of the ERβ–E2 complex but the ERβ–DES complex binds to human pS2 ERE with greater affinity than the ERβ–E2 complex. The ERα–DES complex binds to both EREs with about 1.3-fold less affinity than does the ERα–E2 complex (Fig. 6, Table 3).

Among the compounds tested here, only the 4OHT–ERα complex manifested higher affinity for the ERs than did the ERβ–xenoestrogen complexes. The ERα–4OHT complex has about 1.6-fold higher affinity for Xenopus vit A2 and human pS2 EREs than the ERα–E2 complex. There is also a significant difference in the binding affinities of ERα–4OHT and ERβ–4OHT for each of the EREs (Fig. 6, Table 3).

In general, AdP and AdDP produce higher binding affinities of the ERβ–ligand complex for the EREs. The complexes of ERβ with AdDP and AdP have approximately 1.2-fold lower affinity for Xenopus vit A2 ERE, and 1.5-fold less affinity for human pS2 ERE as compared with the affinity of the ERβ–E2 complex for these ERE. The affinity of ERα–AdDP and ERα–AdP complexes for human pS2 ERE are about 3-fold lower than the affinity of the ERα–E2 complexes. The affinities of ERα–AdDP and ERα–AdP complexes for Xenopus vit A2 ERE are 1.5-fold and 2.6-fold less than the affinity of ERα–E2 complex respectively. AdP triggers differential binding of ERα and ERβ to both EREs, and AdDP differentially affects the binding of ERα and ERβ to human pS2 ERE.

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**Table 2** IC$_{50}$ constants and RBAs of tested synthetic estrogens for human ERα and ERβ from competition experiments. RBA of each competitor was calculated as a ratio of the IC$_{50}$ values of each competitor and E$_2$. RBA value of E$_2$ was arbitrarily set at 100. The data represents the mean IC$_{50}$ values ± S.E.M. from two different experiments.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ERα (IC$_{50}$)</th>
<th>RBA</th>
<th>ERβ (IC$_{50}$)</th>
<th>RBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>E$_2$</td>
<td>13 ± 0.7 nM</td>
<td>100</td>
<td>12 ± 0.5 nM</td>
<td>100</td>
</tr>
<tr>
<td>DES</td>
<td>10 ± 0.5 nM</td>
<td>130</td>
<td>9 ± 0.7 nM</td>
<td>133</td>
</tr>
<tr>
<td>4OHT</td>
<td>96 ± 0.8 nM</td>
<td>14</td>
<td>115 ± 0.7 nM</td>
<td>10</td>
</tr>
<tr>
<td>AdDP</td>
<td>200 ± 1 nM</td>
<td>6.5</td>
<td>80 ± 0.5 nM</td>
<td>15</td>
</tr>
<tr>
<td>AdP</td>
<td>1 ± 1 μM</td>
<td>1.3</td>
<td>200 ± 1 nM</td>
<td>6.0</td>
</tr>
<tr>
<td>AdMP</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</table>

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**Figure 5** Competition binding curves of E$_2$ and various synthetic compounds for ERα–ES2 and ERβ–ES2 complexes. The initial ER–ES2 complexes have high polarization values. When the complex is titrated with competitors, ES2 molecules are displaced from the ER and a gradual decrease in the polarization values is observed. The data points represent the mean percent inhibition values ± S.E.M. from two different experiments.
AdMP does not affect the binding of the ligand-free ERs to Xenopus vit A2 and human pS2 EREs (data not shown). For both of the EREs the affinity of ERα–xenoestrogen complex decreases in the order: 4OHT > DES > AdDP > AdP; and for ERβ–xenoestrogen complex: DES > AdDP > AdP > 4OHT.

There are also differences in the polarization values of ER–ERE complexes at saturation depending on the ligand present (Fig. 6). In the case of Xenopus vit A2 ERE (Fig. 6A), binding of DES to the ERα–ERE complex gives the highest polarization value followed by E2. When ERβ is complexed with Xenopus vit A2 ERE, binding of either DES or E2 gives the same (highest) polarization value.
while the rest of the compounds tested show lower polarization values at saturation. When ERα and ERβ are complexed with human pS2 ERE (Fig. 6B), the polarization values at saturation of the ER–ERE complexes in the presence of all compounds tested are lower than those of ERE–ER–E₂ except ERα–AdDP, which is greater than ERα–E₂.

Discussion

Transcriptional activation mediated by ERs involves binding of a ligand to the receptor and association of the ER–ligand complex to an ERE located within the regulatory region of target genes. The conformation of the ER–ligand complex is crucial for the activation of the transcriptional machinery and the chemical structure of the ligand plays an important role in triggering agonistic or antagonistic effects.

The crystal structure of the ERα LBD complexed with E₂ provides important information about the orientation and the structure of putative ER ligands (Brzozowski et al. 1997, Tanenbaum et al. 1998). The E₂ molecule is oriented by hydrogen bonding and hydrophobic van der Waals’ contacts and it fits well in the LBD. The phenolic hydroxyl group of the A-ring of E₂ lies between α-helices 3 and 6, and makes several direct hydrogen bonds. The polar residue Glu353 (helix H3) has been identified as a putative hydrogen bond partner for the steroidal hydroxyl group at C-3 position of E₂. At the other end of the molecule the 17β-hydroxyl group of the D-ring makes a single hydrogen bond with the δ-nitrogen of His524 in helix H11. Additional stability of this hydrogen-bonding network is provided via salt bridge interactions between Glu353 and Arg394 (helix H5), and Glu419 and Lys531/Asn532 (Tanenbaum et al. 1998, Wurtz et al. 1998). Arg394 is also involved in the orientation of the hormone in the LBD; the side chain of this residue is braced by a hydrogen bond to the carbonyl group of the residue preceding Phe404, which is fixed by its van der Waals’ contact to ring A of the E₂. Thus, the recognition of the 3-hydroxy function of the hormone is coordinated with a van der Waals’ contact to its hydrophobic rings (Tanenbaum et al. 1998). The length and the width of the E₂ skeleton are matched by the receptor’s LBD, but there are large unoccupied spaces opposite the B- and C-rings of E₂.

The E₂ agonist DES fits in the LBD of ERα in a manner that resembles that of E₂; one of the phenolic rings of DES lies in the same position as the E₂ A-ring near helices 3 and 6, and the other phenolic ring of DES rests in a position close to the location of the E₂ D-ring (Shiau et al. 1998). Our competition binding experiments show that DES binds to both ERα and ERβ with affinity even greater than that of E₂ (Fig. 5, Table 2). This can be attributed to the structure of DES; the ethyl groups of DES project perpendicularly from the plane of the phenolic rings and fit into the spaces adjacent to the B- and C-rings of E₂ in the LDB (Shiau et al. 1998). The affinity of DES for the ERs determined by us is similar to that observed by Bolger et al. (1998) using the same FP method, but weaker than the affinity of this ligand measured by Kuiper et al. (1997) using a traditional radiolabeled E₂ binding assay.

Using direct binding experiments we studied the effect of DES on the binding of human ERs to Xenopus vit A2 and human pS2 ERs. The data indicate that the conformational changes in the ERs caused by DES do not differ much from those evoked by E₂ and the ER–DES complexes bind to both ERs with affinity similar to that of ER–E₂ complexes (Fig. 6, Table 3). These results are in agreement with the elucidated crystal structure of the ER–DES complex.

Studies of the crystal structure of the LBD of ERα in a complex with 4OHT show that this compound is bound within the same pocket that recognizes E₂ and DES. The bulky side chain of 4OHT changes the orientation of some of the helices in the LBD and as a result 4OHT binding promotes conformation of the LBD that is distinct from that stabilized by either DES or E₂ binding (Shiau et al. 1998).

Table 3  

<table>
<thead>
<tr>
<th>Compound</th>
<th>Xenopus vit A2 ERE</th>
<th>Human pS2 ERE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ERα</td>
<td>ERβ</td>
</tr>
<tr>
<td></td>
<td>$K_d$</td>
<td>RBA</td>
</tr>
<tr>
<td>E₂</td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td>DES</td>
<td>46</td>
<td>81</td>
</tr>
<tr>
<td>4OHT</td>
<td>25</td>
<td>150</td>
</tr>
<tr>
<td>AdDP</td>
<td>57</td>
<td>66</td>
</tr>
<tr>
<td>AdP</td>
<td>97</td>
<td>38</td>
</tr>
</tbody>
</table>

$K_d$ constants (nM) and RBAs of ERα and ERβ (saturated with xenoestrogens) for Xenopus vit A2 and human pS2 ERs. RBA of each ER saturated with competitor was calculated as a ratio of the $K_d$ values of each competitor and E₂. RBA values of the ERs saturated with E₂ were arbitrarily set at 100.

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The competition binding experiments show that the antiestrogen 4OHT binds with less affinity to the ERs than does DES. The RBAs of 4OHT are much lower compared with the RBAs of E2 for ERα and ERβ, which differ from the findings of Kuiper et al. (1997).

The direct binding FP assay reveals that the conformational changes in the ERs caused by 4OHT result in different binding affinities of the ERα–4OHT and ERβ–4OHT complexes for the EREs, relative to the affinities of ER–E2 complexes. ERα–4OHT complex has the highest affinity for both EREs among the compounds tested here, while ERβ–4OHT has the lowest affinity (Fig. 6, Table 3).

In this study we also investigated the ability of AdP, AdDP and AdMP to associate with human ERs and their effect on the interactions of ERα and ERβ with two different EREs.

The three-dimensional structure of AdDP is similar to that of DES and could exploit hydrogen bonding as well as van der Waals' interactions with the LBD of the ER (Figs 2 and 3). As suggested by our modeling studies AdDP was found to be a more potent ligand for both ERs than AdP (Fig. 5, Table 2). The ERα–AdDP and ERβ–AdDP complexes bind with higher affinities to both Xenopus vit A2 and human pS2 ERE than do the ER–ADP complexes (Fig. 6, Table 3). These results can be explained by the fact that the two phenolic hydroxyl groups of AdDP are situated to function as both the phenolic hydroxyl group and the 17β-hydroxyl group of E2 ligand. The importance of the phenolic hydroxyl groups and their orientation is also illustrated by the observation that AdMP, which lacks para phenolic function, neither binds to the ERs nor affects the binding of ERα and ERβ to the EREs (Table 2).

The direct binding experiments also show that when ERβ is bound to either AdDP or AdP, the resulting complexes have greater affinity for both EREs than does the ERβ–4OHT complex. These results suggest that, compared with 4OHT, the conformational changes in ERβ caused by binding of the three-dimensional AdDP or AdP molecules favor the formation of the ERβ–ERE complexes. These data may be helpful in regard to previous findings that binding affinity is not always directly related to transcriptional activation with certain ER ligands (Sun et al. 1999).

The bulky adamantyl moiety of AdDP and AdP may fill spaces in the LBD that are unoccupied when planar molecules like E2 or DES are bound. These relatively novel structural features of AdDP and AdP may account for their high affinity for ERα and ERβ as compared with other known synthetic estrogens (Bolger et al. 1998, Kuiper et al. 1998). The additional van der Waals' contacts in the hydrophobic cavity of the LBD when adamantyl phenol ligands are bound may also be responsible for an enhanced binding of the ERβ–ligand complexes to the EREs relative to the ERα–ligand complexes.

As we previously noted (Nikov et al. 2000), ER ligands differentially affect the effective molecular volume of ER–ERE complexes, which results in altered speed of rotation of these complexes. The measured polarization values indicate that the Xenopus vit A2 ERα–DES complex has a higher effective molecular volume (higher polarization value, decreased speed of rotation of the fluorescein label) than the Xenopus vit A2 ERα–E2 complex (lower polarization value, increased speed of rotation of the fluorescein label). In contrast, when complexed with Xenopus vit A2, the ERβ–DES and ERβ–E2 complexes have comparable molecular volumes (Fig. 6A). We also observed differential effects of the binding of the ERα–AdP and ERα–AdDP complexes to human pS2 ERE. The presence of a second phenolic group in the molecule of AdDP affects the molecular volume of human pS2 ERE–ERα complex. The effective molecular volume of human pS2 ERE complexed with ERα–AdP (higher polarization value, decreased speed of rotation) is about 1.8-fold higher than the effective molecular volume of human pS2 ERE–ERα–AdP complex 1 (lower polarization value, increased speed of rotation) (Fig. 7). The data demonstrate that AdP and AdDP affect differentially the geometry (bending of the DNA or loosening the end of the DNA that is labeled) of the ERα–human pS2 ERE complex.

Routledge et al. (2000) observed that xenoestrogens might induce conformational changes in the tertiary structure of the ERs that differ from that of E2 and thus affect the ability of ERα and ERβ to recruit coactivator proteins. In this context the conformational changes in the ERs caused by AdDP and AdP may affect the transcriptional activation of ER regulated genes.

We conclude that adamantyl phenols with a distinct three-dimensional structure may function as effective...
ligands for human ERs. AdDP and AdP represent a novel class of synthetic molecules, not based upon the steroidal structure or the planar DES and tamoxifen structures, which may affect the ER signaling pathways. AdDP and AdP should possess biological activity based on their ability to bind the ERs and might be potentially useful for the design of new selective ER modulators.

Acknowledgements

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