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Simulations reveal increased fluctuations in estrogen receptor-alpha conformation upon antagonist binding

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ABSTRACT

Molecular dynamics (MD) simulations have been used to model dynamic fluctuations in the structure of estrogen receptor-alpha (ER-α) upon binding to the natural agonist 17β-estradiol (E2) and to the active metabolite of the breast cancer drug and antagonist, 4-hydroxytamoxifen (OHT). We present the most extensive MD simulations to date of ER-α, with over 1 μs of combined simulations for the monomer and dimer forms. Simulations reveal that the antagonist-bound complex includes significant fluctuations while the agonist-bound complex is tightly restrained. OHT increases dynamic disorder in the loops located to either side of the tail H12 helix; H12 has been associated with the activation status of ER-α. We also report that fluctuations near H12 lead to greater conformational variation in the binding mode of the ethylamine tail of OHT. Both the agonist and antagonist conformations are stable throughout the 240 ns simulations, supporting the hypothesis that there are no transitions between these two states or into intermediate states. The stable position of H12 in the OHT-bound conformation suggests that OHT stabilizes a well-defined antagonist conformational ensemble rather than merely blocking the agonist-driven activation of ER-α. Simultaneously, the increased dynamic properties of the OHT-bound complex is a potential source of binding entropy.

1. Introduction

Estrogen receptor alpha (ER-α) is a transcription factor that mediates the primary physiological response to estrogens. It is a member of the nuclear hormone receptor family, which includes receptors for hormones such as thyroid hormone, androgens, and glucocorticoids [1,2]. Nuclear hormone receptors play a central role in mediating and regulating cell growth and death, development, metabolism, and immune responses. They are the targets of drugs for treating cancer, diabetes, inflammation, and autoimmune diseases [3,4]. Clinically used ER-α agonists include estrogen derivatives for contraception and menopausal symptoms. Drugs with mixed tissue-dependent antagonist and agonist effects are known as selective estrogen receptor modulators (SERMs) and include tamoxifen (used to treat breast cancer), clomiphene (used to treat infertility), and raloxifene (used to treat osteoporosis) [5,6].

ER-α, like other nuclear hormone receptors, contains three structural domains, an N-terminal domain that mediates dimerization and interacts with co-regulator proteins to promote gene expression, a DNA binding domain, and a C-terminal ligand binding domain (LBD) [7]. The crystal structure of full-length ER-α has not yet been determined; however, recent breakthroughs provided crystal structures of intact nuclear hormone receptor complexes bound to DNA that reveal close allosteric interactions between the domains [8].

Ligand interactions are entirely confined to the LBDs. LBDs have been the primary focus of structural studies and drug discovery efforts [9]. Over a hundred crystal structures of LBDs from nuclear hormone receptors bound to ligands have been published including dozens with the ER-α LBD co-crystallized with both agonists and SERMs. The active and inhibited states are associated with two different conformations of the C-terminal H12 helix (residues 538–548) through a "mouse trap" mechanism (Fig. 1) [2]. In the structure of the LBD bound to estradiol (E2), an agonist, H12 closes over the ligand to form part of the interaction surface with co-activators. When bound to antagonist (as in the structure of the LBD bound to SERM 4-hydroxytamoxifen (OHT)), H12 extends away from the ligand and occupies the co-activator binding site. Antagonists are generally larger than agonists (Fig. 2), and their bulk prevents H12 from adopting the active conformation.

Recently, a number of studies have described surprising plasticity in the ER-α ligand binding site, supporting a link between distinct binding orientations and intermediate output states between full agonist and antagonist activities [10,11]. Molecu-

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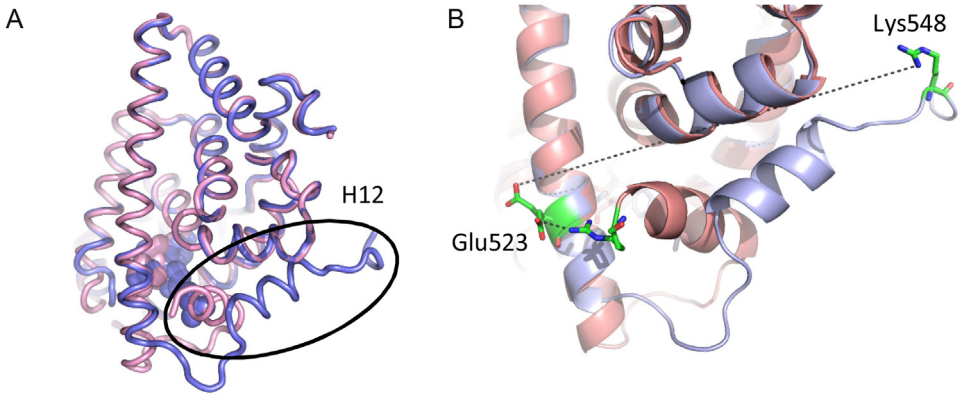

Fig. 1. Crystal structures of ER-α bound to agonist and antagonist. A) Helix H12 (circled) adopts a closed conformation in the agonist (E2) bound structure (pink, PDB 1ere, 3.1 Å). Helix H12 extends away from the ligand in the antagonist (OHT) bound structure (blue, PDB 3ert, 1.9 Å). B) The agonist and antagonist bound conformations are distinguished by the distance between Glu523 and Lys548, which is short in the agonist-bound conformation (pink) and long in the antagonist-bound structure (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

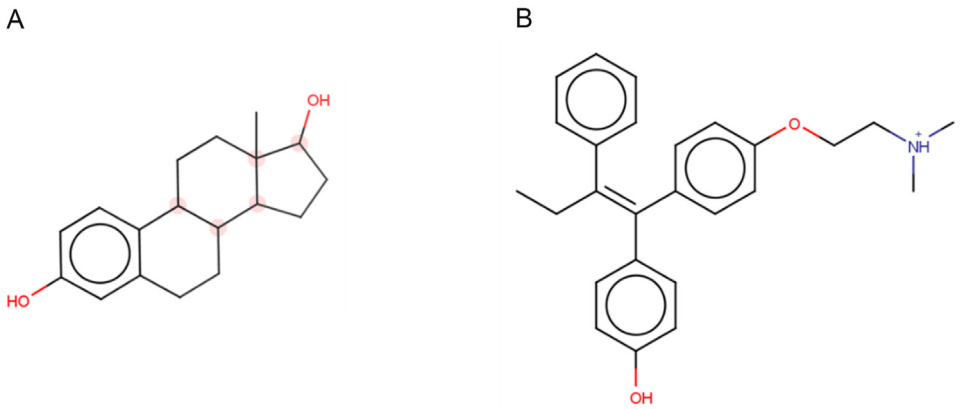

Fig. 2. Structures of ER-α ligands. A) Physiological agonist 17-β-estradiol (E2). B) SERM antagonist 4-hydroxytamoxifen (OHT).

lar dynamics (MD) simulations have yielded further details into the dynamic properties of the agonist- and antagonist-bound conformations [12–17]. These studies indicate that the OHT-bound conformation is compatible with the docking of 11 other antagonists [14], suggest potential mechanisms for ligand release [15,16,18], demonstrate that the agonist-bound conformation is locked by co-activator peptide [12], and suggest the trajectory of the transition between the apo and agonist-bound conformations [12].

We hypothesized that an antagonist-bound complex would show greater dynamic activity than the agonist-bound complex. An agonist must lock the receptor into an active state to bind the co-activator, whereas an antagonist merely needs to interfere with the process. Here we present the results of the most extensive molecular dynamics calculations to date on ER-α with both agonist (E2) and antagonist (OHT) ligands, in monomer and dimer forms. Each monomer system was simulated in three parallel, independent runs for 240 ns. The dimers were simulated in single runs for 240 ns. Our calculations support the hypothesis that the antagonist complex is more dynamic than the agonist-bound conformation and identify receptor regions with increased fluctuation.

2. Methods

The X-ray crystal structures of human ER-α with the physiological agonist, 17β-estradiol, (PDB 1ere) [19] and with the synthetic antagonist, 4-hydroxytamoxifen, (PDB 3ert) [20] were used as initial models for energy minimization and MD calculations. PDB 3ert is a high-resolution crystal structure with diffraction data to 1.9 Å, whereas PDB 1ere is based on a crystal that diffracted to 3.1 Å. Residues missing from the ligand binding domain, such as flexible loops, were modelled with Modeller [21] and Chimera [22]. All solvent and ion atoms located in the crystal structures were removed from the models for further calculations. Ligands were parameterized for the General Amber Force Field (GAFF) [23] using LEaP and Antechamber [24] from AmberTools 15 [25]. Hydrogen atoms missing from the crystal structures were added by LEaP. Each receptor-ligand complex was solvated in a rectangular box of TIP3P water molecules [26] in LEaP extending 10 Å from the complex in 0.15 M NaCl.

The solvated complexes were minimized by PMEMD in Amber 14 using the ff14SB [27] and GAFF force fields with the particle mesh Ewald method [28] with an 8 Å cutoff. Minimization was performed over three cycles in which the atomic coordinates were harmonically restrained with a weight of 5.0, then a weight of 1.0, and finally, were unrestrained. Each cycle included 100 steps of minimization by steepest descent and 900 steps by conjugate gradient.

Energy minimized structures were then equilibrated in MD simulations using the CUDA version of Amber PMEMD to support acceleration with NVIDIA graphics processing units [29,30]. Equilibration was performed in three cycles of 50,000 steps each, with a timestep of 1 fs, at constant pressure using a Berendsen barostat [31]. Temperature was maintained at 298 K with Langevin dynamics. In the equilibration cycles, the atomic coordinates were harmonically restrained with a weight of 5.0, a weight of 1.0, and, finally, a weight of 0.1.