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3D models of human ERα and ERβ complexed with 5-androsten-3β,17β-diol

Michael E. Baker [a,*], Kayla Y. Uh [b], Charlie Chandsawangbhuwana [c]

[a] Department of Medicine, 0693 University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0693, USA
[b] Department of Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0693, USA
[c] Department of Bioengineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0693, USA

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ABSTRACT

Recently, binding of 5-androsten-3β,17β-diol (Δ^5-androstenediol) to human estrogen receptor-beta (ERβ) was found to repress microglia-mediated inflammation, which is associated with various neurodegenerative diseases, such as multiple sclerosis. In contrast, binding of estradiol to ERβ resulted in little or no repression of microglia-mediated inflammation. Binding of Δ^5-androstenediol to ERβ, as well as to ERα, is unexpected because unlike estradiol, Δ^5-androstenediol has a saturated A ring and a C19 methyl group. To begin to elucidate the interaction of Δ^5-androstenediol with both ERs, we constructed 3D models of Δ^5-androstenediol with human ERα and ERβ for comparison with the crystal structures of estradiol in ERα and ERβ. Conformational flexibility in human ERα and ERβ accommodates the C19 methyl on Δ^5-androstenediol. This conformational flexibility may be relevant for binding of other Δ^5-steroids with C19 methyl substituents, such as 25-hydroxycholesterol and 27-hydroxycholesterol, to ERs.

1. Introduction

The physiological actions of estradiol (E2) and other vertebrate steroids, e.g. aldosterone, cortisol, progesterone and testosterone, are mediated by nuclear receptors, a large and diverse group of transcription factors that arose in multicellular animals [1–7]. In mammals, E2 acts through two estrogen receptors (ERs), ERα and ERβ [8], which have about 60% amino acid identity in their estrogen-binding domain. Although E2 is the canonical ligand for these two ERs, other ligands, such as 5-androsten-3β,17β-diol (Δ^5-androstenediol), and 5α-androstane-3β,17β-diol (5α-androstanediol) have nM affinity for ERα and ERβ [8,9], which is surprising because both ligands have important structural differences with E2 (Fig. 1). First, Δ^5-androstenediol and 5α-androstanediol lack a phenolic A ring that is found in E2. The saturated A ring and its 3β hydroxyl on Δ^5-androstenediol and 5α-androstanediol have different chemical properties than the phenolic A ring in E2. Second, both Δ^5-androstenediol and 5α-androstanediol contain a C19 methyl group, which is absent in E2. The C19 methyl group would be expected to interfere with a close packing of the A ring on E2 in ERα and ERβ [5,10–12]. In addition, Δ^5-androstenediol has an unsaturated C5–C6 bond that is lacking in E2 and 5α-androstanediol.

Nevertheless, there is evidence that transcriptional activation of ERβ by 5α-androstanediol and Δ^5-androstenediol is physiologically important [13–15]. Interest in Δ^5-androstenediol has increased due to the recent report [15] that activation of human ERβ in microglia by Δ^5-androstenediol, which is synthesized in brain microglia [16,17], inhibits inflammation by recruitment of the corepressor C-terminal binding protein (CtBP) to the steroid–ERβ complex. This complex binds to activation protein-1 (AP-1) dependent promoters, inhibiting inflammation. Inflammation mediated by microglia is important in a variety of diseases [18,19] including multiple sclerosis [20–22]. Unlike Δ^5-androstenediol, E2 is not a potent inducer of the ERβ/CtBP pathway for repression of inflammation in brain microglia, and 5α-androstanediol has weak activity [15]. These differences motivated us to construct 3D models of human ERα and ERβ complexed with Δ^5-androstenediol for comparison with the crystal structures of E2 in both ERs, with the goal of understanding how, despite the presence of a C19 methyl group in Δ^5-androstenediol, it binds to human ERα and ERβ. Our 3D models indicate that there is flexibility in ERα and ERβ, which allows conformational changes that provide stabilizing van der Waals contacts with the C19-methyl group on Δ^5-androstenediol.

2. Methods

2.1. Construction of 3D model of Δ^5-androstenediol in human ERβ

Previously we used the Biopolymer and Discover 3 options in Insight II to construct 3D models of the steroid-binding domain of human ERα with Δ^5-androstenediol [23]. An obstacle to constructing a 3D model of Δ^5-androstenediol in ERβ is the lack of complete crystal structures of human ERβ in the Protein Data Bank (PDB). The one crystal structure of human ERβ with E2 (PDB: 3OLS)

* Corresponding author. Tel.: +1 858 534 8317; fax: +1 858 822 0873.
E-mail address: mbaker@ucsd.edu (M.E. Baker).

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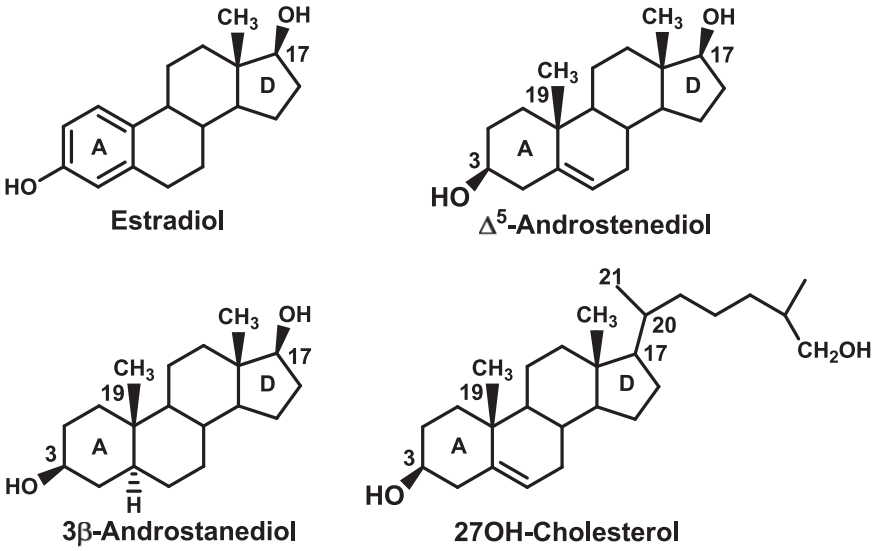

Fig. 1. Structures of E2, Δ^5-androstenediol, 5α-androstanediol and 27-hydroxycholesterol. Despite differences in their structures, all of these chemicals and 25-hydroxycholesterol bind to human ERα and ERβ [8,9,47–49]. E2 has an aromatic A ring and a phenolic hydroxyl at C3. Δ^5-Androstenediol, 5α-androstanediol, 27-hydroxycholesterol and 25-hydroxycholesterol have a saturated A ring, a 3β-hydroxyl, as well as a C19 methyl substituent. Δ^5-Androstenediol, 25-hydroxycholesterol and 27-hydroxycholesterol also have an unsaturated bond between C5 and C6. Although Δ^5-androstenediol and 5α-androstanediol have similar affinities for ERβ, it is Δ^5-androstenediol that forms the most biologically active complex with ERβ and CtBP to bind to AP-1-dependent promoters and inhibit inflammation in brain microglia [15].

[24] lacks coordinates for five amino acids corresponding to residues 416–420. Moreover, the coordinates for these residues are not found in other ERβ structures in the PDB. Alignment of the ligand-binding domains on human ERα and ERβ reveals that these five amino acids are in a loop that does not directly contact E2 (Fig. 2). Nevertheless, their absence in 3OLS could influence the conformation of Δ^5-androstenediol in ERβ. Thus, for the analysis of Δ^5-androstenediol in human ERβ, we first constructed a full 3D model of human ERβ using the Homology option in Insight II with 3OLS and human ERα (PDB: 1G50) [25] as templates. The structure of 1G50 is similar to that of crystallized ERs. The root mean square deviation (RMSD) for the Cα atoms between 1G50 and 1ERE [10] and 1GWR [26] is 0.42 and 0.53 Å, respectively. The RMSD between 1G50 and 3OLS is 1.0 Å. This indicates that 1G50 is a good template for modeling residues 416–420 in human ERβ. A PDB file of the complete ERβ with E2 was refined with Discover 3 with the CVFF force field and a distant dependent dielectric constant of 2 for 50 iterations. We superimposed the 3D structure of ERβ with E2 (PDB: 3OLS) and the energy minimized 3D model of ERβ with E2, and found that the two ERβs had an RMSD of 0.3 Å between their Cα atoms.

Then the Biopolymer option in Insight II was used to convert E2 to Δ^5-androstenediol in ERβ and ERα (1G50). The 3D models of ERα and ERβ with Δ^5-androstenediol were refined through energy minimization with Discover 3 for 10,000 iterations using the CVFF force field, with a distant dependent dielectric constant of 2.

2.2. Docking of Δ^5-androstenediol in human ERα and human ERβ

AutoDock 4 [27,28] was used to dock Δ^5-androstenediol into human ERα and ERβ with the grid centered over the estrogen binding site in ERα and ERβ. AutoDock 4 was run using the Lamarckian Genetic Algorithm with 250 individuals and 25 million energy evaluations. The 100 poses with the lowest energy for Δ^5-androstenediol in human ERα and ERβ were collected for analysis.

3. Results

3.1. Docking of Δ^5-androstenediol to ERα and ERβ

Our previous analysis assumed that the A, B, C and D rings of Δ^5-androstenediol and E2 had the same overall orientation in ERα [23]. However, it is possible that Δ^5-androstenediol has different orientations in ERα and ERβ, as has been found for some ligands [29,30]. That is, Δ^5-androstenediol may flip so that the D ring occupies the region in either ERα or ERβ that is occupied by the A ring of E2, in which case Δ^5-androstenediol assumes an orientation in ERα and ERβ that is opposite to that of E2. To investigate this and other possibilities, we used AutoDock 4 [27,28] to dock Δ^5-androstenediol into ERα and ERβ. The lowest binding energy of Δ^5-androstenediol in ERα was −10.8 kcal/mol (Ki = 12.6 nM) and in ERβ was −11.4 kcal/mol (Ki = 4.8 nM). This docking preference of Δ^5-androstenediol for human ERβ compared to ERα is in agreement with binding studies of Kuiper et al. [8]. The orientation of Δ^5-androstenediol in human ERβ was the same as E2 in all 100 poses, and Δ^5-androstenediol had 76 poses in ERα that were similar to E2 in ERα. In the other 24 poses for Δ^5-androstenediol in ERα, the binding energy was −10.3 kcal/mol (Ki = 29 nM). In these poses, compared to E2, the C3 and C17 positions are reversed and they flip so that both hydroxyls on Δ^5-androstenediol have a different configuration, which does not favor binding to ERα (Supplement Fig. 1). Based on the docking data, we reasoned that the overall orientation of Δ^5-androstenediol upon binding to ERα and ERβ corresponds to that of E2.

3.2. 3D model of Δ^5-androstenediol in human ERα

Fig. 2A and B shows Δ^5-androstenediol and E2, respectively, in human ERα. As described previously [23], Δ^5-androstenediol has stabilizing contacts with Glu-353, Arg-394, Phe-404 and His-524 in human ERα (Fig. 3A) that are similar to that of E2 with human ERα [10,26,31,32] (Fig. 3B). In addition, Met-343 and Met-421 stabilize the D ring in Δ^5-androstenediol (Fig. 3A) and E2 (Fig. 3B),