

How estrogen-specific proteins discriminate estrogens from androgens: a common steroid binding site architecture¹

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SPECIFIC AIMS

The aim of this work was to analyze the structural features of estrogen-specific proteins that underlie the ability to discriminate various ligands from different steroid hormone classes. We have demonstrated the presence of the same overall ligand binding pocket architecture in these proteins, which strongly suggests that such a structural arrangement resulted from a convergent evolution.

1. Human estrogenic 17 β -hydroxysteroid dehydrogenase type 1 and estrogen receptor α

Human estrogenic 17 β -hydroxysteroid dehydrogenase (17 β -HSD1) (EC 1.1.1.62) is an enzyme involved in the biosynthesis and metabolism of steroid hormones. Kinetic experiments performed on 17 β -HSD1 showed that 17 β -HSD1's specificity (k_{cat}/K_m) dropped several thousand times when androgens were used as substrates instead of estrogens. To further understand the C18 vs. C19 steroid discrimination mechanism of this enzyme, we recently showed that 3 β reduction of dihydrotestosterone (DHT) and 17 β oxidation of DHT are catalyzed and share similar K_m values, demonstrating for the first time the ability of 17 β -HSD1 to bind DHT (C19 steroid) in alternative binding modes. Resolution of the 17 β -HSD1–testosterone binary complex confirmed that C19 steroids can bind preferentially in the reverse binding mode. A previous crystallographic study suggested that a leucine residue (Leu149) is involved in the estrogen/androgen discrimination by human estrogenic 17 β -HSD1. This leucine residue present in the steroid binding site and positioned toward the steroid's β -face, together with an opposing valine (Val225) positioned toward the α -face of the steroid, form a sandwich-like structure between which the steroid A-ring is fixed, preventing favorable binding of C19 steroids.

17 β -HSD1 is not the only protein that specifically binds estrogens and for which a sandwich-like structural arrangement has been identified. Similar to the E₂ binding to 17 β -HSD1, E₂ and raloxifen bind to the human estrogen receptor α (ER α) through a combination of specific hydrogen bonds and a complementarity

of the binding cavity to the steroid's nonpolar character. A sandwich between the side chains of the residues Ala350 and Leu387 on the steroid's β -face and Phe404 on its α -face has been described, and it has been suggested that this structural arrangement could impose a requirement on effective ligands to contain an aromatic ring. Moreover, as observed in 17 β -HSD1, both extremities of the steroids establish direct hydrogen bonds with the protein. Mutagenesis studies have shown that in ER α , the residue Glu353 plays an important role in the binding of the A-ring phenolic group of estradiol and in receptor discrimination between estrogens and androgens. As observed in the ER α -E₂ complex, in the available structures of the wild-type 17 β -HSD1 purified from human placenta (PDB entry codes 1JTV, 1IOL, 3DHE) the side chain of the Glu282 residue turns toward the hydroxyl group of the ligand, making a strong hydrogen bond with the steroid. We thus propose that the residue Glu282 of the human placental 17 β -HSD1 plays a similar role to the one played by Glu353 in ER α regarding recognition and discrimination of C18 vs. C19 steroids. Going further in the comparison of the steroid binding site in 17 β -HSD1 and ER α , we have identified that the overall architecture of the binding pocket is conserved in both proteins. Most residues implicated in the recognition and discrimination mechanism of the steroid are present in both structures and are conserved in the other known structures of ER (ER α from rat and human ER β): a glutamic (acceptor) and a histidine (or arginine) residue (donor) that interacts with the O3 of the steroid, a histidine (or a serine) residue (donor) interacting with the O17, a leucine and a phenylalanine residue forming the "sandwich" around the A-ring of the steroid, and finally a leucine stabilizing the C18 methyl group (**Fig. 1**).

¹ To read the full text of this article, go to <http://www.fasebj.org/cgi/doi/10.1096/fj.02-0524fje>; doi: 10.1096/fj.02-0524fje

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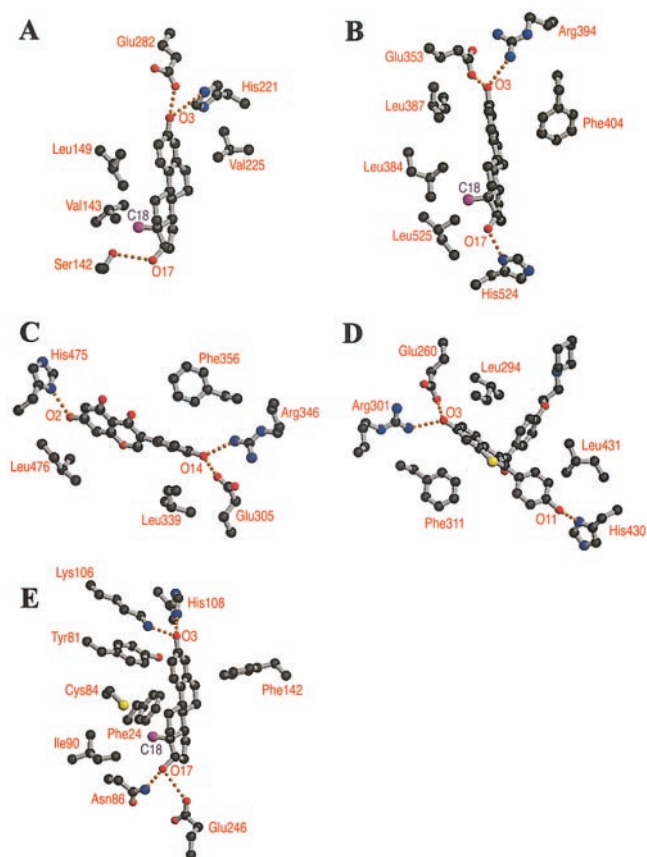


Figure 1. Comparison of the ligand binding pocket architecture in estrogen-specific proteins. *A*) 17β-HSD1-E₂ complex, *B*) human ERα-E₂ complex, *C*) human ERβ-genistein complex, *D*) rat ERβ-raloxifen complex, and *E*) mouse estrogen sulfotransferase (EST)/E₂ complex.

2. Estrogen sulfotransferase

We also analyzed in detail the structure of estrogen sulfotransferase (EST) to verify whether such structural elements are common to all the estrogen-specific proteins. We noticed that, similar to the two other estro-

gen-specific proteins, a glutamic residue (Glu246, proton acceptor) localized at the entrance to the substrate pocket with a proton donor (Asn86) interacts with one steroid end while two proton donors (His108 and Lys106) interact with the other steroid end. On the other hand, three residues interact with the C18-methyl group (Phe24, Cys84, and Ile90). Analysis of the atomic structures of these proteins (part of a large family of proteins responsible for estrogen metabolism) reveals an evolutionary relationship among them that cannot be deduced from the primary sequences of amino acids.

3. Antibodies

The above-mentioned architecture is found to be conserved in four estrogen antibodies for which structures have been determined: anti-estrogen-glucuronide fragment Fv4155 in complex with estriol 3-(β-D-glucuronide) (E13G) and estrone β-D-glucuronide (E3G); anti-estradiol Fab fragment in complex with 17β-estradiol; anti-estradiol monoclonal antibodies 10G6D6 (in complex with estradiol-6-CMO); and 17E12E5 (in complex with estradiol). In these complexes, a similar structural arrangement is found: strong hydrogen bonds with both ligand extremities, at least one hydrophobic residue interacting with the C18, and a sandwich arrangement discriminating the C19.

CONCLUSIONS AND SIGNIFICANCE

We have shown that in all the estrogen-specific proteins for which an atomic structure has been solved, a common steroid binding pocket architecture is found. Such architecture is characterized by the steroid orientation with hydrogen bonding interactions at both the O3 and O17 ends, C19 discrimination by a conserved sandwich like structure, and C18 recognition by several residues. As these different estrogen-specific proteins are not related in overall sequence (10% to 13% of

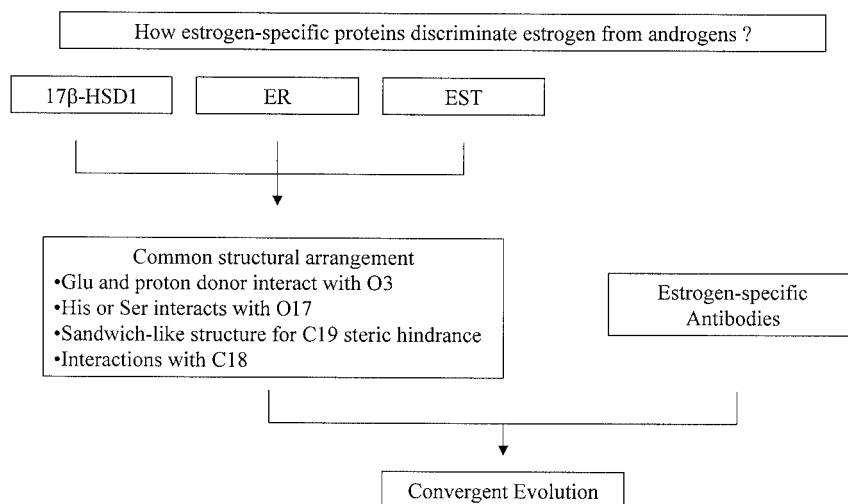


Figure 2. Schematic diagram.

amino acid sequence homology), the inference is that the steroid binding site in these proteins has originated by convergent evolution. We anticipate that this architecture will be present in all the macromolecules that preferentially bind estrogens such as 17 β -HSD type 7, estrogen sulfatase, UGT1A6, and others. We are confi-

dent that our data give valuable insights into the recognition and discrimination mechanism achieved by estrogen-specific proteins and provide the basis for the structure-based design of improved agonists and antagonists in the treatment of estrogen-related diseases. **FJ**