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3D-structure of human estrogenic 17β-HSD1: binding with various steroids ☆

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Abstract

Human estrogenic dehydrogenase (17β-HSD1) catalyses the last step in the biosynthesis of the active estrogens that stimulate the proliferation of breast cancer cells. While the primary substrate for the enzyme is estrone, the enzyme has some activity for the non-estrogenic substrates. To better understand the structure–function relationships of 17β-HSD1 and to provide a better ground for the design of inhibitors, we have determined the crystal structures of 17β-HSD1 in complex with different steroids.

The structure of the complex of estradiol with the enzyme determined previously (Azzi et al., Nature Structural Biology 3, 665–668) showed that the narrow active site was highly complementary to the substrate. The substrate specificity is due to a combination of hydrogen bonding and hydrophobic interactions between the steroid and the enzyme binding pocket. We have now determined structures of 17β-HSD1 in complex with dihydrotestosterone and 20α-OH-progesterone. In the case of the C19 androgen, several residues within the enzyme active site make some small adjustments to accommodate the increased bulk of the substrate. In addition, the C19 steroids bind in a slightly different position from estradiol with shifts in positions of up to 1.4 Å. The altered binding position avoids unfavorable steric interactions between Leu 149 and the C19 methyl group (Han et al., unpublished). The known kinetic parameters for these substrates can be rationalized in light of the structures presented. These results give evidence for the structural basis of steroid recognition by 17β-HSD1 and throw light on the design of new inhibitors for this pivotal steroid enzyme.

1. Introduction

The estrogenic 17β-hydroxysteroid dehydrogenase (17β-HSD1) is a critical enzyme in endocrinology, catalyzing the last step in the biosynthesis of estradiol (E_2), the most potent estrogen, as well as of 5-androstene-3β, 17β-diol, which possesses estrogenic activity [1]. These estrogens stimulate the proliferation of breast cancer cells. The structure function relationships and inhibition of the enzyme have been studied for decades [3–8], however two important disadvantages that have virtually eliminated the therapeutic use of existing inhibitors are their low selectivity and unsuitable estrogenic activity. Of the antiestrogens commonly used in breast cancer therapy, tamoxifen possesses a mixed agonist–antagonist activity that limits its therapeutic potential. In our laboratory of molecular endocrinology, a new series of estradiol derivatives exhibiting both antiestrogenic and 17β-HSD inhibitory activities have recently been synthesized [2,9,10]. However, the K_i of the best inhibitory compound EM139 in these dual site inhibitors is only about 6 μM.

To understand the structure–function relationships and to assist in the inhibitor design of this enzyme, we crystallized 17β-HSD1 and determined the enzyme's structure, the first example of any mammalian steroid-converting enzymes [12,13]. We further determined the structure of the complex between 17β-HSD and estradiol [14].

While 17β-HSD1 is thought to be specific for estrogenic substrates, kinetic data [11] showed that there is

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Fig. 1. A combination of seeding and co-crystallization for 17β-HSD-20α-OH-P: The single crystals of apoenzyme were moved to drops with lower concentrations of PEG and protein than in the original drop. The steroid 20α-OH-P was added to a final concentration of 1 mM in the new drop. The single crystal grew to a typical size of $0.80 \times 0.64 \times 0.18$ mm in 4 weeks.

some ability of this enzyme to bind and use other non-cognate substrates. To further explore the substrate specificity and to get hints for rational design of new inhibitors, we have crystallized various enzyme–steroid complexes. Here we compare the structures and steroid–enzyme interactions of 17β-HSD1 complexes with a cognate estrogen (E_2), an androgen (DHT, i.e., dihydrotestosterone) and a progesterone (20α-OH-P, i.e., 20α-OH-progesterone). The complete structures of these complexes will be published elsewhere.

2. Crystallization of 17β-HSD1 complexes

2.1. Co-crystallization

To accommodate for the low aqueous solubility of steroids, a special method of co-crystallization was applied to the 17β-HSD1-E_2 complex [15]. The complex sample at stoichiometry was prepared at a much higher concentration than the solubility of the free substrate using a gradual concentration of the enzyme–substrate mixture starting with diluted sol-

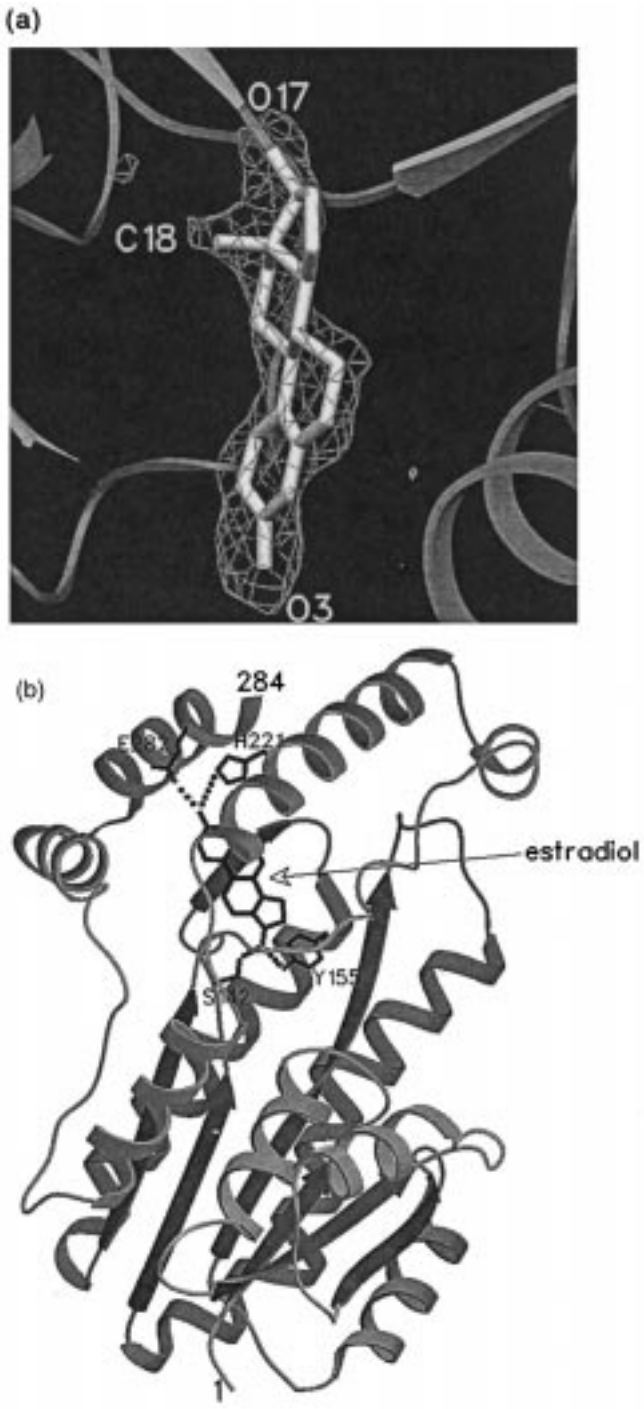

Fig. 2. (A) Electronic density from F_0-F_c difference Fourier map for the active site region of the 17β-HSD1-estradiol structure, omitting the steroid molecule from the calculation of F_c and the phases. The electron density was contoured at 2σ and the refined structure is superimposed. (B) Overall view of 17β-HSD1 monomer from the 17β-HSD1-E2 structure. Alpha helices and beta sheets are presented in light and dark gray respectively. Also shown are the hydrogen bonding interactions between the substrate and residues Tyr155, Ser142, His221 and Glu282 which are believed to be important determinates in substrate binding.

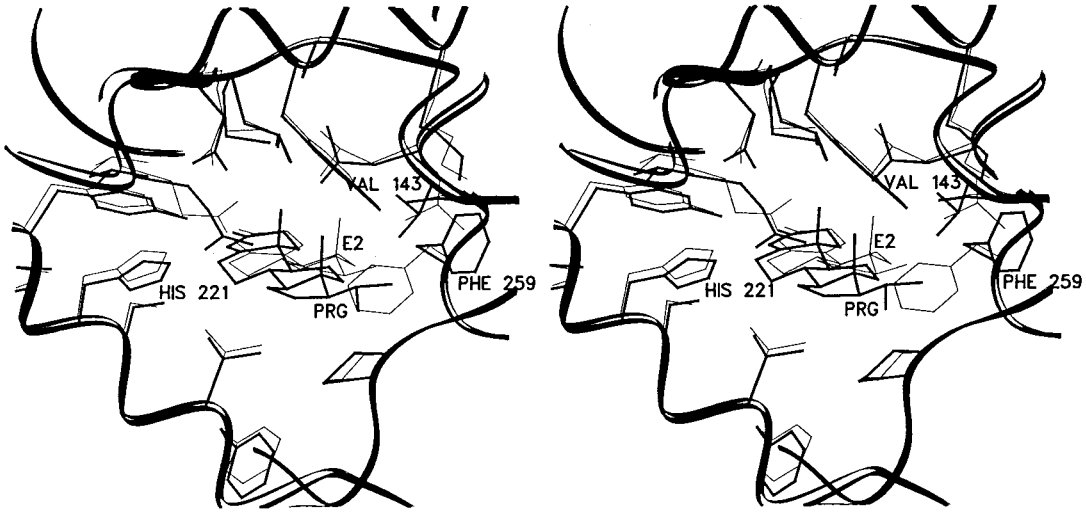

Fig. 3. Stereoview of E_2 (thin lines) and 20α-OH-P complexes (thick lines) with 17β-HSD1: looking towards the C17 of the steroid.

utions. In the course of such a procedure, we observed that the total estradiol concentration in the solution could be higher than its solubility, due to the progressive binding of the substrate to the enzyme molecules. This binding may result from the engagement of estradiol molecule in the hydrophobic binding pocket of the enzyme, while permitting a constant solubility for the free steroid molecules in solution. The high quality of the electronic density map of estradiol supports the full occupancy of the binding site, thus confirming the efficiency of the complex preparation (Fig. 2A). The crystallization of 17β-HSD1-DHT was carried out using the same method, though DHT is a non-cognate substrate for the enzyme (Han et al., unpublished). This method will also be useful in crystallizing other steroid–dehydrogenase complexes, especially when low affinity steroid ligands are concerned.

2.2. Combination of seeding and co-crystallization

To crystallize more steroid complexes with 17β-HSD1, we developed a combined method of seeding and co-crystallization (Zhu et al., unpublished). The apoenzyme crystals are first grown using established conditions [16]. Single crystals of apoenzyme grew to a modest size (~0.2 × 0.16 × 0.06 mm) in 2–3 weeks. Then some of them were removed to reservoirs with lower precipitant and protein concentrations, under which new nucleation was hindered. Steroids, the 20α-OH-P in this case, were added to a final concentration of 1 mM in the presence of PEG. The crystals continued to grow to a typical size of 0.8 × 0.6 × 0.2 mm (Fig. 1). In fact, the small crystals of apoenzyme continued to grow in the presence of the steroid, constituting a combination of seeding and co-crystallization. The final crystals were about 50 times in volume than the starting seeds of apoenzyme. This provides an efficient way to grow crystals of various 17β-HSD1 complexes, as crystal seeds of apoenzyme from the same drop can be used for different complexes without further adapting the original conditions for crystallization.

3. 3D-structures of various 17β-HSD1-steroid complexes: structure–function relationships

3.1. 17β-HSD1 structure with the cognate substrate

The previously solved structure of the 17β-HSD1-E_2 complex showed that the steroid binding site was composed of a primarily hydrophobic tunnel in which E_2 is deeply buried. This tunnel contains residues Ser 142, Val 143, Lys 149, Gly 185, Pro 187, Tyr 218, His 221, Ser 222, Phe 226, Phe 259, Met 279, and Glu 282. Four hydrogen bonds were seen in that structure: two from the O3 of E_2 to His 221 and Glu 282 and two from the O17 of E_2 to Ser 142 and Tyr 155. These hydrogen bonds appear to help hold that steroid in an optimum orientation for reaction with the nicotinamide cofactor and Tyr 155 has also been implicated as acting as an acid-catalyst for the hydride transfer reaction [13]. The hydrophobic portion of the binding site interacts mostly with the A and B rings of the steroid with a high degree of complementarity. This tight com-

plementarity raised the question of how 17β-HSD1 could bind androgens and progesterones.

3.2. 17β-HSD1 structures with an androgen and a progesterone: comparison with the cognate complex of estrogen

We have recently solved the structures of the complexes of DHT and 20α-OH-P with 17β-HSD1 at 2.2 Å with R-factors of 19 and 20% respectively, using the 17β-HSD1-E_2 structure as a starting point. These new structures show that, indeed, 17β-HSD1 is capable of binding these non-cognate substrates in much the same manner as it binds estradiol. Both DHT and 20α-OH-P bind in similar orientations as E_2, though with some differences so as to accommodate for the extra bulk of these substrates.

The steroid-binding region of the active site is very well defined in the complex of 17β-HSD1 with estradiol. Most of the side chains of the aminoacids in this region tend to have relatively low B-factors, indicating a low degree of flexibility. The structures of the complexes of 20α-OH-P and DHT with 17β-HSD1 similarly have well defined steroid-binding sites and the structure of the enzyme is little changed from the E_2-complex (Fig. 3). The RMS difference between the E_2-complex and the 20α-OH-P complex is 0.42 Å for the alpha-carbons of 276 amino acids (excluding the flexible loop formed by residues 191–198 and the C-terminus). The only significant differences in the structure of the enzyme between the E_2- and 20 α-OH-P-complexes (Fig. 3) is a rotation of the chi 1 angle of Phe 259 by about 120 degrees and the rotation of the chi 1 angle of Val 143 by about 90 degrees. Phe 259 appears to be relatively flexible even in the E_2-complex as suggested by its relatively high B-factors, while the side chain atoms of Val 143 have B-factors around the average for the whole structure. These two changes produce a somewhat more open active site and reduce the favorable hydrophobic interactions that the steroid can make with the enzyme. The active site of the DHT-complex also contains some changes in residues Val 143, Phe 226 and Phe 259, but the differences are smaller than for the 20α-OH-P-complex.

The positions of the steroids in the DHT and 20α-OH-P complexes overlap considerably with the position of E_2 in the E_2-complex. Fig. 3 shows the E_2 and 20α-OH-P structures superimposed. In the 20α-OH-P complex, the steroid is shifted 0.4 Å at the O3 end and 1.2 Å at the C17 end, while in the DHT-complex, the O3 end of the steroid is shifted 1.4 Å, and the O17 end is only shifted 0.3 Å.

The shifts of the steroids in the DHT- and 20α-OH-P-complexes relative to the E_2 position are in agreement with kinetic parameters reported previously for these steroids [11]. In that report, the K_m values for DHT and 20α-OH-P are 17 and 30 times higher than E_2, respectively, while the specific activities are 26 and 90 times lower, respectively. The two non-cognate substrates are hindered in binding by the interaction of their C19 methyl group with the side chain of Leu 149. The lack of flexibility of this part of the active site has forced the steroids to shift its position so as to accommodate the increased steric bulk of the C19 group. The 20α-hydroxy group of 20α-OH-P does not make any evident hydrogen bonding interactions, unlike the O17 hydroxyls of E_2 and of DHT, thus further decreasing the favourable binding interactions of this poor substrate. The electron density for these non-cognate substrates is weaker than for E_2, suggesting that they may be bound with somewhat lower occupancy. These results could be correlated to the increased K_m values for DHT and 20α-OH-P.

The shift in position of the C17 end of the steroid might explain a decreased specific activity as the geometry for transfer of a hydride from the nicotinamide cofactor to C17 would be less favorable. In addition, the site of transfer for 20α-OH-P is not at the C17, but at C20, which is 1.6 Å from the position of the C17 in the E_2-complex. This larger shift of the site of hydride transfer in the case of 20α-OH-P than for DHT corresponds to its larger decrease in specific activity.

These results show that the enzyme is not sufficiently flexible to enable binding of C19 and C21 steroids in such a manner as to allow good binding and a high reaction rate. Similarly, they suggest strongly that in order to maximize binding, an inhibitor designed to be specific for 17β-HSD1 must mimic the planar shape of the A-ring of E_2. It should also maintain a large hydrophobic core that can interact with the steroid binding site of the enzyme.

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