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Estrogenic 17 β -hydroxysteroid dehydrogenase may promote tumour growth in some breast carcinomas. The 2.3 Å structure of the human enzyme complexed with 17 β -estradiol gives insight into the specificity of substrate binding.

Sir—Human estrogenic 17 β -hydroxysteroid dehydrogenase (17 β -HSD1) plays a pivotal role in the formation of active estrogens in gonadal and peripheral tissues¹. This enzyme converts estrone to 17 β -estradiol—the most active estrogen—in the presence of the cofactor NADPH or NADH. Because estradiol is a mitogenic factor towards breast epithelium, the interconversion of estrone to estradiol plays a crucial role in stimulating the growth of hormone-dependent breast carcinomas. It has been shown that this enzyme is expressed in breast cancer tissue² and is responsible for maintaining the high intracellular estradiol concentration detected therein³. Suppression of the 17 β -HSD1 activity

may provide a means of reducing tumour estrogen levels and promoting tumour regression⁴.

The human 17 β -HSD1 is a homodimer, each subunit having a molecular mass of 34,500 M_r ⁵. It belongs to the short chain dehydrogenase family⁶ bearing the highly conserved and catalytically crucial Tyr-X-X-X-Lys sequence in the active site⁷. In addition to its activity towards the interconversion of estrone and estradiol this enzyme also shows some activity for the interconversion of dihydroepiandrosterone (DHEA) and Δ^3 -diol, and of progesterone to 20 α -hydroxy-4-pregnen-3-one⁸ but shows no detectable activity for the interconversion of androstenedione

and testosterone (V. Luu-The, personal communication). In the recent structure of 17 β -HSD1 from crystals grown in the presence of saturating NADP⁺ (ref. 9), the electron density corresponding to the cofactor was not reliably identified but the location of the cofactor and estradiol were inferred from molecular modelling. Here we report the 2.3 Å resolution crystal structure of 17 β -HSD1 complexed with estradiol (17 β -HSD1-E2) and describe the important intermolecular interactions relevant to the catalytic mechanism and substrate specificity.

Data collection and refinement statistics are presented in Table 1. The overall structure of 17 β -HSD1 in the complex is essentially the same as the native enzyme structure⁹ (r.m.s.d. of 0.5 Å for all main-chain atoms) except for the segment between residues 192 and 207. No electron density was observed for the 43 C-terminal residues in either structure. The weak electron density for residues 192–207 in both structures preclude accurate refinement of their position. While these residues are near the active site, the rigidly fixed end points prevent the residues in the flexible region from making close interactions with the substrate. The weak electron density reflects a high degree of flexibility of this segment that may permit access of ligands to the binding site. From previous kinetic studies, it has been found that the binding of

Table 1 Data collection, structural and refinement statistics

Unit cell dimensions	a=123.66 Å, b=45.19 Å, c=61.30 Å β =99.1°, α = γ =90.0°									
Space group	C2									
Reflections	35875									
Redundancy	2.37									
Unique reflections with $F > 2\sigma F$	11865									
R_{merge}	0.061									
Resolution range	8.0–2.3									
Resolution (Å)	8–4.37	3.57	3.15	2.88	2.68	2.52	2.40	2.30	overall	
Completeness	93.9	93.4	91.1	86.3	80.2	71.5	67.8	59.6	80.6	
R-value	20.7	16.3	17.3	20.2	21.3	22.7	22.7	25.1	19.6	
Free R-value	29.2	21.4	20.4	28.8	24.1	29.7	31.7	29.4	25.7	
R.m.s. deviation from										
ideal bond length (Å)	0.007									
ideal bond angles	1.53°									
improper angles	1.17°									
dihedrals	23.09°									
Estimated average coordinate error (Luzzati ²²) (Å)	0.29									

correspondence

Table 2 Interactions of 17 β -estradiol with 17 β -HSD1

Type of interaction	Ligand parts	Number of interactions	Ligands atom	17 β -HSD contact residues and atoms	Distance (\AA)
A. Van der Waals interactions¹:					
A. Van der Waals interactions ¹ :	Ring A	7	C1	Phe 259 C ζ	3.6
				Phe 259 C ϵ 1	3.1
				Phe 259 C δ 1	3.8
			C2	Phe 259 C ϵ 1	3.4
				Phe 259 C δ 2	4.0
				His 221 C ϵ 1	3.5
	Ring B	5	C6	His 221 C δ 1	4.1
				Tyr 218 O	3.6
			C7	Ser 222 O γ	4.0
				Ser 222 O δ	4.0
				Tyr 218 O	3.9
	Ring C	6	C8	Leu 149 C δ 1	4.0
			C11	Val 143 C γ 1	3.1
				Val 143 C γ 2	3.4
			C12	Pro 187 C δ	4.0
Pro 187 N				3.8	
Gly 186 C			3.7		
Ring D	1	C15	Gly 186 C α	3.8	
			Met 193 S δ	4.0	
Methyl	5	C18	Ser 142 O δ	3.2	
			Gly 144 C α	3.9	
			Gly 144 N	3.8	
			Tyr 155 C ϵ 1	4.0	
			Leu 149 C δ 1	3.6	
B. Hydrogen-bonds:¹					
B. Hydrogen-bonds: ¹	Ring D	2	O17	Ser 142 O	3.1
				Tyr 155 O	3.5
	Ring A	2	O3	Glu 282 O ϵ 2	2.7
				His 221 Ne2	3.1

¹Cutoffs of 3.4 \AA for hydrogen bonds and up to 4.1 \AA for van der Waals contact were used¹⁹.

cofactor and 17 β -estradiol is unordered¹⁰ so the structures of the enzyme with either estradiol or cofactor are relevant, for example. While only small changes in the structure of the enzyme are necessary to accommodate the binding of the steroid substrate, the complex structure reveals the interactions between the enzyme and the

substrate that are important for substrate specificity.

Estradiol binds close to the catalytically important residues Ser 142, Tyr 155, Lys 159, His 221 and Glu 282 (Fig 1a). The clear electron density for estradiol (Fig. 1b) is consistent with only one binding orientation. The binding site is a narrow hydrophobic tunnel showing a high degree of complementarity to the whole substrate. The total buried surface for estradiol is 229 \AA^2 , which is 92% of the surface area for free estradiol. The buried surface of 17 β -HSD is 340 \AA^2 , 71.5% of which is hydrophobic (carbon atoms). This large hydrophobic surface likely contributes the main thermodynamic force for binding. It is formed by hydrophobic and aromatic residues Val 143, Leu 149, Pro 187, His 221, Val 225, Phe 254, Phe 259, Leu 262, Leu 263, most of which are located in helices α H' (257–271), α H (274–284) and α G' (209–227). There are 24 van der Waals contacts between estradiol and 17 β -HSD, most of them involving rings A, B, C and the methyl group C18 (Table 2). In addition to the hydrophobic interactions, the 17-hydroxyl on the estradiol D ring forms hydrogen bonds with the hydroxyls of conserved Ser 142 (3.1 \AA) and Tyr 155 (3.5 \AA) and the 3-hydroxyl on the A ring forms hydrogen bonds with the Ne2 atom of His 221 (3.1 \AA)

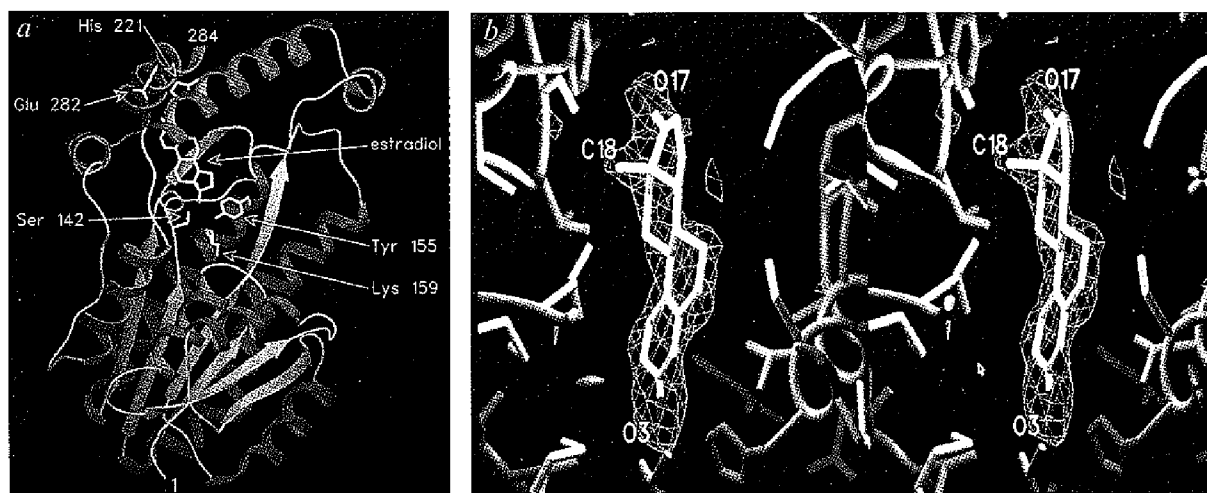


Fig. 1 a, Cartoon representation of a monomer of 17 β -HSD with 17 β -estradiol positioned at its binding site. The estradiol molecule and side chains of residues Ser 142, Tyr 155, Lys 159, His 221 and Glu 282 in the active site are shown in white. This figure and Fig. 2b were prepared using SETOR²⁰. b, Stereo view of the calculated final $|F_o| - |F_c|$ electron density omit map contoured at the 2.8 σ and the final refined model for the 17 β -estradiol molecule. This figure and Fig. 2a were generated using the program O¹⁷.

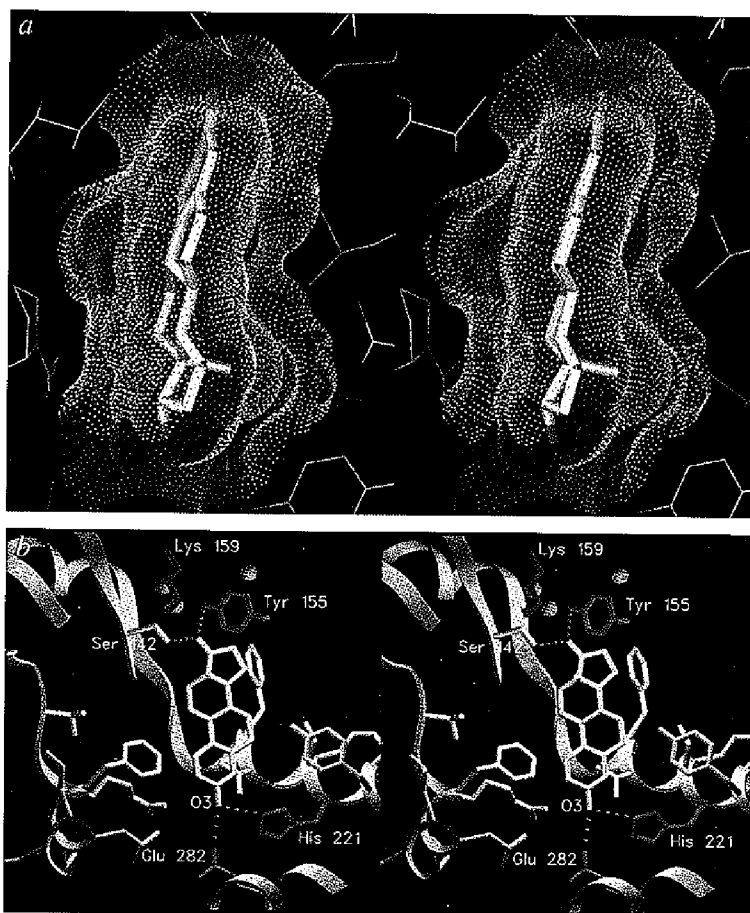


Fig. 2 *a*, Enzyme-substrate complementarity demonstrated by the buried molecular surfaces²¹ of the active site and estradiol. The surfaces were generated with the program MS²² using a probe radius of 1.4 Å. Carbons from estradiol are in cyan, carbons from the amino acid are white, and oxygens are red and nitrogens are green for both molecules. *b*, Detail of the active site stereochemistry of the estradiol bound 17β-HSD1 structure. Hydrogen bonding interactions are represented by red dotted spheres and the water molecules are in blue.

and with a carboxyl oxygen of Glu 282 (2.7 Å) (Fig. 2*a*). This is in agreement with previous observations of the importance of His 221 in catalysis, based on mutagenesis and modelling studies^{7,9}. There is a 100° rotation of the χ_2 angle of residue Glu 282 in comparison to the 17β-HSD1 native structure. The interaction of this residue with the substrate has not been reported previously.

The mechanism of the enzyme is believed to proceed through a direct transfer of a hydride ion from the C4 position of the nicotinamide nucleotide to the acceptor carbonyl C17 of estrone to produce 17β-estradiol¹¹. A proton abstracted from the bulk solvent is provided to the O17 atom to complete the reaction. It has been proposed for other related members of the short chain dehydrogenase family that the conserved tyrosine residue—Tyr 155 for 17β-HSD1—acts as a general acid/base catalyst towards the O17 atom of the substrate, and

that conserved Ser 142 and Lys 159 facilitate this through hydrogen bonding and electrostatic effects with Tyr 155 respectively^{9,12,13}. The interaction between Tyr 155 and the 17-hydroxyl is weaker than between Ser 142 and the 17-hydroxyl, but Tyr 155 is the more likely candidate for the role of acid/base catalyst, because it is only partly buried and its hydroxyl is somewhat accessible to the bulk solvent, while Ser 142 is completely buried by the substrate, and also because the pK_a of Tyr 155 would be lower than that for Ser 142. Therefore the strong Ser 142–estradiol hydrogen bonds suggest that its role is to orient estradiol in the binding cleft. It should be mentioned that mutations of Ser 142 to a cysteine or a glycine residue lead to complete enzyme inactivation (S.-X. L., unpublished results).

There is no evidence of a hydrogen bond between the catalytically important Lys 159 and Tyr 155 in either the native structure⁹ or the

estradiol-bound structure of 17β-HSD1, and the position of Lys 159 does not change on binding estradiol. The positive charge of the lysyl amino group may still be close enough to lower the pK_a of Tyr 155. Inano¹⁴ reported that an oxidized cofactor prevented a lysine residue from being labelled by trinitrobenzene sulphonate. These facts, together with the previously modelled position for NADP⁺ (ref. 9), suggest that Lys 159 may be primarily involved in the binding of the cofactor, since its amino group appears to be in a position to form two hydrogen-bonds with the O2' and O3' hydroxyls of the nicotinamide ribose. This is further supported by Varughese *et al.*¹³ and Tanaka *et al.*¹⁵ who have shown that the conserved lysines of dihydropteridine reductase, carbonyl reductase and 7α-hydroxysteroid dehydrogenase, members of the short-chain dehydrogenase family, form a hydrogen bond with the nicotinamide ribose. This role in cofactor binding does

not preclude the possibility that the lysine has an electrostatic effect that reduces the pK_a of Tyr 155.

The specificity for estradiol binding appears to be due to a combination of hydrogen bonding interactions and the complementarity of the hydrophobic surfaces and estradiol (Fig. 2a). The enzyme's preference for estradiol as substrate could be explained by the complementarity of the narrow cleft to the planar A ring and by the hydrogen bond between the 3-hydroxyl group of estradiol and the carboxylate of Glu 282. In addition to having non-planar A rings, testosterone and DHEA have a C19 methyl group. DHEA has the 3-hydroxyl group while testosterone has a 3-keto group. These structural features are in accord with the enzyme's substrate specificity.

Methods

To overcome the difficulties associated with the low solubility of 17 β -

estradiol, 17 β -HSD1 and estradiol were mixed at low concentration followed by gradual centrifugation (Amicon) concentration. An initial solution of 10–12 μ M protein and 25 μ M estradiol was concentrated to about 220 μ M protein. By following C^{14} -labeled estradiol, we determined that the final concentration of bound estradiol was 440 μ M indicating a stoichiometry of 2:1. The great difference between this total E2 concentration and the solubility of free estradiol (25–30 μ M) indicated the binding of the substrate into the hydrophobic binding pocket. Crystals were obtained in four weeks with 28% PEG (4000), 0.06% β -octylglucoside, 0.16 $MgCl_2$ and 0.1M Hepes pH 7.5 at room temperature. Typical crystal size is 0.15 \times 0.25 \times 0.48 mm (S.-X.L., unpublished results). Data collection was carried out on an R-Axis IIC imaging plate area detector on a Rigaku RU300 rotating anode at room temperature and the data were processed using DENZO and SCALEPACK¹⁶.

The initial electron density map was calculated using the phases and the F_{calc} magnitudes from the native

crystal structure⁹. Fitting to the electron density was performed using the program O¹⁷. All refinement was done with X-PLOR¹⁸. Water positions were determined using the program Peak (M. Cygler and M. Desrochers, personal communication) and checked in the electron density map. The final refined model contained 284 residues, 56 water molecules and the 17 β -estradiol molecule. The R-factor for this model is 0.196 in the 8.0–2.3 Å shell. The coordinates will be deposited in the Brookhaven Protein Data Bank, PDB accession code: 1IOL.

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