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Substrate specificity and inhibitor analyses of human steroid 5β-reductase (AKR1D1)

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Abstract

Human steroid 5β-reductase (Aldo-keto Reductase 1D1) catalyzes the stereospecific NADPH-dependent reduction of the C4-C5 double bond of Δ^4-ketosteroids to yield an A/B *cis*-ring junction. This *cis*-configuration is crucial for bile acid biosynthesis and plays important roles in steroid metabolism. The biochemical properties of the enzyme have not been thoroughly studied and conflicting data have been reported, partially due to the lack of highly homogeneous protein. In the present study, we systematically determined the substrate specificity of homogeneous human recombinant AKR1D1 using C18, C19, C21, and C27 Δ^4-ketosteroids and assessed the pH-rate dependence of the enzyme. Our results show that AKR1D1 proficiently reduced all the steroids tested at physiological pH, indicating AKR1D1 is the only enzyme necessary for all the 5β-steroid metabolite present in humans. Substrate inhibition was observed with C18 to C21 steroids provided that the side-chain at C17 was unsubstituted. This structure activity relationship can be explained by the existence of a small alternative substrate binding pocket revealed by the AKR1D1 crystal structure. Non-steroidal anti-inflammatory drugs which are potent inhibitors of the related AKR1C enzymes do not inhibit AKR1D1 by contrast chenodeoxycholate and ursodeoxycholate were found to be potent non-competitive inhibitors suggesting that bile-acids may regulate their own synthesis at the level of AKR1D1 inhibition.

Keywords

Bile acids; steroid hormones; substrate inhibition; non-steroidal anti-inflammatory drugs

1. Introduction

Steroid 5β-reductase activity is essential for producing 5β-steroids with varied functions in vertebrates and plants. 5β-Reduction of Δ^4-3-ketosteroids introduces a *cis*-configuration at the steroid A/B ring junction. In bile acid biosynthesis, this configuration is required for bile acids to have detergent-like properties to emulsify dietary fats and cholesterol [1]. In steroid hormone metabolism, 5β-reduction generates an array of functionally diverse active 5β-dihydrosteroids that are involved in parturition, erythropoiesis, and the induction of P450 enzymes to protect against xenobiotc insult [2–6]. 5β-Reduction also inactivates androgens,

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progestins and glucocorticoids and could regulate nuclear receptor ligand availability [7–9]. In plants, 5β-reduction produces secondary metabolites, cardenolides, as herbivore deterrents. Glycoside derivatives of cardenolides, *e.g.*, digitalis, are also used as therapeutics for congestive heart failure [10].

All known mammalian 5β-reductases belong to the aldo-keto reductase (AKR) superfamily. The enzyme utilizes NADPH as the hydride donor and stereospecifically transfers the 4-pro-*R* hydride on the nicotinamide ring to the β-face of Δ^4-3-ketosteroids at the C5 position. The first mammalian steroid 5β-reductase characterized was AKR1D2 from rat liver. A highly purified form of this enzyme exhibited activity not only for bile acid intermediates but also sex steroid hormones and glucocorticoids [11]. The broad substrate tolerance of AKR1D2 correlated to the array of 5β-reduced metabolites detected in urine, suggesting that the existence of only one isoform of 5β-reductase is required in rat.

Human 5β-reductase (AKR1D1) was later cloned by Kondo et al. It has a 79% sequence identity to the rat isoform and shares high sequence homology to other AKR members, including aldose reductases and hydroxysteroid dehydrogenases (HSDs) [12]. The structure of AKR1D1 in multiple binary and tertiary complexes has been reported [13–16]. The enzyme harbors a $(\alpha/\beta)_8$-barrel core structure typical to the AKR family where cofactor and steroid substrate sit at the C-terminal end of the β-sheets capped by three long loops [13]. Structural and sequence analyses of AKR1D1 have led to the identification a conserved catalytic tetrad (Y58, K87, E120, and D53) in the active site [12]. Based on the AKR1D1 crystal structure and previous experiments done on the rat liver 3α-HSD (AKR1C9) [13,17–19], a mechanism has been proposed for 5β-reduction, in which Y58 serves as a general acid and E120 activates the Δ^4-double bond through a superacidic hydrogen bond to the C3 carbonyl group.

Contrary to the exciting progress in the structural studies of AKR1D1, kinetic properties of this enzyme have not been thoroughly studied. Earlier studies performed by various groups regarding the substrate specificities of AKR1D1 gave conflicting results. Transient transfection of AKR1D1 cDNA into COS cells by Kondo et al. resulted in the expression of an enzyme with higher activity towards bile acid precursors, 4-cholesten-7α-ol-3-one and 4-cholesten-7α,12α-diol-3-one, than testosterone and cortisol, but no detectable turnover of progesterone and Δ^4-androstene-3,17-dione [20]. Charbonneau et al. stably transfected AKR1D1 into HEK293 cells, resulting in high activity towards progesterone and testosterone but low activity towards steroids containing 11β-hydroxy group substituents such as aldosterone and cortisol [21]. A partial purification of 5β-reductase from human liver by Iyer et al. generated a third set of substrate preference, in which testosterone was reported as an inactive substrate while aldosterone was active [22]. These discrepant results have created uncertainty in substrate preference of AKR1D1 and raised the prospect that multiple forms of 5β-reductase might be necessary to produce the panel of urinary steroid metabolites found in human.

To clarify the confusion provoked by the previous reports, we have systemically examined the substrate specificity of AKR1D1 towards bile acid precursors and Δ^4-3-ketosteroids of the C18, C19, C21, and C27 series (Figure 1) using a homogenous recombinant enzyme preparation. Furthermore, we have performed some long missing basic characterization of the enzyme including the determination of a pH-rate profile and cofactor affinity. In addition, several potent AKR1C inhibitors from non-steroidal anti-inflammatory drugs and bile acids have been evaluated for the inhibition of AKR1D1 (Figure 1).

2. Experimental

2.1. Materials

NADPH was obtained from Roche. Steroids were purchased from Steraloids, Inc. [4-^{14}C]-Testosterone (50 mCi/mmol) was obtained from PerkinElmer Life and Analytical Sciences. Indomethacin, ursodeoxycholic acid, and mefenamic acid were obtained from ICN Biomedicals Inc. 4-Benzoylbenzoic acid was obtained from Sigma-Aldrich.

2.2. Dissociation constant for the cofactor

The dissociation constant (K_d) for AKR1D1-NADPH binary complex was determined by fluorescence titration with NADPH [23]. Briefly, incremental amounts of cofactor was added to 0.16 μM AKR1D1 in 10 mM potassium phosphate (pH 6.0) in a final volume of 1.4 mL at 37 °C. The quenching of the intrinsic tryptophan fluorescence of the protein upon the addition of the cofactor was monitored on a Hitachi F-4500 fluorescence spectrophotometer ($\lambda_{excitation}$ = 295 nm, $\lambda_{emission}$ = 320–500 nm). The data was plotted as a percent change in fluorescence at 340 nm versus cofactor concentration. The data was fit to the Morrison equation [24] using the program GraFit (Erithacus Software) to determine the dissociation constant.

2.3. Product identification of 5β-reduction

The products of Δ^4-3-ketosteroid reduction were identified by thin-layer chromatography. Briefly, reactions were driven to completion in the presence of excess enzyme. Reactions contained 100 mM potassium phosphate buffer (pH 6.0), 4% acetonitrile, 0.5 mM NADPH, 30 μM steroid, and 21.2 μg/mL AKR1D1 in a final volume of 500 μL. For cholestenone reduction, 80 μM steroid and 63.6 μg/mL AKR1D1 were used. The 5β-reduced products except 5β-dihydroaldosterone were extracted twice with 1 mL ethyl acetate, vacuum-dried, redissolved in 50 μL ethyl acetate, and applied to LK6D TLC plates. For 5β-dihydroaldosterone, diethyl ether containing 0.1% triethylamine was utilized in place of ethyl acetate. Authentic synthetic standards were also applied to the same plates. The chromatograms from the reactions with cortisol and epitestosterone were developed twice in toluene/acetone (80: 20 v/v). The chromatograms from the reactions with 11-deoxycorticosterone were developed once in CH_2Cl_2/ethyl acetate (30: 70, v/v). The chromatograms from the reactions with cortisone and aldosterone were developed once in CH_2Cl_2/ethyl acetate (10: 90, v/v) containing 0.1% triethylamine. The chromatograms from the reaction with cholestenone were developed once in CH_2Cl_2/ether (110: 10, v/v). The chromatograms from the reactions with the rest of the steroids including progesterone were developed three times in CH_2Cl_2/ether (110: 10, v/v). The products were identified by co-migration with the standards.

2.4. Steady-State Kinetic Parameters

Initial velocities for AKR1D1 with the series of Δ^4-3-ketosteroids were determined using a continuous fluorimetric assay by monitoring the decrease in NADPH emission at 460 nm ($\lambda_{excitation}$ = 340 nm). Assays were performed in 100 mM potassium phosphate buffer (pH 6.0) containing 4% acetonitrile, 15 μM NADPH, and varied steroid substrate concentrations (0.60 μM – 40 μM) in a final volume of 1 mL at 37 °C. Reactions were initiated by the addition of enzyme and were monitored at 37 °C for 5 min. The initial velocity data were fit to the Henri-Michaelis-Menten equation or the following equation when substrate inhibition was observed:

$$v=\frac{V_{max}[S]}{K_m+[S]+[S]^2/K_i}$$

where K_i is the dissociation constant for substrate from the E-NADP(H) complex.

2.5. pH-Rate Studies

To determine the pH optima for the reduction of testosterone over a range of pH values, a triple buffer system containing equimolar sodium phosphate, sodium pyrophosphate, and 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid was used in place of the potassium phosphate buffer in the continuous fluorimetric assay. The triple buffer system maintained constant ionic strength over the pH range examined and ensured continuity in the pH versus velocity curves. The $k_{cat}^{apparent}$ was determined at 50 mM triple buffer with 4% acetonitrile, 15 μM NADPH, and 10 μM testosterone in a final volume of 1 mL. To ensure the pH stability of the enzyme over the time course of the assay, the enzyme was preincubated in the triple buffer system at varying pH values at 37 °C for 5 min. The remaining enzyme activity was assessed fluorimetrically by diluting the enzyme (50-fold) into 100 mM potassium phosphate buffer (pH 6.0), containing 4% acetonitrile, 15 μM NADPH, and 10 μM testosterone.

2.6. Enzyme Inhibition Studies

Inhibition studies were performed using the radiometric assay by monitoring the reduction of [4-^{14}C]-testosterone in the absence or presence of inhibitor at 37 °C [14]. A family of lines was generated at different fixed substrate concentrations by varying inhibitor concentrations. The pattern of lines was examined to identify the mode of inhibition: competitive, noncompetitive or uncompetitive. K_i values were determined by fitting the lines using GraFit.

3. Results

3.1. Cofactor Binding and K_d Determination

AKR1D1 contains five tryptophan residues and has intrinsic fluorescence at 340 nm when excited at 295 nm. Incremental addition of NADPH to the enzyme quenched the fluorescence emission signal and generated an energy transfer band at 460 nm with an isosbestic point at 405 nm. This energy transfer band likely results from the interaction of the nicotinamide ring with Trp89 based on previous experiments with AKR1C9 (rat 3α-hydroxysteroid dehydrogenases) and homology considerations [25]. The dissociation constant (K_d) of NADPH for wild-type AKR1D1 is 43 ± 16 nM. This tight binding of NADPH is a characteristic property of other steroid transforming AKRs [23,26] but is less than that seen with aldose reductase AKR1B1 (K_d = 9.0 nM) [27].

3.2. Substrate Specificity

The ability of AKR1D1 to catalyze the 5β-reduction of a variety of Δ^4-3-ketosteroids was evaluated. 5β-Reduced products were identified directly with all the C18-C27 steroid substrates except for aldosterone. Chromatograms and R_f values of the Δ^4-3-ketosteroids and their corresponding 5α- and 5β-reduced dihydrosteroinds are provided in the Supporting Information. Enzymatic aldosterone reduction produced a new product with a higher R_f value than that of aldosterone. Even though we were not able to confirm the identity of this product due to the lack of 5β-dihydroaldosterone standard, we conclude that the product is the 5β-reduced isomer based on the product profiles we observed with all other substrates.

The kinetic constants for the 5β-reduction reactions for a wide range of substrates are summarized in Table 1. The k_{cat} values for the steroid substrates ranged from 0.6 min^{-1} to 11.7 min^{-1}. Similarly large differences in K_m values were observed and these were most pronounced with the glucocorticoids, cortisol and cortisone. However, the increase in K_m was not seen with corticosterone suggesting that the presence of the 17α-hydroxyl group has a large effect on K_m. Catalytic efficiencies also varied by 30-fold. The bile acid precursor 4-cholesten-7α-ol-3-one gave a catalytic efficiency of 2.53 min^{-1} μM^{-1}, similar to that seen with other steroid hormones such as testosterone (3.12 min^{-1} μM^{-1}) [13] and corticosterone (0.87 min^{-1} μM^{-1}). Although the catalytic efficiencies obtained for 4-estren-17β-ol-3-one (0.91 min^{-1} μM^{-1}) and 1,4-androstadien-17β-ol-3-one (0.66 min^{-1} μM^{-1}) were lower [14], their turnover demonstrated that while the majority of substrates tested had an angular methyl group at C10 and one double bond in the A-ring, these were not prerequisites for 5β-reduction to proceed. Substrate inhibition was observed for all the C18, C19, and some of the C21 substrates, as described previously for testosterone [13]. There were two substrates missing from our specificity evaluation, progesterone and 11-deoxycorticosterone. Both exhibited excellent k_{cat}/K_m values as a result of their fast turnover rates (> 4 min^{-1}) with nanomolar K_m values (< 0.2 μM). However, there was also significant substrate inhibition (K_i < 2 μM) associated with the two substrates that prohibited accurate determination of either K_m or k_{cat} values.

3.3. pH Optima and Stability

AKR1D1 was stable in the pH range of 5–9 over the time course of the assay (5 min) (Figure 2, ■). These assays were performed with 10 μM testosterone which is the maximum concentration that can be used before there is observable substrate inhibition. Therefore the specific activities observed are apparent V_{max} values which can be converted to $k_{cat}^{apparent}$ values. pH-Rate studies on the reduction of testosterone showed an effect on $k_{cat}^{apparent}$ values that could not be attributed to changes in stability of the enzyme. The pH versus $k_{cat}^{apparent}$ curve exhibited two inflection points, pK_a = 5.5 and pK_b = 7.2, with an optimal pH of 6.0 (Figure 2, ▲). The depressed $k_{cat}^{apparent}$ at both extreme pH values reflected the effect of pH on one or more rate-determining steps. These studies were repeated using cortisone and gave similar results (data not shown).

3.4. Inhibition Studies

Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to inhibit the human hydroxysteroid dehydrogenases (AKR1C1-1C4) [28,29]. The ability of these compounds to inhibit the AKR1D1 dependent reduction of testosterone was measured (Table 2). Indomethacin, an AKR1C3 selective inhibitor, and mefenamic acid and 4-benzoylbenzoic acid, which are nonspecific AKR1C isoform inhibitors, were ineffective. Bile acids, e.g., chenodeoxycholic acid and its 7β-isomer ursodeoxycholic acid, are potent (nanomolar affinity) selective inhibitors of AKR1C2. These compounds were non-competitive inhibitors of AKR1D1 yielding K_i values of 3.2 μM for chenodeoxycholic acid, which is consistent with the previously reported value [30], and 9.8 μM for ursodeoxycholic acid.

4. Discussion

Our study sought to clarify properties of human 5β-reductase, especially its substrate specificity that has not been clearly addressed before. Previous work by different groups to characterize the substrate specificity of AKR1D1 has unnecessarily complicated the problem by using transfected mammalian cells or partially purified enzyme, resulting in conflicting data in substrate preference of the enzyme [20–22]. One drawback to their approach is unforeseen influences of endogenous enzymes that could metabolize either the substrates presented to the cells or the products formed. The presence of substrate inhibition by C18

and C19 Δ^4-3- ketosteroids also likely confounded these earlier attempts that chose to use one single substrate concentration for the evaluation of substrate specificity.

Using homogenous purified recombinant AKR1D1, we have found that the enzyme was capable of reducing a broad range of Δ^4-3-ketosteroids from C18 (e.g., 4-estren-17β-ol-3-one) to C27 (e.g., 4-cholesten-7α-ol-3-one). The ability of AKR1D1 to accept substrates with large size differences was expected since the active site of the enzyme does not strongly discriminate the size of the side-chain at the 17β position. The tertiary structure of AKR1D1 has shown that the steroid binding pocket of AKR1D1 resembles a cylindrical cavity lined largely by hydrophobic residues (Figure 3A) [13]. In the productive binding mode, the steroid A ring is buried deep at the bottom of the active site, while the D ring and the C17 tail protrude toward the opening at the enzyme surface. There is no hindrance from enzyme to limit the size of the 17β substituent. In fact, a long hydrophobic C17 side chain might even provide additional interaction with the enzyme as shown by cholestenone, which binds to the enzyme with the lowest K_m among the substrates tested. The ability of AKR1D1 to reduce a panel of substrates suggests that it is not necessary to invoke the existence of multiple forms of 5β-reductase, which is consistent with the clinical finding that AKR1D1 deficiency severely impaired production of all 5β-reduced steroid metabolites [31]. The reduction of 4-estren-17β-ol-3-one indicates that AKR1D1 may also play a role in the metabolism of 19-nor-Δ^4-3-ketosteroids, which is a characteristic structure found in hormone contraceptives and steroids used in hormone replacement therapy.

Changes in K_m of 50-fold and k_{cat} of 20-fold between the various substrates tested were seen, resulting in a 30-fold difference in catalytic efficiencies. The large fluctuation in k_{cat} suggests that for each reaction there may be differences in the rate-determining step. This is reminiscent of previous studies in AKR1C9 which showed that for the reduction of 5α-dihydrotestosterone the rate-limiting step was the chemical conversion [32], while for the reduction of 5α-androstan-3,17-dione the rate-limiting step was governed by product release with a minor contribution from the chemical step [26].

Substrate inhibition of AKR1D1 has been observed with C18, C19, and some of the C21 steroids. This phenomenon can be readily explained by the structure of AKR1D1 [13,15]. The same principle may be applied to elucidate the absence of substrate inhibition with longer steroids with larger side-chains. As previously reported, the steroids with short C17 substituents might be trapped in a nonproductive binding pocket that is perpendicular to the productive binding orientation with its two extremities tethered by Tyr132 and Thr224-Asn227 cluster respectively [13,15] (Figure 3B). The sides of the pocket are composed of Tyr26, Val309, Trp230, and $NADP^+$, while the length of the pocket is about 15 Å defined by Trp89 and the Thr224-Asn227 cluster. C19 steroids like testosterone and Δ^4-androstene-3,17-dione with a length around 11 Å could tightly fit into the pocket. But the C27 steroids with bulkier C17 substituents cannot be accommodated by this pocket and therefore do not inhibit the enzyme. The only exceptions are the C21 steroids, in which not all steroids elicit substrate inhibition. The fact that progesterone and 11-deoxycorticosterone have low micromolar K_i values, whereas aldosterone has a much higher K_i value and cortisol, cortisone, and corticosterone do not exhibit substrate inhibition suggests that the 11-substituent plays a role in diminishing substrate inhibition. The steric hindrance introduced by the 11-substituent against Tyr26 may prevent steroid from entering the alternative binding pocket. In the crystal structure, progesterone is bound to AKR1D1 in the productive mode. It is likely that while progesterone may adopt both binding modes, the productive binding is thermodynamically favored. Since 5β-reductase is responsible for steroid hormone inactivation, the strong substrate inhibition we observed with progesterone and 11-deoxycorticosterone may play a role in protecting downstream bioactive steroids, such as corticosterone and aldosterone, *in vivo* from inactivation. However, given the

generally low circulating concentrations of Δ^4-3-ketosteroids, substrate inhibition elicited by other substrates may not play a significant physiological role.

Besides substrate specificity, we also tested several potential inhibitors for AKR1D1. AKR1D1 was not inhibited by standard NSAID inhibitors of human AKR1C enzymes. Though AKR1D1 and AKR1C enzymes are highly homologous, the subtle differences in the active site of AKR1D1 impede inhibitor binding. For example, the Leu302-Tyr326 loop in AKR1D1 tightly packs against the steroid binding pocket regardless of substrate binding. A comparison to the tertiary structure of AKR1C3 in complex with cofactor and NSAID inhibitor [33] implies that the loop restricts the size of the active site and prevents inhibitor binding if it stays in the closed conformation. On the other hand, the primary and secondary bile acids chenodeoxycholic acid and ursodeoxycholic acid, respectively, were non-competitive inhibitors of AKR1D1. This inhibition may provide feedback inhibition in bile acid biosynthesis in the liver when concentrations of the bile acids surpass a certain level. It has also been suggested to play a role in modulation of glucocorticoid metabolism [30,34].

A part of our basic characterization of AKR1D1, we evaluated the cofactor affinity and pH-rate profile of the enzyme. AKR1D1 has a nanomolar affinity for NADPH as determined by fluorescence titration studies. This high affinity for cofactor is reminiscent of that seen with AKR1C9 and AKR1C2, where the tight enzyme-NADPH binary complexes were achieved in two isomerization steps [23,26]. In a similar manner, the release of $NADP^+$ from the tight binding AKR1D1-$NADP^+$ complex may place an upper limit on k_{cat}. The K_d is 5-fold higher than AKR1B1 (human aldose reductase), which has a salt bridge that forms a “safety belt” across the pyrophosphate contributing to the higher affinity [35]. The lower affinity of AKR1D1 for NADPH was expected as no salt bridge was observed in the tertiary structure [13]. These findings are in disagreement with Breton and coworkers [15] who reported a weaker affinity in the presence of a salt bridge. The pH-$k_{cat}^{apparent}$ profile of AKR1D1 exhibited two inflection points. Previous studies on 5β-reductase engineered by introducing a H117E mutation into AKR1C9 showed that the mutant utilizes Y58 as the general acid with a single inflection point pK_b of 6.3 in its pH- $k_{cat}^{apparent}$ profile. To assign the titratable groups in AKR1D1, a more complete pH rate profile will need to be determined using steady and transient state kinetic approaches accompanied by site-directed mutagenesis.

In conclusion, our study systemically evaluated the substrate specificity of AKR1D1 using highly purified recombinant enzyme for the first time. The broad substrate tolerance of AKR1D1 suggests that the enzyme is the only form of 5β-reductase in human, consistent with *in vivo* steroid metabolism [31]. The biochemical properties and physiological functions of AKR1D1 are still largely unexplored. Many interesting aspects of the enzyme regarding its kinetic and chemical mechanism, biological activity of 5β-reduced products, and its regulation remain to be addressed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AKR aldo-keto reductase

AKR1B1	aldose reductase
AKR1C1-1C4	human hydroxysteroid dehydrogenases
AKR1C9	rat 3α-hydroxysteroid dehydrogenase
AKR1D1	human steroid 5β-reductase
AKR1D2	rat steroid 5β-reductase
HSD	hydroxysteroid dehydrogenase
NSAID	non-steroidal anti-inflammatory drug
TLC	thin layer chromatography

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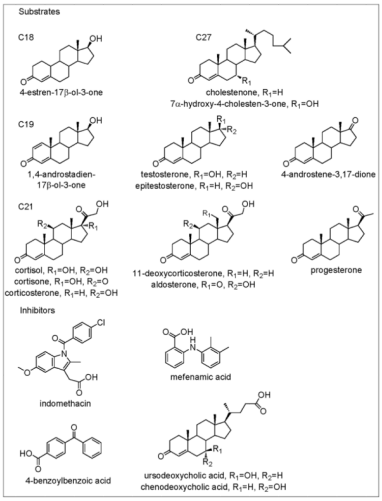

Figure 1.
Substrates and inhibitors evaluated.

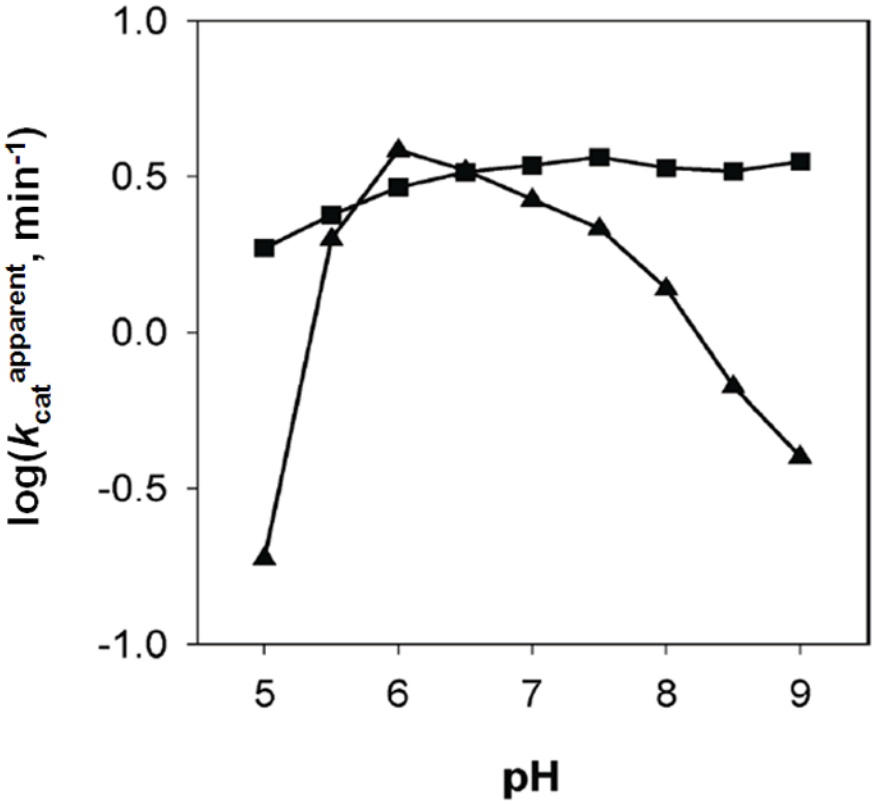

Figure 2.
pH stability and pH-rate optimal of AKR1D1. pH stability (■) and pH optima (▲) of the enzyme was tested from pH 5 to 9 using 10 μM testosterone.

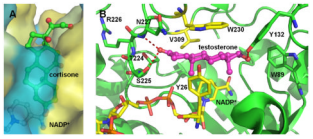

Figure 3.

The active site of AKR1D1 showing (A) the surface of the cylindrical cavity when cortisone is bound (PDB ID: 3CMF). The steroid A ring is buried in the active site, while the C17 side chain protrudes out of the cavity. The surface is colored according to hydrophobicity (yellow corresponds to hydrophobic residues; and blue corresponds to hydrophilic residues). (B) The residues defining the nonproductive binding pocket for testosterone are shown (PDB ID: 3BUR).

Table 1

Substrate specificity of AKR1D1.

Substrate	Type	k_{cat} (min^{-1})[a]	K_m (μM)	K_i (μM)	k_{cat}/K_m (min^{-1} $μM^{-1}$)
4-Estren-17β-ol-3-one	C18	2.7 ± 0.2	3.0 ± 0.5	18.6 ± 3.4	0.91
Δ^4-Androstene-3,17-dione	C19	6.0 ± 0.8	0.9 ± 0.2	4.3 ± 1.3	6.72
Testosterone[b]	C19	8.4 ± 2.0	2.7 ± 1.2	14.5 ± 5.8	3.12
Epitestosterone	C19	6.0 ± 0.7	2.9 ± 0.6	10.0 ± 2.2	2.09
1,4-Androstadien-17β-ol-3-one[c]	C19	2.1 ± 0.1	3.2 ± 0.2	N.A.	0.66
Aldosterone	C21	9.0 ± 0.6	2.5 ± 0.4	66.5 ± 15.7	3.68
Corticosterone	C21	1.9 ± 0.1	2.2 ± 0.5	N.D.	0.87
Cortisol	C21	2.7 ± 0.1	13.1 ± 1.8	N.D.	0.21
Cortisone	C21	11.7 ± 0.1	15.1 ± 0.3	N.D.	0.78
4-Cholesten-7α-ol-3-one	C27	2.0 ± 0.1	0.8 ± 0.2	N.D.	2.53
Cholestenone	C27	0.60 ± 0.04	0.3 ± 0.1	N.D.	1.98

[a] All activity measurements were determined fluorimetrically.

[b] Di Costanzo et al., 2008 [13]

[c] Drury et al., 2009 [14]

N.A., not determined

N.D., not detected

Table 2

Inhibition of AKR1D1 by isoform selective inhibitors for AKR1C enzymes.

Inhibitor	Enzyme(s) inhibited	K_i (μM)	K_i (μM)* (5β-reductase)
Indomethacin	AKR1C3	8.2 (C)†	> 100
Mefenamic acid	AKR1C1, 1C2, 1C3	0.8, 0.22, 0.30 (C)‡	> 100
4-Benzoylbenzoic acid	AKR1C1, 1C2, 1C3	12.5, 13.3, 1.9 (C)‡	> 100
Chenodeoxycholic acid	AKR1C1, 1C2, 1C3	8.3, 0.017, 10 (C)‡	3.2 (NC)
Ursodeoxycholic acid	AKR1C1, 1C2, 1C3	4.9, 0.011, 1.3 (C)‡	9.8 (NC)

* The K_i values were determined by measuring the inhibition of testosterone reduction.

C, competitive inhibition

NC, non-competitive inhibition

† Byrns et al., 2007 [29]

‡ Bauman et al., 2005 [28]