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Structure and catalytic mechanism of human steroid 5 β -reductase (AKR1D1)

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Abstract

Human steroid 5 β -reductase (aldo-keto reductase (AKR) 1D1) catalyzes reduction of Δ^4 -ene double bonds in steroid hormones and bile acid precursors. We have reported the structures of an AKR1D1-NADP⁺ binary complex, and AKR1D1-NADP⁺-cortisone, AKR1D1-NADP⁺-progesterone and AKR1D1-NADP⁺-testosterone ternary complexes at high resolutions. Recently, structures of AKR1D1-NADP⁺-5 β -dihydroprogesterone complexes showed that the product is bound unproductively. Two quite different mechanisms of steroid double bond reduction have since been proposed. However, site-directed mutagenesis supports only one mechanism. In this mechanism, the 4-pro-R hydride is transferred from the re-face of the nicotinamide ring to C5 of the steroid substrate. E120, a unique substitution in the AKR catalytic tetrad, permits a deeper penetration of the steroid substrate into the active site to promote optimal reactant positioning. It participates with Y58 to create a “superacidic” oxyanion hole for polarization of the C3 ketone. A role for K87 in the proton relay proposed using the AKR1D1-NADP⁺-5 β -dihydroprogesterone structure is not supported.

Keywords

aldo-keto reductase; steroid hormone; bile acid biosynthesis; hydroxysteroid dehydrogenase

1. Introduction

The Δ^4 -3-ketosteroid functionality is present in all circulating steroid hormones except estrogens. The first step in their metabolism involves the reduction of the Δ^4 -ene to produce either 5 α -dihydro- or 5 β -dihydrosteroids in reactions catalyzed by steroid 5 α -reductase or 5 β -reductase, respectively (Tomkins, 1956).

Δ^4 -3-Ketosteroid 5 β -reductase is a soluble monomeric NADPH dependent enzyme and a member of the aldo-keto reductase (AKR) superfamily, and in humans is designated AKR1D1 (Kondo et al., 1994; Jez et al., 1997). In utilizing NADPH as the hydride donor, the enzymatic

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reaction introduces a 90° bend at the steroid A/B ring junction, which adopts a *cis*-A/B or β -configuration (Berseus, 1967; Okuda and Okuda, 1984; Onishi et al., 1991). This reaction is unique in steroid enzymology, since the configuration of the product is difficult to achieve by synthetic methods. This change in steroid configuration is essential to establish the emulsifying properties of bile acids. In this regard AKR1D1 catalyzes the pivotal reduction of Δ^4 -cholesten-7 α -ol-3-one and Δ^4 -cholesten-7 α ,12 α -diol-3-one to yield 5 β -cholestan-7 α -ol-3-one and 5 β -cholestane-7 α ,12 α -diol-3-one, respectively on route to the primary bile acids, chenodeoxycholic and cholic acid (Russell, 2003; Russell and Setchell, 1992).

Based on available data on AKRs much can be inferred about the structure-function of AKR1D1. As an AKR enzyme it is predicted to have an (α/β)₈-barrel fold, the cofactor binding site is likely conserved and the stereochemistry of hydride transfer is predicted to be 4-pro-*R* (Jez et al., 1997). Unlike other AKRs that catalyze the reduction of carbonyls, the catalytic tetrad is altered. The conserved AKR tetrad consists of Tyr, Lys, Asp, and His, where Tyr acts as the general acid/base (Grimshaw et al., 1995; Schlegel et al., 1998). However, in AKR1D1 the His is substituted with a Glu. The most thoroughly characterized steroid hormone transforming AKR is the rat 3 α -hydroxysteroid dehydrogenase (AKR1C9), where mutation of the conserved His to Glu to yield the H117E mutant introduced steroid 5 β -reductase activity into the enzyme when none previously existed (Jez and Penning, 1998). Although these studies confirm the importance of the H117E substitution for steroid double bond reduction, without a crystal structure it is difficult to assign a precise role for the Glu residue.

Here we compare the available crystal structures for human AKR1D1; the AKR1D1-NADP⁺ binary complex, and the AKR1D1-NADP⁺-cortisone, the AKR1D1-NADP⁺-progesterone and the AKR1D1-NADP⁺-testosterone ternary complexes (Di Costanzo et al., 2008) with the subsequently determined AKR1D1-NADP⁺-5 β -dihydroprogesterone (5 β -DHP) complex (product complex) (Faucher et al., 2008). Based on these structures two quite different mechanisms of steroid double bond reduction have been proposed. However, only one mechanism is supported by current functional data and that mechanism confirms the critical role of the Glu acid without invoking a role for the conserved Lys residue.

2. Results and Discussion

2.1. AKR1D1-Structures

Recently we determined the crystal structures of AKR1D1 complexed with NADP⁺ at 1.35 Å resolution (HEPES bound in active site (PDB: 3BUV)) and 1.79 Å resolution (PDB: 3BV7), NADP⁺ and cortisone at 1.90 Å resolution (PDB: 3CMF), NADP⁺ and progesterone at 2.03 Å resolution (PDB: 3COT), and NADP⁺ and testosterone at 1.62 Å resolution (PDB: 3BUR) (Di Costanzo et al., 2008). We emphasize that all complexes have been determined with the oxidized form of the cofactor (NADP⁺), and not the reduced form of the cofactor as incorrectly alleged by Breton and colleagues (Faucher et al., 2008).

2.2. AKR1D1-NADP⁺ Binary Complex

The asymmetric unit of the crystal structure of the AKR1D1-NADP⁺ binary complex contains two monomers of AKR1D1, each of which consists of a 325-residue polypeptide chain that adopts an (α/β)₈-barrel fold. The total interface surface area between monomers A and B in the asymmetric unit is 401 Å², and the total interface surface area is 440 Å² between monomers A and B related by the crystallographic symmetry operator -x,y-1/2,-z-1/2. These interface surface areas are too small to be biologically relevant (Postinghl et al., 2005), which is consistent with the monomeric structure of the enzyme detected in solution (Drury and Penning, 2007). In each monomer, the upper part of the β -barrel is capped by loops A (I119-L147), B (Y219-L238), and C (L302-Y326), which enclose and define the active site cleft. Interestingly,

the P133-K139 segment in loop A of monomer A exhibits higher thermal B factors compared with the corresponding segment in monomer B, where this segment appears to be stabilized in part by interlattice contacts. In particular, residues E136 and N137 of monomer B accept hydrogen bonds from residues S290 and K207, respectively, from monomers related by crystallographic symmetry, while E136 and N137 of monomer A are solvent exposed. However, the conformations of loop A in monomers A and B are essentially identical (Di Costanzo et al., 2008).

The NADP⁺ cofactor in the AKR1D1-NADP⁺ complex adopts an extended *anti*-conformation as observed in other AKR structures. It is located in a long tunnel with the adenine group exposed to solvent and the nicotinamide ring located deep inside the tunnel oriented toward the central active site cavity where it participates in a significant number of hydrogen bond interactions (Fig. 1) (Di Costanzo et al., 2008).

2.3. Ternary Complex Structures

The crystal structures of the AKR1D1-NADP⁺-cortisone and AKR1D1-NADP⁺-progesterone complexes (Di Costanzo et al., 2008) reveal that each steroid is positioned with its β -face oriented towards the *re*-face of the nicotinamide ring of NADP⁺, such that the steroid carbon-carbon double bond would be adjacent to the 4-pro-*R* hydrogen of NADPH if the reduced cofactor were bound (Fig. 2A-B). The C3 carbonyl oxygen of each steroid accepts hydrogen bonds from Y58 and the *anti*-oriented conformer of the presumably-protonated carboxylic acid side chain of E120. The C3 ketone of each steroid displaces a water molecule that bridges these residues in the AKR1D1-NADP⁺ complex. Strikingly, the crystal structure of AKR1D1 complexed with NADP⁺ and testosterone reveals an unproductive binding mode for testosterone (Fig. 2C). In this binding mode testosterone lies parallel to the cofactor.

The crystal structures of the AKR1D1-NADP⁺-cortisone and AKR1D1-NADP⁺-progesterone complexes also reveal that steroid substrates bind more deeply in the active site cleft of AKR1D1 in comparison with testosterone binding in the ternary complex of AKR1C9 complexed with NADP⁺ and testosterone (Bennett et al., 1997). This appears to result from the removal of the steric bulk of the H117 imidazole side chain by its substitution with E120 in AKR1D1, which resultantly allows the steroid C=C double bond rather than the steroid C=O bond to be adjacent to the nicotinamide ring of the cofactor (Fig. 3). In this binding mode cortisone and progesterone bind perpendicular to the cofactor.

2.4. Comparison of the AKR1D1-NADP⁺-Progesterone and AKR1D1-NADP⁺-5 β -DHP Complexes

After our report of the first crystal structures of AKR1D1 and its substrate complexes (Di Costanzo et al., 2008), the structure of the AKR1D1-NADP⁺-5 β -DHP complex (PDB: 3CAV) was reported at lower resolution by Breton and colleagues (Faucher et al., 2008). This was an identical crystal form to that reported by us and in general confirmed our structural conclusions. Overall, crystal structures of the AKR1D1-NADP⁺-progesterone complex (Di Costanzo et al., 2008) and AKR1D1-NADP⁺-5 β -DHP complex (Faucher et al., 2008) are essentially identical and the root-mean-square (r.m.s.) deviation of 325 C α atoms between these two structures is 0.30 Å.

To ease structural comparisons, we note that monomers A and B of our structure (Di Costanzo et al., 2008) correspond to monomers B and A of Breton's structure (Faucher et al., 2008). The AKR1D1-NADP⁺-5 β -DHP crystal structure reveals a different binding mode of 5 β -DHP for monomers A and B in the asymmetric unit, and each of these 5 β -DHP binding modes exhibits aspects of unproductive binding. In monomer A of Breton's structure, the A ring of 5 β -DHP points in toward the active site. Significantly, the loop B segment R226-N232 in the AKR1D1-

NADP⁺-5 β -DHP complex shifts slightly (0.8-1.2 Å) to allow the binding of 5 β -DHP in monomer A (Fig. 4). In the AKR1D1-NADP⁺-progesterone structure the side chain of W230 in loop B appears to play a key role in packing against the β -face of the steroid and undergoes a significant conformational change to accommodate the productive binding of this substrate (Di Costanzo et al., 2008). The side chain of W230 is similarly important for packing against the β -face of 5 β -DHP (Faucher et al., 2008). While the progesterone substrate and the 5 β -DHP product bind with similar orientations, progesterone binds more deeply in the enzyme active site such that the C3 carbonyl oxygen occupies the same position as the water molecule that hydrogen bonds with Y58 and E120 in the AKR1D1-NADP⁺ complex (Di Costanzo et al., 2008). The binding of progesterone optimally orients its C5 atom for reduction, which is aligned with C4 of NADP⁺ in such a way the trajectory for the hydride transfer is well defined (presuming that NADPH binds similarly to NADP⁺) (Fig. 5) (Di Costanzo et al., 2008). Notably, however, the distance between the C3 carbonyl oxygen of progesterone in the AKR1D1-NADP⁺-progesterone complex and the corresponding atom of 5 β -DHP in the AKR1D1-NADP⁺-5 β -DHP complex is ~ 2 Å, so the reduced steroid product appears to shift ~ 2 Å out of the active site. The carbonyl oxygen of the product accepts a hydrogen bond from the water molecule bridging Y58 and E120 identified in our earlier structure (Di Costanzo et al., 2008). In our structures this water molecule is only present in the AKR1D1-NADP⁺ binary complex, the AKR1D1-NADP⁺-testosterone complex in which the substrate molecule is bound unproductively (Fig. 2c), and the AKR1D1-NADP⁺-5 β -dihydrotestosterone complex in which the product molecule is also bound unproductively (Fig. 6).

Interestingly, our recent crystal structure determination of the AKR1D1-NADP⁺-5 β -dihydrotestosterone complex at 2.00 Å resolution (PDB: 3DOP; full details of the structure determination will be reported in due course) reveals an unproductive binding mode for this steroid product similar in some respects to that observed for the unproductively bound 5 β -DHP in monomer B of the AKR1D1-NADP⁺-5 β -DHP complex (Faucher et al., 2008), Fig. 6a and 6c. In both these structures 5 β -dihydrotestosterone and 5 β -DHP bind in the active site of AKR1D1 with their A-rings pointing out of the active site cleft, and their binding is backwards (antiparallel) with respect to 5 β -DHP bound to monomer A (Fig. 6). However, it is the β -face of 5 β -dihydrotestosterone that is oriented toward the side chain of W230 (Fig. 6a), whereas it is the α -face of 5 β -DHP that is oriented toward W230 (Fig. 6c). Moreover, the α -face of 5 β -DHP molecule is somewhat rotated away from W230 (Fig. 6). The similarity between the AKR1D1-NADP⁺-5 β -dihydrotestosterone and the AKR1D1-NADP⁺-5 β -DHP structures showing backwards binding of the steroid product, and the unexpected presence of a water molecule in the AKR1D1-NADP⁺-5 β -DHP structure in monomer A raises the possibility that the 5 β -DHP structures are unproductive and offer a view of the steroid product attempting to bind.

2.5. Mechanism of Double Bond Reduction Catalyzed by AKR1D1

Two mechanisms of steroid double bond reduction have been proposed based on these crystal structures. However, when combined with available site-directed mutagenesis data only one mechanism is supported.

In the structures reported, the catalytic tetrad of D53, Y58, K87 and E120 is located in the center of the β -barrel. The cortisone and progesterone ternary complex structures suggest a dual role for E120. First, substitution of E120 for the imidazole side-chain of histidine permits the steroid substrate in both complexes to insert itself deeper into the active site cavity than would otherwise be allowed. This establishes the reaction trajectory between the 4-pro-R hydride of NADPH and the C5 position of the steroid. Second, E120 establishes a hydrogen bond with the C3 ketone of the steroid. Since the side-chain of E120 is in the *anti*-conformation, it is likely fully protonated. Given that carboxylate groups that serve as general bases do so

with their more basic, syn-oriented lone electron pairs (Gandour, 1981), we conclude that the anti-oriented conformer of E120 instead serves an acidic function and creates a “superacidic” oxyanion hole that will enolize the steroid and facilitate hydride transfer. The key components of this first mechanism are shown in Fig. 7A (Di Costanzo et al., 2008).

In the AKR1D1-NADP⁺-5 β -DHP product complex, 5 β -DHP was moved out from the tetrad and a progesterone molecule was manually docked into the cavity so that its C3 ketone displaced the water molecule in the structure. On the basis of this model it was suggested that E120 also serves the same dual function. It allows the steroid substrate to bind deeper into the pocket and it helps polarize the C3 ketone. However, it was also proposed that the pK_b of Y58 was lowered by interaction with K87 which forms a proton relay with D53. It was also suggested that the protonation state of K87 and D53 was altered by the binding of the NADPH cofactor (Fig. 7B), Faucher et al., 2008. Without additional supporting mutagenesis data either mechanism is plausible. However, the available functional data on AKRs strongly support the first mechanism.

Extensive site-directed mutagenesis studies on AKR1C9 speak to the mechanism (Schlegel et al., 1998). Convincing evidence was provided for a “push-pull” mechanism, in which the catalytic Tyr used different partners to alter its pK_i in the reduction and oxidation directions. pH versus k_{cat} profiles for the reduction of 5 α -dihydrotestosterone to 3 α -androstenediol on the wild-type enzyme and its tetrad mutants were performed, in which k_{cat} represents the chemical step. These studies showed that the Y55F mutant eliminated the titratable group with the largest decrease in the pH-independent value of k_{cat} , confirming that Y55 was the general acid. The titratable group was also eliminated in the H117A mutant while it was retained in the K84M mutant. These studies suggest that H117 lowers the pK_b of Y55 in the reduction direction and this function is not performed by K84. By contrast when the oxidation of 3 α -androstenediol to 5 α -dihydrotestosterone was studied the Y55F mutant again eliminated the titratable group with the largest decrease in the pH-independent value of k_{cat} . This was also observed with the K84M mutant but not with the H117A mutant indicating that the pK_a of Y55 was lowered by K84 and this function was not performed by H117. Thus K84 facilitates oxidation by acting as a base to generate a phenolate anion on Y55 for deprotonation of a steroid alcohol. However, a precedent does not exist for K84 acting as a general acid in the AKR mechanism as proposed in Fig. 7B. This conclusion was also reached by extensive site-directed mutagenesis on xylose reductase and chemical rescue of the mutants (Kratzer and Nidetzky, 2005). These studies suggest that K87 is not involved in 5 β -reduction catalyzed by AKR1D1.

Studies on the AKR1C9 H117E mutant were also revealing (Jez and Penning, 1998). This mutant catalyzed 5 β -reduction of testosterone and progesterone when no double bond reduction activity existed before. This observation confirmed the critical importance of E117 in double bond reduction. The H117E mutant also retained 3 α -hydroxysteroid activity, suggesting that hydride transfer could now occur to both the C5 and C3 positions of the steroid confirming that E117 allows hydride transfer to both positions but H117 does not. The same outcome was observed for the reduction of testosterone and progesterone catalyzed by the H117A mutant confirming that the imidazole group prevents steroid 5 β -reduction due to steric hindrance. These studies support the first function of E120 in the mechanism which is to correctly orientate the steroid in the active site cavity. pH versus k_{cat} profiles were also informative where k_{cat} was again governed by the chemistry step. When the reduction of either testosterone or 5 β -dihydrotestosterone was monitored, the H117A mutant eliminated the titratable group, while the H117E mutant caused an acid-shift (0.5 units) in the pK_b suggesting that the second effect of E117 was to lower the pK_b of the general acid. The general acid for 5 β -reduction was Y55 since the double mutant Y55/H117E was devoid of all enzyme activity. These findings support the second function of E120 which is to create a “superacidic” oxyanion hole consistent with the first mechanism (Fig. 7B).

It should be emphasized that in the original mechanism proposed for steroid double bond reduction it was hypothesized that a protonated Glu was hydrogen bonded to the catalytic Tyr to increase the acidity of the Tyr (Jez and Penning, 1998). However, this hypothesis was formulated prior to the crystal structure determination of AKR1D1. The crystal structures reported by Di Costanzo et al., 2008 and by Faucher et al., 2008 do not support hydrogen bond interactions between these two residues. Both reports do support hydrogen bonds between the C3-ketone of the steroid substrate with Y58 and E120. The net effect is, however, to create a more acidic environment to support the chemistry of double bond reduction as proposed (Jez and Penning, 1998).

There are multiple lines of evidence to support the concept that E120 is not the proton donor in the reaction: (i) extensive site-directed mutagenesis on AKR1B1 (aldose reductase) and AKR1C9 (rat 3 α -hydroxysteroid dehydrogenase) support a role of Tyr as the general acid-base (Grimshaw et al., 1995; Schlegel et al., 1998); (ii) wt AKR1C9 performs C3 ketone reduction where H117 is present instead of E117, suggesting that neither H117 or E117 acts as the general acid; (iii) the AKR1C9 H117E mutant performs double bond reduction and this ability is retained in the H117A mutant, whereas the Y55F and Y55F/H117A double mutant abolishes this activity. In further support of the mechanism in Fig. 7A, AKR1D1 Y58F and AKR1D1 E120A mutants are devoid of enzyme activity. It is noteworthy that all the site-directed mutagenesis data support a mechanism without invoking a role for K87 as proposed by Faucher et al., 2008. It should be noted that in the structures of AKR1D1 ternary complexes, the hydrogen bond interaction between K87 and Y58 is weak (3.2 Å) compared to the hydrogen bond interactions between Y58 and progesterone (2.6 Å) and E120 and progesterone (2.76 Å). Therefore the role proposed for K87 may not be possible.

2.6. Comparison with Double Bond Reduction Catalyzed by 2,4-Dienoyl-CoA Reductase

Detailed mechanistic studies have been performed on the mechanism of double bond reduction catalyzed by 2,4-dienoyl-CoA reductase, a short-chain dehydrogenase reductase (SDR) which warrants a comparison. This enzyme catalyzes double bond reduction without a reliance on the “superacidic” environment proposed for AKR1D1 (Alphey et al., 2005). 2,4-Dienoyl CoA reductase reduces a δ,γ -double-bond when it is in conjugation with an α,β -unsaturated thioester and does not reduce the double bond of an α,β -unsaturated ketone. The extended conjugation in the former substrate coupled with the presence of the thioester increases the strength of the electronic sink and makes it easier to perform hydride transfer by a Michael addition. It is proposed that the extra “push” required to reduce the α,β -unsaturated ketone in Δ^4 -3-ketosteroids is provided by the “superacidic” environment of the AKR1D1 active site.

We also note that in the crystal structure of 2,4-dienoyl CoA reductase (Alphey et al., 2008), there is a deviation from the normal catalytic triad of Tyr-X-X(Ser)-Lys of SDRs. While Y199 acts as the general acid, both Y199 and N148 now serve to align the aliphatic tail of the diene in proximity to the cofactor and contribute to the polarization of the carbonyl by stabilizing the dienolate. In AKR1D1, Y58 and E120 perform somewhat similar functions in steroid 5 β -reduction. It is noteworthy that this instance the neutral N148 in the SDR is replaced by the protonated and more acidic E120 in the AKR. We have previously reported that the active site residues of SDRs and AKRs are conserved and superimposable in three-dimensional space suggesting convergent evolution to common enzymatic mechanisms (Bennett et al., 1996).

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Abbreviations

AKR

aldo-keto reductase

5 β -DHP5 β -dihydroprogesterone**SDR**

short-chain dehydrogenase reductase

Fig 1. AKR1D1-NADP⁺ complex. Hydrogen bond interactions are indicated by red dashed lines. A buffer molecule (HEPES) is also bound in the active site. Atoms are color coded as follows: protein carbon atoms are yellow, HEPES carbon atoms are black and its sulfur atom is green, oxygen atoms are red, and nitrogen atoms are blue.

Fig. 2.

AKR1D1-NADP⁺-steroid complexes. Hydrogen bond interactions are indicated by red dashed lines. Atoms are color coded as in Fig. 1 except that steroid carbon atoms are black. (a) Cortisone; (b) progesterone; (c) testosterone; in contrast with the binding of cortisone and progesterone, the A-ring of testosterone is oriented away from the catalytic tetrad and the nicotinamide ring of the cofactor.

Fig. 3.

Comparisons of steroid binding modes in catalysis. Superposition of the AKR1D1-NADP⁺-cortisone complex (red) on the AKR1C9-NADP⁺-testosterone complex (green) showing that the A ring of the substrate binds ~1 Å deeper in the active site of the former enzyme relative to the position of the nicotinamide ring of NADP⁺. Presuming that NADPH binds similarly to NADP⁺, the 4-pro-R hydride of NADPH would be adjacent to the substrate C3 carbonyl carbon in the AKR1C9 active site (green dotted line), whereas the 4-pro-R hydride of the NADPH cofactor would be adjacent to C5 of the substrate carbon-carbon double bond in the AKR1D1 active site (red dotted line). (b) same orientation as (a), but a close-up view showing all atoms.

Fig. 4. Least-squares superposition of Ca atoms of the AKR1D1-NADP⁺-5 β -DHP complex (monomer A; carbon atoms are cyan) Faucher et al., 2008, and the AKR1D1-NADP⁺-progesterone complex (carbon atoms are purple), Di Costanzo et al., 2008. The indole ring of W230 and its associated loop B moves away to accommodate the binding of the product compared with the binding of the substrate.

Fig. 5.

Least-squares superposition of C α atoms of the AKR1D1-NADP⁺ complex (yellow), the AKR1D1-NADP⁺-cortisone complex (red), and the AKR1D1-NADP⁺-testosterone complex (olive green). The distance of ~ 3.7 Å between the C4 of NADP⁺ and the olefinic carbon C5 of cortisone is indicated by a red dashed line, which would represent the trajectory of hydride transfer from NADPH. The position of the water molecule in the unliganded structure is indicated by a yellow sphere. Loops A, B, and C are labeled; note that loop B and W230 in particular must undergo a significant conformational change to accommodate productive substrate binding as represented by cortisone (red). The indole ring of W230 remains in its native, substrate free conformation (yellow) to accommodate the unproductive binding mode of testosterone (green). Reproduced with permission from American Society of Biochemistry and Molecular Biology.

Fig. 6. Comparisons of 5β-dihydrosteroid binding modes. (a) AKR1D1-NADP⁺-5β-dihydrotestosterone complex (PDB: 3DOP); (b) AKR1D1-NADP⁺-5β-DHP complex monomer A; (c) AKR1D1-NADP⁺-5β-DHP complex monomer B (PDB: 3CAV, Faucher et al., 2008); and (d) Least-squares superposition of AKR1D1-NADP⁺-5β-dihydrotestosterone complex monomer B (red), AKR1D1-NADP⁺-5β-DHP complex monomer A (green) and monomer B (blue).

Fig. 7. Mechanism of steroid 5 β -reductase. Mechanism 1, showing the dual role of E120 after Di Costanzo et al., 2008 (J Biol Chem 283, 16830) (Panel A); and mechanism 2 invoking a proton relay to Lys87 after Faucher et al., 2008 (Biochemistry, 47,8261) (Panel B). Note that in Faucher's mechanism, the stereochemistry of the hydrogen bond with E120 is shown incorrectly with syn-orientation rather than the anti-orientation observed in the crystal structures.