Serine 124 completes the Tyr, Lys and Ser triad responsible for the catalysis of human type 1 3β-hydroxysteroid dehydrogenase

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Abstract

Human 3β-hydroxysteroid dehydrogenase/isomerase (3β-HSD) is a key steroidogenic enzyme that catalyzes the first step in the conversion of circulating dehydroepiandrosterone (DHEA), pregnenolone or 17α-hydroxypregenolone to produce the appropriate, active steroid hormone(s): estradiol, testosterone, progesterone, aldosterone or cortisol respectively. Our mutagenesis studies have identified Tyr154 and Lys158 as catalytic residues for the 3β-HSD reaction. Our three-dimensional homology model of 3β-HSD shows that Tyr154 and Lys158 are oriented near the 3β-hydroxyl group of the bound substrate steroid, and predicts that Ser123 or Ser124 completes a Tyr–Lys–Ser catalytic triad that operates in many other dehydrogenases. The S123A and S124A mutants of human type 1 3β-hydroxysteroid dehydrogenase/isomerase (3β-HSD1) were created by PCR-based mutagenesis, expressed in insect cells using baculovirus and purified to homogeneity. The S124A mutant exhibits no 3β-HSD activity and has a $K_m$ value (83·6 µM) for the isomerase substrate that is threefold greater than that of wild-type 1 isomerase. In contrast, S123A has substantial 3β-HSD activity (DHEA $K_m$=11·2 µM; $k_{cat}$=0·8 min$^{-1}$) and utilizes isomerase substrate, 5-androstene-3,17-dione, with a $K_m$ value (27·6 µM) that is almost identical to wild-type. The $K_m$ value (4·3 µM) of S124A for NADH as an allosteric activator of isomerase is similar to that of the wild-type 1 enzyme, indicating that Ser124 is not involved in cofactor binding. S123A utilizes NAD as a cofactor for 3β-HSD and NADH as the activator for isomerase with $K_m$ values that are similar to wild-type. The 3β-HSD activities of S123A and wild-type 3β-HSD increase by 2·7-fold when the pH is raised from 7·4 to the optimal pH 9·7, but S124A exhibits very low residual 3β-HSD activity that is pH-independent.

These kinetic analyses strongly suggest that the Ser124 residue completes the catalytic triad for the 3β-HSD activity. Since there are 29 Ser residues in the primary structure of human 3β-HSD1, our homology model of the catalytic domain has been validated by this accurate prediction. A role for Ser124 in the binding of the isomerase substrate, which is the 3β-HSD product-steroid of the bifunctional enzyme protein, is also suggested. These observations further characterize the structure/function relationships of human 3β-HSD and bring us closer to the goal of selectively inhibiting the type 1 enzyme in placenta to control the timing of labor or in hormone-sensitive breast tumors to slow their growth.

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Introduction

The human type 1 (placenta, skin, mammary gland, prostate and endometrium) and type 2 (gonads and adrenals) isoforms of 3β-hydroxysteroid dehydrogenase (EC 1.1.1.145)/steroid Δ5–Δ4-isomerase (EC 5.3.3.1) (3β-HSD/isomerase) are encoded by two distinct genes which are expressed in a
tissue-specific pattern (Rheaume et al. 1991). 3β-HSD/isomerase catalyzes the conversion of 3β-hydroxy-5-ene-steroids (dehydroepiandrosterone, pregnenolone) to 3-oxo-4-ene-steroids (androstenedione, progesterone) on a single, dimeric protein containing both enzyme activities (Thomas et al. 1989). Androstenedione is converted by placental aromatase to estradiol, which participates in the cascade of events that initiates labor in humans (Kacsoh 2000, Rainey et al. 2002). In addition to placenta and other human peripheral tissues, the type 1 enzyme is selectively expressed in breast tumors (Gingras et al. 1999), where it catalyzes the first step in the conversion of circulating dehydroepiandrosterone to estradiol to promote tumor growth. In human adrenals, type 2 3β-HSD/isomerase is required for the production of cortisol and aldosterone (Rainey et al. 2002). Determination of the structure/function relationships of the type 1 enzyme may lead to the development of specific inhibitors of type 1 3β-HSD/isomerase that can help control the timing of labor and slow the growth of hormone-sensitive tumors without compromising steroidogenesis in the adrenal enzyme.

Mammalian 3β-HSD/isomerase is a member of the short-chain dehydrogenase/reductase (SDR) family of enzymes. The Rossmann-fold coenzyme domain (β-α-β-α-β-α-β) and the Y-X-X-X-K catalytic motif in that domain are highly conserved in the SDR family (Jornvall et al. 1995). Many members of the SDR family utilize a catalytic triad of Ser, Tyr and Lys residues to perform the oxidoreductase enzyme reaction (e.g. 17β-HSD1, UDP-galactose-4-epimerase) (Liu et al. 1997). A Tyr residue acts as the catalytic base, the hydroxyl group of Ser binds to the targeted oxo group to stabilize the substrate, and a Lys residue binds to the nicotinamide ribose and lowers the pKa of the phenolic hydroxyl group of the key Tyr to promote the proton transfer (Filling et al. 2002). In a few species of 3β-HSD (macaque, bovine, rat I, II and IV, guinea pig and chicken), Thr124 is positioned to perform the function of Ser124 (Simard et al. 1996, Morel et al. 1997). Our homology model of the three-dimensional structure of human 3β-HSD1 has been used to predict key amino acid residues involved in catalysis and coenzyme specificity (Thomas et al. 2002, 2003). Our previous mutagenesis study supported roles for Tyr154 and Lys158 in the catalysis of human 3β-HSD1 (Thomas et al. 2002). In the current study, our homology model has predicted a catalytic role for either Ser123 or Ser124. Hence, the S123A and S124A mutants of human 3β-HSD1 have been produced to evaluate the function of these amino acids in the catalysis of the enzyme. Since there are 29 Ser residues in each monomer of the homodimeric enzyme (Thomas et al. 2002), this study also tests the accuracy of our homology model.

Materials and methods

Materials

Dehydroepiandrosterone and pyridine nucleotides were purchased from Sigma (St Louis, MO, USA); 5-androstene-3,17-dione from Steraloids, Inc. (Wilton, NH, USA); reagent grade salts, chemicals and analytical grade solvents from Fisher Scientific (Pittsburgh, PA, USA). Glass-distilled, deionized water was used for all aqueous solutions.

Site-directed mutagenesis

With the Advantage cDNA PCR kit (BD Biosciences Clontech, Palo Alto, CA, USA) and pGEM-3βHSD1 as template (Thomas et al. 1998), double-stranded PCR-based mutagenesis was performed to create the cDNA encoding the S123A and S124A mutant enzymes. The forward and reverse primers used to produce S123A were listed (with the mutant codon in bold italics): 5’-TAC ACCGCTAGCATAGAGGTAGC-3’ (forward); 5’-TATGCTAGGTTAGTAGAAAGACT-3’ (reverse). The primers used to produce S124A were as follows: 5’-CACCAGTGCGCTAGAGGTAGC-3’ (forward); 5’-CGGCTATGGCTAGGGACT-3’ (reverse). The presence of the mutated codon and integrity of the entire mutant 3β-HSD cDNA were verified by automated dideoxynucleotide DNA sequencing using the Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, Foster City, CA, USA). Chou-Fasman and Garnier-Osguthorpe-Robson analysis of each mutant enzyme was used to choose amino acid substitutions that produced no apparent changes in the secondary structure of the protein (Protlyze program, Scientific and Educational Software, State Line, PA, USA).
Expression and purification of mutant and wild-type enzymes

The mutant 3β-HSD cDNA was introduced into baculovirus as previously described (Thomas et al. 1998). Recombinant baculovirus was added to 1·5 × 10^9 Sf9 cells (1 L) at a multiplicity of infection of 10 for expression of each mutant enzyme. The expressed mutant and wild-type enzymes were separated by SDS-polyacrylamide (12%) gel electrophoresis, probed with our anti-3β-HSD polyclonal antibody and detected using the ECL Western blotting system with antirabbit, peroxidase-linked secondary antibody (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Each expressed enzyme was purified from the 100 000 × g pellet of the Sf9 cells (2 L) by our published method (Thomas et al. 1988, 1989, 2002). Each expressed, purified mutant and wild-type enzyme produced a single band (42·0 kDa) on SDS-polyacrylamide (12%) gel electrophoresis that comigrated with the human wild-type 1 control enzyme. Protein concentrations were determined by the Bradford method, using bovine serum albumin as the standard (Bradford 1976).

Kinetics studies

Michaelis–Menten kinetic constants for the 3β-HSD substrate were determined for the purified mutant and wild-type enzymes in incubations containing dehydroepiandrosterone (DHEA, 2–100 µM) and purified enzyme (0·03 mg) at 27 °C in 0·02 M potassium phosphate, pH 7·4. The slope of the initial linear increase with time in absorbance at 340 nm per min (due to NADH production) was used to determine 3β-HSD activity. Kinetic constants for the isomerase substrate were determined at 27 °C in incubations of 5α-androstene-3,17-dione (17–150 µM), NADH (0·05 mM) and purified enzyme (0·01 mg) in 0·02 M potassium phosphate buffer, pH 7·4. Isomerase activity was measured by the initial absorbance increase at 241 nm (due to androstenedione formation) as a function of time. Changes in absorbance were measured with a Varian Cary 219 (Sugar Land, TX, USA) recording spectrophotometer. The Michaelis–Menten constants (K_m and V_max) were calculated from Lineweaver-Burke (1/S vs 1/V) plots and verified by Hanes–Woolf (S vs S/V) plots (17). The k_cat values (min⁻¹) were calculated from the V_max values (nmol/min per mg) and represent the maximal turnover rate (nmol product formed/min per nmol enzyme dimer).

Kinetic constants for the 3β-HSD cofactor were determined for the purified mutant and wild-type enzymes in incubations containing NAD⁺ (13–100 µM), dehydroepiandrosterone (100 µM) and purified enzyme (0·03 mg) in 0·02 M potassium phosphate, pH 7·4, at 27 °C, using the spectrophotometric assay at 340 nm. Kinetic constants for the isomerase cofactor were determined in incubations of NADH (2–50 µM), 5-androstene-3,17-dione (100 µM) and purified enzyme (0·01 mg) in 0·02 M potassium phosphate buffer, pH 7·4, at 27 °C, using the spectrophotometric assay at 241 nm.

pH-dependency studies

The effects of pH on the residual 3β-HSD activities of the Y154F, K158Q, Y269S and K273Q mutants were measured in incubations at 27 °C containing 5α-androstan-3β-ol-17-one (100 µM), NAD⁺ (0·2 mM) and purified enzyme (0·08 mg) in 0·05 sodium phosphate pyrophosphate buffer, pH 7·4 or 9·7, using the 3β-HSD assay at 340 nm.

Modeling and sequence alignment

The three-dimensional structure of human type 1 3β-HSD/isomerase was modeled using the crystal structure (Thoden et al. 1996) of UDP-galactose 4-epimerase from *Escherichia coli* (Protein Data Bank Accession Code 1A9Z) as template, as described previously (Thomas et al. 2003).

Results

Homology modeling targets the 3β-HSD catalytic domain

We recently reported that 40% of the fingerprint residues of *E. coli* UDP-galactose-4-epimerase, an SDR family member with a known crystallographic structure (Thoden et al. 1996), are conserved with respect to human 3β-HSD1 (Thomas et al. 2003). The 3β-HSD and epimerase catalytic events are closely related in that both use a Tyr-X-X-X-Lys motif at the same position in the primary structure for the oxidation of a hydroxyl group (Gerratana et al. 2001). Similar to the catalytic Tyr149 and Lys153 in UDP-galactose-4-epimerase, our site-directed mutagenesis studies have identified Tyr154...
and Lys158 as the catalytic residues for human 3β-HSD1 (Thomas et al. 2002). Another fingerprint residue of UDP-galactose-4-epimerase is the catalytic Ser124, and human 3β-HSD contains a pair of Ser residues (Ser123 and Ser124) that correspond to this position in the homologous proteins. Thus, our model of the catalytic domain predicts that one of these serine residues, most likely Ser124, may complete the catalytic triad for human 3β-HSD1 (Fig. 1).

**Site-directed mutagenesis, expression and purification of the wild-type and mutant enzymes**

The 3β-HSD1 cDNA mutants (S123A and S124A) were produced by double-stranded, PCR-based mutagenesis and inserted into baculovirus, as described in Methods. As shown by the immunoblot in Fig. 2, the baculovirus system successfully expressed the mutant enzyme proteins as well as the human wild-type 3β-HSD1 in Sf9 cells. Each expressed enzyme was purified by our published method (Thomas et al. 1989) to apparent homogeneity, according to SDS–PAGE (Fig. 2).

**Kinetic comparisons of the purified wild-type and mutant enzymes**

The Michaelis–Menten kinetic values measured for substrate utilization by purified wild-type 3β-HSD1 and by the S123A and S124A mutants are summarized in Table 1. The S124A mutant enzyme has no detectable 3β-HSD activity. However, the S123A mutant exhibits a maximal turnover rate ($k_{cat}$) that is 24% of the wild-type 3β-HSD1 activity and a $K_m$ for DHEA that is threefold higher than 3β-HSD1. S123A utilizes the isomerase substrate steroid with a similar $K_m$ value compared with wild-type 3β-HSD1, but the S124A mutant utilizes the isomerase substrate much less efficiently.

The cofactor kinetic values in Table 2 reveal that S123A reduces NAD$^+$ with a $K_m$ value that is very similar to the $K_m$ of NAD$^+$ for wild-type 3β-HSD1. Although S124A has no detectable 3β-HSD activity in the cofactor kinetic study, the $K_m$ values measured for NADH as an allosteric activator of isomerase are similar for S124A, S123A and wild-type 3β-HSD1.

**pH-dependency of the wild-type and mutant enzymes**

The pH dependency of the 3β-HSD activities of the S123A and S124A mutants can help determine whether one of these Ser residues is involved in catalysis. If the residual dehydrogenase activity of the mutant enzyme is pH-dependent, the substituted amino acid must not be catalytic because the residual activity has to be due to a different amino acid in the enzyme (Schlegel et al. 1998). Although 3β-HSD activity is undetectable for S124A using DHEA as substrate (Tables 1 and 2), the use of 5α-androstan-3β-ol-3-one as substrate enhances residual 3β-HSD by threefold to detectable levels. This 3β-hydroxy-5α-reduced steroid is not a substrate for isomerase activity; therefore, feedback inhibition by a 3-keto-4-ene product steroid (e.g. androstenedione) of 3β-HSD/isomerase does not limit the 3β-HSD activity (Milewich et al. 1993). As shown in Table 3, the wild-type 3β-HSD1 and S123A mutant exhibits substantial 3β-HSD activity at pH 7.4, and that activity is increased by 2-7- to 2-8-fold at the optimal pH 9.7. However, even when 5α-androstan-3β-ol-3-one is used as substrate to enhance any residual activity, the S124A mutant enzyme has only trace 3β-HSD activity at pH 7.4, and this trace activity is not affected when the pH is increased to 9.7 (Table 3).

**Discussion**

Human 3β-HSD/isomerase is a unique member of the SDR family. The enzyme sequentially catalyzes the 3β-HSD activity followed by the isomerase activity on a single, dimeric protein. The coenzyme product of the 3β-HSD reaction, NADH, induces a conformational change in the enzyme protein that activates the isomerase reaction (Thomas et al.

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**Figure 1** Ribbon structure of the active site of human 3β-HSD1 based on homology modeling. The primary sequences of 3β-HSD/isomerase and UDP-galactose-4-epimerase were aligned as described in the text. The NAD$^+$ and DHEA structures are included. The catalytic Tyr154 and Lys158 residues (Thomas et al. 2002) as well as the potentially catalytic Ser123 and Ser124 residues are shown. Ser124 is shown hydrogen-binding (dotted bonds) to the 3β-hydroxyl group of DHEA. The Asp36 residue is shown hydrogen-binding (dotted bonds) to the 2β-3-hydroxyl groups of the adenosyl ribose group of NAD (Thomas et al. 2003).
In our previous studies (Thomas et al. 1993, 1997), two tryptic peptides associated with the 3β-HSD and isomerase activities were localized in the primary structure of the type 1 enzyme with affinity-radiolabeled steroids. The Gly250 tryptic peptide contains Tyr253 and Asp257, which we have identified as the catalytic residues for the isomerase reaction (Mason et al. 1998, Thomas et al. 1998, 2003). When this peptide was protected from affinity alkylation by isomerase substrate or NADH (Thomas et al. 1994, 1997), the Glu135 tryptic peptide was identified. The Glu135 peptide contains Tyr154 and Lys158 in a YXXSK motif that is likely to be involved in 3β-HSD catalysis because of its location in the Rossmann-fold domain (Jornvall et al. 1995). Figure 3 localizes these catalytic residues in the primary structure of human 3β-HSD1 and shows the positions of

<table>
<thead>
<tr>
<th>3β-HSDa</th>
<th>Isomeraseb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Km µM</td>
</tr>
<tr>
<td>Purified enzyme</td>
<td></td>
</tr>
<tr>
<td>3β-HSD1</td>
<td>3.7</td>
</tr>
<tr>
<td>S123A</td>
<td>11.2</td>
</tr>
<tr>
<td>S124A</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

aKinetic constants for the 3β-HSD substrate were determined in incubations containing dehydroepiandrosterone (2–100 µM), NAD⁺ (0.1 mM) and purified enzyme (0.03 mg) in 0.02 M potassium phosphate, pH 7.4, 27 °C. kcat values (nmol product formed/min per nmol enzyme dimer) were calculated from the Vmax values. N.D., not determined.
bKinetic constants for the isomerase substrate were determined in incubations of 5-androstene-3,17-dione (15–100 µM) NADH (0.05 mM) and purified enzyme (0.01 mg) in 0.02 M potassium phosphate buffer, pH 7.4, 27 °C. Each Km and kcat value represents the mean of triplicate measurements with a standard deviation ≤7% of single mutant enzyme preparations.
Table 2 Coenzyme kinetics for the 3β-HSD and isomerase activities of the purified S123A, S124A and wild-type 3β-HSD1 enzymes

<table>
<thead>
<tr>
<th></th>
<th>3β-HSDa</th>
<th>Isomeraseb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$</td>
<td>$k_{cat}$</td>
</tr>
<tr>
<td>Purified enzyme</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3β-HSD1</td>
<td>34·1</td>
<td>3·6</td>
</tr>
<tr>
<td>S123A</td>
<td>35·6</td>
<td>0·7</td>
</tr>
<tr>
<td>S124A</td>
<td>N.D.</td>
<td>No activity detected</td>
</tr>
</tbody>
</table>

aKinetic constants for the 3β-HSD cofactor were determined in incubations containing NAD+ (20–100 µM), dehydroepiandrosterone (100 µM) and purified enzyme (0·03 mg) in 0·02 M potassium phosphate, pH 7·4, 27 °C. $k_{cat}$ values (nmol product formed/min per nmol enzyme dimer) were calculated from the $V_{max}$ values. N.D., not determined.

bKinetic constants for the isomerase cofactor were determined in incubations of NADH (2–50 µM) 5-androstene-3,17-dione (100 µM) and purified enzyme (0·01 mg) in 0·02 M potassium phosphate buffer, pH 7·4, 27 °C. Each $K_m$ and $k_{cat}$ value represents the mean of triplicate measurements with a standard deviation ≤6% of single mutant enzyme preparations.

S123 and S124 plus the other 27 Ser residues (underlined).

In agreement with a similar motif (149Y-X-X-X-K153) in UDP-galactose-4-epimerase (Liu et al. 1997), the Tyr154 and Lys158 residues appear to be catalytic residues for 3β-HSD according to the kinetic profiles of the mutant Y154F and K158Q enzymes (Thomas et al. 2002). Our homology model of the active site of 3β-HSD (Fig. 1) localizes these residues in close proximity to the bound A-ring of DHEA and the nicotinamide moiety of NAD+. Identification of the third member of the catalytic triad, Ser, would have been difficult before the development of the homology model. There are 29 Ser residues in the 3β-HSD primary structure (Thomas et al. 2002), but the model predicts that only Ser123 or Ser124 is oriented properly to hydrogen bond to the 3β-hydroxyl group of DHEA and stabilizes the substrate for catalysis by Tyr154 and Lys158.

With DHEA as substrate, the S124A mutant enzyme lacks measurable 3β-HSD activity, but the S123A mutant retains 24% of control 3β-HSD activity. The extremely low residual 3β-HSD activity of the S124A mutant observed with 5α-androstane-3β-ol-17-one as substrate was not increased by raising the pH from 7·4 to the optimal pH 9·7. However, the 3β-HSD activity of the S123A mutant was increased 2·8-fold by this shift in pH, and this pH-dependent increase was mirrored by the wild-type 3β-HSD activity. The lack of pH dependency of the residual 3β-HSD activity of S124A strongly suggests that no other amino acid in the enzyme, even Ser123, can perform the catalytic function of Ser124. According to these data, the Ser124 residue appears to complete the catalytic triad for human 3β-HSD1.

The effects of the mutations on the isomerase activity are also instructive. The S124A mutant utilizes the isomerase substrate steroid with a 3-fold

Table 3 Dependency of the residual 3β-HSD activities of the purified mutant enzymes on pH

<table>
<thead>
<tr>
<th>Purified enzyme</th>
<th>pH 7·4</th>
<th>% WT1</th>
<th>pH 9·7</th>
<th>% WT1</th>
<th>pH 9·7/pH 7·4 activity ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>3β-HSD1</td>
<td>41·6±1·2</td>
<td>100</td>
<td>111±2·1</td>
<td>100</td>
<td>2·7</td>
</tr>
<tr>
<td>S123A</td>
<td>9·2±0·5</td>
<td>22</td>
<td>25·8±0·4</td>
<td>23</td>
<td>2·8</td>
</tr>
<tr>
<td>S124A</td>
<td>2·2±0·2</td>
<td>5</td>
<td>2·1±0·3</td>
<td>2</td>
<td>1·0</td>
</tr>
</tbody>
</table>

13β-HSD activities were measured in incubations at 27 °C containing 5α-androstane-3β-ol-17-one (100 µM), NAD+ (0·2 mM) and purified enzyme (0·08 mg) in 0·05 M sodium phosphate pyrophosphate buffer, pH 7·4 or pH 9·7. WT1 is human wild-type 3β-HSD1. Values are the means±standard deviations of triplicate determinations of single mutant enzyme preparations.
higher $K_m$ and a 15-fold lower maximal turnover rate ($k_{cat}$) relative to the wild-type enzyme. This kinetic profile of S124A suggests that Ser124 participates in the alignment of 5-androstene-3,17-dione for the isomerase reaction. These data support our model for sequential activities of the bifunctional enzyme: the 3β-HSD activity oxidizes DHEA to form 5-androstene-3,17-dione, which remains bound as the substrate steroid of the isomerase activity (Thomas et al. 1989, 1995). In contrast, the S123A mutant utilizes the isomerase substrate with the same $K_m$ and with 25% of the maximal isomerase activity of the wild-type enzyme, which is the same decrease in $k_{cat}$ measured for the 3β-HSD activity of S123A. The kinetic profile of S123A suggests that the removal of this hydroxyl R-group adjacent to the critical Ser124 hinders the proper alignment of DHEA and 5-androstene-3,17-dione in the enzyme active site to some extent, but Ser123 clearly is not a catalytic residue for 3β-HSD or a key substrate-alignment residue for isomerase.

The coenzyme kinetic profiles suggest that the S123A and S124A mutations alter only substrate utilization by the enzyme. The $K_m$ values of S123A measured for NAD$^+$ as the cofactor for 3β-HSD activity and for NADH as the allosteric activator of isomerase are almost identical to those of wild-type 3β-HSD1. Although the complete lack of 3β-HSD activity by S124A prevented the determination of a $K_m$ value for the utilization of NAD$^+$, there was sufficient isomerase activity remaining to measure a $K_m$ value for NADH that was very similar to that of wild-type isomerase. These kinetic data are consistent with Ser124’s serving as a critical residue for substrate, but not for coenzyme utilization.

The correct prediction that Ser124 functions with Tyr154 and Lys158 to complete the catalytic triad responsible for the human 3β-HSD1 activity validates our homology model of the tertiary structure of the enzyme active site. This validation complements our previous study in which the homology model accurately identified Asp36 as the residue responsible for the strict preference of NAD$^+$ as cofactor for the 3β-HSD activity and of NADH as the activator of isomerase instead of NADP$^+$ or NADPH respectively (Thomas et al. 2003). The homology model can be used to target other key amino acids responsible for subunit interactions of the enzyme dimer, coenzyme...
binding, membrane interactions and differences between human 3β-HSD1 and 3β-HSD2 to characterize further the structure/function of this key steroidogenic enzyme.

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