

Molecular Cloning of Human Type 3 3α -Hydroxysteroid Dehydrogenase That Differs from 20α -Hydroxysteroid Dehydrogenase by Seven Amino Acids

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We have isolated, by screening a λ gt11 human prostatic cDNA library, a cDNA clone that shows after transfection into transformed human embryonal kidney (293) cells high 3α -hydroxysteroid dehydrogenase (3α -HSD) activity that catalyzes efficiently the transformation of dihydrotestosterone to 5α -androstane- $3\alpha,17\beta$ -diol. Chronologically, we name this enzyme type 3 3α -HSD (3α -HSD3). Surprisingly, human 3α -HSD3 shares much higher amino acids sequence identity with human 20α -HSD (97.8%) than with human type 1 and type 2 3α -HSD (81.1 and 85.7% identity, respectively). DNA analysis predicts a protein of 323 amino acids with a molecular mass of 36,844. Alignment of the amino acid sequence of 3α -HSD3 with other related 3α - and 20α -HSDs indicates that 3α -HSD3 shares 68.1, 78.3, and 67.4% identity with rat 3α -HSD and rabbit and rat 20α -HSD, respectively. 3α -HSD3 belongs to the aldo-keto reductase family and like almost all the members of this family preferred NADPH as cofactor. © 1996 Academic Press, Inc.

Three α -hydroxysteroid dehydrogenase (3α -HSD) catalyzes the conversion of 3-keto steroid to 3α -hydroxy compounds (1). The most known activity of 3α -HSD is the transformation of the potent natural androgen dihydrotestosterone (DHT) to its inactive form, 5α -androstane- $3\alpha,17\beta$ -diol (3α -diol). Purified 3α -HSDs are often associated with dihydrodiol dehydrogenase (2). The enzyme activity is distributed in various mammalian tissues including the liver (3), prostate (4), brain (5) and epididymis (6). Although chlordecone reductase cDNA has been (7) cloned before rat 3α -HSD cDNA (8, 9), its identification as human 3α -HSD (3α -HSD1) has been realized recently (10,11). A human type 2 3α -HSD (3α -HSD2) have also been reported (11) recently. Sequence analysis indicates that 3α -HSD belongs to the aldo-keto reductase superfamily and is highly homologous to rat (12,13), rabbit (14) and bovine (15) 20α -HSD, bovine prostaglandin f synthase (16), bovine frog lens ρ -crystallin (17), human aldose (18) and aldehyde (19) reductase, dihydrodiol dehydrogenase/bile acid-binding protein (18) and human 3α -HSD related enzymes (20). The wide variety of highly homologous cDNA species related to 3α -HSD agree with the catalytic specificity of these enzymes that are well recognized to be organ-and species-specific (21, 22). The recent isolation of 6 forms of 3α -HSD related cDNAs from rat liver cDNA library (23) strongly suggests that, these activities are catalyzed by separated enzymes. In human, six isoforms of dihydrodiol dehydrogenase have also been identified by chromatofocusing and gel filtration (22, 24, 25). Four types of human 3α -HSD related cDNA (21), and two dihydrodiol dehydrogenases (20, 25) have been isolated from human liver cDNA library. In this study, we report the isolation and expression of human type 3 3α -HSD that is expressed in the prostate. This enzyme could thus play a major role in the control of androgen level in this androgen dependent-organ, and in prostate cancer.

		GAAACATTTGCTAACCGCCAGTGCAGAAA	-1
h3 α -HSD	3:	ATG GAT TCG AAA TAC CAG TST GTG AMG CTG AAT GAT GGT CAC TTC ATG CCT GTC CTG GGA TTT GGC ACC PAT GCG CCT GCA	81
HARKd	:	Met Asp Ser Lys Tyr Gln Cys Val Lys Leu Asn Asp Gly His Phe Met Pro Val Leu Gly Phe Gly Thr Tyr Ala Pro Ala	27
h20 α -HSD	:
h3 α -HSD	3:	GAG GTT CCT AAA AGT AAA GCT CTA GAG GCC GTC AAA TTG GCA ATA GAA GCC GGG TTC CAC CAT ATT GAT TCT GCA CAT GTT	162
HARKd	:	Glu Val Pro Lys Ser Lys Ala Leu Glu Ala Val Lys Leu Ala Ile Glu Ala Gly Phe His His Ile Asp Ser Ala His Val	54
h20 α -HSD	: Thr..... Arg.....
h3 α -HSD	3:	TAC AAT AAT GAG CAG CAG GTT GGA CTG GCC ATC CGA AGC AAG ATT GCA GAT GGC ACT GTG AAG AGA GAA GAC ATA TTC TAC	243
HARKd	:	Tyr Asn Asn Glu Glu Gln Val Gly Leu Ala Ile Arg Ser Lys Ile Ala Asp Gly Ser Val Lys Arg Glu Asp Ile Phe Tyr	81
h20 α -HSD	:
h3 α -HSD	3:	ACT TCA AAG CTT TGG AGC AAT TCC CAT CGA CCA GAG TTG GTC CGA CCA GCC TTG GAA AGG TCA CTG AAA AAT CTT CAA TTG	324
HARKd	:	Thr Ser Lys Leu Trp Ser Asn Ser His Arg Pro Glu Leu Val Arg Pro Ala Leu Glu Arg Ser Leu Lys Asn Leu Gln Leu	108
h20 α -HSD	: Cys.....
h3 α -HSD	3:	GAT TAT GTT GAC CTC TAT CTT ATT CAT TTT CCA GTG TCT GTA AAG CCA GGT GAG GAA GTG ATC CCA AAA GAT GAA AAT GGA	405
HARKd	:	Asp Tyr Val Asp Leu Tyr Leu Ile His Phe Pro Val Ser Val Lys Pro Gly Glu Glu Val Ile Pro Lys Asp Glu Asn Gly	135
h20 α -HSD	:
h3 α -HSD	3:	AAA ATA CTA TTT GAC ACA GTT GAT CTC TGT GCC ACA TGG GAG GCC ATG GAG AAG TGT AAA GAT GCA GGA TTG GCG AAG TCC	486
HARKd	:	Lys Ile Leu Phe Asp Thr Val Asp Lys Cys Ala Thr Trp Glu Ala Met Glu Lys Cys Lys Asp Ala Gly Leu Ala Lys Ser	162
h20 α -HSD	: Val.....
h3 α -HSD	3:	ATC GGG GTG TCC AAC PTC AAC CAC AGG CTG CTG GAG ATG ATC CTC AAC AAA CCA GGG CTC AAG TAC AAG CCT GTC TCC AAC	567
HARKd	:	Ile Gly Val Ser Asn Phe Asn His Arg Leu Leu Glu Met Ile Leu Asn Lys Pro Gly Leu Lys Tyr Lys Pro Val Cys Asn	189
h20 α -HSD	: Arg..... Lys.....
h3 α -HSD	3:	CAG GTG GAA TGT CAT CCT TAC TTC AAC CAG AGA AAA CTG CTG GAT TTC TGC AAG TCA AAA GAC ATT GTT CTG GTT GCC TAT	648
HARKd	:	Gln Val Glu Cys His Pro Tyr Phe Asn Gln Arg Lys Leu Leu Asp Phe Cys Lys Ser Lys Asp Ile Val Leu Val Ala Tyr	216
h20 α -HSD	:
h3 α -HSD	3:	AGT GCT CTG GGA TCC CAT CCA GAA GAA CCA TGG GTG GAC CCG AAC TCC CCG GTG CTC TTC GAG GAC CGA CTT TGT GCT	729
HARKd	:	Ser Ala Leu Gly Ser His Arg Glu Glu Pro Trp Val Asp Pro Asn Ser Pro Val Leu Leu Glu Asp Pro Val Leu Cys Ala	243
h20 α -HSD	:
h3 α -HSD	3:	TTG GCA AAA AAG CAC AAG GCA ACC CCA GCC CTG ATT GCC CTG CGC TAC CAG CTG CAG CGT GGG GTT GTG CTG GCC AAG	810
HARKd	:	Leu Ala Lys Lys His Lys Arg Thr Pro Ala Leu Ile Ala Leu Arg Tyr Gln Leu Gln Arg Gly Val Val Leu Val Leu Lys	270
h20 α -HSD	:
h3 α -HSD	3:	AGC TAC AAT GAG CAG CGC ATC AGA CAG AAC GTG CAG GTG TTT GAA TTC CAG TTG ACT TCA GAG GAG ATG AAA GCC ATA GAT	891
HARKd	:	Ser Tyr Asn Glu Gln Arg Ile Arg Gln Asn Val Gln Val Phe His Gln Leu Thr Ser Glu Glu Met Lys Ala Ile Asp	297
h20 α -HSD	:
h3 α -HSD	3:	GGC CTA AAC AGA AAT GTG CGA TAT TTG ACC CTT GAT ATT TTT GCT GGC CCC CCT AAT TAT CCA TTT TCT GAT GAA TAT TAA	972
HARKd	:	Gly Leu Asn Arg Asn Val Arg Tyr Leu Thr Leu Asp Ile Phe Ala Gly Pro Pro Asn Tyr Pro Phe Ser Asp Lys Ala Ile Asp	323
h20 α -HSD	: Ile.....
		CATGGAGGSCATTGCAATGAGCTCTCCAGAGGCCCTGCGTCTGGATGGTGACACAGAGGATGGCTCTATGCTGGGACTGGACATCGCCCTCTGGTTAAATCTCT	1079
		CCTGCTGGCCACTCAGTACGCTACAGCTAGGCCCTTCGGCGGAAAGAAAGACATATTTTGTGTTTAAAGAAAATTAATGCTCTCTCCTAAAGATT	1186
		CTTCAAAAAA 1198	

FIG. 1. Nucleotide and deduced amino acid sequence of human 3 α -HSD3 cDNA. The nucleotide sequence is numbered on the right in the 5' to 3' direction with the adenosine of the initiation codon (ATG) designated as +1. The translation stop codon (TGA) is indicated by asterisks. Amino acids different from 20 α -HSD and HARKd are indicated below the main sequence. Dots indicate identical residues.

MATERIALS AND METHODS

Cloning of type 3 3 α -HSD cDNA. A human prostatic λ gt11 cDNA library (Clontech Laboratories Inc., Palo Alto, CA) was screened using a ³²P-labeled 3 α -HSD cDNA probe as described (26). Positive recombinant phage plaques were purified and phage DNA were isolated by centrifugation for 90 min at 105 000 \times g followed by a phenol extraction. DNA inserts were obtained by digestion with EcoRI and subcloned into a pCMV expression vector. Plasmids was prepared using the Qiagen Mega kit (Qiagen, Chatsworth, CA, USA). Double-stranded DNA was sequenced according to the dideoxy chain termination method (27).

Transient expression in 293 cells. Transfection was performed using the calcium phosphate procedure (28) with 10 μ g/10⁶ cells of recombinant plasmid. The cells were initially plated at 10⁴ cells/cm² in 6-well culture plates and grown in Dulbecco's modified Eagle's medium containing 10% (vol/vol) fetal bovine serum supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 100 IU penicillin/ml, and 100 μ g streptomycin sulfate/ml.

Assay of enzymatic activity. Determination of activity was performed by adding 0.1 μ M of the indicated ¹⁴C-labeled substrate to a freshly changed culture medium in a 6-well culture plate. After incubation for 1 h, steroids were extracted and separated by thin layer chromatography (TLC) as previously described (29).

RESULTS

Sequence analysis of 3 α -HSD3. Screening about 500 000 recombinant phage plaques allowed to isolate 7 positive clones. Two of the longest clones were sequenced. The DNA sequence contains 30 and 228 nucleotides in the 5' and 3' noncoding region, respectively (Fig.1). The

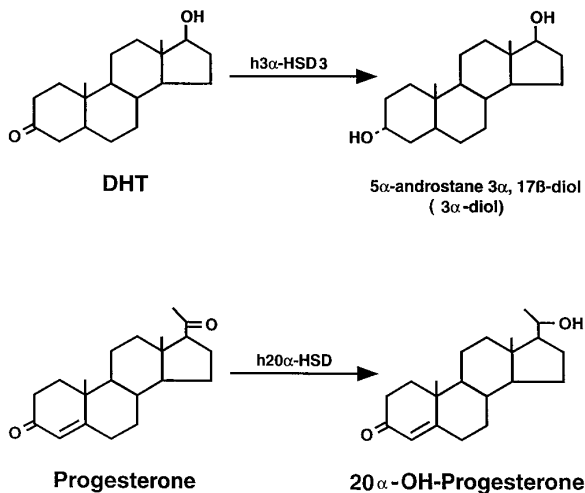


FIG. 2. Example of reaction catalyses by 3 α -HSD and 20 α -HSD.

putative polyadenylation signal aataaa is located 190 nucleotides downstream the TAA stop codon. The coding sequence predicts a protein of 323 amino acids with a calculated molecular mass of 36 844 Da. As illustrated in Figure 1, 3 α -HSD3 contains 7 amino acid differences with 20 α -HSD and 3 amino acid differences with HARKd reported by Qin et al. (19) as a human aldoketo related sequence but the activity is not determined. The importance of these 3 amino acids changes is unknown, but could be crucial since two basic amino acids are replaced by two acidic residus (Lys 179 Glu and Lys 185 Glu). In addition, in the aldoketo reductase superfamily, it is frequently observed that clones with highly conserved DNA sequences could possess different substrate specificity. A typical example is 3 α -HSD3 and

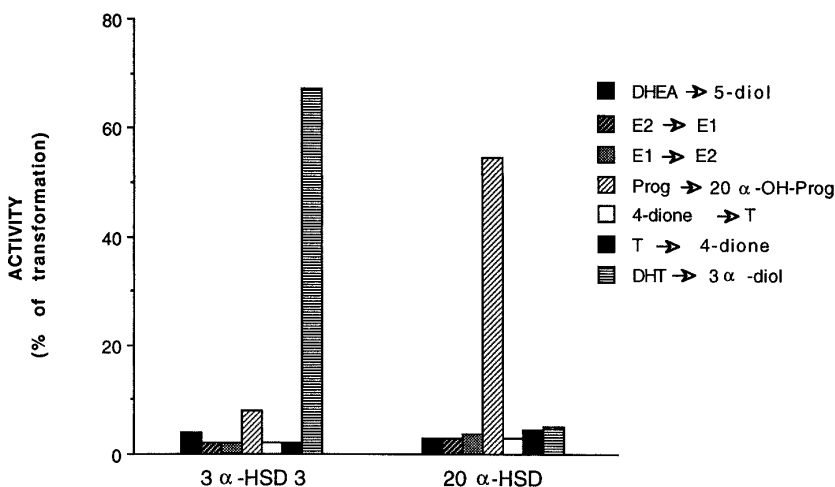


FIG. 3. Substrate specificity of transfected human 3 α -HSD3 and 20 α -HSD. pCMV-3 α HSD3 and pCMV-20 α -HSD vectors were transfected into 293 cells and examined for their ability to transform DHEA, E1, E2, P, Δ 4, T, and DHT. Experimental conditions were as described under Materials and Methods. Data are expressed as the mean of duplicate determinations.

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h3αHSD3 MDSKYQCVKLNDGHFMPVLGFGTYAPAEVPKSKALEAVKLAIEAGFHHIDS AHVYNNEEQVGLAIRSKIADG SVKREIDIFYT SKLWSNSHRPELVRPALERSLKNLQLDY 110
h3αHSD2 ---Q-----P---R---VT-----R-----L-----E-----TF---Q---N---KA---
h3αHSD1 -P--R-E-----P---RNR-V-VT-----R-----YL-----CTFFQ-QM-Q---S---K---
r3αHSD ---ISLR-A---N-I-----TV-EK-A-DEVIK-T-I--DN--R-F---YL-EV-E-Q-----E-T---TF---TC--KT--ST---
h20αHSD -----T-----R-----L-----C-----
rb20αHSD ---F-R-A-S---I-----E-----M-T-I--D--R---YF-K--KE-----T-----CTF---S--D---
r20αHSD -N-I-KM-----SI-----TE-NL-K-SM-ST-I--DV--R---CS-L-Q--EI-Q--V--E-T-----T-----S--N--R--N---
b20αHSD .....AK--I--L--WKSPPGKYTE-VK-AIDLGY...R--C---Q--NE---LQA-LQEKV---L-IV---CTY-DKD--KG-CQKT--SD-K---

h3αHSD3 VDLYLIHFVSVKPGEEVI PKDENGKILFDTVDLCAWEAMECKDAGLAKSIGVSNFNHRLLEMI LNK PGLKYKPVNCVQVECHPYFNQRKLLDFCKSKDIVLVAYSALG 220
h3αHSD2 -----S-M-L---LS-T---VI--I---T-----R-Q--I-----RS-----
h3αHSD1 ---L--MAL---TPL---VI-----S---V-----C-Q-----L--S-----H---
r3αHSD ---IL---MALQ--DIFF-R--H--L-E---I-D--V-----C-Q-R-----L-L-G-M--Y-----I--S-CT--
h20αHSD -----V-----R-Q-----
rb20αHSD ---IL--TAL--V-I--T--H--AI---I---V---R-Q-----L--G--E--G-----
r20αHSD ---L--M-L--D-LL-Q--H-NLIL---D--V-----R-Q--K-----HR-----L-L-S---AY--MN---G---
b20αHSD L---L-W-TGF--KDFL-L--D-NVIPSEK-FVD--TV--ELV-E--V-A-----LQV-K-----AV--I---LT-E-IQY-N--G--VT--P--

h3αHSD3 SHREEPVDWPNFVLLDEPVLCAKAKHKRTPALIALRYLQQRGVVVLAKSYNEQRIRQNVQVFEFQLTSEEMKAIDGLNRNRYLTLDFAGPPNYPFSDEY 323
h3αHSD2 -Q-DKR-----D-----D--LH-FNS-S-ASH---Y---
h3αHSD1 TQ-HKL-----E-I-----D--VLN---NY--VVM-FLMDH-D---
r3αHSD -S-DKT--QK---D---I--Y-Q---V-----P-IR-F-AK--KELT-----A--D-V-L-----F--NNAKY--DH--H--T--
h20αHSD -----
rb20αHSD ---PE--QSA-----LIG---QQ-----I---FT-K--KE-I-----P--D-VV--S---F--V-A-FAI-H-----
r20αHSD TQ-YKYCINEDT---D--I--TM---YQ-----E--I-T-V--F--E--E-L--D---A-DD-ELL-N-D--L--FPANM-KAH--F-----
b20αHSD -.PDR--AK-ED-SI-----RIK-I-D-YNK-T-QVLI-FPI--NLI-IP--VTPE--AE-F--D-E-DK-D-NT-LSY--DW-ACA-VSCASHRD---HE-F

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FIG. 4. Alignment of human 3α -HSD3 and related sequences. The deduced amino acid sequence of human 3α -HSD3 was aligned with amino acid sequences of human 3α -HSD1 and 2 and rat 3α -HSD as well as rabbit (rb), rat (r), and bovine (b) 20α -HSD. Amino acid sequences are given in the conventional single letter code and numbered on the right. Dashes (-) and dots (.) represent identical and missing amino acid residues, respectively.

20α -HSD that catalyze the reduction of DHT at position 3 and progesterone at position 20, respectively, (Fig. 2) are different only by 7 amino acids.

Activities expressed by transfection in 293 cells. The pCMV- 3α -HSD3 and pCMV- 20α -HSD expression vectors were transiently transfected into 293 cells and the activities analyzed for the ability to catalyze the transformation of DHEA, estradiol (E2), estrone (E1), progesterone (Prog), 4-androstenedione (4-dione), testosterone (T), and DHT. As illustrated in Fig. 3, the expressed 3α -HSD3 is highly selective for the transformation of DHT to 3α -diol while 20α -HSD activity is more selective for the conversion of Prog to 20α -hydroxyprogesterone (20α -OH-Prog).

Homology. Alignment of amino acid sequences of human 3α -HSD3 with human 3α -HSD1 (7, 21) and 2 (11), rat 3α -HSD (8, 9), as well as human rabbit (14), rat (12, 13) and bovine 20α -HSD (15) (Fig. 4) indicates that they are highly homologous, sharing 81.1, 85.7, 68.1, 97.8, 78.3 and 49.5% identity respectively

DISCUSSION

In this report, we describe the cloning and characterization of a human 3α -HSD3 from human prostatic cDNA library. This enzyme catalyzes the inactivation of the most potent natural androgen, DHT, to 3α -diol, and thus plays a pivotal role in the regulation of active androgen level in peripheral tissues, especially the prostate, which is the most known androgenic organ and its related-diseases, namely benign prostatic hyperplasia and prostate cancers. Recent study from 5α -reductase knockout mice (30) indicates that 3α -diol, a metabolite of DHT produced by 3α -HSD, plays a major role in parturition. The exact hormonal role of 3α -

diol is not yet defined, but the study suggests that 3α -diol and thus 3α -HSD could play a more crucial role in female physiology than expected. 3α -hydroxysteroid dehydrogenase belong to the aldo-keto reductase superfamily (31) that also includes aldose reductase (18), aldehyde reductase (19), chlordecone reductase (7,10), prostaglandin F synthase (16), eye lens ϵ -crystallin (17). A second most important family of hydroxysteroid dehydrogenases is the short chain alcohol dehydrogenase (32) which is characterized by the presence of a Tyr-X-X-X-Lys sequence at the active site. Rat 3α -HSD (31) contains both, aldo-keto reductase and short chain alcohol dehydrogenase signatures, suggesting a possible implication of Tyr (205)-X-X-X-Lys(209) sequence in the catalytic mechanism of 3α -HSD activity. However, in human 3α -HSD3 such sequence is absent, due to a change of a Tyr at position 205 to a Phe. This observation clearly indicates that the short chain alcohol type mechanism is not involved in the 3α -HSD activity. Although 3α -HSD3 differs from 20α -HSD only by 7 amino acids, these two enzymes exert their activities at diametrically opposite position in the steroid nucleus (Fig. 2). These characteristics offers an unique opportunity to gain better knowledges about a structure-function relationship of these two enzymes using site directed mutagenesis.

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