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Human dehydrogenase/reductase (SDR family) member 11 is a novel type of 17β-hydroxysteroid dehydrogenase

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ABSTRACT

We report characterization of a member of the short-chain dehydrogenase/reductase superfamily encoded in a human gene, *DHRS11*. The recombinant protein (DHRS11) efficiently catalyzed the conversion of the 17-keto group of estrone, 4- and 5-androstenes and 5α-androstanes into their 17β-hydroxyl metabolites with NADPH as a coenzyme. In contrast, it exhibited reductive 3β-hydroxysteroid dehydrogenase activity toward 5β-androstanes, 5β-pregnanes, 4-pregnenes and bile acids. Additionally, DHRS11 reduced α-dicarbonyls (such as diacetyl and methylglyoxal) and alicyclic ketones (such as 1-indanone and loxoprofen). The enzyme activity was inhibited in a mixed-type manner by flavonoids, and competitively by carbenoxolone, glycyrrhetinic acid, zearalenone, curcumin and flufenamic acid. The expression of DHRS11 mRNA was observed widely in human tissues, most abundantly in testis, small intestine, colon, kidney and cancer cell lines. Thus, DHRS11 represents a novel type of 17β-hydroxysteroid dehydrogenase with unique catalytic properties and tissue distribution.

1. Introduction

The *DHRS11* gene is located on human chromosome 17q12, and its encoded 260-amino acid protein is currently annotated as "dehydrogenase/reductase SDR family member 11 (DHRS11)" in the HUGO gene nomenclature database (http://www.genenames.org). The cDNA for the protein was originally identified as that for one of human secreted and transmembrane proteins [1]. DHRS11 belongs to the short-chain dehydrogenase/reductase (SDR) superfamily [2], which encompasses numerous enzymes that play roles in the metabolism of lipids, carbohydrates, vitamins, drugs and xenobiotics. The SDR superfamily is one of the largest protein superfamilies with over 47,000 members, in which DHRS11 is classified as SDR24C1 [2,3]. A crystal structure of tetrameric DHRS11 complexed with $NADP^+$ and acetic acid was previously deposited in the RCSB protein data bank (PDB-ID: 1XG5), and suggested that the enzyme is a soluble oxidoreductase using NADP(H) as coenzymes. However, DHRS11 has not yet been enzymatically characterized. According to current protein databases, DHRS11-like proteins sharing more than 90% amino acid sequence identity are predicted in genomic analyses of nonhuman primates, horses, pigs, cows, dogs, rabbits, rats and mice. This suggests an essential function for human DHRS11 and its orthologs in other animals.

In this study, we analyzed the enzymatic properties of recombinant DHRS11, and show that the enzyme is a novel type of 17β-hydroxysteroid dehydrogenase (HSD) different from known 14 types of 17β-HSD (17β-HSD1 – 17β-HSD14) [4,5]. The expression of DHRS11 in human tissues and cancer cells was also examined by reverse transcription (RT)-PCR, and a spliced isoform of DHRS11 (spDHRS11) lacking the exon 5 was identified.

2. Materials and methods

2.1. Materials

Steroids were obtained from Sigma-Aldrich and Steraloids; 4-oxo-2-nonenal and prostaglandins were from Cayman Chemical; and pCold I expression vectors and total RNAs of human tissues were from Takara Bio.

Abbreviations: SDR, short-chain dehydrogenase/reductase; DHRS11, dehydrogenase/reductase (SDR family) member 11; spDHRS11, spliced isoform of DHRS11; HSD, hydroxysteroid dehydrogenase; RT, reverse transcription; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; bp, base pair..

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2.2. Construction of expression plasmid

The cDNA for DHRS11 (NCBI accession no. NM_024308.3) was isolated from the total RNA sample of human brain by RT-PCR. The RNA and DNA techniques were performed as described by Sambrook et al. [6]. PCR was performed using *Pfu* DNA polymerase (Stratagene) and primers, 5'-ttttcatatgatggccaggcccggcat-3' (forward) and 5'-ttttgtcgacactaggtcacctgctccgt-3' (reverse), which contain underlined *Nde*I and *Sal*I sites, respectively. The PCR products were purified, and ligated into the pCold I vectors that had been digested with *Nde*I and *Sal*I. The insert of the cloned cDNA was sequenced by using a Beckman CEQ8000XL DNA sequencer, to confirm that the 260-amino acid sequence of DHRS11 fused to the N-terminal 6-His tag is encoded.

2.3. Production of recombinant enzymes

Recombinant DHRS11 was expressed in *Escherichia coli* BL21 (DE3) pLysS cells (Invitrogen) transformed with the expression plasmids harboring the cDNA as described previously [7]. The enzyme was purified to homogeneity from the cell extract using a nickel-charged Sepharose 6FF resin (Amersham Biosciences) according to the manufacturer's manual, and its purity was analyzed by SDS-PAGE according to standard procedures. Homogeneous recombinant 17β-HSD5 was prepared as described previously [8]. Protein concentration was determined by Bradford's method using bovine serum albumin as the standard [9].

2.4. Assay of enzyme activity

Reductase and dehydrogenase activities were assayed by measuring the rate of change in NADPH absorbance (at 340 nm) and its fluorescence emission (at 455 nm with an excitation wavelength of 340 nm), respectively, in a 2-ml reaction mixture containing 0.1 M potassium phosphate (pH 7.0), 0.1 mM NADPH or 0.5 mM $NADP^+$, substrate and enzyme. The concentrations of compounds tested as substrates were 5 or 50 μM (for steroids) and 0.1 or 1 mM (for other carbonyl compounds). The reductase activity for all-*trans*-retinal was measured as reported by Parés and Julià [10]. pH dependency of the reaction was determined with 0.1 M potassium phosphate buffers (pH 5.8–8.0), and heat stability of DHRS11 was assessed by assaying the activity after 5-min incubation of the enzyme solution (0.1 mg/ml in 10 mM potassium phosphate buffer, pH 7.0 containing 20% glycerol) at different temperatures. The apparent K_M and V_{max} values were determined over a range of five substrate concentrations at a saturating concentration of coenzyme by fitting the initial velocities to the Michaelis–Menten equation. After the IC_{50} (inhibitor concentrations required for 50% inhibition) values were determined in $NADP^+$-linked oxidation of 10 μM 5α-androstane-3β,17β-diol, the kinetic studies in the presence of three concentrations of an inhibitor ($0.5–2 \times IC_{50}$) were carried out in a similar manner in both 5α-androstane-3β,17β-diol oxidation and NADPH-linked diacetyl reduction. The inhibitor constants, K_{is} and K_{ii}, were calculated from the replots of the slopes and intercepts, respectively, of the double reciprocal plot versus the inhibitor concentration. The kinetic constants are expressed as the means of two or three independent determinations. One milliunit (mU) of enzyme activity was defined as the enzyme amount that catalyzes the oxidation or formation of 1 nmol of NADPH per min at 37 °C.

2.5. Product identification

To identify reaction products, reduction was conducted in a 4.0-ml system containing 0.2 mM NADPH or 1 mM $NADP^+$, 50 μM steroidal substrate, enzyme (100 μg) and 0.1 M potassium phosphate (pH 7.0). The substrate and products were extracted into 8 ml ethyl acetate 30 min after the reaction was started at 37 °C. The steroidal products were identified by TLC [11,12], in which the products and co-chromatographed authentic steroids were visualized by spraying with ethanol/H_2SO_4 (1:1, v/v) solution and heating at 110 °C for 1 h.

2.6. Tissue distribution analysis

Human cultured cells (A172, MCF7, U937 and C32TG) were obtained from ATCC, cultured in the media recommended by the manufacturer, and their total RNAs were prepared [6]. The total RNAs from the human tissues and cells were subjected to RT-PCR with KOD DNA polymerase (Toyobo) and the above primers used in amplification of the cDNA for DHRS11. The cDNA for human β-actin was amplified as an internal control using specific primers (Toyobo). The PCR products were separated by agarose gel electrophoresis, and revealed with ethidium bromide [6].

3. Results and discussion

3.1. Properties of recombinant DHRS11

3.1.1. Optimal pH, coenzyme specificity and heat stability

The purified enzyme showed a single 30-kDa protein band on the SDS-PAGE analysis. To assess the enzymatic properties of recombinant DHRS11, we tested various carbonyl compounds as the substrates using NADPH as the coenzyme at 37 °C, and found that the enzyme reduces 17-ketosteroids (Table 1). Using 50 μM dehydroepiandrosterone (DHEA), optimal pH and coenzyme specificity of DHRS11 were analyzed. The reductase activity showed an optimal pH at 7.0, where no NADH-linked reductase activity was observed. The K_M value for NADPH was 1.1 μM. The strict specificity for NADPH may be attributed to strong hydrogen bond interactions between the 2'-phosphate of $NADP^+$ with residues (Ser20, Arg43 and Thr44) in the crystal structure of DHRS11 (PDB-ID: 1XG5). In the heat stability analysis, the enzyme was stable up to 50 °C, and 70% of its activity was inactivated at 55 °C.

Table 1
Substrate specificity for ketosteroids.

Substrate	$K_{M\ (\mu M)}$	$V_{max\ (mU/mg)}$	V_{max}/K_M (mU/mg/μM)
[17-Ketosteroids]			
Estrone	0.7	43	61
5α-Androstan-3α-ol-17-one	1.3	42	32
5α-Androstan-3β-ol-17-one	2.2	49	22
DHEA	11	118	11
DHEA sulfate	12	60	5.0
4-Androsten-3α-ol-17-one	19	43	2.3
[3-Ketosteroids]			
5β-Pregnan-20β-ol-3-one	0.7	44	63
Dehydrolithocholic acid	1.1	44	40
5β-Cholanic acid-3,7-dione	5.2	85	16
20α-Hydroxyprogesterone	5.1	40	7.8
Taurodehydrocholic acid	44	200	4.5
Dehydrocholic acid	29	119	4.1
5β-DHT	102	389	3.8
[3,17-Diketosteroids]			
5β-Androstane-3,17-dione	5.2	62	12
4-Androstene-3,17-dione	4.6	52	11
5α-Androstane-3,17-dione	12	57	4.8
[3,20-Diketosteroids]			
5β-Pregnane-21-ol-3,20-dione	2.1	49	23
Progesterone	3.4	36	11
5β-Pregnane-3,20-dione	15	123	8.2