

Complete amino acid sequence of human placental 17 β -hydroxysteroid dehydrogenase deduced from cDNA

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cDNA clones for 17 β -hydroxysteroid dehydrogenase (17-HSD; EC 1.1.1.62) were isolated from a placental λ gt11 expression library using polyclonal antibodies against placental 17-HSD. The largest cDNA contained 1325 nucleotides, consisting of a short 5'-noncoding segment, a coding segment of 987 nucleotides terminated by a TAA codon, and a 329 nucleotide long 3'-noncoding segment. The open reading frame encoded a polypeptide of 327 amino acid residues with a predicted M_r of 34853. The amino acid sequence of 23 N-terminal amino acids determined from purified 17-HSD agreed with the sequence deduced from cDNA. The deduced amino acid sequence also contained two peptides previously characterized from the proposed catalytic area of placental 17-HSD.

Estrogen; 17-Hydroxysteroid dehydrogenase; Molecular cloning; Nucleotide sequence; Amino acid sequence

1. INTRODUCTION

17 β -Hydroxysteroid dehydrogenase (17-HSD; EC 1.1.1.62) catalyzes interconversion of neutral and phenolic 17-hydroxy and 17-ketosteroids. It is present in steroid-forming tissues and also in certain steroid target tissues such as human endometrium [1,2]. In the latter tissue the activity of this enzyme is regulated by progestins [3–5], and it possibly has a role in regulating the exposure of the endometrium to estrogen action [1,2,4]. A similar role for this enzyme in breast tissue has been proposed [6]. Hence detailed information of the properties and regulation of this enzyme may be central for our understanding of the hormone dependency of endometrial and breast cancer.

17-HSD has been purified to homogeneity from term placental tissue in this [7] and other [8,9] laboratories. In its native form, placental 17-HSD is composed of two similar if not identical subunits

having an M_r of about 34000 (see [10]). Preliminary data suggest that human endometrium contains a protein showing similar immunological properties as the one purified from placental tissue [7].

As part of our studies directed towards understanding the regulation of 17-HSD in steroid target tissues, we report the cloning of the cDNA of human placental 17-HSD and provide the complete amino acid structure of the enzyme deduced from the cDNA.

2. MATERIALS AND METHODS

2.1. Materials

A human placental cDNA library in the λ gt11 expression vector was obtained from Clontech (Palo Alto, CA). Restriction endonucleases, proteinase K, DNase I, RNase A, and an M13 cloning kit were obtained from Boehringer Mannheim (Mannheim, FRG). A SequenaseTM sequencing kit was purchased from the United States Biochemical Corporation (Cleveland, OH) and [³⁵S]dATP was from Amersham (Amersham, England). Sequence-derived oligonucleotide primers (17 bases) were synthesized with a DNA synthesizer (Applied Biosystems, Foster City, CA). Nitrocellulose filters were obtained from Schleicher and Schuell (Dassel, FRG).

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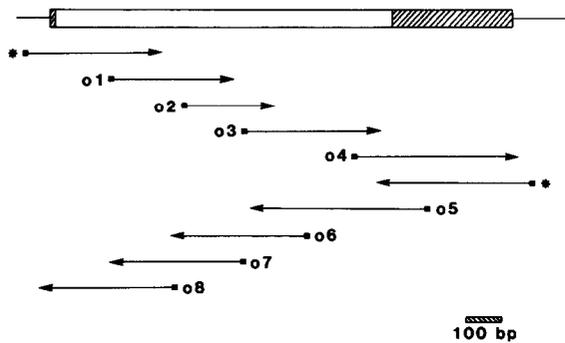


Fig.1. The sequencing strategy. The white area represents the coding sequence of 17-HSD and the hatched areas the 5'- and 3'-untranslated regions. M13 universal primer (*) and sequence specific oligonucleotides (o1-o8) were used to determine the complete sequence of both strands. The bar gives the approximate scale.

2.2. 17-HSD antiserum

Polyclonal antiserum against highly purified placental 17-HSD was raised in rabbits [7]. Antiserum was absorbed with *E. coli* strain Y1090r⁻ lysate to remove antibodies that recognize *E. coli* antigens [11].

2.3. Antibody screening of λ gt11 library

About 5×10^5 plaque-forming units (pfu) of recombinant phages from a placental cDNA library were screened with an anti-17-HSD-antiserum, diluted 1:200 in Tris-buffered saline containing 20% fetal calf serum [11], with the exception that positive plaques were detected by using protein A-peroxidase and 4-chloro-1-naphthol as a substrate. Plaques were further purified by three to five cycles of screening at low plaque density with antiserum until all phages produced positive signals. Eleven positive phage clones were amplified, digested with *EcoRI*, the sizes of the inserts were determined and they were purified.

2.4. cDNA sequence analyses

The *EcoRI* inserts from the λ gt11 clones were subcloned into an M13mp18 vector [12]. The DNA sequence was determined by the dideoxy chain termination method of Sanger [13] using SequenaseTM-enzyme [14] and the universal primer as well as synthetic oligonucleotides for priming (fig.1). The two longest inserts, one later found to have the whole coding area and the other lacking about 40 bases from the 5'-end, were sequenced.

2.5. Protein sequencing

Amino acid sequence analysis of 23 N-terminal amino acids from purified placental 17-HSD was performed with an Applied Biosystems model 477A pulse liquid protein/peptide sequencer with a 120A PTH analyzer (Applied Biosystems, Foster City, CA).

3. RESULTS AND DISCUSSION

A human placental λ gt11 library was screened with antiserum raised against human placental 17-HSD. As a result of screening 5×10^5 individual plaques, 15 clearly positive plaque clones were identified. Eleven plaques remained positive upon three to five rounds of rescreening and they were amplified and digested with *EcoRI*. The sizes of the inserts varied from 0.8 to 1.3 kb and the two longest cDNA inserts (1.2 and 1.3 kb) were sequenced. To compare cDNA-deduced amino acid sequence to the sequence of native placental protein, 23 amino acids from the N-terminus of the purified enzyme were analyzed and the sequences were found to be identical.

The 1.3 kb insert, named λ HSD2A, contained an open reading frame of 987 bp from the initiation codon ATG to the stop codon TAA, and additionally 9 nucleotides of the 5'- and 329 nucleotides of the 3'-noncoding segment (fig.2). The sequence AATAAAA, thought to be required for polyadenylation [15], was located 16 bp upstream from the poly(A) tract. The 1.2 kb insert, named λ HSD5B, contained a sequence from nucleotide 40 to nucleotide 1252 of λ HSD2A, suggesting that these two cDNAs were derived from the same RNA-pieces. The codon ATG, preceding the codon of the first amino acid residue, is obviously the initiator codon, since the sequence surrounding it conforms to Kozak's consensus sequence [16].

Three peptide sequences derived from placental 17-HSD have been published previously. The sequence of five N-terminal amino acids determined by Burns et al. [17] differs in the second amino acid; according to our data it is arginine instead of glutamate. Nicolas and Harris [18] analyzed a tryptic heptadecapeptide labeled to a cysteine residue. This peptide, suggested to be part of the coenzyme binding site in 17-HSD [18], is located between amino acid residues 51 and 67. The peptide of 20 amino acid residues sequenced by Murdock et al. [19] is located between residues 204 and 223 (fig.2). The hydrophobicity profile of the

Fig.2. Nucleotide and deduced amino acid sequence of the subunit of human placental 17-HSD. The numbering of amino acids is based on the analysis of the N-terminal sequence of the purified protein (underlined). Two known internal peptides are also underlined (solid line). The potential cAMP-dependent kinase phosphorylation site (basic-basic-X-Ser) is indicated with a dot. The putative polyadenylation signal site (AATAAAA) is marked with a dashed line.

AGT CTC ACC ATG	GCC CGC ACC GTG GTG CTC ATC ACC GGC TGT TCC TCG GGC ATC GGC CTG	60
	Ala Arg Thr Val Val Leu Ile Thr Gly Cys Ser Ser Gly Ile Gly Leu	16
CAC TTG GCC GTA	CGT CTG GCT TCA GAT CCA TCC CAG AGC TTC AAA GTG TAT GCC ACG TTG	120
His Leu Ala Val	Arg Leu Ala Ser Asp Pro Ser Gln Ser Phe Lys Val Tyr Ala Thr Leu	36
AGG GAC CTG AAA	ACA CAG GGC CGG CTG TGG GAG GCG GCC CGG GCC CTG GCA TGC CCT CCG	180
Arg Asp Leu Lys	Thr Gln Gly Arg Leu Trp Glu Ala Ala Arg Ala Leu Ala Cys Pro Pro	56
GGA TCC CTG GAG	ACG TTG CAG CTG GAC GTA AGG GAC TCA AAA TCC GTG GCC GCT GCC CGG	240
Gly Ser Leu Glu	Thr Leu Gln Leu Asp Val Arg Asp Ser Lys Ser Val Ala Ala Ala Arg	76
GAA CGC GTG ACT	GAG GGC CGC GTG GAC GTG CTG GTG TGT AAC GCA GGC CTG GGC CTG CTG	300
Glu Arg Val Thr	Glu Gly Arg Val Asp Val Leu Val Cys Asn Ala Gly Leu Gly Leu Leu	96
GGG CCG CTG GAG	GCG CTG GGG GAG GAC GCC GTG GCC TCT GTG CTG GAC GTG AAT GTA GTA	360
Gly Pro Leu Glu	Ala Leu Gly Glu Asp Ala Val Ala Ser Val Leu Asp Val Asn Val Val	116
GGG ACT GTG CGG	ATG CTG CAG GCC TTC CTG CCA GAC ATG AAG AGG CCG GGT TCG GGA CGC	420
Gly Thr Val Arg	Met Leu Gln Ala Phe Leu Pro Asp Met Lys Arg Arg Gly Ser Gly Arg	136
GTG TTG GTG ACC	GGG AGC GTG GGA GGA TTG ATG GGG CTG CCT TTC AAT GAC GTT TAT TGC	480
Val Leu Val Thr	Gly Ser Val Gly Gly Leu Met Gly Leu Pro Phe Asn Asp Val Tyr Cys	156
GCC AGC AAG TTC	GCG CTC GAA GGC TTA TGC GAG AGT CTG GCG GTT CTG CTG CTG CCC TTT	540
Ala Ser Lys Phe	Ala Leu Glu Gly Leu Cys Glu Ser Leu Ala Val Leu Leu Leu Pro Phe	176
GGG GTC CAC TTG	AGC CTG ATC GAG TGC GGC CCA GTG CAC ACC GCC TTC ATG GAG AAG GTG	600
Gly Val His Leu	Ser Leu Ile Glu Cys Gly Pro Val His Thr Ala Phe Met Glu Lys Val	196
TTG GGC AGC CCA	GAG GAG GTG CTG GAC CGC ACG GAC ATC CAC ACC TTC CAC CGC TTC TAC	660
Leu Gly Ser Pro	Glu Glu Val Leu Asp Arg Thr Asp Ile His Thr Phe His Arg Phe Tyr	216
CAA TAC CTC GCC	CAC AGC AAG CAA GTC TTT CGC GAG GCG GCG CAG AAC CCT GAG GAG GTG	720
Gln Tyr Leu Ala	His Ser Lys Gln Val Phe Arg Glu Ala Ala Gln Asn Pro Glu Glu Val	236
GCG GAG GTC TTC	CTC ACC GCT TTG CGC GCC CCG AAG CCG ACC CTG CGC TAC TTC ACC ACC	780
Ala Glu Val Phe	Leu Thr Ala Leu Arg Ala Pro Lys Pro Thr Leu Arg Tyr Phe Thr Thr	256
GAG CGC TTC CTG	CCC CTG CTG CGG ATG CGC CTG GAC GAC CCC AGC GGC TCC AAC TAC GTC	840
Glu Arg Phe Leu	Pro Leu Leu Arg Met Arg Leu Asp Asp Pro Ser Gly Ser Asn Tyr Val	276
ACC GCC ATG CAC	CGG GAA GTG TTC GGC GAC GTT CCG GCA AAG GCC GAG GCT GGG GCC GAG	900
Thr Ala Met His	Arg Glu Val Phe Gly Asp Val Pro Ala Lys Ala Glu Ala Gly Ala Glu	296
GCT GGG GGC GGG	GCC GGG CCT GGG GCA GAG GAC GAG GCC GGG CGC AGT GCG GTG GGG GAC	960
Ala Gly Gly Gly	Ala Gly Pro Gly Ala Glu Asp Glu Ala Gly Arg Ser Ala Val Gly Asp	316
CCT GAG CTC GGC	GAT CCT CCG GCC GCC CCG CAG TAA AGG CTT CCT CAG CCG CTG TCT CCC	1020
Pro Glu Leu Gly	Asp Pro Pro Ala Ala Pro Gln ***	327
GCG CCC TTC TTT	GTC CCC TGG GTC TGT GTG GTC CCT GGG GAT GGG GCG GCG GTA GCA GCT	1080
GTG GGT GGC TAA	TTA AGA TAG ATC GCG TTA GCC AGT TTT ACC AGC GCA GCT AGG CGC GAT	1140
GGC GTC GCC TGT	AAT GCC AGC GCT TTG GGA GGC GGA GGC AGG AGG ATC GCT CAA GCC CCG	1200
GAG TTG GAG ACC	AGC CAG AGC AAC ACA GTG AGA CCC CCA TCT CTA CAA <u>AAA TAA AGA AAA</u>	1260
TTT AAA AAT CAA	AAA	1320
AAA AA		1325

deduced amino acid sequence, showing a hydrophobic pocket in this area (fig.3), supports the conclusion of Murdock et al. [19] that this sequence contains the substrate binding site. This kind of structure is shared by several proteins interacting with steroid hormones [20].

The amino acid sequence deduced from cDNA is predicted to contain 327 amino acid residues with a calculated M_r of 34853. This value is in agreement with values obtained in SDS-PAGE using purified enzyme (see [10]). Of the 327 amino acids, there are 6 cysteine residues, 40 acid residues, 32 basic residues, and 20 aromatic residues (table 1). The amino acid composition calculated from the cDNA-deduced sequence agrees well with that obtained by direct chemical analysis of the purified protein [17,21,22].

The amino acid sequence of 17-HSD we have deduced from cDNA does not contain potential Asn-X-Ser/Thr N-glycosylation sites [23], confirming the finding that 17-HSD is not a glycoprotein [10,24]. One potential cyclic AMP-dependent phosphorylation site (base-base-X-Ser) [25] is presented in the 17-HSD sequence at position 131-134 (fig.1). Varying degrees of possible phosphorylation of 17-HSD may explain the microheterogeneity found in isoelectric focusing of the purified enzyme [26].

Table 1

The amino acid composition of the subunit of 17-HSD

	Residues per polypeptide deduced from cDNA	Burns [17]	Jarabak [21] ^a	Nicolas [22] ^a
Ala	38	36	54	54
Arg	23	22	37	34-36
Asn	5			
Asp	17	21	40	54-56
Cys	6	6	10	12
Gln	8			
Glu	23	28	51	68-70
Gly	32	31	50	48
His	7	7	11	14
Ile	4	4	7	20
Leu	42	41	66	66
Lys	9	10	20	30
Met	6	4	5	12
Phe	13	13	20	24
Pro	20	19	31	40
Ser	19	19	34	40
Thr	16	16	29	32-34
Trp	1	1	2	4
Tyr	6	6	9	16
Val	32	32	47	52
N =	327	316	523	624 ± 4

^a Residues per enzyme molecule assuming that 17-HSD is composed of two identical subunits

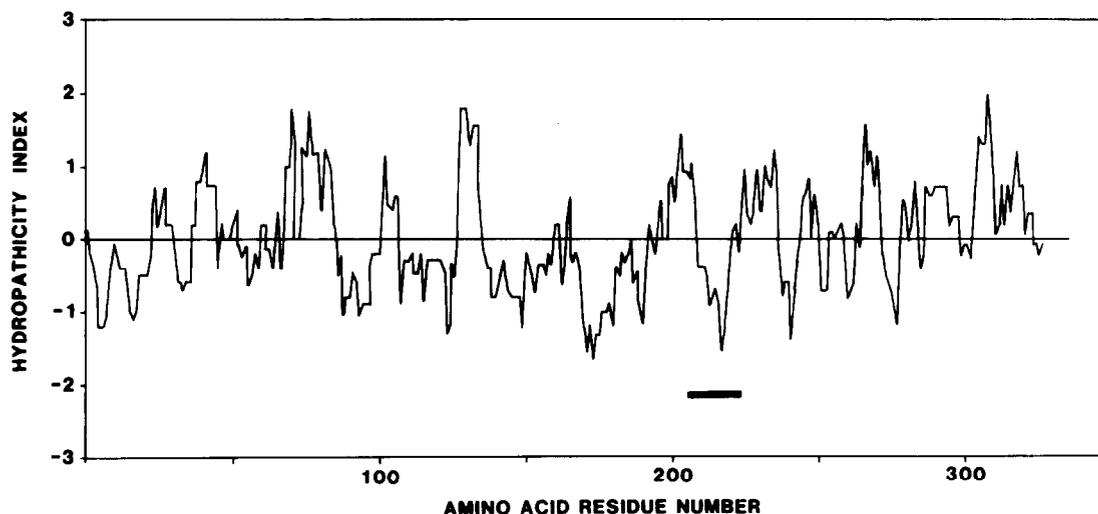


Fig.3. Hydropathicity profile of the deduced amino acid sequence of human placental 17-HSD, determined according to Hopp and Woods [27]. The area thought to be involved in substrate binding is indicated by a bar. Negative values indicate hydrophobic regions of the protein.

A computer homology search of the cDNA of 17-HSD and the amino acid sequence of the derived protein was performed against The Genetic Sequence Data Bank (GenBank) and The National Biomedical Research Foundation (NBRF) Protein Data Bank. No significant homology with other proteins or DNA sequences was found.

Our cloning of the cDNA of human placental 17-HSD will be followed by studies on the gene structure, which should facilitate studies on the hormonal regulation of this enzyme in a number of hormone-responsive tissues, such as human endometrium and breast epithelium. In these tissues, 17-HSD may have important physiological regulator functions, and it may also be important for our understanding of the behavior of cancer in these tissues.

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