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Molecular cloning and sequence analysis of cDNA encoding human cholesterol 7α-hydroxylase

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A complete cDNA clone encoding human cholesterol 7α-hydroxylase has been isolated using a rat P-450$_{ch7\alpha}$ cDNA insert [(1989) FEBS Lett. 257, 97–100] as a probe and totally sequenced. The cDNA contained 1512-base pair open reading frame encoding 504 amino acid residues (M_r 57630), 39-base pair 5′-untranslated region 1322-base pair 3′-untranslated region including 20 nucleotides of poly A tail in the total length of 2873 base pairs. The deduced amino acid sequence showed 82% similarity to rat P-450$_{ch7\alpha}$. Unique amino acid residues were observed in putative binding domains for heme and steroid which are highly conserved in most steroidogenic P-450s.

Cholesterol 7α-hydroxylase; Cytochrome P-450; cDNA cloning; COS cell; Heme binding region; Steroid binding region

1. INTRODUCTION

Cholesterol 7α-hydroxylase (EC 1.14.13.17) is a major regulatory enzyme for bile acid biosynthesis in vertebrates [1], consisting of a microsomal monooxygenase cytochrome P-450$_{ch7\alpha}$ [2] and NADPH-cytochrome P-450 reductase. In view of the importance for the rate of cholesterol elimination in human diseases such as atherosclerosis, hyperlipidemia, gallstone disease, and certain lipid storage disease, an increasing interest has been focused on the mechanism of regulation of this enzyme. Recently, a cDNA clone encoding cholesterol 7α-hydroxylase has been isolated from rat liver cDNA library by the specific antibodies and sequenced in our laboratory [3,4]. The availability of the specific antibodies and the cDNA clone has enabled the exploration of a regulatory mechanism for this important enzyme [4]. Using the cDNA insert as a probe, hybridizable cDNA clones were isolated from human cDNA library in order to characterize human cholesterol 7α-hydroxylase. In this communication, we describe the isolation and the structural determination of a cDNA clone encoding human P-450$_{ch7\alpha}$.

2. MATERIALS AND METHODS

A λgt11 cDNA library and poly(A)$^+$ RNA of human liver were purchased from commercial source (Clontech). The human cDNA library was screened by ^{32}P-labeled insert of rat P-450$_{ch7\alpha}$ cDNA [3] as a probe. Positively reacted clones were selected and subcloned into *Eco*RI site of pBluescript SK(−) plasmid. DNA sequencing was performed by using *Exo*III/Mung bean nuclease deletion system (Takara Co.) [5] and Sequenase kit (United States Biochemical Corp.). Expression of cDNA clone in COS 7 cell system was performed by inserting the 1.9 kbp *Xho*I-*Xho*I fragment into pSVL vector and transfecting COS 7 cells with the constructed plasmid DNA by the DEAE-dextran method [6]. Northern hybridization [7], immunoblotting [8], and determination of 7α-hydroxycholesterol [9] were carried out as described previously.

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3. RESULTS AND DISCUSSION

Out of about 2×10^5 clones of human cDNA library, three positive clones were isolated by the plaque hybridization method and analyzed. The largest clone with about 2.9 kbp insert was subcloned into pBluescript plasmid (pH7α-3) and subjected to restriction mapping and nucleotide sequencing.

Fig. 1 shows the complete nucleotide sequence of pH7α-3 and the predicted amino acid sequence. The overall length of the cDNA was 2873 bp including poly A tail and is coincident to the mRNA size (2.95 kb) estimated by the Northern hybridization as shown in Fig. 2, indicating that the cDNA clone is of full length, whereas the size of rat mRNA was about 3.8 kb [4]. The open reading frame starts at 40th nucleotide, and codes for a peptide consisting of 504 amino acids (M_r 57630). The predicted amino acid sequence of human 7α-hydroxylase exhibited 82% similarity to that of rat 7α-hydroxylase [3], which is higher than the similarity between orthologues of rat/mouse and human P-450s reported to date, e.g. 68% for P-450$_{17\alpha}$ [10,11] and 71% for P-450$_{c21}$ [12,13]. This implies that replacement of amino acid residues has been restricted during evolution to maintain the function of cholesterol 7α-hydroxylase. The nucleotide sequence of the coding region of human cDNA also showed about 82%

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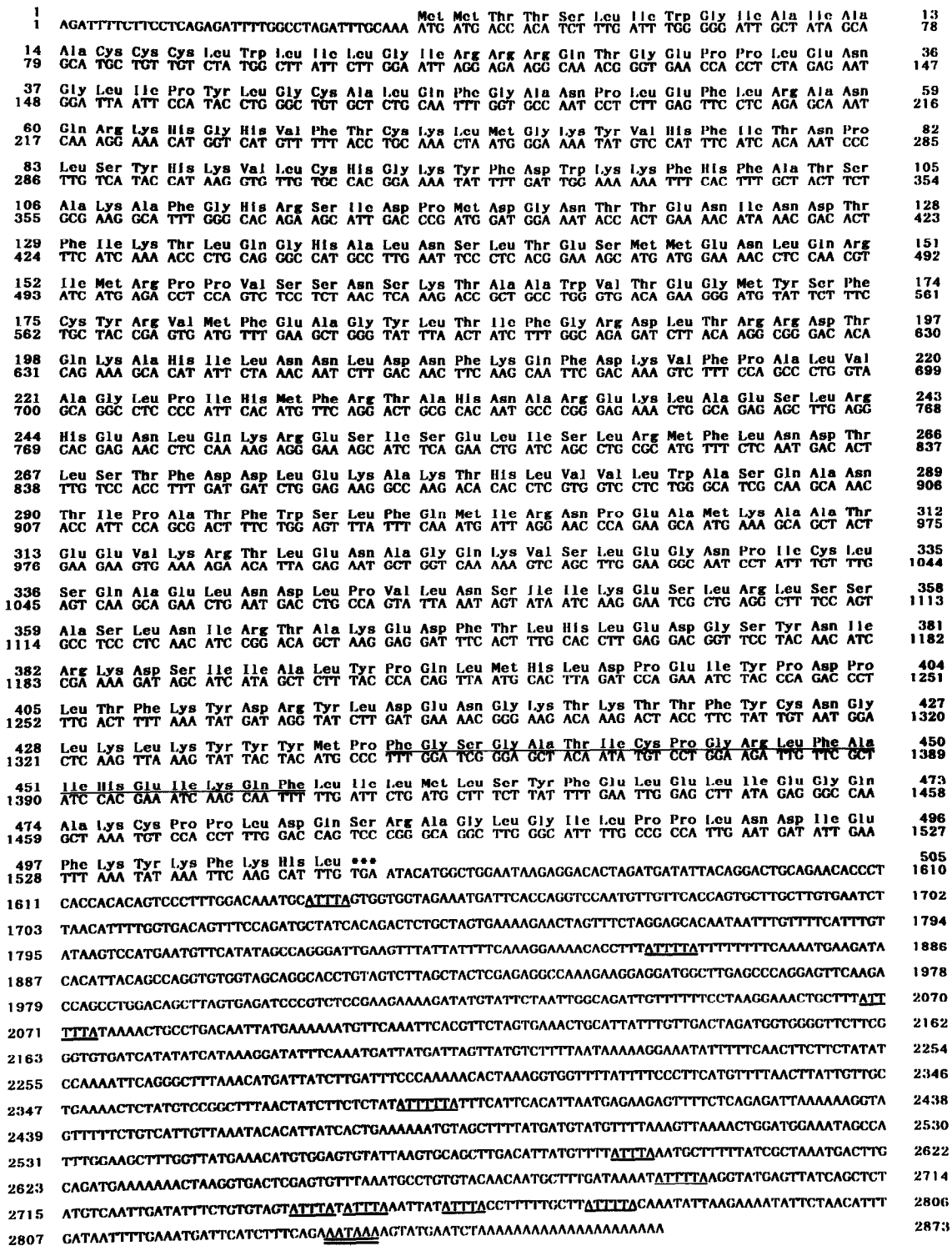

Fig. 1. Nucleotide sequence of the cDNA corresponding to the mRNA for human liver cholesterol 7α-hydroxylase and the predicted amino acid sequence of the protein. The peptide underlined at residues 437–457 indicates the putative heme binding site. ATTTA motif and similar sequences to it within 3′-untranslated region are underlined. A sequence of AATAAA indicates a polyadenylation signal.

similarity to that of the rat cDNA [3], whereas the 3′-untranslated region of human cDNA was shorter than that of the rat cDNA and showed lower similarity to it (46%). In the previous paper [4], it was reported that the 3′-untranslated region of rat cDNA is rich in AT nucleotides and often contained ATTTA motifs [14], 5′-AAT-3′ or 5′-TAA-3′ trinucleotides in single stranded region of the secondary structure [15] which

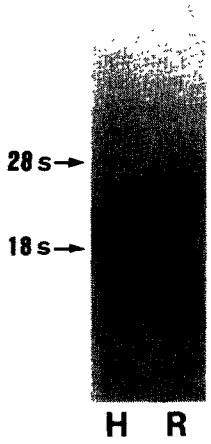

Fig. 2. Northern hybridization of liver poly(A)$^+$ RNA of human and rat. The poly(A)$^+$ RNA samples (10 μg) were electrophoresed on agarose gel containing formaldehyde [7]. A ^{32}P-labeled insert of pH7α-3 was used as a probe. H and R indicate human and rat poly(A)$^+$ RNA samples, respectively.

were reported to be involved in rapidly degrading mRNA. We suggested the possible correlation of the unique structure in 3'-untranslated region with the rapid degradation of 7α-hydroxylase mRNA. A similar unique structure was also often observed in 3'-untranslated region of the human cDNA (Fig. 1), which suggests that the human 7α-hydroxylase mRNA may degrade rapidly as well.

Comparison of the entire amino acid sequence of human 7α-hydroxylase as well as rat 7α-hydroxylase to other forms of sequenced P-450s revealed less than 30% similarity indicating that P-450$_{ch7\alpha}$ constitutes a unique family (family VII [4]) of the P-450 superfamily [16]. The amino acid sequence exhibited two consensus sequences characteristic of the P-450s. The first region is the heme binding site as depicted as underlined at residues 437–457 in Fig. 1. Cys-444 is thought to serve as the fifth ligand to heme [17]. Thr-442 was observed in both the human and the rat, whereas Arg is conserv-

species	No.	Amino Acid Sequence
Human P-450$_{ch7\alpha}$	343	L P V L N S I I K* E S L* R L S S A
Rat P-450$_{ch7\alpha}$	343	L P V L D S I I K E A L R L S S A
Human P-450$_{17\alpha}$	350	L L L L E A T I R E V L R L R P V
Human P-450$_{c21}$	343	L P L L N A T I A E V L R L R P V
Human P-450$_{scc}$	374	V P L L K A S I K E T L R L H P A
Rat P-450$_{11\beta}$	361	L P L L R A A L K E T L R L Y P V
Rat P-450$_{LMT25}$	385	M P L L K A V I K E T L R L Y P V

Fig. 3. Amino acid sequences of a conserved region in steroidogenic P-450s. The numbers are the first amino acid residues of respective sequences depicted in the figure. The amino acid sequences of rat P-450$_{ch7\alpha}$, human P-450$_{17\alpha}$, human P-450$_{c21}$, human P-450$_{scc}$, rat P-450$_{11\beta}$, and rat P-450$_{LMT25}$ are taken from [3,11,13,18–20], respectively. Asterisk indicates amino acid residues conserved in all sequenced cytochrome P-450s [17].

Table I

Expression of cholesterol 7α-hydroxylase cDNA in COS 7 cells

	7α-Hydroxycholesterol (pmol/mg)
pH7α-COS	210
Control COS	ND

Microsomal fractions were prepared from COS cells 48 h after transfection with the recombinant (pH7α) or nonrecombinant (control) expression vector. 7α-Hydroxycholesterol in microsomes was analyzed as described previously [7]. ND, not detectable

ed at this position in almost all other P-450s except for P-450$_{cam}$ which has His instead. Another identical region especially among steroidogenic P-450s is found in a stretch spanning residues 343 to 359 as shown in Fig. 3. Glu-352 and Arg-355 are conserved in all sequenced P-450s [17]. A sequence L-x-A-x-x-x-E-x-L-R-x-x-P is found among steroidogenic P-450s, such as P-450$_{17\alpha}$ [11], P-450$_{c21}$ [13], P-450$_{scc}$ [18], P-450$_{11\beta}$ [19], and P-450$_{LMT25}$ [20]. However, Ala and Pro are substituted by Ser-348 and Ser-358, respectively, in both human and rat 7α-hydroxylase. It is not known at present whether such unique amino acid residues may be related to the unique function [2] and lability of 7α-hydroxylase [4].

The identity of pH7α-3 as cDNA for cholesterol 7α-hydroxylase was confirmed by the expression of *Xho*I-*Xho*I fragment (1.9 kbp) including the coding region of cDNA in COS 7 cells. Table I shows the activity of cholesterol 7α-hydroxylase in the transfected COS 7 cells. The transfected cells accumulated the product, 7α-hydroxycholesterol, 48 h after the transfection by pSVL vector constructed with the cDNA fragment. COS 7 cells contained endogenous NADPH-cytochrome P-450 reductase and the substrate cholesterol, which may have functioned as electron carrier and substrate, respectively [3]. The control cells transfected with only pSVL vector did not show any product at all. This result, taken together with the high sequence similarity to rat 7α-hydroxylase, indicates that pH7α-3 cDNA clone encodes human liver cholesterol 7α-hydroxylase.

In conclusion, we have isolated a cDNA clone of human cholesterol 7α-hydroxylase, which is the most important regulatory enzyme for elimination of body cholesterol. This may pave the way to elucidate the mechanism of cholesterol metabolism on the level of molecular biology, which may ultimately clarify the etiology and exploit the therapy of a number of important human diseases related to disorders of cholesterol metabolism.

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