

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

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A complete cDNA clone encoding human cholesterol 7 α -hydroxylase has been isolated using a rat P-450_{ch7 α} 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 57 630), 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 α} . Unique amino acid residues were observed in putative binding domains for heme and steroid which are highly conserved in most steroidogenic P-450s.

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

1. INTRODUCTION

Cholesterol 7 α -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 α} [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 7 α -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 7 α -hydroxylase. In this communication, we describe the isolation and the structural determination of a cDNA clone encoding human P-450_{ch7 α} .

2. MATERIALS AND METHODS

A λ gt11 cDNA library and poly(A)⁺ RNA of human liver were purchased from commercial source (Clontech). The human cDNA library was screened by ³²P-labeled insert of rat P-450_{ch7 α} cDNA [3] as a probe. Positively reacted clones were selected and subcloned into *Eco*RI site of pBluescript SK(–) plasmid. DNA sequencing was per-

formed 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 7 α -hydroxycholesterol [9] were carried out as described previously.

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 (pH7 α -3) and subjected to restriction mapping and nucleotide sequencing.

Fig. 1 shows the complete nucleotide sequence of pH7 α -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 57 630). The predicted amino acid sequence of human 7 α -hydroxylase exhibited 82% similarity to that of rat 7 α -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 α} [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 7 α -hydroxylase. The nucleotide sequence of the coding region of human cDNA also showed about 82%

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1	AGATTTCCTCTCAGAGATTTTGGCCTAGATTTCGAAA	Met	Met	Thr	Thr	Thr	Ser	Leu	Ile	Trp	Gly	Ile	Ala	Ile	Ala	13
14	Ala Cys Cys Tyr Leu Trp Leu Ile Thr Gly	ATG	ATG	ACC	ACA	ACC	TCT	TGT	ATT	TGG	GGG	ATT	GCT	ATA	GCA	78
79	GCA TGC TGT TGT CTA TGG CTT Ile CTT Gly	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	36
37	Gly Leu Ile Pro Tyr Cys Gly Cys Ala Leu	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	147
148	GGA TTA ATT CCA TAC CTT GGC TGT CTT Ala	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	59
60	Gln Arg Lys His Gly His Val Thr Cys Lys	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	216
217	CAA AGG AAA CAT GGT CAT GTT TTT ACC TGC	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	82
83	Leu Ser Tyr TAC His Cys Val Leu Cys His	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	285
286	TTG TCA TAC TAC His Cys Val Leu Cys His	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	105
106	Ala Lys Ala Phe Gly His Arg Ser Ile Asp	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	354
355	GCG AAG GCA TTT GGG CAC AGA AGC ATT GAC	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	128
129	Phe Ile Lys Thr Thr Cys Gln Gly His Cys	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	423
424	TTT ATT AAA ACC CTT CAG CAC GGC ATT GAA	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	151
152	Ile Met Arg Pro Pro Val Ser Ser Asn Ser	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	492
493	ATC ATG AGA CCT CCA CTT GTC TCC Ser TCT	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	174
175	Cys Tyr Arg Val Phe Gly Glu GCT Gly Thr	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	561
562	TGC TAC CGA GTG Met Phe Glu GCT Gly Thr	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	197
198	Gln Lys Ala His Ile CTA Asn Asn Leu Asp	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	630
631	CAG AAA GCA CAT ATT CTA AAC AAT CTT GAA	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	220
221	Ala Gly Leu Pro Ile His Met Phe Arg Thr	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	699
700	GCA GGC CTC CCC ATT CAC ATG TTC Arg AGG	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	243
244	His Glu Arg Asn Leu Cln Lys Arg Glu Arg	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	768
769	CAC GAG AAC CTA CAC AAG GAA GGC Ser ATC	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	266
267	Leu Ser Thr Phe Asp Asp Leu Glu Lys Ala	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	837
838	TTG TCC ACC TTT GAT GAT CTG GAG AAG GCC	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	289
290	Thr Ile Pro Ala Thr Phe Trp Arg Ser Leu	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	908
907	ACC ATT CCA GCG ACT Thr Phe TGG Ser ATT	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	312
313	Glu Glu Val Lys Arg Thr Leu Glu Asn Ala	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	975
976	GAA GAA GTG AAA AGA CTA TTA GAG AAT GCT	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	335
336	Ser Gln Ala Glu Leu Asn Asp Leu Pro Val	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1044
1045	AGT CAA GCA GAA CTG AAT GAC CTG CCA GTA	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	358
359	Ala Ser Ser Cys Asn Ile Arg Thr Ala Lys	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1113
1114	GCC TCC CTT AAC ATC CGG ACA GCT AAG GAG	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	381
382	Arg Lys Asp Ser Ile Ile Ala Leu Tyr Pro	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1182
1183	CGA AAA GAT AGC ATC ATA GCT CTT TAC CCA	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	404
405	Leu Thr Phe Lys Tyr Asp Arg Tyr CTT Asp	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1251
1252	TTG ACT TTT AAA TAT GAT AGG TAT CTT GAG	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	427
428	Leu Lys Leu Lys Tyr Tyr Tyr Met Pro Phe	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1320
1321	CTC AAG TTA AAG TAT TAC TAC ATG CCC TTT	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	450
451	Ile His Gly Ile Lys Gln Phe Leu Ile Leu	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1389
1390	ATC CAC GAA ATC AAG CAA TTT CTT CTT CTT	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	473
474	Ala Lys Cys Pro Pro Leu Asp Gln Ser Arg	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1458
1459	GCT AAA TGT CCA CCT TTG GAC CAG TCC CGG	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	496
497	Phe Lys Tyr Lys Phe Lys His Leu ***	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1527
1528	TTT AAA TAT AAA TTC AAG CAT TTG TGA	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	505
1611	CACCACACAGTCCCTTTGGACAAATGCAATTAGTGGTGGTAGAAATGATTCACCAGGTCCAAATGTTGTTTCACCAGTGCTTGCTTGGAATCT	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1610
1703	TAACATTTTGGTGACAGTTTCCAGATGCTATCACAGACTCTGCTACTGAAAAGAACTAGTTTCTAGGAGACAAATAATTTGTTTTCATTGT	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1702
1795	ATAAGTCCATGAATGTTTCATATAGCCAGGGATTGAAGTTTATTTATTTTCAAAGGAAAACACCTTTATTATTTTTCAAAATGAAGATA	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1794
1887	CACATTACAGCCAGGTGTGGTAGCAGGCACCTGTAGTCTTAGCTACTCGAGAGGCCAAAGAAGGAGGATGCCCTTGAGCCCAGGAGTTCAAGA	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1886
1979	CCAGCCTGGACAGCTTAGTGAGATCCCGTCTCCGAAGAAAAGATATGTATTCTTAATTCGCACAGATTGTTTTTCTTAAGGAAATCGCTTTATT	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	1978
2071	TTTATAAAACTGCCTGACAATTATGAAAAATGTTCAAATTCACGTTCTAGTGCAAACTGCATTATTTGTTGACTAGATGGTGGGGTTCTTCG	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	2070
2163	GGTGTGATCATATATCATAAAGCATATTTCAAATGATTATGATTAGTTATGTTCTTTAATAAAAAAGAAATATTTTCAACTTCTTCTATAT	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	2162
2255	CCAAAAATTCAGGGCTTTAAACATGATTATCTTGATTTCCAAAAACACTAAAGGTGGTTTATTTTCCCTTTCATGTTTTAACTTATTGTTGC	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	2254
2347	TGAAAACTCTATGTCGGCGTTTAACTATCTTCTATCTTCAAAAAATTTTCATTACATTAAATGAGAAGAGTTTTCTCAGAGATTAAAAAAGGTA	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	2346
2439	GTTTTTCTGTCTATGTTAAATACACATTATCACATGAAAAATGTAGCTTTTATGATGTATGTTTTAAAGTAAAACTGGATGGAATAGCCA	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	2438
2531	TTTGGAGGCTTTGTTTATGAAACATGTGGAGGTGTATTAAGTCAGCTTGACATTATGTTTTTTTAAATGCTTTTTATCGCTAAATGACTTG	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	2530
2623	CAGTCAAAAAAACTAAAGTGACTCGAGTGTTTTAAATGCCTGTGTACAACAATGCTTTTGATAAAATATTTTAAAGGATGAGTTATCAGCTCT	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	2622
2715	ATGTCAAATGATATTTCTGTGTAGTTTATATTTTAAATTTTAACTTTTTCCTTTTTCCTTTTAAATATTAAGAAAAATTTCTAACATT	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	2714
2807	GATAATTTTGAATATGTTTCATCTTTCAGAAATAAAGTATGAATCTTAAAAAATTT	ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	2806
		ATG	ATG	ACC	AGA	AGG	GAA	ACC	Gly	GAA	Pro	CCT	Leu	Glu	Asn	2873

Fig. 1. Nucleotide sequence of the cDNA corresponding to the mRNA for human liver cholesterol 7 α -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

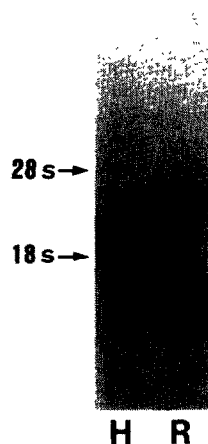


Fig. 2. Northern hybridization of liver poly(A)⁺ RNA of human and rat. The poly(A)⁺ RNA samples (10 µg) were electrophoresed on agarose gel containing formaldehyde [7]. A ³²P-labeled insert of pH7α-3 was used as a probe. H and R indicate human and rat poly(A)⁺ RNA samples, respectively.

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_{ch7α} 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 _{ch7α}	343	LPV LNS I I K E S L R L S S A
Rat P-450 _{ch7α}	343	LPV L D S I I K E A L R L S S A
Human P-450 _{17α}	350	L L L L E A T I R E V I R I 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 _{sc}	374	V P L L K A S I K E T L R L H P A
Rat P-450 _{11β}	361	L P L L R A A L K E T L R L Y P V
Rat P-450 _{LM25}	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_{ch7α}, human P-450_{17α}, human P-450_{c21}, human P-450_{sc}, rat P-450_{11β}, and rat P-450_{LM25} 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_{17α} [11], P-450_{c21} [13], P-450_{sc} [18], P-450_{11β} [19], and P-450_{LM25} [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 *XhoI*-*XhoI* 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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