Molecular Cloning and Expression of Rabbit Sterol 12α-Hydroxylase*

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Sterol 12α-hydroxylase is an important enzyme in bile acid biosynthesis, responsible for the balance between formation of cholic acid and chenodeoxycholic acid. The enzyme has been purified to apparent homogeneity from rabbit liver (Ishida, H., Noshiro, M., Okuda, K., and Coon, M. J. (1992) J. Biol. Chem. 267, 21319–21323), and we here describe the cloning and sequencing of a cDNA coding for this enzyme. After tryptic digestion of purified protein in a polyacrylamide gel, eight different peptides were isolated and sequenced. Using oligonucleotides deduced from the amino acid sequences, clones were isolated from a rabbit liver cDNA library. In addition to several overlapping clones, one full-length clone was obtained that coded for a polypeptide of 500 amino acids, corresponding to a molecular mass of 57 kDa. All of the eight peptides and the reported NH₂-terminal amino acid sequence were matched against the sequence. The peptide sequence showed a 39% similarity with human prostacyclin synthase (CYP1A1) and 31% similarity with the rate-limiting enzyme in over-all synthesis of bile acids, the cholesterol 7α-hydroxylase (CYP7) of the rabbit. The similarity with most other sterol cytochrome P-450 hydroxylases was less. Thus, this species of cytochrome P-450 should belong to a group of its own, here denoted CYP12. Transfection of COS cells with the coding part of the cDNA resulted in a significant expression of sterol 12α-hydroxylase activity toward 7α-hydroxy-4-cholesten-3-one.

Northern blotting showed that the enzyme was exclusively expressed in the liver. The major mRNA fraction in rabbit liver had a size of approximately 2.9 kilobases, and those found in rat and human liver were about 2.5 and 4.5 kilobases, respectively. Fasting of rats and mice led to a severalfold increase in both enzyme activity and mRNA levels. In contrast, starvation of rabbits had little or no stimulatory effect on enzyme activity and mRNA levels.

Sterol 12α-hydroxylase is one of the key enzymes in bile acid biosynthesis, being responsible for the balance between formation of cholic acid and chenodeoxycholic acid (for a general review, see Ref. 1). This balance may be of importance for development of gallstones since such stones are less likely to develop when there is a low ratio between cholic acid and chenodeoxycholic acid in bile. Suppression of sterol 12α-hydroxylase by competitive inhibitors has, therefore, been suggested as a possible therapeutic strategy for dissolution of gallstones (2). Since chenodeoxycholic acid is a more potent suppressor than cholic acid of the rate-limiting enzymes in cholesterol and bile acid synthesis (1), sterol 12α-hydroxylase activity may also be of general importance for over-all cholesterol homeostasis.

In 1973, Björkhem and coworkers (3) solubilized sterol 12α-hydroxylase from rat liver microsomes and demonstrated that the activity could be reconstituted from a crude fraction containing cytochrome P-450 and NADPH-cytochrome P-450 reductase. The relatively low degree of inhibition of the enzyme by carbon monoxide, observed also by other groups (4, 5), made it difficult to completely exclude the possibility that an enzyme other than cytochrome P-450 might be responsible for the action. In fact, one group suggested that a cytochrome P-450 is not the terminal oxidase in 12α-hydroxylation (5).

Sterol 12α-hydroxylase resisted purification for a long time, and for the first time in 1992, two of these authors (6) obtained a homogenous preparation allowing determination of the NH₂-terminal amino acid sequence. A characteristic feature of the sterol 12α-hydroxylase cytochrome P-450 isolated from rabbit liver was that in addition to NADPH and NADPH-cytochrome P-450 reductase, cytochrome b₅ was required for full activity.

A characteristic feature of the sterol 12α-hydroxylase is that the enzyme activity is increased severalfold in rats by starvation or administration of streptozotocin (6–8). It has also been reported from rat studies that the enzyme activity is decreased by treatment with thyroid hormone (9) and phenobarbital (4). In a previous work, we reported that the sterol 12α-hydroxylase in human liver seems to be regulated to some extent by the flux of bile acids, similar to the mechanism for regulation of the cholesterol 7α-hydroxylase (10). The mechanism of 12α-hydroxylation has also been studied with respect to the nature of the rate-limiting step in the hydroxylation, utilizing 12-²H₂-labeled 7α-hydroxy-4-cholesten-3-one as substrate (11).

In the present work, we report on the cloning and sequencing of a cDNA coding for sterol 12α-hydroxylase in rabbit liver. Transfection of COS cells with this cDNA resulted in significant 12α-hydroxylase activity. In addition, we show that the increase in sterol 12α-hydroxylase enzyme activity in rat liver after starvation is associated with increased mRNA levels.

EXPERIMENTAL PROCEDURES

Materials—The following materials were purchased: restriction enzymes, T4 polynucleotide kinase (Boehringer Mannheim GmbH, Mannheim, Germany); Hybond N nylon membranes, Sequenase version 2.0 DNA sequencing kit (Amersham Life Science, Inc., Buckinghamshire, U.K.).
UK); [a-32P]dCTP, [γ-32P]ATP, 5′[a-32S]thio-ATP (DuPont NEN); oligo labeling kit, Taq polymerase, SureCon ligation kit, Ready-to-go T4 DNA ligase kit, pUC18 (Pharmacia Biotechnology, Inc., Upssala, Sweden); pBluescript II SK+ (Stratagene, La Jolla, CA); Fuji RX-L x-ray films (Fuji Photo Film Co., Ltd., Japan).

Animals—Male and female New Zealand White rabbits weighing 2–3 kg were fed standard rabbit chow and fasted for 24 or 48 h. In the experiments evaluating the effects of starvation on enzyme activity and organ distribution, only male rabbits were used. Male Sprague-Dawley rats and male 129/SVJmice were likewise kept on normal chow or fasted for 24 or 48 h, after which liver slices were collected.

Protein Purification—Sterol 12α-hydroxylase was prepared from rabbit liver microsomes as described previously (6).

Generation of Peptides by In-gel Digestion—About 20 μg of the above protein preparation was concentrated (12), and the dried sample was dissolved in 20 μl of sample buffer containing 10 mM dithiothreitol. After boiling for 5 min, the reduced sample was alkylated by incubation at room temperature for 20 min with 1 μl of 4-vinylpyridine and fractionated by polyacrylamide gel electrophoresis in SDS (13) on a 12% gel using a Minigel device (Bio-Rad). After Coomassie Blue staining and destaining, the bands containing the sterol 12α-hydroxylase was excised and trypsin digestion was performed as described by Hellman et al. (14).

Isolation of Peptides by Reversed Phase Liquid Chromatography—The peptides were fractionated by narrow bore reversed phase liquid chromatography on a μRPC C2/C18 SC2 150 column operated in a Smart System (Pharmacia). An Applied Biosystems model 470A sequencer was used.

cDNA Cloning—Oligonucleotides were synthesized on a 380B DNA synthesizer (Applied Biosystems), using the β-cyanophosphitypochymidine chemistry. A cDNA library made from New Zealand White rabbit liver in the phage λgt10 was obtained from Clontech (Palo Alto, CA). The library (5′-stretch plus cDNA library) was constructed with oligo(dT) and random priming, utilizing EcoRI linkers, and was found to contain 2.5 × 106 pfu/ml.1 The library was propagated in Escherichia coli C600 Hfr. Screening of the library was performed with oligonucleotide probes, end-labeled with [32P]P-32P. Positive clones were isolated, and the DNA purified with Wizard λ Prep DNA purification system (Promega, Madison, WI). The inserts were excised from the vector by digestion with EcoRI, extracted from the agarose gel (GenecleanII kit, Bio 101, Inc., La Jolla, CA), and subcloned into pBluescript II or pUC18. Plasmid DNA was purified with the Jetstar plasmid purification system (Genomed). Sequencing of the inserts was either done manually by the dyeoxy sequencing method according to established procedures (15) or automatically on a 373A DNA sequencer stretch (Applied Biosystems).

The abbreviations used are: pfu, plaque-forming units; kb, kilobases; HPLC, high pressure liquid chromatography; bp, base pairs; PCR, polymerase chain reaction.

The procedure was carried out with low stringency (rinsing in 6 × SSC, 0.1% SDS, 50 °C for 5–25 min). In this way, one positive clone (p9) was isolated with the probe 12α-2 as shown in Fig. 2. The most prevailing codons for the amino acids found in the rabbit genome were used (18), and in positions with a high degree of degeneracy, inosine was inserted (Fig. 2). The most prevailing codons for the amino acids found in the 5′-end of the inserts were used (18), and in positions with a high degree of degeneracy, inosine was inserted (Fig. 2).

Isolation of Rabbit Sterol 12α-Hydroxylase Clones and Sequence of a Full-length Clone—Approximately 500,000 pfu were screened with the two oligonucleotides (12α-1 and 12α-2). The procedure was carried out with low stringency (rinsing in 6 × SSC, 0.1% SDS, 50 °C for 5–25 min). In this way, one positive clone (p9) was isolated with the probe 12α-2 while the probe 12α-1 did not pick up any clones. The clone obtained numbered approximately 1,000 bp and coded for an open reading frame of 45 amino acid residues localized in the 5′-end. Within this part, two of the peptide sequences were recognized, one corresponding to the cloning probe. The other peptide (peptide 7) contained only five amino acids, and the C-terminal residue was immediately followed by a stop codon (TGA), suggesting that this represented the C-terminal end of the polyepitope chain. A new screening oligonucleotide consisting using the dye terminator cycle sequencing kit with FS-Amplitaq polymerase (Perkin-Elmer). Quantification of mRNA in Liver Tissue—Total RNA was prepared from frozen tissue slices of various organs using the Ultraspec or Ultraspec II RNA method (Biotec Lab. Inc., Houston, TX). Northern blotting and hybridization of the blots with 32P-labeled cDNA probes were carried out as described (16). Quantitation of the radioactive signals was done by densitometry, using either murine β-actin or human glyceraldehyde-3-phosphate dehydrogenase as an internal standard.

Transfection of COS-M6 Cells—Stock cultures of the cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum and 1% tricine/ethylenediaminetetraacetic acid. Transfection was carried out by electroporation in 0.4-cm cuvettes (Gene Pulsar II without the pulse controller unit, Bio-Rad), using a single pulse of 0.40 kV and 25 μfarad. After transfection, the cells were grown for 48 h, harvested by low speed centrifugation, and homogenized in the incubation medium (cf. below).

Assay of Enzyme Activity—Enzyme activity was routinely assayed as described previously (6) with 7α-hydroxy-4-cholesten-3-one as substrate.

In incubations with transfected cells, the incubations were performed as above with use of homogenates of 2 × 106 cells. In addition to assay by HPLC as above, the extracts of these incubations were also assayed with a more specific technique utilizing combined gas chromatography-mass spectrometry (17). The ion at m/z 380 (M – 2 × 90) was used for quantitation of the silylated product 7α,22-dihydroxy-4-cholesten-3-one, and the ion at m/z 382 (M – 90) was used for quantitation of the silylated substrate 7α-hydroxy-4-cholesten-3-one (see Fig. 8).}

Miscellaneous—Amplification of various regions by PCR of the Agt10 clones were performed with primers deduced both from the vector arms (5′- and 3′-insert screening amplimer as suggested by Clontech) and from the insert sequence. Approximately 103–104 pfu were added to the reaction mixture of 50 μl, and the process was initiated with a hotstart at 98 °C for 8 min, after which the enzyme was added.

RESULTS

Isolation and Sequencing of Tryptic Peptides and Preparation of Oligonucleotides for Screening—Fractionation of the tryptic peptides by HPLC generated a large number of peaks (Fig. 1). Of these, nine were subjected to sequence analysis and found to represent nine peptides, containing between 5–20 amino acids. Two of the peptides were identical except for an amino acid substitution (6). The sequence of two of the peptides obtained after trypsin digestion of rabbit sterol 12α-hydroxylase is the sequence of the corresponding oligonucleotides used for screening.

Fig. 1. HPLC chromatogram of peptides obtained after trypsin digestion of rabbit sterol 12α-hydroxylase.
of 40 bases was synthesized (12a-4), exactly matching one segment located in the 5'-region of clone p9 (corresponding to positions 1513–1552 in the complete sequence). Screening of another 500,000 pfu from the library was performed with both the probes 12a-4 and 12a-2, using separate plaque-lifts and higher stringency for the hybridization with 12a-4 (rinsing in 6×SSC, 0.1% SDS, 63°C for 10–15 min). Approximately 40–50 clones were obtained that hybridized with both oligonucleotides. One of these clones (p41B), with a size of 2,129 bp, was sequenced to completion and was found to contain an open reading frame of 1500 bp, coding for a peptide sequence of 500 amino acids, in which all of the eight peptides and the reported amino-terminal sequence (6) could be identified. Immediately preceding the codon for the amino-terminal valine was the triplet ATG, the codon for a methionine residue. The 5'-part proximal to this numbered 98 bp, in which no triplets coding for methionine were seen. In the 3'-part, the nontranslated region contained 531 bp. The latter part overlapped with another, shorter clone (p12A, also sequenced to completion), in which only 52.9% of the coding sequence was found (Fig. 3). This clone contained an additional 337 nucleotides in its 3'-untranslated part, totaling the number of aligned nucleotides to 2,466. The complete nucleotide sequence and the corresponding amino acids of the full-length cDNA are shown in Fig. 4.

Restriction mapping demonstrated the presence of an EcoRI site in position 262 (see Fig. 3). Thus, when the insert of clone p41B was excised from the vector by digestion with EcoRI, it was cleaved in two fragments (262 and 1867 bp), which were subcloned and sequenced separately. To verify the sequence around the EcoRI site, PCR was utilized to generate a fragment of 693 bp, using a primer deduced from the vector arm upstream (5' insert screening amplimer) and a primer complementary to the plus strand downstream (positions 554–537). The fragment obtained bridged the EcoRI restriction site (see Fig. 3). Sequence analyses of this fragment exactly matched the sequence obtained when the two EcoRI fragments of clone 41B were joined together.

**Structural Similarities with Other Cytochrome P-450 Species**—Utilizing the Kyte-Doolittle plot shows that the N terminus of the sterol 12a-hydroxylase polypeptide is hydrophobic, similar to the case in other microsomal CYPs. It is thought that

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**Fig. 3**. Restriction map of the cDNA and the different independent clones sequenced. The cross-hatched areas indicate the coding region.

**Fig. 4**. Rabbit sterol 12α-hydroxylase cDNA and the sequence of the encoded polypeptide. The nucleotide sequence and translation product (bottom) are derived from the compilation of the above overlapping clones and the full-length clone. The sequences of the eight peptides (peptides 1–4 and 6–9) obtained after tryptic cleavage of the purified sterol 12α-hydroxylase and the NH₂-terminal sequence previously reported (6) are underlined.
this part of the polypeptide is inserted into the membrane of the endoplasmic reticulum. Following the hydrophobic segment is a hydrophilic region (from residue 25). In accordance with other microsomal CYPs, the amino terminus lacks basic amino acids prior to the hydrophobic cores.

The over-all nucleotide sequence homology of the rabbit sterol 12α-hydroxylase was highest with prostacyclin synthase (CYP8) (19) (43.2% homology over the coding region). At the polypeptide level, the corresponding homology was 39.1% (Fig. 5A). The similarity with rabbit 7α-hydroxylase (CYP7) (20), the rate-limiting enzyme in bile acid biosynthesis, was 30.5%. Most other cytochrome P-450 species investigated had a lower degree of homology than that of rabbit CYP7, less than 16%. An exception occurred with a peptide sequence, deduced from a cDNA isolated from mouse brain hippocampus (hct-1) (21), that showed 25.9% homology.

Many CYP polypeptides are characterized by some highly conserved regions corresponding to the heme binding site, a steroidogenic domain, and an O₂-binding pocket (22). The heme binding site has an arrangement of amino acids around a cysteine residue postulated to preserve the three-dimensional structure of this region for ligand binding. This motive was found to be highly conserved in the sterol 12α-hydroxylase (positions 432–445, Fig. 5B). Also, the steroidogenic domain (positions 337–351, Fig. 5C) were relatively conserved. The region corresponding to the O₂-binding pocket (positions 279–295, Fig. 5D) showed a rather high degree of homology with the corresponding section in rabbit CYP7, human CYP8, and hct-1, but these four displayed less homology with the other aligned cytochromes p-450s. Furthermore, the threonine residue, shown to be of fundamental importance for the structural integrity of the O₂-binding pocket (23), is replaced with aspartic acid (position 288 in 12α-hydroxylase) in the former group of peptide sequences.

Evolutionary Age of Sterol 12α-Hydroxylase—Fig. 6 shows a phylogenetic tree showing that CYP7, CYP8, and the cytochrome P-450 responsible for 12α-hydroxylation, here denoted CYP12, are more related than other displayed CYPs. CYP8 and CYP12 seem to have a similar evolutionary age, emerging later during evolution than CYP7.

Expression Pattern of Sterol 12α-Hydroxylase—To visualize the mRNA for 12α-hydroxylase on Northern blots, a probe of 653 bp was made by PCR amplification of the clone p12A, corresponding to positions 926-1578 of the coding cDNA sequence. A strong signal was obtained with rabbit liver total RNA, producing one major band of about 2.9 kb and a weaker fraction of 3.9 kb (Fig. 7). Total RNA from other organs of the rabbit (kidney, lung, adrenal, testis, and thigh muscle) did not generate any signal (not shown). Total liver RNA from rat and mouse generated one band with a size of approximately 2.5 and 2.4 kb, respectively, but the signal was much weaker than with the rabbit RNA. With human liver total RNA, one rather strong band was obtained with a migration velocity corresponding to 4.5 kb (Fig. 7).

![Figure 5. Alignment of rabbit sterol 12α-hydroxylase with other steroidogenic P-450 species](attachment:image.png)

A. alignment of the whole deduced peptide sequence with rabbit 7α-hydroxylase (CYP7) (20) and human prostacyclin synthase (CYP8) (19). B, C, and D, comparison between the residues in the heme binding region (residues 432–444), in the postulated steroidogenic binding domain (residues 337–351), and in the postulated O₂-binding pocket (residues 279–295), respectively. Additional P-450 peptide sequences displayed are murine hippocampal transcript (hct-1) (21), human steroid 11β-hydroxylase (CYP11B1) (32), human 27-hydroxylase (CYP27), human cholesterol monoxygenase (CYP11A1) (28), human steroid 17α-hydroxylase (CYP17) (29), human estrogen synthetase (CYP19) (33), human steroid 21-hydroxylase (CYP21B) (30), human naphthoquinone oxidase (CYP3A4) (31). Identical amino acid residues between the 12α-hydroxylase and the other proteins are indicated by shaded letters, while hyphens in the sequences indicate gaps introduced during the alignment. The regions in A corresponding to B, C, and D are underlined.
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Effect of Starvation on Sterol 12α-Hydroxylase Activity and mRNA Levels—As shown in Table I, starvation of rats and mice led to a severalfold increase in both sterol 12α-hydroxylase activity and mRNA levels. To the contrary, starvation had little or no effect on these parameters concerning rabbit liver.

Expression of Sterol 12α-Hydroxylase Activity in COS cells—To express the sterol 12α-hydroxylase in eucaryotic cells, we utilized the vector pCAGGS, a plasmid vector carrying a modified chicken β-actin promotor (24). A cDNA fragment including the coding sequence of the sterol 12α-hydroxylase was prepared by PCR from the clone p41B. The primers contained a restriction site for XbaI, and the fragment obtained numbered 1557 bp, corresponding to positions 70–1626. The fragment was ligated into the XbaI site of the expression vector pCAGGS, and the construct was propagated in *E. coli* DH5α. DNA from the clones obtained was purified on Jetstar plasmid purification system. The insert could be cleaved out of the vector by digestion with XbaI, and the orientation was established by DNA sequencing. An estimated quantity of 10–15 μg of plasmid DNA, with the cDNA inserted in the correct way, was used to transfect approximately 2 × 10⁶ cells. In a control experiment, a similar quantity of plasmid DNA with the cDNA inserted in the opposite direction was allowed to transfect the same number of cells.

When incubating a homogenate of the transfected cells as described under “Experimental Procedures,” a significant sterol 12α-hydroxylase activity toward 7α-hydroxy-4-cholesten-3-one could be demonstrated (Fig. 8). The degree of conversion was about 3% when assayed with a highly specific assay based on combined gas chromatography-mass spectrometry and utilization of specific ions (m/z 380 and m/z 382). In contrast, homogenates of mock transfected cells (using a construct with the coding region inversely inserted) (Fig. 8) or nontransfected cells had no significant enzymatic activity (below 0.2%).

**DISCUSSION**

The sterol 12α-hydroxylase has long resisted purification, probably in part due to the low level of this cytochrome in the liver of species other than the rabbit and its unusual behavior on anion-exchange chromatography. The previously reported method of purification from rabbit liver (6) was repeated here and resulted in a homogenous preparation of the enzyme. Several attempts to clone the sterol 12α-hydroxylase from a rabbit liver library with use of antibodies directed toward the homogenous enzyme failed. The strategy that proved to be successful involved tryptic digestion in a polyacrylamide gel, followed by HPLC isolation of peptides that were sequenced and used for synthesis of oligonucleotides. The latter were used for screening of a rabbit liver library with a low degree of stringency. Several overlapping clones were obtained and sequenced prior to the finding of one that contained the whole coding sequence.

The results of the present work confirm our previous finding that sterol 12α-hydroxylase is a species of cytochrome P-450. The similarity with other known CYPs was relatively small. It has been recommended that a CYP with a sequence identity of sterol 12α-hydroxylase should be regarded as a new family of cytochrome P-450. Here, CYP12.

It is surprising that CYP8, responsible for synthesis of prostacyclin (26), was the enzyme found to be most similar to CYP12. CYP7, responsible for the rate-limiting step in bile acid biosynthesis, showed a homology with CYP12 lower than that of CYP8 but higher than that of any other cytochrome with known structure. The normal substrate for prostacyclin synthase is prostaglandin H₂, which is converted into prostaglandin I₂. In view of this, it is surprising that prostacyclin synthase has a domain with a structure believed to be "steroidogenic" in other CYPs. This domain had a similarity of 73.3% with the corresponding domain in CYP12.

From an evolutionary point of view, sterol 12α-hydroxylase seems to have appeared at a similar time as prostacyclin synthase but later than cholesterol 7α-hydroxylase. Cholesterol 7α-hydroxylase has a very high substrate specificity, and only cholesterol and the close substrate analogue cholestanol (5α-cholestan-3β-ol) are hydroxylated by the enzyme. Sterol 12α-hydroxylase has a rather broad substrate specificity including a number of 7α-hydroxylated C₂₇ sterols (4). The substrate most efficiently 12α-hydroxylated seems to be 7α-hydroxy-4-cholesten-3-one (4). In this connection, it is interesting to com-

![Figure 6](image-url) Phylgenetic tree of sterol 12α-hydroxylase and other related cytochrome P-450 species.

![Figure 7](image-url) Northern analysis of sterol 12α-hydroxylase expression in livers of normal rabbit (lane 1), mouse (lane 2), rat (lane 3) and human (lane 4). Arrows denote the estimated sizes of the RNA fractions in kilobase pairs.

**TABLE I**

| Enzyme activity and CYP12 mRNA levels in untreated and starved rats | Enzyme activity | Cyp12 mRNA |
|---|---|---|
| pmol/min/mg | Cyp12/Actin | Cyp12/GAP |
| Rat experiment 1 | Control | 9 | 0.11 |
| 24-h starvation | 41 | 1.9 |
| 48-h starvation | 102 | 1.4 |
| Rat experiment 2 | Control | 12 | 0.12 |
| 24-h starvation | 62 | 1.7 |
| 48-h starvation | 100 | 1.2 |
| Rat experiment 3 | Control | 11 | 0.09 |
| 24-h starvation | 61 | 1.0 |
| 48-h starvation | 61 | 1.3 |
| Rabbit | Control | 11 | 0.61 |
| 24-h starvation | 14 | 0.70 |
| 48-h starvation | 11 | 1.4 |
| Mouse | Control | 23 | 1.5 |
| 24-h starvation | 38 | 1.3 |
| 48-h starvation | 101 | 3.2 |
pare the steroidogenic region of the sterol 12α-hydroxylase with the corresponding region of other species of cytochrome P-450 (Fig. 4C). The homology was rather high with some CYPs that utilize C_{27}-substrates (rabbit cholesterol 7α-hydroxylase (20), human cholesterol monoxygenase alternatively denoted “side-chain cleaving enzyme” (27), and human steroid 27-hydroxylase (28) compared with CYPs that utilize C_{19}- or C_{21}-steroids as substrates (human steroid 17α-hydroxylase (29) and steroid 21-hydroxylase (30)). However, a fairly marked homology was also found with nifedipine oxidase (CYP3A4 (31)) and steroid 11β-hydroxylase (32).

It should be noted that the enzyme was found to be expressed in relatively large amounts in rabbit and human liver, which is in accordance with the fact that cholic acid and its 7α-dehydroxylated product, deoxycholic acid, are the dominating bile acids in these species. The expression in rat and mouse liver appeared to be considerably less, possibly explaining previous difficulties to purify the enzyme from rat liver. Starvation of rats and mice, however, greatly increased both enzyme activity and mRNA levels, suggesting that the enzyme is regulated at a pretranslational level in these cases.

Availability of a cDNA for the sterol 12α-hydroxylase should pave the way for further studies on the mechanism of regulation of the enzyme under different conditions. The structural knowledge is also a prerequisite for designing synthetic competitors of the enzyme. Attempts are now being made in our laboratory to characterize the promoter region of the gene. In particular, it is of interest to see if there are structural similarities with the corresponding region of the cholesterol 7α-hydroxylase.

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