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Liver mitochondrial cytochrome P450 CYP27 and recombinant-expressed human CYP27 catalyze 1α-hydroxylation of 25-hydroxyvitamin D_3

(sterol 27-hydroxylase/hepatic 1α-hydroxylation/bacterial expression)

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ABSTRACT **A cytochrome P450 catalyzing 1α-hydroxylation of 25-hydroxyvitamin D_3 was purified from pig liver mitochondria. It also catalyzed 27-hydroxylation of 25-hydroxyvitamin D_3 and 25-hydroxylation of vitamin D_3. The ratio between the 1α-, 27-, and 25-hydroxylase activities remained essentially constant during the purification. Substrates for sterol 27-hydroxylase CYP27 inhibited and a monoclonal antibody raised against CYP27 immunoprecipitated the 1α-, 27-, and 25-hydroxylase activities. Apparently homogeneous preparations of CYP27 from pig and rabbit liver mitochondria catalyzed 1α-hydroxylation. Human liver mitochondrial CYP27 was expressed from its cDNA in *Escherichia coli*. The nucleotide sequence encoding the N terminus of CYP27 was modified in the first eight codons to achieve expression in *E. coli*. The purified recombinant-expressed CYP27 reconstituted with the electron-transferring system of adrenal mitochondria catalyzed 1α-hydroxylation of 25-hydroxyvitamin D_3. Expression of unmodified *CYP27* cDNA in simian COS cells confirmed the 1α-hydroxylase activity toward 25-hydroxyvitamin D_3.**

The activation of vitamin D_3 to its hormonal form, 1α,25-dihydroxyvitamin D_3, involves an initial 25-hydroxylation in the liver. The subsequent 1α-hydroxylation of 25-hydroxyvitamin D_3 is catalyzed mainly by a mitochondrial cytochrome P450 in kidney. Extrarenal 1α-hydroxylase activity toward 25-hydroxyvitamin D_3 has been reported (1–3). Hollis (2) found 1α-hydroxylase activity in both the microsomal and mitochondrial fractions of a pig liver homogenate. The 1α-hydroxylase activity in both subcellular fractions was inhibited by ketoconazole, a known cytochrome P450 inhibitor (2). The enzymes were not purified or further characterized. The pig, rabbit, and rat liver mitochondrial sterol 27-hydroxylase (CYP27) is known to catalyze 24-, 25-, and 27-hydroxylations of C_{27} steroids and vitamin D_3 compounds (4–12). cDNA encoding the human liver CYP27 was isolated by Cali and Russell (13). When expressed in COS-1 cells, the enzyme was able to catalyze multiple oxidation reactions at carbon 27 of sterol intermediates in bile acid biosynthesis. In addition, Guo *et al.* (14) have shown that the human *CYP27* cDNA transfected in COS-1 cells is able to catalyze 25- and 27-hydroxylation of vitamin D_3. The present paper reports that purified liver mitochondrial CYP27 from pig and rabbit and recombinant-expressed human CYP27 catalyze the 1α-hydroxylation of 25-hydroxyvitamin D_3.

EXPERIMENTAL PROCEDURES

Purification of 1α-Hydroxylating Cytochrome P450 from Liver Mitochondria. Mitochondria from 1 kg of pig liver (castrated, otherwise untreated, 6-month-old male pigs) were prepared, cholate solubilized, and applied to octylamine-Sepharose, hydroxylapatite, and anion-exchange chromatography as described by Wikvall (4) with the following modifications. The mitochondria were not frozen and protein concentration during solubilization was 20 instead of 4 mg/ml. All buffers in the purification procedures were potassium phosphate buffers containing 20% (vol/vol) glycerol and 0.1 mM EDTA. 1α-Hydroxylating cytochrome P450 was eluted from the octylamine-Sepharose with 100 mM buffer (pH 7.4) containing 0.4% sodium cholate and 0.08% polyoxyethylene 10 lauryl ether (POELE). The hydroxylapatite column was washed with 35 mM buffer (pH 7.4) containing 0.2% POELE, and 1α-hydroxylating cytochrome P450 was eluted with 200 mM buffer (pH 7.4) containing 0.2% POELE. The eluate was concentrated, dialyzed against 20 mM buffer (pH 8.0) containing 0.1% sodium cholate and 0.4% POELE, and applied to a Q-Sepharose column (1.6 × 14 cm) equilibrated in the same buffer and eluted as listed in Table 1. The flow rate was 2 ml/min. Detergents were removed from the cytochrome P450 preparation as described (4).

CYP27 from pig (7) and rabbit (15) liver mitochondria was purified as described except that the final hydroxylapatite chromatography step to remove nonionic detergent was omitted. The preparations showed a single protein band upon gel electrophoresis with apparent M_rs of 53,000 (pig) and 52,000 (rabbit). The specific cytochrome P450 contents were 7.5 and 10 nmol per mg of protein, respectively. A partially purified CYP27 fraction was isolated from human liver mitochondria as described above using only octylamine-Sepharose and hydroxylapatite chromatography steps.

Bacterial Expression and Purification of Human Liver CYP27 in *Escherichia coli*. Previous work with expression of cytochrome P450 in *E. coli* has shown that the 5′ coding region of the cDNA has to be modified to achieve expression (16). To modify the N terminus of CYP27 for expression in *E. coli*, two synthetic oligonucleotide primers were used. Primer A (5′-GAATTCCATGGCTCTGCCATCCGACAAAGCT-3′) and primer B (5′-GAGCTCCGGTAGCTTTGTCGGA-3′) were annealed and polymerization was performed with the Klenow fragment of DNA polymerase I to generate a modified 5′ fragment. This fragment was subjected to digestion with *Eco*RI and *Sac* I and ligated into the pBSIIKS$^-$ plasmid to construct pBS27H5′. The pBS27H5′ plasmid was digested with *Nco* I and *Sac* I and the modified 5′ fragment was isolated from a 2.5% agarose gel. The plasmid pBSSK$^-$, harboring the full-length human *CYP27* cDNA (a kind gift from David Russell, Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas), was digested with *Sac* I and *Xho* I, generating a 1581-bp fragment of *CYP27* missing the 5′ nucleotide sequence encoding the mitochondrial signal sequence and the first 10

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Abbreviation: POELE, polyoxyethylene 10 lauryl ether.

Table 1. Purification of pig liver mitochondrial cytochrome P450 catalyzing 1α-hydroxylation of 25-hydroxyvitamin D_3

		25-Hydroxyvitamin D_3		Vitamin D_3
	P450*	1α-OHase†	27-OHase†	25-OHase†
Mitochondria	ND	1	3	<1
Octylamine-Sepharose	0.8	13	39	214
Hydroxylapatite				
0.2 M phosphate	1.2	13	48	258
Q-Sepharose				
Nonbound fraction	1.9	<1	<1	8
0–0.1 M sodium acetate	4.3	30	133	1187
0.1 M sodium acetate	2.2	7	18	112
0.1–0.3 M sodium acetate	2.2	<1	<1	20
0.3 M sodium acetate	1.7	<1	<1	7
0.5 M sodium acetate	1.1	<1	<1	2
CYP27	7.5	33	149	498
CYP27 (rabbit)	10.0	30	20	480

ND, not determined.
*nmol per mg of protein.
†pmol per min per mg of protein.

amino acids in the native protein. The *Sac* I/*Xho* I fragment was isolated from a 1% agarose gel. The modified *Nco* I/*Sac* I 5′ fragment and the 1581-bp *Sac* I/*Xho* I fragment of *CYP27* were ligated into the bacterial expression vector pTrc99a (Pharmacia) cleaved with *Nco* I/*Sal* I to construct pTrc27H and the ligation mixture was used to transform JM105 cells (Pharmacia). Plasmids containing the insert were identified by agarose gel electrophoresis and verified by restriction analysis. The sequence of the modified 5′ fragment was determined in the pBS27H5′ plasmid to be that of *CYP27* with the intended substitution by the dideoxynucleotide chain-termination method following the manufacturer's instructions (17).

Glycerol stocks of pTrc27H-transformed JM105 cells served as an inoculum for all expression experiments. In brief, cells were grown overnight with vigorous shaking (225 rpm) at 37°C in terrific broth (18) containing 0.1 mg of ampicillin per ml. A 1:500 dilution was made into 1000 ml of terrific broth containing 0.1 mg of ampicillin per ml in a 2-liter flask and grown at 37°C to an optical density of 0.4–0.6 at 600 nm. Expression of *CYP27* was then induced with 1 mM isopropyl β-D-thiogalactopyranoside, the cultures were moved to room temperature, and the shaking rate was reduced to 150 rpm. After 20–24 h, cells were harvested and membranes were prepared as described by Richardson *et al.* (19) except that the incubation with DNase was omitted. The membranes were suspended in 100 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol, 1 mM EDTA, 0.1 mM dithiothreitol, and 0.25 mM phenylmethylsulfonyl fluoride (PMSF).

All buffers in the purification procedure were potassium phosphate buffers (pH 7.4) containing 20% glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol, and 0.25 mM PMSF. Membranes isolated from 20 liters of culture were diluted to 20 mg/ml with the suspension buffer, solubilized by addition of 0.8% sodium cholate and centrifuged (4). The supernatant was applied to an aminohexyl-Sepharose 4B column (1.5 × 10 cm) equilibrated in 100 mM buffer containing 0.5% sodium cholate. The column was washed with the equilibrating buffer and eluted with 100 mM buffer containing 0.4% sodium cholate and 0.2% POELE. Fractions containing cytochrome P450 were pooled, diluted 1:4 with 20% glycerol, and applied to a hydroxylapatite column (1.5 × 8 cm) equilibrated in 25 mM buffer containing 0.2% POELE. The column was washed with 35 mM buffer containing 0.2% POELE and cytochrome P450 was eluted with 150 mM buffer containing 0.2% POELE. Detergent was removed as described (4).

Transient Transfection of COS-1 Cells. COS-1 cells were transfected with the pCMV expression vector containing the *CYP27* cDNA (13) using the DEAE-dextran procedure (20). Fractions enriched in mitochondria were prepared from COS-1 cells as described (21), solubilized with 0.8% sodium cholate, and centrifuged at 8000 × *g* for 10 min. The supernatant protein fraction was dialyzed against 50 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol, 0.1 mM EDTA, and 0.1 mM dithiothreitol. The enzyme activity of the dialyzed mitochondrial protein was assayed in the presence of adrenodoxin, adrenodoxin reductase, and NADPH as described below. Mitochondrial protein obtained in the same way from COS-1 cells transfected with a plasmid containing the human sterol 27-hydroxylase cDNA in the reverse orientation was used as a control.

Incubation Procedure and Analysis of Enzymatically Formed Products. Incubations were performed as described (22). The concentrations of adrenodoxin and adrenodoxin reductase were 4 and 0.4 μM, respectively. The incubation time was 60 min at pH 7.4. The concentration of substrate was 62.5 μM and that of cytochrome P450 was 0.5 μM if not otherwise stated. Incubations with 5β-cholestane-3α,7α,12α-triol and 5β-cholestane-3α,7α,12α,27-tetrol were analyzed as described (23). Incubations with 25-hydroxyvitamin D_3 and 1α-hydroxyvitamin D_3 were analyzed for 1α,25-dihydroxyvitamin D_3 and 25,27-dihydroxyvitamin D_3 by straight-phase and reversed-phase HPLC (7, 22). The enzymatically formed 1α,25-dihydroxyvitamin D_3 was further identified after the two HPLC steps by combined GC/MS (22, 24).

Other Methods. Adrenodoxin and adrenodoxin reductase from bovine adrenal mitochondria were prepared as described (4). Protein and cytochrome P450 determinations, electrophoresis, silver staining, and incubations with antibody-coupled Sepharose were performed as described (25–27). Immunoblotting was performed as described by Andersson and Jörnvall (28) and visualized by alkaline phosphatase.

RESULTS

Purification of Cytochrome P450 from Pig Liver Mitochondria Catalyzing 1α-Hydroxylation. Incubation of pig liver mitochondria with isocitrate and 25-hydroxyvitamin D_3 resulted in the formation of both 1α,25-dihydroxyvitamin D_3 and 25,27-dihydroxyvitamin D_3 (Table 1). CYP27 is known to catalyze the 27-hydroxylation of 25-hydroxyvitamin D_3 (7) and the 25-hydroxylation of vitamin D_3 (5, 9–11). In further purification of the liver mitochondrial cytochrome P450 catalyzing 1α-hydroxylation, these activities were monitored for comparison. As shown in Table 1, the 1α-, 27-, and 25-hydroxylase activities were enriched in the same cytochrome P450 fraction upon chromatography on octylamine-Sepharose, hydroxylapatite, and Q-Sepharose. Less than 5% of the total 1α-hydroxylase activity was found in side fractions of the first two steps and <20% was found in side fractions of the third step. The ratio between the 1α-, 27-, and 25-hydroxylase activities was about the same in all purified fractions, including the side fractions. The purified 1α-hydroxylase fraction showed a major protein band with an apparent M_r of 53,000. The purified 1α-hydroxylase system required both the cytochrome P450 and the reductase components for activities. The conversion of 25-hydroxyvitamin D_3 into 1α,25-dihydroxyvitamin D_3 increased, although not linearly, with the concentrations of cytochrome P450, adrenodoxin, and adrenodoxin reductase. The conversion increased with time up to 120 min and the system was saturated with 50 μM 25-hydroxyvitamin D_3. The identity of the enzymatically formed 1α,25-dihydroxyvitamin D_3 was confirmed by combined GC/MS.

Substrates for CYP27 Inhibit 1α-Hydroxylation. The findings that the ratio between the 1α, 27-, and 25-hydroxylase activities remained essentially constant during the purification and that the cytochrome P450 preparation catalyzing 1α-hydroxylation showed a protein band with the same apparent M_r as CYP27 from pig liver mitochondria (7) prompted further comparison with this enzyme. Addition of increasing amounts of known substrates for CYP27 to the reconstituted 1α-hydroxylase system decreased both the 1α- and 27-hydroxylase activities toward 25-hydroxyvitamin D_3 in a parallel fashion. 5β-Cholestane-3α,7α-diol or vitamin D_3 together with 25-hydroxyvitamin D_3 in equimolar concentrations decreased the 1α- and 27-hydroxylase activities by 50%, as would be expected if a single enzyme catalyzed both hydroxylations. Higher concentrations did not reduce the activities further, possibly because of insolubility of the lipophilic compounds.

A Monoclonal Antibody Against CYP27 Inhibits 1α-Hydroxylase Activity. A monoclonal antibody raised against the pig liver CYP27 (7) was coupled to Sepharose and incubated with the 1α-hydroxylating cytochrome P450 fraction. After incubation, the antibody-Sepharose was removed and the supernatant was assayed for catalytic activities. Fig. 1 shows that both the 1α- and the 27-hydroxylase activities toward 25-hydroxyvitamin D_3 as well as the 25-hydroxylase activity toward vitamin D_3 were immunoprecipitated in parallel. An irrelevant antibody directed against the microsomal pig liver vitamin D_3 25-hydroxylase (27) did not affect the activities. Thus, the 1α-hydroxylase activity in liver mitochondria was immunologically indistinguishable from the 27- and 25-hydroxylase activities of CYP27. Consequently, apparently homogeneous preparations of CYP27 from pig (7) and rabbit (5, 15) liver mitochondria were analyzed for 1α-hydroxylase activity.

Apparently Homogeneous CYP27 from Pig and Rabbit Liver Catalyzes 1α-Hydroxylation. As shown in Table 1, the CYP27 from pig liver (7) exhibited 1α-hydroxylase activity and ratios among the 1α-, 27-, and 25-hydroxylase activities similar to those found with the 1α-hydroxylase preparation. An apparently homogeneous CYP27 prepared from rabbit liver mitochondria (5, 15) also showed the same specific 1α-hydroxylase activity as the pig liver preparations. The formation of 1α,25-dihydroxyvitamin D_3 by the purified cytochrome P450 preparations was unaffected by the antioxidant 1,2-dianilinoethane; required NADPH, cytochrome P450, and reductase components; and was completely inhibited by 300 μM ketoconazole. Thus, the possibility that 1α,25-dihydroxyvitamin D_3 was formed by nonenzymatic, free radical reactions (3) could be excluded. The results obtained with purified 1α-hydroxylating cytochrome P450 preparations, substrate competition, and immunoinhibition experiments provide strong evidence that 1α-hydroxylation of 25-hydroxyvitamin D_3 in liver mitochondria is catalyzed by CYP27.

Recombinant Expressed Human CYP27 in *E. coli* Catalyzes 1α-Hydroxylation. The expression of human CYP27 was induced by isopropyl β-D-thiogalactopyranoside and membrane proteins were solubilized with sodium cholate. The recombinant CYP27 protein was detected by SDS/PAGE and immunoblotting with an antibody (29) against human CYP27. As shown in Fig. 2, *E. coli* cells harboring pTrc27H expressed a polypeptide with an apparent M_r of 54,000 that was recognized by the antibody (lane 3), whereas cells harboring just pTrc99a did not (lane 4). The antibody recognized a protein with the same apparent M_r in the CYP27 preparation from human liver mitochondria (lane 1) used as a positive control. Solubilized protein from pTrc27H-transformed *E. coli* reconstituted with adrenodoxin and adrenodoxin reductase showed 1α-hydroxylase and 27-hydroxylase activities toward 25-hydroxyvitamin D_3 of about 0.11 and 0.08 pmol per min per mg of protein, respectively. The 27-hydroxylase activity toward 5β-cholestane-3α,7α,12α-triol was 47 pmol per min per mg of protein. In control experiments with solubilized protein from pTrc-

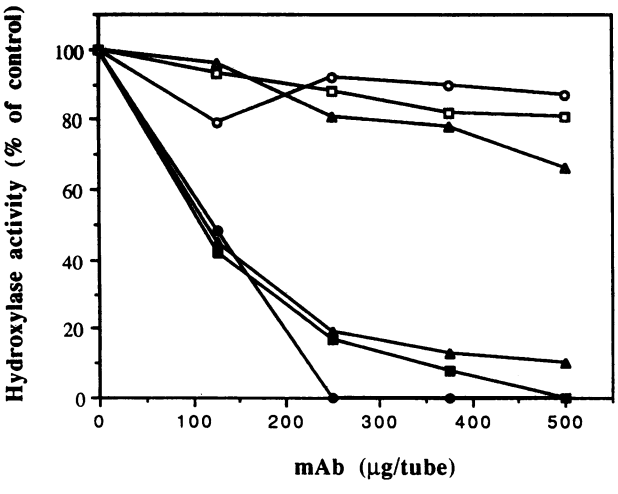

FIG. 1. Effects of a monoclonal antibody (mAb) against pig liver mitochondrial CYP27 (mAb 26C5) on the 1α-hydroxylation (● and ○) and 27-hydroxylation (■ and □) of 25-hydroxyvitamin D_3 and 25-hydroxylation (▲ and △) of vitamin D_3. Cytochrome P450 (0.5 μM) was incubated with the indicated amounts of Sepharose-bound mAb 26C5 (solid symbols) or an irrelevant antibody (mAb 25H6) against pig liver microsomal 25-hydroxylase (open symbols).

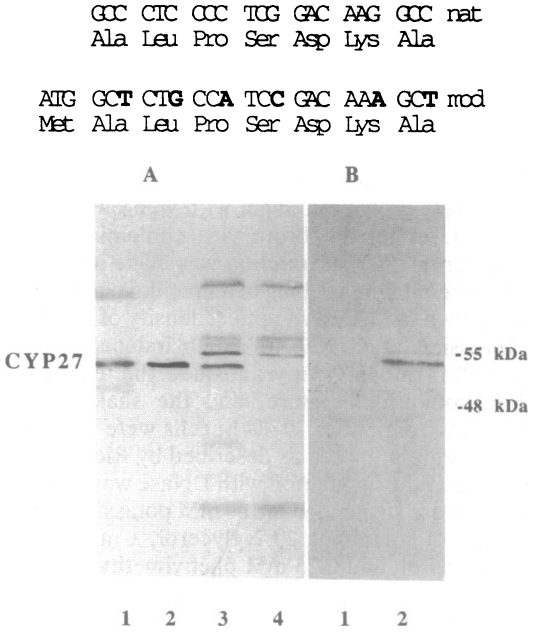

FIG. 2. (*Lower*) SDS/PAGE and immunoblotting of human CYP27. (*A*) Protein fractions in the purification of CYP27 expressed by pTrc27H-transformed *E. coli* compared with a partially purified CYP27 preparation from human liver mitochondria. Lanes: 1, partially purified CYP27 from human liver mitochondria (10 μg); 2, hydroxylapatite eluate from pTrc27H-transformed *E. coli* (10 μg); 3, solubilized membrane fraction from pTrc27H-transformed *E. coli* (30 μg); 4, solubilized membrane fraction from pTrc-transformed *E. coli* used as control (30 μg). (*B*) Lanes: 1, solubilized mitochondrial protein from COS cells transfected with *CYP27* cDNA inserted into the pCMV vector in reversed position used as control (10 μg); 2, solubilized mitochondrial protein from COS cells transfected with *CYP27* cDNA (10 μg). (*Upper*) Modified and native 5′ nucleotide sequences of *CYP27* cDNA used in the pTrc and pCMV vectors, respectively, are shown.

transformed *E. coli*, no 1α- or 27-hydroxylase activity was detected (limit of detection, 0.006 pmol per min per mg of protein). Recombinant-expressed CYP27 was enriched by chromatography on aminohexyl-Sepharose and hydroxylapatite for further characterization. A fraction containing 0.18 nmol of cytochrome P450 per mg of protein was isolated after hydroxylapatite chromatography. SDS/PAGE and immunoblotting (Fig. 2) revealed that the antibody against human CYP27 (29) recognized a single protein with apparent M_r of 54,000 (lane 2). The specific 1α- and 27-hydroxylase activities toward 25-hydroxyvitamin D_3 were 4.3 and 3.4 pmol per min per mg of protein, respectively, representing a 40-fold purification from the cholate-solubilized protein fraction. Table 2 shows that the turnover for 1α-hydroxylation was 24 pmol per min per nmol of cytochrome P450. This turnover is ≈10 times higher than that found in CYP27 purified from pig and rabbit liver (cf. Table 1). The ratio between the 1α- and 27-hydroxylations of 25-hydroxyvitamin D_3 was ≈1:1, which resembles that found with CYP27 from rabbit (cf. Table 1). For comparison, other activities known to be associated with CYP27 are shown in Table 2. As expected, the turnover was highest for 27-hydroxylation of 5β-cholestane-3α,7α,12α-triol. The turnovers for the various reactions were in the following order: 27-hydroxylation of 5β-cholestane-3α,7α,12α-triol, oxidation of the 27-hydroxylated C_{27} steroid into corresponding acid, 25-hydroxylation of 1α-hydroxyvitamin D_3, 25-hydroxylation of vitamin D_3, 1α-hydroxylation of 25-hydroxyvitamin D_3, 27-hydroxylation of 25-hydroxyvitamin D_3. To exclude the possibility that 1α,25-dihydroxyvitamin D_3 was formed by nonenzymatic, free radical reactions (3) a series of experiments were performed. The 1α-hydroxylase activity was unaffected by the antioxidant 1,2-dianilinoethane (10 μM), required NADPH as well as the adrenodoxin and adrenodoxin reductase, and was almost completely inhibited by 100 μM ketoconazole, the cytochrome P450 inhibitor. The 27- and 25-hydroxylase activities showed the same properties.

Recombinant-Expressed CYP27 in COS Cells Catalyzes 1α-Hydroxylation. COS cells were transfected with the pCMV expression vector containing the human *CYP27* cDNA (13), mitochondria were prepared from the cells, and mitochondrial membrane proteins were solubilized with sodium cholate. As a control, COS cells were transfected with the pCMV vector having the cDNA inserted in the reversed position. The recombinant CYP27 protein was detected by SDS/PAGE and immunoblotting with the antibody against human *CYP27* (29). Fig. 2 shows that the pCMV27H-transfected COS cells expressed a protein with apparent M_r of ≈54,000, whereas the pCMV27H reversed-transfected cells did not. Table 3 shows that solubilized mitochondrial protein from COS cells transfected with *CYP27* in the presence of adrenodoxin, adrenodoxin reductase, and NADPH catalyzed 1α-hydroxylation and 27-hydroxylation of 25-hydroxyvitamin D_3 in a ratio of ≈1:1. As expected, the system also showed 27-hydroxylase activity toward 5β-cholestane-3α,7α,12α-triol. Mitochondrial protein from the control cells with pCMV27H reversed did not show 1α- or 27-hydroxylase activities.

Table 2. Catalytic activities of human CYP27 purified from pTrc27H-transformed *E. coli*

Reaction measured	Turnover, pmol per min per nmol of P450
25-Hydroxyvitamin D_3	
1α-Hydroxylation	24
27-Hydroxylation	19
Vitamin D_3	
25-Hydroxylation	72
1α-Hydroxyvitamin D_3	
25-hydroxylation	189
5β-Cholestane-3α,7α,12α-triol	
27-hydroxylation	4337
5β-Cholestane-3α,7α,12α,27-tetrol	
oxidation to acid	363

Incubations with 62.5 μM 5β-cholestane-3α,7α,12α-triol and 12.5 μM 5β-cholestane-3α,7α,12α,27-tetrol were carried out for 120 min. Concentration of cytochrome P450 was 0.26 μM.

Table 3. Hydroxylase activities in solubilized mitochondrial protein from COS-1 cells transfected with human *CYP27* cDNA

	25-Hydroxyvitamin D_3		5β-Cholestane-3α,7α,12α-triol
	1α-OHase	27-OHase	27-OHase
pCMV27H	3.0	2.7	2865
pCMV27HR	<0.2	<0.2	<0.2

As control COS-1 cells were transfected with pCMV27HR containing the cDNA for CYP27 in the reversed position. Incubation mixture contained 240 μg of protein. Results are expressed as pmol per min per mg of protein.

DISCUSSION

From a regulatory point of view the 1α-hydroxylation of 25-hydroxyvitamin D_3 is the most important reaction in the overall metabolism of vitamin D_3. The 1α-hydroxylase in kidney mitochondria has been extremely difficult to purify and no cytochrome P450 enzyme catalyzing 1α-hydroxylation has been previously identified and recombinantly expressed (for review, see ref. 30). In the present communication, it is demonstrated that liver mitochondrial CYP27 and recombinant-expressed human CYP27 in both *E. coli* and mammalian COS cells catalyze 1α-hydroxylation of 25-hydroxyvitamin D_3. The finding that CYP27 is a 25-hydroxyvitamin D_3 1α-hydroxylase was somewhat surprising. On the other hand, it is noteworthy that no attempts to demonstrate 1α-hydroxylase activity have been reported in previous studies on liver mitochondrial CYP27 except for the one by Dahlbäck and Wikvall (5). In that report, the assay conditions were not optimal for the 1α-hydroxylase and the activity was found to be <10 pmol per min per nmol of cytochrome P450. It might be mentioned in this connection that the yield of CYP27 expressed was low compared with other cytochromes P450 expressed in *E. coli*. The reason for this is not known at present. It might be due to extensive degradation of the mRNA of enzyme in *E. coli* or to not optimal modifications of *CYP27* cDNA for expression in *E. coli*. Anyway, the recombinant-expressed human CYP27 catalyzed 1α-hydroxylation with a turnover that was much higher than (31, 32) or comparable with (22) that reported for reconstituted 1α-hydroxylase preparations from kidney mitochondria.

Although the kidney is considered to be the major site of 1α,25-dihydroxyvitamin D_3 production, the relative importance of hepatic and renal 1α-hydroxylation under various conditions is not fully known. It has been suggested that the metabolic controls that affect extrarenal 1α-hydroxylation appear to be very different from the controls that regulate the renal 1α-hydroxylation (3). The results of the present communication identifying CYP27 as a liver mitochondrial 1α-hydroxylase should make it possible to study in more detail the regulation and role of hepatic 1α-hydroxylation in vitamin D_3 metabolism. An even more intriguing possibility is raised by the finding that recombinant-expressed human CYP27 catalyzes 1α-hydroxylation. It is known that mRNA for CYP27 is expressed in several tissues including the kidneys (6), and CYP27 from pig liver and kidney are immunologically indistinguishable (7). Consequently, the results showing that

CYP27 is able to catalyze 1α-hydroxylation of 25-hydroxyvitamin D_3 indicate that renal 1α-hydroxylation is also catalyzed by CYP27. This contention is both supported and not supported by a study on patients with the rare inherited disease cerebrotendinous xanthomatosis having a defective sterol 27-hydroxylation of bile acid intermediates (33). On the one hand, it was demonstrated that extensive osteoporosis and increased risk of bone fractures are components of this disease. On the other hand, the serum concentrations of 1α,25-dihydroxyvitamin D_3 were not significantly lower than in healthy subjects (33). The present study showing that CYP27 is a 1α-hydroxylase of course does not exclude the existence of additional 1α-hydroxylase(s) in the kidney and liver. Anyway, the results open possibilities for further studies that might provide insight into the molecular properties of the mitochondrial 25-hydroxyvitamin D_3 1α-hydroxylation in both liver and kidney.

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