J. Biochem. 127, 1047–1052 (2000)

Characterization of Human Liver Leukotriene B_4 ω-Hydroxylase P450 (CYP4F2)

Yasushi Kikuta,*,[1] Emi Kusunose,† and Masamichi Kusunose*

Department of Applied Biological Science, Faculty of Engineering, Fukuyama University, Fukuyama, Hiroshima 729-0292; and †Department of Molecular Regulation, Osaka City University Medical School, Osaka 537-0025

Received February 28, 2000; accepted March 22, 2000

We previously reported the cloning of a human liver leukotriene B_4 (LTB_4) ω-hydroxylase P450 designated CYP 4F2 [Kikuta *et al.* (1994) *FEBS Lett.* 348, 70–74]. However, the properties of CYP 4F2 remain poorly defined. The preparation solubilized using *n*-octyl-β-D-glucopyranoside from microsomes of CYP 4F2-expressing yeast cells catalyzes ω-hydroxylation of LTB_4, 6-*trans*-LTB_4, lipoxin A_4, 8-hydroxyeicosatetraenoate, 12-hydroxyeicosatetraenoate, and 12-hydroxystearate in the presence of rabbit liver NADPH-P450 reductase. In addition, the enzyme shows ethoxycoumarin *O*-deethylase and *p*-nitroanisole *O*-demethylase activities. The enzyme was purified to apparent electrophoretic homogeneity from yeast cells by sequential chromatography of solubilized microsomes through amino-*n*-hexyl-Sepharose 4B, DEAE-HPLC, and hydroxylapatite HPLC columns. The final preparation showed a specific content of 11.1 nmol of P450/mg of protein, with an apparent molecular mass of 56.3 kDa. CYP 4F2 was distinguished from the closely homologous CYP 4F3 (human neutrophil LTB_4 ω-hydroxylase) by its much higher K_m for LTB_4, inability to ω-hydroxylate lipoxin B_4, and extreme instability.

Key words: CYP4F2, human liver, leukotriene B_4 ω-hydroxylase, lipoxin A_4, P450.

Leukotriene B_4 (LTB_4) is a potent chemotactic and chemokinetic agent, and its ability to recruit and activate neutrophils plays a critical role in inflammatory diseases. Thus, the metabolic inactivation of LTB_4 is thought to be very important for the regulation of its physiological and pathophysiological activities. The liver is the major organ for uptake and elimination of eicosanoids including LTB_4 (*2*, *3*). Earlier work by Murphy's group (*4*, *5*), using isolated rat hepatocytes, demonstrated that LTB_4 is rapidly metabolized by ω-oxidation followed by β-oxidation. Romano *et al.* (*6*) suggested that the ω-and (ω-1)-hydroxylations of LTB_4 are supported by different isozymes of P450 in rat liver microsomes. Furthermore, Sumimoto *et al.* (*7*) provided strong evidence for the identification of rat liver microsomal LTB_4 ω-hydroxylase as a P450, using the photochemical carbon action spectrum and other procedures.

On the other hand, human neutrophil LTB_4 ω-hydroxylase has been investigated more extensively by several groups (*8–10*). In 1993, our laboratory isolated and sequenced a cDNA encoding human neutrophil LTB_4 ω-hydroxylase (*11*). This neutrophil enzyme was demonstrated to be structurally related to the CYP4A subfamily, "fatty acid and prostaglandin ω-hydroxylases" (*12*), and designated CYP 4F3. Both the catalytic properties and genomic organization of CYP 4F3 have recently been characterized (*13*, *14*). As an extension of this work, a second LTB_4 ω-hydroxylase was also cloned from a human liver cDNA library using the CYP 4F3 cDNA as a probe (*15*). This enzyme shows 87.3% sequence similarity with CYP 4F3 and was designated CYP 4F2. Unlike CYP 4F3, CYP 4F2 was found to be expressed in human liver, but not in human neutrophils (*15*). In addition, we observed that human liver microsomes obtained from 14 subjects were capable of converting LTB_4 to ω-hydroxy LTB_4 (*15*). These findings suggested CYP 4F2 to be the major enzyme responsible for LTB_4 ω-hydroxylation in human liver. Recently, Jin *et al.* (*16*) have purified CYP4F2 or a closely related form from human liver microsomes using conventional chromatography combined with immunochemical screening with rat CYP 4A1 antibodies. However, the precise nature of this enzyme remains unknown. In the present study, we have purified for the first time a recombinant CYP 4F2 expressed in yeast cells to electrophoretic homogeneity, and characterized its catalytic properties.

MATERIALS AND METHODS

Materials—Restriction enzymes and T4 DNA ligase were purchased from Takara Shuzo (Kyoto), and PCR kits with r*Tth* DNA polymerase XL from Perkin-Elmer (Norwalk). The 4F2-ATG primer (5′-GGAAGCTTAAAAAAATGTCCC-AGCTGAGCCTGTCCTGGCTGGGC-3′), which contains the *Hin*dIII linker sequence, six A residues, and 30-bp of the sense sequence from the translation starting site of the

[1] To whom correspondence should be addressed. Fax: +81-849-36 2459, E-mail: kikuta@fubac.fukuyama-u.ac.jp

Abbreviations: CYP, a P450 gene(s), cDNA(s) and protein(s), which is the nomenclature recommended by Nelson *et al.* (*1*); CYP 4F2 and CYP 4F3, isoforms of human leukotriene B_4 ω-hydroxylase P450; 5-HETE, [5(*R*,*S*),6*E*,8*Z*,11*Z*,14*Z*]-5-hydroxy-eicosatetraenoic acid; 8-HETE, [5*E*,8(*R*,*S*)9*Z*,11*Z*,14*Z*]-8-hydroxy-eicosatetraenoic acid; 9-HETE, [5*Z*,7*E*,9(*R*,*S*)11*Z*,14*Z*]-9-hydroxy-eicosatetraenoic acid; 11-HETE, [5*E*,8*Z*,11(*R*,*S*)12*Z*,14*Z*]-11-hydroxy-eicosatetraenoic acid; 12-HETE, [5*Z*,8*Z*,10*E*,12(*R*,*S*),14*Z*]-12-hydroxy-eicosatetraenoic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid. RT-PCR, reverse transcription–polymerase chain reaction; HPLC, high-pressure liquid chromatography.

CYP4F2 cDNA, and the 4F2-TGA primer (5'-CCAAGCTTAGTGGGGTCAGAGTGGGTCTCTGCAGAACTCAGC-3'), which contains the anti-sense sequence around the translation termination site of the CYP4F2 cDNA and the *Hin*dIII linker sequence, were synthesized by Espec Oligo Service (Tsukuba). The yeast expression vector pAAH5 carrying the ADH1 promotor and terminator was kindly supplied by Dr. B.D. Hall (University of Washington), and *Saccharomyces cerevisiae*, strain AH22 (*a, leu2, his4, can1, cir+*), by Dr. T. Hatano (Fukuyama University). Emulgen 911 and 913 were kindly provided by Kao Chemicals (Tokyo). All eicosanoids and fatty acids tested were purchased from Funakoshi (Tokyo), *n*-octyl-β-D-glucopyranoside from Nakarai (Tokyo), Sepharose 4B from Pharmacia Fine Chemicals (Uppsala), a DEAE HPLC column (5PW) from Tosoh (Tokyo), a hydroxylapatite HPLC column (KB13-0012) from Koken (Tokyo). Amino-*n*-hexyl Sepharose 4B (AH-Sepharose 4B) was prepared by the method of Cuatrecasas (*17*). NADPH-P450 reductase and cytochrome b_5 were prepared from rabbit liver microsomes by the methods of Taniguchi *et al.* (*18*) and Spatz and Strittmatter (*19*), respectively.

Expression of CYP4F2 in Yeast Cells—The cDNA for CYP4F2 with a *Hin*dIII linker sequence at both ends was constructed by PCR using the CYP4F2 cDNA previously separated (*14*) as the template. The PCR was performed with a primer pair, 4F2-ATG and 4F2-TGA, in a reaction volume of 100 μl that included 20 ng of the CYP4F2 cDNA and r*Tth* DNA polymerase XL (2 units). The PCR product (1.6-kbp) was subcloned to the *Eco*RV site of pBluescript II KS$^+$, and then sequenced. The CYP4F2 cDNA, which did not include any artificial mutations, was separated from the plasmid vector by digestion with *Hin*dIII and subcloned to the yeast expression vector pAAH5 at the *Hin*dIII site. Its orientation was determined by restriction enzyme mapping. Transfection of yeast cells was carried out as described (*20*). Yeast cells with the CYP4F2 expression vector were cultivated in medium containing 0.67% "yeast nitrogen base," 8% glucose, and 2% amino acid mixture without leucine to a cell density of 1–2 × 10^8 cells/ml. The yeast cells were taken up into medium containing 10 mM Tris-HCl buffer (pH 7.5), 0.65 M sorbitol, 0.1 mM dithiothreitol (DTT), 0.1 mM EDTA, and 0.4 mM phenylmethylsulfonylfluoride, and broken with a French pressure cell at 1,500 kg/cm^2. Yeast microsomes were prepared as described (*21*).

Purification of CYP 4F2 from Yeast Microsomes—The microsomes (4,162 mg of protein) from yeast cells cultivated in 30 liters of medium were suspended in 0.1 M potassium phosphate buffer (pH 7.6) containing 20% glycerol, 1 mM DTT, and 1 mM EDTA (designated "0.1 M KP Buffer A") to a final microsomal protein concentration of 5 mg/ml. A 10% sodium cholate solution was added dropwise with stirring to this suspension to give a final concentration of 1% (w/v). After stirring at 4°C for 60 min, the mixture was centrifuged at 140,000 ×*g* for 60 min. The supernatant was diluted with "0.1 KP Buffer A" to a concentration of 0.6% sodium cholate, and applied to a column (2.5 × 21 cm) of AH-Sepharose 4B pre-equilibrated with buffer containing 0.6% sodium cholate. The column was washed with 500 ml of "0.1 M KP Buffer A" containing 0.3% sodium cholate, and then P450 was eluted with "20 mM KP Buffer A" containing 0.4% sodium cholate and 0.08% Emulgen 913. The eluate was concentrated by membrane ultrafiltration on a UK 50 filter (Advantec, Tokyo). The concentrated solution was then diluted 10-fold with 20 mM Tris-acetate buffer (pH 7.5) containing 20% glycerol and 0.4% Emulgen 911 ("DE-A Buffer") and applied to a DEAE HPLC column (0.75 × 7.5 cm) equilibrated with the same buffer. The column was washed with the same buffer, and the P450 was eluted at a flow rate of 0.6 ml/min with a linear gradient of "DE-A Buffer" containing 1 M sodium acetate ("DE-B Buffer") (20%/60 min). The fractions containing P450 that appeared at around 135 mM sodium acetate were pooled and concentrated. This fraction was diluted 10-fold with 10 mM sodium phosphate buffer (pH 6.5) containing 20% glycerol, 0.2% sodium cholate, and 0.4% Emulgen 911 ("HA-A Buffer") and applied to a hydroxylapatite HPLC column (0.78 × 10 cm) equilibrated with the same buffer. The column was washed with the buffer, and P450 was eluted at a flow rate of 0.5 ml/min with a linear gradient of "HA-A Buffer" to 350 mM sodium phosphate buffer (pH 7.5) containing 20% glycerol, 0.2% sodium cholate, and 0.4% Emulgen 911 ("HA-B Buffer") (35%/70 min). P450 eluted at around 80 mM sodium phosphate. The fractions showing high purity on SDS-PAGE were pooled and concentrated on a small hydroxylapatite column (1 × 1 cm). The detergent was removed by washing the column with 20 mM potassium phosphate buffer (pH 7.25) containing 20% glycerol, until the optical density at 208 nm fell below 0.01. P450 was eluted with 350 mM sodium phosphate buffer (pH 7.25) containing 20% glycerol, and concentrated using a Centrisart I (Sartorius, Gottingen). The resuls of a typical purification of P450 are summarized in Table I.

Activity Assays—Omega-hydroxylase activities toward LTB_4 and 6-*trans*-LTB_4 were determined as described by Sumimoto *et al.* (*10*). The reaction mixture containing 10 pmol of P450, 0.1 unit of rabbit liver NADPH-P450 reductase, 20 mM Hepes buffer (pH 7.5) including 340 mM sucrose, 1 mM EDTA, and 1 mM DTT, 1 mM NADPH, and either 60 μM LTB4 or 60 μM 6-*trans* -LTB_4, in a total volume of 0.1 ml, was incubated at 37°C for 15 min. The reaction product was then extracted with ethyl acetate and measured (*10*). Lipoxin A_4 (LXA_4) and lipoxin B_4 (LXB_4) ω-hydroxylase activities were each determined using 40 μM substrate by the methods described by Sumimoto *et al.* (*22*) and Mizukami *et al.* (*23*, *24*), respectively. Omega hydroxylase activities toward various hydroxyeicosatetraenoic acids (HETEs) were determined using 40 μM HETE by measuring the reaction products according to a modification of the procedure described by Marcus *et al.* (*25*). In the present study, the products were separated on an ODS-120A HPLC column (0.46 × 25 cm) (Tosoh, Tokyo) with methanol/acetonitrile/water/acetic acid (27:27:46:0.02) as the eluting solvent at a flow rate of 1 ml/min. Absorbance was monitored at 237 nm. For all the activity assays described above, appropriate amounts of the eicosanoids used as substrates were dissolved in 10 μl of ethanol, dried under nitrogen

TABLE I. **Purification of CYP 4F2 expressed in yeast cells.**

Step	Protein (mg)	P450 (nmol)	Specific content (nmol P450/mg)	Yield (%)
Microsomes	4,162	67.9	0.016	100
Cholate extracts	3,904	53.6	0.014	79
AH-Sepharose 4B	127	8.8	0.07	13
DEAE-HPLC	1.7	3.6	2.12	5.3
Hydroxylapatite-HPLC	0.19	2.1	11.1	3.1

gas, and mixed with the enzyme using a vortex device for 5 s, followed by the addition of other reaction components. The two omega hydroxylase activities toward various fatty acids and prostaglandins (each 100 μM) were determined using 0.1 M potassium phosphate buffer (pH 7.5) and 0.1 M sodium phosphate buffer (pH 7.4), respectively, in a total volume of 0.1 ml (*26*). Ethoxycoumarin *O*-deethylase and *p*-nitroanisole *O*-demethylase activities were determined by the methods of Greenlee and Poland (*27*), and Netter and Seidel (*28*), respectively. The concentration of P450 was determined by the method of Omura and Sato (*29*).

RESULTS

Catalytic Activities of Solubilized Microsomes—Microsomal fractions obtained from yeast cells transfected with the CYP 4F2 expression vector showed 0.02 to 0.04 nmol of P450/mg of protein. In initial studies, we used *n*-octyl-β-D-glucopyranoside to solubilize CYP 4F2 from the microsomes. The detergent was added to the microsomes to a final concentration of 1% with stirring for 30 min at 4°C according to the procedure described by Palmer *et al.* (*30*). Roughly, 60–70% of the P450 was recovered in the supernatant after centrifugation at 100,000 ×*g* for 60 min, with only 20–30% of total microsomal proteins being solubilized. When reconstituted with rabbit liver NADPH-P450 reductase and cytochrome b_5, and NADPH, the solubilized P450 catalyzed ω-hydroxylation of LTB_4 with a turnover rate of 1.7 nmol/min/nmol of P450. With neither NADPH-P450 reductase nor NADPH, no activity was detected. Omission of cytochrome b_5 resulted in a 40% reduction in activity. In addition to LTB_4, the enzyme preparation was found to be capable of hydroxylating various compounds including 6-*trans*-LTB_4, LXA_4, and HETEs under the same conditions (Table II). These hydroxylation reactions were linear over an incubation period of about 10 min. The rates of the reac-

TABLE II. **Catalytic activities of solubilized microsomes.**

Substrate	Activity (nmol/min/nmol P450)
LTB_4	1.7
6-*trans* LTB_4	7.1
Lipoxin A_4	4.8
5-HETE	2.7
8-HETE	2.4
9-HETE	2.3
11-HETE	4.8
12-HETE	4.9
15-HETE	4.7
12-Hydroxystearate	9.7
Oleate	3.3

Note: Values represent the average of four determinations.

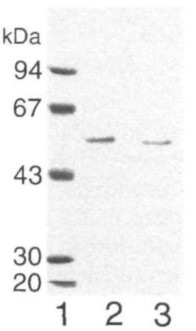

Fig. 1. **SDS–polyacrylamide gel electrophoresis of purified CYP 4F2 and CYP 4F3.** Lane 1 contains molecular mass markers: phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), and soybean trypsin inhibitor (20 kDa); lane 2, CYP 4F2 (6 pmol); lane 3, CYP 4F3 (3 pmol). Electrophoresis was performed with a 10% acrylamide gel, and the proteins were stained with 0.1% Coomassie Brilliant Blue.

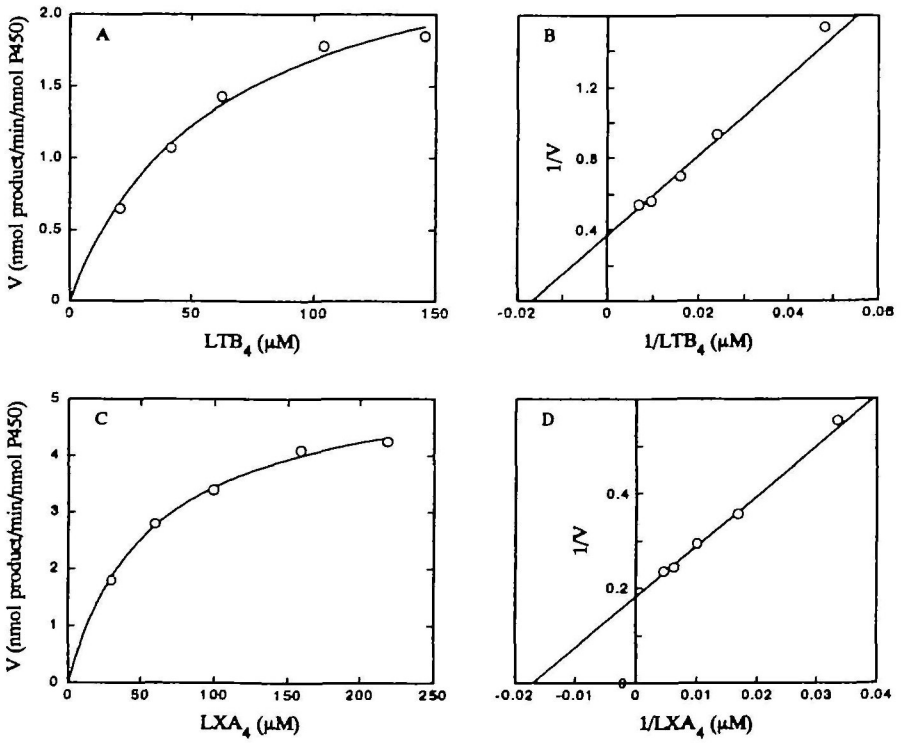

Fig. 2. **Kinetics for the ω-hydroxylations of LTB_4 and LXA_4 by purified CYP 4F2.** Plots of reaction velocity *versus* LTB_4 (panel A) and LXA_4 (panel C) concentrations. Lineweaver-Burk plots for the ω-hydroxylations of LTB_4 (panel B) and LXA_4 (panel D).

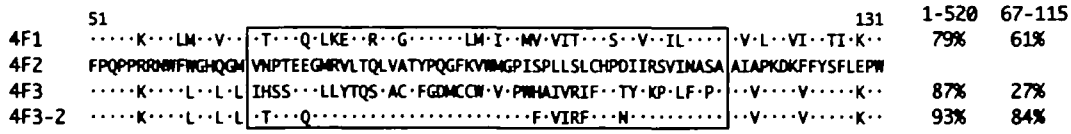

Fig. 3. **Alignment of the amino acid sequences of the unconserved regions of CYP 4F1, 4F2, and 4F3.** Amino acids 49–133 of CYP 4F subfamily members are shown. Amino acids in the unconserved region (67–115) are enclosed in the Box. The amino acid sequence similarities of the whole molecules (1–520) and the unconserved regions (67–115) to CYP 4F2 are also shown to the right. The product derived from the *CYP 4F3* gene with a different exon III sequence is shown as 4F3-2 (*14*).

TABLE III. **Kinetic parameters of purified CYP 4F2-catalyzed ω-hydroxylations.**

Substrate	V_{max} (nmol/min/nmol P450)	K_m (μM)
LTB_4	2.7	60.0
6-*trans*-LTB_4	11.9	55.6
Lipoxin A_4	5.5	58.2
8-HETE	4.0	19.0
12-HETE	2.9	42.3
12-Hydroxystearate	7.0	75.2

tions increased linearly with P450 concentration up to 30 pmol of P450. Furthermore, ethoxycoumarin *O*-deethylase and *p*-nitroanisole *O*-demethylase activities were also detected with turnover rates of 1.4 and 1.2 nmol/min/nmol of P450, respectively. On the other hand, no activity was detected toward laurate, arachidonate, prostaglandin A_1, or LXB_4. All of the activities determined were very unstable; upon storage at –70°C, the loss of activity occurred rapidly.

Properties of Purified CYP 4F2—Attempts to further purify CYP 4F2 were not successful, because the solubilized P450 did not bind to columns such as AH-Sepharose 4B or DEAE, even after extensive dialysis. Therefore, sodium cholate was used to solubilize P450 instead of *n*-octyl-β-D-glucopyranoside, and then CYP 4F2 was purified by sequential chromatography on AH-Sepharose 4B, DEAE analytical HPLC, and hydroxylapatite HPLC columns (Table I). The purified CYP 4F2 had a specific content of 11 nmol of P450/mg of protein, and it showed a single band on SDS-PAGE with an apparent molecular mass of 56.3 kDa (Fig. 1). The carbon monoxide difference spectra of the reduced form of the purified enzyme showed a peak at 450 nm. Table III summarizes the kinetic parameters of the purified CYP 4F2 with several substrates. The reconstituted enzyme showed similar K_m values (~60 μM) for LTB_4 and LXA_4, with the V_{max} for LXA_4 being 2.0-fold higher than that for LTB_4 (Fig. 2). In contrast to the *n*-octyl-β-D-glucopyranoside-solubilized preparation, cytochrome b_5 had no effect on any reactions catalyzed by the purified enzyme. The most striking feature of CYP4F2 was its extreme instability. For example, when stored at –70°C in the presence of 20% glycerol and 1 mM DTT, the purified enzyme lost about 50% of its catalytic activities in 3 days, and 100% in 10 days. In some instances, the enzyme was almost inactive upon final purification.

DISCUSSION

We recently reported that the *CYP 4F2* gene is very similar to the *CYP 4F3* gene, suggesting that the two genes evolved by duplication and alteration of the transcription regulation region (*31*). However, little is known about the enzymatic properties of CYP 4F2. In the present work, we isolated for the first time the recombinant CYP 4F2 in a highly purified state, and characterized its catalytic properties. As in the case of CYP 4F3, CYP 4F2 was found to catalyze the ω-hydroxylation of various lipoxygenase-dependent arachidonic acid metabolites and related compounds. However, there are some substantial differences between CYP 4F2 and 4F3: (i) The apparent molecular mass of CYP 4F2 (56.3 kDa) is somewhat larger than that of CYP 4F3 (55.0 kDa) (see Fig. 1). (ii) The λ_{max} of the reduced CO difference spectra of CYP 4F3 is 449.5 nm. (iii) The K_m value of CYP 4F2 for LTB_4 (60 μM) is 94-fold higher than that of CYP 4F3 (0.64 μM). (iv) CYP 4F3 preferentially ω-hydroxylates LXB_4 over LXA_4. Conversely, CYP 4F2 is only capable of ω-hydroxylating LXA_4. (v) CYP 4F3 is very stable during storage. For example, no activity loss was observed for purified CYP 4F3 even after 4 years at –70°C. In contrast, CYP 4F2 is extremely unstable as described above. To date, no procedure is available that can stabilize CYP 4F2.

The most striking structural difference between CYP 4F2 and 4F3 was observed at positions 67–115 of both amino acid sequences, a region that corresponds to exon III of the genomes (*14*, *31*)(Fig. 3). Although this region accounts for only 9.4% of the entire sequence, 54.5% of the total amino acid replacements are located within the same region. It is possible that the unconserved 49 amino acid sequence contributes to the substantial differences between CYP 4F2 and 4F3. We have recently found that rat hepatic CYP 4F1 (*32*) bears a remarkable resemblance to CYP 4F2 with regard to the sequence of the unconserved region (*31*; Kikuta, Y., Kashu, H., Kusunose, E., and Kusunose, M., unpublished observations). Furthermore, we have demonstrated that CYP 4F1 has catalytic properties common to those of CYP 4F2; for example, the two P450s show similar catalytic efficiencies for both LTB_4 and LXA_4, and both are unable to ω-hydroxylate LXB_4 (*33*). These findings suggest that the unconserved regions have significant effects on the catalytic properties. In addition, recent studies by Christmas *et al.* (*34*) indicating that CYP 4F3 in which the amino acid sequence of this unconserved region is replaced with a sequence similar to that of CYP 4F2, exhibits a 23-fold higher K_m value toward LTB_4 compared with unmodified CYP 4F3, also support this hypothesis.

Most recently, Hardwick *et al.* (*35*) have immuno-localized the expression of the *CYP* 4F2 gene to several human tissues and tumors. They found high levels of CYP 4F2 in the skin, followed by the kidney, prostate, liver, intestine and brain. Among human tumors, the highest amount is found in an undifferentiated hepatic carcinoma followed by various kinds of tumors. These results suggest that CYP 4F2 may play an important role in the control of epidermal cell proliferation.

In this paper, we show that the catalytic efficiency for 8-

HETE (V_{max}/K_m, 0.21) is higher than that for LTB_4 (V_{max}/K_m, 0.045) (Table III). 8-HETE is generated from arachidonic acid by 8(*S*)-lipoxygenase, which is located in the mouse skin and inducible by treatment with phorbol esters, and suggest that 8-HETE may be involved in inflammation or hyperplasia (*36*, *37*). Therefore, it is likely that CYP 4F2 is also responsible for the metabolism of biologically active HETEs such as 8-HETE.

A recent report by Powell *et al.* (*38*), showing that CYP 4F2 catalyzes the ω-hydroxylation of arachidonic acid more efficiently than CYP 4A11 in human liver, conflicts with our results using the recombinant enzyme. The reason for this discrepancy is presently unexplained.

In addition to ω-hydroxylase activities, CYP 4F2 is capable of metabolizing 7-ethoxycoumarin and *p*-nitroanisole. Similar drug-metabolizing activities have been previously observed for purified CYP 4F3 (Kusunose, E., Kikuta, Y., and Kusunose, M., unpublished observations). The physiological significance of these drug metabolizing activities is unknown.

In conclusion, we report here the purification and characterization of recombinant human hepatic CYP 4F2. The results demonstrate that CYP 4F2 catalyzes the ω-hydroxylation of not only LTB_4, but also 6-*trans*-LTB_4, LXA_4, and several HETEs, suggesting that it plays a role in the metabolic inactivation of various lipoxygenase-dependent arachidonic acid metabolites, including both inflammatory and anti-inflammatory mediators. We are currently investigating the physiological function of this enzyme.

We are grateful to Prof. K. Ichihara (Department of Molecular Regulation, Osaka City University Medical School) for helpful discussion. We also thank K. Matsumoto, E. Saito, S. Nakano, and M. Ito (Department of Applied Biological Science, Fukuyama University) for the preparation of the CYP 4F2 expressed yeast microsomes.

REFERENCES

1. Nelson, D.R., Koymans, L., Kamataki, T., Stegeman, J.J., Feyereisen, R., Waxman, D.J., Waterman, M.R., Gotoh, O., Coon, M.J., Estabrook, R.W., Gunsalus, I.C., and Nebert, D.W. (1996) P450 superfamily: update on new sequences, gene mapping, accession numbers and nomendature. *Pharmacogenetics* **6**, 1–42
2. Hagmann, W. and Korte, M. (1990) Hepatic uptake and metabolic disposition of leukotriene B_4 in rats. *Biochem. J.* **267**, 467–470
3. Leier, I., Muller, M., Jedlitschky, G., and Keppler, D. (1992) Leukotriene uptake by hepatocytes and hepatoma cells. *Eur. J. Biochem.* **209**, 281–289
4. Harper, T.W., Garrity, M.J., and Murphy, R.C. (1986) Metabolism of leukotriene B_4 in isolated rat hepatocytes: identification of a novel 18-carboxy-19,20-dinor leukotriene B_4 metabolite. *J. Biol. Chem.* **261**, 5414–5418
5. Hankin, J.A., Clay, C.E., and Murphy, R.C. (1998) The effects of ethanol and acetaldehyde on the metabolism of prostaglandin E_2 and leukotriene B_4 in isolated rat hepatocytes. *J. Pharmacol. Exp. Ther.* **285**, 155–161
6. Romano, M.C., Eckardt, R.D. Bender, P.E., Leonard, T.B., Straub, K.M., and Newton, J.F. (1987) Biochemical characterization of hepatic microsomal leukotriene B4 hydroxylases. *J. Biol. Chem.* **262**, 1590–1595
7. Sumimoto, H., Kusunose, E., Gotoh, Y., Kusunose, M., and Minakami, S. (1990) Leukotriene B_4 ω-hydroxylase in rat liver microsomes: identification as a cytochrome P-450 that catalyzes prostaglandin A_1 ω-hydroxylation, and participation of cytochrome b_5. *J. Biochem.* **108**, 215–221
8. Shak, S. and Goldstein, I.M. (1984) ω-Oxidation is the major pathway for the catabolism of leukotriene B_4 in human polymorphonuclear leukocytes. *J. Biol. Chem.* **259**, 10181–10187
9. Soberman, R.J., Okita, R.T., Fitzsimmons, G., Rokach, J., Spur, B., and Austen, K.F. (1987) Stereochemical requirements for substrate specificity of LTB_4 20-hydroxylase. *J. Biol. Chem.* **262**, 12421–12427
10. Sumimoto, H., Takeshige, K., and Minakami, S. (1988) Characterization of human neutrophil leukotriene B_4 ω-hydroxylase as a system involving a unique cytochrome *P*-450 and NADPH-cytochrome *P*-450 reductase. *Eur. J. Biochem.* **172**, 315–324
11. Kikuta, Y., Kusunose, E., Endo, K., Yamamoto, S., Sogawa, K., Fujii-Kuriyama, Y., and Kusunose, M. (1993) A novel form of cytochrome P-450 family 4 in human polymorphonuclear leukocytes: cDNA cloning and expression of leukotriene B_4 ω-hydroxylase. *J. Biol. Chem.* **268**, 9376–9380
12. Kusunose, M. (1993) Fatty acid omega-oxidation and eicosanoid metabolism in *Cytochrome P-450*, 2nd. ed. (Omura, T., Ishimura, Y., and Fujii-Kuriyama, Y., eds.) pp. 127–141, Kodansha, Tokyo and VCH, Weinheim
13. Kikuta, Y., Kusunose, E., Sumimoto, H., Mizukami, Y., Takeshige, K., Sakaki, T., Yabusaki, Y., and Kusunose, M. (1998) Purification and characterization of recombinant neutrophil leukotriene B_4 ω-hydroxylase (cytochrome P450 4F3). *Arch. Biochem. Biophys.* **355**, 201–205
14. Kikuta, Y., Kato, M., Yamashita, Y., Miyauchi, Y., Tanaka, K., Kamada, N., and Kusunose, M. (1998) Human leukotriene B_4 ω-hydroxylase (CYP4F3) gene: molecular cloning and chromosomal localization. *DNA Cell Biol.* **17**, 221–230
15. Kikuta, Y., Kusunose, E., Kondo, T., Yamamoto, S., Kinoshita, H., and Kusunose, M. (1994) Cloning and expression of a novel form of leukotriene B_4 ω-hydroxylase from human liver. *FEBS Lett.* **348**, 70–74
16. Jin, R., Koop, D.R., Raucy, J.L., and Lasker, J.M. (1998) Role of human CYP4F2 in hepatic catabolism of the proinflammatory agent leukotriene B_4. *Arch. Biochem. Biophys.* **359**, 89–98
17. Cuatrecasas, P. (1970) Protein purification by affinity chromatography. *J. Biol. Chem.* **245**, 3059–3065
18. Taniguchi, H., Imai, Y., Iyanagi, T., and Sato, R. (1979) Interaction between NADPH-cytochrome P-450 reductase and cytochrome P-450 in the membrane of phosphatidylcholine vesides. *Biochim. Biophys. Acta* **550**, 341–356
19. Spatz, L. and Strittmatter, P. (1971) Form of cytochrome b_5 that contains an additional hydrophobic sequence of 40 amino acid residues. *Proc. Natl. Acad. Sci. USA* **68**, 1042–1046
20. Furuya, H., Shimizu, T., Hatano, M., and Fujii-Kuriyama, Y. (1989) Mutations at the distal and proximal sites of cytochrome P-450d changed regio-specificity of acetanilide hydroxylations. *Biochem. Biophys. Res. Commun.* **160**, 669–676
21. Imai, Y. (1988) Characterization of rabbit liver cytochrome P-450 (laurate omega-1 hydroxylase) synthesized in transformed yeast cells. *J. Biochem.* **103**, 143–148
22. Sumimoto, H., Isobe, R., Mizukami, Y., and Minakami, S. (1993) Formation of a novel 20-hydroxylated metabolite of lipoxin A_4 by human neutrophil microsomes. *FEBS Lett.* **315**, 205–210
23. Mizukami, Y., Sumimoto, H., Isobe, R., and Minakami, S. (1993) ω-Hydroxylation of lipoxin B_4 by human neutrophil microsomes: identification of ω-hydroxy metabolite of lipoxin B4 and catalysis by leukotriene B_4 ω-hydroxylase (cytochrome P-450LTBω). *Biochim. Biophys. Acta* **1168**, 87–93
24. Mizukami, Y., Sumimoto, H., Isobe, R., Minakami, S., and Takeshige, K. (1994) ω-Oxidation of lipoxin B_4 by rat liver: identification of an ω-carboxy metabolite of lipoxin B_4. *Eur. J. Biochem.* **224**, 959–965
25. Marcus, A.J., Safier, L.B., Ullman, H.L., Broekman, M.J., Islam, N., Oglesby, T.D., and Gorman, R.R. (1984) 12*S*,20-dihydroxyicosatetraenoic acid: a new icosanoid synthesized by neutrophils from 12*S*-hydroxyicosatetraenoic acid produced by thrombin- or collagen-stimulated platelets. *Proc. Natl. Acad. Sci. USA* **81**, 903–907

26. Sawamura, A., Kusunose, E., Satouchi, K., and Kusunose, M. (1993) Catalytic properties of rabbit kidney fatty acid ω-hydroxylase cytochrome P-450ka2 (CYP4A7). *Biochim. Biophys. Acta* **1168**, 30–36
27. Greenlee, W.F. and Poland, A. (1978) An improved assay of 7-ethoxycoumarin *O*-deethylase activity: induction of hepatic enzyme activity in C57 BL/6J and DBA/2J mice by phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorobenzo-*p*-dioxin. *J. Pharmacol. Exptl. Therap.* **205**, 596–605
28. Netter, K.J. and Seidel, G. (1964) An adaptively stimulated *O*-demethylating system in rat liver microsomes and its kinetic properties. *J. Pharmacol. Exp. Ther.* **146**, 61–65
29. Omura, T. and Sato, R. (1964) The carbon monooxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239**, 2370–2378
30. Palmer, C.N.A., Richardson, T.H., Griffin, K.J., Hsu, M.-H., Muerhoff, A.S., Clark, J.E., and Johnson, E.F. (1993) Characterization of a cDNA encoding a human kidney, cytochrome P-450 4A fatty acid ω-hydroxylase and the cognate enzyme expressed in *Escherichia coli*. *Biochim. Biophys. Acta* **1172**, 161–166
31. Kikuta, Y., Miyauchi, Y., Kusunose, E., and Kusunose, M. (1999) Expression and molecular cloning of human liver leukotriene B_4 ω-hydroxylase (*CYP 4F2*) gene. *DNA Cell Biol.* **18**, 723–730
32. Chen, L. and Hardwick, J.P. (1993) Identification of a new P450 subfamily, CYP4F1, expressed in rat hepatic tumors. *Arch. Biochem. Biophys.* **300**, 18–23
33. Kikuta, Y., Kusunose, E., Ito, M., and Kusunose, M. (1999) Purification and characterization of recombinant rat hepatic CYP4F1. *Arch. Biochem. Biophys.* **369**, 193–196
34. Christmas, P., Ursino, S.R., Fox, J.W., and Soberman, R.J. (1999) Expression of the CYP4F3 gene: tissue-specific splicing and alternative promoters generate high and low K_m forms of leukotriene B_4 ω-hydroxylase. *J. Biol. Chem.* **274**, 21191–21199
35. Hardwick, J.P., Zhang, X., Malmer, M., Donaldson, E., and Chen, L. (1999) Human tissue and tumor localized expression of the CYP4F2 hepatic leukotriene omega hydroxylase P450 gene. *FASEB J.* **13**, Abstract 1344
36. Jisaka, M., Kim, R.B., Boeglin, W.E., Nanney, L.B., and Brash, A.B. (1997) Molecular cloning and functional expression of a phorbol ester-inducible 8 *S*-lipoxygenase from mouse skin. *J. Biol. Chem.* **272**, 24410–24416
37. Hughes, M.A. and Brash, A.R. (1991) Investigation of the mechanism of biosynthesis of 8-hydroxyeicosatetraenoic acid in mouse skin. *Biochim. Biophys. Acta* **1081**, 347–354
38. Powell, P.K., Wolf, I., Jin, R., and Lasker, J.M. (1998) Metabolism of arachidonic acid to 20-hydroxy-5,8,11,14-eicosatetraenoic acid by P450 enzymes in human liver: involvement of CYP4F2 and CYP4A11. *J. Pharmacol. Exp. Ther.* **285**, 1327–1336