Identification of a Meander Region Proline Residue Critical for Heme Binding to Cytochrome P450: Implications for the Catalytic Function of Human CYP4B1†

Yi-Min Zheng,‡ Michael B. Fisher,‡ Noboru Yokotani,§ Yoshiaki Fujii-Kuriyama,|| and Allan E. Rettie*,‡

Department of Medicinal Chemistry, University of Washington, Seattle, Washington 98195, Department of Chemistry, Faculty of Science, Tohoku University, Aoba Sendai, Japan, and Department of Neuropsychiatry, Osaka City Medical School, Osaka, Japan

Received May 29, 1998; Revised Manuscript Received July 1, 1998

ABSTRACT: Alignment of xenobiotic-metabolizing P450 protein sequences highlights an invariant proline residue in the meander region two amino acids N-terminal to the distal arginine of the putative ERR triad thought to be important for heme binding. This occurs as a serine in the sequences derived from human CYP4B1 gDNA and both human lung and placental CYP4B1 cDNAs. Reversion of this serine to the conserved proline residue (Ser427 → Pro) by site-directed mutagenesis conferred the ability to incorporate heme on the human placental enzyme. Mutation of the corresponding proline in rabbit CYP4B1 (Pro422 → Ser) abolished heme incorporation. Membrane preparations of human CYP4B1(Pro) and rabbit CYP4B1(Pro), but not the corresponding CYP4B1(Ser) variants, supported lauric acid hydroxylation preferentially at the ω-position. Purified, reconstituted human CYP4B1(Pro) and rabbit CYP4B1(Pro) formed 12-hydroxylauric acid at rates of 17−21 min^{-1}, and both enzymes were also C-8 to C-10 fatty acid ω-hydroxylases preferentially, with total rates of hydroxylation decreasing in the order C-12 > C-10 > C-9 > C-8. Finally, neither human nor rabbit CYP4B1(Pro) formed detectable levels of any hydroxylated testosterone metabolites. Therefore, the presence of a consensus Pro-X-Arg motif is critical for incorporation of the heme prosthetic group in human and rabbit CYP4B1 proteins expressed in insect cells. Native human CYP4B1, expressed in vivo, is likely to be functionally impaired if Pro427 is required for holoenzyme expression in mammalian cells.

The mammalian CYP[1]4 family of enzymes is composed of the CYP4A, CYP4B, and CYP4F subfamilies (*1*). CYP4B1 is largely an extrahepatic form of P450, having been isolated and characterized first from rabbit lung, where it comprises 50% of the total complement of microsomal P450 (*2*). More recently, CYP4B1 has been cloned from rabbit liver and lung, rat lung, human lung, human placenta, and mouse kidney (*3*−*6*). CYP4B1 proteins from these four species share 84−90% amino acid sequence identities, yet compared to the laboratory animal orthologs, human CYP4B1 has been reported to exhibit significant interspecies differences in substrate specificity.

Mouse CYP4B1, purified initially from kidney microsomes by using *umu* gene expression as an index of mutagenic activation, bioactivates aromatic amines such as 3-methoxy-4-aminoazobenzene, a potent procarcinogen (*6*). The rabbit and rat orthologs have been shown to bioactivate the lung toxin 4-ipomeanol to an electrophilic species which binds covalently to macromolecules (*7*−*9*) and to metabolize the promutagen 2-aminofluorene to its DNA-reactive *N*-hydroxy metabolite (*10*). Moreover, we have shown that rabbit CYP4B1 is the most effective of over a dozen P450 enzymes in the conversion of valproic acid (VPA) to its hepatotoxic 4-ene metabolite (*11, 12*). Therefore, it is clear that animal CYP4B1 isoforms are involved in the bioactivation of several structurally diverse protoxins.

In contrast, human lung CYP4B1, expressed in HepG2 cells, has been reported to be unable to bioactivate 2-aminofluorene (*4*) or 4-ipomeanol (*8*). Furthermore, several reports suggest that human lung CYP4B1 is capable of metabolizing testosterone, and other steroids, to their 6β-hydroxy metabolites (*8, 13*). These latter reactions are characteristic of CYP3A enzymes, but not of animal forms of CYP4B1. This apparent species difference in substrate specificity is particularly curious given the conservation of CYP4B1-mediated enzyme activity across the rat, rabbit, mouse, hamster, guinea pig, and monkey CYP4B1 orthologs (*10*). Clarification of the substrate specificity of human CYP4B1 is of particular significance given the widespread presence of CYP4B1 mRNA in human tumors and in normal human extrahepatic tissues such as placenta, colon, and lung (*5, 14, 15*).

Recently, Yokotani et al. cloned a cDNA for human CYP4B1 from placenta (*5*) and noted a potentially important

† This work was supported by NIH Grant GM49054 (A.E.R.). M.B.F. was supported by NIH Training Grant GM07750.

* To whom correspondence should be sent at: Dept of Medicinal Chemistry, Box 357610, University of Washington, Seattle, WA 98195. Tel: 206-685-0615. Fax: 206-685-3252. E-mail: rettie@u.washington.edu.

‡ University of Washington.

§ Osaka City Medical School.

|| Tohoku University.

[1] Abbreviations: CYP, cytochrome P450; DLPC, dilaurylphosphatidylcholine; BSTFA, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide; GC/FID, gas chromatography/flame ionization detection; GC/MS, gas chromatography/mass spectrometry; VPA, valproic acid, 2-*n*-propylpentanoic acid; 4-ene-VPA, 2-*n*-propyl-4-pentenoic acid.

S0006-2960(98)01280-X CCC: $15.00
Published on Web 08/21/1998

Table 1: Meander Region Sequences of CYP4B Proteins[a]

Microsomal Consensus*	P c -F - P E + a
Rat CYP4B1	P E V F D ^{427}P L **R** F
Mouse CYP4B1	P E V F D ^{427}P L **R** F
Goat CYP4B2*	P E V F D ^{427}P L **R** F
Rabbit CYP4B1	P E V F D ^{422}P L **R** F
Human CYP4B1	P E V F D ^{427}S L **R** F

[a] −, c, +, and a represent any amino acid, charged residue, positively charged residue, and aromatic residue, respectively, as described by Graham-Lorence and Peterson (*17*). The invariant proline residue of the microsomal P450s is underlined and the highly conserved arginine of the putative ERR triad (*16*) is shown in boldface type. * Unpublished goat CYP4B2 sequence was kindly provided by Dr. G. S. Yost.

amino acid change, relative to nine other mammalian P450 sequences available at that time, near the heme-binding site of the enzyme. This mutation caused a Pro427 → Ser transversion in the translated human protein. This locus is now recognized as part of the so-called P450 meander region which, despite its name, displays a high degree of structural organization (*16*). Graham-Lorence and Peterson (*17*) have described a consensus motif for the microsomal P450s at this locus of xPcxFxPE+a, where x represents any amino acid, c is a charged residue, + is a positively charged amino acid, and a is an aromatic residue. The Pro427 → Ser mutation within the human placental cDNA for CYP4B1 maps to the second proline residue of this consensus motif (see Table 1, underlined residues). Indeed, proline is invariant at this position among all of the known xenobiotic-metabolizing P450s (see alignments in ref *18*).

Consequently, the aims of the present study were to express native human placental CYP4B1[2] [human CYP4B1-(Ser)] and the Pro427 mutant [CYP4B1(Pro)] and to compare their substrate specificities toward fatty acids and steroids directly with that of the well-characterized rabbit enzyme. These studies show that Pro427 is a critical determinant of holoenzyme expression and suggest that its absence in the two human cDNAs isolated to date likely explains the confusion surrounding the catalytic properties of human CYP4B1. The data are discussed in terms of the implications for the bioactivation of xenobiotics by human CYP4B1 in vivo.

EXPERIMENTAL PROCEDURES

Chemicals. NADPH, L-α-dilauroylphosphatidylcholine (DLPC), and 12-hydroxydodecanoic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Octanoic acid, nonanoic acid, decanoic acid, dodecanoic acid, valproic acid, and 10-hydroxydecanoic were purchased from Aldrich Chemical Co. (Milwaukee, WI). Testosterone and 6β-hydroxytestosterone were obtained from Steraloids. 11-Hydroxydodecanoic acid, 9-hydroxynonanoic acid, and 8-hydroxyoctanoic acid were synthesized as described previously (*19, 20*). *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Supelco, Inc. (Bellefonte, PA.)

Molecular Biology and Cell Culture Reagents. Restriction enzymes and T4 DNA ligase were purchased from Boehringer Mannheim (Indianapolis, IN) and/or New England Biolabs. The BAC-TO-BAC kit was purchased from Gibco-BRL (Grand Island, NY). TC100 insect cell medium, streptomycin, penicillin G, and amphotericin B were purchased from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum was purchased from Gibco-BRL (Grand Island, NY). cDNAs encoding human CYP4B1 and rabbit CYP4B1 in pUC18 and pBluescript KS(+), respectively, were obtained as described previously (*3, 5*). All plasmids were propagated at 37 °C in *Escherichia coli* XL1-Blue (Stratagene).

Mutagenesis. Site-directed mutagenesis was performed using the Transformer Mutagenesis Kit from Clontech (Palo Alto, CA) according to manufacturer's instructions with the following mutagenic primers (mutation site underlined): human CYP4B1(Ser427→Pro), 5′-GAGGTCTTTGACCCT-CTGCGCTTTT-3′; rabbit CYP4B1(Pro422→Ser), 5′-GGGG-TCTTTGATCCCTGCGCTT-3′.

Briefly, equivalently phosphorylated mutagenic and selection primers (10 pmol) were annealed to the denatured parental plasmids containing the human CYP4B1(Ser) and rabbit CYP4B1(Pro) sequences. Mutated DNA strands were synthesized with T4 DNA polymerase and T4 DNA ligase for 2 h at 37 °C. Parental DNA was selectively linearized with *Sca*I and mutant DNA transformed into competent BMH71-18 *Muts* cells. Following a second *Sca*I digestion the mixed plasmid pool of parental and mutant DNA was transformed into *E. coli*. Mutant plasmids were identified initially by restriction enzyme mapping, and selection confirmed by DNA sequencing.

Expression of CYP4B1 Variants from Recombinant Baculovirus. Human genes (1.56 kb) encoding CYP4B1(Pro) and CYP4B1(Ser) were released from pUC18 by double digestion with *Pvu*II/*Xba*I and the corresponding rabbit CYP4B1 genes (1.52 kb) from pBluescript by digestion with *Eco*RI. The respective DNAs were cloned into pFastBac1 (Life Technologies, MD) at *Stu*I/*Xba*I or *Eco*RI sites. For generation of recombinant baculovirus, 1 ng of transfer vector was used to transpose *E. coli* DH10Bac cells, and bacmid DNA was transfected into insect cells using the Cellfectin Reagent Kit (Life Technologies, MD). For protein expression, *Trichoplusia ni* H5B1-4 (*T. ni*) insect cells were maintained at 27 °C in TC100 media supplemented with 10% fetal bovine serum, penicillin G (10 000 units), streptomycin (10 mg/mL), and amphotericin B (25 mg/mL). *T. ni* cells were inoculated at a density of 1×10^6 cells/mL with recombinant baculoviruses encoding either human CYP4B1(Ser), human CYP4B1(Pro), rabbit CYP4B1(Ser), or rabbit CYP4B1(Pro) at a multiplicity of infection of 10. Heme (2 μg/mL) was added at 24 h postinfection, and the cells were harvested at 72 h postinfection by centrifugation at 8000*g* for 20 min. The supernatant was removed, and the pellet was resuspended in storage buffer (50 mM potassium phosphate, 20% glycerol, 1 mM EDTA, pH 7.4). Insect cell membrane fractions were prepared, and apo- and holoenzyme expressions were evaluated by SDS−PAGE using 9% polyacrylamide resolving gels and by the formation of a reduced-carbon monoxide difference spectra, using methods described

[2] *Native* human CYP4B1 contains a serine residue at amino acid position 427 and is designated CYP4B1(Ser) throughout the manuscript. *Native* rabbit CYP4B1 possesses a proline residue at the corresponding amino acid position 422 and is designated CYP4B1(Pro). Only the CYP4B1(Pro) proteins from either species were found to be catalytically active.

in detail elsewhere (*21*, *22*). Human CYP4B1(Pro) was purified according to the method described previously for native[2] rabbit CYP4B1 (*19*).

Other Enzyme Sources. Insect cell membranes containing recombinant CYP3A1 were prepared as described previously (*23*). Purified recombinant rat liver cytochrome P450 reductase and human cytochrome b_5 were gifts from Dr. Raimund Peter (Department of Medicinal Chemistry, University of Washington).

Lauric Acid Hydroxylation Activity of CYP4B1 Mutants. Rabbit and human CYP4B1(Pro) and CYP4B1(Ser) membrane preparations (0.4−1.2 mg of protein equivalent to 100 pmol of P450 from the respective proline enzyme preparations) were incubated at 37 °C with P450 reductase (200 pmol) for 10 min. Cytochrome b_5 (100 pmol) was then added and incubation continued for a further 10 min. Reaction mixtures were subsequently diluted to 0.9 mL with 0.1 M potassium phosphate buffer, pH 7.4, containing 0.3 mmol of lauric acid and preincubated for a final 5 min at 37 °C prior to initiation of the reaction with 10 mM NADPH (0.1 mL). Reactions were terminated after 20 min by the addition of 1 mL of 10% HCl, and the rates of formation of 11-hydroxy and 12-hydroxy metabolites were determined by GC/FID as described previously (*19*).

Fatty Acid ω-Hydroxylation by Purified Human CYP4B1-(Pro). Purified human CYP4B1(Pro) (100 pmol) was reconstituted with P450 reductase (200 pmol), dilaurylphosphatidylcholine (20 mg), and cytochrome b_5 (100 pmol), and the rates of formation of the ω-hydroxy metabolites of octanoic acid, nonanoic acid, and decanoic acid were measured by GC/FID as described previously (*20*). Analysis of metabolites generated in incubations with VPA as substrate was performed by selected-ion monitoring GC/MS (*11*).

Testosterone Hydroxylase Activity of CYP4B1 and CYP3A1. CYP3A1 membranes (100 pmol of P450, 0.23 mg of protein), human CYP4B1(Pro) membranes (100 pmol, 0.42 mg), or rabbit CYP4B1(Pro) membranes (100 pmol, 1.2 mg) were first combined with cytochrome P450 reductase (200 pmol), and then cytochrome b_5 (100 pmol) was added as described above. Reaction mixtures were subsequently diluted to 0.9 mL with 0.1 M potassium phosphate buffer, pH 7.4, containing 0.5 mmol of testosterone and preincubated for a final 5 min at 37 °C prior to initiation of the reaction with 10 mM NADPH (0.1 mL). Reactions were terminated after 20 min by the addition of 5 mL of dichloromethane, and the formation of 6β-hydroxytestosterone was determined by HPLC as described previously (*23*).

Other Assays. P450 spectra were obtained upon the addition of methyl viologen (1.2 μM) and a few grains of sodium dithionite to insect cell membrane suspensions (1 mg/mL) or purified cytochrome P450s, in 50 mM potassium phosphate, 20% glycerol, 1 mM EDTA, which had been bubbled with carbon monoxide for 30 s. P450 concentrations were estimated using an extinction coefficient of 100 mM cm^{-1}, applied to the maximal change in absorbance observed between 450 and 480 nm. Protein concentrations were determined by the Lowry method.

RESULTS

Recently, we reported the functional expression and purification of native rabbit CYP4B1 from insect cells using the baculovirus expression vector system (*19*). However, all attempts to obtain a reduced-carbon monoxide difference spectrum from *T. ni* cells infected with a recombinant baculovirus containing the cDNA sequence for human CYP4B1(Ser) from placenta (*5*) were unsuccessful. Therefore, on the basis of sequence alignments (Table 1), we mutated human CYP4B1(Ser) to proline [human CYP4B1-(Pro)] at position 427 and mutated rabbit CYP4B1(Pro) to serine at the corresponding position (amino acid 422), in order to evaluate the effect of the Pro-X-Arg motif on heme binding to CYP4B1 isoforms expressed in insect cells.

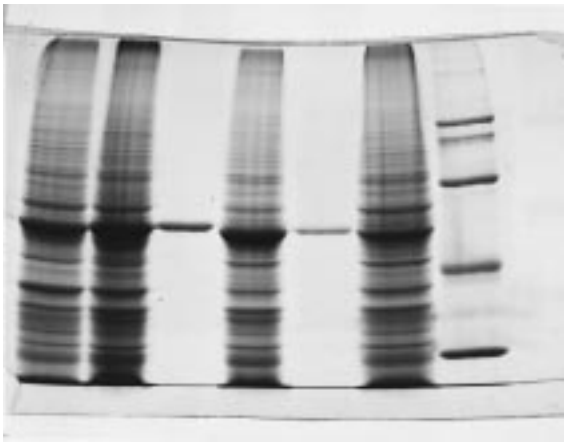

FIGURE 1: SDS−PAGE analysis of human and rabbit CYP4B1 mutants. Lanes 1−7 from left to right contain (1) 10 μg of human CYP4B1(Ser) membranes, (2) 10 μg of human CYP4B1(Pro) membranes, (3) 7 pmol of purified human CYP4B1(Pro), (4) 10 μg of rabbit CYP4B1(Pro) membranes, (5) 7 pmol of purified rabbit CYP4B1(Pro), (6) 10 μg of rabbit CYP4B1(Ser) membranes, and (7) molecular weight markers.

Figure 1 (lanes 1, 2, 4, and 6) depicts the SDS−PAGE analysis of insect cell membrane preparations expressing human CYP4B1(Ser), human CYP4B1(Pro), rabbit CYP4B1-(Pro), and rabbit CYP4B1(Ser), respectively. Protein bands with mobilities corresponding to purified human or rabbit CYP4B1 (lanes 3 and 5) were seen in each case. However, only human CYP4B1(Pro) and rabbit CYP4B1(Pro) membranes exhibited reduced-carbon monoxide binding spectra with peaks at 450 nm (Figure 2) or metabolized lauric acid when supplemented with coenzymes (Table 2). Both rabbit CYP4B1(Pro) and human CYP4B1(Pro) formed the 11-hydroxy and 12-hydroxy metabolites of lauric acid, with the human enzyme exhibiting a higher degree of regioselectivity for hydroxylation at the terminal carbon.

Since rabbit CYP4B1(Pro) characteristically ω-hydroxylates the shorter C-8 to C-10 fatty acids which are not metabolized by CYP4A1 (*20*), we purified human CYP4B1-(Pro) from insect cell membranes (see Figure 1, lane 3), reconstituted the enzyme with P450 reductase, cytochrome b_5, and DLPC, and determined its capacity to metabolize octanoic acid, nonanoic acid, decanoic acid, and dodecanoic acid. With both human CYP4B1(Pro) and rabbit CYP4B1-(Pro), the total (ω + ω−1) rates of fatty acid hydroxylation decreased with decreasing chain length. This effect was much more marked with the human enzyme (Table 3), such that significant levels of octanoic acid metabolites were difficult to detect. In addition, rates of ω-hydroxylation of the isomeric C-8 fatty acid, VPA, by human CYP4B1(Pro) were also extremely low (<1 nmol/nmol/min), and the

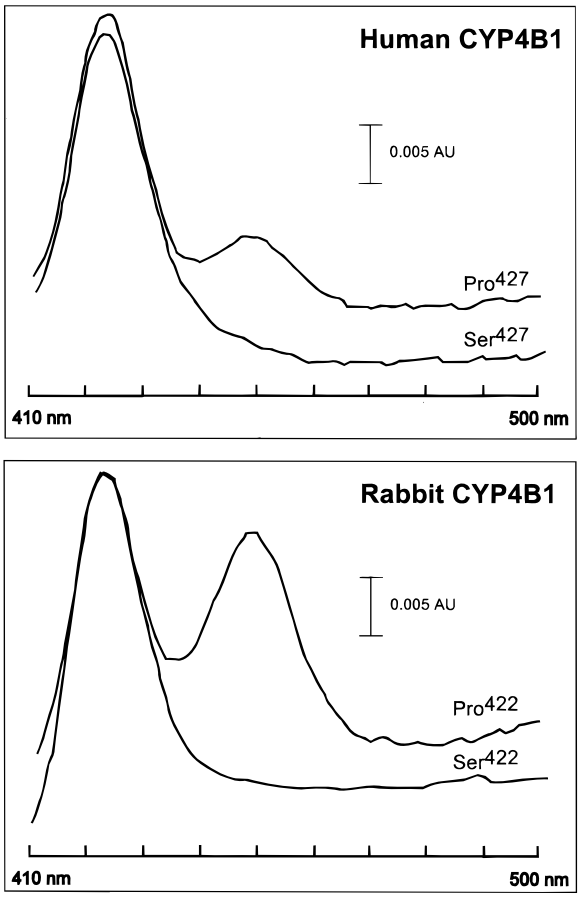

FIGURE 2: P450 spectra of human and rabbit CYP4B1 mutants. Spectra were obtained upon the addition of methyl viologen (1.2 μM) and a few grains of sodium dithionite to 1 mg/mL suspensions of each insect cell membrane preparation in 50 mM potassium phosphate, 20% glycerol, 1 mM EDTA which had been bubbled with carbon monoxide for 30 s.

Table 2: Lauric Acid (LA) Hydroxylase Activity of CYP4B1 Membrane Preparations

	pmol/mg of membrane protein/min		
enzyme	12-OH LA	11-OH-LA	ω:$\omega-1$
rabbit CYP4B1(Ser)	<30	<30	
rabbit CYP4B1(Pro)	708 ± 14[a]	596 ± 30	1.2:1
human CYP4B1(Ser)	<30	<30	
human CYP4B1(Pro)	904 ± 20	260 ± 8	3.5:1

[a] Values are the mean ± SD of triplicate determinations.

Table 3: Rate and Regioselectivity of Fatty Acid Hydroxylation by Human CYP4B1(Pro) and Rabbit CYP4B1(Pro)

	human (nmol/nmol/min)			rabbit (nmol/nmol/min)		
substrate	ω	$\omega-1$	total	ω[a]	$\omega-1$	total
dodecanoic acid	16.5	3.9	20.4	20.6	14.7	35.3
decanoic acid	8.8	2.8	11.6	11.4	10.3	21.7
nonanoic acid	4.0	2.5	6.5	10.3	7.4	17.7
octanoic acid	1.5	<1	<2.5	10.5	1.4	11.9

[a] Data taken from Fisher et al. (*20*).

enzyme failed to generate any detectable amounts of 4-ene-VPA (data not shown).

Finally, testosterone metabolite profiles generated by our membrane preparations of rabbit CYP4B1(Pro) and human CYP4B1(Pro) were compared with those obtained using recombinant CYP3A1 under identical reaction conditions

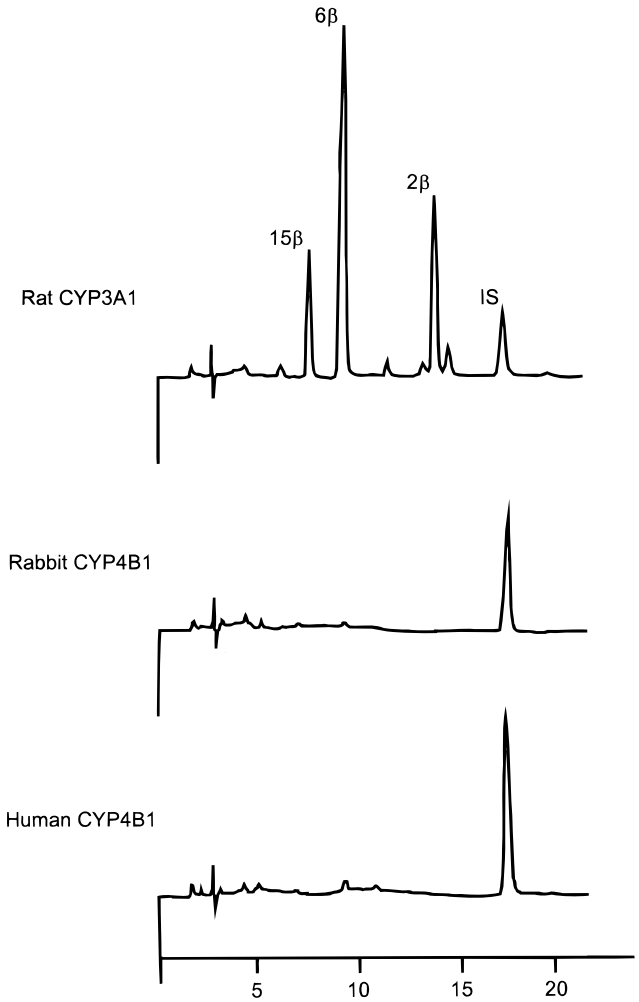

FIGURE 3: Testosterone hydroxylation by baculovirus-expressed CYP3A1 and CYP4B1. Membrane preparations equivalent to 100 pmol of CYP3A1 or human and rabbit CYP4B1(Pro) were reconstituted with P450 reductase and cytochrome b_5 in a ratio of 1:2:1, incubated with testosterone (0.5 mM), and analyzed by reverse-phase HPLC as detailed in Experimental Procedures. The internal standard, 11α-hydroxyprogesterone, eluted at 18 min. The 15β-, 6β-, and 2β-hydroxy metabolites generated by CYP3A1, but not by either CYP4B1 preparation, eluted at 7, 9, and 14 min, respectively.

(Figure 3). Neither human CYP4B1(Pro) nor rabbit CYP4B1-(Pro) formed detectable quantities of any testosterone metabolites. CYP3A1 formed copious quantities of the 6β-hydroxy metabolite and lesser amounts of the 2β-hydroxy and 15β-hydroxy products, as expected.

DISCUSSION

The present studies demonstrate that expression of the holoenzyme of CYP4B1 requires the presence of a critical proline residue located in the meander region at codon 427 or 422, in the human or rabbit sequences, respectively. This is the case for native rabbit CYP4B1, whereas serine is found at position 427 in each of the known sequences of human CYP4B1 (*4*, *5*). Neither native human CYP4B1(Ser) nor the mutated rabbit CYP4B1(Ser) apoenzymes incorporated heme from the baculovirus expression vector system.

Haseman et al. (*16*) have discussed the importance of a conserved ERR triad associated with the meander region which is linked with heme binding to P450. Within the

soluble bacterial forms of the enzyme, this triad forms a set of salt-bridge and hydrogen-bond interactions, thereby providing a conserved structural network in this region despite the absence of obvious helical or β-sheet character. Importantly, the distal arginine of this triad maps to the charged residue within the xPcxPx+a motif, two residues C-terminal to the critical proline identified above (see boldface residues in Table 1.) Therefore, we propose that the serine found at position 427 in human CYP4B1 disrupts the ordered framework of the structure imposed by the ERR triad and results in a loss of heme incorporation.

The above catalytic and spectral observations conflict with data presented previously for the variant of CYP4B1 cloned from human lung,[3] which, upon expression in HepG2 cells, *was* reported to form a typical reduced P450 difference spectrum with carbon monoxide and to metabolize steroids, but not aromatic amines (*4*, *8*, *13*). However, it is notable that upon rigorous plaque purification of the original vaccinia virus for human lung CYP4B1(Ser) and subsequent expression in HepG2 cells, the ability of the preparation to bind carbon monoxide in the reduced state was lost,[4] which suggests the possibility that the original human CYP4B1 vaccinia virus had become contaminated with virus encoding CYP3A5.[5] This would explain the anomalous steroid hydroxylation profiles reported previously for human CYP4B1-(Ser) (*8*, *13*). The preliminary catalytic data we have obtained for human CYP4B1(Pro) suggests that, in general, this enzyme recapitulates the metabolic activity of the rabbit ortholog, at least for fatty acids and steroids. However, human CYP4B1(Pro) clearly exhibits a much lower capacity for the metabolic bioactivation of VPA, a difference we feel reflects the chain-length specificities of the two enzymes for fatty acid substrates, rather than any dramatic species difference which might have been inferred from previous studies.

These findings have important implications for the functional capabilities of human CYP4B1 expressed in vivo. If we can extrapolate from the eukaryotic insect cell expression system to humans, it seems likely that cells expressing native human CYP4B1(Ser) will not have the expected bioactivation capabilities conferred by a functionally competent version of the enzyme. However, these studies raise the intriguing possibility that some segment of the population expresses the functional Pro427 form of the enzyme. Such individuals, if they exist, might be expected to be at enhanced risk for some types of chemical carcinogenesis if the bioactivation capabilities of human CYP4B1(Pro) were similar to those of the laboratory animal orthologs. Genotyping studies and analysis of the metabolic capabilities of functional human CYP4B1 are underway to address these questions.

[3] CYP4B1 cloned from human lung is not identical to the placental clone used in these experiments, since it encodes the Q37R variant. However, this N-terminal mutation seems unlikely to influence heme incorporation and catalytic activity in so dramatic a fashion, because all other CYP4B sequences contain glutamic acid rather than arginine at this position. Therefore, the human lung sequence may represent a minor allelic form of the enzyme, although the relevant genotyping studies have yet to be performed.

[4] K. R. Korzekwa, personal communication.

[5] F. J. Gonzalez, personal communication.

ACKNOWLEDGMENT

The authors wish to thank Drs. R. M. Philpot (NIEHS), F. J. Gonzalez (NIH), and K. R. Korzekwa (University of Pittsburgh) for valuable discussions regarding human CYP4B1 and Stella Thompson (University of Washington) for assistance with the expression of CYP3A1.

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) *Pharmacogenetics 6*, 1−42.
2. Serabjit-Singh, C. J., Wolf, C. R., and Philpot, R. M. (1979) *J. Biol. Chem. 254*, 9901−9907.
3. Gasser, R., and Philpot, R. M. (1989) *Mol. Pharmacol. 35*, 617−625.
4. Nhamburo, P. T., Gonzalez, F. J., McBride, O. W., Gelboin, H. V., and Kimura, S. (1989) *Biochemistry 28*, 8060−8066.
5. Yokotani, N., Sogawa, K., Matsubara, S., Gotoh, O., Kusunose, E., Kusunose, M., and Fujii-Kuriyama, Y. (1990) *Eur. J. Biochem. 187*, 23−29.
6. Imaoka, S., Hiroi, T., Tamura, Y., Yamazaki, H., Shimada, T., Komori, M., Degawa, M., and Funae, Y. (1995) *Arch. Biochem. Biophys. 321*, 255−262.
7. Slaughter, S. R., Statham, C. N., Philpot, R. M., and Boyd, M. R. (1983) *J. Pharmacol. Exp. Ther. 224*, 252−257.
8. Czerwinski, M., McLemore, T. L., Philpot, R. M., Nhamburo, P. T., Korzekwa, K., Gelboin, H. V., and Gonzalez, F. J. (1991) *Cancer Res. 51*, 4636−4638.
9. Verschoyle, R. D., Philpot, R. M., Wolf, C. R., and Dinsdale, D. (1993) *Toxicol. Appl. Pharmacol. 123*, 193−198.
10. Vanderslice, R. R., Domin, B. A., Carver, G. T., and Philpot, R. M. (1987) *Mol. Pharmacol. 31*, 320−325.
11. Rettie, A. E., Sheffels, P. R., Korzekwa, K. R., Gonzalez, F. J., Philpot, R. M., and Baillie, T. A. (1995) *Biochemistry 34*, 7889−7895.
12. Sadeque, A. J. M., Fisher, M. B., Korzekwa, K. R., Gonzalez, F. J., and Rettie, A. E. (1997) *J. Pharmacol. Exp. Ther. 283*, 698−703.
13. Waxman, D. J., Lapenson, D. P., Aoyama, T., Gelboin, H. V., Gonzalez, F. J., and Korzezkwa, K. (1991) *Arch. Biochem. Biophys. 290*, 160−166.
14. Czerwinski, M., McLemore, T. L., Gelboin, H. V., and Gonzalez, F. J. (1994) *Cancer Res. 54*, 1085−1091.
15. McKinnon, R. A., and McManus, M. E. (1996) *Pathology 28*, 148−155.
16. Hasemann, C. A., Kurumbail, R. G., Boddupalli, S. S., Peterson, J. A., and Deisenhofer, J. (1995) *Structure 2*, 41−62.
17. Graham-Lorence, S. E., and Peterson, J. A. (1996) *Methods Enzymol. 272*, 315−326.
18. Nelson, D. R. (1996) in *Cytochrome P450, Structure Mechanism and Biochemistry* (Ortiz de Montellano, P. R., Ed.) 2nd ed., pp 575−606, Plenum Press, New York.
19. Guan, X., Fisher, M. B., Lang, D. H., Zheng, Y.-M, Koop, D. R., and Rettie, A. E. (1998) *Chem.-Biol. Interact. 110*, 103−121.
20. Fisher, M. B., Zheng, Y.-M., and Rettie, A. E. (1998) *Biochem. Biophys. Res. Commun. 248*, 352−355.
21. Haining, R. L., Hunter, A. P., Veronese, M. E., Trager, W. F., and Rettie, A. E. (1996) *Arch. Biochem. Biophys. 333*, 447−458.
22. Haining, R. L., Hunter, A. P., Sadeque, A. J. M., Philpot, R. M., and Rettie, A. E. (1997) *Drug Metab. Dispos. 25*, 790−797.
23. Fisher, M. B., Thompson, S. J., Ribeiro, V., Lechner, M. C., and Rettie, A. E. (1998) *Arch. Biochem. Biophys. 356*, 63−70.

BI981280M