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Multiple Forms of Human P450 Expressed in *Saccharomyces cerevisiae*

SYSTEMATIC CHARACTERIZATION AND COMPARISON WITH THOSE OF THE RAT

Susumu Imaoka,† Takao Yamada,* Toyoko Hiroi,**
*Koji Hayashi,‡ Toshiyuki Sakaki,‡ Yoshiyasu Yabusaki‡ and Yoshihiko Funae**

*Laboratory of Chemistry, Osaka City University Medical School, Osaka, Japan; and
‡Biotechnology Laboratory, Sumitomo Chemical Co., Ltd., Hyogo, Japan

ABSTRACT. We systematically characterized the levels and substrate specificity of P450s from humans and rats to extrapolate drug metabolism data from experimental animals to humans. Human P450s (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2D6, 2E1, and 3A4) were expressed in *Saccharomyces cerevisiae* and purified. Rat P450s were purified from hepatic microsomes of rats. We investigated the catalytic activities of purified P450s in a reconstituted system. Human CYP2B6 and rat CYP2B1 had high lidocaine N-deethylation activity. Human and rat CYP2D forms had high debrisoquine 4-hydroxylation activity. Human CYP3A4 and rat CYP3A2 had high testosterone 2β- and 6β-hydroxylation activities in a modified reconstituted system with a lipid mixture. The hydroxylation site of testosterone by CYP2B6 (16α- and 16β-positions) agreed with that by rat CYP2B1. Human CYP2E1 had the highest lauric acid (ω-1)-hydroxylation activity and also had catalytic properties similar to those of rat CYP2E1. Human CYP2A and 2C forms had catalytic properties in testosterone metabolism different from those of rats. Antibodies raised against purified P450s were used to measure the levels of hepatic P450s. The level of CYP3A4 was the highest in human hepatic microsomes, comprising 30–40% of the total P450. CYP2C9 comprised 10–20% of the total. The levels of CYP1A2, 2A6, 2C8, 2D6, and 2E1 were moderate (5–15% of total P450). CYP2B6 content was very low. The information of this study is useful for drug metabolism and toxicological studies. BIOCHEM PHARMACOL 51;8:1041–1050, 1996.

KEY WORDS. cytochrome P450; yeast expression; human liver; human P450; rat P450; rat liver

Rats are commonly used as experimental animals with which to predict drug metabolism and toxicity in humans, and a considerable amount of data has been accumulated [1]. Extrapolation of the data to humans can be improved by comparing the characteristics and properties of drug-metabolizing enzymes in experimental animals such as rats. Hepatic P450 is a key enzyme in drug metabolism. Several forms of P450 have been purified from human hepatic microsomes and characterized [2–10]. However, these studies have been limited by difficulties associated with obtaining human liver samples. Furthermore, relationships between the DNA sequence coding a P450 and the catalytic specificity of the P450 must be established, because substrate specificities of P450s are sometimes quite different even though they have closely related genes. For example, human CYP2C9 and 2C19 are closely related P450s [8], but they, respectively, metabolize only tolbutamide and mephenytoin.

† Corresponding author: Dr. Susumu Imaoka, Laboratory of Chemistry, Osaka City University Medical School, 1-4-54 Asahimachi, Abeno-ku, Osaka 545, Japan. Tel. 81-6-645-2081; FAX 81-6-646-3922.
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The heterologous expression of single P450 genes has increased the understanding of the roles of individual enzymes, as recombinant human enzymes can be characterized [11]. Heterologous expression makes possible the determination of the catalytic specificity of several P450 forms in individual human samples and the comparison of them with P450s in experimental animals. However, there have been only limited studies on the systematic characterization and comparison of P450s between human and experimental animals such as rats. In addition, the heterologous expression system sometimes does not give sufficient catalytic activities of enzymes. The bufuralol hydroxylation activity of rat P450 2D1 expressed in COS cells is very low even in the presence of exogenous NADPH–P450 reductase [12]. CYP4A1 expressed in yeast cells has low activity toward lauric acid unless exogenous reductase and cytochrome b_5 are added [13]. The catalytic activity of yeast microsomes expressing CYP3A4 is low, but partially purified CYP3A4 exhibits reasonable activity [14]. To compare the substrate specificity of human and rat P450 under the same conditions, the enzymes must be purified, and the possibility of changes in catalytic activities due to factors such as envi-

ronmental lipids and cytochrome b_5 must be ruled out by using a reconstituted system with the purified P450.

Immunochemical studies are important in understanding P450 and in studying various aspects of P450 in human populations. To extrapolate experimental animal data to humans, it is important to know the levels of all the enzymes involved, as well as their catalytic activities. There are marked species differences in P450 expression. For example, CYP3A is the major family of P450s in the human liver, and they metabolize many drugs. However, their level is extremely low in the liver of female rats [3]. The levels of P450s can be determined immunochemically, and antibodies can be raised from purified P450 generated in heterologous expression systems.

We have developed a simple method of purifying P450 expressed in yeast and have compared the catalytic activities of purified human recombinant P450 with those of rats in a reconstituted system. Furthermore, we assayed the levels of P450 in human hepatic microsomes, using a specific antibody raised against recombinant human P450, and compared them with those in the rat liver.

MATERIALS AND METHODS

Chemicals

Dilauroylphosphatidylcholine and dioleoylphosphatidylcholine were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Phosphatidylserine was obtained from PL Biochemicals (Milwaukee, WI, U.S.A.). Lidocaine, MEGX,* and GX were supplied by the Fujisawa Pharmaceuticals Co. (Osaka, Japan). Me-OH Lid and 3-OH Lid were gifts from S. Fujita of Hokkaido University. Desbrisoquine and 4-hydroxydebrisoquine were provided by Dr. S. Narimatsu of Chiba University. Emulgen 911 was a gift from the Kao Chemical Co. (Tokyo, Japan). Cholic acid was obtained from Nacalai Tesque (Kyoto, Japan). Other reagents and organic solvents were obtained from Wako Pure Chemical Industries (Tokyo, Japan).

Preparation of Hepatic Microsomes

Human liver samples were obtained with the agreement of the Institutional Ethical Committee, from cancer patients undergoing liver resection. Tissues were selected from areas of the liver that were visually free of tumor. Hepatic microsomes were prepared from these samples as described for the preparation of rat hepatic microsomes [15]. Rat P450s, NADPH–P450 reductase, and cytochrome b_5 were purified as described [16].

Expression of Human P450s with Yeast Cells

cDNAs coding for human P450s were amplified by PCR from the commercially available human cDNA libraries (Clontech Laboratories, Inc., Palo Alto, CA, U.S.A.). Primers for the PCR amplification of human cDNAs were designed based on published sequences: CYP1A2 [17], CYP2A6 [18], CYP2B6 [19], CYP2C8 [20], CYP2C9 [21], CYP2C18 [22], CYP2D6 [23], CYP2E1 [24], and CYP3A4 [25]. Primers and linkers used for the preparation of human P450 cDNA by PCR are shown in Fig. 1. For CYP2B6 and 2C8, one set of PCR primer was used and the amplified fragment was subcloned into a pUC vector with restriction enzyme sites as indicated in Fig. 1. For CYP2A6, 2C9, 2C18, 2E1, and 3A4, two sets of PCR primer were used and two amplified fragments were subcloned into a pUC vector. For CYP1A2 and 2D6, 5′-regions of cDNA were not able to be amplified by PCR, and synthetic linkers corresponding to 5′-regions of cDNA were used. In the case of CYP2D6, it lacked 0.1 kb of 5′-region of cDNA. Linkers 1 and 2 were subcloned between *Xba* I and *Pst* I sites of a pUC vector, and then linker 3 and two fragments amplified by PCR were inserted into the plasmid containing linkers 1 and 2 with *Sma* I and *Kpn* I sites. Nucleotide sequences of amplified cDNAs were confirmed by sequence analysis to code for the corresponding human P450 without exchange of amino acid residues. *Hind* III sites were introduced into the 5′ and 3′ ends of the coding region of human P450. The human P450 cDNAs cut off from the subcloned plasmids with *Hind* III were introduced into a vector pAAH5N for expression of human P450 cDNAs. The pAAH5N containing the coding sequence of each human P450 cDNA between the alcohol dehydrogenase I promoter and terminator regions was constructed with the *Hind* III site as described previously [26]. The resulting plasmids were used for transformation of the *Saccharomyces cerevisiae* AH 22 strain [27]. The cellular contents of human P450s in the recombinant yeast cells were 1.1, 1.0, 2.5, 4.1, 4.0, 3.4, 1.7, 1.7, and 1.9×10^5 molecules/cell for CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2D6, 2E1, and 3A4, respectively.

Purification of Yeast-Expressed P450

Recombinant *S. cerevisiae* expressing human P450s were cultured as described [28], and the microsomal fraction was prepared from the yeast cells [29]. Yeast microsomes containing 300 mg protein were solubilized at a concentration of 3–5 mg protein/mL in 0.1 M potassium phosphate buffer (pH 7.2), containing 1 mM DTT, 1 mM EDTA, 0.5 mM PMSF, 20% glycerol, and 0.6% sodium cholate for 1 hr at 4°. The solution was centrifuged at 15,000 *g* for 20 min and applied to octylamino-Sepharose 4B in a column (2.0 × 6.0 cm) equilibrated with 0.1 M potassium phosphate buffer (pH 7.2) containing 1 mM DTT, 1 mM EDTA, 0.5 mM PMSF, 20% glycerol, and 0.5% sodium cholate (buffer A). The column was washed with buffer A, and the P450 was eluted with buffer A containing 0.2% or 0.5% Emulgen 911. The eluted fraction (30–40 mL) was dialyzed against 0.02 M Tris-acetate buffer (pH 7.2) containing 20% glycerol overnight, concentrated to 5–10 mL, and applied to

* *Abbreviations:* MEGX, monoethylglycinexylidide; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; GX, glycinexylidide; 3-OH Lid, 3-hydroxylidocaine; Me-OH Lid, methylhydroxylidocaine; and PCR, polymerase chain reaction.

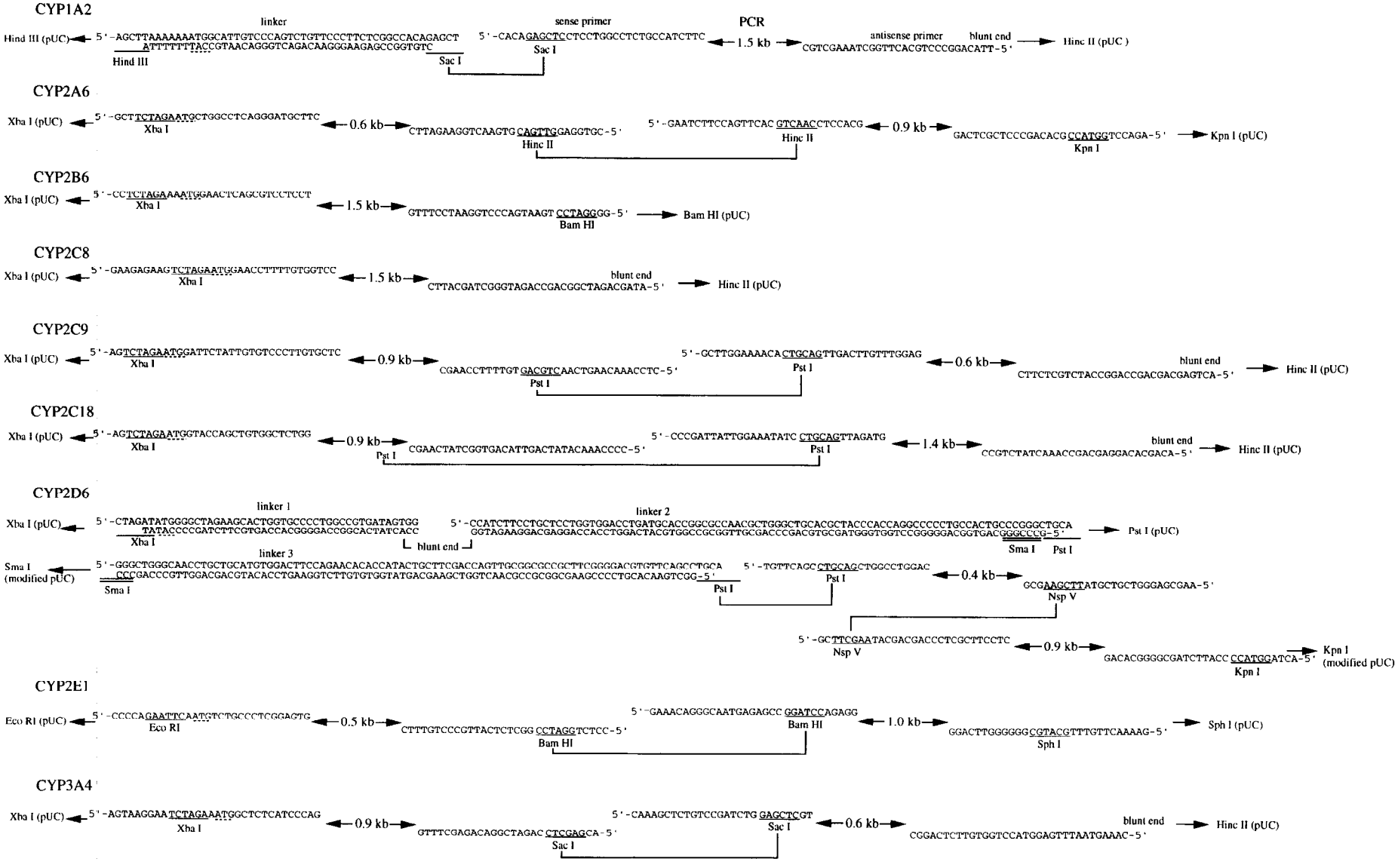

FIG. 1. Strategy for preparation of human cDNAs by PCR. The linkers and primers indicated were used for the preparation of the coding region of human P450 cDNA. The restriction enzyme sites underlined or blunt end sites were used for ligation of oligo nucleotides and a vector. For 2C18, the *Pst* I site is not underlined in the PCR primer and is found within the fragment amplified by PCR but not in the primer. The oligonucleotide sizes indicated between arrows were those amplified by PCR. Except for CYP2D6, one or two fragments of oligonucleotide were inserted directly into a pUC vector. For CYP2D6, cDNA was constructed in two steps because the 5′-region of cDNA could not be amplified by PCR, and three linkers corresponding to the 5′-region of human CYP2D6 cDNA were synthesized. Dotted underlines indicate initiation codon ATG.

HPLC using a DEAE-5PW column (7.5 × 75 mm, Tosoh, Tokyo, Japan). All P450s expressed in this study were eluted in the pass-through fraction. However, this step is efficient to remove other protein derived from yeast. The pass-through fraction was applied directly onto HPLC with a hydroxylapatite column (KB-column, Koken, Tokyo, Japan). P450 was eluted at a flow rate of 0.7 mL/min with a linear gradient of sodium phosphate buffer (pH 7.2) containing 0.2% Emulgen 911, 0.2% sodium cholate, and 20% glycerol from 0.01 to 0.35 M for 50 min. The eluted P450 fraction was diluted five times with 0.01 M sodium phosphate buffer (pH 7.2) containing 20% glycerol and applied to an open column with hydroxylapatite (Biogel HT, Bio-Rad Laboratories, Richmond, CA, U.S.A.) to remove the Emulgen. P450 was eluted with 0.35 M sodium phosphate buffer (pH 7.2) containing 20% glycerol and 0.05% sodium cholate after absorption of Emulgen at 280 nm disappeared. The P450s were purified from yeast microsomes within 2 days.

Preparation of Antibodies against Purified Human P450s

Antibodies against human P450 purified from yeast microsomes were raised under contract service by the Takara Shuzo Co., Ltd. (Kyoto, Japan). The purified P450 (100–500 μg) was mixed with Freund's complete adjuvant and injected intradermally into a male Japanese white rabbit (2.3 to 2.4 kg). The rabbit was given two boosters with half the amount of the antigen at 3 and 5 weeks after the initial injection, and finally bled 2 or 3 weeks after the last injection.

Assays

The metabolic activities of lidocaine, lauric acid, testosterone, and debrisoquine were assayed as described [2, 15, 16]. The levels of P450 were assayed by immunoblotting as described previously [16].

RESULTS

Characterization of Purified Human P450s Expressed in Yeast Cells

cDNA-expressed human P450s in yeast were purified by means of octylamino-Sepharose 4B chromatography, followed by HPLC with anion-exchange and hydroxylapatite columns. CYP1A2 and 2D6 tightly bound to the octylamino-Sepharose column and were eluted with a buffer containing 0.5% Emulgen 911. The others were eluted with a buffer containing 0.2% Emulgen. These chromatographic properties agreed with those reported previously [2]. The eluted P450 was purified further by anion-exchange (DEAE-type) chromatography. All P450s were eluted in the pass-through fraction, and this step was very effective for purification of human P450. The total recovery of P450 except for CYP1A2 was 10–26% from yeast microsomes, and the specific content of purified P450 was 10–15 nmol/mg (Table 1). The recovery of CYP1A2 was low. As described [30], this P450 may be unstable during purification. Purified P450s were applied to SDS–PAGE (Fig. 2). All purified P450 migrated as discrete bands. The molecular weights of each P450 calculated from their mobility on SDS–PAGE were from 47,500 (CYP2A6) to 55,000 (CYP2C9) (Table 1) and were consistent with those of native P450s [31].

TABLE 1. Purification of human P450s from yeast microsomes

P450 (CYP)	Microsomes (nmol/mg)	Purified P450 (nmol/mg)	Recovery (%)	Molecular weight
1A2	0.019	9.5	4	53,000
2A6	0.059	12.1	26	47,500
2B6	0.095	10.7	25	48,000
2C8	0.283	12.9	21	50,000
2C9	0.250	14.4	16	55,000
2C18	0.101	14.6	25	49,000
2D6	0.032	12.2	16	48,500
2E1	0.099	10.2	10	54,000
3A4	0.156	11.5	13	50,500

Each P450 was expressed in yeast microsomes and purified as described in Materials and Methods.

Catalytic Activity of Purified Human P450

Catalytic study of purified P450 was done with lidocaine, debrisoquine, testosterone, and lauric acid (Table 2). Lido-

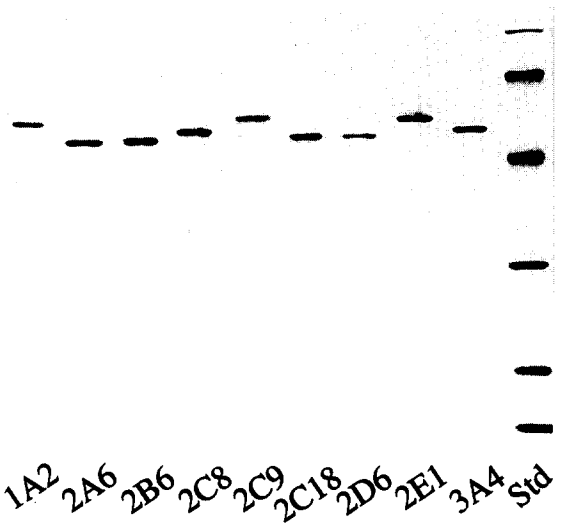

FIG. 2. SDS–PAGE of human P450s purified from yeast microsomes including cDNA-expressed human P450. The P450s were resolved on 10% polyacrylamide gels. Lanes indicated as 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2D6, 2E1, and 3A4 contained purified P450 (0.5 μg each). The lane indicated as Std contained standard proteins, including phosphorylase *b* (97,400), bovine serum albumin (66,200), ovalbumin (42,700), carbonic anhydrase (31,000), trypsin inhibitor (21,500), and lysozyme (14,400). Proteins were stained with Coomassie brilliant blue.

TABLE 2. Catalytic activities of human P450s purified from microsomes of yeast cells

	Activity (nmol/min/nmol P450)								
	1A2	2A6	2B6	2C8	2C9	2C18	2D6	2E1	3A4
Lidocaine									
N-Deethylation	17	0.44	33	2.3	0.50	2.1	0.63	—*	6.0
3-Hydroxylation	0.22	—	—	—	—	—	—	—	—
Methylhydroxylation	—	—	—	—	—	—	—	—	—
Debrisoquine									
4-Hydroxylation	NA†	—	—	—	—	—	0.72	NA	NA
Testosterone									
2α-Hydroxylation	—	—	—	—	—	—	—		—
2β-Hydroxylation	—	—	—	—	—	—	—		0.56
6β-Hydroxylation	—	—	—	—	—	—	—		4.7
7α-Hydroxylation	—	—	—	—	—	—	—	NA	—
15α-Hydroxylation	—	—	—	—	—	—	—		—
16α-Hydroxylation	—	—	0.12	—	—	—	—		—
16β-Hydroxylation	—	—	1.1	—	—	—	—		—
Lauric acid									
ω-Hydroxylation	NA	—	—	—	—	—	—	—	
(ω-1)-Hydroxylation		—	0.39	0.87	8.2	0.47	—	12	NA

Assays proceeded in a reconstituted system containing purified P450 (30 pmol), rat NADPH-P450 reductase (0.3 U), and dilauroylphosphatidylcholine (5 μg). A modified reconstituted system for CYP3A4 contained CYP3A4, NADPH-P450 reductase, cytochrome b_5 (30 pmol), sodium cholate (100 μg), and a lipid mixture consisting of dilauroylphosphatidylcholine, dioleoylphosphatidylcholine and phosphatidylserine (1:1:1). Debrisoquine (100 μM), lidocaine (1 mM), lauric acid (100 μM), and testosterone (0.5 mM) were used as substrates. Lauric acid hydroxylation activity was assayed in the presence of cytochrome b_5 (30 pmol).

* Activities of less than 0.05 nmol/min/nmol are expressed as "—".

† NA = not assayed.

caine is metabolized to MEGX, 3-OH Lid, and Me-OH Lid [32]. CYP1A2, 2B6, and 3A4 efficiently metabolized lidocaine to MEGX. CYP3A4 had low activity in a reconstituted system with dilauroylphosphatidylcholine but high activity in the modified reconstituted system with a lipid mixture [33]. CYP1A2 had lidocaine 3-hydroxylation activity as well as lidocaine N-deethylation activity. None of the P450s studied had lidocaine methylhydroxylation activity. Debrisoquine was metabolized selectively by CYP2D6. Testosterone is metabolized mainly to 2β- and 6β-hydroxytestosterone in human hepatic microsomes [33]. As reported [33], CYP3A4 had high testosterone 2β- and 6β-hydroxylation activity in the modified reconstituted system. CYP2B6 had testosterone 16α- and 16β-hydroxylation activity, whereas the other forms had none. Lauric acid is metabolized mainly to ω and (ω-1)-hydroxylauric acid in rat hepatic microsomes [16]. Lauric acid (ω-1)-hydroxylation was catalyzed efficiently by CYP2C9 and 2E1. Of the three P450s in the CYP2C family, only CYP2C9 had high activity. None of the purified P450s had lauric acid ω-hydroxylation activity.

Immunoassay of Human Hepatic P450 Using an Antibody Against Recombinant Human P450

Antibodies against recombinant P450 purified from yeast microsomes were raised in a rabbit, and their specificity was examined (Fig. 3). Antibodies against CYP1A2, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1, and 3A4 specifically reacted only with their antigen P450. Antibody against CYP2C18 reacted with CYP2C18 and weakly with CYP2C8 and 2C9. He-

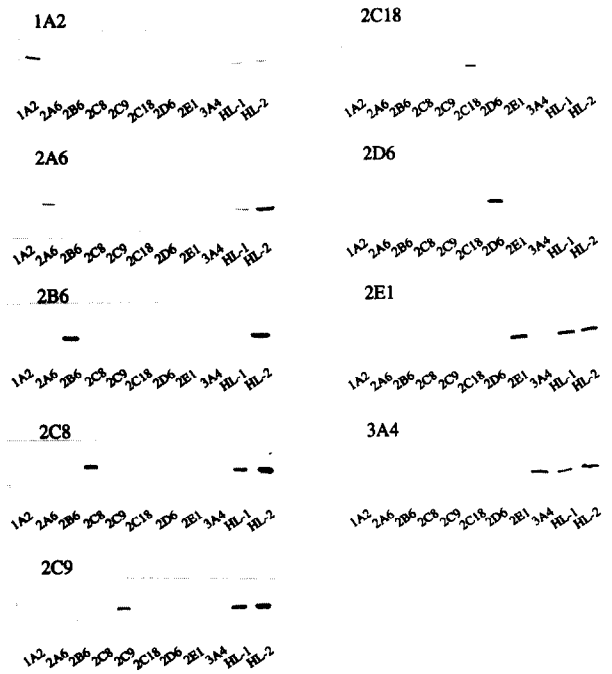

FIG. 3. Immunoblots of purified human P450 and human hepatic microsomes with antibodies against human P450. Each lane indicated as 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2D6, 2E1, and 3A4 contained purified human P450 (0.5 pmol). Lanes indicated as HL-1 and HL-2 contained hepatic microsomes from individual humans. Lanes HL-1 and HL-2 contained 2.5 μg of microsomal protein for CYP3A4 antibody, 5 μg for CYP2C8, 2C9, and 2E1 antibodies, and 10 μg for CYP1A2, 2A6, 2B6, 2C18, and 2D6 antibodies. Purified P450s and hepatic microsomes were resolved in 7.5% polyacrylamide gels and were transferred electrophoretically to a nitrocellulose membrane. The membrane was stained immunochemically with the antibody shown at the top of the membrane.

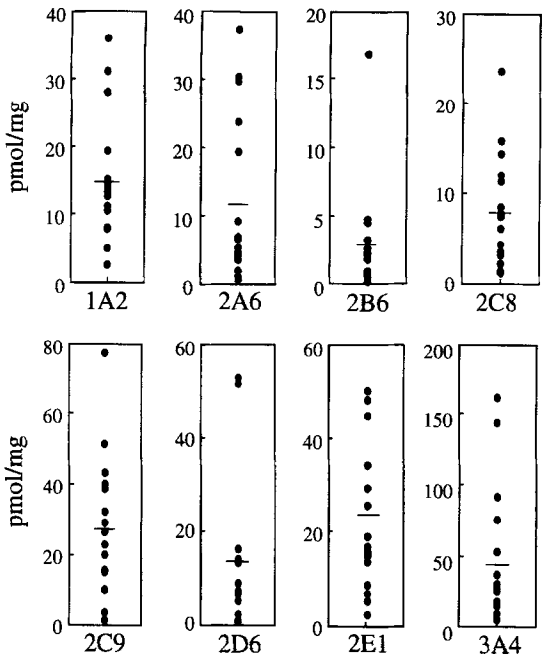

FIG. 4. Levels of individual P450s in human hepatic microsomes. The levels of human P450s were assayed by immunoblotting with the antibodies against purified human P450. The purified human P450 was used as a standard for measurement of P450 levels. Sixteen human samples were assayed, and bars represent the average values.

patic microsomes gave a single band on immunoblots with antibodies against CYP1A2, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1, and 3A4 (Fig. 3). On immunoblot with CYP2C18 antibody, hepatic microsomes gave a band corresponding to CYP2C9, but the band corresponding to CYP2C18 was hardly detected. Individual P450 levels were measured by densitometry of membranes on which human hepatic microsomes were blotted as a standard with purified P450s (Fig. 4). CYP3A4 and 2C9 were the major forms in human hepatic microsomes. CYP3A4 comprised 30–40% of total P450 in human hepatic microsomes, and CYP2C9 accounted for 10–20%. Individual variations were 37-fold for CYP3A4 and 65-fold for CYP2C9. CYP1A2, 2A6, 2C8, 2D6, and 2E1 levels were moderate in human hepatic microsomes. Each of these forms comprised 5–15% of the total P450. The CYP2B6 content was very low. Liver samples used in this study were from liver tumor patients, and it cannot be ruled out that expression of P450 in these samples is different from that in human liver in physiological situations. However, our results were consistent with published reports [31, 34, 35], and the composition of P450 obtained in this study was not different from that in normal human liver.

Comparison of Human and Rat P450s

The catalytic activities of purified human and rat P450s were compared (Fig. 5). Human and rat CYP2B, 3A, and 1A2 forms had high lidocaine N-deethylation activity, of

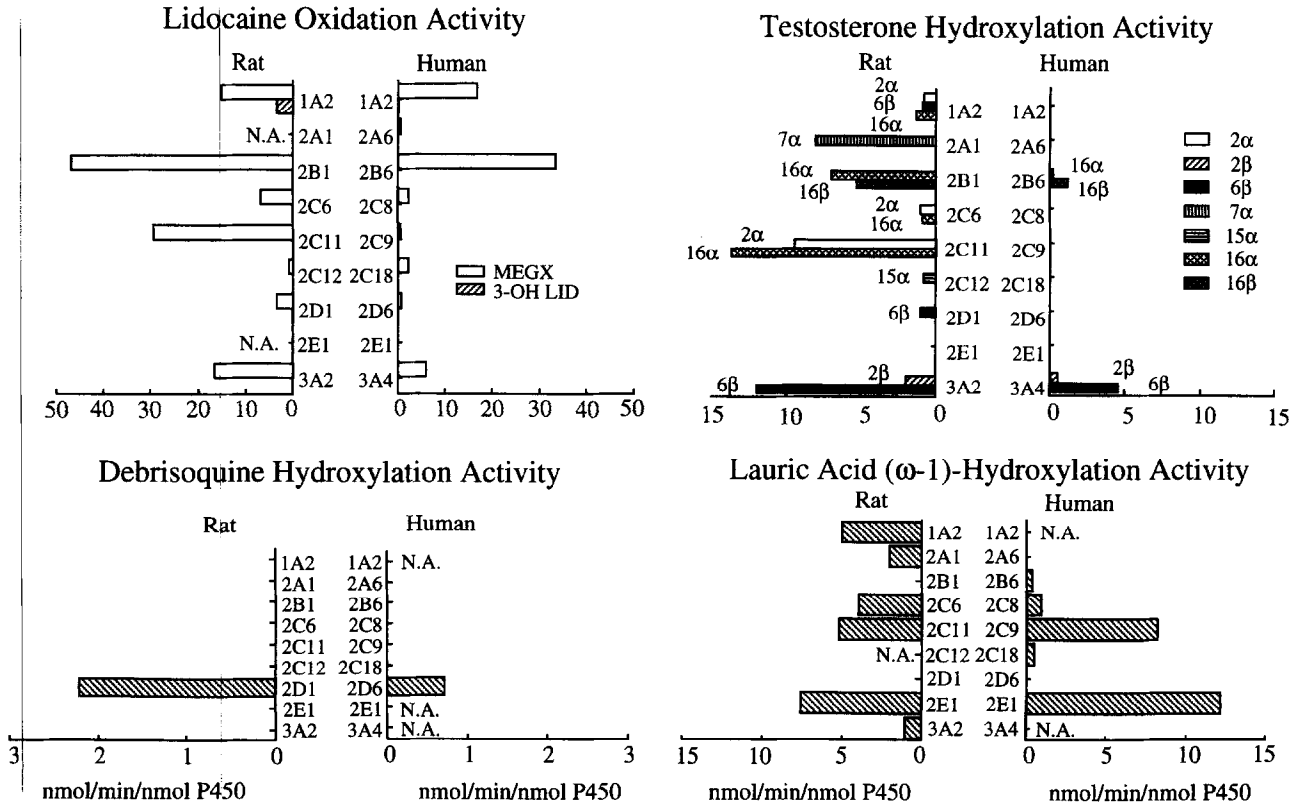

FIG. 5. Catalytic activities of P450s purified from human and rat hepatic microsomes. Assay conditions were the same as those described in the footnote to Table 2. Abbreviations: MEGX, monoethylglycinexylidide; 3-OH LID, 3-hydroxylidocaine; and N.A., not assayed. 2α, 2β, 6β, 7α, 15α, 16α, and 16β indicate hydroxylation sites of testosterone.

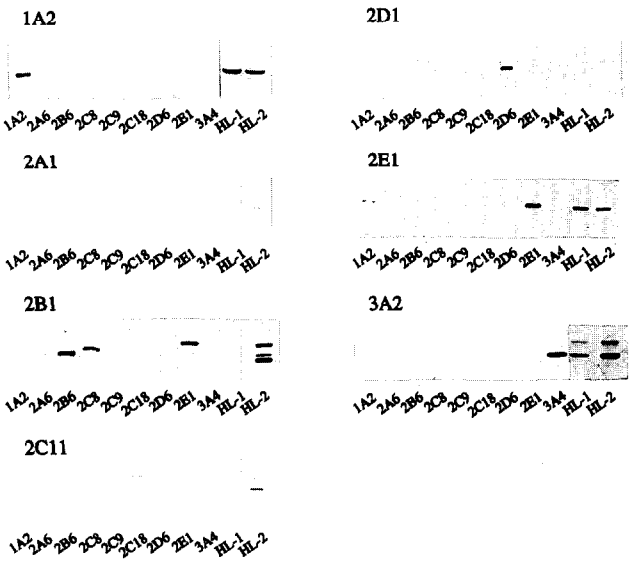

FIG. 6. Immunoblots of purified human P450 and human hepatic microsomes with antibodies against rat P450. Each lane indicated as 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2D6, 2E1, and 3A4 contained purified P450 (0.5 pmol). Lanes indicated as HL-1 and HL-2 contained individual human hepatic microsomes. Lanes HL-1 and HL-2 contained 2.5 μg of microsomal protein for CYP3A2 antibody, 5 μg for CYP2C11 and 2E1 antibodies, and 10 μg for CYP1A2, 2A1, 2B1, and 2D1 antibodies. Purified P450s and hepatic microsomes were resolved by electrophoresis in 7.5% polyacrylamide gels and were transferred electrophoretically to a nitrocellulose membrane. The membrane was stained immunochemically with the antibody shown at the top of the blot.

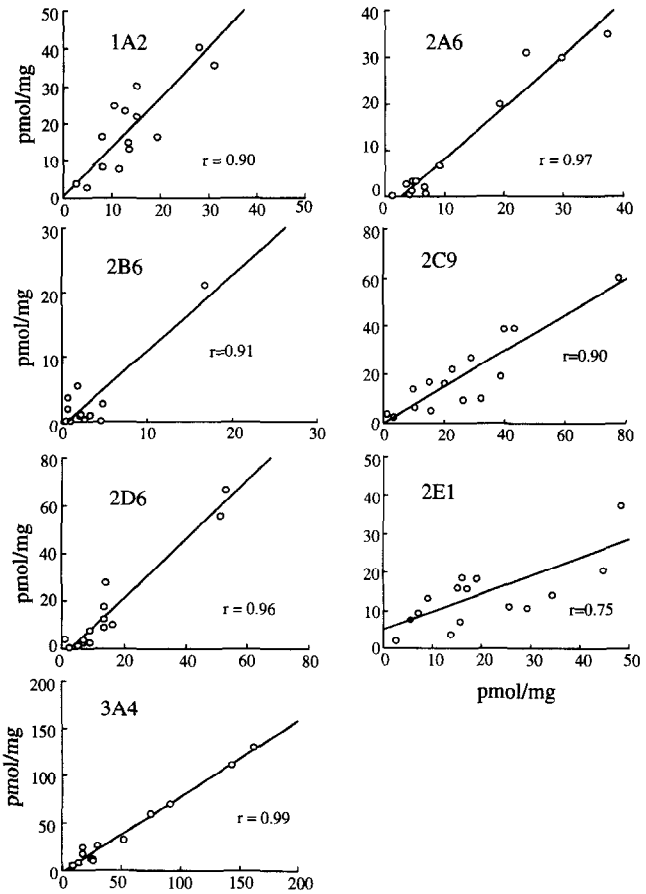

FIG. 7. Correlation between levels of P450s in human hepatic microsomes assayed with antibodies against human and rat P450s. Levels of individual P450s were assayed by immunoblotting as described in the legend of Fig. 4. The x- and y-axes show the levels of P450s in human hepatic microsomes detected with antibodies against human and rat P450s, respectively.

which the CYP2B form was the highest. In humans and in control rats, lidocaine is N-deethylated mainly by CYP3A forms [32]. Since the levels of CYP2B forms in human and control rats are very low, their contribution to this activity may be low, although this P450 had high activity. There were also species differences between human and rat P450 forms. Rat CYP2C11 had high lidocaine N-deethylation activity, whereas none of the human CYP2C forms did, and rat CYP2D1 had lidocaine 3-hydroxylation activity but human CYP2D6 did not. Human and rat CYP2D forms had high debrisoquine 4-hydroxylation activity. Human P450s had lower testosterone hydroxylation activity than rat P450s. Only human CYP2B6 and 3A4 had testosterone hydroxylation activity, and the hydroxylation site for these forms in testosterone agreed with those of rat CYP2B1 and 3A2, respectively. Rat CYP2A and 2C forms had high activity, but their human counterparts had no activity toward testosterone. Human and rat CYP2C and 2E forms had similarly high lauric acid (ω-1)-hydroxylation activity.

Immunoblots of human recombinant P450 with antibodies against rat P450s are shown in Fig. 6. Antibodies against CYP1A2, 2A1, and 3A2 cross-reacted only with their human counterparts, CYP1A2, 2A6, and 3A4, respectively. CYP2B1 antibody reacted with CYP2C8 and 2E1 as well as CYP2B6. CYP2C11 antibody cross-reacted with CYP2C9 and 2C18 and weakly with 2C8. CYP2D1 antibody reacted with CYP2D6 and weakly with CYP2C8. CYP2E1 antibody cross-reacted with human 2E1 and weakly with CYP1A2. Human hepatic microsomes were immunoassayed using antibodies against rat P450s (Fig. 7). The human individual P450 levels assayed with antibodies against human CYP1A2, 2A6, 2B6, 2C9, 2D6, and 3A4 correlated well with those assayed with antibodies against rat counterpart P450s. The individual P450s levels detected with antibodies against human and rat CYP2E1 indicated a low correlation.

The contents of human and rat P450 in hepatic microsomes are shown in Fig. 8. In hepatic microsomes of human and mature male rats (7 weeks old), which are often used in drug metabolism studies, CYP3A and 2C were the major forms; other forms such as CYP2E1 and 1A2 were found at low levels. The level of CYP2C11, which can metabolize many drugs [36], was extremely high, and CYP2C11 seemed to have quite different catalytic properties from human CYP2C forms. The mature male rat may give a different metabolic profile of drugs from that of the human when CYP2C11 contributes to metabolism. In mature female rats (7 weeks old), CYP3A2 was a minor form, and the content

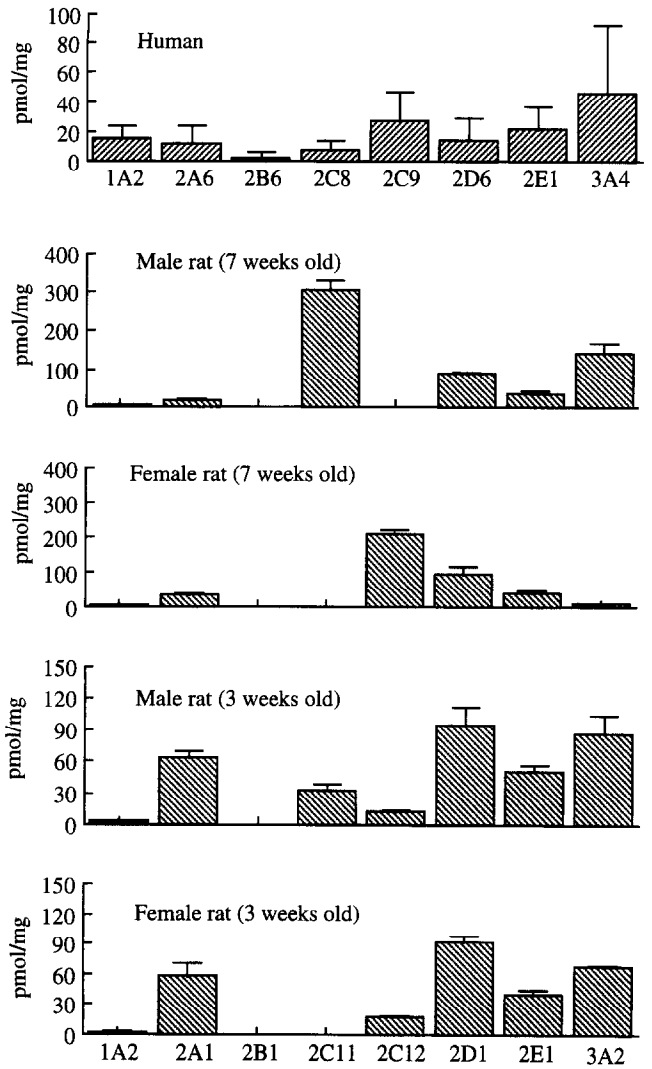

FIG. 8. Levels of P450s in human and rat hepatic microsomes. The levels of P450 in human hepatic microsomes were expressed as means ± SD from the values of Fig. 4. The levels of P450 in rat hepatic microsomes were expressed as the means ± SD of three to five rats.

of individual P450s in mature female rats differed from that of human P450s. In immature female rats (3 weeks of age), the levels of CYP2A, 2D, and 3A forms were high, and CYP2C11 was not detectable. Rat CYP2A1 had catalytic properties in testosterone metabolism different from those of human CYP2A6, but CYP2A1 did not metabolize many drugs, unlike CYP2C11 [36].

DISCUSSION

The levels and catalytic activities of several P450 forms in individual human samples have been determined and compared with P450s in experimental animals [36]. However, there have been only limited efforts to systematically characterize P450 levels and activities in samples from humans and experimental animals. We therefore compared several forms of P450 in human and rat livers.

Like rat hepatic microsomes, CYP2C and 3A forms were the major P450s in human hepatic microsomes. These results are consistent with other findings [31, 34, 35]. Human and rat CYP3A forms had similar characteristics; they require phospholipid mixtures and cytochrome b_5 for efficient catalytic activity in a reconstituted system, as reported [33]. The native CYP3A4 (originally named P450NF) purified from human hepatic microsomes also had low activity toward nifedipine but exhibited enough activity in the presence of a lipid mixture extracted from human hepatic microsomes [3]. Also, these forms had similar catalytic activities such as testosterone 2β- and 6β-hydroxylation activities and erythromycin N-demethylation activity [3]. However, rat CYP2C11 had catalytic properties different from those of human CYP2C8, 2C9, and 2C18. CYP2C11 had high activity toward testosterone and some drugs [37], but human CYP2C forms did not. Therefore, differences in drug metabolism between humans and rats may occur when CYP2C11 contributes to metabolism. In the rat, there is a sex difference in the content of CYP2C11 and 3A2; mature female rats do not have CYP2C11 and 3A2. Instead, they have CYP2C12 which has low activity toward testosterone and drugs that CYP2C11 can metabolize [37]. CYP2C12 may correspond to human CYP2C forms. Mature female rats also have a different composition of P450s than humans. On the other hand, immature female rats had CYP2A1, 2D1, and 3A2 as major forms. CYP2A forms in human and rat had different catalytic properties. These findings suggest that mature and immature rats must be used for drug metabolism to extrapolate the data from rats to humans.

Rats have five kinds of CYP2D forms, whereas humans have only CYP2D6. In drug metabolism, CYP2D6 is important because it exhibits genetic polymorphism, and metabolism of some drugs such as debrisoquine and spartein is delayed in poor metabolizers with a mutated *CYP2D6* gene [38, 39]. In rats, each CYP2D form seems to have different catalytic activity. For example, Dark-Agouti (DA) rats have low activity toward debrisoquine, so it is though to be a model of human genetic CYP2D6 polymorphism; only CYP2D1 is deficient in the DA rat [12]. Human and rat CYP2D forms may have different catalytic properties, although CYP2D1 and 2D6 seem to have similar activities. CYP2E1 is ethanol inducible in both rats and humans. Human and rat CYP2E1 have similar catalytic activities including oxidation of nitrosoamine and ethanol [40]. In this study, we also found that human, as well as rat, CYP2E1 had lauric acid (ω-1)-hydroxylation activity.

Rat CYP2B1 and human CYP2B6 have high activity toward several drugs including lidocaine. Rat CYP2B1 is induced by phenobarbital and by many other drugs [41]. Human CYP2B6, as well as CYP3A4, may be induced by barbiturates [2]; rat CYP3A2 is also barbiturate inducible. When the level of CYP2B6 is high, the rate of drug metabolism may increase.

In conclusion, CYP3A and 2C forms were the major P450s in human hepatic microsomes. Human CYP1A2, 2B6, 2D6, 2E1, and 3A4 had properties similar to those of their rat counterparts, but human CYP2C forms and

CYP2A6 had different catalytic properties from the rat CYP2C and 2A forms. Mature male rats had a high level of CYP2C11, which can metabolize many drugs, and a difference in drug metabolism between humans and rats may occur when CYP2C11 contributes to metabolism. To extrapolate drug metabolism from rats to humans, both mature and immature rats must be used. These results give useful information for drug metabolism and toxicological study.

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