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Molecular Cloning and Functional Analysis of Cynomolgus Monkey CYP1A2*

*Tsutomu Sakuma,†¶ Minako Hieda,† Tomoko Igarashi,† Satoru Ohgiya,‡ Ryoichi Nagata,§ Nobuo Nemoto‖ and Tetsuya Kamataki†**

†Division of Drug Metabolism, Faculty of Pharmaceutical Sciences, Hokkaido University, N12 W6, Sapporo, Hokkaido 060; ‡Hokkaido National Industrial Research Institute, Agency of Industrial Science and Technology, Sapporo, Hokkaido 062; §Shin Nippon Biochemical Laboratories, Ltd., Yoshida, Kagoshima 891-13; and ‖Department of Toxicology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

ABSTRACT. Complementary DNA fragments encoding cynomolgus monkey CYP1A2 were amplified by the reverse transcriptase–polymerase chain reaction (RT-PCR) method from the liver total RNA of a 3-methylcholanthrene (3-MC)-treated cynomolgus monkey. The nucleotide sequence determined was 1630 bp long and contained an open reading frame for a polypeptide of 516 residues. The nucleotide and the deduced amino acid sequences of cynomolgus monkey CYP1A2 showed 95.1 and 92.8% identities to those of human CYP1A2, respectively. The level of CYP1A2 mRNA in the liver of untreated cynomolgus monkey was very low. Treatment with 3-MC increased it. Still, it was one-fortieth that of CYP1A1. Cynomolgus monkey CYP1A2 expressed in recombinant yeasts activated 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) at efficient rates in the *umu* mutagenicity test. This cytochrome P450 (CYP) also activated 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), but less efficiently. These results indicate that cynomolgus monkeys have a functionally active *CYP1A2* gene, but its expression level is very low in the liver of untreated cynomolgus monkeys. BIOCHEM PHARMACOL **56**;1:131–139, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. cytochrome P450; mutagenic activation; MeIQx; induction, 3-methylcholanthrene

CYP†† consists of a superfamily of ubiquitous monooxygenases associated with the metabolism of a broad group of structurally unrelated compounds, including drugs, environmental pollutants, and endogenous substrates. It is also known that CYP catalyzes the metabolic activation of promutagens and procarcinogens, leading to their genotoxicities [1]. Therefore, it is believed that the activity and expression level of each CYP are important factors to determine the consequences of exposure to chemical procarcinogens.

To date, many heterocyclic amines such as IQ, MeIQx, and PhIP have been isolated from cooked meat and fish products, and identified as procarcinogens in rodents [2–5]. These carcinogenic heterocyclic amines show mutagenicity only after undergoing metabolic activation by so-called drug-metabolizing enzyme systems. This is accomplished via a two-step process involving the *N*-hydroxylation of the exocyclic amino group by CYP followed by phase II esterification [6, 7]. One of the candidates of CYP responsible for the *N*-hydroxylation of heterocyclic amines is CYP1A2 [8–11]. Nevertheless, the real carcinogenic potential of these heterocyclic amines in humans has not been proven, since it is difficult to extrapolate to humans from the rodent data on procarcinogen metabolism due to the large species differences in the properties of CYPs.

The monkey is recognized as the secondarily nearest species to humans on the evolutionary tree and is supposed to be a suitable animal model for pharmacological and toxicological studies [12]. Thus, cynomolgus monkeys (*Macaca fascicularis*) have been used for carcinogenicity testing for over 30 years [13]. However, the molecular aspects of the metabolism of procarcinogens and promutagens in monkey livers have not been clarified. We have already

* The nucleotide sequence reported in this paper appears in the GSDB, DDBJ, EMBL, and GenBank database with accession number "D86474," definition "*Macaca fascicularis* adult liver mRNA for cytochrome P-450."

¶ Corresponding author and present address: Tsutomu Sakuma, Ph.D., Department of Toxicology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan. Tel. 81-764-34-2281; FAX 81-764-34-5046; E-mail: tsakuma @toyama-mpu.ac.jp.

** Correspondence may also be addressed to: Dr. Tetsuya Kamataki, Division of Drug Metabolism, Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo, Hokkaido 060, Japan. Tel./FAX 81-11-706-4978; E-mail: kamataki@yakugaku.pharm.hokudai.ac.jp.

†† *Abbreviations:* CYP, cytochrome P450; CYP reductase, NADPH–cytochrome P450 reductase; DTT, dithiothreitol; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; 3-MC, 3-methylcholanthrene; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMLV, Moloney murine leukemia virus; PCR, polymerase chain reaction; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; and RT, reverse transcriptase.

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reported that forms of CYP belonging to the *CYP2C*, *CYP2E*, and *CYP3A* gene subfamilies are expressed constitutively in the liver of cynomolgus monkeys, whereas expression of CYP1A1 is not detectable in the liver of untreated cynomolgus monkeys [14, 15].

Recently, some investigators reported data showing the lack of constitutive expression of CYP1A2-like protein and only marginal induction of CYP1A2-like protein in the liver of cynomolgus monkeys [16–21]. They concluded from these results that the contribution of CYP1A2 to carcinogenesis in cynomolgus monkeys is negligible [18, 20]. However, these reports raised the question of whether the cynomolgus monkey has a functionally active *CYP1A2* gene. Thus, the aim of this study was to ascertain with certainty, using a molecular cloning technique, whether the cynomolgus monkey has any constitutive expression and induction of CYP1A2.

MATERIALS AND METHODS

Materials

Restriction enzymes, S1 nuclease, T_4 polynucleotide kinase, and other DNA-modifying enzymes were obtained from Takara Shuzo. MMLV reverse-transcriptase was purchased from United States Biochemical, native *Pfu* DNA polymerase from Stratagene, *Taq* DNA polymerase from Pharmacia Biotech, and 3-MC from Sigma. Radioactive reagents, a pMOS blue T-vector kit, and a sequencing kit were obtained from Amersham, and IQ, MeIQx, and PhIP from Wako Pure Chemicals. Yeast expression vector pAAH5 was provided by Dr. Ryo Sato of Osaka University. *Salmonella typhimurium* TA1535/pSK1002 was donated by Dr. Tsutomu Shimada of the Osaka Prefectural Institute of Public Health. Emulgen 911, a non-ionic detergent, was provided by the Kao Co. Other chemicals were of the highest grade commercially available.

Animals

An adult female and three adult male cynomolgus monkeys (*M. fascicularis*) were used in this study. The male cynomolgus monkeys, weighing about 5 kg, were given a single i.p. injection of 3-MC in corn oil at a dose of 20 mg/kg and were killed 3 days later. Livers were excised immediately and used for the preparation of RNA and microsomes.

Amplification of Cynomolgus Monkey CYP1A2 cDNA

The primers used in this study were synthesized with a DNA synthesizer (model 381A; Applied Biosystems). The sequences of the synthesized primers were as follows:

RT-1S, 5′-TTACAACCCTGCCAATCTCAAG-3′;
RT-1AS, 5′-AAGTCCTGCTCGTGTTCCTGG-3′;
RT-2S, 5′-CCCTCAACACCTTCTCCATC-3′;
RT-2AS, 5′-AGCCATTCAGCGTTGTGTCC-3′;
RT-3S, 5′-AGGAACACTATCAGGACTTTG-3′;
RT-3AS, 5′-GAAGAGAAACAAGGGCTGAGT-3′;
RT-4S, 5′-TGGCGTCTTCTTCACTTGAT-3′;
RT-4AS, 5′-GGCCGGCCCTTGAAATCGTC-3′.

Total RNA was prepared from livers of untreated and 3-MC-treated cynomolgus monkeys according to the method of Chirgwin *et al.* [22]. An aliquot (3.5 μg) of total RNA prepared from the liver of a 3-MC-treated male cynomolgus monkey was reverse-transcribed into cDNA in a reaction mixture (30 μL) containing 50 mM of Tris–HCl (pH 8.3), 75 mM of KCl, 3 mM of $MgCl_2$, 10 mM of DTT, 0.4 mM of dNTPs, random hexamer (120 ng), and MMLV reverse-transcriptase (150 U). Before the addition of MMLV reverse-transcriptase, the reaction mixture was heated to 70° for 2 min, and then cooled to room temperature. After the addition of MMLV reverse-transcriptase, the reaction mixture was incubated at 37° for 1 hr and boiled for 10 min. An aliquot (5 μL) of the cDNA solution was added into 50 μL of the reaction mixture containing 20 mM of Tris–HCl (pH 8.8), 2 mM of $MgCl_2$, 10 mM of KCl, 6 mM of ammonium sulfate, 0.1% Triton X-100, 0.1 mg/mL of nuclease-free bovine serum albumin, 0.1 mM of each dNTP, 1 μM of each primer and 2 U of native *Pfu* DNA polymerase, and then was amplified by 30 cycles including 1 min at 94°, 2 min at the optimized temperature of annealing for each primer set, and 2 min at 72° using a DNA Thermal Cycler (Perkin–Elmer Cetus). The RT–PCR products were purified by electrophoresis in a 1% agarose gel followed by ligation into the pMOSblue T vector (RT-1P, RT-2P, RT-3P) or the pAAH5 yeast expression vector (RT-4P). In the case of RT-4P, both ends were blunted by T_4 DNA polymerase before ligation, and the resulting plasmid (pMK1A2) was used to express cynomolgus monkey CYP1A2 in the recombinant yeast. The nucleotide sequences of the amplified cDNA fragments were determined by the dideoxy method [23] with Sequenase ver. 2 and an ABI 377 automated DNA sequencer.

S1 Nuclease Protection Assay

For the S1 nuclease protection assay, a 141 nucleotide-long single-strand DNA encompassing positions 251 to 352 of cynomolgus monkey CYP1A2 was prepared. The 40 nucleotide-long segment in this probe, which was located at 3′-downstream of CYP1A2 cDNA, was an unhybridizable segment that came from the M13 vector. This segment can be cut off by S1 nuclease. The probe labeled at the 5′-end with [γ-^{32}P]ATP was hybridized at 49°. S1 nuclease digestion was performed using 25 U/20 μg of total RNA at 30° for 40 min. After reaction, the mixture was applied to an 8% polyacrylamide gel containing 42% urea. The polyacrylamide gel was dried and visualized autoradiographically.

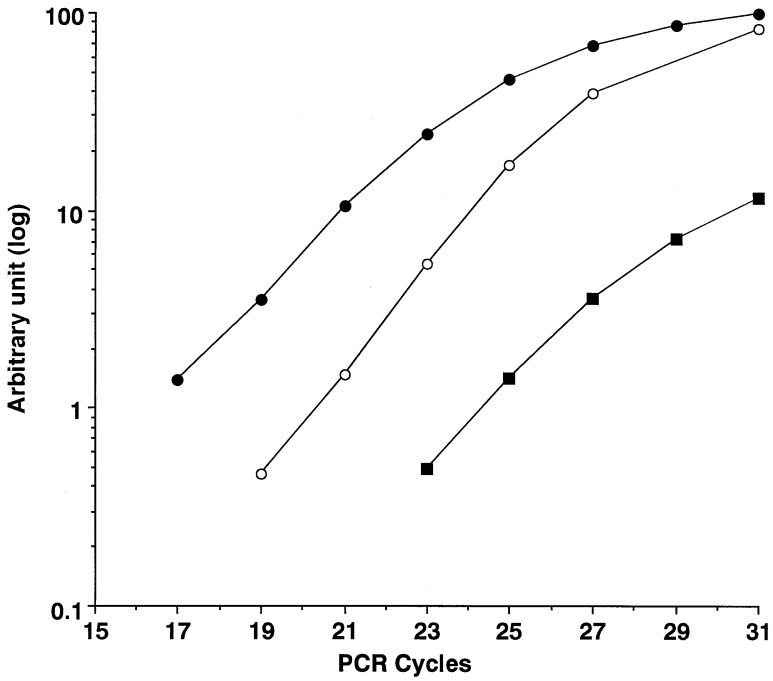

FIG. 1. Product formation during PCR cycling with the common primers for CYP1A1 and CYP1A2 cDNAs. Hepatic total RNA (0.08 μg) was reverse-transcribed, and then submitted to PCR with the following amplifying conditions: at 94° for 60 sec, 51° for 120 sec, and then 72° for 120 sec. The reaction mixtures after 17, 19, 21, 23, 25, 29, and 31 cycles were digested with *Msp*I and applied to polyacrylamide gel electrophoresis. Amounts of the products were measured with a densitometer and are shown as values relative to that of CYP1A1 after 31 cycles. Each cycle represents the mean of each cDNA of two 3-MC-treated monkeys. Key: (●) CYP1A1; (○) GAPDH; and (■) CYP1A2.

Comparison of the mRNA Level between CYP1A1 and CYP1A2 using Semiquantitative RT–PCR

RT–PCR was performed as described above with *Taq* DNA polymerase instead of *Pfu* DNA polymerase. The forward and reverse primers were 5′-TGACCCAGCCTCCT CAT-3′ and 5′-TTCGCTACCTGCCCAAC-3′, respectively. Both primers completely matched to CYP1A2 and CYP1A1 of cynomolgus monkeys. The PCR was performed with 94° for 1 min, 51° for 2 min, and then 72° for 2 min. To determine the amounts of CYP1A1 and CYP1A2 cDNA separately, the PCR products were digested with *Hha*I or *Msp*I, which can digest only CYP1A1 or CYP1A2 cDNA, respectively, and then were resolved by 8% polyacrylamide gel electrophoresis. The amounts of PCR products increased exponentially to 27 cycles (Fig. 1). The efficiency of amplification seemed to be equal between CYP1A1 and CYP1A2. The product length was 228 bp, spanning the region from 501 to 789 of cynomolgus monkey CYP1A2 cDNA. When the mixture of RT reaction was incubated without RT as a negative control, no RT–PCR product was amplified (see Fig. 4A, extreme right lane). These observations indicate that the amounts of RT–PCR products reflect the initial amount of CYP1A1 and CYP1A2 mRNA. To normalize the quantity of RNA applied, portions of the cDNA samples were amplified simultaneously by PCR using primers for human GAPDH (Clontech). The forward and reverse primers were 5′-ACCACAGTCCATGCCATCAC-3′ and 5′-TCCAC CACCCTGTTGCTGTA-3′, respectively. The PCR condition for GAPDH was the same as that of CYP1A2. Exponential amplification of GAPDH cDNA was also confirmed (Fig. 1). The length of the PCR products was 452 bp. While the nucleotide sequence of cynomolgus monkey GAPDH is not available thus far, cDNA fragments with the expected size were amplified, using this primer set from the cynomolgus monkey. The expression level of both mRNA species in the liver of cynomolgus monkeys was shown as a ratio to GAPDH.

Expression of Cynomolgus Monkey CYP1A2 in Recombinant Yeast

An expression plasmid for cynomolgus monkey CYP1A2 was constructed as described above. Another expression plasmid containing human CYP1A2 cDNA, pHM1A2, was constructed with the same strategy. The nucleotide sequences of both expression constructs were confirmed before use. Both expression plasmids were used to transform the High-red yeast (M8 line) by the lithium acetate method [24]. The High-red yeast is a recombinant strain of *Saccharomyces cerevisiae* YPH500, which shows a high CYP reductase activity (M8 line; 635 nmol of cytochrome *c* reduction/min/mg of protein) by the integration of the expression unit of hamster CYP reductase into yeast genome [25]. Transformed yeast cells were selected on a plate containing a synthetic minimum medium with added histidine, lysine, adenine, and uracil. Cultivation of recombinant yeast cells and preparation of microsomal fractions were carried out as described previously [26]. The concentrations of CYP and protein were determined as reported by Omura and Sato [27] and Lowry *et al.* [28], respectively.

Mutation Assay

CYP-mediated activation of heterocyclic amines was measured by determination of the expression of the *umu* gene in

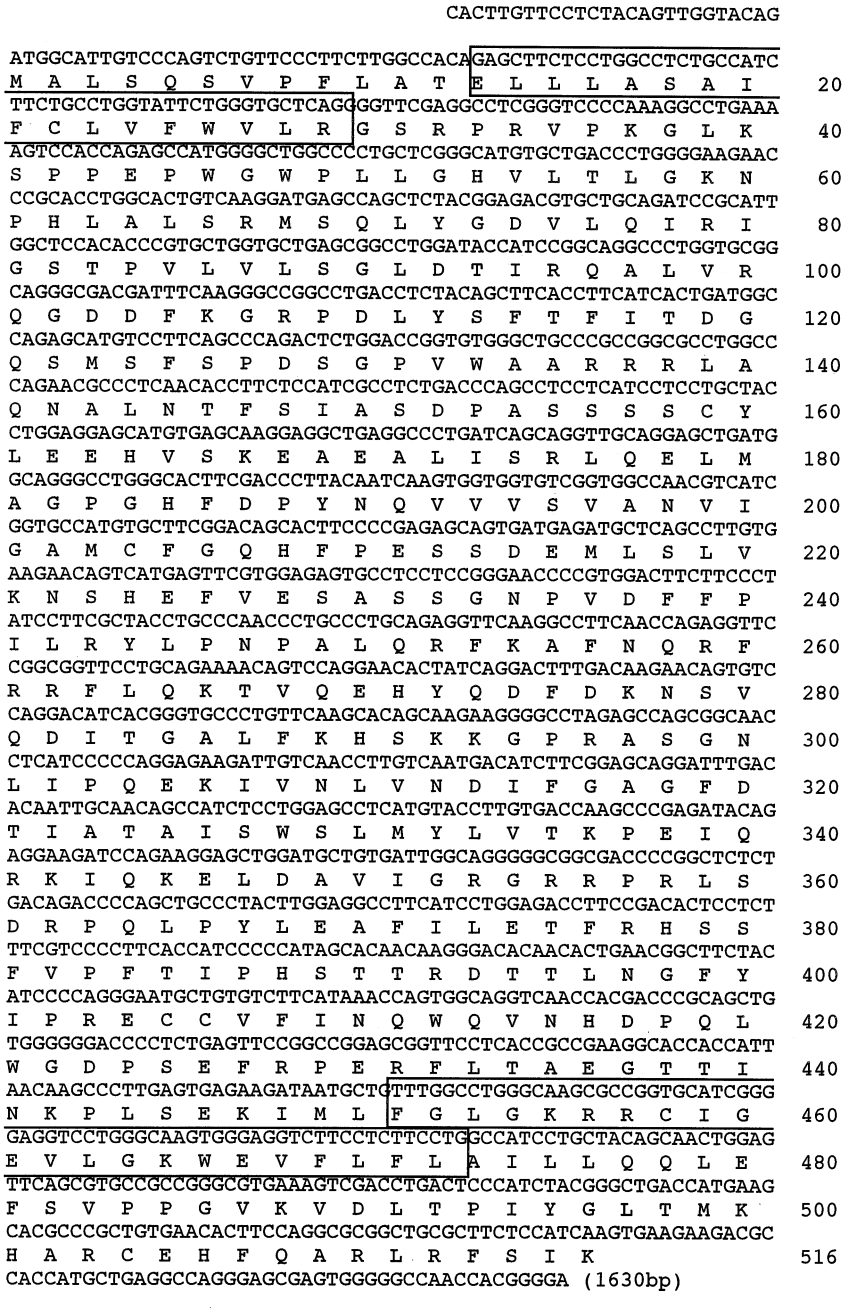

FIG. 2. Nucleotide and deduced amino acid sequences of cynomolgus monkey CYP1A2. The number of the deduced amino acid sequence is shown on the right. Boxed sequences are two predicted functional regions: the former is the signal-anchor sequence and the latter is the heme-binding region.

Salmonella typhimurium TA1535/pSK1002, as reported previously [29].

RESULTS

Amplification and Characterization of Cynomolgus Monkey CYP1A2 cDNA

Complementary DNA of cynomolgus monkey CYP1A2 was amplified using the RT–PCR method. We previously showed that cynomolgus monkey CYPs shared over 95% identities in nucleotide sequences with corresponding forms in humans [14, 15]. Based on this finding, we expected that cynomolgus monkey CYP1A2 would show very high identities to human CYP1A2 if cynomolgus monkeys have a *CYP1A2* gene. Therefore, we also expected that cDNA for cynomolgus monkey CYP1A2 must be amplified by primers for human CYP1A2 under appropriate conditions. Thus, we designed PCR primers adapting human CYP1A2 sequences. We designed the primers considering the following: one should be a well-conserved sequence among mammalian CYP1A2, and the other should be a sequence showing low identity to cynomolgus monkey CYP1A1. Amplification was carried out through two steps. First, we amplified three cDNA fragments with overlapping regions. Complementary DNA was synthesized from the liver total RNA of a 3-MC-treated cynomolgus monkey, and PCR was

TABLE 1. Comparison of the nucleotide and the deduced amino acid sequences of cynomolgus monkey CYP1A2 with other mammalian *CYP1A* genes

	Cynomolgus monkey	Human	Dog	Rabbit	Guinea pig	Rat	Hamster	Mouse
CYP1A1								
Nucleotide	79.6	79.5	78.1	78.7	74.4	75.5	74.9	75.6
Amino acid	73.6	72.5	73.1	72.3	69.9	69.6	68.0	69.8
CYP1A2								
Nucleotide	100	95.1	83.6	84.1	80.2	80.2	80.0	79.2
Amino acid	100	92.8	81.9	80.4	77.4	75.7	74.7	73.7

The coding regions of forms of the *CYP1A* gene subfamily were compared with those of cynomolgus monkey CYP1A2. Each value indicates percent identity.

carried out with native *Pfu* DNA polymerase and RT-2S and RT-2AS primers. As a result, one cDNA fragment corresponding to the middle portion of the coding region of cynomolgus monkey CYP1A2 (RT-2P) was amplified. Based on the nucleotide sequence of this cDNA fragment, we designed two primers: one was an antisense primer (RT-1AS) for amplification of the N-terminal half of the coding region, and the other (RT-3S) was for the C-terminal half of the coding region. We also synthesized the other two primers, RT-1S and RT-3AS, which corresponded to the 5′- and 3′-noncoding region of human CYP1A2, respectively. Using these primers, two cDNA fragments encompassing the portion of the N-terminal half (RT-1P) and the C-terminal half (RT-3P) were amplified. The nucleotide sequences of these cDNA fragments were determined after subcloning into the pMOSblue T-vector. In the next step, in order to confirm the presence of mRNA containing the entire coding region and to express the cynomolgus monkey CYP1A2 in recombinant yeast, a cDNA fragment (RT-4P) containing the entire coding region of cynomolgus monkey CYP1A2 was amplified. The nucleotide sequences of two independent clones of RT-4P were confirmed. Their nucleotide sequences were completely identical. Figure 2 shows the nucleotide and the deduced amino acid sequences of the cynomolgus monkey CYP1A2. The nucleotide sequence determined in this study was 1630 bp long and contained an open reading frame (position from 27 to 1574) encoding a polypeptide of 516 amino acid residues. The estimated molecular weight of cynomolgus monkey CYP1A2 was 58,196. The heme-binding cysteine was present at the 458th residue. A signal-anchor sequence also existed at the N-terminus. Table 1 shows the result of a comparison between cynomolgus monkey *CYP1A2* and *CYP1A* genes from Mammalia. The amino acid sequence of the cynomolgus monkey CYP1A2 showed the highest identities of 92.8% to human CYP1A2 and showed lower identities (less than 73.6%) to mammalian *CYP1A1* genes. Thus, we confirmed that RT–PCR products obtained in this study were cDNA fragments encoding the cynomolgus monkey CYP1A2.

Expression of CYP1A2 in the Liver of Cynomolgus Monkeys

To further confirm the expression of CYP1A2 in the liver of cynomolgus monkeys, an S1 nuclease protection assay was carried out. As shown in Fig. 3, a strong and a weak signal were detected in the liver of a 3-MC-treated and an untreated cynomolgus monkey, respectively. This result indicates that a trace amount of CYP1A2 mRNA was expressed in the liver of the untreated cynomolgus monkey, and its level was strongly induced by 3-MC.

Comparison of the mRNA Level between CYP1A1 and CYP1A2

We have demonstrated previously the expression of CYP1A1 mRNA in the liver of 3-MC-treated cynomolgus monkeys [14]. To compare the mRNA level between

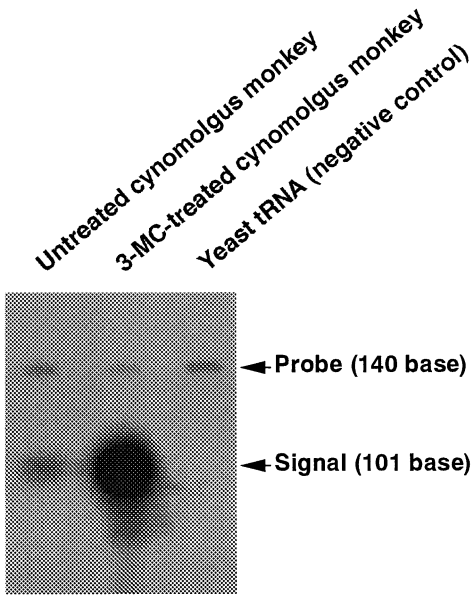

FIG. 3. Expression of CYP1A2 mRNA in the liver of an untreated cynomolgus monkey and induction by 3-MC treatment. A portion (20 μg) of total RNA was allocated to S1 nuclease protection assay, as described under Materials and Methods.

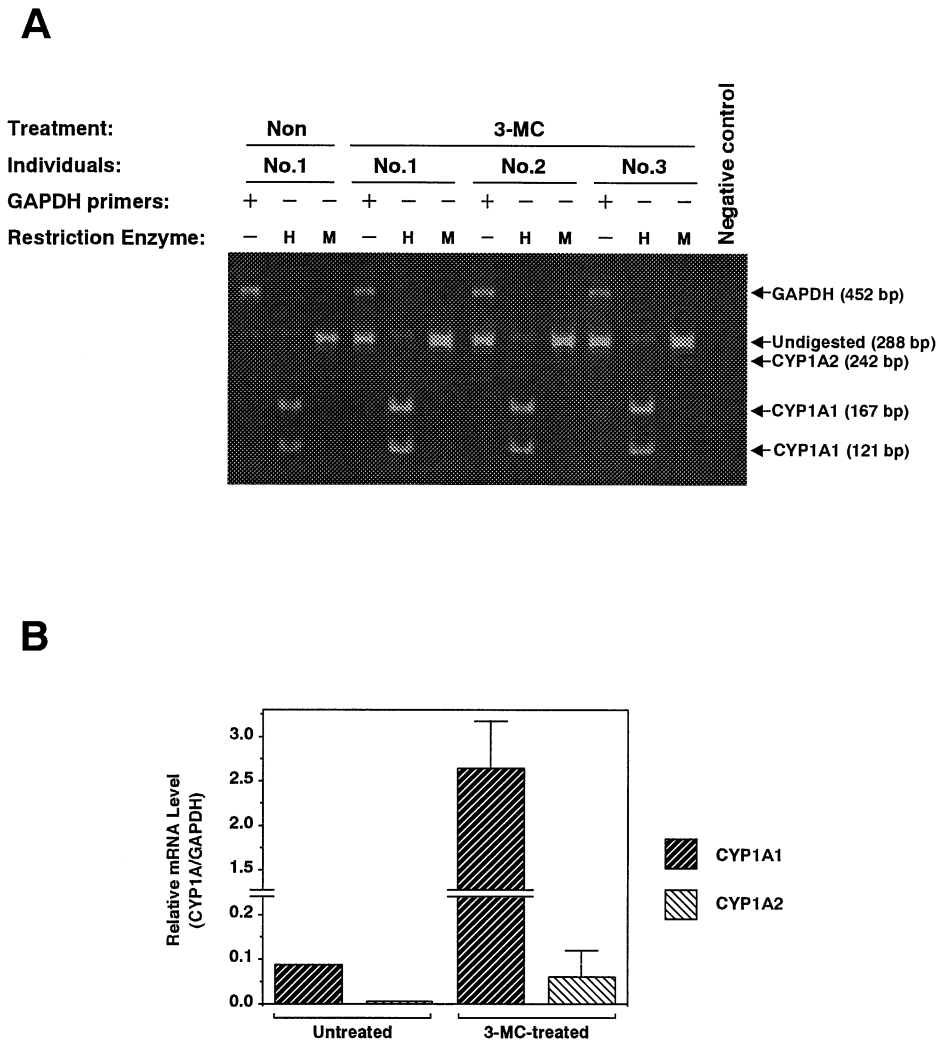

FIG. 4. Comparison of the mRNA level between CYP1A1 and CYP1A2 in the liver of cynomolgus monkeys using quantitative RT PCR. (A) Development of the RT PCR products. Undigested products and products digested with *Hha*I or *Msp*I were developed on 8% polyacrylamide gel. Two *Hha*I digests (167 and 121 bp) corresponding to CYP1A1 cDNA and one *Msp*I digest (242bp) corresponding to CYP1A2 cDNA were detected. To normalize the quantities of RNA applied, GAPDH cDNA was simultaneously amplified (left lane of each sample). The amount of total RNA used was 0.08 μg except for 0.4 μg in the lanes of digested products from the untreated cynomolgus monkey. Abbreviations used are: H, *Hha*I; and M, *Msp*I. (B) The level of CYP1A1 and CYP1A2 mRNA in the liver of cynomolgus monkeys. The level of each mRNA species was shown as a ratio to that of GAPDH. Each untreated sample was determined in duplicate, and the columns of 3-MC-treated cynomolgus monkeys represent the mean ± SD (N = 3).

CYP1A2 and CYP1A1, quantitative RT–PCR analysis was carried out (Fig. 4). Both CYP1A2 and CYP1A1 mRNA were detected in the liver of either the untreated monkey or the 3-MC-treated cynomolgus monkeys. Treatment with 3-MC strongly induced both CYP1A1 and CYP1A2 mRNA. The level of CYP1A2 mRNA in the liver of 3-MC-treated cynomolgus monkeys was one-fortieth of that of CYP1A1. This result indicates that most of CYP1A enzyme expressed in cynomolgus monkey livers is CYP1A1.

Functional Analysis of Cynomolgus Monkey CYP1A2

To examine whether or not cynomolgus monkey CYP1A2 had the capacity to activate carcinogenic heterocyclic amines such as IQ, MeIQx, and PhIP, cynomolgus monkey CYP1A2 was expressed in recombinant yeast. The expression plasmid pMK1A2 was constructed by insertion of RT-4P into the region downstream of the yeast ADH promoter in the pAAH5 yeast expression vector. The pMK1A2 was introduced into the High-red yeast, which has high CYP reductase activity by insertion of a hamster CYP reductase expression unit into yeast genome. The expression plasmid pHM1A2 for human CYP1A2 was also constructed and introduced into the recombinant yeast to compare catalytic properties. Yeast cells transformed with pMK1A2, pHM1A2, or pAAH5 vector were cultivated, and the microsomal fractions were prepared. The expression of cynomolgus monkey and human CYP1A2 proteins in yeast cells was confirmed by immunoblot analysis using the antibodies raised against rat CYP1A2. The results

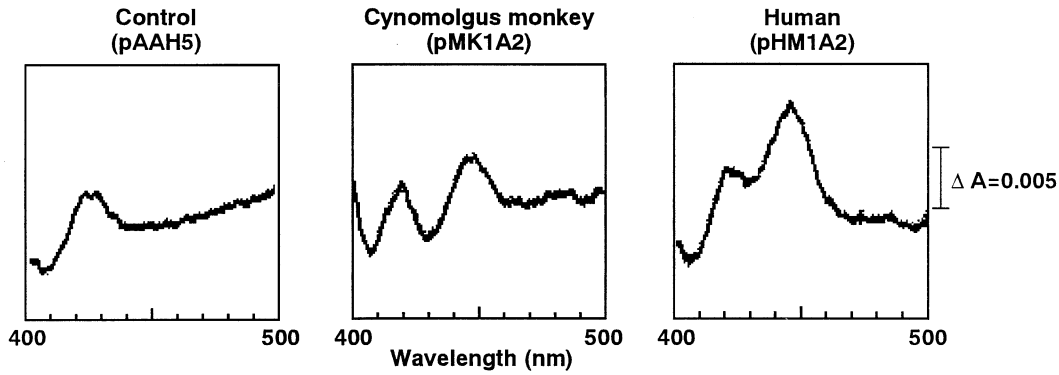

FIG. 5. Reduced carbon monoxide-difference spectra of microsomes of recombinant yeast cells. The spectra were measured after solubilization with 0.2% Emulgen 911, a non-ionic detergent.

showed that weak immunoreactive bands were detectable around 52 kDa (data not shown). Figure 5 shows the reduced carbon monoxide-difference spectra of microsomes from the recombinant yeast cells. An absorption peak was seen at 447 nm in the difference spectra of microsomes of the yeast cells transformed with pMK1A2 and pHM1A2, although both microsomes showed an absorption peak at 420 nm to a similar level. On the other hand, no absorption peak around 450 nm was seen in the spectrum of microsomes from the control yeast cells, which were transformed with pAAH5. These results indicate that cynomolgus monkey CYP1A2 and human CYP1A2 were expressed as the holo-enzymes. The contents of CYP in microsomes of yeast cells were estimated to be 0.022 and 0.046 nmol/mg of protein for the cynomolgus monkey CYP1A2 and human CYP1A2, respectively.

The capacity of cynomolgus monkey CYP1A2 to activate promutagens was examined by the *umu* mutagenicity test. The results are summarized in Table 2. Microsomes containing the cynomolgus monkey CYP1A2 enzyme activated IQ and MeIQx efficiently, and PhIP at a lower rate. The activation rates were comparable to those of human CYP1A2. Microsomes from yeast cells transformed with pAAH5 as a control did not activate these promutagens.

TABLE 2. Activation of promutagens by cynomolgus monkey and human CYP1A2 expressed in recombinant yeast cells

Promutagen	Control (pAAH5)	Cynomolgus monkey (pMK1A2)	Human (pHM1A2)
		units/min/mg protein (units/min/pmol P450)	
IQ	ND*	143	324
	(–)	(986)	(707)
MeIQx	ND	37.7	47.3
	(–)	(260)	(103)
PhIP	ND	1.79	5.20
	(–)	(0.246)	(0.227)

The concentration of promutagens added to the incubation mixture was 10 μM. Each value represents the mean of duplicate determinations.

*ND = not detectable.

This result indicates that cynomolgus monkeys have a functionally active *CYP1A2* gene.

DISCUSSION

The aim of this study was to ascertain with certainty whether the cynomolgus monkey has any constitutive expression of CYP1A2. To resolve this question, we constructed a liver cDNA library from a 3-MC-treated cynomolgus monkey. Although some CYP1A1 cDNA clones were obtained, no CYP1A2 cDNA clone could be isolated using cDNA fragments of human CYP1A2 or cynomolgus monkey CYP1A2 (RT-2P), which was amplified in this study, as probes (data not shown). Therefore, we amplified the cDNA fragment containing the entire coding region and determined the primary structure. Cynomolgus monkey CYP1A2 consisted of 516 amino acid residues. This is the same as human CYP1A2 reported by Quattrochi *et al.* [30] and one residue larger than that reported by Jaiswal *et al.* [31].

In this study, we were able to detect CYP1A2 mRNA in the liver of the untreated cynomolgus monkey by an S1 nuclease protection assay and semiquantitative RT–PCR analysis. However, no signal was detectable in Northern blot and immunoblot analyses (data not shown). In addition, it was reported that no CYP1A2 protein was detected in liver microsomes of untreated cynomolgus monkeys by immunoblot analysis [18–20]. The reason for this apparent discrepancy is likely due to the high sensitivity of the S1 nuclease protection assay and the semiquantitative RT–PCR analysis. Our results indicate that CYP1A2 is expressed in the liver of untreated cynomolgus monkeys, although the expression level must be too low to be detected by Northern blot and immunoblot analyses.

In this study, we were able to use only four cynomolgus monkeys, and we divided them into two groups: three males were put in a 3-MC-treated group and one female was untreated. Thus, we do not have any direct information about gender-difference of monkey CYP1A2. However, there are no reports showing remarkable gender-difference in the expression level of CYP1A2 in other species,

including humans, to our knowledge. Thus, we presume that there is no such difference in cynomolgus monkeys.

Concerning the relative mRNA level of CYP1A2 and CYP1A1 in the liver of cynomolgus monkeys, it appeared that mainly CYP1A1 was expressed in the liver of the untreated monkey as well as the 3-MC-treated monkeys. This is in contrast to an observation in humans.

In the *umu* mutagenicity test, cynomolgus monkey CYP1A2 expressed in recombinant yeast cells was capable of activating carcinogenic heterocyclic amines such as IQ, MeIQx, and PhIP at rates comparable to human CYP1A2. The result indicates that the cynomolgus monkey has functionally active CYP1A2. We were interested in comparing the activity toward heterocyclic amines between cynomolgus monkey CYP1A2 and CYP1A1. However, the strain of yeast used in this study was different from that used in our previous study [14]. In this study, we used a recombinant yeast that shows very high P450-reductase activity. On the other hand, the yeast used in our previous study had low P450-reductase activity. Thus, we cannot directly compare these activities. Successful expression of the recombinant cynomolgus monkey CYP1A2 in yeast cells, coupled with the extremely low level of CYP1A2 mRNA in the liver, suggests that the low level of CYP1A2 protein in the liver is not due to stability of monkey CYP1A2 protein. Thus, analysis of the gene sequence may help us to understand why this species shows such low levels of CYP1A2.

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