Molecular Cloning and Expression of CYP2J2, a Human Cytochrome 
P450 Arachidonic Acid Epoxygenase Highly Expressed in Heart*

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Shu Wu†, Cindy R. Moomaw‡, Kenneth B. Tomer§, John R. Falck¶, and Darryl C. Zeldin¶

From the †Laboratories of Pulmonary Pathobiology and §Molecular Biophysics, NIEHS, National Institutes of Health, Research Triangle Park, North Carolina 27709 and the ¶Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, Texas 75235

A cDNA encoding a human cytochrome P450 arachidonic acid epoxygenase was isolated from a human liver cDNA library. Sequence analysis revealed that this 1,876-base pair cDNA contained an open reading frame and encoded a new 502-amino acid protein designated CYP2J2. Blot hybridization analysis of RNA prepared from human tissues revealed that CYP2J2 was highly expressed in the heart. Recombinant CYP2J2 protein was prepared using the baculovirus expression system and purified to near electrophoretic homogeneity. The enzyme metabolized arachidonic acid predominantly via olefin epoxidation to all four regiosomeric cis-epoxyeicosatrienoic acids (catalytic turnover 65 pmol of product formed/nmol of cytochrome P450/min at 30 °C). Epoxydation of arachidonic acid by CYP2J2 at the 14,15-olefin was highly enantioselective for (14R,15S)-epoxyeicosatrienoic acid (76% optical purity). Immunoblotting of microsomal fractions prepared from human tissues using a polyclonal antibody raised against the recombinant hemoprotein confirmed primary expression of CYP2J2 protein in human heart. The in vivo significance of CYP2J2 was suggested by documenting the presence of epoxyeicosatrienoic acids in the human heart using gas chromatography/mass spectroscopy. Importantly, the chirality of CYP2J2 products matched that of the epoxyeicosatrienoic acid enantiomers present, in vivo, in human heart. We propose that CYP2J2 is one of the enzymes responsible for epoxidation of endogenous arachidonic acid pools in human heart and that epoxyeicosatrienoic acids may, therefore, play important functional roles in cardiac physiology.

The role of P4501 in the NADPH-dependent epoxidation of arachidonic acid is well documented (1–3). The primary products formed are four regioisomeric cis-epoxyeicosatrienoic acids (5,6-, 8,9-, 11,12-, and 14,15-EET) (1–3). Thus far, arachidonic acid epoxygenase activity has been demonstrated in microsomal fractions prepared from several organs including liver, kidney, lung, and pituitary (4–8). Studies utilizing both purified and recombinant P450 enzymes have shown that (a) the epoxygenase reaction is enantioselective, (b) the reaction asymmetry is P450 enzyme specific, and (c) the predominant epoxygenase isoforms belong to the CYP2 gene family (4, 6, 9–13). The chiral nature of endogenous EET pools in liver, kidney, lung, and plasma confirms the biosynthetic origin of these eicosanoids and documents an endogenous role for microsomal P450 in the bioactivation of arachidonic acid (6, 11, 14, 15).

The potential physiological significance of the epoxygenase reaction is highlighted by the fact that the EETs possess numerous biological activities including modulation of membrane ion fluxes, stimulation of peptide hormone release, and effects on airway smooth muscle (Refs. 6 and 16–19 and references therein). Recent studies demonstrating that (a) the rat renal epoxygenase is under regulatory control by dietary salt, (b) alterations of the rat renal epoxygenase induce hypertension in rats fed a high salt diet, and (c) urinary excretion of epoxygenase metabolites is increased during pregnancy-induced hypertension in humans have supported the hypothesis that P450-derived arachidonic acid metabolites may be involved in the pathophysiology of hypertension (5, 19–21). The EETs have also been shown to have other cardiovascular effects. For example, EETs cause renal artery vasoconstriction (14), cerebral, intestinal, and coronary artery vasodilation (22–25), inhibition of platelet aggregation (26), and cardiac myocyte shortening (27). Importantly, the EETs have been shown to exacerbate the response of the heart to ischemia and reperfusion (27).

P450 has been identified using spectral, immunologic, and/or monooxygenase activity assays in heart microsomal fractions from several vertebrate species including scup, rat, rabbit, and pig (28–31). In addition, aromatic hydrocarbons have been shown to induce P450 activity in scup, chick embryo, and rabbit heart (30, 32, 33). Despite these studies, the identity of the P450 isoforms present in heart tissues has not been reported. Furthermore, the function of this ubiquitous enzyme system in the heart remains unknown. In this report, we describe the cloning and cDNA-directed expression of a new human P450 arachidonic acid epoxygenase that is highly expressed in human heart. We also show that human heart contains substantial quantities of endogenous EETs and that the chirality of these EETs matches that of those produced by the recombinant enzyme.

**EXPERIMENTAL PROCEDURES**

Materials—[α-32P]ATP, [γ-32P]ATP, and [1-14C]arachidonic acid were purchased from DuPont NEN. Restriction enzymes, Escherichia coli polymerase I, and T4 polynucleotide kinase were purchased from New England Biolabs. Triphenylphosphine, N,N-diisopropylethylamine, N,N-dimethylformamide.
and diazepam were purchased from Aldrich. All other chemicals and reagents were purchased from Sigma unless otherwise specified.

Isolation of RNA, Synthesis and Screening of the cDNA Libraries—Normal human tissues obtained through the Cooperative Human Tissue Network (National Disease Research Interchange, Philadelphia) were rapidly frozen in liquid nitrogen and maintained at −80 °C for up to 6 months prior to use. Rabbit tissues were obtained from male New Zealand White rabbits sacrificed by lethal intravenous injection of sodium pentobarbital. Poly(A)+ mRNA was prepared from the guanidinium thiocyanato/dıg(dT)-cellulose method using total RNA extraction and mRNA purification kits supplied by Pharmacia Biotech Inc. Oligo(dT)-primed Uni-Zap cDNA libraries were synthesized from human and rodent tissues using poly(A)+ mRNA. Human lung and liver poly(A)+ mRNA was prepared from normal human tissues obtained through the Cooperative Human Tissue Network (National Disease Research Interchange, Philadelphia) and mRNA (50 ng) was reverse transcribed into cDNA using the MAXBAC protocol (35). The cDNA library used in this study consists of 105 plaques, containing an average of 1.5 kb of cDNA fragment containing parts of the published sequence of human CYP2C10, including the untranslated 3′-end regions (34, 35). Approximately 5 × 10⁶ plaques were plated, employing XL-1 Blue E. coli as host, at a density of 10⁶ pfu/ml. Nucleic acid hybridizations were done at 57 °C in 0.9 M NaCl containing 0.05% NaH₂PO₄, Na₂HPO₄ (pH 7.0), 0.5% SDS, 0.01 μg of E. coli DNA, 5 × Denhardt's solution, and 0.1 mg of heat-denatured salmon sperm DNA/ml. Approximately 140 duplicate positive plaques were identified, of which 50 were identified, of which 50 were selected on the basis of hybridization with a cDNA insert synthesized from cDNA that was isolated from rabbit lung. This cDNA insert (human CYP2J2) was accomplished using the MAXBAC protocol described previously (36). Hybridizations were performed in 50% formamide, 5× SSC, 10% SDS, 0.1 mg/ml of heat-denatured salmon sperm DNA/ml. Double-stranded cDNA probes were labeled by nick translation using E. coli polynucleotide kinase and [γ-32P]ATP. Plasmid DNAs were replicated in DH5α E. coli. Positive clones were identified, of which 35 clones, selected at random, were grown in Luria's Broth containing 0.1 mg of ampicillin/ml and isolated using a Qiagen plasmid purification kit (Qiagen Inc., Chatsworth, CA). Insert sizes were determined by agarose electrophoresis after being excised from their plasmid DNA inserts using SmaI restriction endonuclease. The entire sequence was confirmed using the MAXBAC protocol described previously (36). Sequences that were identical and shared homology with several human and rodent CYP2 family members were identified. The entire sequence was confirmed using the MAXBAC protocol described previously (36).

For purification of recombinant CYP2J2, the P450 and sodium cholate concentrations of the crude cell lysate were adjusted to 1 μM and 0.4%, respectively. The resulting suspension was loaded by gravity at room temperature onto a 3 × 5 cm ω-aminooxy-agarose (Sigma) column equilibrated with 20% (v/v) glycerol, 0.1 M potassium phosphate (pH 7.4) containing 0.1% (v/v) dithiothreitol, 0.1 μM EDTA, and 0.1 μM dithiothreitol (buffer A). The column was washed with 4 column volumes of buffer A, and the bound CYP2J2 was eluted with buffer A containing 0.4% (v/v) Emulgen 911 (Kao Chemical Co., Tokyo). After dialysis versus 200 volumes of Tris-Cl buffer (pH 7.4) containing 20% (v/v) glycerol, 0.1% (v/v) sodium cholate, the CYP2J2 was eluted with 0.1 M sodium phosphate (pH 7.4) containing 20% (v/v) glycerol, 0.1% (v/v) sodium cholate, 0.1 μM EDTA, and 0.1% (v/v) dithiothreitol (buffer B). The CYP2J2 was eluted as a single peak with a k of −0.26 with 0.1 M sodium phosphate (pH 7.4) containing 20% (v/v) glycerol, 0.1% (v/v) sodium cholate, and 0.4% (v/v) Emulgen 911. The column was washed with 6 column volumes of equilibration buffer, 4 column volumes of 0.04 M sodium phosphate (pH 7.4) containing 20% (v/v) glycerol, 0.1% (v/v) sodium cholate, and 0.1% (v/v) Emulgen 911, and the bound CYP2J2 was eluted with 0.1 M sodium cholate, dialyzed versus 400 column volumes of sodium cholate-free buffer B, and concentrated using a Centricon-30 microconcentrator (Amicon). Recombinant CYP2J2 was subjected to N-terminal amino acid analysis and molecular mass determination by mass spectrometry. For N-terminal amino acid analysis (38), partially purified CYP2J2 was electrophoresed on SDS, 10% (w/v) polyacrylamide slab gels (200 × 200 × 1 mm), electroblotted onto Immobilon-P polyvinylidene difluoride membranes (Millipore), stained with Coomassie Brilliant Blue R-250 (Bio-Rad), and sequenced directly in the membrane after removal of the protein stain using an Applied Biosystems 4730 protein sequence (Perkin-Elmer Corp.). Cycle yields were calculated by comparison with internal standards. For molecular mass determination, recombinant CYP2J2 was analyzed on a PerSeptive Biosystems Voyager RP matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (PerSeptive Biosystems, Houston, TX) using bovine serum albumin (M₉ = 68,500) and cytochrome b₅ (M₉ = 54,000) as standard mass calibrants. The CYP2J2 was analyzed on a PerSeptive Biosystems Voyager RP matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (PerSeptive Biosystems, Houston, TX) using bovine serum albumin (M₉ = 68,500) and cytochrome b₅ (M₉ = 54,000) as standard mass calibrants.

Production and Isolation of Polyclonal Antibody, Protein Immuno blotting—Polyclonal antibodies against human CYP2J2 were prepared by immunizing two New Zealand White rabbits with partially purified CYP2J2 protein in Freund's complete adjuvant as described (39). After the rabbits were administered with the following antigens: high molecular weight CYP2J2, CYP2J2 C170A mutants in Freund's incomplete adjuvant. Pre-immune serum, collected from the rabbits prior to immunization, did not cross-react with CYP2J2 or with microsomal fractions prepared from human liver. Immune serum from both rabbits gave identical patterns on immunoblots of human...
and/or partially purified CYP2J2 were electrophoresed in SDS-10% PAGE. Recombinant CYP2C8 baculovirus were used as a source of human CYP2J2. Masahiko Negishi (NIEHS, National Institutes of Health) determined the catalytic activity of CYP2J2 toward arachidonic acid was determined by the formaldehyde colorimetric assay. The sensitivity of the formaldehyde colorimetric assay was 10^{-3} M. The EET internal standard was synthesized from [1-14C]arachidonic acid (55–55 μCi/μmol, 100 μCi, final concentration). Reaction were initiated with NADPH (1 μM, final concentration) and continued at 30°C with constant mixing.

RESULTS AND DISCUSSION

Molecular Cloning of Human CYP2J2—Although there has been extensive studies of the rat and rabbit P450 arachidonic acid epoxygenases (4–6, 9, 11–13, 17–19), substantially less is known about the human enzyme system. In humans, EETs have been detected in the kidney (8), urine (21), reproductive tissue (51), and endothelium (52, 53). Laniado-Schwartzman and co-workers (54) have purified a P450 isoform (P450-AA) from human liver and demonstrated that it catalyzed the epoxidation of arachidonic acid. More recently, several purified and recombinant human P450s of the CYP2C subfamily have been shown to metabolize arachidonic acid with a high degree of regio- and enantiofacial selectivity (10, 55). Importantly, pregnancy-induced hypertension in humans results in significant increases in the urinary excretion of several epoxygenase metabolites, suggesting a role for this enzymatic pathway in human disease (21).

Studies on the importance and functional role of the human P450 arachidonic acid epoxygenases in human physiology and pathophysiology required a detailed knowledge of the molecular and catalytic properties of the enzymes involved and access to biospecific probes to study the regulation of the relevant enzymes at the gene and/or protein level. With these goals in mind, we screened a human liver library with a 0.9-kb human CYP2J2 IgG, goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad), and the ECL Western blotting detection system (Amersham Life Sciences, Buckinghamshire, United Kingdom).

Reconstitution of Recombinant CYP2J2 Activity, Product Characterization—Purified rat liver NADPH-P450 reductase was a gift from Dr. Masahiko Negishi (NIEHS, National Institutes of Health). The catalytic activity of CYP2J2 toward arachidonic acid was determined by mixing purified, recombinant CYP2J2 with NADPH-P450 reductase (1 μM each, final concentration) in the presence of sonicated, 1,1-di- lauroyl-1-sn-glycero-3-phosphocholine (50 μg/ml, final concentration). After 2 h min at room temperature, the enzyme mixture was diluted to the final reaction volume with 0.05 M Tris-Cl buffer (pH 7.5) containing 0.15 M NaCl, 0.01 M MgCl2, 8 mM sodium isocitrate, and 0.5 IU of isocitrate dehydrogenase/ml and equilibrated at 30°C for 2 min prior to the addition of 1,14-[1-14C]arachidonic acid (25–55 μCi/μml, 100 μCi, final concentration). Reactions were initiated with NADPH (1 μM, final concentration) and continued at 30°C with constant mixing.

At different time points, samples of the reaction mixture were extracted into ethyl ether, dried under a nitrogen stream, resolved by reversed-phase HPLC, and quantified by on-line liquid scintillation using a Radiomatic Flo-One β-detector (Radiomatic Instruments, Tampa, FL) (40). For chiral analysis, the reaction product was collected from the HPLC eluent, resolved by chiral-phase HPLC, and quantified by liquid scintillation as described previously (41, 42). The benzphetamine—PFB esters were resolved into the corresponding antipodes by chiral-phase HPLC, resolved into the corresponding antipodes by chiral-phase HPLC, and quantified by liquid scintillation as described previously (41, 42). The benzphetamine—N-demethylase activity of recombinant CYP2J2 was assessed using the same purification enzyme preparation under identical reaction conditions but employing benzphetamine (2 μM, final concentration) as the substrate. The reaction product (formaldehyde) was quantified according to the method of Nash (43). The sensitivity of the formaldehyde colorimetric assay was 10^{-8} M.

Quantitation of Endogenous EETs in Human Heart—Methods used to quantify endogenous EETs present in human heart were similar to those used to quantify EETs in rat liver (11) and rabbit lung (6). Briefly, paraffin-embedded human heart tissue was cut into 50-μm sections, and those used to quantify EETs in rat liver (11) and rabbit lung (6). Ligand extracted-twice, under acidic conditions, with two volumes of liquid nitrogen and immediately homogenized in 15 ml of phosphate-buffered saline containing triphenylphosphate (5–10 mg). The homogenate was extracted twice, under acidic conditions, with two volumes of chloroform/methanol (2:1) and once more with an equal volume of chloroform, and the combined organic phases were evaporated in tubes containing mixtures of 8:9, 11:12, and 14:15-[1-14C]EET internal standards (55–57 μCi/μmol, 30 ng each). Saponification to recover phospholipid-bound EETs was followed by silica column purification. The eluent, containing a mixture of radiolabeled internal standards and total endogenous EETs, was resolved into individual regioisomers and analyzed by gas chromatography/mass spectroscopy on a Kratos Concept ISQ mass spectrometer (Kratos Analytical, Inc. Ramsey, NJ) operating under selected ion monitoring (SIM) conditions. The EET internal standard was synthesized from [1-14C]arachidonic acid (55–57 μCi/μmol) by nonspecific oleosynthesis as described previously (44). Racemic and enantiomerically pure EETs were prepared by total chemical synthesis according to published procedures (45–48). Methylation were performed using an ethereal solution of diazomethane (49). PFB esters were formed by reaction with pentfluorobenzyl bromide as described (11). Protein determinations were performed according to the method of Bradford (50).

Other Methods—The EET internal standards were synthesized from [1-14C]arachidonic acid (55–57 μCi/μmol) by nonspecific oleosynthesis as described previously (44). Racemic and enantiomerically pure EETs were prepared by total chemical synthesis according to published procedures (45–48). Methylation were performed using an ethereal solution of diazomethane (49). PFB esters were formed by reaction with
CYP2J2 cDNA probe. As shown in Fig. 2, the CYP2J2 probe hybridized with heart RNA as a single 1.9-kb band demonstrating abundant CYP2J2 message in human heart. In contrast, CYP2J2 transcripts were present at lower levels in human liver, ileum, jejunum, and colon, at barely detectable levels in human kidney, and were not detected in human brain, lung, ovary, or testes (Fig. 2). Blot hybridization analysis of RNA prepared from several donor hearts and livers confirmed the primarily cardiac expression of human CYP2J2 and showed remarkably low interindividual variation in the expression of CYP2J2 message (data not shown). Guengerich and Mason (29) have shown that untreated rat heart microsomal fractions contain spectrally evident P450 and possess 7-ethoxycoumarin O-deethylase and benzo(a)pyrene hydroxylase activities. More recently, other investigators have provided evidence for the constitutive expression of P450 monooxygenases in scup, rabbit, and pig heart, although the identity of the P450 isoforms present in these heart tissues remains unknown (28, 30, 31). Pretreatment with \(\beta\)-naphthoflavone, 2,3,7,8-tetrachlorodibenzo-p-dioxin, and/or 3-methyl-cholanthrene has been shown to induce P450 monooxygenases in the heart and liver of these animals (29, 30, 32, 33). We are unaware of previous reports showing constitutive or inducible expression of a P450 in human heart. This is also the first report of a P450 isoform that is predominantly expressed in heart.

Kikuta and co-workers (36) reported that CYP2J1 was selectively expressed in rabbit small intestine with barely detectable levels in human kidney, and were not detected in human brain, lung, ovary, or testes (Fig. 2). Blot hybridization analysis of RNA prepared from several donor hearts and livers confirmed the primarily cardiac expression of human CYP2J2 and showed remarkably low interindividual variation in the expression of CYP2J2 message (data not shown). Guengerich and Mason (29) have shown that untreated rat heart microsomal fractions contain spectrally evident P450 and possess 7-ethoxycoumarin O-deethylase and benzo(a)pyrene hydroxylase activities. More recently, other investigators have provided evidence for the constitutive expression of P450 monooxygenases in scup, rabbit, and pig heart, although the identity of the P450 isoforms present in these heart tissues remains unknown (28, 30, 31). Pretreatment with \(\beta\)-naphthoflavone, 2,3,7,8-tetrachlorodibenzo-p-dioxin, and/or 3-methyl-cholanthrene has been shown to induce P450 monooxygenases in the heart and liver of these animals (29, 30, 32, 33). We are unaware of previous reports showing constitutive or inducible expression of a P450 in human heart. This is also the first report of a P450 isoform that is predominantly expressed in heart.

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human CYP2J2 cDNA probe, we detected mRNA transcript levels, albeit at low levels, in rabbit liver, lung, and kidney. The expression of CYP2J1 in rabbit heart was detectable but was clearly lower than in other rabbit tissues (data not shown).

Based on these data, we conclude that rabbit CYP2J1 and human CYP2J2 have different tissue-specific distributions and that only the human CYP2J2 cDNA is predominately expressed in the heart.

Heterologous Expression and Purification of Recombinant Human CYP2J2—Several systems have been used to express mammalian P450s including yeast, bacteria, COS cells, vacinia virus, and baculovirus (57–61). We chose the baculovirus system because it consistently gives high expression levels of mammalian P450s including yeast, bacteria, COS cells, vacinia virus and baculovirus. The baculovirus system because it consistently gives high expression levels of recombinant protein and it requires minimal manipulation of the cloned cDNA (57). To express the protein encoded by the CYP2J2 cDNA, we cotransfected SF9 insect cells with the pBBlueBacII transfer vector containing the cloned CYP2J2 insert and with wild-type baculovirus DNA. The resulting recombinant virus was plaque purified and utilized for the high titre infection of SF9 cells grown in the presence of 5 μM hemin as described previously (10, 57). Using these conditions, the level of expression of recombinant CYP2J2 was 100–150 nmol of P450/mg of infected SF9 cells

For purification of recombinant CYP2J2, infected SF9 cells were lysed in the presence of 1% sodium cholate, and the crude protein lysate (specific content, 0.33 nmol of P450/mg of protein) was loaded onto an ω-aminooctyl-agarose column as described previously (10, 57). The major component of the recombinant protein remained bound to the ω-aminooctyl-agarose, while most insect proteins eluted during sample loading and washing with the column equilibration buffer. The CYP2J2 was eluted as a single broad band after addition of 0.4% Emulgen 911 to the washing buffer. As shown in Fig. 3, this simple chromatographic step produced a substantially purified protein in 85% yield. Following dialysis, the ω-aminooctyl-agarose purified protein was loaded onto a hydroxylapatite column, washed with a buffer containing 0.04 M sodium phosphate, and the recombinant P450 eluted as a narrow band with a 0.1 M sodium phosphate-containing buffer affording a slightly more purified protein in 35–40% overall yield (Fig. 3).

Alternatively, the recombinant P450 was eluted using a stepwise gradient from 0.02 to 0.1 M sodium phosphate-containing buffer affording a protein that was nearly electrophoretically pure in 30% overall yield (Fig. 3). The purified protein was dialyzed, passed over a second hydroxylapatite column to remove free Emulgen 911, dialyzed against detergent-free buffer, and concentrated. The resulting protein had a specific content of 7.14 nmol of P450/mg of protein and was obtained in 10% overall yield. Purified CYP2J2 migrated as a discrete band on SDS-polyacrylamide gels with an estimated molecular mass of 57,000 Da (Fig. 3). Based on the P450 specific content, recombinant CYP2J2 was estimated to be approximately 40% pure. The molecular mass of the purified, recombinant protein was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to confirm that the protein expressed was, indeed, recombinant CYP2J2. The experimentally obtained molecular mass (57,666 Da) was within 0.02% of the derived molecular mass (57,653 Da). We further confirmed that the recombinant protein was identical with CYP2J2 by performing N-terminal amino acid sequence analysis on the purified protein. The experimentally obtained amino acid sequence (MLAAMGSLAALWAVVHPRT) was identical to the amino acid sequence predicted from the nucleic acid sequence of clone SW2–14 (Fig. 1).

Metabolism of Arachidonic Acid by Recombinant CYP2J2—Previous studies utilizing purified and/or recombinant hemoproteins have demonstrated that several members of the CYP2 family are capable of epoxidizing arachidonic acid (4, 6, 9, 10, 12, 13, 55). To reconstitute CYP2J2 activity and to ascertain the catalytic properties of the recombinant hemoprotein, we incubated purified CYP2J2 with NADPH-P450 reductase and arachidonic acid. As shown in Fig. 4, CYP2J2 metabolized arachidonic acid (catalytic turnover: 65 pmol of product formed/nmol of P450/min at 30 °C) generating all four regioisomeric EETs and 5,6-DHET as the main products (76% of the total reaction products). Insofar as, 5,6-DHET formation must be preceded by 5,6-EET formation (62, 63), the chromatograms in Fig. 4 demonstrate that recombinant CYP2J2 is an arachidonic acid epoxidase. We identified each of these metabolites by comparing their HPLC properties with those of authentic standards and by gas chromatography/mass spectroscopy (40). None of these metabolites were formed in the absence of NADPH or in the absence of NADPH-P450 reductase showing that the reaction was P450 mediated (Fig. 4). The catalytic turnover of recombinant CYP2J2 is similar to that of CYP2B4 but lower than that of other CYP2 family arachidonic acid epoxigenases (6, 10, 12, 13, 55).

The regio- and stereochiral composition of EETs produced by recombinant CYP2J2 is shown in Table I. Metabolism of arachidonic acid by the hemoprotein was only moderately regioselective with epoxidation occurring preferentially at the 14,15-olefin (37% of total EET products) and less often at the 11,12-, 8,9-, and 5,6-olefins (18, 24, and 21% of total EET products, respectively). Thus, CYP2J2 is less regioselective than rabbit CYP2C2 and human CYP2C8, which generate only 14,15- and 11,12-EETs (10, 13, 55). The regioselectivity of CYP2J2 also is different from (a) rat CYP2C23, which generates primarily 11,12-EET (9, 12); (b) rabbit CYP2B4, which has a unique preference for the 5,6-olefin (6); and (c) human CYP2C9 and CYP2C10, which do not generate significant quantities of 5,6-EET (10, 55). Epoxidation by CYP2J2 at the 14,15-olefin was highly enantioselective for (14R,15S)-EET (ratio of antipodes 3:1) (Table I). In contrast, epoxidation at the 11,12- and 8,9-olefins was non-enantioselective and thus generated racemic EETs (Table I). The stereoselectivity of epoxidation at the 5,6-olefin could not be evaluated because 5,6-EET underwent rapid, chemical hydration to 5,6-DHET and the δ-lactone of 5,6-DHET. The stereoselectivity of CYP2J2 is different than that previously reported for several purified and recombinant rodent P450 epoxigenases including CYP1A1, CYP2B1, CYP2C11, and CYP2C23 (4, 9). The stereoselectivity

Fig. 3. SDS-polyacrylamide gel electrophoresis of fractions obtained during the purification of recombinant CYP2J2 from insect cell lysates. Fractions obtained during the purification of recombinant CYP2J2 from insect cell lysates (6–8 pmol of P450/lane) were electrophoresed on SDS, 10% polyacrylamide gels as described under “Experimental Procedures.” Gels were stained for 2 h in a 10% solution of Coomassie Brilliant Blue R250 dye and destained in 0.7% acetic acid containing 10% methanol. STD, molecular weight standards in Da; lane 1, CYP2J2-infected SF9 cell lysate; lane 2, aminooctyl-agarose purified CYP2J2; lane 3, hydroxylapatite-purified CYP2J2; lane 4, sodium phosphate stepwise gradient (0.02–0.1 M) hydroxylapatite-purified CYP2J2.
of CYP2J2 is also different from that reported for the human epoxygenases CYP2CB8, CYP2C9, and CYP2C10 (10, 55).

Kikuta and co-workers (36) have previously reported that rabbit CYP2J1 rapidly catalyzed the N-demethylation of benzphetamine to formaldehyde. The same group also found that CYP2J2 did not catalyze lauric acid or arachidonic acid to formaldehyde. The same group also found that CYP2J2 IgG also produced a discrete band, albeit much less intense, with microsomal fractions prepared from human lung, heart, jejunum, liver, and skeletal muscle (50 μg of microsomal protein/lane) were electrophoresed on SDS, 10% polyacrylamide gels, and the resolved proteins were transferred to nitrocellulose membranes as described under “Experimental Procedures.” Values shown are averages of at least four different experiments with S.E. <5% of the mean. ND, not determined.

| Regioisomer | Distribution | Enantioselectivity |
|-------------|--------------|--------------------|
|             | % total      | R,S                |
| 14,15-EET   | 37           | 76                 |
| 11,12-EET   | 18           | 49                 |
| 8,9-EET     | 24           | 47                 |
| 5,6-EET     | 21           | ND                 |

In contrast, anti-CYP2J2 IgG immunoreacted with three electrophoretically distinct bands in microsomal fractions prepared from human liver: (a) a predominant band electrophoretically similar to the CYP2J2 immunoreactive band observed in extrahepatic human tissues, (b) a lower mobility band of slightly lower intensity, and (c) a higher mobility band of least intensity. Control studies using microsomal fractions prepared from cells expressing recombinant human CYP1A1, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP2E1 demonstrated that anti-CYP2J2 IgG did not cross-react with these human CYP1 and CYP2 family P450s (data not shown). Based on these data, we concluded that (a) CYP2J2 protein is highly expressed in human heart and at lower levels in extracardiac tissues, (b) in addition to CYP2J2 protein, human liver microsomal fractions contain two additional proteins that share immunochemical determinants with CYP2J2, and (c) the cross-reactive liver proteins appear to be different from previously characterized P450s of the CYP1A, CYP2A, CYP2B, CYP2C, CYP2D, and CYP2E subfamilies. Further work will be necessary to determine if human liver contains multiple CYP2J2 isoforms or if anti-CYP2J2 IgG detects other, previously uncharacterized, human liver P450s. All five duplicate positive clones isolated from the human liver cDNA library and characterized by partial nucleic acid sequence analysis and restriction enzyme digestion were identical, suggesting that if human liver contains multiple CYP2J2 isoforms, then done SW2–14 encodes the most abundant one. To our knowledge, the expression of a P450 in skeletal muscle has not been reported.

With the exception of human kidney, CYP2J2 protein levels correlated well with CYP2J2 mRNA levels (Figs. 2 and 5).
human kidney, CYP2J2 protein was expressed at moderate levels despite low mRNA expression. These results are particularly interesting given the potential relevance of the epoxygenase enzyme system to kidney salt and/or water metabolism and to the pathophysiology of human hypertension (5, 8–10, 14, 19–21). Other investigators have noted the lack of correlation between protein and mRNA levels for some human P450s and have proposed that translation rate and/or protein turnover may be important in determining human P450 hemoprotein levels (64). Fig. 5 also demonstrates that the purified, recombinant CYP2J2 protein produces an immunoreactive band that migrates with a slightly lower mobility (higher molecular mass) than the bands produced by endogenous CYP2J2 present in human tissues. We have determined that the molecular mass of the purified, recombinant CYP2J2 protein is nearly identical to that calculated from the amino acid sequence derived from the CYP2J2 cDNA. The differences in electrophoretic mobility between recombinant CYP2J2 and endogenous CYP2J2, although minor, suggest that (a) the endogenous hemoprotein is produced in a truncated form, (b) the endogenous protein is post-translationally modified, or (c) the clone that we isolated (clone SW2–14) encodes a P450 that shares antigenic determinants with a related, slightly lower molecular weight protein that is more abundant in human tissues and is predominately expressed in heart. Further investigation will be necessary to determine if these explanations, or others, can account for the minor differences in electrophoretic mobility between the endogenous and the recombinant proteins.

To evaluate interindividual differences in expression of CYP2J2 protein, we performed immunoblotting on microsomal fractions prepared from an additional seven human liver and three human heart specimens. Protein immunoblotting of the human liver microsomal fractions revealed remarkably low interindividual variation in the expression of CYP2J2 protein (middle band) in human liver tissue (Fig. 6A). In contrast, the expression of the cross-reactive liver proteins (upper and lower bands) were more variable (Fig. 6A). Thus, while some livers contained roughly equal amounts of CYP2J2 and the two cross-reactive proteins (e.g. L3-L7), others primarily expressed CYP2J2 and the lower mobility protein (upper band) (L1-L2).

Protein immunoblotting of the human heart microsomal fractions revealed that, while CYP2J2 was expressed at high levels in each of the heart tissues, there was greater interindividual variation in CYP2J2 expression in human heart than in human liver (Fig. 6B). In a given individual, however, CYP2J2 expression was always significantly higher in the heart than in extracardiac tissues. Many factors are known to alter the levels of expression of human P450 genes including genetic polymorphism, enzyme induction, and/or inhibition and developmental factors (65–67). A number of investigators have reported large (3–115-fold) interindividual variation in expression of human P450s of the CYP1, CYP2, CYP3, and CYP4 gene families (64, 68–70).

### Table II

Regio- and stereochemochemical composition of human heart EETs

| Regioisomer | Concentration (ng/g heart) | Distribution | Enantioselectivity |
|-------------|---------------------------|--------------|-------------------|
| 14,15-EET   | 23                        | 39           | 63                |
| 11,12-EET   | 14                        | 24           | 44                |
| 8,9-EET     | 22                        | 37           | 43                |
| 5,6-EET     | ND                        | ND           | ND                |

The enantiomers of human heart 14,15-, 11,12-, and 8,9-EET were extracted, purified, and quantified as described under “Experimental Procedures.” Concentration values shown are averages of three determinations on different heart tissues with S.E. <15% of the mean. For enantioselectivity, S.E. <5% of the mean, ND, not determined.
central role in the biosynthesis of biologically active EETs and, consequently, may have important roles in cardiac function.

Compared with human kidney cortex, human heart contains approximately 5-fold less total EETs and has a distinctly different regio- and stereochromatic profile (76). Thus, whereas both tissues favor epoxidation at the re, si face of the 14,15-diene and produce racemic 8,9-EET, only human heart produces racemic 11,12-EET. The chirality of endogenous EETs recovered from human heart also differs from those isolated from rat liver and rabbit lung in which (14R,15S), (11S,12R), and (8S,9R)-EET were the predominant antipodes (6, 11). In rat liver and rabbit lung in which (14R,15S), (11S,12R), and (8S,9R)-EET were the predominant antipodes (6, 11).

In our study, the fact that CYP2J2, an epoxygenase highly expressed in heart, also produces racemic 11,12- and 8,9-EETs supports the contention that these eicosanoids are formed enzymatically.

The P450 monoxygenases have long been thought to function primarily in the metabolism of exogenous compounds including drugs and carcinogens (66, 67). Over the past 10–15 years, there has been an increased awareness that this ubiquitous enzyme system may also be involved in the bioactivation of endogenous substrates such as steroids and fatty acids (16–18). The documentation of P450 monoxygenases in vertebrate heart tissue (28–33), together with the known cardiovascular effects of P450 arachidonic acid epoxygenase metabolites (14, 18). The documentation of P450 monoxygenases in vertebrate heart tissue (28–33), together with the known cardiovascular effects of P450 arachidonic acid epoxygenase metabolites (14, 18).

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Shu Wu, Cindy R. Moomaw, Kenneth B. Tomer, John R. Falck and Darryl C. Zeldin

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