Adaptations for the Oxidation of Polycyclic Aromatic Hydrocarbons Exhibited by the Structure of Human P450 1A2

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Microsomal cytochrome P450 family 1 enzymes play prominent roles in xenobiotic detoxication and procarcinogen activation. P450 1A2 is the principal cytochrome P450 family 1 enzyme expressed in human liver and participates extensively in drug oxidations. This enzyme is also of great importance in the bioactivation of mutagens, including the N-hydroxylation of arylamines. P450-catalyzed reactions involve a wide range of substrates, and this versatility is reflected in a structural diversity evident in the active sites of available P450 structures. Here, we present the structure of human P450 1A2 in complex with the inhibitor α-naphthoflavone, determined to a resolution of 1.95 Å. α-Naphthoflavone is bound in the active site above the distal surface of the heme prosthetic group. The structure reveals a compact, closed active site cavity that is highly adapted for the positioning and oxidation of relatively large, planar substrates. This unique topology is clearly distinct from known active site architectures of P450 family 2 and 3 enzymes and demonstrates how P450 family 1 enzymes have evolved to catalyze efficiently polycyclic aromatic hydrocarbon oxidation. This report provides the first structure of a microsomal P450 from family 1 and offers a template to study further structure-function relationships of alternative substrates and other cytochrome P450 family 1 members.

Enzymes of the cytochrome P450 (CYP) family play a significant physiologic role in the detoxication of foreign compounds and the biosynthesis of endogenous compounds, including steroid hormones, bile acids, and cholesterol. The enzymes comprising P450 families 1, 2, and 3 contribute most extensively to the biotransformation of xenobiotics to more polar metabolites that are more readily excreted. In humans and most mammals, family 1 contains three well characterized P450 monooxygenases: 1A1, 1A2, and 1B1. These enzymes are generally distinguished from P450s in other families by their capacity to oxidize a variety of polynuclear aromatic hydrocarbons (PAHs). Moreover, the expression levels of the three enzymes are induced by exposure to PAHs (1). The induction is mediated by a ligand-activated transcription factor, the aryl hydrocarbon receptor, which is a basic-loop-helix PAS domain protein that binds to enhancer elements flanking the CYP1A1, CYP1A2, and CYP1B1 genes and stimulates transcription.

The oxidation of PAHs is generally protective. However, some P450-catalyzed reactions can transform these relatively inert compounds into genotoxic metabolites that can initiate mutagenesis and cancer. Human P450 1A2 is notable among family 1 enzymes for the capacity to N-oxidize arylamines, the major metabolic process in the bioactivation of arylamines to potent mutagenic or carcinogenic compounds (2). α-Naphthoflavone (ANF), a prototype flavonoid, is known to competitively inhibit P450s of family 1, albeit at different concentrations, and has been used to discriminate between P450 family 1 enzymes (3). Flavonoids have gained recent interest in view of their potential therapeutic and prophylactic effects on P450-mediated chemical carcinogenesis (4). CYP1A2 is the principal family 1 enzyme expressed in human liver, and CYP1A2 contributes significantly to the hepatic metabolism of drugs, as recently reviewed (5). Among liver P450 drug-metabolizing enzymes, P450 1A2 plays a predominant role in the metabolic clearance of caffeine and melatonin as well as of marketed drugs such as flutamide, lidocaine, olanzapine, tacrine, theophylline, triamterene, and zolmitriptan. Large inter-individual differences in CYP1A2 expression levels and catalytic activity contribute to significant differ-
ences between individuals in drug clearance. The basis for this variation is only partially understood.

This report provides the first structural characterization of human CYP1A2 and is the first structure of a microsomal P450 from family 1. The structure reveals an enzyme that is highly adapted for the oxidation of relatively large, planar molecules such as (heterocyclic) arylamines and PAHs and provides a basis for understanding the unique roles this enzyme plays in the biotransformation of xenobiotics. CYP1A2 exhibits less than 40% amino acid sequence identity when compared with other structurally characterized mammalian microsomal P450s. The reverse primer encoded a HindIII restriction site, a stop codon, four histidine codons, and seven amino acids immediately upstream of the polyproline motif at residue 42. The multiple cloning sites inserted at this position contribute to the overall capacity of P450s to oxidize a vast array of structurally dissimilar substrates.

**EXPERIMENTAL PROCEDURES**

**Expression and Purification of hP450 1A2**—Plasmid vectors were constructed to express modified forms of human P450 1A2 in *Escherichia coli*. The modifications were designed to facilitate crystallization by removing the N-terminal transmembrane helical domain to reduce aggregation, increase solubility, and eliminate a flexible appendage that is not part of the catalytic domain. In the expression construct, the native sequence upstream of the polyproline motif at residue 42 was modified to correspond to a modified, truncated N-terminal sequence successfully employed for crystallization of P450s 2C5/3LVdH (8, 15), 2C8dH (9), 2B4dH (16), and 2A6dH (6). This was achieved using a forward PCR primer that encoded a 5′-NdeI restriction site, the modified N-terminal sequence MAKKTSSKGKL, and sequence encoding the native protein beginning at codon 42. The reverse primer encoded a HindIII restriction site, a stop codon, four histidine codons, and seven C-terminal codons of the protein. Following PCR amplification from a plasmid template containing the human CYP1A2 cDNA (17) kindly provided by Robert Tukey, University of California San Diego (UCSD), using high fidelity Platinum Taq polymerase (Invitrogen), the PCR fragment was isolated and digested with the appropriate endonucleases for ligation into the pCWori vector. A series of alternative constructs was also generated that deleted the transmembrane helical domain but retained different portions of the native linker region preceding the proline-rich motif at the beginning of the catalytic domain. For the D24 construct used in this study, double-stranded oligonucleotides with appropriate overhangs were ligated into the initial expression vector designated G1, which had been digested with the endonucleases NdeI and NcoI. The D24 construct encodes residues 26–515 of the protein, preceded by the proline-rich motif at the beginning of the catalytic domain. The D24 construct was crystallized by hanging drop vapor diffusion. The vapor diffusion experiments were conducted at 298 K over a standard well solution (0.5 ml) composed of 10% PEG 3350, 20% glycerol, 100 mM NaCl in a Hepes buffer, pH 7.4. Protein drops were set up in a ratio of 1:1 protein detergent solution to precipitant solution. The setup allowed us to fine-tune the equilibration rate by only changing the precipitant solution in the experiment while holding the well solution constant. The protein detergent solution contained 1.25 μl of a concentrated protein solution mixed with detergent solution to contain 7.5 mM CYMAL-6 and 0.01 mm C12E8 (Anatrace). A precipitant solution containing 100 mM Tris-HCl buffer (pH 8.5), 10% PEG 3350, and 50 mM ammonium iodide yielded crystals for the G1 construct from a protein solution at a concentration of 390 μM ANF, 500 mM NaCl, and 20% glycerol. Crystals of the D24 construct were obtained by mixing a protein solution (400 μM) in N-(2-acetamido)-iminodiacetic acid buffer (pH 6.5) containing 10 mM ANF, 600 mM NaCl, and 20% glycerol with precipitant solution containing 11% PEG 3350 and 75 mM ammonium nitrate in 100 mM Tris-HCl buffer (pH 8.5).

**Structure Determination**—The G1 construct was crystallized initially. A complete data set to 2.9 Å resolution was collected on a single crystal, using synchrotron radiation at the Stanford Synchrotron Radiation Laboratory beamline 9-1. Prior to x-ray exposure, the crystal was short-soaked in a cryoprotectant solution containing 0.05 m NaCl, 0.08 m NH₄Cl, 0.1 m potassium phosphate buffer (pH 7.4), 8% PEG 3350, 5% glycerol, and 25% ethylene glycol before being flash-frozen in liquid nitrogen. Data integration and scaling employed HKL2000 (20), MOSFLM, and SCALA (21). A molecular replacement solution in space group P1 could be obtained with Phaser (22) using the structure of CYP2A6 (PDB accession number 1Z10) as a probe. Four CYP1A2 molecules were independently positioned in the unit cell. Model building and comparison with electron density maps was done with the computer program O (23) and iterated with refinement of the atomic coordinates and grouped B-factors by simulated annealing and/or minimization using CNS (24) with the application of non-crystallographic symmetry restraints. This allowed construction of an initial CYP1A2 model for residues 42–513 of the native protein with the exception of an exterior loop encompassing residues 294–305. Statistics on data collection, processing, and refinement are provided in Table 1. Although the G1 construct produced crystals
Human CYP1A2 Structure

| TABLE 1 |
| --- |
| Data collection and refinement statistics |
| Human P450 1A2 construct | G1 | D24 | D24 |
| --- | --- | --- | --- |
| Data collection |
| Space group | P1 | P2,2,2 | I222 |
| Used wavelength (Å) | 0.98 | 0.98 | 0.98 |
| Resolution limit (Å) | 2.90 (2.98–2.90) | 2.45 (2.51–2.45) | 1.90 (1.95–1.90) |
| Cell parameters |
| a, b, c (Å) | 63.45, 101.22, 103.35 | 63.48, 181.78, 220.08 | 79.63, 80.82, 175.82 |
| α, β, γ (°) | 81.97, 90.48, 88.12 | 81.97, 90.48, 88.12 | 81.97, 90.48, 88.12 |
| X-ray source | BL9–1 SSRL | BL11–1 SSRL | BL11–1 SSRL |
| Total observations | 207212 | 391400 | 192055 |
| Unique reflections | 53266 | 89465 | 41139 |
| Completeness of all data (%) | 94.4 (74.0) | 99.2 (99.6) | 92.2 (95.2) |
| Mean I/σ | 15.6 (1.9) | 7.9 (1.3) | 4.0 (1.5) |
| Rsym value (%) | 9.6 (60.6) | 7.8 (53.0) | 9.2 (39.4) |
| Refinement |
| Resolution range (Å) | 25.0–2.90 | 40.0–2.45 | 29.5–1.95 |
| Reflections used | 50512 | 88876 | 38022 |
| Reflections used in Rfree set | 2684 | 4702 | 1871 |
| Rcryst/Rfree | 0.250/0.282 | 0.236/0.281 | 0.226/0.267 |
| Protein atoms | 14840 | 15090 | 41139 |
| Solvent atoms | 0 | 129 | 160 |
| Heme atoms | 172 | 172 | 43 |
| ANF atoms | 84 | 84 | 21 |
| r.m.s. deviation | 1.34 | 1.30 | 2.00 |
| Bond angles (°) | 0.007 | 0.007 | 0.019 |
| Bond lengths (Å) | 0.007 | 0.007 | 0.019 |

Values between parentheses indicate data in the highest resolution shell.

### RESULTS AND DISCUSSION

Protein Design and Structure Determination—CYP1A2 was modified to remove the N-terminal transmembrane domain to improve solubility and to reduce aggregation as described previously for the crystallization of other microsomal P450s (6, 9, 15, 16). Expression of CYP1A2 as a conditional membrane protein suitable for crystallization was achieved in two ways. In the G1 construct, a short hydrophilic sequence that has been successfully employed previously for the crystallization of other human P450 enzymes was substituted for the native N-terminal sequence upstream of the proline-rich motif at the beginning of the catalytic domain (18). The alternative D24 construct employed a truncation of the wild-type CYP1A2 sequence (GenBank™ P05177) for which residues 3–26, including the transmembrane signal anchor domain, were deleted. Both proteins crystallized under similar conditions but produced crystals that belonged to three different space groups. This appears to reflect shifts in the crystal packing to accommodate the longer N-terminal sequence of the D24 construct, resulting in denser intermolecular packing interactions, a lower crystal solvent content, and a better diffraction quality. Nevertheless, conformational differences in the catalytic domain were not evident for structures obtained from crystals belonging to space groups I222 or P2,2,2, for the D24 construct and space group P1 for the G1 construct. Root mean square deviation values for the pairwise superimposition of Co traces of the three respective models do not exceed 0.55 Å. The final model was refined against data to a resolution of 1.95 Å obtained for a single crystal in space group I222 and encompasses residues 34–513 of the native protein (PDB: 2HI4). Data collection and refinement statistics are shown in Table 1.

that exhibited higher symmetry, the diffraction quality could not be extended to higher resolution.

The D24 construct yielded crystals both in the orthorhombic space group P2,2,2, and in the orthorhombic space group I222. All diffraction data were collected at 100 K on crystals that were flash-frozen in liquid nitrogen upon harvesting. The CCP4 programs MOSFLM and SCALA (21) were used to process the data. Structures for both space groups could be solved with the CYP1A2 model initially obtained for the G1 construct as a probe in molecular replacement searches using Phaser (22). Crystals in space group P2,2,2, generally diffracted to 2.5 Å resolution and have four copies of P450 1A2 in complex with ANF in the asymmetric unit. Statistical analysis on a data set of one of these crystals collected to a limiting resolution of 2.45 Å is presented in Table 1. A high resolution data set to 1.95 Å resolution, collected at the Stanford Synchrotron Radiation Laboratory beamline 11–1 on a single crystal belonging to space group I222, was used to build and refine the final model (PDB: 2HI4). The CYP1A2 model encompasses residues 34–513 of the native protein and includes the missing loop that could not be built in the initial model determined for the G1 construct. Nine residues at the N terminus and 6 residues at the C terminus of the truncated and His-tagged construct could not be traced in the electron density maps. During later stages of refinement, 160 water molecules were added, and the inhibitor ANF was modeled in the distal active site pocket above the heme prosthetic group, as evidenced by 2|Fobs| – |Fc| σA-weighted electron density maps contoured at 1σ. Data collection and refinement statistics are provided in Table 1 together with statistics describing the stereochemical features of the protein model.
Human P450 1A2 Structure—The structure of P450 1A2 exhibits the 12 α-helices and 4 β-sheets designated A–L and 1–4, respectively, which are generally seen for the canonical P450 fold (Fig. 1). Several additional helices, some of which are also seen in other P450 structures, are present and indicated by prime or double prime designations. When compared with mammalian P450s of known structure, the most conserved regions are the core of the protein forming the heme binding site and the proximal surface that is thought to provide binding sites for the redox partners, NADPH cytochrome P450 oxidoreductase and cytochrome b5 (25). The most divergent regions between known P450 structures are the portions that form the distal surfaces of the substrate binding cavity, the helix B-C and F-G regions and the C-terminal loop following helix L. Mammalian microsomal P450 enzymes generally have a large insertion between helices F and G, which exhibits a reverse amphipathicity that constitutes part of a hydrophobic surface on the tip of the protein close to the transmembrane domain. This hydrophobic surface forms part of the juxtamembrane surface of the catalytic domain of eukaryotic P450s (8, 26). Two short α-helices, F’ and G’, are generally observed in this region for other mammalian P450 structures. In the P450 1A2 structure, both helix F’ and G’ are 310 helical fragments rather than α-helices. Nevertheless, a reverse amphipathicity is maintained. This insertion and hydrophobic surface are not generally seen in soluble prokaryotic P450 structures.

P450 1A2 shares less than 40% amino acid sequence identity with either P450 3A4 or structurally characterized family 2 P450s. Therefore, as would be expected based on the low sequence identities, the P450 1A2 structure differs from those of families 2 and 3 in the lengths and local structures of loop regions connecting conserved secondary structure elements. A structure-based sequence alignment of P450s 1A2, 2A6, and 3A4 is provided in supplemental Fig. S1. An additional β-sheet (β3’) is present between helices H and I, as well as an additional small α-helix (K’) residing at the proximal surface. Furthermore, the region connecting helices C and D shows a serine-rich insertion, which forms a loop that extends into the solvent.

Another remarkable feature of the P450 1A2 structure is the disruption of helix F as it crosses the distal surface of the active site cavity (Fig. 2). P450 family 2 structures (6–12) display an intact helix F for this region (Fig. 2). Also, in the structure of P450 3A4 (13, 14), a coiled structure connecting helices F and F’ crosses above the active site cavity (Fig. 2). In the P450 1A2 structure, the α-helical hydrogen-bonding pattern is lost at Val-220 and Lys-221, causing one helical turn in the middle of helix F to...
unwind. Two water molecules fill the space thus created, giving rise to water-bridged contacts between Val-220 carbonyl oxygen and Thr-223 O\(^-\), and Lys-221 carbonyl oxygen and His-224 amide nitrogen, respectively. The bending of helix F brings the C-terminal portion of the helix in toward the core of the protein, closing down the active site cavity. Substrates bind in the cavity located above the distal surface of the heme prosthetic group. In the P450 1A2 structure of the ANF complex, the active site is closed without evident solvent or substrate access channels. The volume of the cavity was estimated to be 375 Å\(^3\), which is larger than that of P450 2A6 (260 Å\(^3\)) (Fig. 2). The rather compact, closed active site cavity topologies of P450 1A2 and P450 2A6 contrast with the much more open active site architectures displayed by P450 3A4 (Fig. 2) and P450 2C8, with volumes of 1385 Å\(^3\) and 1438 Å\(^3\), respectively.

**Human P450 1A2 in Complex with α-Naphthoflavone**—The protein was purified and crystallized in the presence of ANF. The structure and numbering of ANF are shown in Fig. 3. The presence of ANF in the active site cavity was well defined by \(2|F_o| - |F_c|\) \(\sigma\)A-weighted electron density maps calculated for a model that did not include ANF (Fig. 4). The orientation of the ANF molecule is clearly indicated by the curvature of the electron density map and further supported by electron density for the carbonyl group and the phenyl group, which were defined by the narrowing of the map at the bond connecting the phenyl ring to the benzo(h)chromen-4-one moiety. This indicates that ANF binds in a single preferred orientation, which places the phenyl ring close to the heme iron.

ANF is reported to be a potent, competitive inhibitor of human P450 1A2-catalyzed reactions with an estimated \(K_i\) of 1–50 nM (3, 27). Several structural features are likely to contribute to the high apparent binding affinity of P450 1A2 for ANF. First, ANF closely fits the size and shape of the substrate binding cavity (Fig. 4), leading to dense and extensive Van der Waals interactions arising mainly from non-polar side chains. Also, the hydrophobic effect is likely to contribute to a favorable free energy difference for ANF binding. In addition, both orthogonal and parallel aromatic interactions between ANF and phenylalanine side chains 125 and 226 contribute to a tight binding affinity. Finally, the presence of an ordered water molecule near the carbonyl group of ANF provides an extra binding interaction. The water molecule appears to be hydrogen-bonded to the carbonyl of ANF as well as to the carbonyl of Gly-316 on helix I (Fig. 5). This water molecule is the only one present in the active site, and no apparent solvent channels that connect the active site cavity with the protein surface are evident.

The distance from the heme iron to C\(^4\) of ANF is 4.7Å. This distance falls at the long end of the range of distances observed for productive substrate binding in other P450 structures and is rather large for the energetically more favorable direct \(\pi\)-attack of the compound I intermediate on the C\(^4\) aromatic carbon (28). The capacity of human CYP1A enzymes to oxidize ANF has been reported (29). Although ANF was readily metabolized by CYP1A1 to form ANF-5,6-diol and ANF-5,6-oxide, CYP1A2 oxidation of ANF was too limited for products to be analyzed. The single preferred orientation for ANF observed in the P450 1A2 structure suggests that the site of limited CYP1A2 oxida-

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**FIGURE 3.** Structure and numbering of α-naphthoflavone.

**FIGURE 4.** Detailed view of the binding of α-naphthoflavone in the human P450 1A2 active site. A shows the position of ANF and the heme group defined by \(\sigma\)A-weighted \(2|F_o| - |F_c|\) electron density maps (blue mesh). B and C show two views of the amino acid residues constituting the active site cavity depicted in sticks, with portions of the protein backbone represented as a ribbon schematic. The inhibitor ANF and the heme prosthetic group are shown in sticks, with carbons colored yellow and pink, respectively. Oxygen, nitrogen, and iron atoms are colored red, blue, and orange, respectively. A water molecule is represented as a red sphere.
tion would be at the other end of the molecule relative to the site of CYP1A1 oxidation. The low rate of oxidation could reflect the high affinity binding of ANF in a suboptimal position for oxidation and a concomitant slow off-rate of the products. These factors are likely to contribute to the tight binding competitive inhibition of P450 1A2 by ANF.

**Active Site Architecture**—The substrate binding cavity is uniformly narrow throughout its extent and is lined by residues on helix F and helix I that define a relatively planar substrate binding platform on either side (Fig. 5). Helix I bends as it crosses the heme prosthetic group. As a consequence, the helix I residues constituting one side of the substrate binding cavity adopt a relatively flat conformation of the peptide backbone, resulting in a near perfect coplanarity for the Ala-317 side chain, the Gly-316—Ala-317 peptide bond, and the Asp-320—Thr-321 peptide bond (Fig. 5). The occurrence of a water molecule within hydrogen-bonding distance from the backbone carbonyl group of Gly-318 on the backside of helix I helps to stabilize this deformation. On the other side of the cavity, the side chain of Phe-226 on helix F produces a parallel substrate binding surface. The distortion of helix F as it passes over the substrate binding cavity contributes to the narrow, extended cavity architecture. Not only is the C-terminal end of the helix positioned to produce a compact active site cavity, the observed bend in helix F also positions Phe-226 for the observed \( \pi-\pi \) stacking with ANF. The shape of the active site cavity is further stabilized by a strong hydrogen-bonding interaction between the side chain of Thr-223 on helix F with the side chain of Asp-320 on helix I, connecting both helices at the roof of the cavity. Both Thr-223 and Asp-320 participate in an extensive network of hydrogen-bonded water molecules and side chains, including Tyr-189, Val-220, Thr-498, and Lys-500. Taken together, these interactions produce a binding cavity that fits closely with planar compounds such as ANF and typical CYP1A2 substrates such as caffeine, melatonin, tacrine, olanzapine, arylamines, and alkoxyresorufins. In this regard, the structure of the P450 2A6 active site is most similar with that of P450 1A2, which is roughly 50% larger in volume. As CYP2A6 preferentially oxidizes small alkylamines, and alkoxyresorufins. In this regard, the structure of the P450 2A6 active site is most similar with that of P450 1A2, which is roughly 50% larger in volume. As CYP2A6 preferentially oxidizes small alkylamines, and alkoxyresorufins. In this regard, the structure of the P450 2A6 active site is most similar with that of P450 1A2, which is roughly 50% larger in volume.

The planar active site topology leads to CYP1A2 mutants at this position that are relatively inefficient for most substrates.

Human CYP450 family 1 enzymes show overlapping specificities for which the molecular planarity of substrates and inhibitors is of great importance. Thus, the planar active site topology observed in the P450 1A2 structure, which is well adapted for the oxidation of relatively large aromatic compounds, is likely to be conserved among the family 1 enzymes. Of the 22 residues lining the active site cavity in the P450 1A2 structure described here, 13 are identical, and an additional 7 are (semi)-conserved among human family 1 enzymes (supplemental Fig. S2). Relatively small changes in the enzyme active site residues indeed

![Image](https://example.com/image.png)

**FIGURE 5.** The active site topology of human P450 1A2 is well adapted for the binding of relatively large planar aromatic compounds. ANF is sandwiched between the flat surfaces formed by Gly-316 and Ala-317 on helix I and Phe-226 on helix F. The atom color code is the same as described in the legend for Fig. 4.
Human CYP1A2 Structure

assist in the rationalization of CYP1A specificities for the O-dealkylation of alkoxyresorufins. Although wild-type CYP1A1 versus CYP1A2 shows a clear preference for 7-ethoxyresorufin versus 7-methoxyresorufin O-dealkylation, the reciprocal CYP1A1 V382L and CYP1A2 L382V mutants display interchanged specificities (34). In our structure, the distance between Leu-382 C6 and C‘3 and C‘4 of ANF is only 3.9 and 4.1 Å, respectively, which demonstrates the restricted architecture at the base of the CYP1A2 active site cavity and explains the preference of CYP1A2 for shorter alkoxyresorufins. Differences in residues that are expected to have more profound changes on P450 family 1 active site architecture are clustered around the central portion of helix F and in the B’-C loop region. It is possible that the disruption of helix F observed in the P450 1A2 structure is not a conserved feature of P450 family 1 enzymes. The Thr-223 side chain plays a crucial role in the unusual disruption of helix F by its involvement in water-bridged contacts with solvent molecules that fill the void created by the unwinding of one helical turn and by its contribution in the firm connection between helices F and I. Human CYP1A1 and CYP1B1 both display an asparagine at the equivalent position of Thr-223, which may alter folding interactions within helix F as well as between helices F and I. The absence of a disruption in helix F would essentially result in a less compact active site topology. The B’-C region in the P450 1A2 structure is characterized by the presence of 3 polar residues (Thr-118, Ser-122, and Thr-124) pointing into the active site cavity, in contrast to the more hydrophobic nature of the majority of amino acids lining the cavity. Both threonines are unique to CYP1A2. Based on homology modeling, Lewis and Lake (35) proposed that Thr-118 and Thr-124 would be implicated in two hydrogen bonds to both carbonyl oxygen atoms of caffeine. The P450 1A2 structure indicates that at least for Thr-124, this is likely to be true. The position of Thr-118 appears to be too distant to directly position smaller CYP1A2 substrates such as caffeine but may contribute to substrate binding and positioning by playing a prominent role in stable hydration that is likely to occur in the distal portion of the active site cavity upon binding of smaller substrates. The importance of Thr-124 in substrate binding was also observed in the equivalent reciprocal CYP1A1 S122T mutant, which displayed significantly increased O-dealkylation activity for both 7-ethoxy and 7-methoxyresorufin over the CYP1A1 wild-type enzyme (34). In contrast, O-demethylation activity for 7-methoxyresorufin of the CYP1A2 T124S mutant dropped by 80% relative to the wild-type activity. The occurrence of Thr-124, with a demonstrated influence on substrate binding and its position at the base of the P450 1A2 active site cavity, may also assist in the orientation for N-hydroxylation of heterocyclic arylamines. Based on the observed aromatic stacking and staggering interactions seen for ANF, mutagens such as 2-amino-3-methylimidazo[4,5-f]quinoline and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine can be docked in the P450 1A2 structure in a position suitable for N-hydroxylation, in which the heterocyclic nitrogen atom next to the site of metabolism is ideally positioned for a hydrogen-bonding interaction with Thr-124. Taken together, the planarity of the active site cavity, the compact and restricted active site architecture, the ability to provide an H-bonding partner in close proximity to the heme, and the potential for stable hydration of the distal part of the active site cavity can all be identified as potentially important determinants of human CYP1A2 specificity. The predicted less compact P450 1A1 active site topology, in which hydrogen-bonding interactions have less of an influence on substrate binding and orientation, is concordant with the CYP1A1 preferred substrates that are usually more lipophilic and slightly bulkier than CYP1A2 substrates and may explain the differences in catalytic activities for overlapping substrates observed with these enzymes.

Conclusions—The human P450 1A2 structure exhibits a relatively narrow, planar substrate binding cavity that is highly adapted for the size and shape of substrates that are oxidized by the enzyme. The structure assists in the rationalization of P450 1A2 specificity for substrates that are of great importance in drug metabolism as well as in procarcinogen activation. The topology of the active site is mainly characterized by residues on helix F and helix I that produce two parallel substrate binding platforms on either side of the cavity. The unique active site architecture demonstrates how P450 family 1 enzymes have evolved to catalyze efficiently polycyclic aromatic hydrocarbon oxidation and delineates structural properties that define a distinctive substrate binding site that has not been observed in P450 family 2 and 3 structures. The recent increase in the number of available human P450 structures provides a clearer understanding of how the diversity displayed in the active site structures is complementary and forms the basis for the capacity of these enzymes to oxidize an extremely wide range of structurally distinct substrates. In this respect, the first structure of a human microsomal P450 from family 1, described here, will aid in unraveling the molecular basis of drug recognition by human P450s and provide a rational platform for exploring P450 1A2 ligand interactions.

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REFERENCES
1. Nebert, D. W., Dalton, T. P., Okey, A. B., and Gonzalez, F. J. (2004) J. Biol. Chem. 279, 23847–23850
2. Kim, D., and Guengerich, F. P. (2005) Annu. Rev. Pharmacol. Toxicol. 45, 27–49
3. Shimada, T., Yamazaki, H., Foroozesh, M., Hopkins, N. E., Alworth, W. L., and Guengerich, F. P. (1998) Chem. Res. Toxicol. 11, 1048–1056
4. Hodek, P., Trefil, P., and Siborova, M. (2002) Chem. Biol. Interact. 139, 1–21
5. Agundez, J. A. (2004) *Curr. Drug Metab.* 5, 211–224
6. Yano, J. K., Hsu, M. H., Griffin, K. J., Stout, C. D., and Johnson, E. F. (2005) *Nat. Struct. Mol. Biol.* 12, 822–823
7. Scott, E. E., White, M. A., He, Y. A., Johnson, E. F., Stout, C. D., and Halpert, J. R. (2004) *J. Biol. Chem.* 279, 27294–27301
8. Williams, P. A., Cosme, J., Sridhar, V., Johnson, E. F., and McRee, D. E. (2000) *Mol. Cell* 5, 211–224
9. Scott, E. E., White, M. A., He, Y. A., Johnson, E. F., Stout, C. D., and Halpert, J. R. (2004) *J. Biol. Chem.* 279, 27294–27301
10. Williams, P. A., Cosme, J., Vinkovic, D. M., Ward, A., Angove, H. C., Day, P. J., Vonrhein, C., Tickle, I. J., and Jhoti, H. (2004) *Science* 305, 683–686
11. Schoch, G. A., Yano, J. K., Wester, M. R., Griffin, K. J., Stout, C. D., and Johnson, E. F. (2004) *J. Biol. Chem.* 279, 9497–9503
12. Williams, P. A., Cosme, J., Ward, A., Angove, H. C., Matak, V. D., and Jhoti, H. (2003) *Nature* 424, 464–468
13. Wester, M. R., Yano, J. K., Schoch, G. A., Yang, C., Griffin, K. J., Stout, C. D., and Johnson, E. F. (2002) *Methods Enzymol.* 357, 73–79
14. Scott, E. E., He, Y. A., Wester, M. R., White, M. A., Chin, C. C., Halpert, J. R., Johnson, E. F., and Stout, C. D. (2003) *Proc. Natl. Acad. Sci. U. S. A.* 100, 13196–13201
15. Quattrochi, L. C., Pendurthi, U. R., Okino, S. T., Potenza, C., and Tukey, R. H. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 6731–6735
16. Wester, M. R., Stout, C. D., and Johnson, E. F. (2002) *Methods Enzymol.* 357, 73–79
17. Sandhu, P., Guo, Z., Baba, T., Martin, M. V., Tukey, R. H., and Guengerich, F. P. (1994) *Arch. Biochem. Biophys.* 309, 168–177
18. Otwinowski, Z., and Minor, W. (1997) *Methods Enzymol.* 276, 307–326
19. Collaborative Computing Project Number Four (1994) *Acta Crystallogr. Sect. D Biol. Crystallogr.* 50, 760–763
20. McCoy, A. J., Grosse-Kunstleve, R. W., Storoni, L. C., and Read, R. J. (2005) *Acta Crystallogr. Sect. D Biol. Crystallogr.* 61, 458–464
21. Jones, T. A., and Kjeldgaard, M. (1997) *Methods Enzymol.* 277, 173–208
22. Bruenger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszweski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonon, T., and Warren, G. L. (1998) *Acta Crystallogr. Sect. D Biol. Crystallogr.* 54, 905–921
23. Wester, M. R., Johnson, E. F., Marques-Soares, C., Dijols, S., Dansette, P. M., Mansuy, D., and Stout, C. D. (2003) *Biochemistry* 42, 9335–9345
24. Parikh, A., Josephy, P. D., and Guengerich, F. P. (1999) *Biochemistry* 38, 5283–5289
25. Gerber, N. C., and Sligar, S. G. (1994) *J. Biol. Chem.* 269, 4260–4266
26. Liu, J., Ericksen, S. S., Sivaneri, M., Besspiata, D., Fisher, C. W., and Szklarz, G. D. (2004) *Arch. Biochem. Biophys.* 424, 33–43
27. Lewis, D. V., and Lake, B. G. (1996) *Xenobiotica* 26, 723–733
28. Keyworth, G. J., and Jones, T. A. (1994) *Acta Crystallogr. Sect. D Biol. Crystallogr.* 50, 178–185