Properties of cytochrome P450 isoenzymes and their substrates Part 1: active site characteristics

Dennis A. Smith, Mark J. Ackland and Barry C. Jones

Cytochrome P450 isoenzymes are pivotal in drug clearance. Protein homology modelling combined with studies on the structure and physico-chemistry of substrates is allowing the key factors governing selectivity for substrates to be ascertained. The knowledge gained will be used in future in the drug design process to produce compounds more resistant to metabolism or with lowered potential to inhibit the enzyme. Ultimately, therefore, the progress being made will result in the discovery and development of safer and more effective therapies.

Xenobiotic metabolism is a process that changes the molecular structure of a xenobiotic from one that is absorbed (lipophilic, capable of crossing the membrane lipid core) to one that can be readily voided (incapable of crossing the lipid core of membranes, hydrophilic), particularly by the kidney. Various enzyme systems, classically divided into phase I and phase II, exist to facilitate this. Phase I normally involves oxidative attack, while phase II involves the conjugation of the molecule with glucuronic acid, sulphuric acid, etc.

Although there are a number of oxidative enzymes capable of undertaking xenobiotic metabolism (e.g. alcohol dehydrogenase, xanthine oxidase and the amine oxidases), the pivotal oxidative enzyme system in xenobiotic metabolism is cytochrome P450 (P450). This enzyme system can oxidize compounds on carbon and on heteroatoms such as nitrogen and sulphur. Products of oxidation include carbinols, phenols, epoxides, nitrogen oxides, sulphoxides, sulphones and, via oxidation α to a heteroatom, dealkylation products. This rich diversity of possible products underlines the pivotal role of the enzyme system, which also has an unrivalled ability to metabolize xenobiotics of huge diversity in structure[1,2].

P450 is a superfamily of similar proteins (isoenzymes) that have the same porphyrin–haem complex as the catalytic centre, but different amino acid sequences, which alters the topography of the active site. The identity of each protein is based on amino acid sequence homology such that all members of a particular family or subfamily are at least 40% similar to all the other members of that family or subfamily. Metabolism of a compound by each isoenzyme is determined by three factors:

- The topography of the active site.
- The degree of steric hindrance that impedes the access of the iron–oxygen complex to the possible sites of metabolism.
- The ease with which electrons or hydrogens can be abstracted from the various carbons or heteroatoms of the substrate.

The mechanism of P450 catalysis is constant across most of the isoenzymes. As stated by Guengerich and

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MacDonald[3], the chemistry is determined by the ability of a high-valency, formal $(FeO)^{3+}$ species to carry out one-electron oxidations through the abstraction of hydrogen atoms or electrons. Because of the diversity of possible substrates, this review will focus on pharmaceuticals rather than other xenobiotics.

Analysis of the literature indicates that six major forms of P450 isoenzymes are involved in the metabolism of pharmaceuticals in man: CYP1A2, CYP2D6, CYP2C9, CYP3A4, CYP2E1 and CYP2A6 (Ref. 2). The metabolic process for P450 enzymes has been divided into a recognition phase, an accessibility phase and a reactivity phase[4]. Recognition is a long-distance (up to 8 Å), predominantly electrostatic interaction between the substrate and the enzyme. Access to the buried active site of the enzyme is by a channel leading from the outside of the protein to the haem centre. The overall dimensions of the channel plus its relative hydropathy are potentially important in substrate discrimination. The final phase of the process is actual oxidation once the substrate has gained access to the site. The recognition of the three stages indicates how protein structure and physicochemical or chemical properties of the substrate may influence the overall process by impacting on one of the stages. For instance, a substrate's overall lipophilicity may allow entry to the active site via the channel. The actual hydrophobic functions, say planar aromatic rings, may undergo π–π interactions with aromatic amino acid side chains. These specific interactions may govern orientation of a molecule in the active site.

The review will focus on the major P450 isoenzymes, in terms both of enzyme structure and function (Part 1) and of substrate structure and physicochemical characteristics (Part 2), thus demonstrating the use of this understanding in drug discovery.

Structure of the active site

The main approaches used to date to study P450 active site topography and structure include template/pharmacophore modelling, protein homology modelling, quantitative structure–activity relationships (QSARs) and molecular orbital techniques. These techniques have made a significant contribution to our understanding and should be regarded as essential and complementary methods in P450 research.

Protein homology modelling of the human P450s continues to be dependent upon crystal structures derived from prokaryotic sources, such as $P450_{cam}$ (CYP101)[5], $P450_{terp}$ (CYP108)[6] and $P450_{BM-3}$ (CYP102)[7]. A comparative analysis of these structures[8] suggests that all P450s will be found to possess the same tertiary structure and that $P450_{BM-3}$ is closest to eukaryotic P450s for modelling purposes. CYP102 appears to exhibit higher sequence homology with mammalian P450s than do the other bacterial P450 crystal

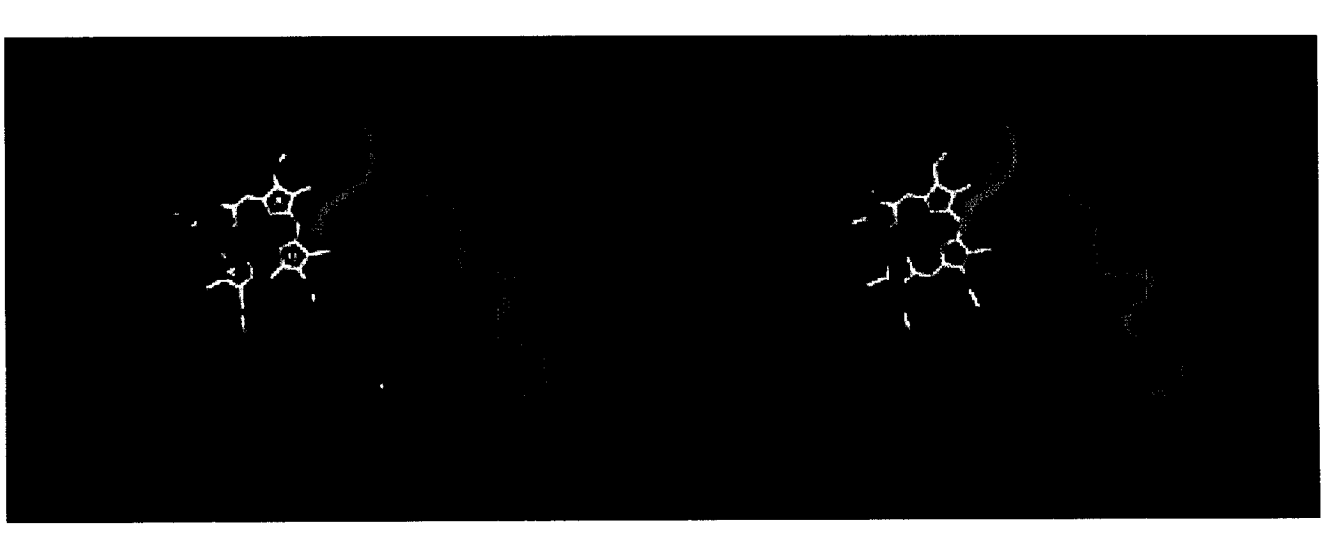

Figure 1. *Active site region of the X-ray crystal structure of $P450_{BM-3}$ illustrated from above the plane of the porphyrin ring. Secondary structural elements include the A (light blue), A′ (red), F (dark blue), I (magenta) and 3_{10} helices, the B′ helix plus associated loop regions (yellow), and the β1–4 (dark green) and β4–1/β4–2 (pink) β sheet regions. Rings A, C and D of the haem are labelled.*

structures, and it shares a common redox partner with the microsomal P450s (oxidoreductase flavoprotein).

There is now a measure of confidence that membrane-bound eukaryotic P450s can be modelled using structures such as $P450_{BM-3}$, although with some caveats. For instance, there is likely to be spatial variation (1–4 Å) between the various secondary structural elements in different enzymes, depending upon the particular isoform. In terms of the active site regions, it is apparent from the work of Hasemann and coworkers[8] that any model is likely to be speculative with respect to precise positioning of the upper (A and F helices, β4–1 sheet) regions of an active site model (Figure 1) as well as one of the active site faces (B′ helix plus associated loop region), but acceptable with respect to the other active site faces as defined by the I helix, the β1–4 sheet region and the remaining β sheet regions. This offers the opportunity to develop active site models, interpret site-directed mutagenesis data and speculate on likely amino acids specifically involved in substrate interactions. Experimental approaches to probing the active site have included various spectral techniques[7], X-ray crystallography[5,7,9,10], site-directed mutagenesis[7] and active site chemical probes[11,12].

CYP1A2

Early modelling studies[13,14] on the CYP1A family (then referred to as cytochrome P448) characterized the substrates as lipophilic planar polyaromatic/heteroaromatic molecules defined by a small depth and a large area-to-depth ratio. Sanz and coworkers[15] have published a theoretical study on the metabolism of caffeine by CYP1A2 in which a 'biophore' for the demethylation of caffeine is proposed on the basis of molecular electrostatic potentials (MEPs). Further detailed studies on three xanthines identified a common feature expressed in a planar triangular electrostatic pattern between three MEP minima, illustrated here for caffeine (Figure 2). The three minima arise from heteroatom lone pairs situated on the main ring. These data were significant because they offered the possibility that there may be three hydrogen bonding sites within the active site of CYP1A2 responsible for interactions at the three locations.

Fuhr and coworkers[16] were able to substantiate this work by studying the relationship between structure and *in vitro* inhibition of caffeine 3-demethylation for a series of quinolone antibacterial agents. Comparison of the MEPs of caffeine with those of the competitive inhibitors showed a strong resemblance. It was found that the negative potential

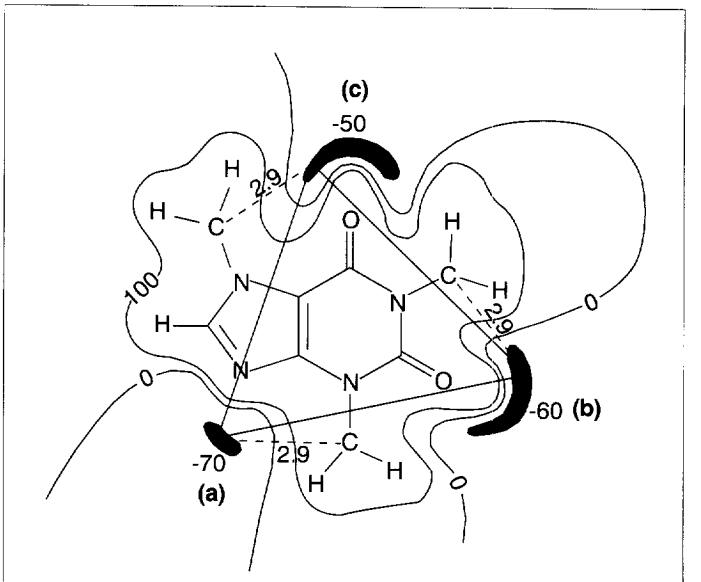

Figure 2. *Molecular electrostatic potential map (MEP) of caffeine, showing the planar triangular electrostatic pattern between the three MEP minima (a, b, c). Reproduced with permission of Verlagsgesellschaft.*

arising from the carbonyl oxygens at positions C-2 and C-6 of caffeine (Figure 2) and the positive potential arising from the methyl hydrogen atoms at positions N-1 and N-7 of caffeine overlapped with similar profiles in the quinolone molecules.

The active site of CYP1A2 has been explored using aryl-hydrazine probes[17], which demonstrated that the active site of the enzyme was relatively open directly above all four pyrrole rings of the haem. This is unusual; it has been shown that pyrrole ring B of the haem is masked by the I helix in most of the P450 enzymes. Lewis and coworkers[18] have studied a homologous series of alkoxyresorufins in an attempt to explain the specificity of CYP1A2, CYP1A1 and CYP2B1 for methoxy-, ethoxy- and pentoxyresorufins, respectively. The increase in chain length of the alkoxy substituent increased hydrophobicity and was found to affect enzyme affinity for CYP1A as a result of steric hindrance at the binding site. The orientation of methoxyresorufin in the active site of CYP1A2 was also studied using a protein homology model based on the X-ray crystal structure of CYP102.

A further, more extensive, study has been reported by Lewis and coworkers[19] in which the active site interactions of caffeine, 2-amino-1-methyl-6-phenylimidazo(4,5-*b*) pyridine (PhIP), oestradiol, 2,4- and 2,5-diaminotoluene,

glucose-1-phosphate, phenacetin, acetanilide, 7-methoxy- and 7-ethoxyresorufin, 11-methylcyclopenta[*a*]phenanthren-17-one, 7-ethoxycoumarin, aflatoxin B_1, benzo[*a*]pyrene, benzo[*a*]pyrene-7,8-diol and 1′-hydroxy-3-methylcholanthrene were studied. Specific amino acid residues were proposed for rat CYP1A1, rat CYP1A2 and human CYP1A2 that contributed to the active site environment and consequently substrate binding and specificity for the enzymes.

The active site of the CYP1A enzymes was modelled as an approximately rectangular slot composed of several aromatic side chains, including the coplanar rings of Phe181 and Tyr437, which restrict the size and shape of the cavity such that only planar structures (approximately 6 Å in width) are able to occupy the binding site. This feature could explain the preference of CYP1A enzymes for hydrophobic, planar aromatic species that are able to partake in π–π interactions. The cavity dimensions appear to be further influenced by two aromatic residues that lie 'end-on' either side of the active site, namely Phe88 and Phe266. In addition to the flanking aromatic residues, there were several strategically placed hydrogen bond donor side chains that were involved in various key substrate interactions, namely Thr78, Thr87 and Asn184 (Thr184 in human CYP1A2). The model was able to explain the fact that caffeine is *N*-demethylated at the 1, 3 and 7 positions by CYP1A2, with N-3 demethylation being the major pathway.

Figure 3 shows caffeine orientated for *N*-demethylation at position N-3 of rat CYP1A2 such that metabolism can occur,

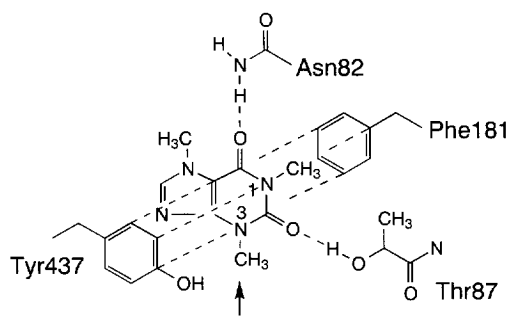

Figure 3. *Proposed key interactions of the CYP1A2 active site showing caffeine orientated for* N*-demethylation at position N-3 (indicated by the arrow). The substrate lies approximately perpendicular to the haem and is sandwiched between Phe181 and Tyr437. Specific hydrogen bonding interactions include Asn82 and Thr87. Residue numbering is as in the original article*[19].

and includes the critical residues thought to be responsible for binding. The molecule is orientated approximately perpendicular to the haem and lies sandwiched between Phe181 and Tyr437. Specific hydrogen bonding interactions include Asn82 and Thr87. This work appears to be consistent with the electrostatic potential maps discussed earlier for caffeine[15] and the quinolone antibacterials[16]. Sequential overlay of the aforementioned substrates by Lewis and coworkers[19] resulted in a composite template model inserted into the protein active site. A comprehensive picture of the CYP1A2 active site is therefore emerging in which the molecular dimensions and hydrophobicity of the active site, together with its key hydrogen bonding and π–π interactions, have been defined.

CYP2C9

Mancy and coworkers[20] have published a molecular modelling study of CYP2C9 based on a series of tienilic acid (ticrynafen) derivatives and known substrates. Analogues of tienilic acid in which the carboxylic acid group has been removed or replaced with non-acidic functional groups do not interact with the isoenzyme. It was proposed, therefore, that a corresponding cationic group possessing a positive charge at physiological pH may exist in the active site of CYP2C9. The cationic group would be positioned at a position approximately 7.8 Å, and at an angle of 82°, from the sites of oxidation for the known substrates. The presence of an aromatic ring above the haem moiety appeared to be an important feature of the majority of substrates studied.

Jones and coworkers[21] proposed an active site template model of CYP2C9 based on known substrates and using phenytoin as the initial rigid template. In this study the ability of substrates to act as hydrogen bond donors was stressed. The binding of sulfaphenazole involved a bridging water molecule to account for the relatively short interatomic distances involved between the ligating pyrazole nitrogen and the hydrogen bonding site. X-ray crystallography studies on P450eryF (Ref. 22) have shown that water molecules can indeed be used by P450s to bind substrates.

Jones and coworkers[23] have suggested a three-dimensional QSAR for CYP2C9 based on a comparative molecular field analysis (CoMFA) of overlaid structures. The approach assumed that a π-stacking interaction would be the primary aligning factor for the template rather than the site of metabolic attack. All the calculations were performed on neutral molecules, and it was found that this approach gave a highly predictive model for the determination of binding constants.

The hemiketal form of (*S*)-warfarin was used as it is known that this is the preferred form in lipophilic environments. The study included a number of compounds that would not be ionized at physiological pH and did not have strong dipoles. Furthermore, it was noted that normally anionic molecules at physiological pH can have higher pK_as in a lipophilic environment such as an active site, and consequently exist as neutral species. The model indicated the presence of at least one and possibly two electrostatic interactions involved in binding; hence, compounds able to present an alcohol, carbonyl or amine to the primary binding site were liable to be strong binders. The model also suggested the presence of a phenyl (π) binding site above the plane of the haem group.

While the above modelling studies are in agreement that there is at least one binding site for substrates/inhibitors of CYP2C9, there is some controversy as to the nature of the site, namely whether it exists as a highly charged species such as a histidine or lysine residue, or whether it is essentially a hydrogen bonding residue such as a serine or asparagine residue. Assuming there is a single binding site, the weight of evidence thus far suggests that the residue is likely to be a hydrogen bonding group. However, it is interesting to note that the Jones study[23] implicated a second electrostatic site lying closer to the CYP2C9 oxidation site and that the apparently contradictory data revealed by Mancy and coworkers[20] might be adequately explained by invoking a second site as well. The Mancy data could be explained by the existence of a short-range hydrogen bonding site and a long-range cationically charged site.

Lewis[24] has generated a protein homology model of CYP2C9 based on CYP102 and has modelled tolbutamide, mephenytoin, tienilic acid, (*S*)-warfarin and sulfaphenazole[10], as well as tamoxifen[25], in the active site. These studies propose that a serine aligned at position 331 of CYP102, and present in 2C9 and 2C19, is responsible for binding tolbutamide, sulfaphenazole and mephenytoin; this lies approximately 7.86 Å from the site of oxidation. In the case of tienilic acid, Lewis proposes that the ether oxygen hydrogen bonds to Ser331 and that the carboxylic acid hydrogen bonds to a second residue, namely Ser69, which lies approximately 9.43 Å from the site of oxidation in the active site. A similar argument was used for the binding of (*S*)-warfarin (Figure 4) and tamoxifen. The paper proposes that the aromatic ring of (*S*)-warfarin is able to undergo a π–π stacking interaction with Phe74, a residue that could be implicated in the earlier π–π interactions proposed by Jones and coworkers[23]; however, there are no site-directed mutagenesis data to confirm these proposals. CYP2C9, therefore, has probably two hydrogen bonding sites situated approximately 7.8 and 9.4 Å from the site of oxidation. Additionally, there is an aromatic residue able to enhance binding of aromatic substrates via a π–π stacking interaction, and there is evidence that water molecules may play a part in the binding of sulfaphenazole.

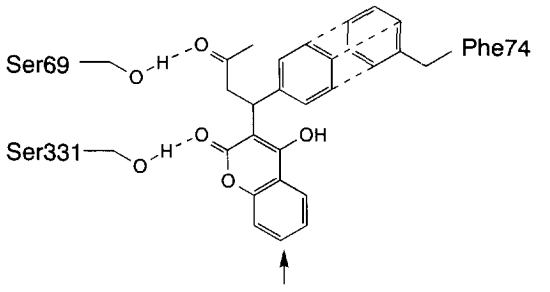

Figure 4. *Proposed key interactions of the CYP2C9 active site showing warfarin orientated for aromatic oxidation. The substrate is thought to undertake hydrogen bonding interactions with Ser69 and Ser331 and π–π interactions with Phe74. Residue numbering is as in the original article[10]. The arrow indicates the site of oxidation.*

CYP2D6

This enzyme is perhaps the best studied isoform to date. The first model of CYP2D6, by Wolf and coworkers[26], was based on a study of known substrates using space-filling models. It was proposed that many of the substrates were oxidized in the vicinity of an aromatic or methylene group and were therefore binding to a lipophilic pocket adjacent to the iron atom of the haem moiety. It was also noted at the time that CYP2D6 substrates had in their structure at least one nitrogen atom that could be protonated at physiological pH and that the active site could possess a complementary anionic group responsible for substrate binding. A distance of 5 Å was proposed between the site of oxidation and the cationic group.

Further work by Meyer and coworkers[27] at first appeared to contradict the previous work by suggesting that an interatomic distance of 7 Å was required for binding. However, Koymans and coworkers[28], using molecular modelling, were able to show that both substrates could be accommodated by a 'bidentate' hypothesis. It was proposed that

the 5 and 7 Å substrates were binding at either oxygen of a single carboxylic acid residue within the active site. It was also suggested that a coplanar conformation of substrates was required.

Islam and coworkers[29] produced a template model using X-ray coordinates of the known substrates, and these were orientated relative to the known position of oxidation of camphor, obtained from the X-ray coordinates of CYP101 (Ref. 1).

More recently, Strobl and coworkers[30] published a pharmacophore model based on potent inhibitors of CYP2D6. This group identified a cationic nitrogen group, two potential hydrogen bonding groups and a flat hydrophobic region perpendicular to the N–H axis as important criteria for binding.

Ackland[31] was able to show a qualitative correlation between the site of oxidation for aromatic CYP2D6 substrates and a molecular orbital term, electrophilic superdelocalizability. This work suggested that the electronic properties of the substrates also played a part in reactivity and regiospecificity. A molecular template was also constructed and evaluated with respect to its ability to predict substrate regiospecificity in a series of aminotetralins[32]. Figure 5 illustrates one of the compounds, *N,N*-dipropyl-2-aminotetralin, orientated in the active site.

Figure 5. *View of the CYP2D6 active site as determined by template and protein homology modelling. The substrate,* N,N-*dipropyl-2-aminotetralin (yellow), and site of oxidation (white) are shown within the active site cavity (blue). Red areas indicate the optimal spatial positioning for binding of charged functional groups within the substrate.*

Koymans and coworkers[33] have published a protein homology model based on the X-ray crystal structure of CYP101 (Ref. 5). Automated and manual alignment procedures were used to match the sequences, and then regions with no apparent homology were removed from the model. This paper was the first to propose two potential protein residues that might be responsible for substrate binding, namely Asp100 and Asp301.

In site-directed mutagenesis experiments, Ellis and coworkers[34] and Mackman[35] were able to demonstrate that Asp301 plays an important role in determining the substrate specificity of CYP2D6. The influence of amino acid residue 374 on the regio- and enantioselective metabolism of metoprolol was also studied[36], and it was shown experimentally, and by using an active site homology model, that both Val374 and Asp301 indeed lie in the active site of the enzyme and are substrate contact residues.

The active site topology of CYP2D6 and a series of position 301 mutants have been explored recently using aryldiazines[37]. It was shown that there were differences between the P450s in the region of the active site cavity above the catalytic haem moiety. Of the known P450 crystal structures, CYP102 appeared to be the most similar to CYP2D6, although there were still minor differences.

A recent development[38] has involved the use of proton NMR to study the orientation of a substrate (codeine) in the active site of CYP2D6. The interatomic distances between the substrate hydrogen atoms and the iron atom of the catalytic site were used to position the molecule in the active site; protein homology modelling was then undertaken using this information. CYP2D6 can now be understood in terms of a lipophilic cavity that is at least 15 × 7.5 Å in dimensions and 990 cubic Å in volume[32]. The primary determinant in binding is an acidic residue (Asp301), which lies approximately 5–7 Å from the site of oxidation and has been precisely positioned in protein homology models of the active site. There is evidence from template as well as homology modelling that there are at least one, and possibly two, aromatic residues present within the active site, close to the haem, and these are responsible for key π–π interactions with aromatic substrates.

CYP3A4

Of the cytochrome P450s discussed in this paper, CYP3A4 is probably the least well understood enzyme in terms of its active site structure and function, despite being one of

the most prevalent in human liver and important in terms of the metabolism of pharmaceuticals. The active site has to accommodate a wide range of differing substrate classes[2,39,40], from relatively small molecules such as steroids (e.g. testosterone, MW 288) to large molecules such as the macrolides (e.g. cyclosporin A, MW 1202). Recent literature[40] indicates that α-naphthoflavone is able to activate the metabolism of 6β-testosterone[41] and aflatoxin B_1 (Ref. 42). This raises the possibility[43] that activation may arise out of the simultaneous occupation of the active site by two substrate molecules, supporting the proposed size of the active site. The regioselectivity of oxidation in CYP3A4 substrates appears to be dependent more on the site of chemical reactivity within the molecule than on specific interactions with the active site. For instance, the 6β-oxidation of steroids occurs at an allylic carbon that is likely to be chemically activated, as are *N*-demethylation reactions (see later). Protein homology models have been attempted by Ferenczy[44] and, more recently, by Lewis and coworkers[24,45], using CYP101 and CYP102, respectively, as templates. The sequence identity between CYP3A4 and CYP102 is 27%, and the overall homology is 52% when similarity between amino acid residues is accommodated, and this appears to be the highest for all the hepatic P450s (Ref. 45). On this basis, Lewis has identified a number of potential sites of interaction within the CYP3A4 active site for the substrates testosterone (Asn74), granisetron (Asn74), cyclosporin A (Asn74, Phe72), nifedipine (Asn74, Phe72), tamoxifen (Asn74, Phe72), omeprazole (Asn74, Phe74), ondansetron (Asn74), salmeterol (Thr73), gestodene (Asn74) and ketoconazole (Asn74, Phe72), although these have not been confirmed by site-directed mutagenesis. The active site pocket proposed was large and open and comprised predominantly of hydrophobic and some neutral amino acids together with a small number of polar side chains. Aromatic side chains appeared to be present, allowing for the possibility of π–π interactions with aromatic substrates. Apart from general hydrophobic interactions, the most important interaction proposed involved Asn74, which was 6.8 Å from the site of oxidation in the substrates investigated (see schematic representation in Figure 6). Figure 7 gives an example of the key interactions of testosterone in the putative active site in which Asn74 and Ser271 hydrogen bond with the D-ring hydroxyl and the C-3 ketone on the steroid, respectively. A further interaction is proposed between the A-ring carbonyl and Ser271, as well as a number of hydrophobic interactions.

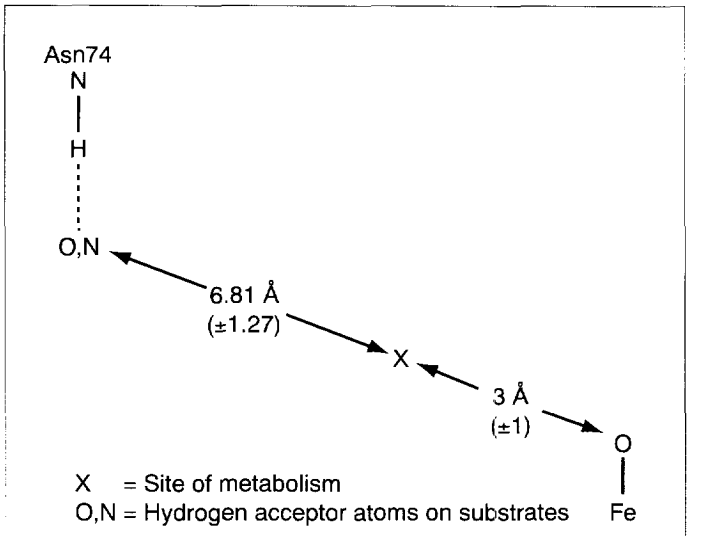

Figure 6. *Schematic representation of optimal distance requirements for CYP3A4 substrate binding. Redrawn from Ref. 10.*

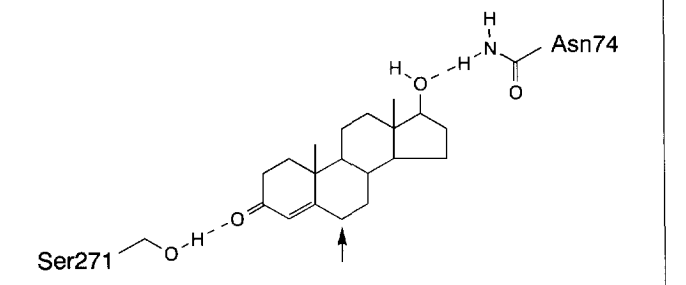

Figure 7. *Proposed key interactions of the CYP3A4 binding site. Residue Asn74 hydrogen bonds to the D-ring hydroxyl group of testosterone and Ser271 hydrogen bonds to the C-3 ketone of the steroid. Residue numbering is as in the original article[45]. The arrow indicates the site of oxidation.*

CYP2E1

This enzyme has been studied extensively in experimental animals and in humans because of its possible relevance to alcoholism, chemical carcinogenesis and other diseases[40]. Some drugs are substrates, such as acetaminophen, zoxazolamine and gaseous anaesthetics (halothane and enflurane); other substrates include *N*-nitrosodimethylamine, aniline, ethanol and carbon tetrachloride. Also, 2E1 appears to be responsible for the metabolic activation of low molecular weight carcinogens such as chloroform, vinyl chloride/

bromide, ethylene dibromide/dichloride, vinyl and ethyl carbamate, benzene and styrene[46].

There is limited information on site-directed mutagenesis; studies on rabbit CYP2E1 showed that replacement of a conserved threonine, Thr303, with serine modified the regioselectivity of fatty acid hydroxylation[47].

No template models have been attempted, perhaps because of the small and variable nature of the substrates; however, Wang and coworkers[48] have probed the active sites of rat and human CYP2E1 using a series of alcohols and carboxylic acids of increasing chain length in order to gain information on the size, hydrophobicity and charge accommodation of the site. The K_i values for the alcohol series suggested an optimal chain length of seven carbon atoms for binding in both the rat and human enzyme. Optimal binding for straight-chain carboxylic acids was achieved for dodecanoic acid, suggesting that the acid group lies outside the active site, possibly at the mouth of the channel. Experiments with ω-fatty acids resulted in high K_i values, suggesting that the active site is very hydrophobic in nature.

On the basis of these results, a simple model was proposed, consisting of a hydrophobic channel of approximately 8 Å, leading to an active site of approximately 7 Å in length. Lewis[10,24] has proposed a protein homology model based on the crystal structure of CYP102. According to this model, the active site is hydrophobic and reflects the relatively small size of the substrates as it is conformationally restricted by bulky amino acid side chains such as Phe78, Phe88, Phe181 and Phe263, which form two pairs of π–π stacked phenyl rings, and Ile436, Ile438, Leu327 and Val328. Residues Thr266 and Arg82 are proposed as possible binding sites for substrates via hydrogen bonding and electrostatic interactions, but these have not been experimentally confirmed. Experiments with arylhydrazine probes suggest that the CYP2E1 active site is open above pyrrole ring D (Ref. 49).

CYP2A6

This enzyme is the only member of the 2A subfamily that is expressed in man; it is also present in relatively low levels (4% in human liver). The purified enzymes have been shown to catalyse the *O*-de-ethylation of 7-ethoxycoumarin and the activation of aminochrysene and aflatoxin B[1]; also coumarin 7-hydroxylation is exclusive to CYP2A6 (Ref. 40).

The application of computational techniques to CYP2A6 is currently confined to a protein homology model[50] based on CYP102 crystal structure. The model suggests that orientation of coumarin for 7-hydroxylation is achieved by hydrogen bonding between the substrate carbonyl and Thr184, together with a π–π interaction of the substrate with Phe181 (Figure 8). The active site is again hydrophobic in nature.

Summary

There has been a significant expansion of our understanding of the active sites of the cytochrome P450s in the last few years. This has been due, in large part, to the increased availability of prokaryotic P450 X-ray crystal structures. Moreover, the secondary and tertiary structures of these proteins have largely been conserved throughout evolution. Although no crystal structures are available for the membrane-bound P450s involved in the metabolism of drugs, this conservation allows predictive models to be built. The isoforms covered by this review, the key isoforms in the metabolism of drugs, all have hydrophobic active sites, with at least one aromatic amino acid residue close to the catalytic haem capable of undertaking π–π interactions with substrates. Other residues more distant from the catalytic centre can partake in further substrate binding, including additional hydrophobic (CYP3A4), hydrogen bonding (CYP1A2, CYP2C9 and CYP2A6) or ion-pair (CYP2C9, CYP2D6) interactions. This theme will be further developed in Part 2, where the particular structural and physicochemical characteristics of the substrates will be discussed.

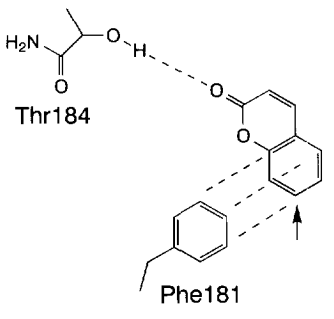

Figure 8. *Proposed key interactions of the CYP2A6 binding site showing coumarin orientated for oxidation at the 7-position (indicated by the arrow). The substrate lies approximately perpendicular to the haem. Key interactions include hydrogen bonding of the carbonyl to Thr184, and π–π interactions with Phe181. Residue numbering is as in the original article[50].*

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