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Designing better drugs: predicting cytochrome P450 metabolism

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Many 3D ligand-based and structure-based computational approaches have been used to predict, and thus help explain, the metabolism catalyzed by the enzymes of the cytochrome P450 superfamily (P450s). P450s are responsible for >90% of the metabolism of all drugs, so the computational prediction of metabolism can help to design out drug–drug interactions in the early phases of the drug discovery process. Computational methodologies have focused on a few P450s that are directly involved in drug metabolism. The recently derived crystal structures for human P450s enable better 3D modelling of these important metabolizing enzymes. Models derived for P450s have evolved from simple comparisons of known substrates to more-elaborate experiments that require considerable computer power involving 3D overlaps and docking experiments. These models help to explain and, more importantly, predict the involvement of P450s in the metabolism of specific compounds and guide the drug-design process.

Cytochrome P450s (P450s, CYPs [1,2]) constitute a large superfamily of heme-containing enzymes. The superfamily is divided into families for which the pairwise amino acid sequence identity between individual members is >40%, and then into subfamilies for which the pairwise amino acid sequence identity between members is usually ≥55% [2]. P450s can metabolize (either oxidize or reduce) a large number of structurally different endogenous and exogenous compounds. Seven of the 57 known human isoforms of P450s are responsible for >90% of the metabolism of all pharmaceuticals in current clinical use: CYP1A2, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 [3]. Several of the P450 isoforms show polymorphisms (e.g. CYP2D6 and CYP2C9) that can result in the poor metabolism of drugs [4]. It is therefore vitally important for the pharmaceutical industry to be able to predict at an early stage whether or not a drug candidate will interact with the P450s, which isoform the drug candidate will preferentially interact with and, consequently, whether it is worthwhile spending time and money taking that candidate through to development.

During the past decade or so, a variety of computational chemistry methods have been applied to cytochrome P450s, either concentrating on the small molecules that are metabolized by or inhibit P450s, the P450 proteins themselves, or a combination of the two. These approaches have provided us with a vast selection of models of P450s that have improved our understanding of the function and selectivity of these important enzymes. This review focuses on 3D models created for P450s.

Models capable of predicting the possible involvement of P450s in the metabolism of endogenous compounds, drugs or drug candidates are important tools in drug discovery and development, primarily because of the clinical importance of P450s in the metabolism of xenobiotics and endogenous compounds and identification of potential drug–drug interactions [5]. The computational approaches that can be used are varied and dictated by the availability of structural data.

Ligand-based models for cytochrome P450: 3D-QSAR models

All ligand-based models provide indirect information about a protein's active site on the basis of the shape, electronic properties and conformations of substrates, inhibitors or metabolic products. These models are often dependent on the availability of experimental data for a sufficiently large number of substrates (and their conformations). Ligand-based models can be constructed using a variety of methods and commercially available software.

The simplest approach to predicting the activity and/or binding of a compound is via quantitative SAR (QSAR) using experimental

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and calculated properties. Such methods attempt to link chemical structures with observed activities in a quantitative manner. Measured or calculated properties are termed 'descriptors' and can be subdivided in many ways (e.g. spatial, electronic, thermodynamic, conformational, topological, structural, and so on). The potential number of descriptors available is immense, and can be calculated using a range of commercially available software [6]. Many of the descriptors are correlated (interdependent) so usually a representative subset is chosen. An overview of the selection and calculation of appropriate descriptors is available elsewhere [6].

There are several 3D-QSAR techniques that do not involve the superposition of specific atoms. These techniques have been used to derive a variety of models for P450 substrates and inhibitors using small molecules. 3D-QSAR techniques are often based on molecular field analysis (MFA) or receptor surface analysis (RSA), both of which involve calculating the energy of interaction between a probe and a molecule. In MFA the energy of interaction for each point in a rectangular or spherical grid around the small molecule is calculated, whereas in RSA it is calculated for a set of points on the receptor (or enzyme) surface. Overviews of 3D-QSAR models derived for P450s have recently been published [4,7].

Ligand-based models for cytochrome P450: pharmacophore models

The most straightforward ligand-based model is a pharmacophore model. Pharmacophore modelling overlays structures of ligands (Figure 1) or properties of these ligands in 3D space in an attempt to describe the physical, spatial and chemical properties of the active (or binding) site. Knowledge of the 3D strucure of the binding site can help with the construction of a pharmacophore by introducing excluded volume (i.e. regions of space occupied by the protein and therefore not available for the ligand to occupy) as an additional pharmacophoric feature, although this is not essential. Pharmacophore modelling approaches remain important because 3D structures are publicly available for only a few human P450s. The model obtained is a consensus structure that describes the size and electrostatic properties of the active site. When applied to P450s, the technique is dependent on the selection of a suitable set of compounds that ideally: (i) are specifically metabolized by the P450 isoform being studied, (ii) are large and rigid (thus limiting the number of conformations), and (iii) comprise compounds containing representatives of all functional groups known to be metabolized by the isoform. In general, the pharmacophore approach assumes that the substrates will all be oriented in a similar manner (both electronically and sterically) in the active site of the enzyme. This, of course, is not always true [8], especially if compounds are metabolized through pathways atypical for the given isoform or are metabolized in more than one location, for example 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in CYP2D6 [9]. The pharmacophore models developed for human P450s are summarized in Table 1. More details on ligand-based models (including pharmacophore and 3D-QSAR models) developed for cytochrome P450s are available elsewhere [4].

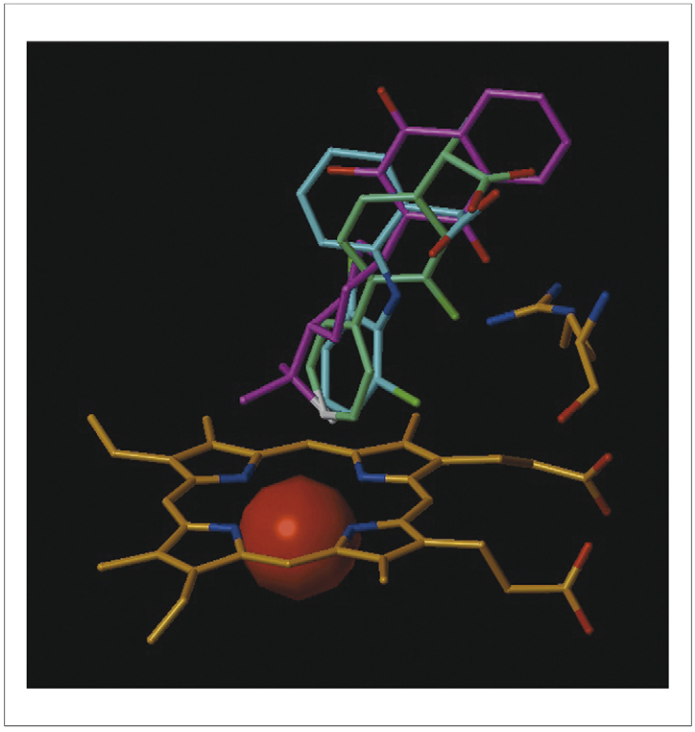

FIGURE 1

A pharmacophore overlay (based on structure) for human CYP2C9. The overlay of several compounds, the heme moiety and Arg108 are shown [11]. Colours used: heme (with Fe ion shown in space filling) and Arg108, orange carbon atoms; 58C80, purple carbon atoms; diclofenac, cyan carbon atoms; flurbiprofen, green carbon atoms. Sites of metabolism are white; other heavy atoms are coloured by atom type.

Structure-based models for cytochrome P450s

Experimental techniques, such as X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy and electron microscopy are used to determine the 3D structure of proteins to high resolution. Unfortunately, most proteins are currently not amenable to these techniques because they are difficult to crystallize, insufficiently soluble or too large for NMR studies, or unable to withstand the required pre-treatment of samples. These factors, together with the rapid expansion and success of the various genome projects, have resulted in a large gap between the knowledge of a protein amino acid sequence and the ability to relate this to its 3D structure. Alternative methods have been developed to help understand the functional role of a protein in terms of its 3D structure in those cases for which experimental information is lacking. One such technique is homology (or comparative) modelling.

The observation that proteins with similar amino acid sequences have a tendency to adopt similar 3D structures forms the basis of homology modelling [10]. The 3D structure of a protein can therefore be predicted on the basis of its amino acid sequence and the 3D structures of proteins with similar sequences. Although such models will be less accurate than those derived experimentally, they are invaluable because they provide testable hypotheses in the absence of experimental data. Until recently, structural models of human P450s were based on the known, distantly related, bacterial P450s (Table 2). The recent determination of the crystal structure of rabbit and human cytochrome P450s has improved the reliability of homology models for human P450s (Table 2). Figure 2 shows the homology model of CYP2C9 [11], which is in very good agreement with the more recently obtained crystal structure of CYP2C9 [12,13].

TABLE 1

Pharmacophore models for human P450s

	Description	References
CYP1A2	A model based on an overlay of molecular electrostatic potentials [41] of heterocyclic amines (substrates and inhibitors).	[42]
CYP2C9	An update of an earlier model with 14 additional compounds. Inclusion of these compounds in the initial model did not seem to change the model appreciably.	[26]
	A pharmacophore model based on 27 substrates constructed within the active site of a homology model (based on the CYP2C5 X-ray structure [15]). The model has been used successfully to predict the metabolism of a variety of compounds. The interaction of substrates with Arg108 (within active site) was recently confirmed by the first wild-type X-ray structure of CYP2C9 with flurbiprofen bound in the active site [13].	[11]
	A comparison of fingerprints/pharmacophores based on complementary properties on substrates and in active site of homology model validated 43 diverse substrates (87 metabolic pathways). In 90% of the cases the experimental site of metabolism was in the top three predicted sites of metabolism.	[43]
	A heteroactivator model based on 37 compounds includes two hydrophobic regions, a hydrogen bond acceptor and an aromatic ring.	[44]
CYP2D6	A model based on 24 substrates capable of ranking predicted versus 28 observed K_m values. The pharmacophore consists of a hydrogen bond acceptor, two hydrophobic regions and a positive ionisable feature and was docked into a CYP2D6 homology model.	[45]
CYP3A4	A model based on testosterone, α-naphtoflavone, progesterone, artemisinin, quinidine and felbamate, consisting of two hydrogen bond acceptors separated by two hydrophobes.	[46]
	A pharmacophore for inhibitors including two aromatic rings, a hydrogen bond acceptor and a hydrophobic region.	[47]
	A manual pharmacophore generated on the basis of 15 CYP3A4 substrates and docked into a homology model.	[48]

Homology modelling is an iterative process. A detailed analysis of the individual steps involved in the production of a homology model is available elsewhere (e.g. Kirton *et al.* [14]). Once the amino acid sequence alignment is produced, the next step is to derive a 3D model. In the case of the P450s, the target often shares low sequence identity with the known crystal structures. Using more than one template therefore generates a set of models that hopefully provide a better representation of the target protein than if a single template was used. The results of homology modelling are crucially dependent on the choice of the structural template(s) and the sequence alignment used.

TABLE 2

Available P450 crystal structures

Bacteria		**Fungi**	
CYP51B1 (14α-sterol demethylase)	[49]	CYP55A1 (nor)	[50]
CYP101A1 (cam)	[51]	CYP119	[52]
CYP102A1 (BM-3)	[53]		
CYP105A3 (sca-2)	[54]		
CYP107A1 (eryF)	[55]		
CYP108A1 (terp)	[56]	**Mammals**	
CYP121	[57]	CYP2A6 (human)	[32]
CYP152A1 (BSβ)	[58]	CYP2B4 (human)	[18–20]
CYP154A1	[59]	CYP2C5 (rabbit)	[16,17]
CYP154C1	[60]	CYP2C8 (human)	[31]
CYP158A2	[61]	CYP2C9 (human)	[12,13]
CYP165B3 (OxyB)	[62]	CYP2D6 (human)	[21]
CYP165C4 (OxyC)	[63]	CYP3A4 (human)	[22,23]
CYP167A1 (epoK)	[64]		
CYP175A1	[65]		

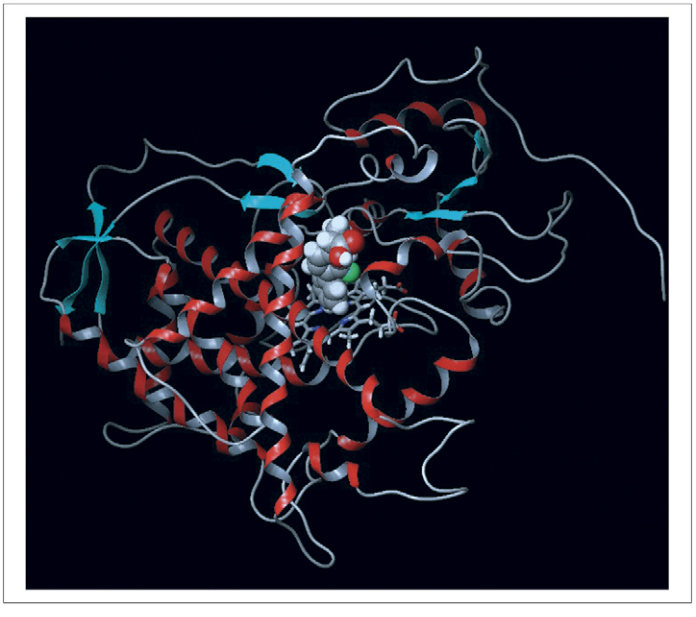

FIGURE 2

A homology model for human CYP2C9 [11] based on the crystal structure of rabbit CYP2C5 [15]. α-Helices and β-sheets are shown. Flurbiprofen is shown in CPK style (atoms shown with van der Waals radii) above the heme moiety.

In the absence of experimental data, homology models can be used to flag up possible protein–drug interactions by providing an insight into which residues in the protein seem to be important in the binding of the ligand and how the candidate could be modified to increase or decrease its affinity for a particular target. Several homology models of different human isoforms have been produced in a variety of ways [4], mainly on the basis of crystal structures of soluble bacterial and fungal P450s of which there are currently 15 available in the protein data bank (PDB; Table 2). The identity between the amino acid sequences of mammalian P450 sequences and the sequences of these crystal structures is, however, low (generally <20%) so any model produced is inherently limited by the fact that an accurate amino acid sequence alignment between templates and target is difficult to obtain.

The solution of the first mammalian crystal structure (rabbit CYP2C5 [15]) was a major leap forward in the ability to model the human P450 isoforms because of the structure's increased sequence identity to the target human proteins compared with the bacterial and fungal P450s. Additional crystal structures of rabbit CYP2C5 with bound substrates [16,17] and the structure of engineered rabbit CYP2B4 [18–20] have become available. More recently, several additional human P450s have been crystallized (Table 2) including CYP2C9 [12,13], CYP2D6 [21] and CYP3A4 [22,23], three of the major drug-metabolizing P450s in humans. All currently available crystal structures for P450s are summarized in Table 2, while an overview of 3D homology models derived for P450s has been published elsewhere [4,7].

Future prospects

The number of P450s examined using pharmacophore and 3D-QSAR models is mainly limited to those few P450s of significant interest to drug metabolism [4]. Pharmacophore- and 3D-QSAR-derived approaches have evolved from crude comparisons of molecules to highly advanced models combining features of ligand-based models with protein homology models (e.g. models for CYP2B6 [24], CYP2C9 [11,25–28] and CYP2D6 [29,30]). The more advanced models produce qualitative, quantitative and visual approximations of P450 ligands that have both increased our insight into the P450 active sites and supported the design of novel drug candidates. Such models demonstrate the potential for predicting the possible involvement of specific P450 isoenzymes in the metabolism of selected substrates or predicting drug–drug interactions. The data available for generating ligand-based models for various P450s are not always of sufficient quality (or quantity), however, to warrant their use in pharmacophore modelling.

In the absence of crystal structures, homology modelling has been shown to be a valuable tool for gaining an insight into the interaction between substrates and P450s. The success of homology modelling is based on the continual determination of high-quality X-ray structures. The use of homology models has shown that a valuable insight can be gained into drug–receptor interactions before crystal structures become available. For example, docking studies for CYP2D6 predicted an acidic residue, Glu216, as a major determinant in the binding of basic substrates and suggested that Asp301, thought previously to be a direct binding determinant, had an indirect role. Both of these hypotheses have been supported by experimental work. The recent crystal structure of CYP2D6 [21] implicates both residues and helps to explain how these residues act as substrate-binding residues.

With the availability of mammalian P450 crystal structures [12,13,15–19,21–23,31,32] and the prospect that additional crystal structures will be available in the near future, the generation of homology models for P450s has received a boost, increasing reliability in the more diverse regions of the P450s. So far, the availability of the various human crystal structures has not had much of an impact on early-phase drug discovery. The homology model of CYP2C9 (Figure 2 [11]) is in very good agreement with the CYP2C9 crystal structure [12,13] and, as a consequence, the pharmacophore model (Figure 1 [11]) can be easily accommodated within the CYP2C9 crystal structure, identifying the appropriate interactions between substrate and protein.

A single crystal structure for a given enzyme–substrate interaction might not, however, provide a good description of the binding mode responsible for product formation, as was noted for the bacterial CYP101A1–nicotine complex [33]. Flexibility of the P450 active sites [18,34] also has to be taken into account when constructing binding models. An additional complicating factor is the possibility that more than one substrate binds simultaneously within the active site of a single P450 [23,35]. For CYP3A4, at least three different binding sites were identified with testosterone, α-naphtaflavone and midazolam occupying these sites [36], which eventually led to all three of the substrates being used in CYP3A4 inhibition experiments. Consequently, as more high-quality crystal structures become available for biotransformation enzymes, homology models with and without pharmacophore and/or 3D-QSAR models within their active sites will become more reliable and further enhance computational chemistry techniques.

Models for all the key P450s involved in drug metabolism have already been constructed. Adding models for more P450s will aid our understanding of P450-mediated metabolic pathways of molecules. More impact might be made by using ligand-based and homology modelling when the focus from metabolism and inhibition is widened to include the prediction of induction [37] and clearance [38] for individual P450s.

Combined with other ADME–Tox models – for example hERG (human ether-a-go-go-related gene) [39], solubility [40], and so on – the P450 models will be a valuable tool to assess ADME–Tox-related issues at a very early stage in the drug discovery process, providing an insight into drug–drug interactions using *in silico* methodologies. As the software used for generating these models moves towards the desktop, chemists will have the ability to dock substrates into P450s or assess the potential for drug–drug interactions without the intervention of a computational chemist or computational ADME scientist.

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