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Cytochrome P450 Enzymes in Drug Metabolism and Chemical Toxicology

AN INTRODUCTION

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Cytochrome P450 (P450) enzymes include a family of related enzymes that are involved in metabolism of vitamins, steroids, fatty acids, and other chemicals. This review presents a brief historical overview of the discovery and characterization of P450 enzymes extending from intermediary metabolism to the fields of drug metabolism and toxicology. Introductions to P450 enzyme structure and function are also presented. The goals of this review are to 1) provide an introduction to a few of the many aspects of P450 research relating to humans, 2) introduce additional ways of thinking about metabolism, 3) provide some basic examples of P450 enzymology, and 4) provide applications to topics widely taught in undergraduate courses in biochemistry.

Keywords: Cytochrome P450 enzyme, drug metabolism, chemical toxicity, P450 mechanism.

Cytochrome P450 (P450)[1] enzymes are a family of heme-containing proteins found from bacteria to human. In mammals, the enzymes are membrane-bound (usually in the endoplasmic reticulum, although the seven in Families 11, 24, and 27 are found in mitochondria; Table I). P450s serve a variety of functions including steroid, fatty acid, eicosanoid, and vitamin A and D metabolism as well as metabolism of foreign compounds including natural products, pharmaceuticals, and carcinogens. P450s are found in every tissue except skeletal muscle and red blood cells [1]. With the completion of the human genome sequence project, there appear to be 57 distinct *P450* genes in humans [2]. Of these, about 13 are "orphan" P450s for which the metabolic function is not yet known [3].

There is wide interest in P450s due to applications in a variety of fields including biochemistry, biotechnology, chemistry, environmental sciences, enzymology, microbiology, physiology, pharmacology, plant sciences, and toxicology. Indeed, in the last decade alone, there were more than 1000 publications per year relating to P450s. This review will highlight the roles of human P450s in drug metabolism and chemical toxicity and applications to undergraduate studies. References to more in-depth reviews are also provided.

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[1] The abbreviations used are: P450, cytochrome P450 enzyme; PDB, Protein Data Bank.

DISCOVERY AND PURIFICATION

The history of P450 can be traced back to questions related to the metabolism of steroids, drugs, and carcinogens in the 1940s. Early studies of difficult alkyl oxidations of amino acids and fatty acids, such as oxidation at the terminal methyl group of a fatty acid, allowed for the isolation of cell-free extracts capable of performing NAD(P)H-dependent oxidation reactions [4]. The "mixed-function oxidase" stoichiometry.

$$NAD(P)H + H^+ + O_2 + RH \rightarrow NADP^+ + H_2O + ROH$$

REACTION 1

that is now recognized as the basic reaction catalyzed by a P450 was not immediately obvious. In particular, the requirement for a reduced pyridine nucleotide to achieve an overall oxidation was surprising at the time. The concept of mixed-function oxidation was developed by O. Hayaishi *et al.* in Japan [5] and H. Mason in the U.S [6]. Subsequently, such reactions were shown to occur with steroids and xenobiotics, particularly drugs (J. Axelrod and B. Brodie *et al.*) [7, 8] and carcinogens (Mueller and Miller) [9]. For an interesting set of perspectives on the history of metabolism of xenobiotics (chemicals not normally found in the body), see the web site www.issx.org/pages/page04a.html.

P450 was characterized as a pigment in liver extracts by D. Garfinkel [10] and M. Klingenburg [11] in the 1950s and then by T. Omura and R. Sato in Japan [12]. The pigment absorbed light maximally at 450 nm when reduced in the presence of carbon monoxide. Thus, the term P450 was

TABLE I
Classification of human P450s based on major substrate class [1-3]

Sterols	Xenobiotics	Fatty acids	Eicosanoids	Vitamins	Unknown
1B1	1A1	2J2	4F2	2R1	2A7
7A1	1A2	4A11	4F3	24	2S1
7B1	2A6	4B1	4F8	26A1	2U1
8B1	2A13	4F12	5A1	26B1	2W1
11A1	2B6		8A1	26C1	3A43
11B1	2C8			27B1	4A22
11B2	2C9				4F11
17A1	2C18				4F22
19A1	2C19				4V2
21A2	2D6				4X1
27A1	2E1				4Z1
39A1	2F1				20A1
46A1	3A4				27C1
51A1	3A5				
	3A7				

coined for "pigment-450" [13]. This phenomenon is unusual since all other known heme-containing proteins, such as hemoglobin, absorb light at 420 nm. The difference with P450s is due to the identity of the fifth ligand to the heme formed by a cysteine residue in P450s as opposed to a histidine fifth ligand with proteins such as hemoglobin.

A role of P450 in steroid hydroxylation was established by D. Cooper *et al.* [14], and the work was carried over to other substrates. A major advance in the study of these mixed-function oxidase reactions occurred in the 1960s with the discovery of a soluble (non-membrane-bound) P450 from the bacterium *Pseudomonas putida* by I. Gunsalus and co-workers [15]. This protein was termed $P450_{cam}$ (now P450 101A1) because it was isolated from a pseudomonad selected to grown on the terpene camphor as a sole carbon source, and the protein had a high rate of camphor oxidation, thus allowing for growth. With large quantities of a soluble P450 available, purification of the enzyme for kinetic, structural, and mechanistic studies was more readily possible. Indeed, much of the early P450 mechanistic and structural work was accomplished with P450 101A1 (*vide infra*).

In the late 1960s, evidence began to accumulate that multiple forms of P450 might exist in the mammalian liver endoplasmic reticulum. A major limitation in this era was the difficulty in separating and purifying P450 from mammalian tissues. In 1968, M. J. Coon and A. Y. H. Lu, working at the University of Michigan, utilized detergents to separate the components of the P450 system from rabbit liver microsomes, namely P450 enzyme, NADPH-P450 reductase, and phospholipids. As noted, P450s are membrane-associated proteins that require reducing equivalents, *i.e.* electrons, from NADPH. Those electrons are usually provided indirectly via an accessory enzyme, NADPH-P450 reductase, that is also bound in the membranes of the endoplasmic reticulum (Fig. 1). When recombined, these isolated components together "reconstituted" a system that was catalytically active in the 12-hydroxylation of lauric acid [16]. This work led to the isolation of multiple forms of P450 from rabbit and rat liver in Coon's laboratory and others. Subsequently, several of the major human liver P450s involved in drug metabolism were purified. The work was technically challenging, and the recovery of purified P450 3A4 ($P450_{NF}$), the most abundant human P450, was only ~3 mg from 400 g of human liver [17].

In the 1980s, recombinant DNA technology became available, and the sequences of many of the P450s were obtained. Later, it became possible to produce recombinant P450s in heterologous vector systems expressed in *Escherichia coli* or other expression systems, and large amounts of individual P450s can now be produced and rapidly purified (for instance, from 1 liter of bacterial culture, it is routinely possible to get 25 mg of recombinant human P450 in less than a week in some cases). Another, more recent, development is the delineation of the human genome, which reveals 57 human *P450* genes, as judged by the presence of the signature F*XX*G*XXX*C*X*G sequence near the C terminus involved in heme binding (via the indicated Cys).

CLASSROOM APPLICATION NOTE

The movement of electrons in the P450/reductase system is shown schematically in Fig. 1. Students could be asked to explain this direction of electron movement based on what they have learned from the mitochondrial electron transport system covered in most undergraduate biochemistry courses, *i.e.* movement from lowest to highest standard reduction potential ($\xi^{o\prime}$) with O_2 being the final electron acceptor. In addition, they could be asked to show the differences in the electronic structures of the pyridine nucleotide, flavins, and iron during the electron movement.

NOMENCLATURE AND DISTRIBUTION

In the initial purification of P450 enzymes, names relating to substrate specificities or product specificities were useful. However, as it became clear that there were multiple and overlapping substrate specificities among the P450s, a more systematic naming system was required. P450 nomenclature now follows a consistent method, developed by Nebert *et al.* [18], that also provides information relating to enzyme sequence similarity. P450 names are given with a number-letter-number designation with the first number indicating the P450 family based on 40% or greater sequence identity, the letter indicating the P450 subfamily based on 55% or greater sequence identity, and

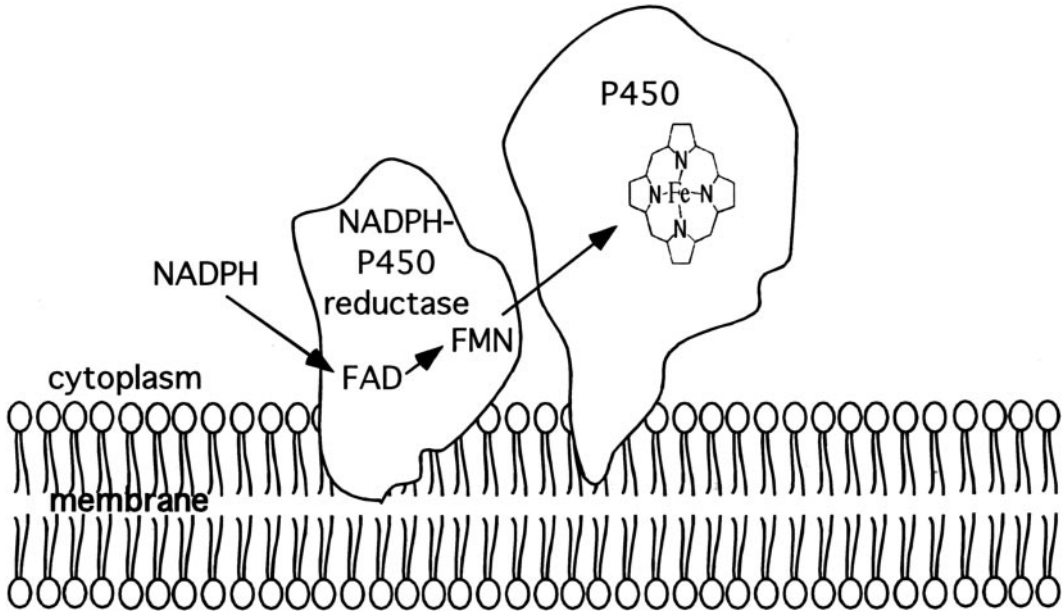

FIG. 1. **Schematic of P450 and NADPH-P450 reductase system and the route of electron transfer.** Electrons for the reduction of molecular oxygen are provided by the cofactor NADPH via the flavoprotein NADPH-P450 reductase. NADPH-P450 reductase contains two redox prosthetic groups, FAD and FMN. Electrons are passed from NADPH to the FAD and then the FMN of NADPH-P450 reductase and finally to the heme of P450. Both P450 and NADPH-P450 reductase are membrane-associated proteins. NADPH is located in the cytoplasm. With the seven P450s normally found in the mitochondria, electrons are transferred from NADPH to the flavoprotein NADPH-adrenodoxin reductase to a non-heme iron protein called adrenodoxin and then to the P450s (not shown). Electrons can also be passed from NADH to P450 via cytochrome b_5, another membrane-bound protein not shown.

the final number representing the individual P450 within that family/subfamily (Fig. 2). Thus, for example, in humans, the P450 1 family has two subfamilies, A and B. There is only one member in the 1B family (1B1), and there are two members in the 1A subfamily (1A1 and 1A2). All three individual members of the P450 1 family have >40% sequence identity with the two members in the 1A subfamily having >55% identity.

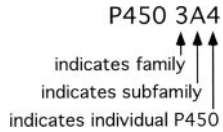

FIG. 2. **P450 nomenclature.** P450s are numbered by family, subfamily, and individual members within subfamilies. P450s are placed in families based on ≥40% sequence identity and in subfamilies based on ≥55% sequence identity.

P450 enzymes are expressed throughout nature. Some enterobacteria do not have P450s, and the yeast *Saccharomyces cerevisiae* has only three. More complex bacteria that synthesize complex natural products (*e.g.* antibiotics) have more P450s, *e.g. Streptomyces avermitilis* has 32. The role of P450s in synthesis of antibiotics is currently being exploited as an approach for development of new antibiotics [19]. As mentioned earlier, humans have 57 *P450* genes, in the range of some other mammals. Plants have much larger numbers, *e.g. Arabidopsis thaliana* has 273 and rice has 452. These P450s are involved in the formation of complex secondary metabolites such as terpenes, alkaloids, and pigments (drnelson.utmem.edu/CytochromeP450.html).

Within mammals, about one-third of the P450s are found in the liver, and most of these function in the degradation of xenobiotics. Many of the P450s involved in the metabolism of sterols are found in steroidogenic tissues such as the adrenal glands, testes, and ovaries. Other P450s with specialized functions are found in appropriate sites, *e.g.* thromboxane synthase (P450 5A1) in platelets.

CLASSROOM APPLICATION NOTE

Instructors may provide students with two or more human P450 sequences and ask students to determine whether the two enzymes are in the same family or subfamily using bioinformatics tools such as CLUSTAL-W at European Bioinformatics Institute (www.ebi.ac.uk/clustalw) or at Pôle Bioinformatique Lyonnais (pbil.univ-lyon1.fr). If sequences from P450s with known structures are used, students could then identify specific regions of sequence similarity using the bioinformatics tool ConSurf (consurf.tau.ac.il) in conjunction with Protein Explorer (www.umass.edu/microbio/rasmol). Students may then compare the output of the two sequence comparison tools (CLUSTAL-W *versus* ConSurf). Tutorials for the use of these free bioinformatics programs are available from a variety of sources. At Kalamazoo College, the program developed by Feig and Jabri is used [20]. Students could also be asked to predict which amino acids in P450 are most likely to be conserved (as noted, the heme binding sequence is signature of a P450).

ROLES OF P450s IN INTERMEDIARY METABOLISM

One way to classify the P450s, particularly in mammals, is by the nature of the substrates they oxidize, as shown for the human P450s in Table I [1]. About one-fourth of the P450s are involved in sterol metabolism. Most of these P450s are localized in extrahepatic tissues, although some involved in cholesterol oxidation are in the liver (*e.g.* P450 7A1, which catalyzes the first step in bile acid formation).

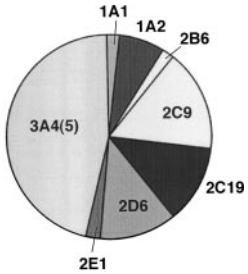

FIG. 3. **P450s involved in drug metabolism.** The chart shows approximate contributions of individual P450s to the metabolism of drugs currently marketed. The figure was adapted from Ref. 22.

Genetic deficiencies in most of these P450s are usually debilitating [21]. Five of the human P450s have roles in the metabolism of prostaglandins, including two unusual P450s that synthesize thromboxane and prostacyclin (5A1, 8A1). Six of the P450s have defined roles in the metabolism of the fat-soluble vitamins (A and D).

There is still limited information about one-fourth of the human P450s (Table I), and these can be described as "orphans" in this sense [3]. Presently, there is little information about how essential these P450s are. More knowledge of substrates can be developed from biochemical studies with isolated enzymes and from studies with transgenic animals in which the apparent orthologs have been deleted.

Unlike most enzymes covered in traditional biochemistry courses, P450s generally accommodate many substrates and form a variety of products. This is particularly true of the "xenobiotic-metabolizing" P450s, which may accommodate possibly thousands of different substrates. Furthermore, although most of the metabolic pathways and metabolism topics taught in traditional biochemistry courses were discovered nearly half a century ago or more, metabolism mediated by P450 enzymes of exogenous compounds is more recent and still unfolding. This is especially true in relation to metabolism of pharmaceuticals and potential carcinogens, and many interesting aspects of these enzymes have been revealed from this work.

ROLE OF P450s IN DRUG METABOLISM

As roles for P450 in metabolism of endogenous compounds were being elucidated, roles for P450s in the metabolism of exogenous compounds, particularly drugs, natural products, and other compounds were also being discovered. It is now recognized that P450s are involved in the metabolism of most pharmaceutical compounds (Fig. 3). In humans, P450s 3A4, 2D6, 2C9, 1A2, and 2C19 perform the majority of drug metabolism, with some contribution from P450s 2E1, 2A6, 2C8, and 2B6 [22] (Fig. 3).

The majority of P450 drug metabolism occurs in the liver and the small intestine. P450 3A4 and P450 2C9 are the two most abundant P450s in these tissues, so it is not surprising that they account for ~70% of the metabolism of pharmaceuticals [22] (Fig. 3). P450s 3A4 and 2C9 also accommodate substrates larger in size than those of the other P450s involved in drug metabolism. For instance, P450 2E1 is involved in the oxidation of small molecules such as ethanol (molecular mass 46) and chlorzoxazone (molecular mass 169), whereas P450 3A4 metabolizes drugs such as lovastatin (molecular mass 423), finasteride (Propecia®) (molecular mass 372), and sildenafil (Viagra®) (molecular mass 475). Many pharmaceutical companies prefer to develop larger drug molecules due to increased target specificity and affinity of larger compounds [1], tending to favor drug metabolism by P450s 3A4 and 2C9.

The Food and Drug Administration requires information relating to specific P450s involved in the metabolism of each new drug very early in the testing and registration process. In addition, data are required relating to any possible inhibition of other P450s by the new drug. Thus, pharmaceutical companies have groups assigned to these tasks, which allow for prediction of drug-drug interactions and variations in bioavailability. Upon drug approval for market, this information is made available for patients and physicians. Even drug advertisements in lay magazines include a drug information sheet (the "fine print") that will usually specifically mention individual P450s involved in the metabolism of the drug or those P450s possibly inhibited by the drug. This information may also be relayed indirectly by listing possible drug interactions or contraindications for other drugs.

For instance, individuals taking birth control pills, which all contain 17α-ethynylestradiol as the active estrogenic component, are advised to avoid certain other drugs such as rifampin (antibiotic) and phenytoin (Dilantin®, an antiepileptic drug related to the barbiturates). The biochemical explanation for these contraindications requires an understanding of the role of P450s in drug metabolism. The 17α-ethynylestradiol used in oral contraceptives is metabolized to a form that is more rapidly cleared from the body, the 2-hydroxy product, by P450 3A4 (most of the progestins in birth control pills are also metabolized by this enzyme). Both rifampin and phenytoin induce expression of P450 3A4 enzyme. More enzyme present means more catalyst for the metabolism of 17α-ethynylestradiol to its rapidly cleared metabolite. Thus, the steroid is excreted from the body at a rate faster than normal, and the blood plasma concentration of 17α-ethynylestradiol will fall when taken in conjunction with rifampin or phenytoin (or other P450 3A4 inducers). This may lead to ovulation and unexpected pregnancy. It is of interest to note that the same response occurs with some herbal medicines that are not regulated by the Food and Drug Administration (and vary considerably in their active ingredients, which have not been characterized in many cases). St. John's wort, which has been used to alleviate depression, contains a potent P450 3A4 inducer, hyperforin [23], that has led to unexpected pregnancies as well [24].

Thus, because P450 3A4 is involved in the metabolism of many different drugs (almost 50% of those on the market; Fig. 3), modulation of its activity can lead to changes in plasma concentrations, *i.e.* bioavailability, of any drugs for which it is the primary metabolizer. The same principle can be applied to other P450s involved in drug metabolism and to drugs that inhibit P450 activity. In the case of P450 inhibition by a drug, plasma concentrations of another drug may greatly increase, leading to possibly severe side effects or even death. A now classic case is the inhibition of P450 3A4 by azole drugs and erythromy-

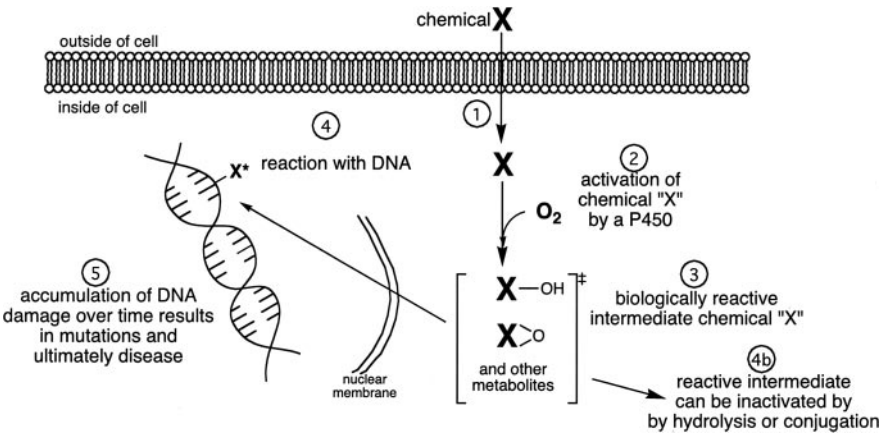

FIG. 4. **Role of P450s in chemical toxicity/carcinogenesis.** *1*) Absorption of chemical; *2*) biotransformation of chemical to biologically active intermediate by P450; *3*) active intermediates (possibly multiple); *4*) covalent attachment of intermediate with DNA (or protein); 4*b*) inactivation of intermediate by other enzymes; *5*) DNA injury, and ultimately cancer, or other disease.

cin, which resulted in decreased metabolism of terfenadine (Seldane®) and some deaths due to arrhythmia [25, 26]. At first, the drug required a warning label about these contraindications, but subsequently, terfenadine was withdrawn and replaced with its metabolite, fexofenadine (Allegra®), which does not have these issues.

Furthermore, levels of drug-metabolizing P450s vary considerably among individuals, whereas those involved in metabolism of endogenous substrates generally do not. This knowledge helps to explain partly differential responses to drugs by different individuals. In addition, P450s 2D6, 2C9, and 2C19 involved in drug metabolism also display common polymorphisms, *i.e.* differences in amino acid sequence that produce altered and usually lowered enzyme activity. Polymorphisms in these enzymes can therefore lead to poor drug metabolism and more severe side effects with drugs that are metabolized by these three P450s. With this knowledge, pharmaceutical companies are now very cautious in the development of drugs that are known to interact with these P450s.

CLASSROOM APPLICATION NOTE

A classic example of a P450 3A4 inhibitor is grapefruit juice, specifically the furanocoumarins related to bergamottin in the juice. Inhibition of 3A4 by grapefruit juice components was discovered rather serendipitously in a study with an antihypertensive drug, felodipine. The purpose of the study was to determine whether drinking ethanol affected the clearance of felodipine from the body. The ethanol was mixed with grapefruit juice to disguise the taste. A strong effect was noted (slower clearance of the drug), but the same effect was seen with grapefruit juice without ethanol [27]. No effect occurred with orange juice. (The importance of controls should be stressed here.)

Many of the students in class may know or find out that their parents or grandparents are instructed by their physicians not to take medications with grapefruit juice. Students could be asked to explain how an inhibitor of P450 3A4 taken with a drug metabolized by P450 3A4 could cause severe side effects. Alternatively, students could suggest a method for isolation of the active ingredient of grapefruit juice that causes the inhibition (this has not been accomplished as easily as might be expected).

The account of terfenadine is usually of interest to students, particularly premedical and medical students. One approach is to ask students how many regularly take antihistamines and then how many use Allegra®. The background on terfenadine is of interest and emphasizes the practical use of biochemistry and enzymology in medical practice and drug development.

ROLES OF P450s IN CHEMICAL TOXICITY

Although P450s are involved in the metabolism of pharmaceutical chemicals, they are also the primary catalysts for the metabolism of other foreign compounds including natural products, pollutants, and other chemicals. Not surprisingly, many of the P450s involved in drug metabolism are also the same P450s involved in metabolism of other xenobiotic chemicals [1]. Oxidation of some compounds is beneficial for removal of hydrophobic, potentially toxic, chemicals. However, in some cases, oxidation by P450 can result in production of a toxic metabolite, *e.g.* carcinogen. Indeed, chemical carcinogens including polycyclic aromatic hydrocarbons (from incomplete combustion), heterocyclic amines (from charbroiled meats), aromatic amines (dyes, pesticides, tobacco smoke, pharmaceuticals), nitrosamines (tobacco smoke and diet, formed from nitrites and nitrates), and nitropolycyclic hydrocarbons (from incomplete combustion) are transformed to their carcinogenic forms by P450 enzymes. In this model, a chemical carcinogen is converted by a P450 to a biologically reactive intermediate, usually an electrophile such as an epoxide, hydroxylamine, acyl halide, etc. [28]. (Fig. 4). Reactive intermediates, even those that are short-lived, are able to become covalently attached to nucleophiles such as found in proteins, lipids, or DNA causing cell damage (Fig. 4). Scavenger systems (such as glutathione) that conjugate the electrophiles and reduce the toxic effect of the reactive metabolite also operate, but these can be depleted in the presence of high doses of electrophile.

Because some chemicals can be activated to toxic or carcinogenic products via P450 oxidation by P450s, there is interest in developing inhibitors of P450s as “chemopreventive” agents. Such inhibitors have long been known to be able to prevent acute toxicity or cancer in experimental animal models [29]. Precedence exists in the use of inhibitors of P450 19A1, which converts androgens to estrogens, as therapeutic agents in the treatment of estrogen-dependent tumors [30]. The drug oltipraz can block the activation of the mycotoxin aflatoxin B_1 in humans [31, 32]. Although inhibition of P450s has potential in chemoprevention strategies, the application is still very limited.

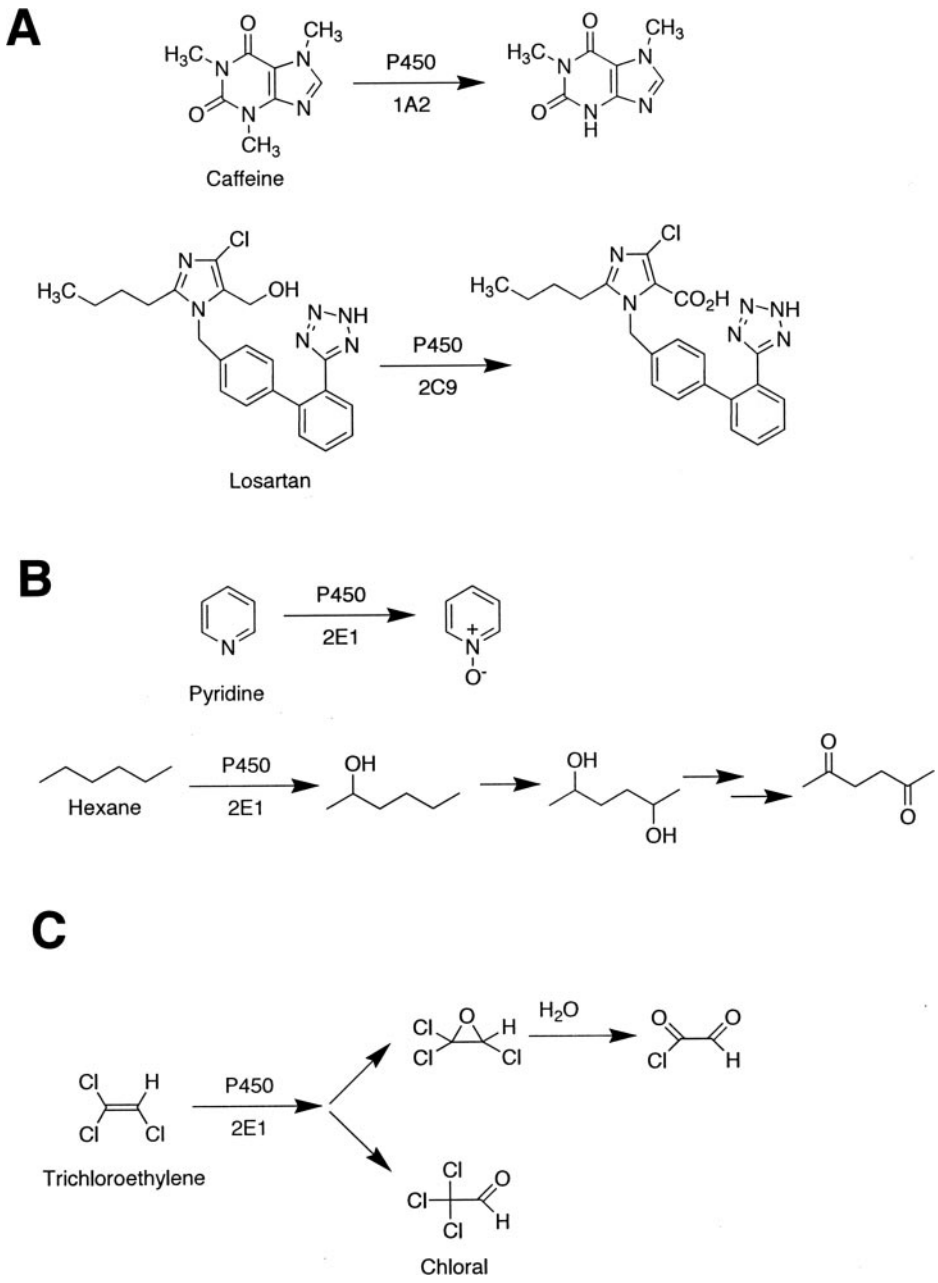

FIG. 5. **Example P450 chemical transformations.** P450s are involved in the metabolism of drugs and other xenobiotics and use many reaction types. *A*, during drug metabolism, P450s most commonly inactivate a drug, as is the case with caffeine (stimulant) (heteroatom dealkylation) [35]. P450s may also convert a drug into a more highly active form, as is the case with losartan (antihypertensive) (oxidation). *B*, during metabolism of other chemicals, P450s may inactivate a toxicant, such as observed with pyridine (solvent) (heteroatom oxygenation), or activate a chemical to a toxic metabolite, as observed with hexane (solvent) (hydroxylation and further oxidation of the alcohols to ketones). *C*, in some cases, P450s may produce a mixture of deactivated and toxic products, as is the case with trichloroethylene (solvent, common contaminant in waste dumpsites) (epoxidation followed by rearrangement to a more reactive product [36], and olefin oxidation accompanied by a 1,2 migration of chloride ion to the deactivated product chloral, which is actually a sedative [37]). Despite the differences in reaction types, all reaction mechanisms proceed via the generalized P450 catalytic cycle shown in Fig. 6.

CATALYSIS BY P450s

The basic mixed-function oxygenase reaction presented earlier suggests that the products of P450 reactions are alcohols. However, reactions are not limited to carbon hydroxylation reactions (Fig. 5). Typical P450 reactions, reviewed in detail in [33, 34], include hydroxylation, dealkylation, epoxidation, heteroatom oxygenation and dealkylation, group migration, oxidation of olefins and acetylenes, and heme inactivation. Furthermore, there is an even larger number of seemingly unusual reactions also characterized for P450 enzymes including ester cleavage, ring expansion, ring formation, rearrangements, isomerization, desaturation, and reduction (reviewed in [33] and [34]). These seemingly unusual reactions can be rationalized using the same general catalytic mechanisms as the major reactions. Furthermore, these reactions are not particular to activation or deactivation pathways, and the prediction of metabolic pathways (*i.e.* activation or deactivation) for a compound is not always obvious. That is, P450s may deactivate a drug to increase its clearance from the body, as is the case with heteroatom dealkylation reaction with caffeine [35] (Fig. 5*A*). Alternatively, P450s may increase the intrinsic (pharmacodynamic) activity of a drug, as in the case of oxidation of an alcohol to an aldehyde and then a carboxylic acid for losartan (Fig. 5A). The xenobiotics vary, *e.g.* the solvents pyridine and hexane (Fig. 5B). The oxidation product of pyridine is inactive, but the final product of hexane oxidation, 2,5-hexanedione, can cross-link proteins. To complicate matters, it is also not unusual for multiple products with varying metabolic activity to be formed from P450 reactions on a single substrate as with trichloroethylene [36, 37] (Fig. 5*C*). Thus, understanding of the P450 catalytic mechanism is one part of rationalizing and predicting the roles of P450s in drug and xenobiotic metabolism.

The basic catalytic cycle for cytochrome P450s involves

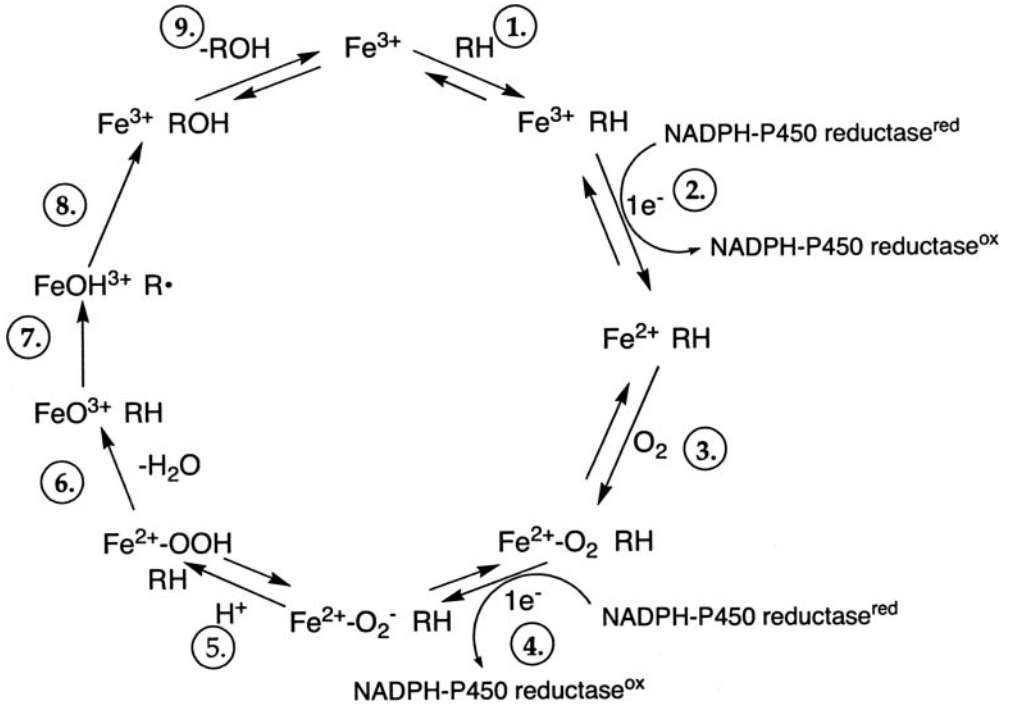

FIG. 6. **Generalized P450 catalytic cycle.** Only the heme iron of P450 is shown to represent the active site of the P450. the abbreviations are as follows: *Fe*, iron atom in P450 heme; *RH*, substrate; ROH, product; *ox* and *red*, the reduced and (1-electron) oxidized states of P450 reductase involved in electron transfers (under "Catalysis by P450s"). The figure was adapted from [1, 34].

several steps, outlined in Fig. 6 [33]. The cycle begins with substrate binding to oxidized P450 (step 1). Following substrate binding, the first electron is provided to the P450 from NADPH-P450 reductase, to reduce the P450 heme to the ferrous state (step 2). Molecular oxygen then binds to the ferrous P450 followed by transfer of a second electron and activation of oxygen (steps 3 and 4). The addition of a proton (step 5) is followed by O–O bond cleavage and release of H_2O (step 6). An electron-deficient FeO^{3+} formal complex is left behind. This complex then abstracts a hydrogen atom or an electron from the substrate to produce a substrate radical (step 7), and the "second" atom of oxygen is transferred to the incipient substrate radical (step 8). Finally, product is released with the heme iron returned to the ferric state (step 9). Although this mechanism accounts for much of the observed chemistry of P450 enzymes, it does not show important protein conformational changes that have also been observed during the P450 catalytic cycle or interactions with individual amino acids that may be important for catalysis. I. Schlichting *et al.* [38] have provided a complete atomic resolution view of the P450 catalytic cycle based on trapping techniques and time-resolved crystallography of P450 101A1, a soluble bacterial P450 (*vide supra*).

The general reaction mechanism of P450s presented in Fig. 6 can be applied to specific P450 reaction types such as carbon hydroxylation (Fig. 7). In carbon hydroxylation, after the electron-deficient P450 FeO^{3+} complex is formed in association with substrate (Figs. 6 and 7, *step 6*) a hydrogen atom is abstracted from the substrate (Figs. 6 and 7, *step 7*). This abstraction process generates a transient radical followed by the rapid addition of a hydroxyl group (oxygen rebound) (Figs. 6 and 7, *step 8*). At the same time, desaturation reactions are also often observed, although at slower rates [34] (Fig. 7, *step 8b*). The desaturation reaction begins in the same way as the carbon hydroxylation but follows an alternative ending (that is, the process involves bifurcation of an enzyme intermediate). In this case, a second hydrogen atom is abstracted by the P450 $FeOH^{3+}$ complex to generate H_2O, a desaturated product, and regenerated ferric P450 (Fig. 7, *step 8b*). There are many more applications of the P450 catalytic mechanism to specific and diverse reactions that have been well characterized, and the reader is referred to more examples in [33] and [34].

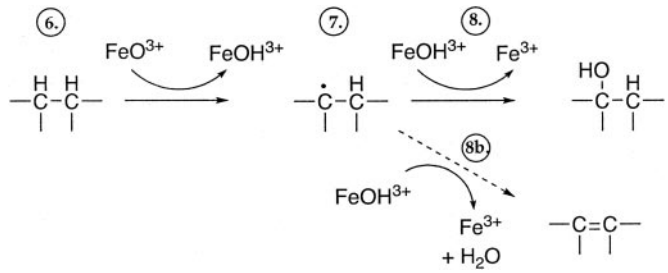

FIG. 7. **Carbon hydroxylation and desaturation reactions catalyzed by P450.** The basic P450 carbon hydroxylation reaction is shown in relation to the catalytic cycle of P450 shown in Fig. 6. The hydroxylation product and the desaturation product are both formed from the same radical intermediate generated by hydrogen abstraction by the electron-deficient P450 FeO^{3+} complex formed at the end of step 6 of the P450 catalytic cycle depicted in Fig. 6. The figure was adapted from [34, 39].

CLASSROOM APPLICATION NOTE

The basic reaction scheme of P450s can be used to remind students of the difference between cofactors (which show up in the reaction stoichiometry, *e.g.* NADPH) and prosthetic groups (which do not, *e.g.* the heme in

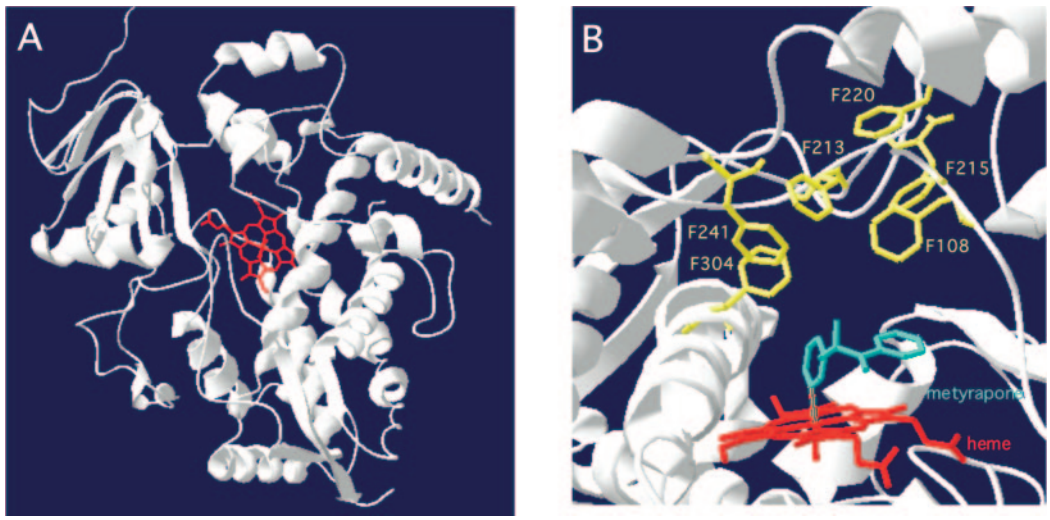

FIG. 8. **Structure of human P450 3A4 with the inhibitor metyrapone bound.** *A*, overall fold of human P450 3A4. *B*, major interactions of metyrapone (*blue*) in the P450 3A4 active site. Metyrapone is bound directly to the (ferric) iron of the heme (shown in *red*) via the alkyl pyridine nitrogen. The non-polar inhibitor is further stabilized within the active site through interactions with the phenylalanine residues that make up the "Phe cluster" (shown in *yellow*). Structures prepared in Swiss Deep View (PDB entry 1WOF [47]).

P450). This cycle can also be used to remind students that prosthetic groups must be regenerated as part of the reaction sequence, *e.g.* the heme iron returns back to the ferric state at the end of the cycle.

STRUCTURES OF P450s

Substrate binding pockets of individual P450s are able to accommodate a range of substrates, unlike most enzymes. P450s also display a range of kinetic activities with different substrates, even cases including both negative and positive cooperativity. Thus, interest in details of the spatial interaction of P450s with different substrates has been great.

The first P450 structure was the soluble prokaryotic P450 101A1, published in 1985 [40]. Until recently, most information relating to structure of human (and all mammalian enzymes) was deduced from modeling human P450s against P450 101A1 or from mechanism-based ("suicide") inhibition studies that allowed for predication of active site amino acids important for substrate binding. Crystallization of mammalian P450s for structural analysis by x-ray crystallography has been difficult due to the hydrophobic nature of these membrane protein samples, complicated by low solubility of many substrates and inhibitors [41]. With advances in cloning and expression of mammalian P450s as well as development of new crystallography software (primarily multiwavelength anomalous diffraction phasing), structures of mammalian P450s have recently been solved, including several human P450s by E. Johnson and the Astex group: 2A6, 2C8, 2C9, and 3A4 [42–47]. Based on crystal structures of P450s now available, it appears that despite differences in substrate selectivity, species origin, or metabolic roles, all P450s display a similar overall fold that consists mostly of α-helices (A-L) and some β-sheet (β1–β4) (Fig. 8*A*). The more variable active site and the heme are located in the α-helical region (Fig. 8). With structures of mammalian P450s now available and more becoming available, it is possible to address some questions relating to substrate and inhibitor binding and kinetics and to better predict interactions of drugs with particular P450s.

CLASSROOM APPLICATION NOTE

Students can use the PDB entries for human P450s 2C9 and 3A4 to manipulate the three-dimensional structures in Protein Explorer, Swiss Deep View, or other molecular visualization tools. Fig. 8 was prepared in Swiss Deep View [48] by undergraduates as part of a course assignment in which students were required to produce a figure that showed an important aspect of P450 3A4 interaction with substrate or inhibitor. Prior to producing this figure, the students had done a brief tutorial with Swiss Deep View available at chemweb.calpoly.edu/chem/lindert/PSE/Proteinstructure-FS.htm.

Alternatively, students can build structural alignments of some of the 17 unique P450 sequences in the PDB using the bioinformatics tool VAST (www.ncbi.nlm.nih.gov/Structure/VAST/vast.shtml). Students could also be asked to propose and discuss models for how a monomeric protein, P450 3A4, can display positive and negative cooperativity.

CONCLUSIONS

P450 enzymes are a family of monooxygenases that participate in the metabolism of not only steroids, fatty acids, eicosanoids, and vitamins but also the many and diverse xenobiotics that humans encounter every day. Catalysis by P450s is important in drug metabolism and chemical toxicology, and the types of reactions are diverse, although all follow the same general chemistry. The findings from structural studies of human P450s are also increasing our understanding of the catalytic activities of these enzymes. Finally, the study of P450 enzymes provides a rich history of traditional biochemistry and enzymology coupled to modern interests in drug metabolism and chemical toxicology that is of interest to undergraduates and can be made accessible to students with a variety of brief exercises in most undergraduate biochemistry courses.

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