Reactions and Significance of Cytochrome P-450 Enzymes*

F. Peter Guengerich

From the Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146

Cytochrome P-450 enzymes (EC 1.14.14.1, non-specific monooxygenase) catalyze the oxidations of many chemicals (1). The mass of the substrates ranges from that of ethylene (M, 29) to that of cyclopasin A (M, 1201). The classification of a hemoprotein as a P-450 is defined by its absorption spectrum; the Fe(II)-CO complex has a characteristic absorption maximum (Soret band) near 450 nm due to axial ligation with a cysteine thiolate of the protein (with or without substrate present). This cysteine residue is present in a relatively well conserved region, ~80% into the protein from the N terminus. Collectively there are thousands of potential substrates for the P-450s; each of the P-450s may have a rather strict limitation of catalytic specificity (e.g. P-450s involved in steroid anabolism) or be a catalyst for the oxidation of many substrates (e.g. some of the inducible P-450s utilized in xenobiotic oxidation). There are many different P-450 enzymes; we now realize that there can be >30 P-450 (or so-called “CYP”) genes expressing their products in a single organism, and many of these are concurrently produced in a single tissue. These genes have been classified on the basis of their coding sequences (2).

Functional Roles of Cytochrome P-450 Enzymes

There is broad interest in the P-450s because of the significance of these enzymes in a wide variety of disciplines, ranging from medical genetics to inorganic chemistry. Historically the P-450 gene family has been considered to be a very large one, with at least 38 genes identified in the rat to date (2). However, numerous other cases of large gene families now exist, such as the steroid receptors, interferons, and the glutathione S- and UDP-glucuronosyl transferases involved in drug metabolism (3, 4). There have been two major views on the function of P-450 enzymes: (i) the enzymes have critical and specific roles in the metabolism of endogeneous chemicals and (ii) the enzymes process the burden of natural products (and, in today’s world, chemicals such as drugs and other xenobiotics, i.e. “foreign” chemicals) in a relatively non-selective manner (5). There is certainly ample evidence for the latter view, and a special case can occur when microorganisms are selected for growth on a particular chemical (or for growth in the presence of a toxic chemical). Thus, Gunsalus and his colleagues (6, 7) were able to develop a useful bacterial model system with a pseudomonad that harbors a plasmid coding the redox system containing P-450 101 (P-450cam), an enzyme which allows growth of the organism on the terpene camphor as the carbon source. More recently, Gunsalus and his colleagues (8) have also isolated the distantly related P-450cam, from bacteria selected for growth on another terpene, linalool. As has been pointed out elsewhere (5, 9), mammals consume a considerable daily burden of natural products such as terpenes, steroids, and alkaloids, and P-450 enzymes are involved in much of the clearance. In the same way, the P-450s encounter numerous new drugs and pollutants each year. The lack of complete selectivity of some of the P-450s and the number of the individual forms are thus an advantage. However, in some cases the oxidation of these chemicals can generate dangerous electrophiles that are detrimental to the host and may cause toxicity or cancer (10).

The other major hypothesis, that of the importance of P-450s in normal metabolism, also has validity. Even in yeasts, lanosterol 14α-demethylase is a key P-450 activity (11). In mammals many steroidogenic tissues contain critical P-450s. The lack of functional P-450 21A2 (catalysing 21-hydroxylation of progesterone and pregnenolone) constitutes a serious congenital disease (12), and P-450s are also known to play important roles in vitamin D homeostasis (13). Most of the eukaryotic P-450s have been considered to be located in the endoplasmic reticulum (i.e. microsomal), except for the two P-450 11 products in steroidogenic tissues (14). These mitochondrial P-450s and their bacterial counterparts utilize electron transport systems consisting of a flavoprotein and an iron-sulfur protein (adrenodoxin), instead of the single flavoprotein (containing FMN and FAD) in the endoplasmic reticulum. Further, the mitochondrial and bacterial P-450s are usually much more selective in terms of the range of substrates each will oxidize. In recent years Avadhani and others (13, 15) have found evidence that some hepatic P-450s are found in mitochondria and have significant catalytic activities toward xenobiotics as well as steroids (13, 15).

What do we know about the significance of the P-450s involved in the processing of normally endogeneous chemicals? While it is clear that some of the P-450s such as P-450 21A2 are critical for humans, the lack of other P-450s such as P-450 2D6 does not generally appear to be a detriment to health (16, 17). Sometimes particular P-450s can be targets for drugs that function by mechanism-based inactivation, such as the estrogen-forming aromatase P-450 19 in breast cancer (18, 19). ω-Hydroxylation of fatty acids was the assay that Lu and Coon (20) first used in the isolation and reconstitution of hepatic microsomal P-450s, but the physiological contribution of this activity does not appear to be dramatic in the case of short-chain fatty acids; with longer fatty acids and eicosanoids the processes of ω-, ω-1, and ω-2-hydroxylation may be more important (21). While it is accepted that some steroid hydroxylations are extremely critical (22), the situation with the “non-specific” hepatic microsomal enzymes that also oxidize xenobiotics is unclear; while many can hydroxylate androgens and other steroids regio- and stereoselectively (23), it is not obvious that these reactions are particularly critical (24). A low Kd for a particular reaction is not strong evidence of inherent selectivity or function, and a low Kd can actually work against catalysis by increasing the activation energy (25).

Recently Spector and others (26) have identified metabolic pathways in mammals leading to the de novo synthesis of the analogic morphine, and it is highly likely that at least several of these steps are catalyzed by P-450s, as might be expected from the known roles of P-450s in oxidizing these (27) and other nitrogen-containing drugs and alkaldoids (28).

There is considerable interest in the function of eicosanoids and other long-chain fatty acid derivatives as messengers and the function of P-450s in relation to the metabolism of these compounds. Arachidonic acid is converted to alcohols and to epoxides by P-450s (21, 29, 30), and these epoxides can even be incorporated into phospholipids (31). There is significant evidence that the epoxides of arachidonic acid can exert important physiological responses at low concentrations. Recently Kauser et al. (32) have shown that P-450 inhibitors can attenuate the myogenic response of dog renal arteries in vivo, and such eicosanoid-linked pathways may be involved. Such arachidonic acid epoxides have been implicated in pregnancy-induced hypertension (33). Although some of the less selective hepatic P-450s have been shown to account for much of the epoxidation of arachidonic acid in microsomal systems (34), the contributions of individual P-450s to in vivo function remain to be defined. Iwai and Inagami (35) have found that genetically hypertensive rats fail to express...
unusual reactions such as dehydrogenation have been discussed in detail elsewhere (45). Pending upon the substrate presented ever, seen with the plant growth hormone jasmonic acid. This activity has also been noted for the other oxygenated complexes, which operate via specialized mechanisms involving rearrangement of oxidized chemicals as opposed to oxygen activation per se. These enzymes are critical in influencing many functions. Recently Song and Brash (37, 38) have characterized a flaxseed allene oxide synthase as a P-450 (vide infra). The hydroperoxide substrates are found in high amounts in plants, where the enzyme catalyzes a key step in the biosynthesis of the plant growth hormone jasmonic acid. This activity has also been demonstrated in lower animals, including coral and starfish oocytes. These are but a few examples of situations where P-450 enzymes may be considerably important.

**Mixed Function Oxidation Reaction Chemistry**

Most P-450 reactions proceed with the stoichiometry characteristic of monoxygenases (39).

\[ \text{NAD(P)H} + \text{O}_2 + \text{RH} \rightarrow \text{NAD(P)}^+ + \text{H}_2\text{O} + \text{ROH} \]

In many cases the product does not appear to be a simple alcohol because rearrangement has occurred. P-450 has not been shown to act as a lipoxigenase or other type of dioxygenase, e.g. with a reaction of the type

\[ \text{RH} \rightarrow \text{R}^+ \rightarrow \text{RO}^- \rightarrow \text{ROOH}, \]

except perhaps in the case of unusually labile compounds. However, P-450s often have the capability to utilize hydroperoxides in various modes (vide infra).

The catalytic mechanism of monoxygenase may be considered in two parts, oxygen activation and substrate oxidation (Scheme 1). Current views of the details of both aspects have been discussed in detail elsewhere (39,43). The Fe(II)-O_2/substrate complex is unstable but has been characterized both with the bacterial P-450 101 (P-450_mmo) and mammalian P-450s (7, 44, 45). Some evidence for the other oxygenated complexes has been seen with the P-450 enzymes (46, 47), but much of our inference is based upon studies of diagnostic rearrangements catalyzed by the enzymes and biometallic metalloporphyrin models (39, 48, 49). The overall oxygenation reactions include such processes as hydroxylation at carbon and the heterostoms N, S, and I, dealkylation of amines and ethers, and epoxidation (39, 40). These reactions can all be rationalized in terms of two steps, abstraction of a hydrogen atom (or electron) and oxygen rebound (radical recombination).

\[ (\text{FeO})^{2+} + \text{RH} \rightarrow (\text{FeOH})^{3+} \rightarrow (\text{FeO})^{2+} + \text{ROH} \]

The chemistry in the different reactions is thought to be rather invariant, and the key influence on catalytic specificity is the apoprotein (40). Further, the different P-450 enzymes should not individually be considered as strictly being epoxidases, N-demethylases, etc.; the reaction effected is a function of the fit of the substrate (or, more properly, its transition state) with the protein. A single protein can catalyze all of the types of reactions, depending upon the substrate presented (39). Moreover, seemingly unusual reactions such as dehydrogenation (42, 50), ester cleavage (51), ring expansion, N-hydroxylation, exchange of protons with solvent (52), and modification of the heme prosthetic group (53, 54) may be understood in these paradigms (39, 40, 42).

**Other Mechanistic Modes**

P-450 enzymes can catalyze reduction reactions as well as oxidations. Notable examples include carbon tetrachloride (55) and azo dyes (56). Other reported reductions (e.g. of epoxides) (57) are poorly understood (1). P-450 enzymes can form H_2O_2 when the oxidation of substrates is not tightly coupled to electron flow. Evidence has been accumulated that most of this H_2O_2 is formed from the nonenzymatic dismutation of superoxide anion, O_2^- which is a breakdown product of the Fe(II)-O_2 complex (58).

\[ \text{Fe(II)-O}_2 \rightarrow \text{Fe(III)} + \text{O}_2^- + \text{H}_2\text{O} \]

However, in some cases superoxide anion has not been detected in the decomposition of P-450 Fe(II)-O_2 complexes (45). The complete reduction of O_2 to H_2O by P-450 has also been documented (59).

\[ 2\text{NADPH} + 2\text{H}^+ + \text{O}_2 \rightarrow 2\text{H}_2\text{O} \]

This reaction, analogous to that of the mitochondrial cytochrome oxidase, appears to be exacerbated in the presence of uncoupling agents such as perfluoroalkanes whose C-F bonds are not easily broken (58). The in vivo significance of these nonproductive reactions is still vague; in liver microsomes and with some purified P-450s a large fraction of the reducing equivalents of NADPH is used nonproductively, and it is not clear that such a stress on the reduced pyridine nucleotide pool is incurred in intact tissues. P-450s can also utilize H_2O_2, hydroperoxides, and peroxides, and in this regard they have some relationship with the peroxidas, which also utilize formal Fe(IV) porphyrin radical cation intermediates (FeO^{4+})(43, 60). The fungal enzyme chloroperoxidase is by spectral definition a P-450; it catalyzes the following reaction (61).

\[ \text{H}_2\text{O}_2 + \text{HCl} + \text{RH} \rightarrow \text{RCI} + 2\text{H}_2\text{O} \]

The turnover number for chlorination of dimedone is on the order of 10^9 min^{-1}. The enzyme can also carry out some of the oxidations diagnostic of the more typical P-450s (drug N-demethylation, oxygenation, etc.) in the presence of H_2O_2 (62, 63). Oxidations by the hepatic P-450s can also be supported by artificial "oxygen surrogates" such as iodosylbenzene (64)

\[ \text{RIO} + \text{R}^+ \rightarrow \text{R'O} + \text{RI} \]

and hydroperoxides. The hydroperoxide-supported reactions are complex because P-450s can apparently cleave hydroperoxides either homolytically or heterolytically, depending upon the particular protein,

\[ \text{Fe}^{2+} + \text{ROOH} \rightarrow \text{RO}^- + (\text{FeOH})^{3+} \]

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and in the latter case the alkoxide anion may propagate radical reactions (43, 49). P-450s can also catalyze "reductive" β-scission of hydroperoxides to yield alkanes and carbonyl products (65). The flaxseed P-450 isolated by Song and Brash uses a linolenic hydroperoxide to generate an allene oxide in what appears to be an internal rearrangement (Scheme 2). Other interesting rearrangements of prostaglandin H_2 are catalyzed by the important P-450s thromboxane synthase and prostacyclin synthase (Scheme 3).

**What Regulates Rates of Catalysis?**

Most of the P-450 reactions are relatively slow, and rates of ~1 nmol of product formed/nmol of P-450/min (or min^{-1}) are common for many substrates. Rates this slow can explain in vivo drug disposition in many cases. Rates with some other substrates

\[ \text{ALL rates are expressed as turnover numbers, in units of min}^{-1} (\text{i.e. nmol of product formed/nmol of P-450 or metalloporphyrin/min, usually under optimal conditions).} \]

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\[ 6 \text{W.-C. Song, and A. R. Brash, submitted for publication.} \]
supply can actually limit the rates of P-450 reactions (72).

Ultimately all of these features may be understood at a molecular level. The three-dimensional structures of several kinds of bacterial P-450cam (P-450 101) crystals are known at high resolution (73, 74). However, none of the intrinsic membrane-bound P-450s has been crystalized to date. Extrapolation of the domains of the soluble bacterial protein to the other enzymes may be useful in illuminating several features of function but will probably not reveal high resolution details of catalytic specificity, which can be highly sensitive to small amino acid replacements (75). The structure of the bacterial protein, however, has revealed a general domain structure and that the iron spin state is controlled by the accessibility of an H2O residue (or OH-) as a distal iron ligand (76). The same principle probably holds in the other P-450s, but the correlation between spin state, Fe3+/Fe2+ oxidation-reduction potential, and catalysis is clearly not universal among P-450s (77). Negishi and his associates (78) have also shown that minor variations in a single residue (position 209 of a mouse P-450) can produce dramatic changes in the catalytic specificity toward a pair of substrates, coumarin and testosterone, and in the iron spin state, but there is no obvious correlation. Side-ionized mutagenized model systems may implicate the basic amino acids (Lys and Arg) clustered in two regions of rat P-450 1A2 (in the vicinity of residues 100 and 450) in electron transfer from NADPH-P-450 reductase (79). Ishimura's laboratory (80) has used site-directed mutagenesis to show that (in bacterial P-450 101) the Thr-252 residue is near the distal heme ligand region and is critical for appropriate heterolytic scission of the oxygenated iron complex.

\[
\text{Fe}^{3+} + \text{O}_2 \rightarrow \text{Fe}^2+\text{OH} \rightarrow (\text{Fe}^3+ + \text{OH}^-)
\]

In the absence of this residue the enzyme is "uncoupled," that is it acts as an oxidase.

\[
\text{NADH} + \text{H}^+ + \text{O}_2 \rightarrow \text{NAD}^+ + \text{H}_2\text{O}
\]

This finding is surprising in that the appropriate heterolytic cleavage was thought to be largely a function of the axial Cys thiolate ligand (73). The question can then be raised as to what the function of the universal Cys ligand really is in catalysis. Does it have a function as such? The biomimetic models seem to work at least somewhat effectively without it, although the presence of imidazole often improves these systems (49). However, the ability of a Thr hydroxyl to assist in such a protonation has been questioned by Raag and Poulos, who feel that the role of Thr-252 is better understood in the context of a network of residues near the distal face of the heme (73, 74).

\[\text{Cys} \quad \text{Cys} \quad \text{Cys}\]

**Other Aspects of P-450 and Prospects for the Future**

In this brief review it is really not possible to deal with all aspects of P-450 biochemistry or to adequately acknowledge all studies that have led to our current views. The reader is referred to lead reviews and articles on P-450 protein structure (73), nomenclature and sequence similarity (2), biomimetic and other models (49), catalytic mechanism (39–42), molecular biology studies (16) and regulation (81), features of structure/activity relationship (74, 82), and roles of P-450s in steroidogenesis (14), drug metabolism (83, 84), and carcinogenesis (10).

Great progress has been made in the understanding of these complex enzymes through the efforts of many individuals and the availability of new technologies. Some areas in which research needs remain include the following. (i) Analysis of more protein structures and their interactions with substrates, inhibitors, and other domains, is needed. The P-450s are too large for the application of global NMR methods, and a need exists for crystallization of the membrane proteins. (ii) Further details of catalytic mechanisms remain to be elucidated. For instance, some points regarding N-oxidation and epoxidation remain controversial. (iii) The regulation of levels of P-450 enzymes is complex, and much remains to be learned. The individual P-450s appear to be regulated in different manners, with some elements of similarity among cer-
tains genes. While much attention has been focused on transcription, there are other points of control as well. (iv) There are still important reactions for which the P-450s remain to be isolated and characterized. (v) Finally, in vivo and in vitro systems are needed to determine the significance of the individual P-450s in influencing humans to risk and disease from xenobiotics and also endogenous chemicals.

Acknowledgments—I thank Drs. J. H. Capdevila, L. J. Mannett, M. J. Coon, T. D. Porter, T. Shimada, and A. Brash for their comments on the manuscript. The work referred to in this review is based on the P-450s appeared in late 1991.

REFERENCES


