Review

Cytochrome P450: Molecular Architecture, Mechanism, and Prospects for Rational Inhibitor Design

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Cytochromes P450 catalyze the insertion of one $O_2$-derived oxygen atom into an aliphatic or aromatic molecule. P450s are best known for the metabolism of xenobiotic molecules, where hydroxylation renders insoluble hydrocarbons more soluble for easier elimination. In addition to this important catabolic function, P450s catalyze key steps in steroid and plant growth regulator metabolism. A variety of therapeutic, fungicidal, and agrochemical agents that perturb these metabolic pathways very likely operate by binding in the lipophilic P450 active site and coordinating with the heme iron atom. Recent determination of a bacterial P450 crystal structure, P450cam from *Pseudomonas putida*, in addition to the crystal structure of four inhibited complexes, has provided some insight into the potential use of P450 as a model system for the rational design of therapeutic agents. The crystal structure has also shed light on the P450 catalytic mechanism. P450cam operates differently from peroxidase or catalase in cleaving the $O-O$ bond, since unlike these other enzymes, P450 contains no acid–base catalytic groups near the oxygen binding site. Instead, the $O_2$ pocket is lined with aliphatic and aromatic residues. This strongly suggests that the catalytic push required to cleave the $O-O$ bond resides with the ability of the Cys heme ligand to donate electron density to the heme–oxy system. A comparison of the substrate-free and -bound P450cam crystal structures has revealed some interesting aspects regarding the dynamics of substrate binding. The structures of both forms of P450cam are the same except that in the substrate-free enzyme, the active-site pocket fills with a network of water molecules, one of which coordinates with the iron atom. Despite this lack of any significant conformational rearrangement of protein groups, a careful analysis of crystallographic temperature factors shows dynamical differences. Segments of the protein that are separated in the sequence but that lie close to one another in the structure and that define a small entrance to the substrate pocket undergo significantly higher thermal motion in the substrate-free enzyme. This suggests that dynamical fluctuations at the molecular surface play an important role in controlling substrate binding.

KEY WORDS: cytochrome P450; crystal structure; inhibitor design; catalytic mechanism.

INTRODUCTION

Specific and nonspecific hydroxylation reactions play an essential role in a number of metabolic and catabolic processes. Cytochrome P450, an enzyme of central importance in such reactions, catalyzes the pyridine nucleotide-dependent insertion of one $O_2$-derived oxygen atom into an aliphatic or aromatic substrate. Cytochrome is perhaps a misnomer since this implies only an electron transfer function when, unlike cytochromes and globins, P450s catalyze bond making and breaking reactions. At the time of its discovery, P450 was recognized as an unusual pigment and not as an enzyme (1,2). The discovery of P450 is itself an interesting story. Initially it was found that when liver microsomes were reduced and purged with carbon monoxide, a strong absorption band at 450 nm resulted, which was most unusual for the then-known pigments (1,2). Thus the name P (for pigment) 450. What seems remarkable, especially considering that P450 is the major heme protein of liver microsomes, comprising about 1.0 nmol/mg microsomal protein (3), is that P450 was not discovered until the late 1950s and was not established as a heme protein until 1964 (4).

Since its discovery, research on P450 has undergone an explosive growth, due primarily to three key observations. First, Estabrook and co-workers established the role of P450 in the metabolism of steroids (5); second, Remmer and Merker found that P450 was induced in animals pretreated with various drugs (6); and third, Mason and co-workers discovered a unique electron paramagnetic resonance signal in liver microsomes which (7) later was found to be due to P450. Thus, P450 attracted the interest of both biological and biophysical disciplines including many forms of spectroscopy, enzymology, pharmacology, toxicology, and molecular biology. Perhaps the single property of P450s that ties these disciplines together is the inducibility of specific P450s by different pharmacological agents. The classic example is the phenobarbital and 3-methylcholanthrene induction of microsomal P450s (8), with more recent advances centered on the molecular biology of the murine Ah locus involving induction of P450s by aromatic hydrocarbons such as...
as 2,3,7,8-tetrachlorodibenzo-p-dioxin (9). The importance of P450 induction to pharmacologists and molecular biologists is obvious but P450 also is of interest to the enzymologist and biophysical chemist. The unusual spectroscopic properties of P450 and the changes in these properties that occur upon substrate or inhibitor binding have provided fruitful terrain for many spectroscopic probes. The nature of how the enzyme cleaves the O–O bond and then inserts an oxygen atom into an unactivated C–H bond has been a particularly challenging problem. Perhaps most intriguing is the large number of structurally unrelated organic molecules hydroxylated by various P450s despite the fact that the basic redox, spectral, and O–O bond cleavage properties are similar in all P450s.

One additional area of considerable interest is the use of P450 inhibitors as therapeutic agents. The structure of several such agents is shown in Fig. 1. The most well-known agent is metyrapone, used to treat hypercortisolism (Cushing’s syndrome; 10). Metyrapone operates by binding at the active site of mitochondrial P450s involved in steroid metabolism, thereby lowering the production of cortisol. A variety of triazole and imidazole compounds blocks ergosterol biosynthesis in fungi, most likely as P450 inhibitors, and are used as medical and agricultural fungicides (11). As with any set of drugs, insecticides, or herbicides, however, the key problem is specificity. The goal is to kill the invading organism but leave the host unaffected. Toward this end, there is growing optimism in the area of rational drug design. If one possesses detailed three-dimensional structural information about the receptor macromolecule, hopefully of both the invader and the host, one is in a position to use computer molecular modeling techniques to design specific inhibitors. P450 offers some promise in this area. After reviewing the structural and catalytic properties of P450 we return to this question of the prospects for the rational design of P450 inhibitors.

**MOLECULAR PROPERTIES OF P450**

P450s consist of a single polypeptide chain on the order of 45,000 to 55,000 daltons and contain a single, noncovalently bound heme. Most P450s exhibit an absorption maximum near 418 nm, which shifts to near 390 nm when substrate binds. This represents a low-to-high spin transition where, in the substrate-free form, the heme iron atom has all 5 d electrons maximally spin paired to give a net spin of 1/2, while when substrate binds the spin shifts to 5/2. This shift is a classic trademark of P450–substrate interactions and typically is used to follow substrate binding.

Owing to recombinant DNA technology, the number of available P450 sequences continually grows. All P450s thus far sequenced (over 60) exhibit strong homology even when the only complete bacterial P450 sequence is compared with its eukaryotic counterparts. The most striking homology occurs around the heme ligation environment. Table I shows the sequence around one such active-site peptide. P450cam represents the camphor monooxygenase P450 from *Pseudomonas putida*, which currently represents the only complete bacterial sequence (12) and the only known crystal structure of a P450 (13). Cys357 of P450cam operates as one of the axial heme ligands (Fig. 2) and the strong sequence homology around Cys357 provides compelling evidence that the corresponding Cys in all P450s provides one of the heme ligands. The degree of homology extends to the microenvironment surrounding the Cys ligand. For example, Phe350 thusfar is invariant, while a branched aliphatic side chain always occurs at position 359 (P450cam numbering). An invariant Gly (Gly353) occurs at a strategic location for reversing the direction of the polypeptide chain just beneath the heme. It therefore appears that not only is the homologous Cys used as a ligand in all P450s but that the three-dimensional structure surrounding the Cys ligand is conserved in all P450s. The reason for such strict conservation very likely relates to the critical role that the Cys–Fe bond plays in the catalytic mechanism which we consider further.

**Overall Molecular Architecture**

Figure 3 shows the overall topography of P450cam derived from the highly refined 1.63-Å X-ray structure (13). Helices account for about half the residues and are divided among 13 different helical segments. The beta structure occurs mainly as isolated antiparallel beta pairs with no extended sheet structure. Thus, P450 is similar to the globins and cytochrome c peroxidase, where helices dominate the overall fold. Notice that P450cam divides into two domains, with most of the helices clustered together on the right half of the molecule while most of the beta structure occurs on the left half.

The heme is completely sequestered, with only the carboxylates of the heme propionates solvated. Helix I lies directly over the heme and, together with helix C and one strand of beta 3, provides hydrophobic heme contacts. As