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Book review

"The Ubiquitous Role of Cytochrome P450 Proteins", Vol. 3 of Metal Ions in Life Sciences, A. Sigel, H. Sigel, R.K.O. Sigel (Eds.). Wiley, New York (2007). Hardcover, 678 pages, May 2007; US$ 360.00, ISBN: 978-0-470-01672-5

This volume, which is edited by the Sigel family, covers a variety of aspects on Cytochrome P450 (CYP) enzymes. These enzymes are present in the entire tree of life where they perform metabolism of xenobiotics, detoxification of poisons, and biosynthesis of hormones as well as signaling molecules. The active site of the enzyme contains an iron protoporphyrin-IX complex that binds an atmospheric dioxygen molecule and activates it using two proton and two electron equivalents to form the reactive species which is a high valent iron-oxo porphyrin cation radical complex, so called Cpd I (Compound I). Using this active species, the enzyme catalyzes the 'insertion' of a single oxygen atom into almost any imaginable organic molecule (RH):

$$RH + O_2 + 2H^+ + 2e^- \rightarrow RHO + H_2O$$

The enzyme performs a few other interesting transformations, like C–C cleavage, etc, and is thought to be using to these ends other oxygen-reactive species in the catalytic cycle.

The book involves 17 chapters that were written by a select group of experts well known in the field. These chapters provide an overview of the field with an accent on the biological aspects, but also considerable coverage of chemical aspects. The book shows the interdisciplinary nature of the field, and at the same time, it highlights the central role of chemistry and chemists in this emerging area.

The first chapter (by Schuler and Sligar) gives an overview that serves as a preamble for the book by stressing the ubiquity of P450s in all life forms; altogether there are thousands of P450 isozymes that have the same active species (the heme cysteinate complex) and different proteins. The chapter gives a concise review of the field from structure through function to the multitude of the biotransformations that are catalyzed by this superfamily of enzymes. This chapter as well as Chapter 3 offer very concise and useful summaries of the method of nomenclature used to label the various isozymes, based on the extent of primary acid sequence identity, using the benchmarks of >40%, >55% and the individual loci designation to label the isozyme by a sequence of a number, a capital letter, and a number, respectively (e.g., CYP1A2), while the >97% sequence identity is labeled with a lower case letter and number (e.g., v1), respectively. In addition, the authors of Chapter 1 describe the main features of the enzymes and their diversity; structural diversity, diversity of mechanisms, and diversity in regulations. It is shown that despite the immense diversity of the superfamily, the essential features of the catalytic cycle, the structure of the active site, and the mechanism of organic reactions are all highly conserved across the tree of life.

Structural aspects of the enzyme appear almost in all chapters, but are emphasized systematically in Chapters 3 (by Poulos and Meharenna) and 8 (by von König and Schlichting). These two chapters along with Chapter 4 cover a wide spectrum of P450 isozymes, such that the reader can have a good glimpse into the structural features of the family members. Chapter 3 deals with the structures of P450 enzymes and the phylogeny. The chapter emphasizes the conservation of the P450 fold, the hydrogen bonding to the sulfur atom in the cysteinae ligand loop, the putative role of the propionate side chains, the oxygen binding region, the substrate binding regions, the electron transfer site, the binding of P450 to the membrane, and the potential role of oligomeric structures of P450s. There are short subsections on related thiolate enzymes that have different function, CPO and NOS, on P450nor that converts NO to N_2O, and on the comparison of P450 to peroxidases. The last subsection is dedicated to the archaeon P450s, which are thermophilic enzymes that survive in temperatures as high as 100 °C or higher. Chapter 8 reviews the structural elements of the workhorse of P450s, CYP101 (known also as P450CAM) with an attempt to rationalize the impact of these features on function, by use of structural information and activity of point mutants. The O–O activation role mediated by the acid–alcohol pair (Asp251 and Thr252) in the distal side is thoroughly discussed, and the radiolytic and cryogenic methods used to generate some of the intermediates in the catalytic cycle and provide the X-ray structure are described. Other enzymes, CYP107A and CYP102 and their mutants are discussed too, but more briefly than CYP101. Chapter 4 (by Snyder) is dedicated to aquatic P450 species with a focus on invertebrates and a lesser focus on mammals. The chapter provides a summary of what is currently known about the CYP gene families in these aquatic species.

Chapters 2, 5, 6, 7,11, 12, 13, 14, 15 and 16 provide the chemical and molecular-biological aspects of P450s with emphasis on the products of the chemical reaction, and in a few cases also on the reactions mechanism: Chapter 2 (by Woggon) reviews the

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efforts of the bioinorganic and bioorganic communities to synthesize structural and functional mimics of the active species of the enzyme using a plethora of metal-oxo prophyrin complexes with a variety of sixth ligand groups. The author describes the rich structural, electrochemical and mechanistic chemistry in the field and points out what elements are still missing to generate a true functional mimic. Electrochemical studies of the electron transfer step in the catalytic cycle of P450 are described in Chapter 5 (Bond et al.). The factors that govern the electron transfer to P450 are discussed in Chapter 6 (Gray et al.), where it is argued that the simplest way of replacing the reductase machinery is by means of a suitably modified electrode and electron wires, such as Ru^{II} and Re^{I} complexes that shuttle electrons to the active site. Chapter 7 (by Jung) reviews how spectroscopy has been used to study the mechanisms of 'failures' of the action of P450 due to the generation of uncoupling products such as water, H_2O_2 and protein radical formation, or due to the loss of the cysteineate ligand or heme loss. The discussion in Chapter 7 is based on thermodynamic and kinetic principles that are outlined at the beginning of the chapter and provide a rational framework for understanding the data. Jung emphasizes the critical role of water content. Chapter 11 (Perera et al.) is an updated review of the chemical aspects of P450 catalysis, starting from the catalytic cycle, through the probing of the intermediates in the cycle, to the mechanistic hypotheses about the reactivity of the enzymes. In addition, the chapter describes the attempts to trap and probe the active species Cpd I, and reconciles the conflicting results from a few laboratories; it is shown that Cpd I is present and performs typical reactions with peroxidase substrates. The role of the substrate on the reactivity patterns of P450 and its last seen intermediate species, the ferric hydroperoxide, are discussed. Chapter 11 is updated in the sense that it devotes some space to reviewing the contributions of quantum chemical calculations and theoretical models to the understanding of various aspects of the enzyme. Chapter 13 (De Voss and Cryle) reviews the diverse C–C bond cleavage reactions mediated by P450 during biosynthesis, e.g., of pregenolone from cholesterol in CYP11A1, or the conversion of progesterone to 17-*O*-acetyltestosterone by CYP17A, etc. The chapter reviews the subject with mechanistic insight into this fascinating reaction. A molecular-biological perspective is given in Chapter 12 (by Bernhardt and Waterman), which reviews the P450-mediated syntheses of sterols, bile acids, steroid hormones, vitamin D as well as secondary metabolites, and the steroidogenic P450s that perform these transformations, as well as the glands and/or organs where these transformations take place. The chapter also discusses the inhibition of some of these enzymes that have become drug targets. Chapter 15 (by Gilliam and Hunter) reviews extensively the metabolism of xenobiotics as a defense mechanism in organisms across the tree of life. Thus, the authors show that considerable genetic resources have been invested in P450s that serve primarily to enhance the detoxyfication mechanisms of the organism. What typify these isozymes are their small numbers, their versatility in terms of substrate catabolism, their considerable overlap in activity, and their poor catalytic rates. The authors review these enzymes and their substrates in mammals, non-mammalian invertebrates, birds, reptiles, fishes, insects, and so on and so forth. Chapter 16 (by Guengerich) reviews the topic of drug metabolism by P450s. The author shows that a small subset of human P450s (3A4, 3A5, 1A2, 2B6, 2C9, 2C19, 2D6, and 2E1) is responsible for most of the metabolism of drugs on the market. These P450s are briefly described including structural aspects wherever available (at the time of writing the structure of 2D6 was not known, but recently it was solved and published. See: PNAS 2006, 281, 7614). Subsequently, the author takes a very didactic approach and discusses five cases of drug metabolism (Tylenol, Seldane, EE2, Tylenic acid, and Perhexiline) to demonstrate key issues and especially the type of chemistry that leads to toxicity of these drugs. Chapter 14 (by Bell et al.) reviews the design and engineering aspects of P450s by mutations including directed-evolution approaches, with an aim to use these enzymes as benign means for 'green' and efficient chemistry. The authors review the attempts to use site directed mutations to increase the yield of substrate oxidation, by controlling the size and shape of the pocket to increase substrate binding and juxtaposition (vis-à-vis the heme). Directed evolution methods were shown to improve the yields of hydroxylation by 101A2, and combinations of directed and rational evolutions were shown to improve the rate and selectivity of propane hydroxylation using the peroxide shunt instead of coupling the enzymes to reductase, ethane hydroxylation has nee achieved, etc. However, while the structures of the mutants are known, the mutations, e.g., in the ethane-hydroxylase mutant of CYP101A2, are rather remote from the active site or the substrate binding, and hence, do not really explain the source of the improvement. Similar attempts in mammalian P450s are reviewed and the same conclusion is reached that the structure may not provide a basis for rationalizing the improved catalysis and selectivity.

Chapter 9 (by Lu and Pfister) reviews briefly the secondary coordination-shell effects on the distal and proximal sides of the heme and the functions of the interactions with the heme. Specifically, in the proximal side there are hydrogen bonding interactions around the cysteine ligand, and the hydrophobic interaction with the conserved Phe residue, while in the distal side the residues in the secondary coordination shell form a hydrogen-bonding network that shuttles protons to the reaction center, and some residues that bind the substrate. The authors further review some related insight from model systems. Chapter 10 (by Munro et al.) provides an overview of the features associated with the interactions of P450s with NO and other ligands and the modes of regulation and inactivation of P450s by these ligands. Some of these ligands (e.g., NO) are reversible inhibitors, while others (e.g., thiophene derivatives) are irreversible inhibitors that deactivate P450s.

The last chapter 17 (by Carver) deals with clinical aspects of P450 activity. The reader who is interested in these issues will find here many interesting discussions of the effect of diet on P450s, for example, the effect of grapefruit juice that acts as an inhibitor of CYP3A4, the effect of garlic and herbal medications, the effect of drug–drug interactions and so on and so forth.

In summary, this collective volume is extremely rich, as rich as the chemistry of P450 enzymes. It shows the vast field, its many facets and unresolved issues. It will be highly interesting primarily to the P450 community, medicinal chemists, and biochemists. And while it is a bit 'heavy' on the biological side, still it would be interesting for mechanistic chemists in the field of oxidative chemistry and worthwhile also for chemists interested in catalysis and green chemistry.

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