Models and Mechanisms of Cytochrome P450 Action

John T. Groves

1. Introduction

The reactions catalyzed by the cytochrome P450 family of enzymes have challenged and intrigued chemists for more than three decades. Alkane hydroxylation and olefin epoxidation, particularly, have attracted a sustained worldwide effort, the allure deriving both from a desire to understand the details of biological oxygen activation and transfer and, as well as the sense that the development of new, selective catalysts, based on these principles could be of considerable economic value. The focus of this chapter is on the advances in our understanding of the mechanisms of the remarkable oxygenation reactions mediated by oxometalloporphyrins in both enzymatic and in small molecule model systems. Particular emphasis is on the period since the publication of second edition of this monograph in 1995.

The activation and transfer of molecular oxygen into its substrate by an iron-containing enzyme was first demonstrated by Hayaishi in the 1950s. It was shown, in some of the first mechanistically informative oxygen isotopic measurements, that both the inserted oxygen atoms in the conversion of catechol to cis-muconic acid derived from O2 and not water. These findings challenged the then firmly held view that oxygen in biological molecules was derived exclusively from water via hydration processes. The biosynthesis of cholesterol and its precursor, lanosterol, from the hydrocarbon squalene were also shown to derive their oxygen functionality from molecular oxygen. Here, a single oxygen atom derived from molecular oxygen while the other was transformed to water. Later, the prostaglandins were shown to derive from the incorporation of two molecules of oxygen to form, initially, an alkyl hydroperoxide-endoperoxide. Thus, what appeared at first to be an obscure process of bacteria and fungi became recognized as a major theme of aerobic metabolism in higher plants and animals. The subsequent search for “active oxygen species” and efforts to elucidate and understand the molecular mechanisms of oxygen activation and transfer have been richly rewarding. Novel and unusual iron redox chemistry, particularly those of high-valent metal–oxo and metal–peroxo species, has appeared as our understanding of enzymatic oxidation strategies has developed.

2. Oxygen Activation by Heme-Thiolate Proteins

The heme-containing metalloenzymes cytochrome P450, chloroperoxidase (CPO), nitric oxide synthase (NOS), and their relatives catalyze a host of crucial biological oxidation reactions. Highly specific P450s are involved in the selective oxygenations of steroid and prostaglandin biosynthesis. Myeloperoxidase, which is a CPO, is an...
integral part of the immune response, and NOS is the source of the highly regulated signal transducer, nitric oxide (NO). Certain fungal CPOs and bacterial P450s have been genetically engineered for large-scale biotransformations\(^6\)-\(^10\). The active sites of these three protein families, known in detail from a number of X-ray crystal structures\(^4\), \(^11\)-\(^13\), are remarkably similar. All three have an iron protoporphyrin IX center coordinated to a cysteine thiolate. All of them are oxidoreductases that activate molecular oxygen (O\(_2\)), in the cases of P450 and NOS, or hydrogen peroxide in the case of CPO, at the iron center and incorporate one of the oxygen atoms into a wide variety of biological substrates. The other oxygen atom is transformed into H\(_2\)O. All three proteins are proposed to initiate their chemistry through the oxidation of a resting iron(III) state (1) to a reactive oxoiron(IV) porphyrin cation radical intermediate (2) (Figure 1.1). A depiction of the CPO active site derived from the crystal structure of this protein from *Caldariomyces fumago* is shown in Figure 1.2. The structure, biochemistry, molecular biology, and the chemistry of cytochrome P450 and related model systems have been extensively reviewed\(^{14}\)-\(^{22}\).

Our understanding of the mechanism of action of these heme proteins comes from the direct

![Figure 1.1](image1.png)

**Figure 1.1.** Iron(III) protoporphyrin IX with a cysteinate as the axial ligand (1), which is typical of cytochrome P450, chloroperoxidase (CPO), and nitric oxide synthase (NOS) enzymes. The active oxygen species of these proteins and related heme enzymes is an oxoiron(IV) porphyrin cation radical (2), often called compound I.

![Figure 1.2](image2.png)

**Figure 1.2.** Crystal structure of the active site of chloroperoxidase (CPO) (EC 1.11.1.10) from *C. fumago*. Protein framework is shown as ribbons. The heme is buried in a hydrophobic binding pocket containing the iron-coordinating cysteinate ligand. Adapted from the X-ray atomic coordinates of CPO\(^4\).