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Review

Complex reactions catalyzed by cytochrome P450 enzymes

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Abstract

Cytochrome P450 (P450) enzymes are some of the most versatile redox proteins known. The basic P450 reactions include C-hydroxylation, heteroatom oxygenation, heteroatom release (dealkylation), and epoxide formation. Mechanistic explanations for these reactions have been advanced. A number of more complex P450 reactions also occur, and these can be understood largely in the context of the basic chemical mechanisms and subsequent rearrangements. The list discussed here updates a 2001 review and includes chlorine oxygenation, aromatic dehalogenation, formation of diindole products, dimer formation via Diels–Alder reactions of products, ring coupling and also ring formation, reductive activation (e.g., aristolochic acid), ring contraction (piperidine nitroxide radical), oxidation of troglitazone, cleavage of amino oxazoles and a 1,2,4-oxadiazole ring, bioactivation of a dihydrobenzoxathiin, and oxidative aryl migration.

Keywords: Cytochrome P450; Oxidation reactions; Rearrangement reactions; Bioactivation; Metabolism of drugs

1. Introduction

Cytochrome P450 (P450) enzymes are some of the most versatile redox proteins known. Collectively they use substrates ranging in size from ethylene (M_r 28) to cyclosporin A (M_r 1201). Some of the P450s are indispensable, being essential for normal development and homeostasis in mammalians or allowing microorganisms to live on particular carbon sources [1] or to produce compounds for defense [2,3]. The so-called xenobiotic-metabolizing P450s are generally not considered to be individually critical for life but collectively serve as defense against the detrimental effects of natural products, e.g. alkaloids, terpenes that would accumulate and be harmful. The relatively low specificity of these P450s thus provides a general defense system, and this broad specificity carries over to drugs and other synthetic chemicals.

2. P450 catalytic mechanisms

The P450 catalytic cycle is usually considered in the general form shown in Fig. 1. The course of events follows the order of substrate binding, 1-electron reduction, O_2 binding, a second 1-electron reduction, and then a series of less well-defined steps understood as protonation, homolytic scission of the O–O bond to yield an active perferryl FeO species (depicted as FeO^{3+}), reaction with the substrate, and release of the product. The kinetics of these systems have been discussed elsewhere [4,5]. Two points should be made, in that the literature often contains some misunderstanding. First, which step is rate-limiting in the cycle varies with the P450 and the substrate. In some cases the slowest step can be C–H bond-breaking [5] or product release [6]. The second point is that the cycle shown in Fig. 1 is an over-simplification. The binding and electronic steps appear to be associated with conformational changes as shown by X-ray crystallography [7,8]. Further, some of the steps do not necessarily proceed in a defined linear manner. For instance, some P450s are reduced rapidly with or without substrate present [9], and work with human P450 2A6 indicates that the substrate can dissociate and reassociate with ferrous enzyme [10].

Steps 7 and 8 of Fig. 1 can be considered in more detail. In the general mechanism, the chemical step with the substrate can be considered in the context of "odd-electron" chemistry [11–13]. The first step is the abstraction of either a hydrogen atom or, in

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cases where the effective redox potential (modified by distance) is appropriate, an electron (Fig. 2). Another variation is the formation of an O–C bond intermediate by electrophilic addition to a π bond or aromatic system (Fig. 2). Rearrangements within the context of these systems occur and will be prominent in the discussion of seemingly unusual reactions later.

Proposals have been made that (i) the different reactions shown in Fig. 2 may be differentially ascribed to high- and low-spin FeO^{3+} complexes [14,15] and (ii) that some or even many of the P450 reactions may involve FeO_2^- or FeOOH species (instead of FeO^{3+}) [16,17]. Theoretical support for the first proposal has been published [18] although experimental biochemical or chemical studies have not been done. The concept has an attraction in explaining some of the issues but will not be employed in the reactions considered here. The second proposal [19] cannot be discarded, at least for some reactions (theoretical support in favor and against the proposal has been published [18,19]). Much of the evidence for the use of FeO_2^- and FeOOH lies in the effects of Thr to Ala mutants [20] and rearrangements of strained cycloalkane substrates [21,22] but alternate explanations for observations are possible [13,18]. Some of the reactions covered here can be rationalized with the Fe–O_2 forms but the explanations will be only done with FeO^{3+}. Even one of the P450 reactions most widely cited as a case for the FeO_2(H) mechanism, the third step of androgen aromatization by P450 19A1 [23,24], has been also recently proposed to be more likely to involve FeO^{3+} chemistry [25].

Most P450 reactions are oxygenations, described with the steps depicted in Figs. 1 and 2. P450s can catalyze some reductions [12]. Some P450s (e.g., 5A1, 8A1, 74A subfamily) catalyze rearrangements of unstable oxygenated species but the mechanisms involve redox chemistry at the iron atom [26]. Several P450s can also catalyze various rearrangements of hydroperoxides [27].

3. Simple P450 reactions and previously reviewed rearrangements

The basic P450 reactions include C-hydroxylation, heteroatom oxygenation, heteroatom release (dealkylation),

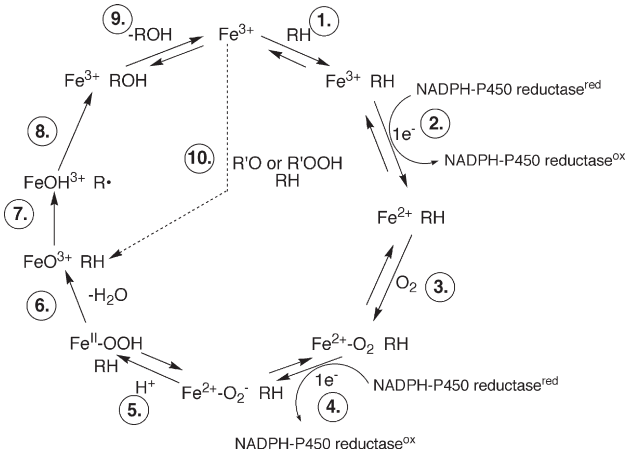

Fig. 1. Generalized catalytic cycle for P450s.

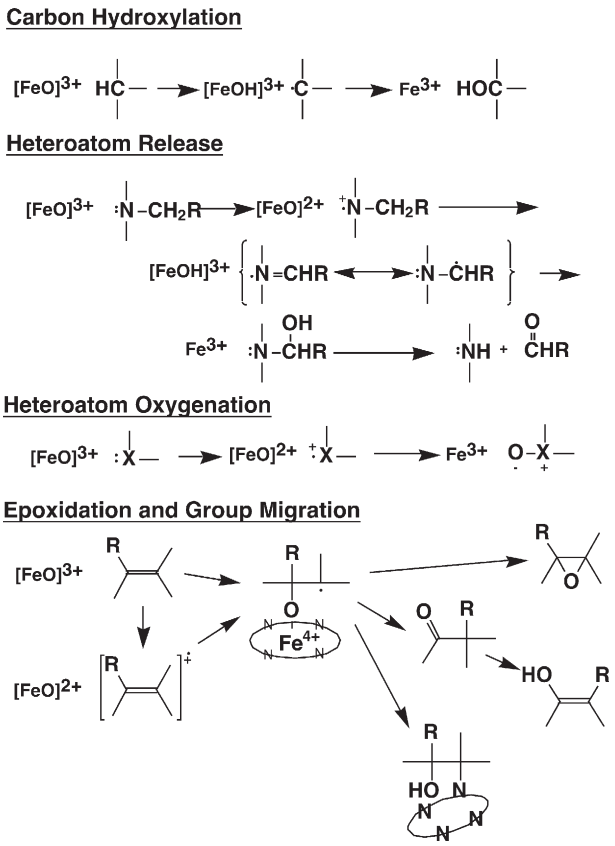

Fig. 2. Mechanisms of major reactions in P450 catalysis [11,12].

epoxide formation, and 1,2-migration (Fig. 2) [11]. Mechanistic explanations for these reactions have been advanced, and a relatively high level of understanding is available [12,13].

A number of more complex P450 reactions were reviewed in an article written in late 2000 [12]. For the sake of brevity, examples (or general descriptions) of each are shown in Fig. 3, along with a word description, and the rest of this review will be focused mainly on the literature since 2000. The reader is referred to an excellent review of P450 chemistry and reactions by Ortiz de Montellano and DeVoss [13], a review of transformation of 5-membered heterocyclic rings [28], and two other reviews on reactions involved in bioactivation [29,30].

4. More complex P450 reactions

4.1. Chlorine oxygenation

As pointed out above, heteroatom oxygenation is a relatively well-documented P450 reaction [11,13,31,32]. The P450-catalyzed oxygenation of halides had been proposed [33,34] and demonstrated with iodine and possibly bromine [35]. Recently Ortiz de Montellano and his co-workers [36] provided clear evidence that P450 4A1 can catalyze the oxygenation of alkyl iodides, bromides, and chlorides (Fig. 4). The reaction with an alkyl chloride is somewhat