Coordination Chemistry Reviews 256 (2012) 1137–1150

Contents lists available at SciVerse ScienceDirect

Coordination Chemistry Reviews

journal homepage: www.elsevier.com/locate/ccr

Review

Recent density functional theory model calculations of drug metabolism by cytochrome P450

Dongmei Li[a,b], Yong Wang[a], Keli Han[a,*]

[a] State Key Laboratory of Molecular Reaction Dynamics, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China
[b] College of Pharmacy, Nankai University, Tianjin 300071, China

Contents

ARTICLE INFO

Article history:
Received 3 August 2011
Accepted 28 January 2012
Available online 5 February 2012

Keywords:
Density functional theory
Compound I
Hydrogen atom transfer
Two-state reactivity

ABSTRACT

Cytochrome P450 (P450) enzymes are the major catalysts involved in the oxidative metabolism of most drugs, steroids, carcinogens, and other chemicals. They catalyze a variety of reactions and convert chemicals to potentially reactive products as well as make compounds less toxic. More than 75% of drugs in clinical use are metabolized by P450s. Understanding the mechanism of drug metabolism by P450, in particular the chemical process, is indispensable in the early phases of drug discovery process. In this review, we discuss our recent theoretical studies on the mechanism of some specific compounds catalyzed by P450. Density functional theory (DFT) is used as the quantum mechanical (QM) tool to explore the fundamental mechanism of these reactions. These DFT calculations provide structures, energies, and some other properties of transition states and intermediates and thus shed light on the electronic factors that govern the stability and reactivity. These theoretical studies provide a complementary insight to experiment and suggest some new features. DFT serves a powerful tool to explore the chemical mechanism of drug metabolism by P450. The revealed fundamental mechanism concerning how the enzyme catalyzes the drug metabolism, especially the transition state of the rate-determining reaction step, could provide a valuable mechanistic base for rational design of novel drugs.

1. Introduction

Cytochrome P450 (P450) enzymes are the major catalysts involved in the oxidative metabolism of most drugs, steroids, carcinogens, and other chemicals [1,2]. They have been stated to be among "the most versatile biological catalysts known" [3]. P450s are found predominantly in endoplasmic reticulum and mitochondria, and in greatest abundance in liver. They catalyze a variety of reactions and convert chemicals to potentially reactive product as well as make compounds less toxic. More than 75% of drugs in clinical use are metabolized by P450s in phase I metabolism [4]. The oxidation of xenobiotic substances (e.g. drugs) by P450s is a significant focus of scientists in the areas of toxicology, drug metabolism, and pharmacology. The effects of these oxidations can be manifested in poor drug bioavailability and various acute and chronic toxicities, including adverse drug interactions, cancer susceptibility, and birth defects [5]. Understanding the mechanism of drug metabolism by P450, in particularly the chemical reaction process, is indispensable in the early phases of drug discovery process.

P450s show a great diversity in the chemical reactivity on different drugs or substrates. They can catalyze a series of reactions, e.g. C—H hydroxylation, C=C epoxidation, heteroatom oxidation,

* Corresponding author. Tel.: +86 411 84379293; fax: +86 411 84675584.
E-mail address: klhan@dicp.ac.cn (K. Han).

0010-8545/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ccr.2012.01.016

aromatic oxidation, dealkylation, etc. Normally, P450s use molecular oxygen, insert one of its oxygen atoms into a substrate, and reduce the other oxygen to water. Thus, P450s belong to the monooxygenase class of enzymes. The general reaction catalyzed by P450s is

$$S + O_2 + 2e^- + 2H^+ \xrightarrow{P450} SO + H_2O \quad (1)$$

in which S represents a drug or some other typical substrate, SO represents the product.

The catalytic cycle of P450s is quite complicated [3,6–9]. After the substrate bind to the heme center, a series of events are triggered, leading to two reduction and two protonation steps and the formation of a high-valent oxo-ferryl species. This oxo-iron species (Compound I, Cpd I) in P450 enzymes is an elusive oxidant due to holding a ultrashort lifetime and degenerate multiple ground spin states. It is difficult to detect experimentally and after several decades of effort, it was finally determined by Green's group in 2010 [10]. As theoretical studies by Shaik's group revealed, Cpd I of P450 is an elusive "chameleon" [11,12], for its ability to adapt its electronic structure as a response to the variation of the environment, making the chemical process catalyzed by P450 much more complicated. Therefore theory comes into play and offers a complementary tool to aid experiment. Theoretical studies can provide an essential and complementary insight of the short-lived intermediates which cannot be characterized and studied experimentally. They afford structures, energies, electron and spin density distributions, and other properties of intermediates and transition states and provide insight about the electronic factors that govern stability and reactivity. Such knowledge obtained from theoretical study then feeds back into experiment by offering the missing insight and revealing new features.

Density functional theory (DFT) is based on functions that use electron density as the basic variable to describe the ground state of a multiple-electron system. For a system comprising n electrons, $\rho(r)$ represents the total electron density at a particular point in space r. The electronic energy of the system can be expressed as a functional $E(\rho)$ of the electron density function. For a system comprising n electrons, the traditional electronic wavefunction depends simultaneously on the coordinates of all the electrons ($3n$ variables) and one more per electron if the spin is included, i.e., a total of $4n$ variables, whereas the electron density ρ in DFT depends only on three variables, independently of the number of electrons that constitute the system [13]. Hence, DFT increases the efficiency a lot over the traditional electronic structure theory. Because of its relative cheapness and relative accuracy, DFT computations have become an accepted tool for analyzing structure, bonding, reactivity and properties.

Many DFT studies have been performed to understand the fundamental aspects of P450. In the late 1990s, Green [14,15] and Harris [16] calculated some intermediates in the catalytic cycle of P450 and revealed the electronic structure, spin state and spectra of the intermediates. DFT calculations on the reaction mechanism of P450-catalyzed substrate metabolisms were started by the Shaik group. They suggested the two-state reactivity (TSR) mechanism [17–20], originating from the degenerate high-spin (HS) quartet and low-spin (LS) doublet ground spin states of Cpd I. In this mechanism, the reaction proceeds via two spin states (i.e. the HS and the LS). The HS and LS routes are close to each other during the H-abstraction step and then bifurcate, while the HS forms a radical that has a significant barrier for rebound, the LS rebound is virtually barrierless. The TSR mechanism corroborated the conventional Groves' rebound mechanism [21,22] and resolved the "rebound controversy" by rationalizing the experimental ultrafast radical clock data [23–25]. Later, the Yoshizawa group studied the kinetic isotope effect of the C—H bond activation of alkane [26,27] and the mechanism of camphor hydroxylation by Cpd I [28]. Since then, P450-catalyzed substrate metabolism has been widely studied using the DFT method, including alkane hydroxylation [28,29], alkene epoxidation [30–33], aromatic hydroxylation [34–38], *S*-, *N*-, *O*-oxidation and dealkylation [39–44], dehalogenation of perhalogenated benzene [45], prostaglandin H_2 isomerization [46], and so on.

In this review we will discuss our recent DFT studies on the mechanism of some specific compounds catalyzed by P450 in order to achieve a "molecular view" on the mechanisms of xenobiotic and drug metabolism by P450.

In all the studies we used HS^- as the proximal ligand of Cpd I in the model system since it was proven to be a better mimic of the full cysteinato ligand in the real protein environment, compared with CH_3S^-. Cpd I with HS^- ligand in the gas phase can produce the correct A_{2u} ground state with radical mainly on porphine ring, while Cpd I with CH_3S^- produces a Π_S state with radical mainly on the HS^- ligand [20,47]. All our calculations were carried out with B3LYP functional [48–51]. We had noted that B3LYP was considered to be dubious, in general, on its reliability for transition metal complexes [13,52,53]. It was suggested [53,54] that the safest way to approach a problem requiring the identification of the ground spin state of a species was to calculate this property with more than one functional, preferably a GGA-type functional and a hybrid functional. In case of large differences between different sets of results, caution was required, and in the absence of other evidence, the most reliable way to make the prediction would be to use a functional such as B3LYP* with 15% Hartree–Fock exchange [55,56]. Nevertheless, there were extensive exceptions, B3LYP functional performed well for hexacoordinate Fe complexes [57,58], especially for heme derivatives [59–63]. In order to further examine the computational results, we also used some other density functionals, including BP86, BLYP, B3LYP*, and B3PW91, to evaluate the energy barriers for the rate-determining steps in nicotine reactions. It turned out that BP86 and BLYP overestimated the stability of the quartet state of the reactant complex, whereas the hybrid functionals B3LYP*, B3LYP, and B3PW91 gave similar energy barriers [64]. Furthermore, the B3LYP* functional tended to exaggerate the destabilization of the high-spin states relative to that of the low-spin states and was less suitable than B3LYP for predicting the ferric aqua resting state and the product ferric alcohol complexes during camphor hydroxylation [59]. Hence, the B3LYP functional was used for all our studies. For geometry optimization, we used the Los Alamos effective core potential coupled with the double-ζ LACVP basis set [65,66] on iron and 6-31G basis set on the remaining atoms (hence, LACVP for short). Larger basis sets (LACVP* and onward to LACV3P+*) corroborated the LACVP results and predicted the same state ordering of Cpd I [20,47]. All electron 6-311+G* basis set also predicted similar A_{2u}–Π_S and $^4A_{2u}$–$^2A_{2u}$ energy gaps for the HS^- truncated model of Cpd I [47]. The Cpd I model, the computational method, and the basis sets used in our studies had been extensively used and proved to be sufficient to obtain at least qualitatively reliable results for the reactions catalyzed by cytochrome P450 enzymes [20,47,59,62,63].

2. DFT studies on the chemical process of drug metabolism catalyzed by P450

2.1. Ethanol oxidation

Ethanol is an intoxicating ingredient found in beer, wine, and liquor. It is a central nervous system depressant that is rapidly absorbed from the gastrointestinal tract into the bloodstream and is oxidized primarily in the liver. Alcohol dehydrogenase (ADH) was early and widely believed to be the only significant enzyme involved in ethanol metabolism. Oxidation of ethanol via ADH explains various metabolic effects of ethanol but does not account