Drug Metabolism Modeling

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Understanding P450-mediated Bio-transformations into Epoxide and Phenolic Metabolites

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Abstract: *Adverse drug reactions are commonly the result of cytochrome P450 enzymes (CYPs) converting the drugs into reactive metabolites. Thus, information about the CYP bioactivation of drugs would not only provide insight into metabolic stability, but also into the potential toxicity. For example, oxidation of phenyl rings may lead to either toxic epoxides or safer phenols. Herein, we demonstrate that the potential to form reactive metabolites is encoded primarily in the properties of the molecule to be oxidized. While the enzyme positions the molecule inside the binding pocket (selects the site of metabolism), the subsequent reaction is only dependent on the substrate itself. To test this hypothesis, we used this observation as a predictor of drug inherent toxicity. This approach was used to successfully identify the formation of reactive metabolites in over 100 drug molecules. These results provide a new perspective on the impact of functional groups on aromatic oxidation of drugs and their effects on toxicity.*

Most administered drugs are processed in the liver in order to be more efficiently excreted from the human body. In phase 1 metabolism, molecules are modified by a set of enzymes, primarily oxidases and hydrolases. Of the drugs currently on the market, 75% are metabolized by one of the 57 human cytochrome P450s (CYPs), which constitute a large family of heme-containing monooxygenases.[1] The metabolites produced in this process have their own pharmacological effects and intrinsic toxicity that may differ from those of the parent compound. For example, the transfer of oxygen to aromatic compounds may lead to the production of either the hydroxylated and/or epoxidized products (Figure 1 A). While the hydroxides are often safely processed in the next stages of metabolism (for example, conjugation to glucuronic acid or sulfation) or excretion, epoxides are often reactive and may covalently bind to macromolecules (such as DNA and liver proteins), leading to toxicity. Severe or even fatal complications have led to drug withdrawals or clinical stage failures, tragic outcomes, or a potentially enormous financial burden. Detailed information about the P450-mediated bioactivation of drugs would provide knowledge of their metabolic stability and half-life, as well as of their potential to induce toxicity.[2]

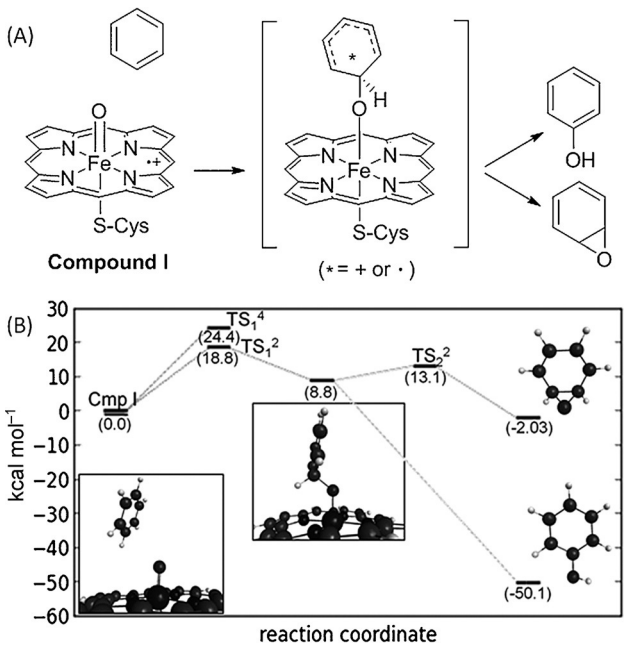

Figure 1. A) Oxidation of phenyl rings by CYP Cmp I. B) Full reaction pathway of benzene oxidation by P450.

What are the factors for inducing toxicity? Are both the substrate and enzyme equally affecting the outcome of the oxidation process? "Predictive" approaches often assume that the drug molecule alone is to be considered; approaches based on toxic alerts do not consider the protein. A compound is analyzed to identify potentially dangerous functional groups, patterns, and other molecular descriptors known to lead to toxicity experimentally.[3] However, the P450 enzyme is not a mere spectator in the metabolism of its substrates since binding and positioning of the substrates in the enzymatic cavity depends on the protein environment.[4] In this context, the reactivity of P450 active sites have been studied experimentally,[5] by DFT[6] and QM/MM[1b], in order to derive activation energies, force field parameters,[7] and rules for site of metabolism (SoM) prediction.[1b,3d,7–8] Many programs, based on diverse approaches, are available for SoM prediction, yet very few of them tackle the chemical nature of the metabolites produced.[9]

Herein we report on the key factors responsible for biotransformation of aromatic groups in drugs into toxic epoxides or safer phenolic derivatives.

To fully understand the reaction mechanism and analyze further a number of key electronic and structural properties,

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we started an in-depth computational study of the oxidation reaction. The CYP catalytic site comprises an iron-heme complex bound to the enzyme through a cysteine-iron coordination. The oxidized form of this complex, demonstrated to be the oxidant of the substrate[10] and commonly referred to as compound I (Cmp I), is a radical species where iron is coordinated to atomic oxygen as a distal ligand (Figure 1 A).

Experimental and computational studies revealed that aromatic oxidation goes through a tetrahedral intermediate common to both epoxide and hydroxide pathways.[11] As a fundamental demonstration, we first investigated the oxidation of benzene and the transition state (TS) structure leading to the tetrahedral intermediate and then the second step of the reaction, which leads to the aforementioned products. In accordance with previously reported results,[12] the formation of the TS leading to the tetrahedral intermediate using the quartet spin structure (TS_1^4) was found to be higher in energy than the one formed by the doublet spin state complex (TS_1^2) by 5.6 kcal mol^{-1} (Figure 1 B). Therefore, only the pathway through TS_1^2 was further investigated. Our calculations suggested that the oxidation of benzene by Cmp I would go to hydroxylation by the proton-shuttle mechanism.[12] Conversely, the epoxidation of benzene was observed also starting from the same tetrahedral intermediate, going through a small energy barrier (below 5 kcal mol^{-1}) to form the second carbon-oxygen bond (Figure 1 B).

Based on the energies of activation computed for multiple aromatic systems, the rate determining step is the formation of the tetrahedral intermediate common to both pathways. A closer look at these computed intermediates revealed that those resulting in epoxides had more of a radical character while those yielding hydroxides were more cationic in nature. For example, the bromobenzene intermediate, experimentally leading to epoxidation, had spin density on the aromatic ring (Figure 2 A), while phenol had none (Figure 2 B). The phenol intermediate is thus more cationic, which influences the acidity of the hydrogen on the reacting carbon, favoring the proton-shuttle mechanism and resulting in a hydroxylation. On the other hand, bromobenzene is less likely to transfer a hydrogen onto the heme since its intermediate is more radical than cationic. In this case, the molecule is further oxidized into an epoxide. Thus, the information that encodes which metabolite (hydroxide or epoxide) will be formed must be contained within the features of the substrate, while the primary role of the enzyme is to position the molecule within the reactive site and select the SoM.

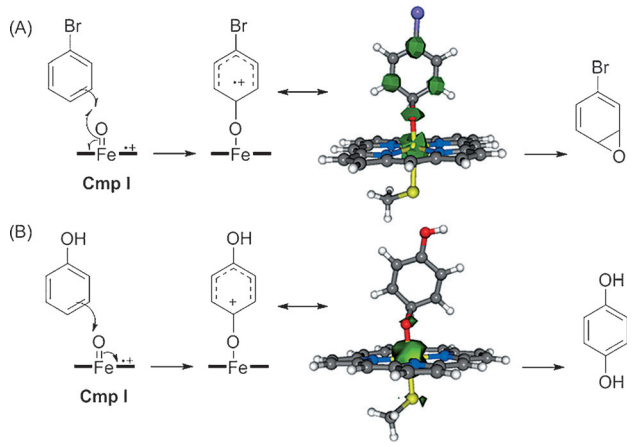

Figure 2. Schematic of reaction mechanism and spin density surfaces (unpaired electrons) of tetrahedral intermediates of CYP aromatic oxidations: A) bromobenzene, B) phenol. (see the Supporting Information for table of values).

Inspired by these results, we sought to identify a molecular property of the substrate that could relate the reaction mechanism obtained by QM with the metabolite obtained after CYP aromatic oxidation. One such property is the molecular orbitals (MOs): they describe the reactivity of the molecule, are influenced by its conformation, and are rapidly computed. MOs have previously been used to determine the SoMs on aromatic rings.[13] Instead, we made a connection between the MOs of the substrate, the preferred mechanism of oxidation, and consequently the metabolite formed. According to the frontier molecular orbital (FMO) theory, the density of the highest occupied molecular orbital (HOMO) indicates the valence electron distribution.[14] We believe that carbons with high HOMO density (ρ) will be more polarized and have a weaker bond with its hydrogen. Furthermore, occurrence of nodes of the HOMO near the oxidized position seems to influence the strength of carbon–carbon bonds in the aromatic ring. For example, if the carbons adjacent to the reactive site have MO coefficients of opposite signs, these C–C bonds are less conjugated. As a result, such a bond would be easier to break and use both electrons for the oxygen–carbon bond formation leading to a hydroxylation at this SoM.

Reported experimental studies (Supporting Information) revealed that a given CYP-catalyzed oxidation yields either metabolite and not a combination of both. Similarly, our model yields a binary output, predicting either an epoxidation or a hydroxylation at any given SoM. These ideas became the basis of our approach to evaluate the ability of a compound to form reactive metabolites. In fact, as we analyzed the HOMOs of a set of drug molecules, a trend became clear: the ability to transfer electrons to the newly formed C–O bond is directly related to the density and coefficients of the reacting and adjacent carbons.

Using the anti-inflammatory drug diclofenac (Figure 3) as an example, we will illustrate how the electron density of the HOMO at the SoM indicates which metabolite will be produced by CYP oxidation. The initial structure of the drug was obtained from the DrugBank[16] and optimized using the AM1 semiempirical method. The optimized conformation was then docked into CYP 2C9 using our program Impacts[17] to obtain possible reactive conformations of diclofenac/CYP complexes. Impacts combines docking, transition state modeling routines, and an energy of activation predictor to model the transition state of P450-mediated oxidations of xenobiotics. The MOs of each docked conformation pose were then obtained using B3LYP/6-31G(d) method by single point calculation from which the metabolites produced at each SoM were evaluated. The protocol used for diclofenac and all other substrates is summarized in Figure 4 (see the Supporting Information for details). Occasionally, the HOMO of a com-

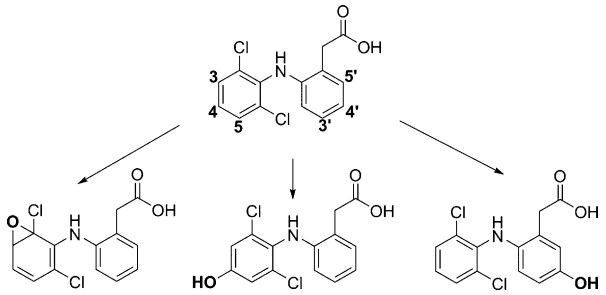

Figure 3. Schematic CYP-mediated metabolism of diclofenac, experimental results.[15]

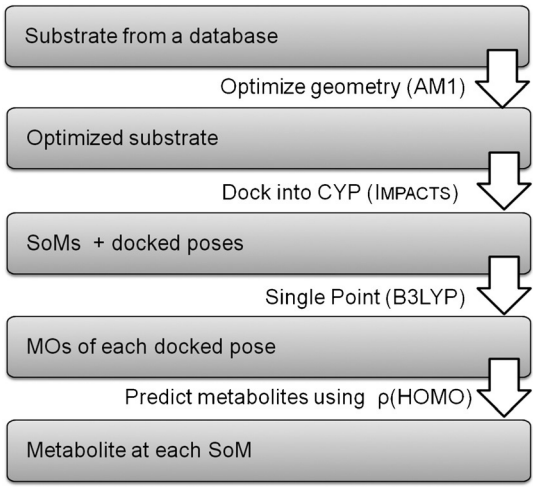

Figure 4. Procedure used to predict the metabolites formed by CYP aromatic oxidation for all 118 drug molecules, 173 SoMs tested (see the Supporting Information, sections S4, S5).

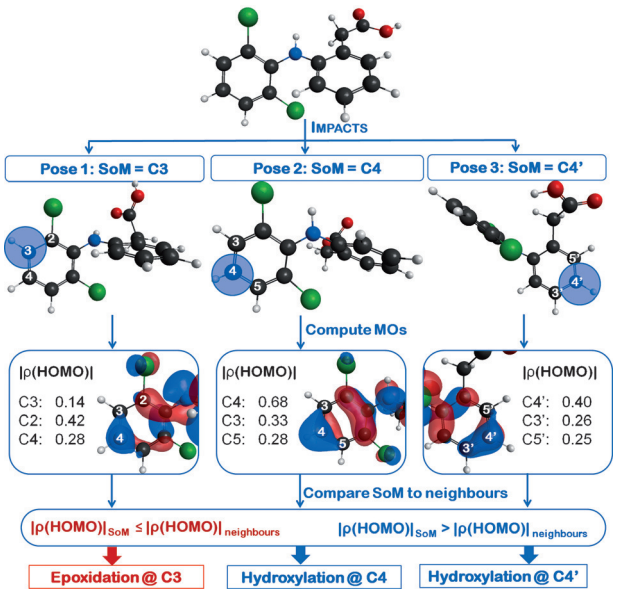

Figure 5. The model applied to diclofenac as an example: 1) the substrate is docked into the corresponding CYP (2C9 in this case) using IMPACTS and the SoMs are labelled (circled in blue); 2) ρ(HOMO) for each carbon is computed for each binding mode (poses 1–3); 3) ρ(HOMO) at the SoMs are compared to that of the adjacent carbons; and 4) the metabolites are predicted for each SoM.

Table 1: Validation of the method on 118 drug molecules/173 SoMs: accuracy by method compared to experimental results (see the Supporting Information, section 5 for details).

Method	Benzene-derivative SoMs	All SoMs	SoMs leading to epoxidation only
Docked	83%	84%	94%
Non-Docked	68%	71%	90%

pound did not correspond to the SoM. In these cases, the next occupied MO was used. As demonstrated previously, the shapes and energies of the occupied MOs obtained from DFT are well described.[18]

Experimentally, diclofenac is metabolized at several positions, three of which are aromatic (Figure 3).[15] After oxidation at these SoMs, the metabolites produced are one epoxide (toxic) and two hydroxides (safer). IMPACTS successfully identified these SoMs and output the conformations that diclofenac would take while reacting with Cmp I (Figure 5, poses 1–3). To determine the metabolite formed at each oxidized position, the HOMO density (ρ(HOMO)) on the SoM is compared to that of its neighboring carbons. The first SoM, C3, has ρ(HOMO) lower than that of C2 and C4 so, according to our model, its oxidation would lead to epoxidation (Figure 5 left). In contrast, carbons C4 and C4′ have ρ(HOMO) greater than that on adjacent carbons, enabling the proton shuttle mechanism to take place, resulting in hydroxylation (Figure 5, center and right).

We then tested this approach on a set of diverse drug molecules taken from open online databases and compared our predictions to experimental results. Out of 145 oxidations on aromatic positions on 6-membered rings, we were able to predict the correct metabolite in 83% of the cases using this theory (Table 1). To verify whether our approach was transferable to five-membered aromatics and rings with heteroatoms, we increased the testing set. Our predictions were correct in 84% of cases in a total of 173 SoMs. This approach identifies drugs leading to reactive epoxide metabolites in 94% of the cases if the SoM is known. However, experimental hydroxylations labeled as epoxidations (false positives) were also observed. This may be explained by the nature of the experimental results mined from the literature; our method assessed the first oxidation of a molecule by CYP enzymes while the data collected often identified the products of oxidation in urine, after other enzymes such as epoxide hydrolases may have altered the metabolite or the molecule may have undergone a second oxidation.

To further analyze the depth of our understanding, the method was then tested on conformations of molecules that have not been docked prior to the evaluation of their MOs, validating our perception of the key role played by the enzyme. As expected, the accuracy dropped to 71%, demonstrating the importance of determining the correct geometry of the substrate in the binding site of the enzyme before evaluating its properties.

In a drug discovery project, the SoMs of a lead compound may not be known, so a good screening method should be able to identify molecules leading to reactive metabolites without this information. We tested our approach on its ability to flag drugs forming epoxides using the SoMs found by the docking program. This approach was applied to the 118 molecules making up the set of 173 SoMs. If at least one of the metabolites of a given drug is an epoxide, it should be red-flagged while others should be green-flagged. When applied to the two most likely SoMs (top 2 as predicted by IMPACTS), the method was able to red-flag correctly 62% of drugs having reactive metabolites and green-flagged 77% of the safe drugs. Taking into account the 70–80% accuracy of IMPACTS to predict the correct SoMs with this metric, the combinatorial predictive power is excellent.

In conclusion, we have shown that drugs can be inherently toxic at given SoMs while only the selection of the SoM is made by the P450s. This explains the reasonable accuracy of ligand-based toxicity prediction methods as well as the isoform dependence of the drug metabolism and the genetic predispositions of some patients. This study demonstrates that metabolite prediction of aromatic oxidation by CYP enzymes can be done using only the molecular orbitals of the docked substrate. Our data reveals that the electron density on the HOMO at each SoM directly influences the reaction mechanism and determines which metabolite, the epoxide or the hydroxide, will be formed. Other properties, such as Fukui functions[19] or local ionization potentials,[20] could be used to fine tune the results, however with a significantly lower throughput. One application of this method is the prediction of toxicity of potential drug candidates, prior to experimental testing. This approach is dependent on the quality of the docking used to find the SoMs, but it does not rely on any training set, making it highly transferable. Our model reproduces experimental results, shortens computational time from weeks to minutes, and can offer insights to eliminate drugs that would lead to reactive metabolites once bioactivated.

Experimental Section

From the heme structure extracted from the crystal structure of the most widely found CYP isoform, 3A4 (pdb: 3NXU), was obtained the ground state geometry of Cmp I in both doublet and quartet spin, the two possible states, through optimization. As in most QM models of this system,[1b] Cmp I was truncated: the carboxylate side arms of heme were deleted, and cysteine was replaced by thiomethyl. All structures were optimized in gas phase using the PBE0 functional with a custom basis set (see the Supporting Information) and the vibrational analysis was performed when necessary. GAMESS-US and ORCA computational software were used for the calculations. Detailed experimental procedures can be found as supporting information.

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