N- and S-Oxidation Model of the Flavin-containing Monooxygenases

Peter Walton, Mario Öeren, Peter Hunt, Matthew Segall

Optibrium Limited, Cambridge, UK; info@optibrium.com

Introduction

Existing computational models of drug metabolism are heavily focused on predicting oxidation by cytochrome P450 (CYP) enzymes, because of their importance in phase I drug metabolism, reactive metabolite formation, and drug–drug interactions. Due, in part, to the success of these models, new drug candidates are typically well-optimised with respect to CYP metabolism. However, novel metabolites are observed due to other, less-studied, enzyme families such as the flavin containing monooxygenases (FMOs).[1] FMOs are found in multiple tissues, including the liver, and have five active isoforms (FMO1-5). In common with CYPs, FMOs are responsible for phase I, oxidative metabolism and catalyse a variety of reaction types, including N- and S-oxidation, demethylation, desulphuration and Bayer-Villiger oxidation.

The objective of this study was to elucidate the reaction mechanism of FMO-mediated oxidation to inform the development of models to predict the metabolism of novel substrates.

N- and S-oxidation

N- and S-oxidation account for approximately 99% of known FMO-mediated metabolism. An oxygen atom is transferred to the substrate from a flavin adenine dinucleotide (FAD) peroxide reaction centre in the protein (see Figure 1). The formation of the peroxide and regeneration of FAD are independent of the substrate, thus we focus on the product-formation step highlighted in red.
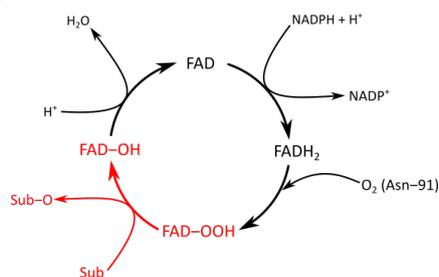

Figure 1. The catalytic cycle of N- and S-oxidation by FMOs. Sub stands for substrate.

Methods

Density functional theory (B3LYP/def2-SVP) was applied to study the reaction mechanism of oxidation using NWChem[2]. The FAD peroxide molecule is too large to permit routine calculations at this level of theory, therefore a simplified analogue (Figure 2b) was used as the basis for the calculations.
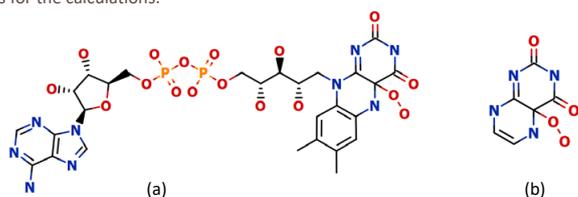

Figure 2. FAD peroxide (a) and the FAD analogue used to study the reaction mechanism (b).

To confirm that the simplified analogue reproduced the potential energy surface of the full FAD-peroxide around the peroxide reaction centre, we compared the peroxide bond-stretching energy in the two systems and found that they were indistinguishable, as shown in Figure 3.
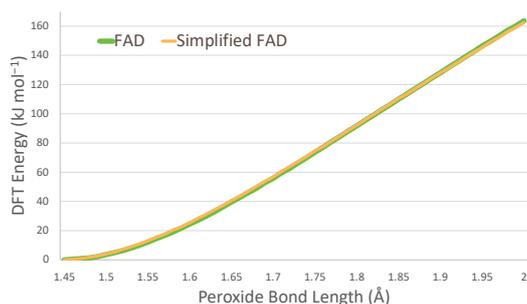

Figure 3. Comparison of peroxide bond-stretching energy between FAD peroxide and the simplified FAD peroxide analogue used herein.

Reaction Mechanism

A rigorous search was performed for transition states for radical and S_N2 reaction mechanisms using the simplest FMO substrate, trimethylamine. A transition state could not be found for a radical mechanism, but a transition state was confirmed for an S_N2 mechanism, illustrated in Figure 4. Detailed analysis of charge density distributions further supported an S_N2 mechanism.
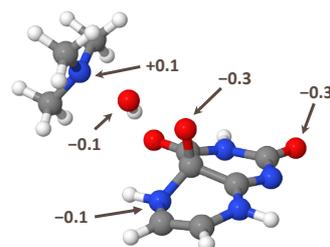

Figure 4. Transition state geometry for trimethylamine oxidation with illustrative Hirshfeld charges.

The full reaction mechanism is illustrated in Figure 5. During the reaction, the proton of the peroxide is anchored to the carbonyl group of the FAD, stabilising the transition state, and is subsequently transferred to the peroxide oxygen remaining on the FAD. An exploration of the energy profile of the reaction indicates that this final step is barrierless.
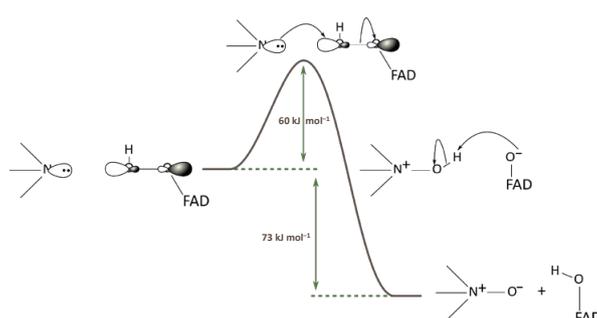

Figure 5. Reaction mechanism for N- and S- oxidation.

Application to Diverse Substrates

Having identified a putative reaction mechanism for FMO-mediated N- and S-oxidation, transition state calculations were performed for five drug-like substrates of FMO, for which the sites of metabolism have been determined experimentally. In every case, the site with the lowest activation energy corresponded to the observed site of metabolism, further supporting the proposed mechanism, as shown in Figure 6.
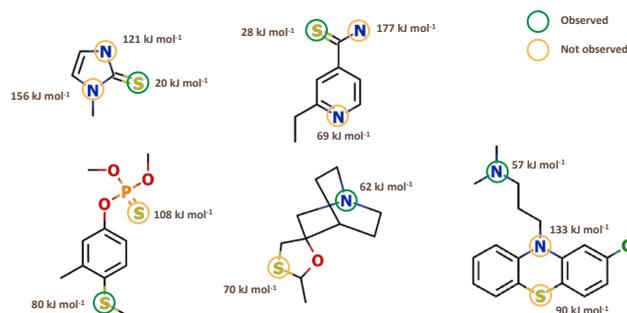

Figure 6. Five substrates of FMO, the potential sites of metabolism and activation energy values.

Conclusion

We proposed an S_N2 reaction mechanism for N- and S-oxidation by FMOs and tested the applicability of the transition state model on five additional FMO substrates. The results indicate that the activation energy for the oxidation reaction is an important factor in the regioselectivity of FMO metabolism. This work will enable further efforts to build a computational model for prediction of FMO metabolism.

References

[1] Dixit et al. (2017) WIREs Comput. Mol. Sci. 7:e1323

[2] Valiev et al. (2010) Comput. Phys. Commun. 181, 1477