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Detoxification of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by cytochrome P450 enzymes: A theoretical investigation

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ABSTRACT

Two types of detoxification routes, *N*-demethylation to form 4-phenyl-1,2,3,6-tetrahydropyridine (PTP) and aromatic hydroxylation to generate 4-(4′-hydroxyphenyl)-1-methyl-1,2,3,6-tetrahydropyridine (MPTP-OH), for 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mediated by Compound I (Cpd I) of cytochrome P450 are investigated theoretically using hybrid density functional calculations. Quantum chemical results reveal that for the *N*-demethylation, the initial C–H bond activation is achieved *via* a hydrogen atom transfer (HAT) mechanism. This is followed by a subsequent O-rebound to yield the carbinolamine intermediate. Due to the nature of pericyclic reaction, the generated carbinolamine decomposes in a non-enzymatic aqueous environment with the assistance of water molecules, forming amine and hydrated formaldehyde. For the aromatic hydroxylation, an initial addition of Cpd I to the substrate occurs mainly through a side-on approach with a subsequent proton shuttle to form the phenol product. A comparison of the energy barriers for both routes indicates that the *N*-demethylation (7.5/5.7 kcal/mol for the quartet/doublet state in solvent) is thermodynamically more favorable than the aromatic hydroxylation process (14.9/14.8 kcal/mol for the quartet/doublet state in solvent). This trend is in good agreement with the experimental product distribution, *viz.*, the *N*-demethylation product PTP is more than the aromatic hydroxylation product MPTP-OH. Taken together, these observations not only enrich our knowledge on the mechanistic details of the *N*-dealkylation and the aromatic hydroxylation by P450s, but also provide certain insights into the metabolism of other analogous toxins.

1. Introduction

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as a neurotoxic byproduct formed in the chemical synthesis of heroin, is able to induce a Parkinson-like syndrome in humans and in animals [1–6]. It has been extensively used to understand how chemicals cause Parkinson's disease [7]. The neurotoxicity of MPTP is mainly due to its activation by monoamine oxidase to yield 1-methyl-4-phenyl-2,3-dihydropyridinium cation, which is selectively absorbed by dopaminergic neurons leading to cell death [5,6,8,9]. However, plenty of experimental evidence indicates that as a consequence of the structural features of cyclic tertiary allylamine, MPTP should be one of the substrates for cytochrome P450 (P450) enzymes [8,10]. The metabolism of MPTP mediated by P450s (mainly CYP2D6) is a major detoxification pathway *in vivo* [5,7,9,11–13]. The toxic outcome caused by MPTP might result from the balance between the rate of metabolism to toxic product (activation) and the rate of detoxification (inactivation) [9]. The detoxification, which can protect neurons against chemical damage, is a critical aspect of MPTP neurotoxicity within the central nervous system [5]. Generally speaking, increasing the detoxification efficiency will be able to efficiently alleviate the neurotoxicity. Thus, it is desirable to thoroughly understand the mechanistic details of the detoxification to provide guidance for the protection against such chemical damage.

Two main metabolites have been obtained experimentally during the detoxification of MPTP by P450s in the presence of NADPH-P450 reductase. Therein, as shown in Scheme 1, one is the *N*-demethylation product, 4-phenyl-1,2,3,6-tetrahydropyridine (PTP), and the other is the aromatic hydroxylation product, 4-(4′-hydroxyphenyl)-1-methyl-1,2,3,6-tetrahydropyridine (MPTP-OH). Modi et al. [12] have proved the existence of two alternative binding modes for MPTP in P450 through physicochemical methods, including paramagnetic relaxation techniques. One is a binding mode with the *N*-methyl group close to the heme iron, and the other is a binding mode with the *para* H of the phenyl ring closest to the heme iron. Meanwhile, the latter with a lower dissociation constant (K_d) was more favorable than the former. However, kinetic studies of Herraiz et al. revealed that the *N*-demethylation process ($K_m = 69.6 \pm 2.2$ μM) was more efficient than the aromatic hydroxylation one ($K_m = 79.36 \pm 3$ μM), resulting in more PTP [5,9]. This might be due to the intrinsic chemical reactivity of the various positions of the substrate [14] rather than the allosteric

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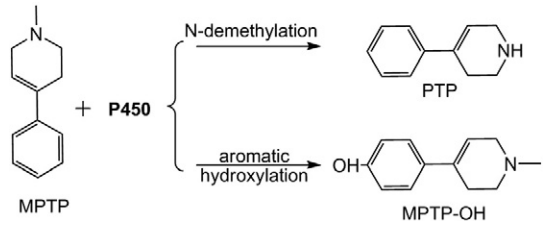


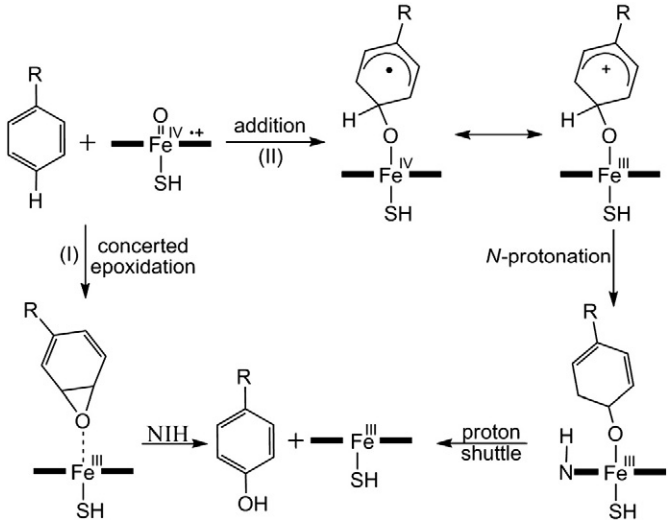
Scheme 1. Mechanistic pathways of MPTP mediated by P450s.

effect of the P450 reductase [15]. Since the binding of MPTP to P450 2D6 was not affected by the P450 reductase, which was proved by the superimposable spectral titration results of Hanna et al. [15]. There has been considerably more experimental and theoretical work on the mechanism of the *N*-dealkylation [16–36] as well as the aromatic hydroxylation [14,37–45] mediated by P450s, and great progress has been made.

For the initial process in the reaction of P450-mediated *N*-dealkylation of amines (Scheme 2), two alternative mechanisms (single electron transfer, SET and hydrogen atom transfer, HAT mechanism) have survived for several decades [16–36], which have been described in detail by Wang et al. [30]. The hydrogen atom transfer mechanism is the long presumed and generally accepted one so far.

For the aromatic hydroxylation, early studies revealed that the observation of NIH-shift [46,47], *i.e.*, the migration of a substituent from the site of hydroxylation to the adjacent carbon, suggested the existence of the arene oxide intermediates (path I in Scheme 3) [14,48,49]. Whereas, the following studies [14,39–41,50,51] ruled out the concerted epoxidation mechanism, but supported an alternative one, *i.e.*, an initial addition of the oxidant Compound I (Cpd I) to a substrate carbon to give a tetrahedral σ-complex intermediate (path II in Scheme 3), followed by rearrangement to different products. However, some observations confirm that the specific reactivity patterns are also affected by the nature of the substrate. For instance, P450-mediated hydroxylation of aromatic compounds containing a halogen substituent is achieved *via* expelling the halogen as an anion, rather than by an NIH shift [47,52–56]. Schyman et al. [57] have revealed that instead of the addition process, an initial hydrogen atom transfer with a subsequent ring-π radical rebound step occurred to achieve the hydroxylation of tyramine.

To the best of our knowledge, there is no theoretical investigation on the mechanism of P450s-meditated detoxification of MPTP by now. Thus, in the present study, density functional theory (DFT) calculations were employed to address the mechanistic details of such detoxification process. Both aromatic hydroxylation and *N*-demethylation have been explored from a chemical point of view. Our computational results reveal that the *N*-demethylation route is more favorable than the aromatic hydroxylation route, which is in line with experimental product distribution [5,9]. It is shown that the initial C–H activation of the *N*-

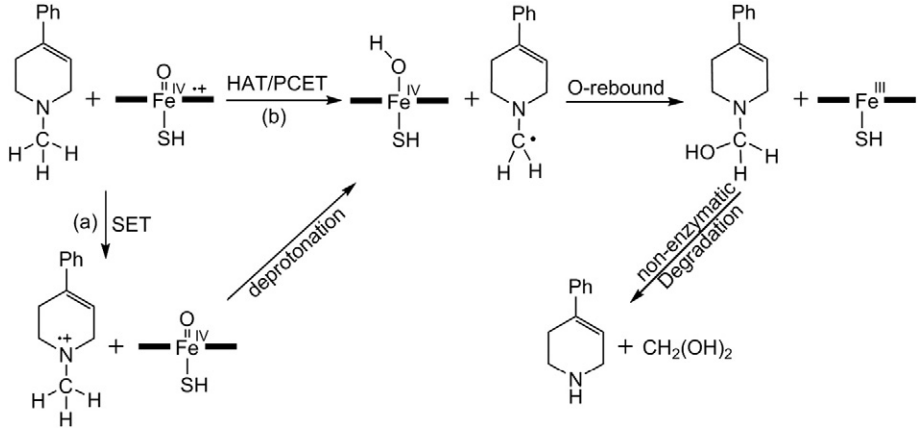


Scheme 3. Alternative mechanistic hypotheses for the aromatic hydroxylation by P450s.

demethylation proceeds *via* a rate-limiting HAT step and the generated carbinolamine degrades with the assistance of water molecules in an aqueous solution yielding hydrated formaldehyde product. Furthermore, the transition state of the addition of Cpd I to the substrate has a mixed cationic and radical characteristic and the phenol product is obtained from the σ-complex intermediate *via* a proton shuttle mechanism.

2. Computational methods

The active species of P450 was simplified to the model complex (SH)FePor(L) with L = O, which has been proven a better representation of this enzyme [58,59]. MPTP was selected as the substrate. The spin-unrestricted B3LYP (UB3LYP) [60–63] hybrid density functional was employed for all calculations using the Gaussian 09 package of programs [64]. The LACVP(Fe)/6-31G(C, H, O, N, S) basis set [65], *i.e.*, LACVP (denoted B1) was used for geometry optimizations without symmetry constraint and frequency calculations. The transition states were characterized with the sole imaginary frequency for a correct single mode along the reaction path, while all local minima were verified with real frequencies. Single point energy (SPE) calculations on the optimized geometries were performed using a larger basis set, which describes Fe by LACV3P and all the rest of the atoms by 6–311+G** basis set, *i.e.*, LACV3P+** (denoted B2). All calculations mentioned above were performed in vacuum. In addition, the self-consistent reaction field (SCRF) method in a polarizable continuum model (PCM) of a nonpolar



Scheme 2. Alternative mechanistic hypotheses for the oxidative *N*-demethylation of MPTP by P450s.