Tetrahedron 72 (2016) 2858–2867

Contents lists available at ScienceDirect

Tetrahedron

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

Quantum chemical exploration on the metabolic mechanisms of caffeine by flavin-containing monooxygenase

Yuan Kang [a,†], Jing Tao [a,†], Zhiyu Xue [a], Yan Zhang [a], Zeqin Chen [a,*], Ying Xue [b]

[a] College of Chemistry and Chemical Engineering, Chemical Synthesis and Pollution Control Key Laboratory of Sichuan Province, China West Normal University, Nanchong 637002, PR China
[b] College of Chemistry, Key Laboratory of Green Chemistry and Technology in Ministry of Education, Sichuan University, Chengdu 610064, PR China

ARTICLE INFO

Article history:
Received 9 December 2015
Received in revised form 17 March 2016
Accepted 28 March 2016
Available online 4 April 2016

Keywords:
Flavin-containing monooxygenase
Caffeine
N-demethylation
C-8 oxidation
Homolysis

ABSTRACT

Caffeine, a ubiquitous natural product, is widely consumed by humans. The metabolic mechanisms of caffeine by flavin-containing monooxygenase (FMO) were systematically investigated in this study by quantum mechanics calculations. Four main metabolic pathways were characterized, including N-demethylations at N_1-, N_3-, and N_7- sites (paths I–III) and C-8 oxidation (path IV). N-demethylation proceeds via the concerted homolytic cleavages of C–H and O–O bonds, while C-8 oxidation is an oxygen atom transfer mechanism. It shows that C-8 oxidation predominates over N-demethylations and trimethyluric acid is therefore the optimum metabolite of caffeine by FMO. Additionally, N_3-demethylation is more favorable than N_1 and N_7-demethylations. This study can offer important clues for the biodecaffeination techniques.

1. Introduction

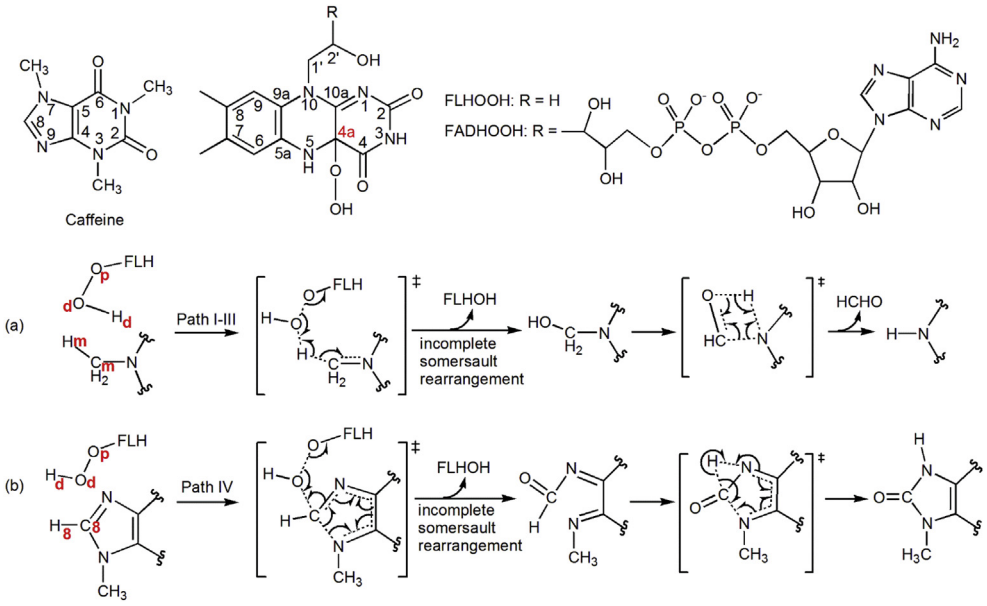
Caffeine, known as 1,3,7-trimethylxanthine (Scheme 1), is a commercially important purine alkaloid existing mainly in the seeds, leaves or fruits of some plants.[1,2] It is endowed as the world's most popular psychoactive substance because of its activity at adenosine receptors,[3,4] which makes it widely used as a respiratory stimulant, a diuretic in pharmaceuticals, and an analgesic enhancer in headache, etc.[5–11] However, heavy coffee consumption may increase the risk of bladder cancer.[12] The implications of caffeine on human health have caused great scientific interests in its biodegradation.[13,14]

Metabolized by biological oxidation, caffeine in humans can produce at least 12 metabolites which have been detected in the urine.[15] The four important primary metabolites of caffeine are theophylline (TP, 1,3-dimethylxanthine), paraxanthine (PX, 1,7-dimethylxanthine) and theobromine (TB, 3,7-dimethylxanthine), formed by N-demethylation at each of the three tertiary amine nitrogen atoms, as well as 1,3,7-trimethyluric acid (TMU) formed by C-8 oxidation.[16,17] Bioconversion of caffeine into its metabolites is an enzymatic process and occurs primarily in the liver.[18] Two main types of enzymes presented in the liver microsomes of human can potentially catalyze the metabolism of caffeine, namely, flavin-containing monooxygenase (FMO) and cytochrome P450 (CYP).[19,20] Both enzymes are monooxygenases and involve in the oxygenation of a wide range of heteroatom-containing compounds, by which the lipophilic compounds are converted into more hydrophilic metabolites for rapid excretion.[21] The mechanism by which each monooxygenase operates, however, is quite distinct.[22–24] Drug toxicity thus far observed in the clinic is mainly the result of CYP-dependent oxidation because CYP can also oxidize compounds to electrophilic reactive metabolites that can have significant consequences for toxicity. Our previous study has systematically explored the metabolic mechanisms of caffeine by CYP.[25] In contrast, FMO generally converts lipophilic nucleophiles into more polar, readily excreted and harmless metabolites and is rarely inhibited. Nevertheless, the oxidation metabolic mechanisms of caffeine by FMO have remained elusive up to now yet.

FMO is a class of monooxygenases capable of oxygenating nucleophilic oxygen, nitrogen, halide, selenium, and phosphorous atoms of a wide range of substrates, such as amines, amides, thiols, and sulfides.[26–30] As the oxygenant of FMO, the electrophilic C-4α-flavinhydroperoxide (FLHOOH, Scheme 1) intermediate is a covalent adduct between the C-4α-carbon of flavin and dioxygen molecule,[31] which is a short-lived ($t_{1/2}$=2.5 ms) intermediate oxidant and can break down into oxidized flavin (Flox) and HOOH rapidly in the absence of the stabilizing influence.[32,33] In the past years, some theoretical attempts have been performed to identify

* Corresponding author. Tel.: +86 817 2568081; e-mail address: chenzeqin@cwnu.edu.cn (Z. Chen).
† These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.tet.2016.03.091
0040-4020/



Scheme 1. Metabolic mechanisms of caffeine catalyzed by FMO.

the oxidation mechanism of FMO.[34–41] Canepa and his co-workers identified the oxidation mechanism of dimethyl sulfide using a series of bicyclic and tricyclic models of FLHOOH and proposed a S_N2-like attack mechanism.[34] Subsequently, Bach and his co-workers confirmed the S_N2-like attack mechanism of the heteroatom on the distal oxygen of the hydroperoxide based on the investigation on the oxidation of N-, S-, P-, Se-containing nucleophiles catalyzed by FMO.[35] More recently, Bach formulated another new mechanism for FMO oxidation of *N*- and *S*-nucleophiles, namely, a concerted emolytic O—O bond cleavage in concert with hydroxyl radical transfer from the flavin hydroperoxide.[36,37] Additionally, the oxidation mechanisms of *p*-hydroxybenzoate (*p*-OHB) and its derivatives mediated by *p*-hydroxybenzoate hydroxylase (PHBH) were also characterized based on the FLHOOH model. Three possible mechanisms have been indicated, including a OH radical transfer mechanism, an electrophilic aromatic substitution mechanism, and an oxygen atom transfer reaction with intramolecular 1,2-proton transfer.[38–41]

This work aims at uncovering the oxidation metabolic mechanisms of caffeine by FMO. The obtained results show that N-demethylation proceeds via the concerted homolytic cleavages of C—H and O—O bonds, while C-8 oxidation proceeds via an oxygen atom transfer mechanism. C-8 oxidation is more favorable than N-demethylations and trimethyluric acid therefore is the optimum metabolite of caffeine by FMO. To date, this is the first time to report the involvement of FMO in the metabolism of caffeine at the theoretical level, which can offer important understandings for the bio-decaffeination techniques.

2. Computational details

The computational reaction model adopted in this work consists of the two parts: (a) caffeine and (b) the reactive FLHOOH of FMO enzyme which comprises three segments, the tricyclic isoalloxazine moiety, the C-4α-hydroperoxide functionality, and the β-hydroxyethyl group to model the effect of the 2′-OH group of the ribityl side chain of native FADHOOH (Scheme 1).[42] The coordinate of FLHOOH was established from the crystallographic structure of FMO enzyme (PDB code: 2GVC).[43] Standard procedures within the Gaussview program were used to incorporate the coordinate of caffeine to build the suitable initial structure to search for transition state geometries at the DFT level.

All the quantum chemistry calculations were performed using the Gaussian 09 program.[44] Geometric structures for all the stationary points, including the reactant complexes, product complexes, intermediates, and transition states, were optimized in the gas phase using the dispersion corrected hybrid functional of B3LYP-D3[45,46] in conjunction with the standard 6-31+G(d) basis set. Vibrational frequency calculations were performed to confirm the stationary point as a minimum with all positive frequencies or as a transition state with only one imaginary frequency. The connectivity between the stationary points was established by intrinsic reaction coordinate (IRC) calculations.[47,48] Natural Population Atomic (NPA) charges were determined with the Natural Bond Order (NBO) theory.[49] The binding energies of caffeine and FLHOOH were corrected using basis set superposition error.

Single-point calculations were performed both in the gas phase and in the protein environment using B3LYP-D3 functional with a higher basis set, 6-311++G(d,p), which has be proved to have consistent results with B3PW91-D3 and PBE1PBE-D3 functionals.[50] The weak polarization effect of a protein environment was modeled using conductor-like polarizable continuum model (CPCM)[51] with dielectric constant of ε=5.62 (chlorobenzene). The value (ε=5.62) was taken as a reasonable compromise for the enzyme active site.[25,52,53] All of the single-point energies were corrected by the gas-phase thermodynamic quantities. The thermodynamic data reported in this paper are at 298.15 K and 1 atm. Cartesian coordinates for the optimized geometries and energies of all stationary points along the potential energy profiles are provided in the Supplementary data document.

3. Results and discussion

Mediated by FMO, the degradation of caffeine can take place along two classes of mechanisms: demethylation and oxidation, which were divided into four reaction pathways (Scheme 1). Paths I—III involve the demethylation of caffeine at N_1-, N_3-, and N_7- sites, yielding theophylline, paraxanthine, and theobromine, respectively, whereas path IV concerns the oxidation of caffeine at C_8