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Biomimetic oxidation of acetaminophen prodrugs catalyzed by iron porphyrins: Effect of nitrogen and thiolate axial ligands on drug and metabolite formation

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ABSTRACT

Metalloporphyrins (MP) are often employed in biomimetic catalysis due to their structural and functional similarity to the heme prosthetic group of oxygenating enzymes. In these enzymes, the iron porphyrin is primarily protein-bound at the active site by cysteine (cytochrome P450, CYP), histidine (peroxidase), and tyrosine (catalase), acting as axial ligands. The diverse functionality and variable oxidizing power exhibited by different oxygenating enzymes is [in part] attributed to the ligating amino acid, and the relationship between iron and the heteroatom. Attempts to mimic this relationship by developing MP systems that behave similarly to CYP enzymes have mainly focused on incorporating nitrogen (*N*)-heterocycles. Although it is widely known that different enzyme families favor distinctive products, sulfur is easily oxidized and prone to form disulfide bonds, and as such, thiolates for the most part remain unexplored. Herein, we report our findings from the biomimetic oxidation of phenacetin, methacetin, and acetanilide by *meta*-chloroperoxybenzoic acid (*m*-CPBA), catalyzed by hindered iron (III) porphyrins that are chelated by different axial ligands. Nine axial ligands that vary in pK_a (2.8–11.2), size, and heteroatomic identity were examined, to more accurately determine the factors that contribute to function and effect. The [three] thiolate ligands afforded the greatest yields of acetaminophen in the majority of cases. Furthermore, nitrogen- and sulfur-containing ligands were observed to promote different reaction pathways, regardless of pK_a or ligand size.

1. Introduction

Metalloporphyrins (MP) are often used as biomimetic catalysts for phase I cytochrome P450 (CYP)—mediated metabolism [1–4], as they structurally mimic the active site analogous to all CYP isozymes, featuring an iron protoporphyrin IX known as heme B. The super-family of CYP oxygenating enzymes is estimated to be responsible for over 90% of pharmaceutical biotransformations [5–7]. Although many oxygenating enzymes contain a heme-active site, none possess the catalytic efficiency of CYP enzymes, the major differentiating factor being the amino acid the active site is bound by.

In CYP enzymes, heme B is protein-bound by cysteine (Cys), whereas other enzymes are histidine (His)- or tyrosine (Tyr)-bound, i.e. peroxidase and catalase, respectively. This relationship between iron(heme B) and sulfur(Cys) creates the "push–pull" effect that is attributed for the oxidizing power of CYP [8–10]. Moreover, other oxygenating enzymes have different mechanisms, create a dissimilar "push" onto iron, and have significantly slower auto-oxidation rates than CYP enzymes [11,12]. Attempts to more closely mimic CYP oxidation has primarily involved the incorporation of imidazole, or other nitrogen (*N*)-heterocycles, that ligate to

Abbreviations: AcOH, acetic acid; APAP, acetaminophen; CAT, catalase enzyme; CH_2Cl_2, dichloromethane; Cpd I, compound I; CYP, cytochrome P450; Cys, cysteine; EA, electron affinity; EtOAc, ethyl acetate; FeP, iron(III) porphyrin; GC–MS, gas chromatography–mass spectrometry; h, hours; His, histidine; HPLC-UV, high performance liquid chromatograph-ultraviolet; HRP, horseradish peroxidase enzyme; IPA, 2-propanol; *m*-CPBA, *meta*-chloroperoxybenzoic acid; MeCN, acetonitrile; MeOH, methanol; MeOH-d_3, (1,1,1)-trideuteromethanol; NAPQI, N-acetyl-p-benzoquinone imine (OMe: methoxy, OEt: ethoxy); PhMe, toluene; QM, quantum mechanical; (r)t, (retention) time; TDCPP, *meso*-tetrakis(2,6-dichlorophenylporphyrin); TDCPPβBr_8, *meso*-tetrakis(2,6-dichlorophenylporphyrin-β-octabromo); TDFPP, *meso*-tetrakis(2,6-difluorophenylporphyrin); THF, tetrahydrofuran; TLC, thin-layer chromatography; Tyr, tyrosine.

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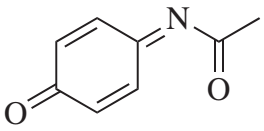

Fig. 1. NAPQI.

the MP in situ. As sulfur is easily oxidized and prone to dimerization, thiols largely remain unexamined [12].

In recent years, MP catalysts have primarily been used to increase reaction stereo- or regio-selectivity, as well as produce and/or scale-up metabolites. However, targeting prodrugs offers an even greater range of applicability. Biomimetic systems provide a simpler platform to assess a compound's efficiency as a prodrug, and streamline the process of evaluating prodrug libraries, as opposed to employing time-consuming in vitro or in vivo models. In order to tangentially explore these areas, the effects of different sulfur (*S*)- and/or (*N*)-containing axial ligands were assessed in the biomimetic oxidation of acetaminophen (paracetamol, APAP) prodrugs, using iron (III) porphyrin catalysts, a general example for which is illustrated in Scheme 1.

APAP is one of the most widely used analgesic and antipyretic agents, particularly with children where sudden adverse reactions from alternatives have been reported [13,14]. Unfortunately, APAP is associated with dose-dependent toxicity, stemming from the electrophilic metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI, Fig. 1), generated during first-pass metabolism by CYP2E1, 1A2, and 3A4. At therapeutic doses, NAPQI accounts for less than 10% of the metabolic products, a small amount that then undergoes glutathione (GSH)-conjugation and is excreted [15]. Conversely, at high concentrations, GSH-stores deplete and excess NAPQI becomes covalently bound to cellular proteins via Cys [16]. NAPQI-protein adducts have made APAP the leading cause of acute liver failure [17]. To circumvent NAPQI-induced liver toxicity, many have taken the promoiety approach to prodrug design, capping the APAP phenol-group using ether- [18,19], amino acid- [20], and/or other similar derivatizations [21]. Moreover, this approach has proven to be beneficial for improving the poor solubility and bioavailability [22] that is commonly exhibited by drugs containing phenolic functional groups [23].

Here, we report our findings from iron(III) porphyrin (FeP)-mediated biomimetic oxidations of APAP (**1**) prodrugs, with particular emphasis on the effect of axial ligands. Phenacetin (**2**), methacetin (**3**), and acetanilide (**4**) are examined (Fig. 2), (**2**-**4**) being chosen as representative compounds that reportedly undergo different biotransformations to (**1**). The activity of three hindered FeP complexes (Fig. 3) are compared in this work, as recent literature has demonstrated variable catalytic efficiency from β-pyrrole substituted MPs [24–28]. To examine the role of sterics and electronics, Fe(TDCPPβBr$_8$)Cl and Fe(TDCPP)Cl are used to assess the effect of increased electronegativity at the β-positions, compared to potential steric hindrance from the bulky β-pyrrole bromines surrounding the iron active site. Employing Fe(TDFPP)Cl then allows a more direct comparison of the effects of electronegativity. In one aforementioned study assessing the effect of β-pyrrole bromination [25], *meta*-chloroperoxybenzoic acid (*m*-CPBA) was found to afford the largest conversion of carbamazepine, regardless of the degree of bromination (0–8). Based on these findings, *m*-CPBA decidedly provided the most equal starting point for the FeP catalysts.

While a number of other variables are discussed, axial ligands are the primary focus (Table 1), as our aim is to identify chelating agents that promote behavior more similar to what is observed of Fe—S(Cys) in CYP enzymes. We show that optimal FeP/axial ligand combinations are different for each respective transformation, providing a starting point for potential prodrugs of (**1**) derived from similar starting materials. Furthermore, reactions in which *S*-containing reagents are employed afford greater yields of (**1**), and generate different products than reactions with *N*-containing reagents.

2. Experimental

2.1. Materials and methods

2.1.1. Reagents

The iron porphyrin complexes, iron (III) *meso*-tetrakis (2,6-difluorophenylporphyrin) chloride [Fe(TDFPP)Cl], iron (III) *meso*-tetrakis (2,6-dichlorophenylporphyrin) chloride [Fe(TDCPP)Cl], iron (III) *meso*-tetrakis (2,6-dichlorophenylporphyrin-β-octabromo) chloride [Fe(TDCPPβBr$_8$)Cl], were purchased from Frontier Scientific (Logan, UT, U.S.A.). Catalyst purity was assessed by UV–vis and compared to literature [35,36]. All analytical standards, axial ligands, oxidants, substrates, and solvents were purchased from Fisher Scientific, Sigma–Aldrich, or T.C.I. America, and used without further purification. Preparative thin-layer chromatography (TLC) plates were purchased from Silicycle (Québec, Canada).

2.1.2. Equipment

Qualitative analysis was performed by gas chromatography-mass spectrometry (GC–MS) using Agilent Technologies (Wilmington, DE, U.S.A.) HP 6890 Series GC system G1530A with an Agilent HP 7683 autosampler coupled to Agilent HP 5973 quadrupole mass-selective detector (transfer line 275 °C, source 230 °C, ionization potential 70 eV, scan range 30–400 amu). Helium was used for carrier gas flow at a rate of 1.4 mL/min. Separations were performed on an Agilent 19091Z-002HP-1 methyl siloxane capillary column (25.0 m, 200 μm internal diameter, 0.11 μm film thickness). GC–MS data acquisition was controlled by MSD ChemStation G1701DA Software.

The samples were quantitated by HPLC-ultraviolet (UV) (Agilent 1100 Series, G1322A Degasser, serial: JP73021014), using a prepared calibration curve of each standard (λ = 254 nm). Separation of acetaminophen and its precursors was carried out on a Phenomenex Aqua C18 Column, particle size 5 μm (150 mm × 4.6 mm). HPLC-UV data acquisition was controlled by ChemStation for LC 3D Software Rev.A.10.01(1635), Agilent Technologies (1990–2003). Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 400 MHz on a Varian NMR instrument; spectra were prepared using ACD/Labs (Toronto, Canada).

The iron porphyrin complexes, Fe(TDFPP)Cl, Fe(TDCPP)Cl, Fe(TDCPPβBr$_8$)Cl, were examined by UV–vis Spectrophotometry

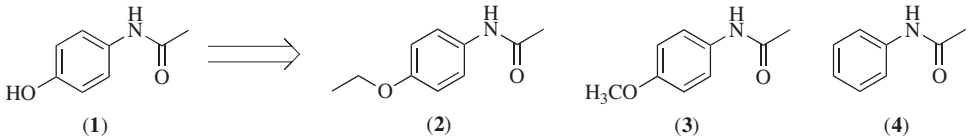

Fig. 2. Acetaminophen (APAP, **1**) and prodrugs analyzed in this study: phenacetin (**2**), methacetin (**3**), and acetanilide (**4**).