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Structural basis for cooperative binding of azoles to CYP2E1 as interpreted through guided molecular dynamics simulations

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ABSTRACT

CYP2E1 metabolizes a wide array of small, hydrophobic molecules, resulting in their detoxification or activation into carcinogens through Michaelis–Menten as well as cooperative mechanisms. Nevertheless, the molecular determinants for CYP2E1 specificity and metabolic efficiency toward these compounds are still unknown. Herein, we employed computational docking studies coupled to molecular dynamics simulations to provide a critical perspective for understanding a structural basis for cooperativity observed for an array of azoles from our previous binding and catalytic studies (Hartman et al., 2014). The resulting 28 CYP2E1 complexes in this study revealed a common passageway for azoles that included a hydrophobic steric barrier causing a pause in movement toward the active site. The entrance to the active site acted like a second sieve to restrict access to the inner chamber. Collectively, these interactions impacted the final orientation of azoles reaching the active site and hence could explain differences in their biochemical properties observed in our previous studies, such as the consequences of methylation at position 5 of the azole ring. The association of a second azole demonstrated significant differences in interactions stabilizing the bound complex than observed for the first binding event. Intermolecular interactions occurred between the two azoles as well as CYP2E1 residue side chains and backbone and involved both hydrophobic contacts and hydrogen bonds. The relative importance of these interactions depended on the structure of the respective azoles indicating the absence of specific defining criteria for binding unlike the well-characterized dominant role of hydrophobicity in active site binding. Consequently, the structure activity relationships described here and elsewhere are necessary to more accurately identify factors impacting the observation and significance of cooperativity in CYP2E1 binding and catalysis toward drugs, dietary compounds, and pollutants.

1. Introduction

CYP2E1 metabolizes a wide array of biologically important small, hydrophobic molecules (molecular weight < 100) comprised mainly of drugs, dietary compounds, and especially pollutants [1]. Substrates include monocyclic compounds such as styrene, acetaminophen, and isoniazid, as well as bicyclic compounds chlorzoxazone and caffeine. These CYP2E1 substrates undergo oxidation to various metabolites that facilitate their elimination from the human body. Nevertheless, the biological consequences for these events range from detoxification to carcinogen activation [2]. The prediction of these outcomes is hampered by gaps in our knowledge of the molecular determinants for CYP2E1 specificity and metabolic efficiency toward these compounds. Consequently, advances in interpreting and predicting the biological significance of CYP2E1 metabolism requires improvements in our understanding of the mechanisms underlying interactions between CYP2E1 and its substrates.

The Michaelis–Menten mechanism underlies the generally accepted paradigm for CYP2E1 metabolism of substrates and their resulting impact on health outcomes. Nevertheless, growing evidence implicates the importance of more complex cooperative mechanisms for CYP2E1 [3–10]. Those kinetic profiles deviate from the hyperbolic relationship predicted by the Michaelis–Menten mechanism. For 4-nitrophenol, metabolic rates of turnover increase and then decrease as a function of substrate concentration indicating substrate inhibition [3,6]. Alternatively, many CYP2E1 substrates, including phenacetin, *m*-xylene [5], styrene [7,8], and

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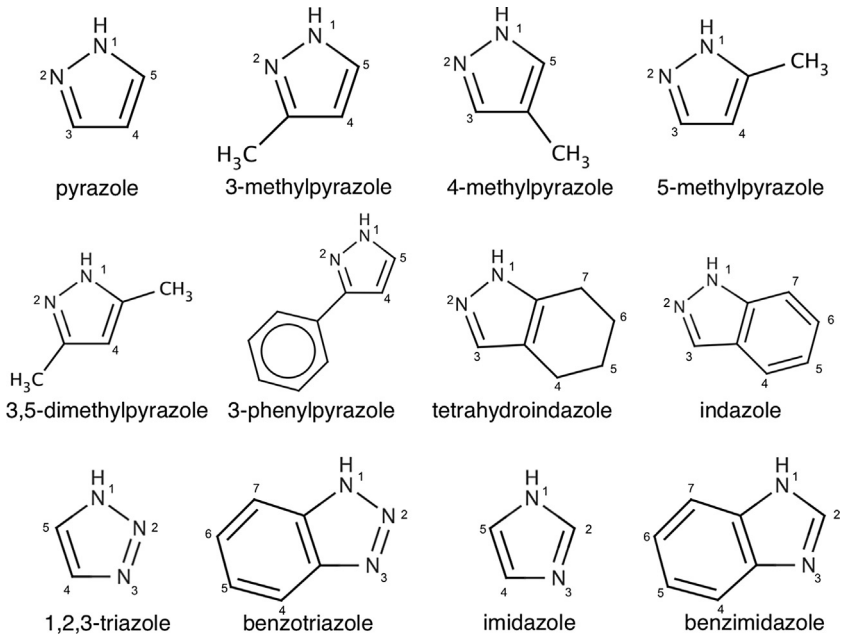

Fig. 1. Azoles used for computational docking to CYP2E1 active and cooperative sites.

7-ethoxycoumarin, demonstrate a poor efficiency in turnover at low substrate concentrations that rapidly improves at higher concentrations through a positive cooperative mechanism. Recent studies have further shown that aniline metabolism by CYP2E1 metabolism involves negative cooperativity in which higher substrate concentrations inhibit the ability for the enzyme to reach a maximal rate [9]. While the Hill equation is commonly used to qualify the degree of cooperativity, it reveals nothing of the mechanism underlying the observed kinetic profile. As an alternative, we have identified and validated mechanistic models involving two binding site to explain non-hyperbolic kinetic profiles for CYP2E1 substrates and inhibitors through the use of binding and catalytic experiments coupled with computational structural studies [6–10].

Recently, we investigated the selectivity of both catalytic and cooperative sites for rabbit CYP2E1 through binding and catalytic studies using an array of ten azole inhibitors (Fig. 1) [10]. Data from spectral binding studies for monocyclic azoles were consistent with two binding events, while bicyclic azoles implicated only one. Pyrazole affinity toward the CYP2E1 catalytic site improved upon introduction of a single methyl group at either position 3 or especially 4 of the azole ring. The presence of two methyl groups at positions 3 and 5 precluded any spectral binding event suggesting a lack of interaction with the P450 heme and possibly the catalytic site. A large hydrophobic phenyl ring located at position 3 did not improve pyrazole binding. By contrast, fusion of the pyrazole ring to benzene or cyclohexane greatly increased affinity. The consequences of these binding events on CYP2E1 catalysis were studied through inhibition studies with 4-nitrophenol, a substrate known to bind both sites [6,11]. Most pyrazoles shared a common mixed cooperative inhibition mechanism in which pyrazole binding rescued CYP2E1 from substrate inhibition. Overall, inhibitor affinities toward the CYP2E1 catalytic site were similar to those reported for binding studies, and the same trend was observed for binding at the cooperative site. Taken together, these studies identified contributions of ring substituents and fusions on the stoichiometry and affinity of azoles for catalytic and cooperative sites; nevertheless, the complementary role that the CYP2E1 protein structure plays in these interactions remains unknown.

Insights on the function of protein structure have been greatly advanced through computational and X-ray crystallographic methodologies. Initial computational homology models provided the first predictions of CYP2E1 contacts with substrates and inhibitors [12]. The first X-ray crystallographic structures were reported for human CYP2E1 complexes with either 4-methylpyrazole or indazole [13]. These structures validated the importance of amino acid residues in the catalytic site as suggested by computational [12] and site-directed mutagenesis studies [14,15]. Nevertheless, all crystallographic CYP2E1 structures reported to date include only those with one molecule bound to the enzyme [13,16,17], and thus they provide no information on the role of CYP2E1 structure on cooperative interactions. Computational methods provide a viable solution for identifying CYP2E1 residues responsible for mediating contacts with compounds bound at catalytic and cooperative sites. We were the first to describe docking of 4-nitrophenol, 4-methylpyrazole and indazole to both sites in the binding cavity of rabbit CYP2E1 to explain experimental binding and catalytic data [11]. Subsequent studies by others introduced molecular dynamic simulations to improve predictions of complexes with 4-nitrophenol [18] and aniline [19].

Herein, we employed computational docking studies to identify the structural basis for rabbit CYP2E1 binding and cooperativity toward twelve azoles possessing diverse methyl and ring substituents (Fig. 1). The array of molecules included all azoles from the previously published experimental studies [20] as well as 5-methylpyrazole in order to provide insights on CYP2E1 structure activity relationships relationships. We modeled interactions between these azoles and CYP2E1 through a guided molecular dynamics approach using SYBYL-X 1.3 (Tripos, Inc.). This technique has been used successfully to find exit channels [21] as well as dock ligands from the outside of the protein to the inner catalytic site [22]. In brief, azoles were initially placed at the entrance of the substrate access channel and were then guided to the binding site. Meanwhile, intermolecular interactions were sampled along the path for the molecule through the protein structure. Complexes were then subjected to multiple cycles of molecular dynamics and energy minimization. When bound at the catalytic site, binary azoles were tethered to the heme iron based on reported Type II binding spectra [6,10], which in the case of triazoles involved