ORIGINAL ARTICLE

UNVEILING ALPHA-NAPHTHOFLAVONE MEDIATED CYP1A2 SUPPRESSION AND ANALYSIS OF CONSEQUENT STRUCTURAL DYNAMICS

Zahra Zahid Piracha[1], Umar Saeed[2], Naila Azam[3]

[1]International Center of Medical Sciences Research (ICMSR), United States of America (USA). [2] AssociateProfessor Biocamistry, Clinical and Biomedical Research Centre (CBRC) and Multidisciplinary Lab (MDL), Foundation University School of Health Sciences (FUSH), Foundation University, Islamabad, Pakistan. [3]Professor, Community Medicine, Foundation University School of Health Sciences (FUSH), Foundation University, Islamabad, Pakistan

ABSTRACT

Background: Cytochrome P450 enzymes play vital roles in metabolizing drugs, endogenous compounds, and environmental pollutants. Among them, Cytochrome P450 1A2 (CYP1A2), CYP1A1 and CYP1B are particularly important for activating carcinogens. Computational modeling of CYP1A2 is essential for understanding its interactions with various molecules, substrates, and inhibitors.

Objective: To characterize the structure of CYP1A2 and explore the binding of alpha-naphthoflavone to its active site.

Study Design: In Silico study (Computational modeling).

Place of Study: International Center of Medical Sciences Research (ICMSR), Islamabad, Pakistan.

Material and Methods: Using the Swiss PDB Viewer, the structural features of CYP1A2 were assessed, focusing on key residues, motifs, helices, and conserved regions.

Results: Our findings identified specific binding sites for Alpha-Naphthoflavone (ANF), highlighting its potential as a potent inhibitor of CYP1A2. This research contributes to our knowledge of the clinical and toxicological implications associated with CYP1A2.

Conclusion: Structural differences were found between CYP1A2 and related enzymes, with less than 40% sequence identity compared to several other P450s. The study predominantly compares CYP1A2 with CYP 2A6 and CYP3A4 due to these differences. The developed structural models offer a fast and precise method for studying CYP1A2, aiding in understanding its role in drug metabolism and toxicology.

Key words: *Alpha-naphthoflavone Cytochrome P450, Computational modeling, CYP1A2, Structural characterization*

How to cite this article: Piracha ZZ, Saeed U, Azam N. Unveiling Alpha-Naphthoflavone Mediated CYP1A2 Suppression and Analysis of Consequent Structural Dynamics. HMDJ. 2024; 04(01): 16-20. doi: 10.69884/hmdj.4.1.3590

INTRODUCTION

Cytochrome P450 (CYP) enzymes, a diverse superfamily of hemeproteins is found in both prokaryotes and eukaryotes, playing critical roles in the metabolism of various compounds, including lipophilic xenobiotics, procarcinogens, drugs, and environmental toxins[1]. They are pivotal in drug metabolism, where they catalyze oxidation, reduction, and hydrolysis reactions that significantly influence the bioavailability and efficacy of drugs[2]. Genetic variations in CYP enzyme activity due to polymorphism can lead to differences in drug response and metabolism among individuals. Beyond drug metabolism, cytochrome P450 enzymes are central to detoxification and elimination of xenobiotics, including environmental pollutants and carcinogens. By converting the lipophilic substances into more hydrophilic forms, CYP enzymes facilitate their excretion from the body, providing a crucial defense mechanism against toxic insults [3]. In addition to xenobiotics, CYP enzymes are also important in metabolizing some compounds formed in the body, like bile acids, steroids and fatty acids. This metabolic activity is essential for maintaining physiological homeostasis and regulating biological processes like hormone synthesis and cholesterol metabolism.

Certain cytochrome P450 enzymes, including CYP1A1, CYP1A2, and CYP1B1, are implicated in the activation of procarcinogens to reactive intermediates that can initiate DNA damage and promote carcinogenesis[4]. Conversely, other CYP enzymes contribute to detoxification of carcinogens, helping to mitigate their harmful effects. Cytochrome P450 enzymes are also critical in mediating drug-drug interactions due to their involvement in the metabolism of multiple medications.

Correspondence to: Zahra Zahid Piracha, International Center of Medical Sciences Research (ICMSR), United States of America (USA).

Email: piracha.zahra@gmail.com

Conflict of Interest: None
Financial Disclosure: None

Received: 20-05-2024
Accepted: 22-06-2024

doi: 10.69884/hmdj.4.1.3590

Inhibition or induction of specific CYP enzymes by one drug can alter the metabolism of co-administered drugs, potentially leading to adverse effects or therapeutic failure. Genetic polymorphisms in cytochrome P450 genes contribute to significant inter-individual variability in enzyme activity[5]. This variability can impact drug response, predisposition to drug-induced toxicity, and susceptibility to certain diseases. For example, polymorphisms in CYP2D6 and CYP2C19 are linked with altered metabolism of psychotropic drugs and cardiovascular medications. The study of cytochrome P450 genetics and their impact on drug metabolism forms the basis of pharmacogenomics, a field focused on tailoring drug treatments based on individual genetic profiles. Understanding CYP enzyme polymorphisms enables the prediction of drug responses and facilitates personalized therapeutic approaches [6].

CAPSULE SUMMARY

There are significant structural differences between cytochrome P450 1A2 and other cytochrome P450 enzymes (2A6 and 3A4). The developed structural models in the study show that cytochrome 1A2 has a compact and closed active site cavity, well-suited for oxidizing large planar substrates providing a better understanding its role in drug metabolism and toxicology.

CYP1A2, known as Phenacetin-O-deethylase, constitutes approximately 15% of total liver P450 enzymes and is constitutively expressed in the liver, metabolizing 8-10% of clinically significant drugs[4,7,8]. The CYP1A2 *gene*, located on chromosome 15, comprises six introns and seven exons, including a 55bp non-coding exon[5]. It shares a 5'-flanking region with *CYP1A1*, indicating evolutionary linkage. Genetic as well as environmental factors influence *CYP1A2* expression & activity, with over 15 variant alleles and numerous SNPs identified [6, 7-10]. Substrates of CYP1A2 are typically aromatic, flat, lipophilic, and neutral, with planar structures that fit well into its active site [10,11]. Together with CYP1A1 and CYP1B, CYP1A2 plays a critical role in activating carcinogens[12-14]. Inhibitors of CYP1A2, such as alpha-naphthoflavone, often contain chloro, fluoro, or methyl groups and can impact drug metabolism [15-17]. Understanding the structure and function of CYP1A2 through computational modeling and in silico studies is crucial for identifying potential inhibitors and understanding their clinical implications in drug metabolism and toxicity [18-20].

MATERIAL AND METHODS

An in-silico study was conducted to investigate the structural features of CYP1A2. The analysis aimed to characterize the molecule's architecture, focusing on important residues, motifs, helices, and functional regions. Using the Swiss PDB Viewer, CYP1A2 was observed to consist of twelve Alpha helices and four Beta sheets.

The secondary structural elements were designated from A - L for helices and from 1 - 4 for beta sheets, sequenced from the N-terminus. The prosthetic group (heme) and the substrate binding cavity were prominently identified. The structural analysis highlighted CYP1A2's specialization in oxidizing large planar, molecules such as Poly Aromatic Hydrocarbons (PAHs), laying the groundwork for an efficient xenobiotic biotransformation. Supplementary helices were also identified, beyond the twelve Alpha-helices and four Beta-sheets. Substrates got bound within the cavity, above the heme prosthetic group's distal surface.

This study underscores the structural adaptability of CYP1A2 for its biological function, elucidating key features essential for its role in metabolizing a variety of substrates, particularly PAHs, crucial for understanding its impact on xenobiotic metabolism.

RESULTS

In the structural analysis of CYP1A2, certain regions emerge as highly conserved and functionally critical within the protein's architecture. Among the most conserved regions were the heme binding site, nestled deep within the protein core, and the binding sites for redox partners. Notably, the proximal surface of the CYP1A2 redox partner has the binding site situated on it. NADPH-cytochrome P450 oxidoreductase & cytochrome b5(Redox partners) play pivotal roles in CYP1A2's enzymatic activities. Cytochrome b5, for instance, enhances specific cytochrome P450 monooxygenase reactions, augmenting the catalytic efficiency of CYP1A2. Meanwhile, NADPH-cytochrome P450 oxidoreductase (CYPOR) serves as the primary electron donor, facilitating electron transfer to all microsomal cytochromes P450, including CYP1A2. Furthermore, important motifs within CYP1A2 were identified through motif scanning techniques, unveiling their strategic positions within the protein structure. The accompanying diagram illustrates the precise locations of these motifs, providing valuable insights into their functional roles and contributions to CYP1A2's enzymatic capabilities.

Structural Variations in CYP1A2 and Alpha-Naphthoflavone (ANF) binding to Active Site Mediated Suppression

Within the structure of Cytochrome P450 1A2 (CYP1A2), certain regions exhibit notable divergence, particularly influencing substrate binding and catalytic activity. The most divergent regions turned out to be the C-terminal loop and the distal binding sites of the substrate binding cavity, imparting significant flexibility to this enzyme's ability to oxidize a broad range of structurally distinct substrates. A unique feature of CYP1A2 is observed in the F-G region, characterized by a reverse amphipathic nature resulting from substantial insertions between helices F and G. This structural alteration leads to a hydrophobic surface near the protein's tip, just about the transmembrane domain. This hydrophobic surface typically interfaces with a membrane, juxtaposed to the catalytic site. Despite existing as a helical fragment rather than a typical alpha-helix, this region maintains crucial reverse amphipathicity, critical for CYP1A2 function. In the B-C region of CYP1A2, three polar residues—Thr118, Ser122, and

Thr124—are strategically positioned, pointing towards the active site cavity. Notably, both Thr118 and Thr124 play unique roles in substrate interactions.

Thr118 promotes stable hydration within the distal active site, particularly with smaller substrates, while Thr124 aids in orienting substrates for specific enzymatic activities, including N-hydroxylation of heterocyclic aryl amines and dealkylation of resorufins. Additionally, both Thr118 and Thr124 contribute to the formation of hydrogen bonds with caffeine's carbonyl oxygen atoms. The active site is enriched with amino acid residues residing on helices F and I, that constitute dual substrate-binding platforms, flanking the cavity. Helix- F undergoes disruption across the distal surface by losing the pattern of hydrogen bonding at Val220 and Lys221, resulting in the unwinding of the helix.

Water-bridged contacts involving Thr223 play a pivotal role in this disruption, forming key interactions with solvent molecules and ultimately causing a bend in helix- F that closes down the cavity of the active site. An estimated volume of the active site, approximately 375 angstroms, underscores the critical involvement of these structural alterations in modulating substrate-binding and efficiency of catalysis. The analysis highlights the dynamic and functionally crucial regions within CYP1A2, shedding light on the structural adaptations that enable its broad substrate specificity. Understanding these structural variations is essential for elucidating the enzyme's diverse enzymatic capabilities and its role in xenobiotic metabolism.

The visual representation of key hydrogen bonds between amino acids and the heme moiety within close proximity, crucial for stabilizing the substrate binding cavity and optimizing enzymatic activity. Alpha-naphthoflavone (ANF) acts as a competitive inhibitor of CYP1A2 catalytic reactions by closely matching size & shape of the substrate -binding cavity, resulting in Van der Waals forces primarily from the non-polar side chains. According to the scientists' belief, ANF is metabolized rapidly by CYP1A1 to form ANF-5,6-diol & ANF-5,6-oxide. However, there happens a limited oxidation of ANF by CYP1A2.

ANF inhibits P450 1A2 in part because of the higher-affinity binding shown by the reduced oxidation rate. This property makes ANF useful for distinguishing the P450 family 1 enzymes. Alpha-naphthoflavone binds within CYP1A2's active site, positioned right above heme's distal surface. The electron density map's curvature can be used to determine this inhibitor's orientation. ANF forces the phenyl ring to be near the heme iron by binding in a single favoured configuration. This binding process is supported by the electron density map narrowing at the interface between the phenyl ring and the benzo-h-chromen-4-one moiety, as shown in Fig. 1 with red-colored heme and yellow ANF.

The substrate-binding site within the CYP1A2 complex features a uniformly narrow structure throughout its extent. Residues from helices F and I play a critical role in defining this planar substrate binding site. Particularly, Phe226 on helix F contributes to creating a parallel substrate-binding surface; any distortion in this structure results in a narrower substrate-binding cavity. Having no solvent channels in the CYP1A2 complex linked with ANF is indicative of this stable and narrow arrangement, which lowers the probability for water to occupy the cavity. Only one molecule of water is observed within the active site cavity, and there doesn't appear to be any solvent routes joining the protein surface to the cavity. This water molecule makes hydrogen bonding with the carbonyl groups of ANF & Gly316 on helix- I. The peptide backbone causes substrate-binding cavity to adopt a rather flat conformation, resulting in near-perfect coplanarity of Ala317 side chain, Gly316-Ala317 peptide link & Asp320-Thr321 peptide bond.

Strong hydrogen bonding between Thr223 on helix- F and Asp320 on helix- I, which connect the two helices at the cavity's roof, help to stabilise the active site's structure. Hydrogen-bonded water molecules, as well as the side chains of Tyr189, Val220, Thr498, and Lys500, influence active site's stability. A comprehensive understanding of the interactions involving these helices is illustrated in Figure 1.

DISCUSSION

The term "In Silico" refers to work conducted via computer simulation or computational methods. By integrating scientific experiments with theoretical and mathematical modeling, researchers can gain valuable insights. This interdisciplinary

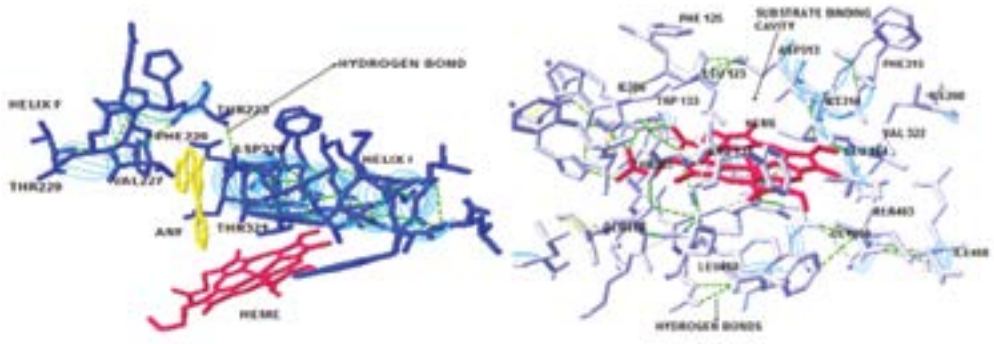

Figure 1: Structural Motifs of CYP1A2 and hydrogen bond interactions between amino acids and heme.

approach combines vivid computational imagery with biochemical experiments, offering critical information on the interactions between various intracellular components[18]. Collaborative investigations, combining physical techniques with chemical modification, immuno-localization studies, and site-directed mutagenesis experiments have increasingly verified the hypotheses based on P450 enzyme homology modelling.

Studies have found some common features of CYP1A2 ligands. These typically consist of one to two hydrophobic areas, one aromatic ring & one hydrogen bond acceptor [14]. Mutagenesis investigations have identified many amino acids within the substrate recognition sequence (SRS) sections of CYP1A2 that play critical roles in substrate-enzyme interactions, which is supported by homology modelling. Notable residues include Arg108, Thr124, Thr223, Glu225, Phe226, Lys250, Arg251, K253, Asn312, Asp313, Glu318, Thr319, Asp320, Thr321, Val322, Leu382, Thr385, and Ile386 [19]. Furthermore, research on non-SRS areas has shown that residues critical for ligand-CYP1A2 interactions include Lys99, Arg137, Gln141, Phe186, Phe205, Val227, Lys453, Arg455, and Thr501 [20].

Mammalian CYPs share a conserved core structure encompassing a heme-binding site & a proximal surface believed to interact with cytochrome b5 and other cytochrome P450 proteins. The structure of CYP1A2 differs from other family members due to variations in loop lengths connecting conserved secondary structures. Interestingly, CYP1A2 exhibits the F-helix disruption but CYP2A6 does not, suggesting a potentially less compact active site. On the other hand, a coil that joins F and F' in CYP3A4 extends above its active site. For CYP1A2, the active site cavity volume is about 375 Å, while the reported volumes for CYP2A6, CYP2C8, and CYP3A4 are 260 Å, 1438 Å, and 1385 Å, respectively [8]. This work supports ANF to be a potent inhibitor of CYP1A2, while Coumarin and Ketoconazole are identified as potent inhibitors for CYP2A6 and CYP3A4 respectively[10,11]. It is noteworthy that CYP1A2 cannot oxidize ANF, a capability possessed by CYP1A1[13]. The active site of P450 2A6 resembles the active site of P450 1A2, being approximately 50% bigger in volume though[14]. Typically, P450 1A2 exhibits a high-spin iron configuration in its native state, with no apparent solvent channels observed in crystal form. Mutants such as D320A, F226Y, and F226I display reduced catalytic efficiency, possibly due to alterations in the iron-oxygen intermediate [15]. Notably, mutations like CYP1A1 V328L and CYP1A2 L382V have been observed to alter substrate specificity. Cytochrome P450 enzymes are predominantly found in the hepatocytes but are also distributed throughout various cells in the body. They are localized inside the endoplasmic reticulum (ER) and mitochondria. Enzymes in the ER typically metabolize the foreign substances, especially drugs & environmental pollutants, while those in mitochondria are involved in synthesizing and metabolizing internal substances. These enzymes play a crucial role in drug metabolism, accounting for 70 to 80 percent of the enzymes involved.

Among the various P450 families, including 1, 2, and 3, enzymes within families 1, 2, and 3 are particularly significant in transforming xenobiotics into more polar metabolites that can be excreted. Recent studies indicate a strong link between exposure to dietary carcinogens like polycyclic aromatic hydrocarbons and colorectal cancer risk. Family -1 enzymes oxidize the polycyclic aromatic hydrocarbons efficiently. This study reports on the structural characterization of cytochrome P450 1A2, exploring its connection with substrates & inhibitors and emphasising structural distinctions. The developed models provide fast and precise tools for understanding CYP1A2's structure and comparing it with other cytochrome P450 family members. Notably, CYP1A2 shows a<40% amino acid sequence identity as compared to enzymes like 2A6, 2B4, 2C5, 2C8, 2C9, 2D6, and 3A4, making comparisons primarily with CYP2A6 & CYP3A4 more relevant. Analysis of cytochrome P450 1A2 structure serves as a foundational platform for further exploring its clinical and toxicological significance. Understanding the unique structural features of CYP1A2 enhances insights into its function and impact on drug metabolism and toxicology.

CONCLUSION

The structural analysis of cytochrome P450 1A2 provides valuable insights into its substrate interactions and inhibitor binding, shedding light on its unique features compared to other cytochrome P450 family members. The study highlights the adaptation of CYP1A2 with a compact and closed active site cavity, well-suited for oxidizing large planar substrates. Key findings include the significant structural differences between CYP1A2 and related enzymes, with less than 40% sequence identity compared to several other P450s. The study predominantly compares CYP1A2 with CYP2A6 and CYP3A4 due to these differences. The developed structural models offer a fast and precise method for studying CYP1A2, aiding in understanding its role in drug metabolism and toxicology. These findings provide a foundation to further explore the clinical & toxicological implications associated with cytochrome P450 1A2, for future research and potential therapeutic interventions.

AUTHORS' CONTRIBUTION

Zahra Zahid Piracha, Umar Saeed, Naila Azam	Drafting the Article
Zahra Zahid Piracha, Umar Saeed, Naila Azam	Analysis and interpretation of data
Zahra Zahid Piracha, Umar Saeed	Conception and design
Zahra Zahid Piracha, Umar Saeed, Naila Azam	Acquisition of data
Zahra Zahid Piracha, Umar Saeed, Naila Azam	Critical revision

REFERENCES

1. Granfors MT, Backman JT, Neuvonen M, et al. Ciprofloxacin greatly increases concentrations and hypotensive effect of tizanidine by inhibiting its cytochrome P450 1A2-mediated presystemic metabolism. Clin Pharmacol Ther. 2004;76(6):598–606.
2. Vasanthanathan P, Taboureau O, Oostenbrink C, et al. Classification of cytochrome P450 1A2 inhibitors and noninhibitors by machine learning techniques. Drug Metabolism and Disposition. 2009; 37 (3) 658-664.
3. P05177 · CP1A2_HUMAN [Internet].Cambridgeshire(CB): Elixir, Welcome Genome Campus Hixton.[cited 2024 Feb 26]. Available from http://www.uniprot.org/uniprot/P05177.
4. Nebert DW,Russell DW. Clinical importance of the cytochromes P450. Lancet. 2002; 360(9340):1155-1162.
5. Bauer E, Guo Z, Ueng YF Bell, et al, P. Oxidation of benzo[a]pyrene by recombinant human cytochrome P450 enzymes. . Chem Res Toxicol. 1995;8(1):136-42.
6. Shimada T, Yamazaki H, Foroozesh M, et al. Selectivity of polycyclic inhibitors for human cytochrome P450s 1A1, 1A2, and 1B1. Chem Res Toxicol. 1998;11(9):1048–1056.
7. Gunes A, Dahl ML. Variation in CYP1A2 activity and its clinical implications: influence of environmental factors and genetic polymorphisms. Pharmacogenomics. 2008;9(5):625–37.
8. Zhou SF, Wang, B, Yang, LP, et al. Structure, function, regulation and polymorphism and the clinical significance of human cytochrome P450 1A2. Drug Metab Rev. 2010; 42(2): 268–354.
9. Ikeya K, Jaiswal AK, Owens RA, et al. Human CYP1A2: sequence, gene structure, comparison with the mouse and rat orthologous gene, and differences in liver 1A2 mRNA expression. Mol Endocrinol. 1989;3(9):1399–1408.
10. Lewis DF, Lake BG, Dickins M, et al. Homology modelling of human CYP1A2 based on the CYP2C5 crystallographic template structure. Xenobiotica. 2003;33(3):239–254.
11. Rendic S. Summary of information on human CYP enzymes: human P450 metabolism data. Drug Metab Rev. 2002;34(1-2):83–448.
12. Parker AC, Preston T, Heaf D, et al. Inhibition of caffeine metabolism by ciprofloxacin in children with cystic fibrosis as measured by the caffeine breath test. Br J Clin Pharmacol. 1994;38(6):573–576.
13. Caldwell J. The current status of attempts to predict species differences in drug metabolism. Drug Metab Rev.1981;12(2): 221–237.
14. Shimizu T, Tateishi T, Hatano M,et al. Probing the role of lysines and arginines in the catalytic function of cytochrome P450d by site-directed mutagenesis. Interaction with NADPH-cytochrome P450 reductase. J Biol Chem. 1991;266(6):3372–3375.
15. Smith DA, Ackland MJ, Jones BC, et al. Properties of cytochrome P450 isoenzymes and their substrates part 2: properties of cytochrome P450 substrates. Drug Discov Today. 1997;2(11):479 – 486.
16. Saeed U, Waheed Y, Anwar A, et al . In-Silico Binding of ATP to Protein Kinase C Delta: Insights into the Structure and Active Site. Eur J Sci Res.2011; 52 (2) :177-187.
17. Liu J, Ericksen SS, Sivaneri M, et al. The effect of reciprocal active site mutations in human cytochromes P450 1A1 and 1A2 on alkoxyresorufin metabolism. Arch Biochem Biophys. 2004;424(1):33–43.
18. Zhou SF, Yang LP, Zhou ZW, et al. Insights into the substrate specificity, inhibitors, regulation, and polymorphisms and the clinical impact of human cytochrome P450 1A2. APPS J. 2009;11(3):481-494.
19. Sansen S, Yano JK, Reynald RL, et al. Adaptations for the oxidation of polycyclic aromatic hydrocarbons exhibited by the structure of human P450 1A2. J Biol Chem. 2007;282(19):14348-14355.
20. Cho US, Park EY, Dong MS, et al. Tight-binding inhibition by α-naphthoflavone of human cytochrome P450 1A2. Biochim Biophys Acta. 2003;1648(1-2):195–202.
