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Importance of hydrophobic parameters in identifying appropriate pose of CYP substrates in cytochromes

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ABSTRACT

Cytochromes are catalytic enzymes which perform oxidative metabolic reactions on drugs. To determine the primary binding forces of CYP-substrate complex, molecular docking studies were carried out. Molecular docking analysis of several drugs with the enzymes CYP2C9, CYP2D6 and CYP3A4 revealed that hydrophobic interactions play a major role in determining the pose selection between substrates and enzymes. GOLD software with hydrophobic and hydrogen bond constraint was employed to identify the specific interactions which play deterministic role in the pose selection.

1. Introduction

Drugs are catalyzed into metabolites by cytochromes. Cytochromes are heme containing mono-oxygenase enzymes involved in a variety of functions including the metabolism of exogenous and endogenous substances [1,2]. Cytochromes are the major drug metabolizing enzymes which metabolize over 80% of therapeutic drugs. CYP2C9 metabolizes 15–20% of drugs which are weakly acidic in character. Substrates of CYP2D6 generally contain protonated basic nitrogen and CYP2D6 is involved in the metabolism of 25% of drugs. CYP3A4 has low substrate specificity, metabolizes about 50% of drugs [3–6]. The substrates of CYP3A4 are highly hydrophobic in nature. Fig. 1 shows representative examples of drugs metabolized by CYP2C9, CYP2D6 and CYP3A4 [7–9]. The active site of cytochrome P450 contains a heme, is the reactive center for oxidative reaction. The iron atom is located at the center of heme and is tethered to the P450 system via thiolate ligand derived from a cysteine residue. The substrate binds to the active site of the enzyme in close proximity to the heme group and opposite to cysteine. The bound substrate induces changes in the conformation of the protein and often displacing the water molecules from heme. During metabolism, the heme-porphyrin unit gets excited to a high spin state after binding to an oxygen molecule. The active species in the catalytic cycle is Compound-I, a heme-porphyrin radical containing (Fe=O) bond. There are various types of metabolic reaction pathways for the oxidative metabolic cycle and some of the proposed pathways are (i) rebound mechanism, (ii) electron transfer process, (iii) concerted oxidation process and (iv) proton shuttle mechanism [1,2]. X-ray crystal structures for CYP2C9, CYP2D6 and CYP3A4 were determined, these help to establish the enzyme–substrate interactions through molecular docking. Several attempts are made to explore the means of achieving best SOM prediction using molecular docking algorithms, a few details are given in the next paragraph.

Sykes et al. carried out studies on the prediction of site of metabolism (SOM) for CYP2C9 substrates using molecular docking and alignment methods. The alignment method was based on the shape and known chemistry of the CYP2C9 substrates. Molecular docking results were found to be less successful than the results based on alignment method. The results indicated the advantage of ligand based approaches over target based approaches for CYP2C9 [10]. Zhou et al. performed molecular dynamics simulations and molecular docking study to explore structure activity relationship of CYP2C9*13 and identified the trans configuration of the bond between Pro90 and Asp89 in CYP2C9*13 is mainly responsible for the reduction in the catalytic activity of the enzyme [11]. Prusis et al. performed molecular docking studies for CYP2D6 substrates and concluded that treating protein atoms in the structure defined

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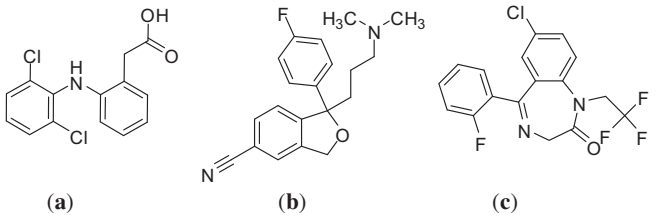

Fig. 1. Representative substrates of (a) CYP2C9: diclofenac (b) CYP2D6: citalopram and (c) CYP3A4: 2-oxoquazepam.

by 2F9Q with reduced van der Waals radii is important for SOM prediction using docking [12]. Hritz et al. carried out molecular dynamics to define protein structure and performed ligand docking for CYP2D6 substrates; the work explored the impact of plasticity and flexibility of the protein structure on docking studies for CYP2D6 substrates. The method was investigated by docking 65 of CYP2D6 substrates to an ensemble of 2500 protein structures. The effect of induced fit, conformation of Phe483 and SOM prediction was analyzed. The results were found to be successful in predicting SOM of 80% of CYP2D6 substrates [13]. Oh et al. developed a combined model (molecular docking and QSAR) MLite, for the regioselective SOM prediction of CYP3A4 substrates. This approach used molecular docking for the accessibility prediction and activation energy for the reactivity prediction. MLite predicted the SOM with an accuracy upto 76% [14]. Kjellander et al. developed a new computational tool to interpret the docking poses. It helped to identify the importance of anchoring amino acids in enzyme–ligand complex. For the CYP3A4 structures, this method was compared with consensus principal component analysis [15].

Predicting SOM of drugs is an important challenge; molecular docking methods are being used to predict SOM and to identify the active disposition of drug in the cavity of cytochromes. Though molecular docking methods do not always offer deterministic solution, they are ideally suited methods to achieve the above objectives. Several docking algorithms are available to perform molecular docking of substrate with CYPs; each suffers from limitations in one form or the other. To overcome these limitations, unified molecular docking protocol is necessary. As discussed in the previous paragraph, several protocols were developed to predict the SOM using molecular docking methods. The success rate from each of the cases varies from 50 to 80%. Above described protocols are specific to a given CYP isoform, cannot be generalized and presume an organic molecule as substrate for specific CYPs. There is a need to explore further and to establish common successful protocols. In this work, we describe the protocols for molecular docking methodology to predict the various metabolic profiles like (a) ligand binding mode (b) sterically accessible SOM and (c) interaction pattern and are acceptable for all isoform specific conditions, the details are given in the following sections.

2. Materials and methods

Molecular modeling studies were carried out using GOLD and Maestro (GLIDE) [16–18] software on desktop computers, running under Linux operating system.

2.1. Collection of CYP substrates

A database of known CYP substrates was collected from literature. Substrate selection was carried out on the basis of availability of experimental information on CYP catalyzed product formation. The compounds were included as substrates in the databases for CYP2C9, CYP2D6 and CYP3A4 enzymes only when the following information are available: (i) SOM specifically identified, (ii) Characterized the metabolites, (iii) CYP isoform involved in metabolism specified, (iv) Kinetic data (K_m/V_{max}) for metabolism and (v) Defined chirality of the substrate. The chosen compounds were separately stored in respective CYP databases. Data set of CYP2C9, CYP2D6 and CYP3A4 respectively constitute 30 (Training set: 24 + Test set: 6), 37 (Training set: 30 + Test set: 7) and 75 (Training set: 65 + Test set: 10) substrates.

2.2. Ligand preparation

The 3D structures of CYP substrates were built using *Build* panel implemented in Maestro. The 3D structure of the substrates must be in the best representation of the actual ligand structure as they appear at physiological condition for molecular docking. To obtain physiological states of ligand, ligand preparation was carried out using ionizer option of *ligprep* module implemented on Maestro. Desalting of the substrate was carried out whereas tautomers and stereoisomers were not generated using *ligprep* module. Finally, the structures were energetically minimized using OPLS2001 force field.

2.3. Protein selection and preparation

The crystal structures of human cytochromes were downloaded from Brookhaven protein data bank (CYP2C9-PDB ID: 1R9O, CYP2D6-PDB ID: 3QM4 and CYP3A4-PDB ID: 3NXU) [19–21]. The disordered missing residues were modeled [22] and the selected 3D crystal structure of CYPs subjected to the protein treatments as defined in the protein preparation wizard of Maestro. The bound ligand and water molecules present in protein structure were deleted as there was no information regarding conserved water molecules. Hydrogen atoms were added and the bond orders were assigned to the entire protein system. The crystal structure of the protein was processed for the refinement to fix the side chain information using PRIME module of Maestro software package. Then, it was subjected to metal treatment to delete the covalent bonds between protein and metal. The localized charge of iron was assigned as Fe^{+3} with appropriate atom type. Hydrogen bonds were optimized through exhaustive sampling and the entire protein was minimized to avoid the steric clashes of added hydrogen. The energy minimization was carried out using OPLS2001 force field. These prepared protein structures for CYP2C9, CYP2D6 and CYP3A4 were independently saved in .pdb format.

2.4. Analysis of the active site of CYPs and the importance of anchoring residues for hydroxylation mechanism

Active sites of three CYPs were visually inspected through 3D visualizer to find the key active site residues. The base of the active site of CYPs is defined by heme prosthetic group. Active site region above the heme of the CYPs composed mostly of hydrophobic residues (Fig. 2).

For simplicity, the active sites of cytochromes may be defined in terms of three subunits: the base region, the wall region (Region-1) and the dome region (Region-2) (Fig. 3). The base is occupied by the heme prosthetic group. The region-1 consists mostly of hydrophobic residues (CYP3A4: 73%, CYP2C9 78%, CYP2D6: 78%). On the other hand the dome region consists of relatively less percentage of hydrophobic residues (except in CYP3A4) – (CYP3A4: 80%, CYP2C9: 60%, CYP2D6: 50%) (Table S1, Supporting Information). Also oxygen transfer takes place at the hydrophobic center of the substrates.