Structural and energetic analysis to provide insight residues of CYP2C9, 2C11 and 2E1 involved in valproic acid dehydrogenation selectivity

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A B S T R A C T

Docking and molecular dynamics (MD) simulation have been two computational techniques used to gain insight about the substrate orientation within protein active sites, allowing to identify potential residues involved in the binding and catalytic mechanisms. In this study, both methods were combined to predict the regioselectivity in the binding mode of valproic acid (VPA) on three cytochrome P-450 (CYP) isoforms CYP2C9, CYP2C11, and CYP2E1, which are involved in the biotransformation of VPA yielding reactive hepatotoxic intermediate 2-*n*-propyl-4-pentenoic acid (4*n*VPA). There are experimental data about hydrogen atom abstraction of the C4-position of VPA to yield 4*n*VPA, however, there are not structural evidence about the binding mode of VPA and 4*n*VPA on CYPs. Therefore, the complexes between these CYP isoforms and VPA or 4*n*VPA were studied to explore their differences in binding and energetic stabilization. Docking results showed that VPA and 4*n*VPA are coupled into CYPs binding site in a similar conformation, but it does not explain the VPA hydrogen atom abstraction. On the other hand, MD simulations showed a set of energetic states that reorient VPA at the first ns, then making it susceptible to a dehydrogenation reaction. For 4*n*VPA, multiple binding modes were observed in which the different states could favor either undergo other reaction mechanism or ligand expulsion from the binding site. Otherwise, the energetic and entropic contribution point out a similar behavior for the three CYP complexes, showing as expected a more energetically favorable binding free energy for the complexes between CYPs and VPA than with 4*n*VPA.

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1. Introduction

Valproic acid (VPA, Fig. 1) is an important antiepileptic drug that has been widely used in the management of various types of seizure disorders [1]. Although VPA is considered a relatively safe drug, in some occasions, it may associate with severe and sometimes fatal liver injury [2]. Although the mechanism of VPA hepatotoxicity is not understood so far, there are multiple factors that could contribute to the toxicity, including (1) formation of reactive metabolites of VPA [3] and the subsequent

covalent binding to cellular proteins [4–6], and (2) development of oxidative stress [7].

In the course of the characterization of the metabolism of VPA, it was discovered that there were multiple metabolic pathways involved in its biotransformation which give rise to more than 50 known metabolites of the parent drug [8]. However, from these multiple pathways, the oxidative metabolism of VPA by cytochrome P450 (CYP) constitutes a minor metabolic pathway, but it represents the one which generates the hepatotoxic metabolite 2-*n*-propyl-4-pentenoic acid (4*n*VPA) in liver microsomes from rats, mice, rabbits and humans [9]. Experiments using deuterated analogs of VPA to obtain evidence about the dehydrogenation reaction demonstrated that this reaction proceeds via hydrogen atom abstraction of the C4-position [9]. The metabolism of VPA to 4*n*VPA represents a dehydrogenation reaction of a sp^3 hybridized center, which is not adjacent to any sp^2 hybridized carbon center. The metabolite arises by either abstraction of a second hydrogen atom by the Fe(IV)–OH intermediate and subsequent collapse of the diradical or via abstraction of an electron and deprotonation
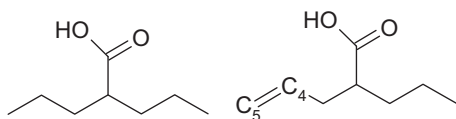
Fig. 1. Chemical structures of VPA (left) and 4nVPA (right).

of the cationic intermediate to yield the alkene [10,11]. However, although this kind of biochemical reaction is unusual in nature; there are several cases where this reaction has been observed [10–16].

CYP is a superfamily of cysteinato-heme enzymes [10,17]. Its active site contains a heme, which is deeply buried inside the protein at the bottom of a large, internal binding cavity. Heme unit consists of an iron protoporphyrin IX complex with a cysteine (Cys) residue as the proximal axial ligand. The active catalytic species, with a Fe(IV)-oxo moiety, is commonly denoted as Compound I. These enzymes are potent oxidants involved in oxidizing reactions such as the hydroxylation of saturated carbon-hydrogen bonds, the epoxidation of double bonds, the oxidation of heteroatoms, dealkylation reactions, and oxidations of aromatics using dioxygen to catalyze a great variety of stereospecific and regioselective processes of oxygen insertion into a variety of organic compounds, both of endogenous (such as steroids) and exogenous (xenobiotics) origins [18–23]. Furthermore, CYP enzymes can also catalyze the dehydrogenation of some organic molecules instead of carrying out the oxygenation of the substrate as observed for VPA and other organic molecules [24]. In this manner, CYP converts all the dioxygen into water and generates an olefin, thus exhibiting a mixed function as an oxidase-dehydrogenase enzyme.

The CYP enzymes share common structural characteristics such as a common overall fold despite less than 20% sequence identity across the CYP gene superfamily [23]. In recent years, the elucidation of several complexes of CYP crystal structures has provided further insight into the important structural aspects of these critical interactions, pointing out that steric and electronic parameters of the enzyme active site residues facilitate dehydratation or dehydrogenation reactions by positioning the substrate and/or stabilizing transition states [25,26]. On the other hand, although we have a general understanding about how 4nVPA is formed [9], the precise mechanisms or factors that direct dehydrogenation versus oxygenation are not well understood. Because human CYP2C9, CYP2E1 and rat CYP2C11 have been reported as VPA metabolizing in liver microsomes and also involved in 4nVPA formation [27], elucidating the factors that control its dehydrogenation is crucial to the design of analog drugs with reduced risks of metabolic activation.

Molecular models are valuable tools for studying substrate orientation within the CYP active site [28–31]. Nevertheless, the predictive power of these procedures is highly dependent on several factors, such as the accurate of the three-dimensional (3D) structure used as target [32,33] and the force field parameters for accurately determining the interactions between a compound and its target [34]. Bioinformatics methods have been widely used to predict ligand conformation into CYPs binding sites [13,35]. Moore et al. performed docking studies to model the metabolism of raloxifene, a CYP3A4-mediated dehydrogenated substrate [13]. Molecular docking is a method that predicts the binding mode of a ligand to a protein, and has been extensively used in rational design of drug [36–38]. However, some researcher have mentioned that the conformations obtained through docking procedures need to be examined by molecular dynamic (MD) simulations [39,40]. Therefore, in order to propose a plausible binding conformation between VPA or 4nVPA and CYPs that would allow to explain the binding orientation of both ligands into CYPs binding site as to be susceptible to undergo a dehydrogenation reaction, we carried out

automatic molecular docking using AutoDock software. Afterward, the dynamic stability of these complexes conformations were further analyzed using MD simulations, together with binding free energy estimation using the Molecular Mechanics Generalized Born Surface Area (MMGBSA) method.

To develop this study, we utilized two X-ray crystal structures of human beings (CYP2C9 and CYP2E1) and a model from rat (CYP2C11), which was obtained through homology modeling procedures. Structural analysis shows that CYP2C9, CYP2C11, and CYP2E1 share a similar overall folding structure (Fig. 2A), where the substrate binding cavity is formed by similar secondary structure elements: helices I, F, G, and F–G, B–C loops (Fig. 2B). These CYPs structures were chosen because it has been reported that both human enzymes catalyze the dehydrogenation of VPA [41–43], whereas that CYP2C11 is one of the most active and versatile CYP in rat liver microsomes [44–47] and the one used widely in our laboratory to evaluate the VPA metabolism. Thus, in overall our aim in this study is to determine whether or not the dehydrogenation mechanism was consistent among CYP enzymes or differences were possible among the isoforms. Finally, our results demonstrate the utility of these combined techniques in evaluating the intricate interactions between dehydrogenated substrates and CYP enzymes.

2. Methods

2.1. Homology modeling of CYP2C11

Since the crystal structure of CYP2C11 is not available so far in the Protein Data Bank (PDB), the 3D model of CYP2C11 used in the present contribution was constructed based on a homology modeling method using the known 3D structures of CYP2C9 (1R9O) and CYP2E1 (3KOH) as templates, and under the following procedure. First, we explored the NCBI protein data base [http://www.ncbi.nlm.nih.gov/sites/entrez] to search the amino acid sequences of rat CYP2C11 (Swiss-prot entry code: P08683). The ClustalW program version 2.0 [48] was used to identify similarity of this sequence to other CYP structures, and the I-TASSER server [49] was used to generate the 3D model. Once obtained the latter, this was minimized using MD simulations and its dihedral angles were validated by checking its Ramachandran map. Afterward, a protein sequence alignment among the CYP2C11 sequence, and those of templates (CY2C9 (1R9O) and CYP2E1 (3KOH)) was performed using ClustalW program [48] to evaluate the percentage of identity among the three CYP sequences, whereas that the level of conservation for each residue was calculated using PRALINE server [50].

2.2. Automated docking

AutoDock version 4.0.1 [51] was used to perform the automated docking studies between VPA or 4nVPA versus CYPs. Both substrates were treated as flexible ligands by modifying their rotatable torsions, but CYP2C9 (Fig. 2C), CYP2C11 and CYP2E1 (Fig. 2D and E) template were considered to be a rigid protein. 3D coordinates of the CYP2C9 and CYP2E1 structures 1R9O and 3KOH, respectively, were acquired from the PDB, whereas CYP2C11 was constructed using homology modeling procedures (see Section 2.1). X-ray structures were cleaned of both water molecules and co-crystallized ligands.

The substrate structures were drawn using the Isis/Draw program [52], and then were converted to a 3D format using WebLab Viewer and Molekel Visualization Package [53,54]. A geometric pre-optimization was carried out by using HyperChem 6.0 [55]. The minimum energy structure of the ligands was obtained using a semi-empical method at the AM1 level by