Molecular Dynamics Simulations of Phenylimidazole Inhibitor Complexes of Cytochrome P450<sub>cam</sub>

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Abstract. Molecular dynamics simulations have been performed on three phenylimidazole inhibitor complexes of P450<sub>cam</sub>, utilizing the X-ray structures and the AMBER suite of programs. Compared to their corresponding optimized X-ray structures, very similar features were observed for the 1-phenylimidazole (1-PI) and 2-phenylimidazole (2-PI) complexes during a 100 ps MD simulation. The 1-PI inhibitor binds as a Type II complex with the imidazole nitrogen as a ligand of the heme iron. Analysis of the inhibitor-enzyme interactions during the MD simulations reveals that electrostatic interactions of the imidazole with the heme and van der Waals interactions of the phenyl ring with nearby hydrophobic residues are dominant. By contrast, 2-PI binds as a Type I inhibitor in the substrate binding pocket, but not as a ligand of the iron. The interactions of this inhibitor are qualitatively different from that of the Type II 1-PI, being mainly electrostatic/H-bonding interactions with a bound water and polar residues. Although the third compound, 4-PI, in common with 1-PI, also binds as a Type II inhibitor, with one nitrogen of the imidazole as a ligand to the iron, the MD average binding orientation deviates significantly from the X-ray structure. The most important changes observed include: (1) the rotation of the imidazole ring of this inhibitor by about 90° to enhance electrostatic interactions of the imidazole NH group with the carbonyl group of LEU244, and (2) the rotation of the carbonyl group of ASP251 to form a H-bond with VAL254. An analysis of the H-bonding network surrounding this substrate in the optimized crystal structure revealed that there is no H-bonding partner either for the free polar NH group in the imidazole ring of 4-phenylimidazole or for the polar carbonyl group of the nearby ASP251 residue. The deviation of the dynamically averaged inhibitor-enzyme structure of the 4-PI complex from the optimized crystal structure can therefore be rationalized as a consequence of the optimization of the electrostatic interactions among the polar groups.

Key words. P450<sub>cam</sub>, phenylimidazole inhibitor, molecular dynamics simulation

Introduction

Poulos et al. have obtained [1] and refined [2] the crystal structures of three inhibitor complexes of the ferric form of cytochrome P450<sub>cam</sub>, with 1-phenylimidazole (1-PI), 2-phenylimidazole (2-PI) and 4-phenylimidazole (4-PI) shown below and found that they bind in the substrate binding site and inhibit the function of this enzyme. The free energies of binding of these inhibitors are significant with dissociation constants in the μM range [3].

![1-phenylimidazole](image1.png)  ![2-phenylimidazole](image2.png)  ![4-phenylimidazole](image3.png)

1-phenylimidazole  2-phenylimidazole  4-phenylimidazole

Despite their similarities, comparisons of the crystal structures of these phenylimidazole inhibitor-P450<sub>cam</sub> complexes reveal a number of qualitatively different features. First, as shown in Figure 1, the binding orientations of the inhibitors are different. Specifically, the 1-PI and the 4-PI isomer both form Type II complex with a N of the imidazole ring acting as a ligand of the heme iron atom of the P450<sub>cam</sub> enzyme, but the orientations of the phenyl ring and the imidazole ring differ in these two inhibitors. In addition, there is no free N atom to interact with other residues in the 1-isomer while in the 4-isomer, there is one. The 2-PI isomer forms a Type I complex, binding in the substrate binding site but not as a ligand to the heme iron atom. Also, there are two bound waters in the site, one acting as a ligand to the iron, with the phenyl ring of the 2-isomer located nearby, and the other interacting with one of the two N atoms of the imidazole ring of 2-PI. This water serves as a H-bond bridge to the ASP251. The other N of the imidazole ring in the 2-PI isomer is H-bonded to the hydroxyl group of TYR96. In addition to the different orientations of the inhibitors, the surrounding residue structures, especially those in the central region of helix I, in the three complexes are perturbed to different degrees from the substrate free form, with the 1- and 4-isomer environments more similar to each other.

In this paper, we have performed molecular dynamics simulations for all three phenylimidazole complexes of P450<sub>cam</sub>. Our goals were: (i) to use the dynamic behavior to obtain additional insight into the mode of inhibitor enzyme interactions; (ii) to identify and characterize the key inhibitor-enzyme interactions for each complex; (iii) to elucidate the origin of the observed differences in binding of the three inhibitors; and (iv) to provide knowledge for future design of more potent inhibitors for the P450<sub>cam</sub> enzyme.

**Method**

The crystal structures of the 1-phenylimidazole, 2-phenylimidazole, and 4-phenylimidazole complexes with cytochrome P450<sub>cam</sub> used were those in the Brookhaven Crystallographic Database as documented in the published work of Poulos and Howard [1,2]. The neutral form of each inhibitor was used, and for the 2-PI, the only inhibitor for which there was a choice of the two imidazole nitrogens, the proton was assigned to N3 after consideration of optimization of hydrogen bonding interactions in its crystallographic orientation. Each of the inhibitors was then fully geometry optimized using the GAUSSIAN92 programs and a 6-31G* basis set [4]. Charge parametrization for each of the inhibitors was developed from CHELPG charges fit to the molecular electrostatic potential for each of the inhibitors derived from a 6-31G**//6-31G* single point calculation. The intramolecular harmonic vibrational parametrization was taken principally from AMBER [5] and MSI/Quanta [6]. The charges for the heme were taken to be those of the resting state heme as determined from ZINDO Mulliken charges from that state [7]. Intramolecular/vibrational force constants for the heme were derived from the standard AMBER parameter set.

The P450<sub>cam</sub>/inhibitor energy minimization and molecular dynamics simulations were performed using AMBER 4, employing the all atom representation and full protein dynamics including all crystallographic waters. Initial hydrogen positions