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# Prediction of three-dimensional structures and structural flexibilities of wild-type and mutant cytochrome P450 1A2 using molecular dynamics simulations

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ABSTRACT

In this study, we investigated the effect of genetic polymorphism on the three-dimensional (3D) conformation of cytochrome P450 1A2 (CYP1A2) using molecular dynamics (MD) simulations. CYP1A2, a major drug-metabolizing enzyme among cytochrome P450 enzymes (CYPs), is known to have many variant alleles. The genetic polymorphism of CYP1A2 may cause individual differences in the pharmacokinetics of medicines. By performing 100 ns or longer MD simulations, we investigated the influence of amino acid mutation on the 3D structures and the dynamic properties of proteins. The results show that the static structures were changed by the mutations of amino acid residues, not only near the mutated residues but also in distant portions of the proteins. Moreover, the mutation of only one amino acid was shown to change the structural flexibility of proteins, which may influence the substrate recognition and enzymatic activity. Our results clearly suggest that it is necessary to investigate the dynamic property as well as the static 3D structure for understanding the change of the enzymatic activity of mutant CYP1A2.


## 1. Introduction

Cytochrome P450 (CYP), superfamily of heme-containing multifunctional enzymes, widely exists in bacteria, plants, and animals [1]. CYP plays many important roles, including synthesis of biologically active substances, metabolic activation of some mutagenic compounds, and detoxification of xenobiotics [2–4]. The biologically active substances synthesized by CYPs include steroids, fatty acids, lipid soluble vitamins, and eicosanoids [2]. However, CYPs have attracted clinical attention not only for their biological regulation function but also for their drug metabolism function [3,4]. Drug metabolism is divided into two phases: phase I (oxidation, reduction, and hydrolysis) and phase II (conjugation reaction). During the phase I oxidation reaction, CYPs catalyze the monooxygenation of drugs using molecular oxygen by interacting with cytochrome P450 redox partner (CPR).

CYPs have many variant alleles, and their genetic polymorphism is viewed as a pharmacotherapeutic challenge as it sometimes affects their efficacy and results in adverse side effects of medicine [5]. Major drug-metabolizing CYPs such as CYP2C9, CYP2D6, and CYP3A4/5 show genetic polymorphism, which is recognized to be important from the view of pharmacokinetics [6]. Furthermore, CYP1A2 is known as a major drug-metabolizing enzyme. The genetic polymorphism of CYP1A2 may cause individual differences in the pharmacokinetics of clinically important medicines such as asthma remedies (theophylline), antipsychotics (amitriptyline, imipramine, and olanzapine), and antiarrhythmic drugs (mexiletine and propranolol).

It has previously been too difficult to compare the enzymatic activity of mutant CYP1A2 because this activity has in several past studies been measured under varying experimental conditions [7–9]. Previously, we investigated the enzymatic activities of 20 variant alleles of CYP1A2 under a unifying experimental condition

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[10]. In that study, the enzymatic activities of CYP1A2 were measured against 7-ethoxyresorufin and phenacetin, which are widely used as standard reagents for *in vitro* experiments. These compounds are oxidized by CYP1A2 at the 1-position carbon atom of the ethoxy group.

Recently, the influence of amino acid substitution on 3D structure of CYPs has been investigated by computational studies [11–19]. Oda et al. predicted the 3D structure of wild-type and mutant CYP2C19 by homology modeling and MD simulations [11]. Their result suggests that the hydrogen bond between the ligand, (*S*)-mephenytoin, and CYP2C19 detected in the wild type cannot form in mutants. Moreover, Kobayashi et al. investigated the influence of single nucleotide polymorphisms of CYP2B6 on the conformation by docking and MD simulations [12]. Their result suggests that not only the static structure but also the structural flexibility of mutant CYP2B6 changes from that of wild-type CYP2B6, which results in the substrate, in this case artemether, not being recognized by some mutants. Regarding the genetic polymorphism of CYP1A2, the CYP1A2.11 Phe186Leu mutation was investigated [13,14]. Zhang et al. investigated the effect of the peripheral mutation Phe186Leu on the enzymatic activity by performing 20 ns MD simulations [13]. The Phe186Leu mutation is located away from the catalytic pocket within the wild type CYP1A2 structure. Their result suggests that the Phe186Leu mutation results in a change of protein flexibility, and the collective protein motion causes the main substrate access channel to be mostly closed. To date, the 3D structures for mutant CYP1A2 have not been experimentally determined. Only the 3D structure for CYP1A2.11 has been computationally predicted. For most of the mutant CYP1A2, except for CYP1A2.11, the causes for the activity changes have not been explained.

There are some computational studies suggesting that the structural flexibilities of CYP proteins have large influence on the substrate recognition or the enzymatic activity [12,13,16,20]. In actual fact, experimental studies suggested that CYP proteins have structural flexibility or conformational plasticity in mammalian CYPs [21–25] as well as bacterial CYPs [26–29]. Scott et al. reported that the structure of ligand-free CYP2B4 is different from that of 4-(4-chlorophenyl) imidazole-bound CYP2B4 in B′ to C and F to G helices [21,22]. In their studies, the ligand-free CYP2B4 was obtained as an open conformation [21], whereas the ligand-bound CYP2B4 was obtained as a closed conformation [22]. These investigations suggest that the static structure of the CYP protein undergoes dynamic change induced by ligand binding.

In the current study, we investigated the influence of genetic polymorphism on the 3D structure and structural flexibility of CYP1A2 by MD simulations, referring to the enzymatic activity data previously determined by us [10]. As a first step, more than 100 ns MD simulations were performed, which is longer than the MD simulations of most previous studies on CYPs [11–15,17–20]. Only the CYP2D6 study by de Waal et al. similarly performed 100 ns MD simulations [16]. Our long time MD simulations are expected to be more useful for prediction of the dynamic property of CYP protein.

In the current study, the metabolism by CYPs has been investigated by *in silico* methods, and the results are expected to enhance the structure-based drug design [30,31]. The current investigation will facilitate understanding of the metabolic mechanism of CYPs and provide useful information for structure-based drug design.

## 2. Methods

Wild-type and mutant CYP1A2 used in the current study are shown in Table 1. The amino acid mutations and the differences of the enzymatic activities previously experimentally determined by us are shown in Table 1. The initial 3D structure of the wild-type CYP1A2 (CYP1A2.1) was constructed using experimentally determined structures registered in the Protein Data Bank (PDB ID: 2HI4) [32]. At the present time, 2HI4 is the only crystal structure of CYP1A2 deposited in the Protein Data Bank. The crystal structure of CYP1A2, except for the N-terminal membrane-binding domain, was determined by X-ray crystallography at 1.6 Å resolution, and Arg34 to Arg503 were observed. As a first step, we refined the 3D structure of CYP1A2.1 because the crystal structure was obtained as a complex structure with the inhibitor, α-naphthoflavone (ANF), which would have caused conformational changes by ligand binding. To construct the initial structure of CYP1A2.1, ANF and crystal waters were deleted from the crystal structure. Subsequently, the crystal structure was refined by minimization. Temperature-increasing and equilibrating MD simulations were then performed. The structures of eight mutants were constructed from the 3D structure of CYP1A2.1 refined after MD simulation.

More than 100 ns MD simulations were performed for each CYP1A2 protein. The temperature-increasing MD simulations were performed for 20 ps in which the temperature was raised from 0 K to 300 K. Equilibrating MD simulations under constant pressure were then performed at 300 K. 100 ns equilibrating MD was conducted for CYP1A2.1, CYP1A2.4, CYP1A2.6. CYP1A2.13, and CYP1A2.14 to reach the conversion. On the other hands, 150 ns simulations were required for CYP1A2.15 and CYP1A2.16, and 175 ns simulations were needed for CYP1A2.8 and CYP1A2.11. The system was solvated by TIP3P model and calculated under cyclic boundary conditions. The cyclic boundary box was spaced by a margin of at least 8 Å from the protein surface. The cut-off distance for van der Waals interactions was set at 10 Å, and Particle Mesh Ewald method was used for calculating the electrostatic interactions. For the neutralization of the system, eight chloride ions were arranged. For the force field parameter around the heme iron, our previously determined parameters [33] were used, which represents the sextet state with five-coordinate iron (III). When the substrate is recognized by most CYPs, formation of this spin and coordinate

**Table 1**
Wild-type and mutant CYP1A2 used in the current study and the experimentally determined enzymatic activities [10].

| Gene | Protein | Mutation | Activity[a] | Time/ns[c] | RMSD/Å[d] |
|---|---|---|---|---|---|
| ***CYP1A2*1*** | CYP1A2.1 | – | Normal | 100 | 1.73 |
| ***CYP1A2*4*** | CYP1A2.4 | Ile386Phe | N. D.[b] | 100 | 1.56 |
| ***CYP1A2*6*** | CYP1A2.6 | Arg431Trp | N. D.[b] | 100 | 1.70 |
| ***CYP1A2*8*** | CYP1A2.8 | Arg456His | N. D.[b] | 175 | 1.28 |
| ***CYP1A2*11*** | CYP1A2.11 | Phe186Leu | Decreased | 175 | 1.60 |
| ***CYP1A2*13*** | CYP1A2.13 | Gly299Ser | Normal | 100 | 1.22 |
| ***CYP1A2*14*** | CYP1A2.14 | Thr438Ile | Increased | 100 | 1.80 |
| ***CYP1A2*15*** | CYP1A2.15 | Pro42Arg | Decreased | 150 | 1.65 |
| ***CYP1A2*16*** | CYP1A2.16 | Arg377Gln | N. D.[b] | 150 | 1.75 |

[a] 7-ethoxyresorufin *O*-deethylation activities.
[b] not detectable.
[c] time taken for MD simulation.
[d] RMSDs between the starting and final structures of MD simulations.