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Molecular dynamics mechanism to generate species differences in inhibition of protoporphyrinogen oxidase by flumioxazin

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ABSTRACT

Flumioxazin is an *N*-phenylimide herbicide and shows a remarkable species difference in developmental toxicity between rats and rabbits. The species difference is corresponded well to the inhibitory potency on protoporphyrinogen oxidase (PPO), an enzyme involved in chlorophyll and heme biosynthesis. *In vitro* experiments have shown that rat PPO is more sensitive to flumioxazin than human PPO and rabbit PPO. However, it remains unknown how the large difference in sensitivity to flumioxazin is generated in PPOs with highly conserved amino acid sequences. In order to determine the molecular dynamics (MD) mechanism responsible for these species differences, we performed MD simulations of human, rat, and rabbit PPO–flumioxazin complexes and found that rat PPO exhibited a higher binding affinity of flumioxazin than human and rabbit PPOs. A sophisticated comparative analysis of MD trajectories demonstrated that differences in the dynamics of the 107–120 loop region derived from amino acid sequence variants generated differences in the PPO–flumioxazin interactions via changes in the dynamics of Arg97. Moreover, a change in the shape of the flumioxazin-binding pocket in human PPO weakened the van der Waals forces contributing to the human PPO–flumioxazin interaction. Additionally, the positional and orientational shift in flumioxazin weakened the Coulomb force contributing to the rabbit PPO–flumioxazin interaction. These findings support the involvement of an MD mechanism in species differences in flumioxazin sensitivity.

1. Introduction

Flumioxazin (also known as S-53482) is an *N*-phenylimide herbicide that has beneficial effects on broadleaf weeds. Notably, however, this herbicide causes embryonic lethality, teratogenicity (mainly ventricular septal defects [VSDs] and wavy ribs), and growth retardation in fetuses in the absence of maternal toxicity at a dose of 30 mg/kg/day in rat developmental toxicity study. In contrast to rats, flumioxazin caused no developmental toxicity in rabbits, even at a maternal toxic dose of 3000 mg/kg/day [1]. Moreover, there are compound-specific differences in developmental toxicity among *N*-phenylimide compounds structurally similar to flumioxazin (Table 1) in rats and rabbits [2]. At 20 mg/kg/day, S-23121 induces the same pattern of developmental toxicity in rats as 30 mg/kg/day flumioxazin. In contrast, the developmental toxicity is not observed in rats treated with S-23031, even at a level of 1500 mg/kg/day. Neither S-23121 nor S-23031 cause developmental toxicity in rabbits at the highest dose levels tested, indicating that no tested *N*-phenylimide herbicides have been shown to have teratogenic potential in rabbits. Kawamura et al. demonstrated that species- and compound-specific differences in developmental toxicity of *N*-phenylimide herbicides correspond well to the potency of protoporphyrinogen oxidase (PPO) inhibition [3]. Rat PPO is the most sensitive to flumioxazin, followed by human PPO; in contrast, rabbit PPO is relatively insensitive to flumioxazin. This species difference in flumioxazin sensitivity is apparent, despite that the amino acid homology of PPOs is very well conserved across species (Fig. 1). These findings have increased our interest in trying to understand how small differences in amino acid sequences of PPOs can cause large differences in sensitivity to flumioxazin.

Due to the high sequence similarity of PPOs, particularly the identical sequences in the active site, both static and dynamic

Abbreviations: MD, molecular dynamics; VDW, van der Waals; VSD, ventricular septal defect; ANOVA, analysis of variance; PPO, protoporphyrinogen oxidase; NMR, nuclear magnetic resonance; TI, thermodynamic integration; *NVT*, constant number (*N*), volume (*V*), and temperature (*T*); *NPT*, constant number (*N*), pressure (*V*) and temperature (*T*).

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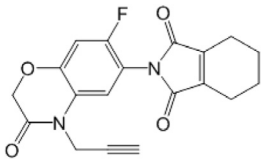
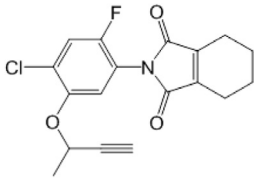
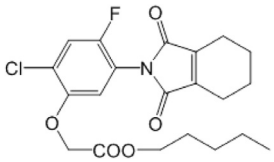
Table 1
N-Phenylimide derivatives including flumioxazin and their inhibitory activities against protoporphyrinogen oxidase. The pIC_{50} value are calculated from minus common logarithms of IC_{50} values (M) quoted from Kawamura et al. [3].

Name	Structure	pIC_{50} (M)		
		Human PPO	Rat PPO	Rabbit PPO
Flumioxazin		7.76	8.95	6.86
S-23121		–	7.97	5.81
S-23031		–	6.10	5.32

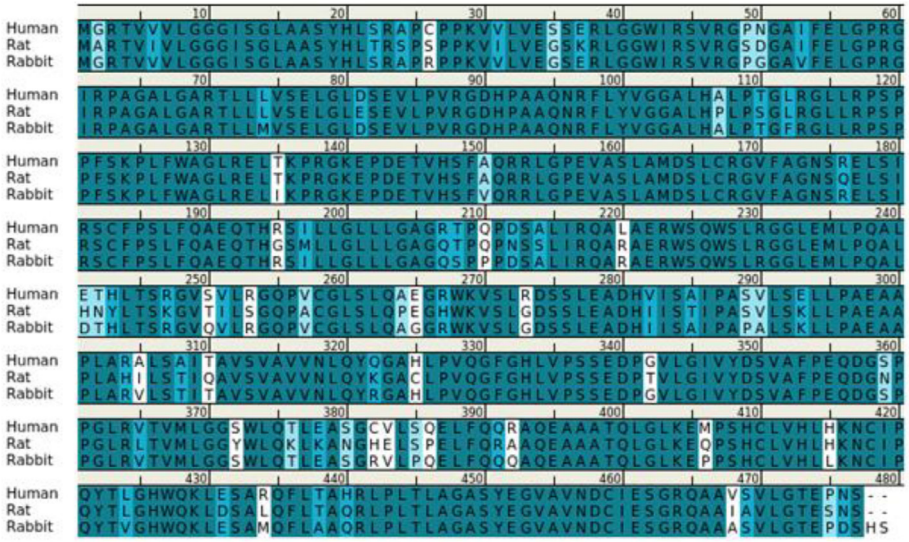


Fig. 1. Multiple sequence alignment of human, rat, and rabbit PPOs. Color strength represents a similarity among the three species: Dark blue shows a consensus of amino acid residues, while white shows discordance of amino acid residues.

structures of PPO–flumioxazin complexes should be considered in order to resolve this research issue. Nuclear magnetic resonance (NMR) spectroscopy is an experimental method used to analyze molecular dynamics (MD) behaviors. However, NMR is unable to capture proteins consisting of more than 300 amino acids, such as PPO. In MD simulations, the positions and velocities of all atoms, including environmental molecules such as water and inorganic ions, are periodically determined by solving equations of motion for all atoms, and all trajectories of MD simulations correspond exactly to the statistical ensemble in statistical mechanics. Actually, MD simulation with the binding free energy calculation in implicit water solvent have revealed the interaction mode and binding affinity of some ligands for the PPO [4–9]. On the other hand, the thermodynamic integration (TI) method, which integrates the energy required for protein–ligand binding processes based on MD simulation in explicit water solvent, is an effective method for calculating the binding free energy (ΔG_{bind}), considering the effect of environmental molecules [10]. Theoretically, the binding free energy calculated by the TI method is correlated with pIC_{50} values, logarithms of the half-maximal inhibitory concentration, measured in biological experiments [3]. Thus, MD simulations make it possible to analyze dynamics directly linked to the binding affinity of protein–ligand complexes.

In this study, we performed MD simulation of flumioxazin in complexes with human, rat, and rabbit PPOs and calculated ΔG_{bind} by the TI method. In order to ensure higher reliability of the simu-