Weed Turf. Sci. 6(1):28~31
http://dx.doi.org/10.5660/WTS.2017.6.1.28 Print ISSN 2287-7924, Online ISSN 2288-3312

Research Article Weed & Turfgrass Science

Weed & Turfgrass Science was renamed from formerly both Korean Journal of Weed Science from Volume 32(3), 2012, Korean Journal of Turfgrass Science from Volume 25(1), 2011 and Asian Journal of Turfgrass Science from Volume 26(2), 2012 which were launched by The Korean Society of Weed Science and The Turfgrass Society of Korea founded in 1981 and 1987, respectively.

Sulfonylurea Herbicide Resistance Mechanism of Some Acetohydroxy Acid Synthase Mutants and New Designed Herbicides Specific to the Mutants

Mun Myong Choe[1], Hun Chol Kang[1], In Chul Kim[1], Hai Su Li[2], Ming Gen Wu[2]*, and Im Shik Lee[1,3]

[1]*Department of Agriculture and Life Science, Pyongyang University of Science & Technology, Pyongyang, DPRK*
[2]*College of Agriculture, Yanbian University, Yanji, Jilin, China*
[3]*Institute of Physics, Nankai University, Tianjin, China*

ABSTRACT. The mutation rate of proline in the position 197 (Pro197) in acetohydroxy acid synthase (AHAS) is highest among sulfonylurea (SU) herbicide-resistance mutants. Therefore, it is significant to investigate the resistance mechanism for the mutation and to develop the herbicides specific to the mutants. SU herbicide resistance mechanism of the mutants, 197Ser, 197Thr and 197Ala, in AHAS were targeted for designing new SU-herbicide. We did molecular dynamics (MD) simulation for understanding SU herbicide-resistance mechanisms of AHAS mutants and designed new herbicides with docking and MD evaluations. We have found that mutation to 197Ala and 197Ser enlarged the entrance of the active site, while 197Thr contracted. Map of the root mean square derivation (RMSD) and radius gyrations (Rg) revealed the domain indicating the conformations for herbicide resistant. Based on the enlarging-contracting mechanism of active site entrance, we designed new herbicides with substitution at the heterocyclic moiety of a SU herbicide for the complementary binding to the changed active site entrances of mutants, and designed new herbicides. We confirmed that our screened new herbicides bonded to both AHAS wild type and mutants with higher affinity, showing more stable binding conformation than the existing herbicides.

***Key words*:** Acetohydroxy acid synthase, Herbicide designs, Herbicide resistance, Molecular dynamics

Received on November 10, 2016; Revised on February 27, 2017; Accepted on March 8, 2017

***Corresponding author:** Phone) +86-0433-2435566, Fax) +86-0433-2435600; E-mail) 2819896@hanmail.net

Introduction

Herbicides make major contributions to global food production by easily removing weeds. SU herbicides are regarded as one of the major herbicides to global food production because of their eco-friendly advantages in extremely low toxicity towards mammals, ultra-low dosage application and high selectivity (Park et al., 2014). While herbicides have the beneficial point, persistent application of herbicides to weeds in many areas over the world can result in the rapid evolution of herbicide resistance (Choe et al., 2015). If mutations occur in a herbicide target enzyme, the structure and function of the target enzyme change to form weak enzyme-herbicide binding (Mulwa et al., 2006). In many weed species, herbicide resistance to several herbicides have developed and become great challenge to agriculture in the world (Price and Watkins, 2003). Investigation for herbicide resistance and developing new herbicides are necessary for sustainable weed control.

To provide a rational molecular basis for understanding herbicide-resistant mutations, the crystal structure of the catalytic subunit of *Arabidopsis thaliana* L. AHAS in complex with chlorimuron ethyl (CIE), four other SU herbicides and imazaquin (IQ), was elucidated (McCourt et al., 2006). Although the crystal structures give an understanding of the molecular structure and function, especially in wild-type AHAS–herbicide interactions, they are insufficient to elucidate the interaction mechanism in the mutated states. Therefore, various herbicide-resistant AHAS mutations are still speculative (Duggleby et al., 2008).

In this work, using molecular modeling techniques and molecular dynamics (MD) simulations of the wild type and three mutants (197Ser, 197Thr and 197Ala) (Mccammon et al., 1997; Scott, 1998), we investigated the conformational and dynamical interactions between mutant AHAS and herbicide. The herbicide-resistance mechanism of resistant mutants was

characterized at molecular level and used for designing and evaluating new herbicides specific the resistant mutants.

Materials and Methods

The sequences of three mutated AHAS were obtained from *K. scoparia* and *R. raphanistrum*. X-ray structure of AHAS, 1YBH (PDB ID), was selected as a template structure for building the mutants, Ser, Thr and Ala at the point 197 Pro (called 197Ser, 197Thr and 197Ala, respectively). Conformational analysis of the wild and mutated AHASs was performed with the Discovery Studio 3.5 (Accelrys Inc., San Diego). The MD simulations and trajectory analysis were carried out with GROMACS 4.5.5. The topology file of SU (CIE) and two cofactors [FAD (flavin adenine dinucleotide) and ThDP (thiamine diphosphate)] consistent with GROMOS 96 force field was generated by the public access PRODRG online server.

All simulations were run under periodic boundary conditions with NPT (constant number, constant pressure and constant temperature) ensemble and V-rescale thermostat. All the post-dynamic analyses of the trajectories were carried out using the auxiliary analysis package available in the GROMACS, and Discovery Studio 3.5.

For designing new herbicides, the CIE structure was obtained by using the CIE template structure of 1YBH structure. The benzoate region of template structure was focused to redesign using ReCore of Leadit.

The designed herbicides were evaluated by molecular docking using FlexX. The wild and mutated AHASs were used as initial receptor structure and the designed herbicides as ligand with ThDP and FAD as cofactor and Mg^{2+} as metal ion.

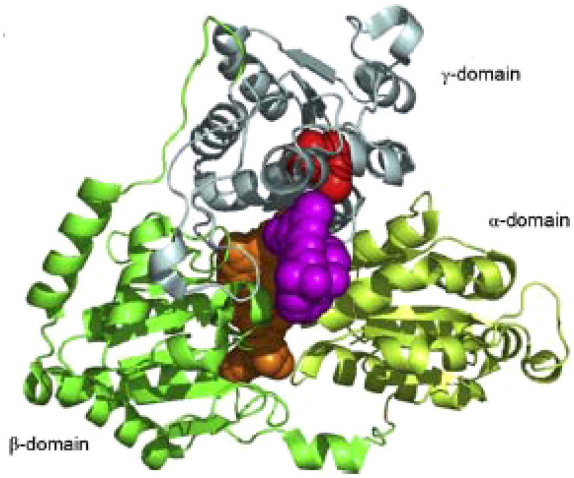

Fig. 1. X-ray crystallographic structure of the *A. thaliana* AHAS catalytic subunit with the herbicide CIE (purple). CIE binding pocket is formed by three domains of AHAS. There were also additional two cofactors, FAD (brown) and ThDP (red) with Mg^{2+} ion.

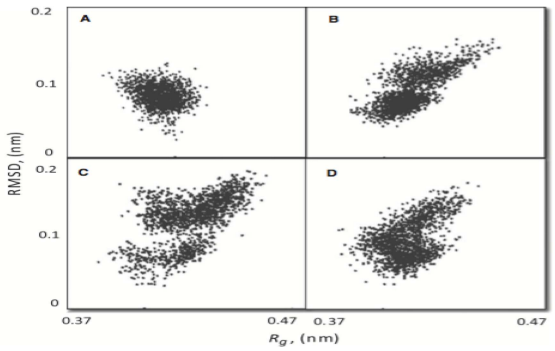

Fig. 2. Two-dimensional distribution between RMSD and Rg of CIE. For the wild type (A), mutant with 197Thr (B), mutant with 197Ala (C) and 197Ser(D).

Result

Conformational characteristics of CIE bindings

Conformation of AHAS was used for molecular dynamics with /without CIE as shown in Fig. 1. CIE RMSD against X-ray crystallographic conformation of CIE in the binding pockets was also calculated for wild type and the three mutants models.

The relation between RMSD and radius of gyration (Rg) of CIE was plotted as shown in Fig. 2. The distribution for the wild type was accumulated as a single domain (Fig. 2A), but that for the mutated types were scattered forming two domains corresponding to the two possible CIE binding conformations (Fig. 2B-D).

The additional clusters for the mutants were located at a higher RMSD and Rg compared with the cluster for the wild type. This indicated that the binding interactions between CIE and the mutated AHASs decreased. The additional CIEs binding conformation in the three mutated systems would be destabilized at the active site, assuming that the herbicide would be separated from the active site. 197Ala mutant AHAS was the most changed among the three mutants. In contrast, the single-domain distribution of the wild- type system indicated that the binding formation of CIE formed stable complementary interaction bindings with its enzyme. Thus, the single point mutants with 197Pro substituted by 197Thr, 197Ala or 197Ser induced significant conformational changes in the pyruvate-binding pocket of AHAS.

Herbicide design

In AHAS mutants of herbicide-resistant weeds, the binding affinity of enzyme and herbicide was weakening due to the structural changes of herbicide binding resulted from mutation (Price et al., 2003). Therefore, herbicides were designed by changing the benzoate ring of SU, to make

Table 1. Comparison of molecular docking scores (Gibbs free energy, kJ/mol) between designed herbicides and normal herbicides.

Receptor		197Ser		197Ala		197Thr	
Rank		Model Name	Score	Model Name	Score	Model Name	Score
1		SU_443	-46.666	SU_443	-45.438	SU_358	-43.734
2		SU_421	-44.839	SU_422	-43.562	SU_476	-43.732
3		SU_422	-43.197	SU_423	-41.922	SU_425	-43.382
4		SU_423	-42.772	SU_425	-41.485	SU_421	-42.420
5		SU_419	-41.975	SU_475	-41.420	SU_443	-41.544
6		SU_425	-41.225	SU_445	-41.244	SU_294	-41.333
7		SU_475	-41.086	SU_420	-41.110	SU_386	-41.013
8		SU_420	-40.692	SU_406	-40.619	SU_411	-40.087
9		SU_445	-40.542	SU_405	-40.446	SU_67	-38.701
10		SU_294	-40.315	SU_421	-40.439	SU_350	-38.042
Herbicides		CIE	-30.873	CIE	-32.208	CIE	-32.875
		Bensulfuron	-32.822	Bensulfuron	-32.376	Bensulfuron	-34.741
		Chlorsulfuron	-31.925	Chlorsulfuron	-31.925	Chlorsulfuron	-31.925
		Cyclosulfamuron	-30.897	Cyclosulfamuron	-33.148	Cyclosulfamuron	-28.240
		Ethoxysulfuron	-37.327	Ethoxysulfuron	-37.455	Ethoxysulfuron	-36.993
		Imazosulfuron	-30.873	Imazosulfuron	-32.208	Imazosulfuron	-32.875
		Metsulfuron	-32.822	Metsulfuron	-32.376	Metsulfuron	-34.741

complementary binding between herbicide and altered entrance of active site channel. Using CIE template structure obtained from X-ray crystal structure 1YBH, the benzoate region of template structure was substituted using program ReCore of Leadit. In this case, the bond between sulfonyl atom of SU bridge and the neighbor carbon atom of benzoate ring was pointed as cutting bond. We could obtained ~ 505 possible models. 505 of new SU herbicides were designed and named as SU_1, SU_2, SU_3, ···, SU_505 by substituting of benzoate ring of CIE.

All possible models were screened by using FlexX molecular docking. Top 10 herbicides in docking score in every mutant were compared (Table 1). SU_420, SU_421, SU_422, SU_423, SU_425, SU_475, SU_443, SU_445 and SU_475 belonged commonly to top 10 herbicides in both 197Ser and 197Ala.

The molecular docking results of SU_443 with top score in 197Ser and 197Ala are shown in Fig. 3 A-B. As can be seen, SU_443 had similar conformations in two mutants. The herbicide docked to 197Ser made complementary binding with enzyme forming many hydrogen-bonds with active residues.

Summary

Three mutated AHAS at the residue 197Pro with 197Ser, 197Ala and 197Thr showed significant changes at the entrance of active site channel, the herbicide-binding region. The herbicide resistance was illustrated by the conformational changes in terms of RMSD and Rg. These structural characteristics were used to evaluate the newly designed herbicides. We proposed 10 candidates respectively for three AHAS mutants to overcome herbicide-resistance.

Acknowledgments

This work was supported by the NSFC (31160370).

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