

## ASSESSMENT OF PESTICIDES INTERACTIONS WITH *BACILLUS PASTEURII* UREASE. A COMPUTATIONAL STUDY\*

D. L. VLĂDOIU<sup>1,2</sup>, M.N. FILIMON<sup>1,2</sup>, V. OSTAFE<sup>1,2</sup>, A. ISVORAN<sup>1,2</sup>

<sup>1</sup> Department of Biology-Chemistry, West University of Timisoara, 16 Pestalozzi, 300316 Timisoara, Romania, E-mail: dyana\_vladoiu@yahoo.com, nicoleta.filimon@e-uvvt.ro, vostafe@cbg.uvt.ro, adriana.isvoran@e-uvvt.ro

<sup>2</sup> Laboratory of Advanced Researches in Environmental Protection, West University of Timisoara, 4 Oituz, 300086 Timisoara, Romania

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Two herbicides (chlorsulfuron and nicosulfuron) and one fungicide (difenoconazole) are considered in this work and molecular docking studies have been implemented to evaluate their interactions with *Bacillus pasteurii* urease, a soil microorganism enzyme using a bimetallic nickel active center. Structural characterization of herbicides and fungicide is also performed by comparison to urea and known inhibitors of urease and taking into account the protein surface description. The two herbicides, chlorsulfuron and nicosulfuron, and the fungicide difenoconazole have higher dimensions than urea and known inhibitors of *Bacillus pasteurii* urease. Both herbicides and the fungicide are evaluated to strongly interact to *Bacillus pasteurii* urease, the most favorable interaction being predicted for chlorsulfuron. Moreover, all the pesticide molecules provide the capacity to bind to urease in several places to the enzyme surface, but only chlorsulfuron is proficient to bind to the catalytic site. It may be explained by the fact that chlorsulfuron contains a charged region that is assumed to interact electrostatically with the active site of the enzyme, the known inhibitors also exhibiting such a region.

*Key words:* urease, pesticides, molecular docking.

### 1. INTRODUCTION

Urea is an important organic mineral soil fertilizer. Urease (EC 3.5. 1:5) is the enzyme performing urea's ammonification and it is found in a group of soil microorganisms, such as *Micrococcus sp.*, *Bacillus sp.*, *Pregnancy sp.* and *Urobacterium sp.*. Urease belongs to the class of amidases and is responsible for the hydrolysis of urea fertilizers in NH<sub>3</sub> and CO<sub>2</sub> resulting into the increase of soil pH and a rapid losses of nitrogen by volatilization of NH<sub>3</sub> [1].

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Soil urease comes mainly from plant residues, animal dejections and microorganisms present in the soil, suggesting that a significant part of ureolytic activity is performed by the extracellular urease, stabilized by immobilization of soil mineral and organic colloids [2]. Urease activity is considered an important factor in the decrease of efficiency of urea administrated as a soil fertilizer [3] and some urease inhibitors have been identified [4, 5].

Urease activity in the soil is influenced by many factors including the organic matter content of soil, crop plants, soil depth, soil works, heavy metal content, as well as environmental factors (temperature, pH, humidity) [6–10]. Since urease plays a vital role in the hydrolysis of urea fertilizer, it is also important to discover other unknown factors that may reduce the effectiveness of this enzyme in the ecosystem [11].

Studies on the effects of some herbicides (glyphosate, glufosinate, sulphonylurea) on soil enzymes activity (including urease) highlight their inhibitor potential [12, 13]. A recent experimental study concerning the effects of chlorsulfuron and 2-methyl-4 chlorophenoxyacetic acid (MCPB-Na) herbicides on soil microorganism communities shows that the urease activity is the most sensitive to these herbicides action [14]. Other studies concerning the effects of some soil fungicides on the urease activity reflect both stimulating and inhibiting potential of the fungicide depending on the dose, as well as the duration of incubation [15, 16]. The fungicide may be toxic to certain species of microorganisms resulting in inhibited activity of some enzymes, but elevated urease activity shows that it is very likely that fungicide acts as a carbon source for some species of microorganisms leading to an increase in the microbial biomass and consequently in the activity of urease.

Taking into account all these data it becomes important to assess the inhibitor potential of some commonly used pesticides in Romania on soil enzymes activity. Within this study we focus on the assessment of effects of two herbicides (nicosulfuron, chlorsulfuron) and a fungicide (difenoconazole) on soil urease activity by computational methods.

## 2. METHOD

There are 4 entries concerning *Bacillus pasteurii* urease in the *Protein Data Bank* (PDB) [17], the corresponding codes entry being: 2UBP for the native enzyme in complex with  $\text{Ni}^{2+}$  ions [18], 3UBP for the enzyme in complex with the inhibitor diamidophosphate [18], 1UBP for the enzyme in complex with the inhibitor  $\beta$ -mercaptoethanol [4] and 4AC7 for the enzyme in complex with the inhibitor citrate [5].

The *Simplified Molecular-Input Line-Entry System* (SMILES) linear formula [19] for every considered pesticide molecule (chlorsulfuron, nicosulfuron and difenoconazole) has been extracted from ChemSpider chemical structure free

database (<http://www.chemspider.com/>) and used as input by Frog2 webserver [20] to obtain the spatial structure of the ligands.

The interactions between ligands and proteins usually occur via the cavities and/or protrusions present at the protein surface. There are some computational tools that allow the identification of cavities and also characterization of their local geometric and chemical properties. Within this study we have used the Fpocket tool [21].

In order to compare the structural properties of the considered enzyme when it interacts with inhibitors we may use structural alignment based on the superposition of the atomic coordinate sets of two or more protein structural files and a minimal *Root Mean Square Deviation* (RMSD) between the structures is subtracted reflecting the degree of dissimilarity of two three-dimensional structures. There are different possible subsets of the protein atoms that can be used in generating the structural alignment and computing the corresponding RMSD values, but usually the alpha carbon (CA) positions are considered [22]. A zero value for the RMSD indicates identical structures and it increases as structures are dissimilar. Structural similarity of enzyme and its complexes is compared using the structure matching tool in the UCSF Chimera software [23]. The same software is used to compute the area and volumes of considered molecules of pesticides and compared to those of urea (the natural substrate of urease) and its known inhibitors [4, 18, 5].

The computation of the interactions of urea (the natural substrate of urease and used here as a control ligand) and considered pesticides with *Bacillus pasteurii* urease are obtained using SwissDock tool [24]. SwissDock is a web-based interface handling all aspects of molecular docking using EADock tools [25]. It outcomes the most favorable position and orientation of the ligand on a protein surface (the binding mode) and the interaction energy expressed as FullFitness score:

$$E_{\text{full}} = E_{\text{ligand}} + E_{\text{receptor}} + E_{\text{vdw}} + E_{\text{elec}} + \Delta G_{\text{elec,solv}} + \sigma \cdot \text{SASA} \quad (1)$$

where  $E_{\text{ligand}}$  is the internal energy of the ligand (the sum of the internal bonded and non-bonded interactions terms),  $E_{\text{receptor}}$  is the internal energy of the receptor (which is constant since it is kept fixed during the simulation),  $E_{\text{vdw}}$  is the van der Waals interaction energy,  $E_{\text{elec}}$  is the electrostatic interaction energy,  $\Delta G_{\text{elec,solv}}$  is the electrostatic solvation free energy and the term  $\sigma \cdot \text{SASA}$  refers to the nonpolar contribution to the solvation energy (assumed to be proportional to the solvent accessible surface area, SASA, that is buried upon complexation) [25]. The first 5 terms in equation 1 are computed using CHARMM force field [26]. The obtained binding modes are clustered and clusters are ranked by averaging the FullFitness scores of their elements. Visualization of the results obtained using the SwissDock server is made using Chimera package [23]. Protein and ligand structures can be

inputted directly from databases on the web interface of SwissDick, but for a better accuracy we have prepared the structures of urease and pesticides using „DockPrep” facility under Chimera package [23]. Selected docking type was „accurate” and we have performed flexible docking.

### 3. RESULTS

First of all, we have characterized the surface of *Bacillus pasteurii* urease using the Fpocket tool. Fpocket identifies 32 cavities for the catalytic domain of the urease and the superposition of the identified cavities of the catalytic domain with the 3D structure of the protein reveals that the active site is represented by the biggest polar cavity of the protein, as presented in figure 1. Characteristics of this cavity are: the charge score is -7, the polarity score is 22 and the volume is 920 Å<sup>3</sup>.

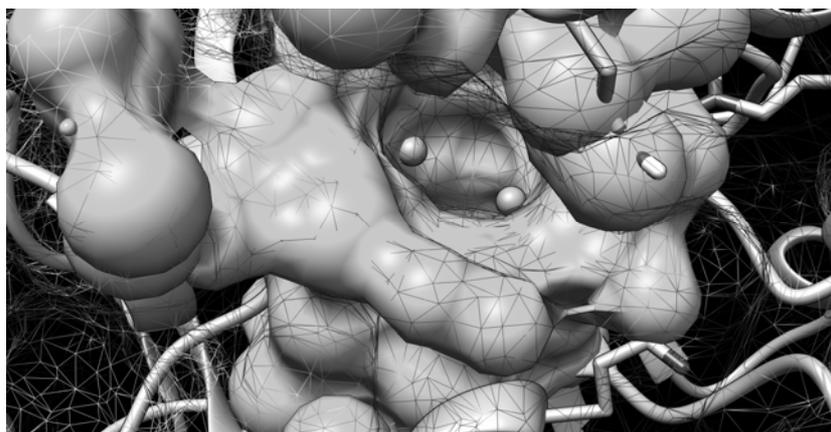


Fig. 1 – Focus on the cavity containing the active site of *Bacillus pasteurii* urease (solid surface) superposed to the 3D structure of the protein (mesh surface). The two spheres inside the cavity represent the nickel ions.

Characterization of the 3D structures of considered pesticides by comparison to the substrate urea and the known inhibitors reveals that pesticides are bigger than substrate and inhibitors (Table 1). Table 1 also contains the logP values both for the urea inhibitors (extracted from ChemSpider data base) and the pesticides (extracted from Pesticide Properties DataBase, <http://sitem.herts.ac.uk/aeru/ppdb/en/>). Urea and the known inhibitors of urease reflect a hydrophilic character. It is also true for the herbicide chlorsulfuron, while the pesticide difenoconazole reflect a pronounced hydrophobic character.

Table 1

Surfaces and volumes of investigated pesticides by comparison to urea and known inhibitors of *Bacillus pasteurii* urease

Molecule	Molecule role	Area (Å <sup>2</sup> )	Volume (Å <sup>3</sup> )	logP
urea	substrate	69.46	47.05	-2.11
beta-mercaptoethanol	known inhibitor	159.10	132.40	-0.23
diamidophosphate	known inhibitor	83.18	61.87	-2.03
citrate	known inhibitor	81.73	59.25	-1.72
difenoconazole	fungicide	325.00	330.70	4.92
nicosulfuron	herbicide	324.80	309.60	0.61
chlorsulfuron	herbicide	278.70	261.50	-0.99

The superposition of the three dimensional structures of native urease and those of its complexes with inhibitors reveals only small conformational changes that occur when the urease interacts with the inhibitors in good correlation with the ligands small dimensions: 0.181 Å<sup>2</sup> for the superposition of native urease to its complex diamidophosphate, 0.113 Å<sup>2</sup> for the superposition of native urease to its complex with beta-mercaptoethanol and 0.154 Å<sup>2</sup> for the superposition of native urease to its complex citrate respectively.

Utilization of SwissDock server for assessment of the putative interactions of pesticides with *Bacillus pasteurii* urease outcomes the binding modes characterized by the interaction energy expressed as FullFitness scores and solvation energy ( $\Delta G$ ), both presented in the Table 2. The values presented in Table 2 reveal high FullFitness scores and solvation energies for the interaction of both substrate and pesticides with *Bacillus pasteurii* urease meaning that all these molecules are able to bind to the protein (Figures 2).

Table 2

Interaction energies of pesticides with *Bacillus pasteurii* urease in comparison to the interaction energy with natural substrate (urea)

Substrate/pesticide	FullFitness (kcal/mol)	$\Delta G$ (kcal/mol)	Cluster rank
urea	-3866.45	-6.51	1
difenoconazole	-3750.12	-7.86	1
nicosulfuron	-3974.46	-8.61	1
chlorsulfuron	-4010.47	-7.90	1

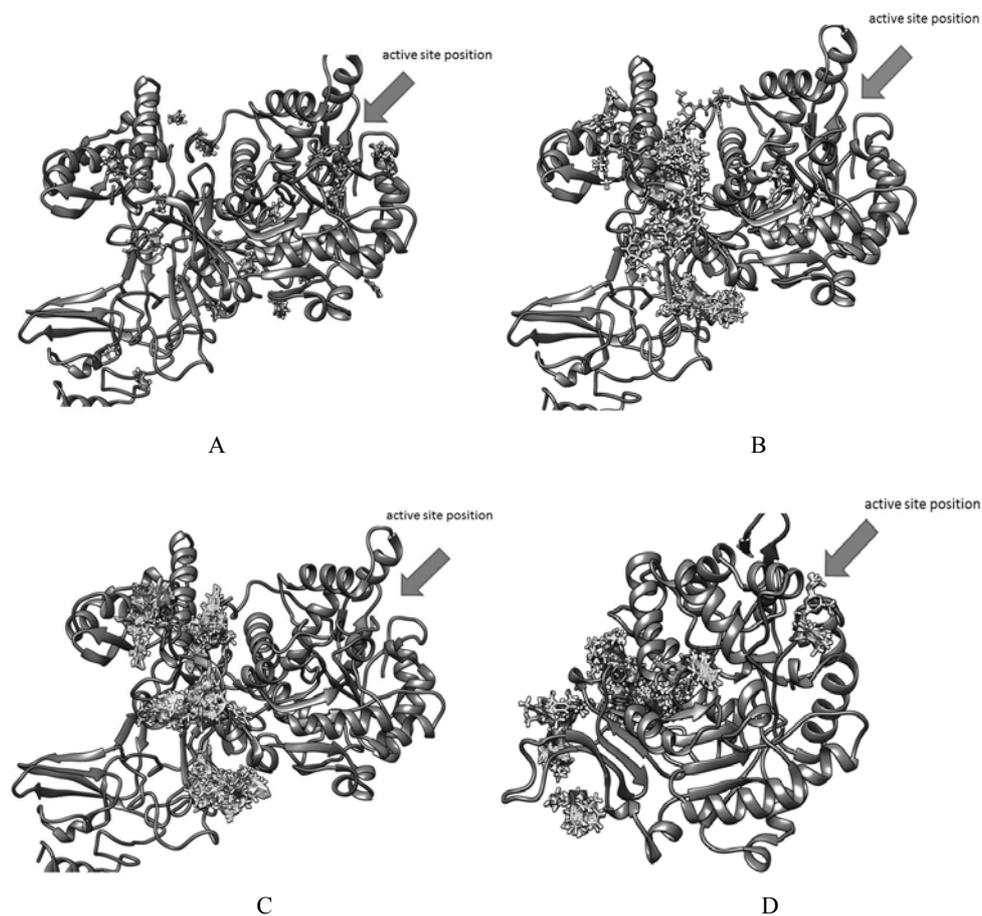


Fig. 2 – Ligands-urease interaction SwissDock profiles: urea-urease interaction profile (A), nicolsulfuron-urease interaction profile (B), difenoconazole-urease interaction profile (C), chlorsulfuron-urease interaction profile (D).

Analysis of the binding modes obtained for every molecule of ligand exposes that only urea and chlorsulfuron binds to the active site. Urea enters deeply in the active site and it is also true for a part of the chlorsulfuron molecule. It is illustrated in Figure 3 where we visualize the binding of urea (very deep, not visible), known inhibitors (black, dark gray and gray respectively) and chlorsulfuron (bigger and light grey) to the active site of *Bacillus pasteurii* using Chimera package.

A most detailed picture illustrating the chlorsulfuron binding to the active site of enzyme is provided in Figure 4.

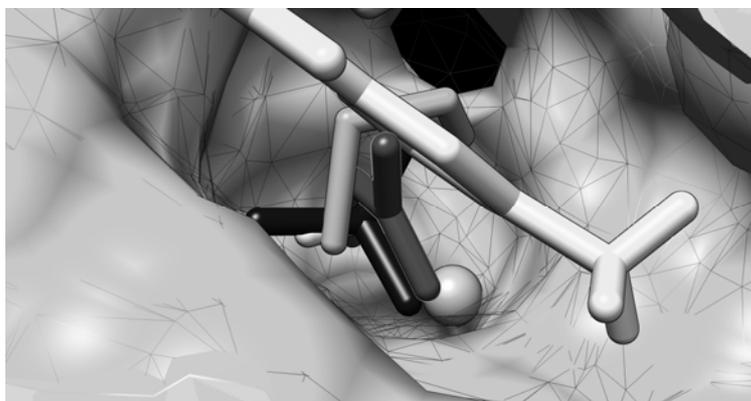


Fig. 3 – Focus on the binding of inhibitors (diamidophosphate - black, citrate -dark gray, β-mercaptoethanol – gray) and chlorsulfuron (light gray) to the active site of *Bacillus pasteurii* urease (solid light gray surface). The sphere inside the cavity represent the nickel ion.

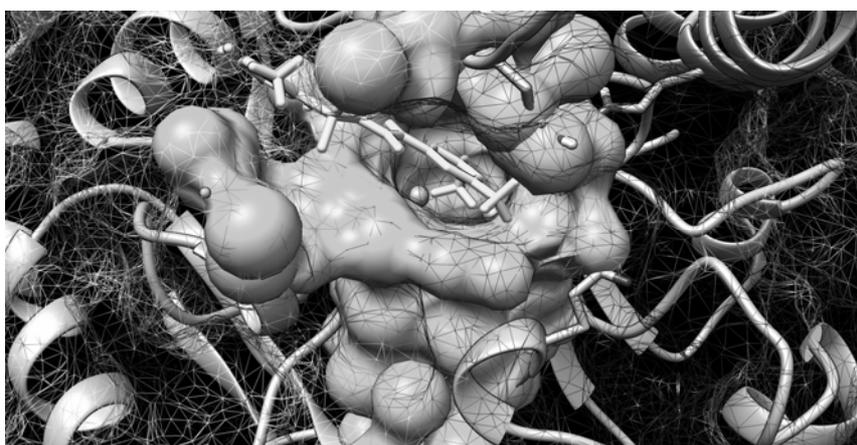


Fig. 4 – Detailed picture of chlorsulfuron (light gray, sticks) binding to the active site (solid light gray surface) of *Bacillus pasteurii* urease (mesh surface and cartoon representation).

It can be noticed that the  $H_3Cl$  group of atoms of the chlorsulfuron molecule enters deeply inside the active site of *Bacillus pasteurii* urease meaning that it may inhibit urease's activity. The *Coulombic surface colouring* facility under Chimera package allow to highlight the electrostatic nature of the interaction as the cavity surface emphasizes a negative electrostatic potential and the  $H_3Cl$  group of atoms belonging to chlorsulfuron reveals a positive electrostatic potential (data not shown as color pictures are not allowed).

Our group has experimentally proved that the presence of chlorsulfuron strongly affects the soil urease activity [14] and, as it is able to bind to the active site of urease we may conclude that it has inhibitory potential.

#### 4. CONCLUSIONS

The two herbicides, nicosulfuron and chlorsulfuron, and the fungicide difenoconazole are evaluated to strongly interact to *Bacillus pasteurii* urease, providing the capacity to bind to the enzyme in several places to its surface. More than it, chlorsulfuron is proficient to bind to the catalytic site of the enzyme as the known inhibitors do and the contribution electrostatic interaction to the complex formation is important. Urea and known inhibitors of urease have a hydrophilic character. It is also true for chlorsulfuron, but nicosulfuron and difenoconazole reflect a hydrophobic character. All these observations suggest that urease inhibitors must be hydrophilic.

Taking into account that the values of FullFitness and the solvation energies for the difenoconazole and nicosulfuron binding to urease are comparable with those obtained for chlorsulfuron binding, we may predict that nicosulfuron and difenoconazole also influence the soil urease activity. Further experimental studies are needed to evaluate these effects.

Our results are in good correlation with experimental data proving inhibitor or stimulator effects of some herbicides and fungicides to soil urease activity [12, 13, 15, 16, 14]. Moreover, it underlines that docking scoring approaches are useful for predicting enzyme-ligands interactions being also able to identify the binding region for every pesticide and to reflect the contribution of specific types of interaction to the binding.

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