CAPÍTULO 7

IN-SILICO DETOXIFICATION EVIDENCE OF THE HERBICIDE BISPYRIBAC SODIUM BY A TEORETHICAL MODEL OF GLUTATHIONE S-TRANSFERASE TAU 5 FROM *Oryza sativa* L.

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ABSTRACT: Rice (*Oryza sativa* L.) is one of the most important crops in the world, and it has being the primary source of nutritional layout in developing countries in Asian. Despite this remarkable importance, there are few studies about the development of techniques that minimizes the potential problems inherent of this grain cultivation, such as competition with weeds, making necessary the use of herbicides. The glutathione S-transferases (GSTs) superfamily confers to rice protection against biotic and abiotic stress, and herbicide resistance. However, the three-dimensional structure of a GST Tau class, is unsolved. The objectives of this work were to develop a reliable comparative model for the s-transferase glutathione class Tau 5 from rice, and simulate docking interactions, against herbicides bentazon and metsulfuron. Results showed that the predicted model is reliable and has structural quality. Ramachandran plot set 95,4% of the residues in the most favored regions. All complexes showed negative binding energies values; and bispyribac sodium docked to the glutathione tripeptide, and it represents an in-silico evidence of glutathione conjugation with this herbicide.

KEYWORDS: Molecular Modeling, Docking, OsGST, Herbicide.

1 I INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most importante crops in world, being 90% of the mundial rice cultivation arising from Asia, supplying 60% of the rice mundial demand (DOGARA; JUMARE, 2014). This means an importance against it impact on the agrobusiness, since its nutritional value consists in the primary source of nutritional layout in developing countries in Asian (DOGARA; JUMARE, 2014). According to FAO, and the International Institute of Rice Research (IRRI), rice needs more production to supply the alimentation demand,

since for the year 2050 it is estimated that the demand increases at least 60% in relation to 2016 production level (ORGANIZACIÓN DE LAS NACIONES UNIDAS PARA LA ALIMENTACIÓN Y LA AGRICULTURA, 2016). However, there is a few studies about development of techniques with the potential to minimize the problems inherent to the cultivation of this cereal, and competition with weeds, (SANTOS, 2006), without despising the damage caused by many herbicides.

Among detoxification enzymes, the most known and studied are glutathione transferases, which conjugates these xenobiotics and turn them into a water soluble complex (WILCE e PARKER, 1994; KREUZ, TOMMASINI, MARINOIA, 1992). This enzimes confers to rice catalitic action, and protection agains biotic and abiotic stress including herbicides resistence/tolerance (FROVA, 2003, 2006), and its main reaction consists in the conjugation of the tripeptide glutathione to a hydrophobic compound, making it more soluble and less (MARRS, 1996), maintaining cellular homeostasis. Tau class GSTs are extremely important as they are involved in the metabolization to a broad spectrum of important commercial herbicides (EDWARDS, DIXON, WALBOT, 2000). A study made by (Lajmanovich et al., 2013) suggest that Bispyribac Sodium has a correlation with Glutathion S-trasnferase expression. The aim of this study was to construct a theoretical model for a tau 5 *Oryza sativa* glutathione S-transferase (*OsGSTU5*) and perform docking simulations against Bispyribac Sodium herbicide.

2 I MATERIAL AND METHODS

2.1 Data mining

At first, the primare *OsGSTU5* (*Oryza sativa* L. glutathiona S-transferase tau 5) sequence were obtained in *.fasta* extension in NCBI data bank, a good quality structure model were searched for template using the “BLASTp” (Basic Local Alignment Search Tool for proteins - http://blast.ncbi.nlm.nih.gov/Blast.cgi) in *.pdb* extension from PDB (Protein Data Bank*, http://www.rcsb.org/)* data bank.

2.2 Homology modeling and model validation

The *OsGSTU5* primary sequence was obtained from NCBI database (https://www.ncbi.nlm.nih.gov/). The SWISS-MODEL server Automated Mode tool (http://swissmodel.expasy.org/) was used to generate the model (ARNOLD et al., 2006; KIEFER et al., 2009) The model validation process used the Ramachandran graph analysis through the PROCHECK program (LASKOWSKI et al., 1993) to verify the stereochemical quality of the structure. Local quality was accessed by ANOLEA (MELO, 1998) and GROMOS force fields (van GUNSTEREN et al., 1998). All the generated docked complexes were visualized with Visual Molecular Dynamics software (SURHONE et al., 2010).

2.3 Protein pockets identification, docking simulation and anchor residues

identification

The protein pockets identification where using the ghecom 1.0 finder server used to find multi-scale pockets on protein surfaces using mathematical morphology (KAWABATA 2007, 2010).

The as ligands structure (Table 1.0) were obtained from the ZINC database (http://zinc.docking.org/) in *.mol2* extension files. These files were converted to *.pdbqt* in Autodock 4.2.1 (MORRIS et al., 2009) (https://www.chpc.utah.edu/documentation/software/autodock.php), polar hydrogens were removed and their molecules were assigned with the Gasteiger parameters (GASTAIGER, 1980).

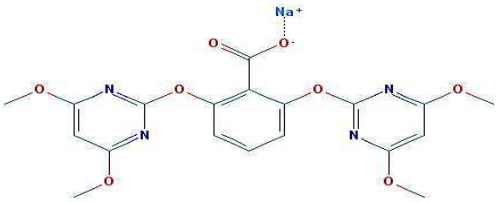
Name	Zinc ID	Class	HRAC	2D
Bispyribac sodium	ZINC 04098944	Ácido Pirimidiniloxibenzóico	B	

Table 1. Herbicide used in docking simulation

The *OsGSTU5* theoretical model, was converted to *.pdbqt* file in Autodock, hydrogens and Kollman parameters were added (WEINER et al., 1984). The GTX was treated as a cofactor. Docking simulations were run on the Autodock 1.5.6 program (MORRIS et al., 2009) and the Lamarckian genetic algorithm (LGA) whas choosen. The simulations had the following parameters: 10,000 replicates, energy analyzes per 1,500,000 and 27,000 generations, population size of 150 and mutation rates and crossing-over of 0.02 and 0.08 respectively. The 10 conformations were generated that were ranked based on the lowest energy and important residues interaction were analyzed in the VMD (SURHONE et al., 2010) (http://www.ks.uiuc.edu/Research/vmd/).

3 I RESULTS AND DISCUSSION

3.1 Data mining

The template used was a *crystal structure of a glutathione S-transferase PtGSTU30* from *Populus Trichocarpa,* in complex with GSH (PDB ID: 5J4U) presented 1.249 Å resolution (THOM et al., 2002), and high 55.09% identity value, revealing a homology between the *OsGSTU5* and *PtGSTU30* proteins appropriate for the modeling what was perceived by the results with other Tau class proteins used in researches (KILILI et al., 2004).

Target Protein		Template data				
Protein	ID	Protein	(PDB-ID)	Metod	Identy	Resolution Å
OsGSTU5	AAG32470.1	PtGSTU30	5j4u	XRD	55.09	1.249

Table 2. Target and template proteins data description

3.2 Sequences alignment and conserved regions identification

The Figure 1 shows conserved and semiconserved regions between *OsGSTU5* and PtGSTU30 (template) sequences (labeled), and some important anchor residue in the catalitic pocket HIS 51 (green arrow) other important residues like LYS53, LYS 111 and LYS 112 (red arrows) were not conserved residues.

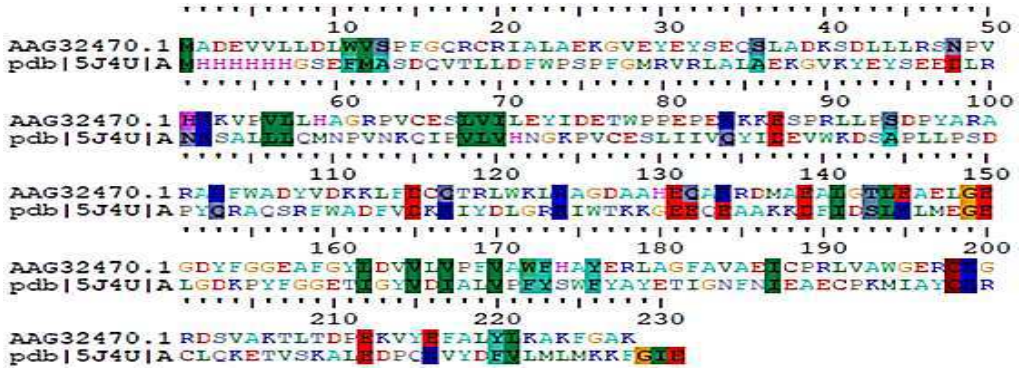


Figure 1. Sequences alignment, identification of conserved and semiconserved regions (labeled) and achor residues (arrows)

3.3 Theoretical model construction, quality evaluation and validation

According to Laskowski (1993, 2012), a reliable predicted model are supposed to display over 90% of residues in core regions of Ramachandran plot (A, B and L), for Ho (2005) some residues as glycine and proline has predictable and distinct distribution on the Ramachandran plot, as they present different stereochemical patterns. The stereochemical quality was accessed considering the Laskowski (2012) critters, the Ramachandran plot showed 95.4% of the residues (black squares and triangles) in regions that were more favorable (red), 4.1% in allowed regions (yellow), 0.5% in generously allowed regions (cream) and 0.0% in regions (white) as shown in Figure 2, a model validation results by Maia and Nadvorny (2014) had 100.0% of the residues in allowed regions.

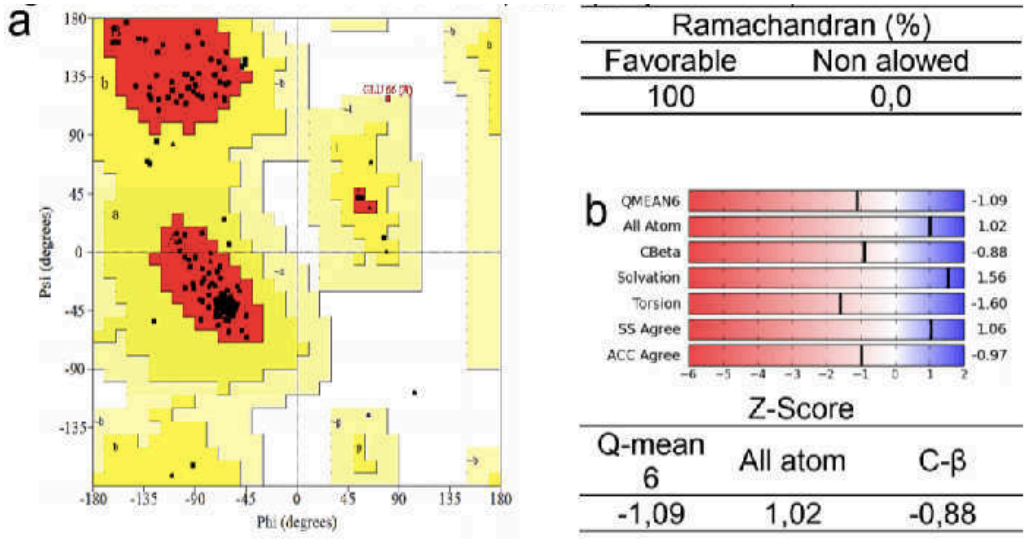


Figure 2. Model validation a. Ramachandran plot, b. quality mean for 6 paramethers.

Quality mean for 6 parameters Z-score (Qmean6) was between 1 and 2 and considered appropriated, meaning that the evaluation of the theoretical native structure protein in comparison with experimental models of the similar size (residues) in databanks had it stereochemical and atom parameters average considered good, showed by the red arrow/star situed in Figure 3.

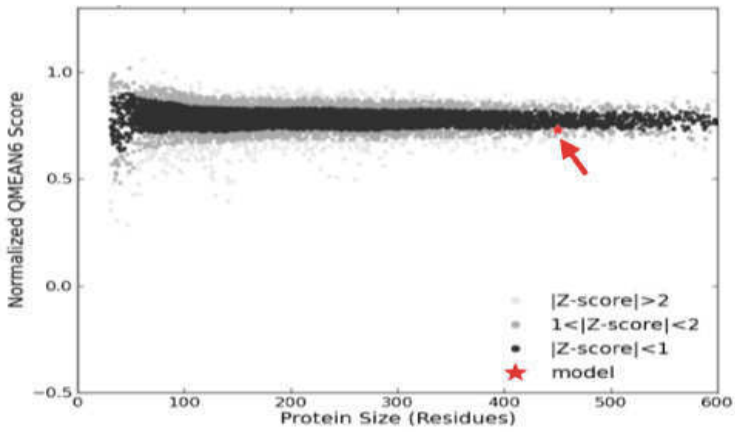


Figure 3. Theoretical model quality comparison among experimental models quality.

The results from ANOLEA (MELO, 1998), and for GROMOS (van GUNSTEREN; BERENDSEN, 1990), were generally negative values, revealing a model with stable energy, and negative values for majority of the residues, corroborating with the results showed by Hamid (2013), Maia and Nadvorny (2014). The generated and validated theoretical model had the atomic coordinate considerated satisfactory for it native structure and appropriated for Docking test.

3.4 Docking and anchor residues and protein pockets identification

The protein pockets were predicted (Figure 4. and Figure 4. A) by a multi-scale, mathematical morphology algorithm (KAWABATA 2007, 2010). The docking anchor

residues were compared throug the Ghecom results, and was perceived that they belonged to the best pocket (in red), the catalytic cleft (Figure 4.B), near the detoxifying subunit.

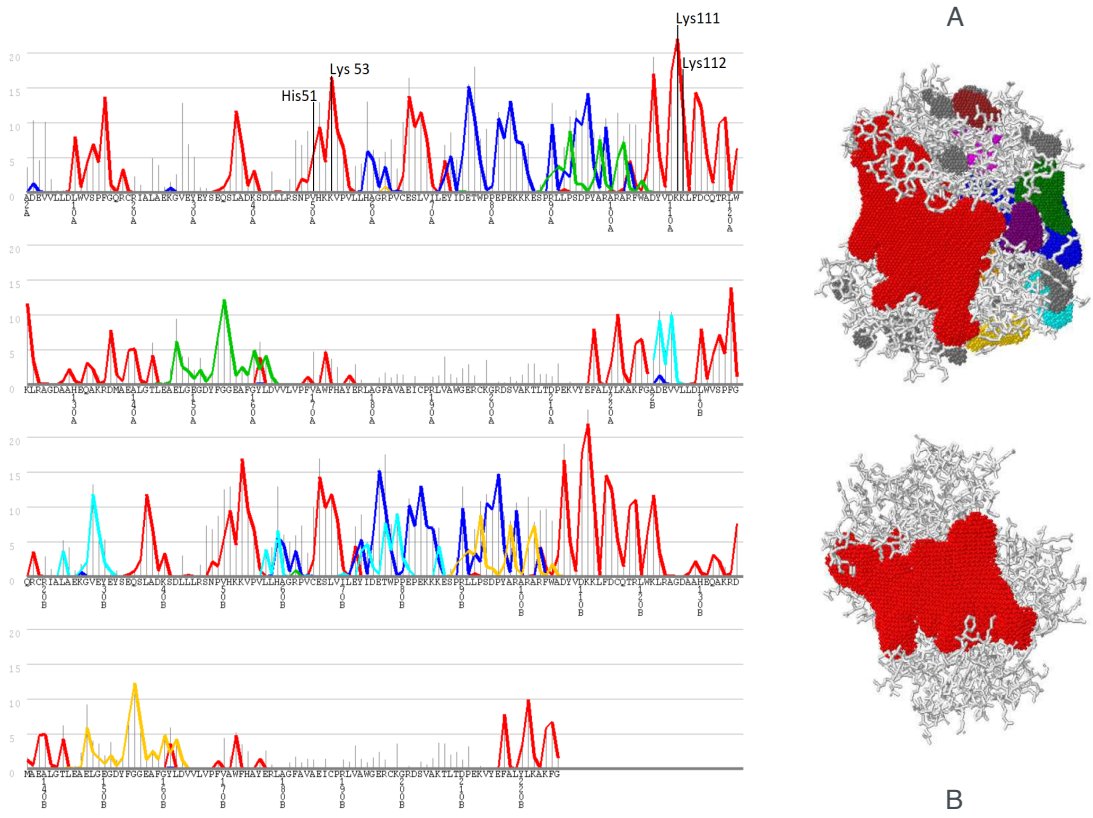


Figure 4. Pockets and aminoacids sequence description, and anchor resides described Figure4A All ranked pokets, Figure4 B Best pocket described (red)

The docking results for the *Bispyribac Sodium* "2,6-bis[(4,6-dimethoxypyrimidin-2-yl)oxy]benzoic acid", processed by the autodock program ranked ten possible complexes based on intermolecular energy scores, binding energy, and H-bonds that reveals atom (and residues) of the docked region binding of the ligand and the protein (MORRIS *et al*. 2009) that present favorable interation information of the model described in Table 3.

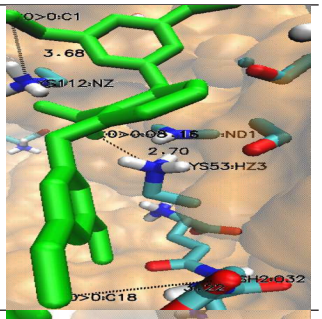
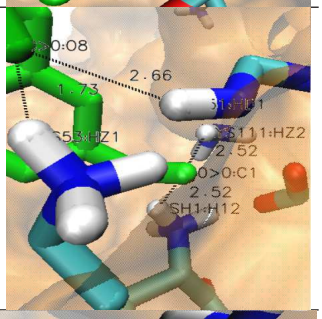
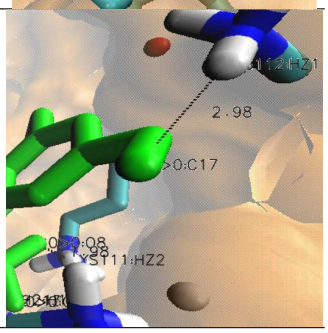
Bispyribac Sodium Herbicide “2,6-bis[(4,6-dimethoxypyrimidin-2-yl)oxy]benzoic acid”					
Protein	Ranking	Sistem Energy	Inhibition Constant (Ki)	Binding Atoms Ligand_Protein residue _distance Å	Image
U5	1	-4,71	354,22	<0>O:C1_ LYS112:NZ1_3.68 <0>O:O8_ LYS53:HZ3_2,70 <0>O:O8_ HIS51:ND1_3,16	
	2	-4,01	1,16	<0>O:O8_ HIS51:HD1_2,66 <0>O:O8_ LYS53:HZ1_1,73 **<0>O:C1_ GSH1:H12_2,52** <0>O:C1_ LYS111:HZ2_2,52	
	3	-3,62	2,21	<0>0:C17_ LYS112:HZ1_2,98 <0>0:O8_ LYS111:HZ2_1,98 **<0>0:O3_ GSH2:H11_2.13**	

Table 3. Best three dockings ranked by the autodock, with descriptions of the interaction.

For clarification the non GSH ligand atoms distribution on the protein topology is described in the Figure 5, and can be perceived theyr belonging to the catalitic cleft of the protein (in White).

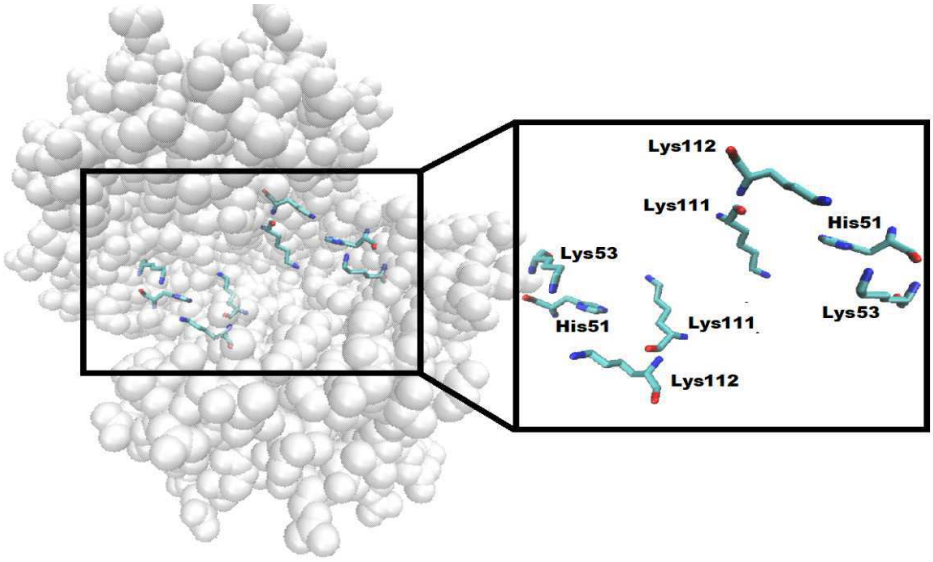


Figure 5. Description of anchor residues location.

The docking results for herbicide Bispyribac Sodium and *OsGSTU5* are displayed on the Table 3. and suggest that Lysins (LYS) situed on the catalitic pocket are importante residues for the interaction, representing anchor residues. The anchors residues seems to be three lysines at positions 53, 111 and 112 and a histidine at the 51 position, located on the catalytic H-site of the enzyme (Figure 5) and a GSH bound listed in Table 3, the results of the docking still reveals that the herbicide can dock to U5 protein, in the tripeptide glutathione (GSH) subunit showed in Figure 3, suggesting a possible detoxifying process (KILILI, 2004). It is probably that *OsGSTU5* metabolizes bispyribac sodium herbicide through GSH-conjugation (YAMAMOTO et al., 2012). A work done by Lajmanovich and Junges (2013) and another done by Lu (2013) showed a correlattion between Bispyribac Sodium and the superfamily of GST expression, its possible that *OsGSTU5* is one of the superfamily members that suffer the same expression effect indicating the correlation between the herbicide metsulfuron and *OsGSTU5*.

4 I CONCLUSIONS

The results lead to conclude that the theoretical model developed (OsGSTU5), presentes quality and is a representative native model suitable to the docking test. Our insights could be used appoint possible molecular markers, for futher marker-assisted selection tolerance/resistance to herbicide in plant studies.

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