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In Silico Site-Directed Mutagenesis of Ser^{11} and Lys^{107} on the Predicted 3D Structure of glutathione s-transferase from *Acidovoras sp.* KKS102

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ABSTRACT

Bacterial glutathione s-transferases (GSTs) are known to have variety of functions in detoxification processes. It is familiar that this detoxification ability is achieved through the attack of the thiolate form of glutathione on the electrophilic centres of toxic compounds. Indeed, cytosolic glutathione s-tranferase from *Acidovorax* sp. KKS102 is now known to have a dehalogenation function. However, little is known about the specific amino acids involved in this catalytic process. In this study, we investigated the effect of *in silico* site-directed mutagenesis of the evolutionarily conserved amino acids, Ser^{11} and Lys^{107}, on the theoretical 3D structure of GST from *Acidovorax* sp. KKS102 (GST-KKS102) using Deep View/Swiss-Pdb Viewer. The substitution of Ser^{11}, with aromatic amino acids, Tyr, Phe and Trp and positively charged amino acids, Arg, His and lys produced the greatest effect on the stability of the 3D structure of GST-KKS102. Indeed, at Lys^{107} position, substitution with nonpolar amino acids, Pro and Gly produced the highest structural stability effect on the theoretical 3D structure of the GST-KKS102. This *in silico* analysis suggests that Deep View rotamer scores could aid in planning *in vitro* site-directed mutagenesis studies in protein engineering.

Keywords: Glutathione s-transferase, *Acidovoras sp.* KKS102, Site-directed mutagenesis, 3D structure, Ser^{11}, Lys^{107}

INTRODUCTION

Glutathione s-transferases (GSTs) constitute a family of ubiquitous enzymes that have a vital role in detoxification processes on a wide range of exogenous and endogenous electrophilic substrates (Simarati *et al.,* 2016). It is familiar that GSTs catalyse the nucleophilic attack of the sulfohydryl group of glutathione (GSH) on the electrophilic centres of various xenobiotic and endobiotic compounds, making them more soluble and less toxic (Wilce and Parker, 1994; Edwina *et al.,* 2007). GSTs also show peroxidase and isomerase activities and are capable of binding several substrates non-catalytically (Mannervik *et al.,* 1988; Armstrong, 1997). GSTs are divided into at least three major families of proteins, namely cytosolic, mitochondrial and microsomal GSTs (Hayes *et al.,* 2005). Indeed, GSTs catalyze reductive dehalogenation (also termed thiolytic dehalogenation), in which a chlorine atom from the substrate is replaced by the thiol group from the reduced glutathione (Warner *et al.,* 2005; Fortin *et al.,* 2006).

A cytosolic GST of beta class from the biphenyl/polychlorobiphenyl degrading organism, *Acidovoras sp.* KKS102 (GST-KKS102) is recently known to have a dechlorination function on various organochlorine substrates (Shehu and Zazali, 2018a). A homolog of BphK (biphenyl upper pathway K), the gene of GST-KKS102 was named BphK-KKS. GST-KKS102 reacted towards 1 -chloro -2,4 dinitrobenzene (CDNB), ethacrynic acid, hydrogen peroxide and cumene hydroperoxide. *Acidovorax* sp. KKS102 is a biphenyl/polychlorobiphenyl degrading organism isolated from the soil near a refinery in Japan (Ohtsubo *et al.,* 2012). Database information suggests that *Acidovorax* sp. KKS102 contained at least eleven putative GSTs. These bacterial cytosolic GSTs are shown to be dimeric proteins with average molecular weight of 25,000 Da (Ohtsubo *et al.,* 2012). The dimeric protein is composed of two domains, the N-terminal and C-terminal domains. The GSH binding site (G site) is located in the N-terminal thioredoxin-like domain and the hydrophobic electrophilic substrate binding site (H site) in the C-terminal α-helix domain (Federici *et al.,* 2007; Allocati *et al.,* 2012).

Several mutagenesis studies have been carried out on bacterial GSTs in order to identify specific amino acids involved in structural stability and catalysis (Inoue *et al.,* 2000; Gilmartin *et al.,* 2005). Many site-directed mutagenesis studies have reported that both a conserved tyrosine residue in

class *alpha, mu, pi* and *sigma* GSTs and a serine residue in class *theta* GSTs have been identified in stabilizing the thiolate form of GSH for GST catalysis (Armstrong, 1997; Favaloro *et al.,* 1998; Wilce and Parker, 1994). Also the site-directed mutagenesis analysis on the functional role of Tyr12 in zeta-like GST from *Acidovoras sp.* KKS102 reported a remarkable alteration in substrate specificity of the enzyme (Shehu and Zazali, 2018a). Also Shehu and Zazali (2018b), reported the kinetic parameters of wild type and mutants BphK-KKS using CDNB and GSH as substrates, showing that mutation of Cys10 to Phe resulted in the alteration of substrate binding domain with 1.16 - and 1.51 - fold increase in the Km toward CDNB and GSH respectively, when compared with wild type.

In-silico side-directed mutagenesis is an essential tool for predicting the effect of mutation on the theoretical 3D structure and function of protein prior to the actual *in-vitro* side-directed mutagenesis. This could give a direction to the intended protein engineering work, particularly in searching for the amino acid residues that have potentiality in altering the structure and stability of the target protein entity. This study is aimed to investigate the *in silico* effect on substitution of the conserved amino acids, Ser^{11} and Lys^{107} on the predicted 3D structure of GST-KKS102 in an attempt to understand the effect of these amino acids on the protein's structural stability.

MATERIALS AND METHODS

Protein Homology Studies

The protein sequence homology analysis of cytosolic GST-KKS102 was carried out using European Molecular Biology Laboratory and European Bioinformatics Institute (EMBL-EBI) Clustal Omega Online Resources (https://www.ebi.ac.uk/Tools/services/web). Ten protein sequences, *Bphk*LB400, *Sphingomonas paucimobilis*, *Escherichia coli*, *Proteus mirabilis*, *Ralstonia sp.* B15, *Pseudomonas pseudoalcaligenes*, *Pseudomonas sp.* B4, *Paraburkholderia xenovoras* LB400, *Cyclolasticus oligotrophus* and *Sphingomonas sp.* P2 were used as templates for the sequence homology studies (Edwina *et al.,* 2007; Shehu and Zazali, 2018a). Both the target, GSTKKS102 and the templates protein sequences were loaded onto the alignment space bar and online protein sequence alignment was done.

3D Structure Prediction

The theoretical 3D structure of GST-KKS102, with a 202 amino acid residues was predicted using the SWISS-MODEL homology modelling alignment interface approach mode (Guex and Peitsch, 1997; Schwede *et al.,* 2003). Homology modelling determines structure based on the target sequence, GST-KKS102, possessing homology with template sequence in the structural database. *Bphk*LB400 (PDB file ABE37052.1), *Sphingomonas paucimobilis* (PDB file 1f2eB), *Escherichia coli* (PDB file 1a0fB), *Proteus mirabilis* (PDB file 1pmT), *Ralstonia sp.* B15 (PDB file Q9RAF0), *Pseudomonas pseudoalcaligenes* (PDB file Q52037), *Pseudomonas sp.* B4 (PDB file Q9RBS6), *Paraburkholderia xenovoras* LB400 (PDB file Q59721), *Cyclolasticus oligotrophus* (PDB file Q46153) and *Sphingomonas sp.* P2 (PDB file Q83VK4) were used as template sequences (Gilmartin *et al.,* 2005). The PDB file of the protein, GSTKKS102 was loaded onto the loading space bar provided by the online SWISS MODEL software system which perfectly modelled the GSTKKS102 3D model. The predicted theoretical 3D structure was viewed and manipulated using DeepView/Swiss-PdbViewer version 3.7 (SP5) (Guex and Peitsch, 1997). The protein data bank (PDB) template files were obtained from the National Center for Biotechnology Information (NCBI) protein data resources (www.ncbi.nlm.nih.gov).

In Silico Site-Directed Mutagenesis

The effect of *in silico* site-directed mutagenesis of Ser^{11} and lys^{107} on the predicted 3D structure of GST-KKS102 was carried out using the DeepView/Swiss-PdbViewer version 4.1 (SP5) molecular graphics program. The 3D model of GSTKKS102 was subjected to *in-silico* mutation by manipulating the protein model at the target sites. New amino acid side chains were selected by opting for the mutate tool in accordance with DeepView/Swiss-PdbViewer user guide protocols (Guex and Peitsch, 1997).

RESULTS AND DISCUSSION

Cytosolic GST-KKS102 possesses approximately above 40% sequence identity with all the templates ABE37052.1, 1f2eB, 1a0fB, 1pmT, Q9RAF0, Q52037, Q9RBS6, Q59721, Q46153 and Q83VK4 in the structural database (Table 1). In bacteria, one major criterion for classifying cytosolic GSTs is the use of percentage sequence similarity, which is generally agreed that proteins which shared 40% and above of the sequence similarity belongs to the same class, while those with less than 20% are classified into a different class (Brennan *et al.,* 2009). All the templates used in this study were beta class GSTs. Thus, going by the above criterion, cytosolic GST-KKS102 belongs to the same class with all the templates used.

Table 1: Percentage sequence similarity between various bacterial GST templates and cytosolic GST-KKS102

Template	% Sequence similarity with GST-KKS102
ABE3705.1	47.26
1F2EB	42.50
1A0FB	42.00
IPMT	44.28
Q9RAF0	44.28
Q52037	46.77
Q9RBS6	46.67
Q59721	47.26
Q46153	42.00
Q83VK4	42.50

The templates are in form of Protein Data Bank (PDB) designation files (NCBI, 2018)

The two evolutionarily conserved amino acids in cytosolic GST-KKS102, Ser^{11} and Lys^{107}, as shown in figures 1, which were selected in the present study, are located in the core region of the enzyme protein entity. Ser^{11} was selected due to its possible capacity to conserve network of hydrogen-bond interactions, mediated by the presence of a structural water molecule that links the Ser^{11} to other residues and possibly confers more stability and enhancement of catalytic efficiency as reported in *Ochrobactrum anthropi* glutathione transferase by Federici *et al.* (2007). Even though Ser^{11} is not involved in glutathione (GSH) binding, site-directed mutagenesis of this residue may either partially or fully disrupt the conserved network of hydrogen-bond interactions and consequently decrease catalytic efficiency. Lys^{107} was selected due to its presence in the cluster of amino acids that participate in the substrate binding. Various interactive forces including hydrogen bonding and hydrophobic interactions were predicted to bind the substrates to the G and H-site in GST-KKS102.

Figure1: Sequence homology of cytosolic GST-KKS102 and various GST templates, indicating evolutionarily conserved Ser^{11} (S11) and Lys^{107} (K107) residues shown in bars.

Ser^{11} and Lys^{107} of cytosolic GST-KKS102 were substituted using all the proteinogenic amino acids. Rotamer and score values were allocated for each substitution depending on how much disruption the new amino acid was predicted to affect the theoretical 3D structure of cytosolic GST-KKS102 (Tables 2 and 3). DeepView/Swiss-PdbViewer molecular graphics program selects the best rotamer or conformation of the new amino acid side chain from libraries of rotamers within the program. The best rotamer is defined as one that produces the lowest score indicating the least amount of effect on the structure of the protein and is based on minimization of energy. However, any rotamer with a high score of positive integer is likely to affect the 3D structure of the protein by affecting its energy minimization criteria.

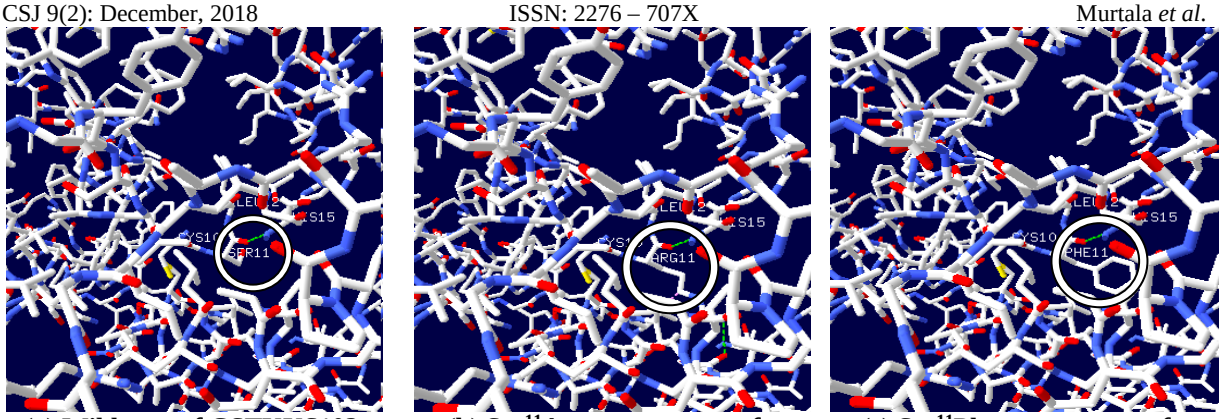
Table 2: Rotamer and score values of *in-silico* site-directed mutagenesis of Ser^{11}

Amino acid	Ser^{11} Mutation	Rotamer	Score
Alanine	Ala	1/1	-1
Arginine	Arg	15/26	+12
Asparagine	Asn	5/5	-2
Aspartic acid	Asp	3/4	+1
Cysteine	Cys	3/4	-3
Glutamine	Gln	4/14	+4
Glutamic acid	Glu	3/3	-3
Glycine	Gly	1/1	0
Histidine	His	5/6	+7
Isoleucine	Ile	4/5	+1
Leucine	Leu	2/4	+1
Lysine	Lys	15/16	+6
Methionine	Met	2/12	+2
Phenylanine	Phe	1/5	+15
Proline	Pro	1/2	-1
Serine	Ser	-	-
Threonine	Thr	2/2	-3
Tryptophan	Trp	1/6	+15
Tyrosine	Tyr	1/5	+28
Valine	Val	1/3	-3

Amino acid substitution with a positive score index shows great effect on the 3D structure of the protein

Ser^{11} is located at the N-terminal, within the outer loop of the core region in GSTKKS102 reflecting the polar nature of the amino acid. Of all the proteinogenic amino acid substitutions carried out on Ser^{11}, only Ser^{11}Arg, Ser^{11}Phe, Ser^{11}Trp and Ser^{11}Tyr (Table 2) were found to produce the greatest effect on the overall 3D structure of GSTKKS102, with rotamer score values of +12, +15, +15 and +28 respectively. Also the other amino acids that showed tendency to affect the 3D structure of GSTKKS102 protein were His, Lys, Gln, Asp, Ile and Leu with rotamer scores of +7, +6, +4, +1, +1 and +1 respectively. Here, we have discussed the four amino acids with highest rotamer scores and possibly greatest effect on the structure of GSTKKS102. Ser^{11} is involved in GSH stabilization by contributing to the formation of a conserved network of hydrogen-bond interactions, mediated by the presence of a structural water molecule that links Ser^{11} to Glu^{198} (Mannervik and Danielson, 1988; Federici *et al.*, 2007). The Ser^{11} to Arg substitution, Ser^{11}Arg (Figure 2) may likely interfere with integrity of the conserved network of hydrogen-bond interactions contributed by the Ser^{11} side chain.

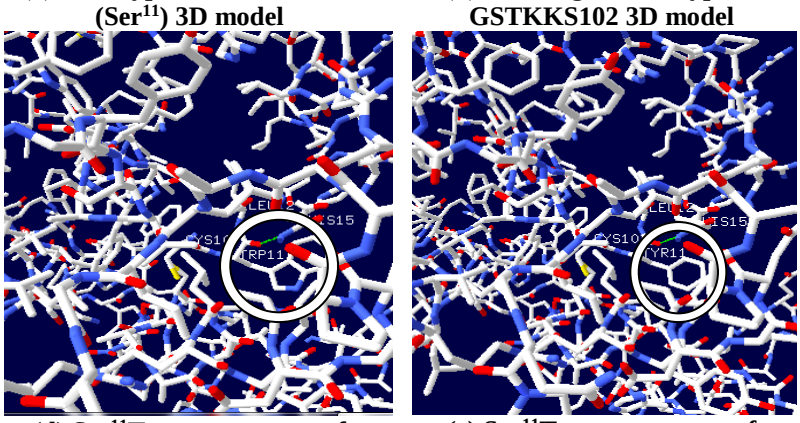
The aromatic amino acids (Phe, Trp and Tyr) substitutions of Ser^{11}; Ser^{11}Phe, Ser^{11}Trp and Ser^{11}Tyr (Figure 2) greatly affect the 3D structure of GSTKKS102 and likely affect the function of the protein. Since phenylalanine, tyrosine and tryptophan are large aromatic residues that are normally found buried in the interior of a protein and are important for protein stability (Federici *et al.*, 2007; Brennan *et al.*, 2009). Among the aromatic amino acid substitutions in GSTKKS102, Ser^{11}Tyr has special properties since the hydroxyl side chain of Tyr may function as a nucleophile or contribute to additional hydrogen bond interactions and greatly affect the 3D structure of the protein. However, the aromatic amino acid side chains are bulkier than the serine side chain. This may cause steric clashes and perhaps, create an effect that may distort the conformation of the 3D structure and perhaps, the function of GSTKKS102.



(a) Wild type of GSTKKS102 (Ser[11]) 3D model

(b) Ser[11]Arg mutant type of GSTKKS102 3D model

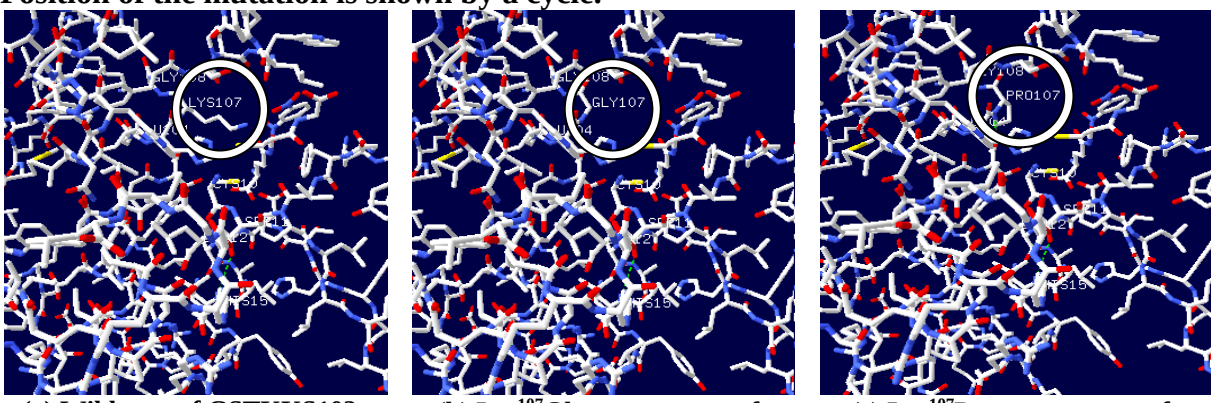
(c) Ser[11]Phe mutant type of GSTKKS102 3D model



(d) Ser[11]Trp mutant type of GSTKKS102 3D model

(e) Ser[11]Tyr mutant type of GSTKKS102 3D model

Figure 2: Wild type and Ser[11] substituted mutants of GSTKKS102 protein models. Position of the mutation is shown by a cycle.



(a) Wild type of GSTKKS102 (Lys[107]) 3D model

(b) Lys[107]Gly mutant type of GSTKKS102 3D model

(c) Lys[107]Pro mutant type of GSTKKS102 3D model

Figure 3: Wild type and Lys[107] substituted mutants of GSTKKS102 protein models. Position of the mutation is shown by a cycle.

Table 3: Rotamer and score values of *in-Silico* site-directed mutagenesis of Lys^{107}

Amino acid	Lys^{107} Mutation	Rotamer	Score
Alanine	Ala	1/1	-1
Arginine	Arg	6/26	-3
Asparagine	Asn	2/5	-2
Aspartic acid	Asp	1/4	-1
Cysteine	Cys	1/3	-2
Glutamine	Gln	8/14	-2
Glutamic acid	Glu	6/14	-2
Glycine	Gly	1/1	0
Histidine	His	1/6	-1
Isoleucine	Ile	1/5	-1
Leucine	Leu	1/4	-1
Lysine	Lys	-	-
Methionine	Met	9/12	-2
Phenylanine	Phe	1/5	-1
Proline	Pro	1/2	+3
Serine	Ser	1/3	-2
Threonine	Thr	2/2	-2
Tryptophan	Trp	1/6	-1
Tyrosine	Tyr	2/5	-2
Valine	Val	1/3	-1

Amino acid substitution with a positive score index shows great effect on the 3D structure of the protein

Lys^{107} is located in C-terminal of the α-helix of GSTKKS102 and was found to be conserved in all the ten templates used for the homology studies, indicating a possibility of critical role of this amino acid in the structure and function of the GST protein entity. Several blind molecular docking studies reported that the binding pocket of GSTKKS102 was found to be occupied by a cluster of amino acids including Lys^{107} which form various interactive forces, including hydrogen bonding and hydrophobic interactions that were predicted to bind the substrates to the G and H – sites (Shehu and Zazali, 2018b). Analysis of hydrogen bond interactions in 2 -chlorobenzoate, 3 - chlorobenzoate, and 4 -chlorobenzoate all predicted the presence of two hydrogen bonds between Lys^{107} and the ligand oxygen. Of all the proteinogenic amino acid substitutions, Lys107 with Gly (Lys^{107}Gly) and Pro (Lys^{107}Pro) showed great effect on the 3D structure of GSTKKS102 as indicated by the rotamer scores of 0 for Gly and +3 for Pro (Table 3).

Lys is positively charged amino acid capable of forming hydrogen bond-interaction with other ligands. Lys^{107}Gly substitution (Figure 3) may disrupt this interact since Gly lacks a positively charged side chain. This may interfere with predicted hydrogen bond interaction between Lys and the electrophilic substrate binding reported in GSTKKS102 (Shehu and Zazali, 2018b). Glycine is a non-polar aliphatic amino acid and was reported to introduce kinks into a chain (Gilmartin *et al.,* 2005).

Proline is a non-polar amino acid with unique structural properties such as turning points in β -sheets and structural disrupter of α-helices (Edwina *et al.,* 2007). Lys^{107}Pro substitution (Figure 3) may thus, affect the predicted 3D structure and function of GSTKKS102 as indicated by the positive score of +3.

CONCLUSION

Using the Swiss Model, DeepView/Swiss-PdbViewer molecular graphics program, the *in silico* side-directed mutagenesis of Ser^{11} and Lys^{107} by some aromatic, Ser^{11}Phe, Ser^{11}Trp and Ser^{11}Tyr, positively charged Ser^{11}Arg and nonpolar amino acids in Lys^{107}Pro and Lys^{107}Gly revealed higher positive rotamer score index which may produced great structural effects on the theoretical 3D structure and likely the function of GSTKKS102 protein.

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