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In silico Phylogenetics and Molecular Docking Studies of Rhodanese from Yeast (*Saccharomyces cerevisiae*)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DMS and OTL designed the study and OTL wrote the introduction and methodology while author THF performed the in silico analyses. All the authors interpreted the results. Authors DMS and SOS edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the *in silico* phylogenetics, binding energy and poses of rhodanese from Yeast (*Saccharomyces cerevisiae*) with known substrates and inhibitors.
Study Design: The three categories of ligands which include substrates, salts and effectors, were used against the phylogenetically conserved rhodanese from yeast.
Place and Duration of Study: The study was carried out at the Enzyme Biotechnology and Bioinformatics Unit, Department of Biochemistry, Federal University of Technology Akure, Nigeria. The research was carried out from January to March 2018.
Methodology: The properties of rhodanese from *S. cerevisiae* was evaluated using EMBOSS server, phylogeny was constructed through Blastp and ClustalO server, and molecular docking was carried out using AutoDock Tools and AutoDock Vina software.
Results: Evolutionary divergence was observed for two rhodanese (RDL1 and RDL2) from two

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gene loci of yeast, with different active site amino acid residues that showed deletion of ten amino acids residues during paralogue event, and optimum pH of 5 and 9 respectively.RDL2 was further analyzed based on the sequence length, phylogeny and the characteristic of the active site similar to previous experimental reports on rhodaneses. The binding energy obtained from molecular docking were -3.0, -5.2, -5.7, -4.2, -2.5, and -1.1 kcal/mol for thiosulphate, sodium metabisulphite, EDTA, ascorbic acid, $MgCl_2$ and ammonium persulphate respectively. However, it was observed that yeast rhodanese (RDL2) has three other binding domains apart from its active site, that functions in pre-catalysis, ionic sensitivity and acid-base sensitivity for other sulphur-containing substrates, salts and other agents respectively.

Conclusion: This study provides an insight to the mechanism of rhodanese by showing the presence of a critical catalytic cysteine residue and the relationship that possibly exists between the catalytic site and other allosteric sites, and that pre-catalysis occur for sulphur-containing substrates.

Keywords: Saccharomyces cerevisiae; rhodanese; thiosulfate cyanide sulfurtransferase; phylogenetics; molecular docking; in silico.

1. INTRODUCTION

Cyanide compounds are widely distributed on earth and are produced naturally by many organisms. They are synthesized by plants as a defense mechanism, secreted by fungi and bacteria as an antimicrobial compound [1,2,3], and synthesized by insects as a control over mating behavior [4]. Cyanide is cytotoxic and kills the cell by inhibiting cytochrome oxidase which functions in electron transport chain in the mitochondrial, and that leads to cellular suffocation[5]. Numerous numbers of plants were found containing cyanide glycoside [1]. Several attempts to develop biological processes for cyanide detoxification have been concentrated on cyanide-degrading enzymes obtained from animals, plants, fungi, and prokaryotes [6,7,8].

Yeast are unicellular fungi, characterized by a wide dispersion of natural habitats. Yeasts multiply as single cells that divide by budding such as *Saccharomyces*, or direct division (fission) such as *Schizosaccharomyces*, or they may grow as simple irregular filaments (mycelium). The whole genome of *Saccharomyces cerevisiae* is available [9], and for the past three decades, *Saccharomyces cerevisiae* has been the model system for majority of molecular genetic research because the basic cellular mechanics of replication, recombination, cell division and metabolism are generally conserved between yeast and larger eukaryotes, including mammals. However, duplication of large chromosomal fragments in *S. cerevisiae* occasionally results in the formation of supernumerary chromosomes that are highly unstable during mitosis [10,11] and gene duplication in a specie during evolution leads to paralogue.

Rhodanese, is an enzyme officially named as thiosulfate:cyanide sulfurtransferase (TST, EC 2.8.1.1), based on the reaction catalyzed in vitro. Rhodanese is in the mitochondrial, where it catalyses the detoxification of cyanide by sulphuration in a double displacement (ping pong) mechanistic reaction [12]. Two forms of rhodanese identified are dephospho – and phospho– rhodanese. These forms are with respect to their kinetic parameters, amino acid composition amino terminal amino acid, sulphahydryl content, tryptic maps and molecular weights [12].

Studies on the rhodanese from *Klebsiella edwardsii*, *Pseudomonas aeruginosa* and *Bacillus brevis* showed effective cyanide detoxification [13,14]. Existence of two isoforms of rhodanese was reported in *Arabidopsis thaliana* [15]. However, two isoforms of rhodanese were established in mitochondrial bovine liver and observed to use reduced thioredoxin as a sulphur-acceptable substrate [16]. Moreover, the mechanism of rhodanese activity is not fully known, and that serve as the basic aim of this work, to evaluate the *in silico* phylogenetics and molecular docking of rhodanese from *Saccharomyces cerevisiae* with known substrates and inhibitors.

2. MATERIALS AND METHODOLOGY

2.1 Phylogenetic Analysis

The sequence of rhodanese from two loci of yeast was obtained from saccharomyces genome database (www.yeastgenome.org/locus) with ID of RDL1 (S000005811) and RDL2 (S000005812). The sequence physicochemical statistics was obtained using EMBOSS Pepstat

at default setting. Local and global alignment of RDL1 and RDL2 sequence was done using EMBOSS Matcher and EMBOSS Stretcher respectively. The sequence of RDL2 was selected for further analysis. The homologs having minimum of 60% identity to RDL1 and RDL2 were obtained through Blastp of NCBI (www.blast.ncbi.nlm.nih.gov/Blast.cgi) and stored as FASTA format for further analysis. Multiple sequence alignment was carried out using ClustalO, at default setting, and phylogenetic tree was constructed. The real phylogeny was visualized at www.phylo.io using tree data obtained from Clustal O [17]. The evolutionary variance of RDL1 and RDL2 was also validated through prediction of binding site amino acid residues using 3D Ligand Site (http://www.sbg.bio.ic.ac.uk/3dligandsite/) [18].

2.2 Molecular Docking Studies

2.2.1 Protein preparation and identification of binding site residues

The tertiary structure of RDL2 was modeled separately using Swiss Model (https://swissmodel.expasy.org) [19]. Three templates with the high quality and identity were selected from Protein Data Bank (www.rcsb.org) and used to build the model structures of RDL2. The modeled structure of RDL2 with best quality was used as the target structure. The 3DLigandSitewas used to predict the active site of the three modeled structures of RDL2[18] while potential binding sites amino acid residues were noted for docking analysis.

2.2.2 Ligands preparation

The pose and binding energy of three different categories of ligands on rhodanese were evaluated. The three categories of ligands used were; the salts ($MgCl_2$, $CaCl_2$, $NiCl_2$, $MnCl_2$, and KCl), substrates (thiocystine, sodium thiosulphate, 2-mercaptoethanol, ammonium persulphate, and sodium metabisulphite), and effectors (EDTA, ascorbic acid, and urea). The structure of the ligand was obtained from Pub Chem database (https://pubchem.ncbi.nlm.nih.gov/compound/) in sdf format. All file conversions required for the docking study were performed using the open source chemical toolbox Open Babel version 2.4.1 (www.openbabel.org) [20]. All rotatable bonds present on the ligands were treated as non-rotatable to perform the rigid docking. The Gasteiger charge calculation method was used and partial charges were added to the ligand atoms prior to docking [21].

2.2.3 Grid box preparation and docking

Blind docking parameters with the grid size of 110, 110, and 110 along the three axes X, Y, and Z respectively, 0.375 Å spacing and the grid centre of 4.737 x 5.506 x 12.974 Å. were set by using AutoDock Tools (ADT), a free graphic user interface of MGL software packages (version 1.5.6) [22]. The grid box was generated that was enough to capture the entire protein's catalytic site and can accommodate all abovementioned ligands to move freely during docking. Other docking parameters were set to the software's default values. The molecular docking program AutoDock Vina (version 1.1.2) [23], was employed to perform the docking experiment from the Command Prompt. After docking, the ligands were ranked according to their binding energy and close interactions of binding of the target with the ligands were analyzed through Auto Dock Tools.

3. RESULTS AND DISCUSSION

The rhodanese has nearly the same sequence length but buffers in sequence identity and similarity. They possessed different isoelectric point and will give different fluorescence peak in spectrophotometer as shown in Tables 1 and 2. RDL1 predicted binding site consist of Glu51, Cys98, Ala99, Ser100, Lys102, Arg103 and Asn127, while that of RDL2 consists of Glu59, Tyr63, Cys106, Ala107, Lys108, Gly109, Val110, Arg111 and Thr135. Based on the sequence length, the variation in the active site showed that eight amino acids residues and two amino acids residues were deleted from N-terminal and C-terminal of RDL1 respectively during gene duplication process. RDL2 of *S. cerevisiae* showed close homology to those from *Saccharomyces eubayanus* (XP_018219484.1), *Saccharomyces cerevisiae x Saccharomyces kudriavzevii* VIN7 (EHN00117.1), *Saccharomyces arboricola* H-6 (EJS41604.1), *Saccharomyces kudriavzevii* IFO 1802 (EJT43242.1), *Lachancea quebecensis* (CUS20326.1), *Lachancea thermotolerans* CBS 6340 (XP_002553271.1), but diverged from *Lachancea fermentati* (SCW01750.1), *Kazachstania naganishii* CBS 8797 (XP_022465600.1), and *Zygosaccharomyces bailii* ISA1307 (CDH15677.1) as shown in Fig. 1.

Table 1. Theoretical physicochemical properties of rhodanese RDL1 and RDL2 of *Saccharomyces cerevisae*

Properties	RDL1	RDL2
Residues	139	149
Molecular weight	15413.27	16697.38
Average residue weight	110.887	112.063
Charge	-0.5	11.0
Isoelectric point	6.3280	10.2594
A_{280} Molar extinction coefficients	25440 (reduced) 25440 (cystine bridges)	12950 (reduced) 12950 (cystine bridges)
A_{280} Extinction Coefficients 1 mg/ml	1.651 (reduced), 1.651 (cystine bridges)	0.776 (reduced), 0.776 (cystine bridges)
Improbability of expression in inclusion bodies	0.842	0.691

Table 2. Local and global alignment of rhodanese RDL1 and RDL2

Alignment properties	EMBOSS matcher	EMBOSS stretcher
Matrix	EBLOSUM62	EBLOSUM62
Gap penalty	14	12
Extend penalty	4	2
Length	110	150
Identity	47/110 (42.7%)	53/150 (35.3%)
Similarity	66/110 (60.0%)	77/150 (51.3%)
Gaps	0/110 (0.0%)	10/150 (6.7%)
Score	248	210

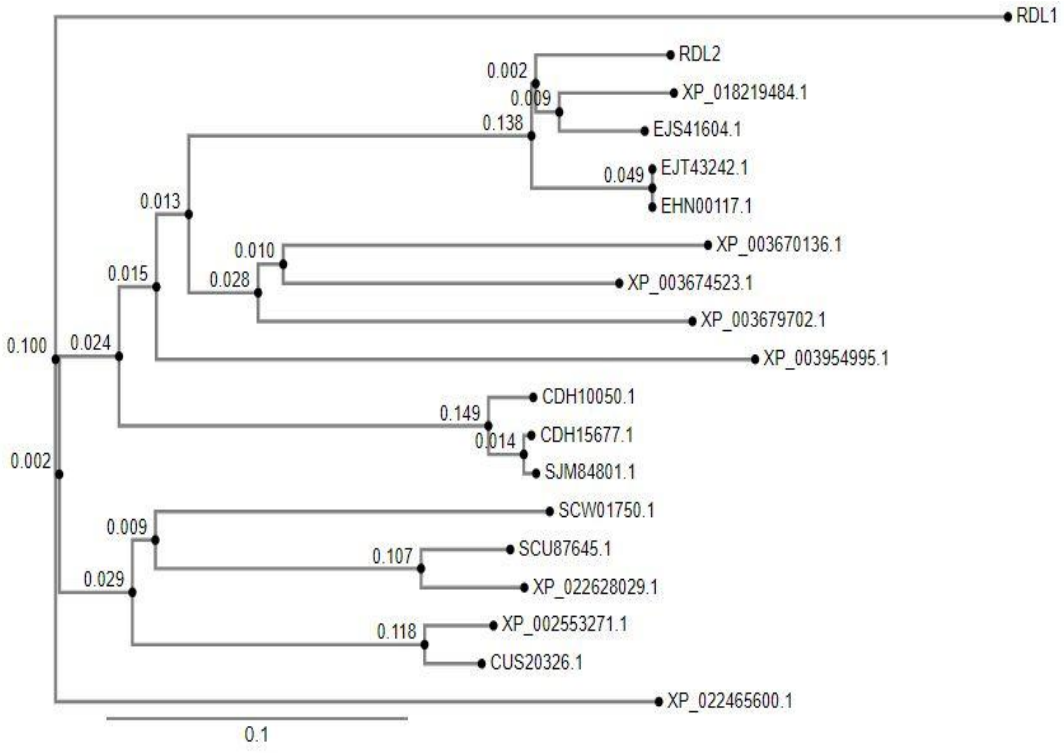

Fig. 1. Phylogenetic tree of rhodanese (RDL1 and RDL2) of *Saccharomyces cerevisiae*
The numbers indicate the branch length from the closest node, calculated by neighbor joining method.

RDL2 was further analyzed based on the sequence length and phylogeny. Rhodanese RDL2 of yeast is expected to have low thermal stability. An optima pH within pH 5 for RDL1 and pH 9 for RDL2 is expected based on the theoretical isoelectric point shown in Table 1. The variation in the pH could have possibly resulted from deletion of eight amino acid residues that occurred at the N-terminal of RDL1. However, an optimum pH and optimum temperature of pH 9.0 and 35°C for rhodanese from the liver of fruit Bat (*Eidolon helvum*) [24], pH 8.0 and 50°C for rhodanese from the hepatopancreas of *Limicolaria flammea* [7] and pH 6.0 and 60°C for rhodanese from *Klebsiella edwardsii* [14] Schlesinger and Westley [25] reported pK values of 5.4 and 9.9 for rhodanese from bovine liver, and that its activation enthalpy was very small while the activation entropy was large and negative.

The model structures of rhodanese RDL2 have above average global model quality estimation (GMQE) with highest equals to 1, and the high quality in these models was from the template protein of 3d1p.1A which has the least negative QMEAN score as shown in Table 3 and Fig. 2. The stronger the negative QMEAN the more deviated the model from the experimental structures of similar size. The binding site prediction for the RDL2 models were similar and include cysteine residue which is very essential for catalytic function.

The molecular docking scores were calculated as the predicted binding free energies in kcal/mol (Table 4). The lowest binding free energy (i.e. best docking score) indicated the highest ligand/protein affinity. The interactions between the active site and other sites nearby amino acid residues the modelled RDL2 structure for each ligand was also reported. Among the ligands, thiosulphate with -3.0 kcal/mol proved to be the most natural substrate for rhodanese by its interaction with amino acid residues that matched the predicted active site of RDL2 consisting of Lys108, Val110 and Arg111 (Table 4).

According to Cipollone et al. [26], only the C-terminal domain of rhodanese carries the seven or six-amino acid active site loop hosting at the first position the Cys residue involved in the catalytic process while the counterpart of the Cys residue in the N-terminal domain is an Asp residue which has no involvement in catalysis. This active site loop has been found also in the yeast arsenic-resistance protein Acr2, yeast protein YG4E, and rhodanese-like proteins from *mycobacterium tuberculosis* and *Streptomyces peucetis* [27]. Thiosulphate thus serve as the reference ligand for categorizing the impact of other ligands in this study. Thiosulfate has been reported having good catalytic parameter (K_m and V_{max}) as a substrate for rhodanese with single domain, between pH 8.5 and 9.0 and between 20.0°C and 25.0°C [26].

It can also be observed from the result shown in Table 4, that there are other regulatory sites or cavities on the rhodanese which may account for relatively high pH stability and ionic activation. However, despite the report on thiocystine (bis-[2-amino-2-carboxyethyl] trisulfide) as a natural substrate for rhodanese [28,29], the results of this study showed that it binds to a site different from that of thiosulfate. This may indicate the possibility of pre-catalysis reaction by rhodanese on sulphur-containing substrates aside thiosulphate, which also corroborate the earlier report [25].

The catalytic rhodanese domain is often combined with other characterized protein domains. The involvement of a rhodanese domain in a given process seems to be related to the functional properties of the accompanying domain [26]. The cationic site has been reported present in the rhodanese of bovine liver, and that binding at this site by ligands decreases the enzyme-substrate interaction [25]. The impact of the ligand binding to the regulatory site will depend on its effect on the active site conformation. Thus, it may be expected that ascorbic acid, $NiCl_2$, $MgCl_2$, $CaCl_2$, $MnCl_2$, and Urea will have inhibitory effect while EDTA, ammonium persulphate and KCl will have activating effect due to their interaction with proline residue, thereby provide structural stability to the enzyme. Although, previous experimental report showed that cations such as K^+, Ni^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, Ca^{2+}, and Co^{2+}did not significantly affect the activity of the rhodanese from the liver of fruit Bat [24], *T. cucumerina* mesocarp [8] and *Klebsiella edwardsii* [14] but rhodanese from the seed of *T. cucumerina* was inhibited by the metal ions [8]. The variation observed in rhodaneses of *T. cucumerina* may be due to differences in the amino acid composition because of functional requirement at different parts of plant.

Table 3. Protein structure and binding site

RDL2 models	Structural parameters					Predicted binding site residues
	Template (PDB ID)	Sequence identity (%)	Sequence similarity	GMQE	QMEAN	
A	3d1p.1A	42.73	0.42	0.59	-0.27	Glu59, Cys106, Ala107, Lys108, Val110, Arg111, Lys113, Thr135
B	6bev.1.A	38.86	0.38	0.53	-2.09	Arg55, Glu59, Cys106, Ala107, Lys108, Arg111, Thr135
C	1dp2.1A	25.86	0.31	0.53	-2.44	Glu59, Ala107, Lys108, Val110, Arg111, Lys113, Thr135

Table 4. Predicted binding free energies (docking scores) and detailed interactions observed between the ligands and the target enzyme

Ligands	PubChem CID	Predicted binding energy (kcal/mol)	Interaction with amino acid residues	Site functions
Thiosulphate	1084	- 3.0	Lys108, Val110, Arg111	Catalytic, natural substrate region
Sodium metabisulphite	656671	- 5.2	Pro45, Asn48, Lys49, Leu54, Thr67	Pre-catalysis, other substrates region
Sodium thiosulphate	24477	- 4.1	Leu41, Val42, Asn48, Lys49, Leu51	
Thiocystine [Bis (2-amino-2-carboxyethyl) trisulfide]	167019	- 3.9	Asn46, Lys49, Leu50, Leu51, Thr67, Ile69	
2-Mercaptoethanol	1567	- 2.5	Asn46, Lys49	
EDTA	6049	- 5.7	Val42, Pro45, Asn46, Lys49, Thr67	Regulatory, acidic - alkaline sensitive region
Ascorbic acid	54670067	- 4.2	Asp47, Lys49, Leu50, Leu51, Lys100	
Urea	1176	- 2.8	Asn48, Lys49	
Ammonium persulphate	16211170	- 1.1	Glu84, Pro96	
Magnesium chloride	5360315	- 2.5	Val38, Leu51, Met65, Ile103, Tyr130	Regulatory, ionic sensitive region
Manganese chloride	24480	- 2.5	Val38, Leu51, Met65, Ile103, Tyr130	
Nickel chloride	24385	- 2.5	Val38, Leu51, Met65, Ile103, Tyr130	
Calcium chloride	5284359	- 2.5	Val38, Leu51, Met65, Ile103, Tyr130	
Potassium chloride	4873	- 1.8	Ala76, Pro77, Gly78, Ala79, Leu80, Leu118	

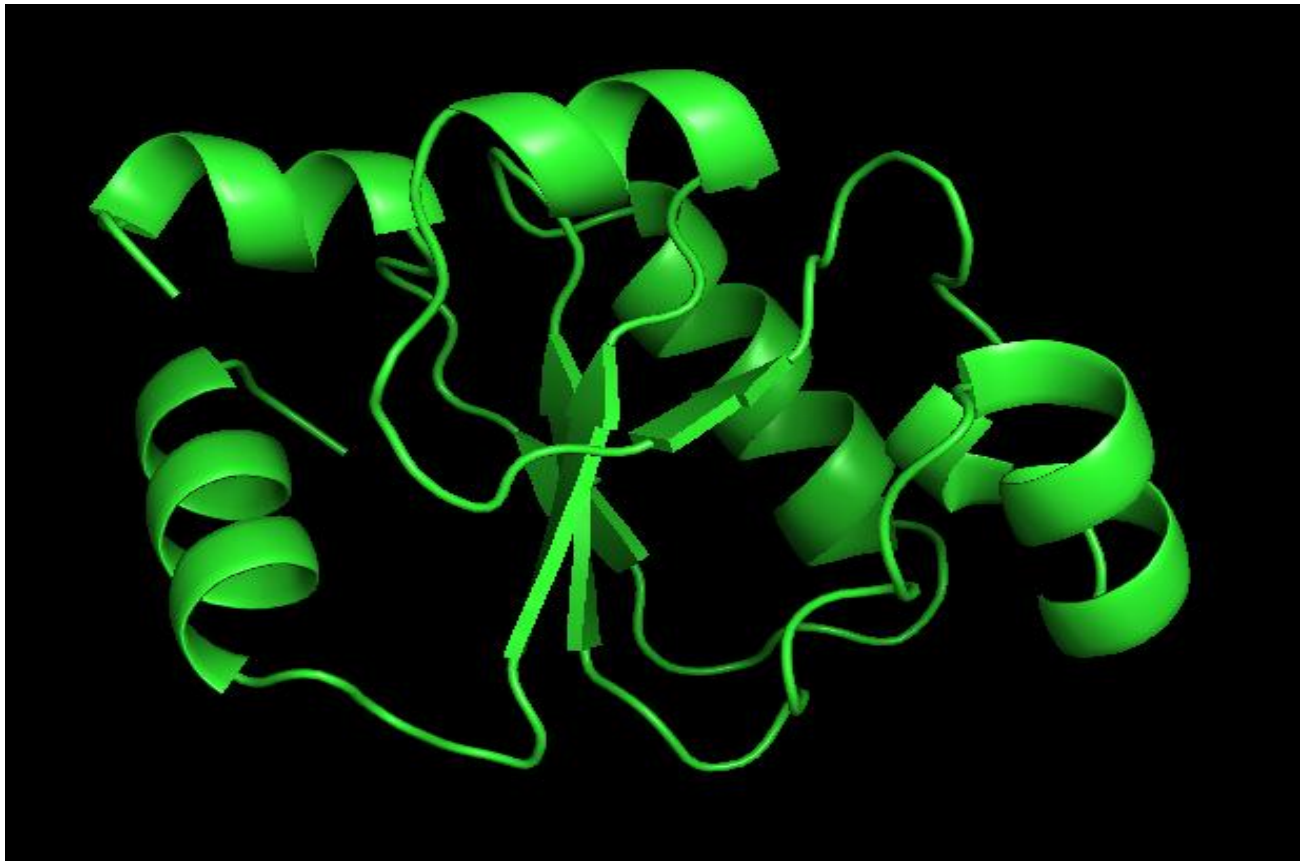

Fig. 2. A modeled structure of Rhodanese RDL2 of *Saccharomyces cerevisiae,* visualized by PyMOL (version 2.0.7)

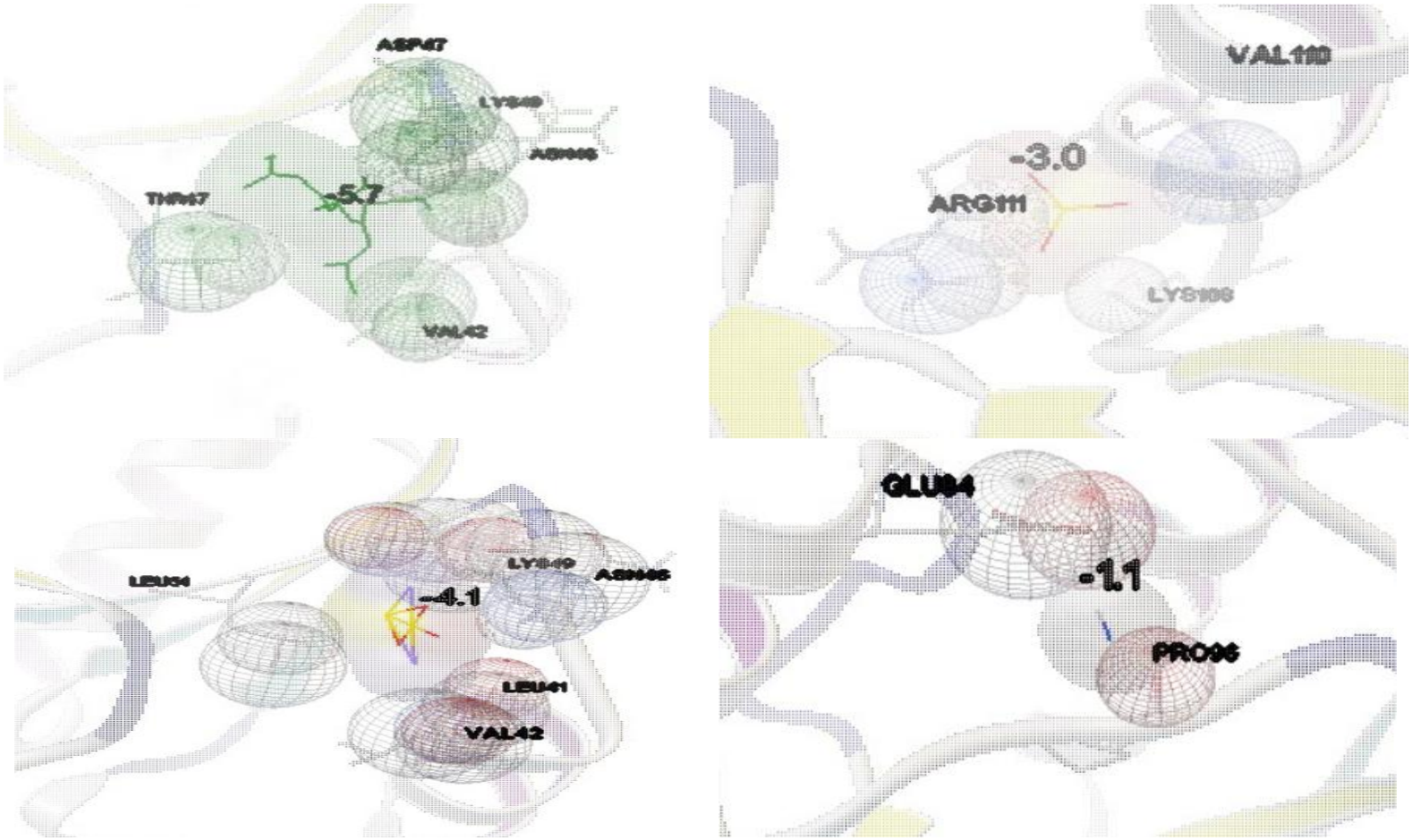

Fig. 3. Molecular interactions between the *S. cerevisiae* (Yeast) rhodanese RDL2 and EDTA (-5.7), thiosulphate (-3.0), Sodium thiosulphate (-4.1) and ammonium persulphate (-1.1), generated by AutoDock Tools

4. CONCLUSION

Knowledge on the molecular interactions of rhodanese with array of substrates and salts is potentially useful tool for the understanding of its mechanism of action, for biotechnological applications which include cellular detoxification and environmental bioremediation and degradation. This *in silico* study thus provides an insight to the mechanism of rhodanese by showing the relationship that possibly exists between the catalytic site and other allosteric sites, and that pre-catalysis occur for sulphur-containing substrates to ensures cleavage and proper orientation of the sulphur group to the catalytic center for the formation of enzyme-substrate complex.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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