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Structural Analysis of the Mode of Interactions of SoxB Protein with SoxYZ Complex from *Allochromatium vinosum* in the Global Sulfur Oxidation Cycle

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Abstract Microbial redox reaction is a very essential reaction to maintain for the recycling of sulphur to maintain the environmental sulphur balance. These oxidation process is conducted by a large number of phylogenitically diversed sulphur oxidizing bacteria. The sox gene cluster of α-proteobacteria, *Allochromacium vinosum* (*A.vinosum* or *A.vino*) are mainly responsible for microbial redox reaction. The main proteins of this process are SoxY, SoxZ and SoxB. SoxY binds to sulfur anions with the help of SoxZ. SoxB is a heterodimeric protein, which then hydrolytically releases one molecule of sulfate to yield a SoxY-persulfide. In the present work, homology modeling has been used to build the three dimensional structures of SoxY, SoxZ. Due to large sequence length only 5'-nucleotidase C-terminal domain of SoxB has been modelled by homology modeling. With the help of protein-protein docking complex structure of SoxYZB is formed and using Protein interaction calculator (P.I.C) webserver the amino acid residues of these proteins involved in the interactions have been identified. The interactions between the SoxY, SoxZ and 5'-nucleotidase, C-terminal domain of SoxB proteins are mediated mainly through hydrogen bonding. The probable biophysical mechanism of SoxB interaction with SoxYZ complex has been identified.

Keywords Docking simulations; Environmental sulphur balance; Homology modelling; Sox operon; Sulphur oxidation

Background

Oxidation-reduction reaction is an important mechanism to maintain natural sulphur cycle. The thiosulphate , tetrathionate ,sulphide are the major sources of sulphur for oxidation-reduction reaction. Thiosulphate is an environmentally abundant sulphur compound which fulfils an important role to maintain environmental sulphur balance. Thiosulphate oxidation is mainly governed by multienzyme complex (Sox) which is present in phylogenetically diverse set of microorganism (Meyer et al., 2007; Freidrich et al., 2005). However the bio-molecular mechanism of the sulphur oxidation process by the Sox multi-enzyme complex is not well understood.

Allochromatium vinosum (*A.vino*) is a α-proteobacteria accumulates water insoluble sulphur inside the periplasm during the oxidation of reduced sulphur compounds such as thiosulfate or sulphide. Three periplasmic Sox proteins encoded by the *SoxB*, *SoxXAK*, and *SoxYZ* genes are mainly responsible thiosulphate oxidation in the *A.vino*. The molecular mechanism proposed for the oxidation of thiosulfate in *A.vino* reveals that thiosulfate gets coupled to the carboxy terminal cysteine residue of SoxY bound to SoxZ and the whole process is facilitated by SoxXA. SoxB then comes into play and cleaves the SoxY-thiosulfate hydrolytically adduct to release a molecule of sulphate (Bagchi, 2012). It was documented that SoxB of *A.vino* reacted productively with SoxYZ complex (Welte et al., 2009). However the details of interactions between SoxB and SoxYZ protein complex at the residue level are yet to be explored.

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In the present study, the three dimensional structures of SoxY, SoxZ and 5'-nucleotidase C-terminal domain of SoxB from *A.vino* obtained by homology modeling have been described. Molecular docking simulations have been performed in order to find out the possible modes of binding of these proteins. Binding sites of SoxY, SoxZ and SoxB have been predicted and analyzed. These studies provide a detailed structural view of the acceptable residue level interaction of these proteins in the global sulfur oxidation reaction cycle.

1 Results

1.1 Description of the structure of SoxY

The modelled structure of SoxY is a 111 amino acid residue long protein. The predicted structure is very similar to the structure of the Sulfur Carrier Protein SoxY from *Chlorobium Limicola F Thiosulfatophilum* (PDB code: 2NNC A chain for SoxY). The most of the structure is made up of β strand and coil regions (7~12, 23~26, 37~44, 51~56, 64~68, 78~83, 91~94 and 107~111 positions are mainly β strand structure). The rest of the portion is helical structure interspersed with coil regions. The structure is presented in Figure 1.

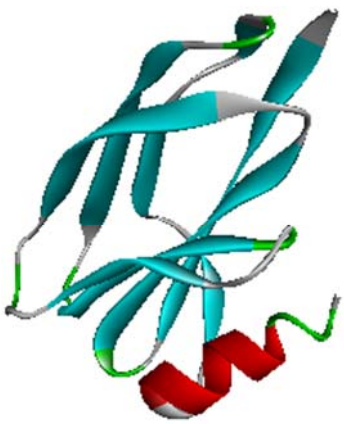

Figure 1 Model structure of SoxY protein from *A.Vinosum*
Note: With distinct secondary structure showing as α-helix, β sheet and random coil

1.2 Description of the structure of SoxZ

The modelled structure of SoxZ is a 104 amino acid residue long protein. The predicted structure is very similar to the structure of the SoxZ protein from the SoxYZ complex from *Paracoccus denitrificans* (PDB code: 2OXG; Z chain for SoxZ). The protein is mainly composed of β strand and coil structure. The structure is presented in Figure 2.

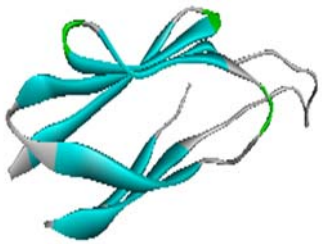

Figure 2 Model structure of SoxZ protein from *A.Vinosum*
Note: With distinct secondary structure showing as α-helix, β sheet and random coil

1.3 Description of the structure of C-terminal domain of SoxB

The modelled protein structure of 5'-nucleotidase, C-terminal domain of SoxB is a 138 amino acid residue long protein. The predicted structure is very similar to the structure of the SoxB.

Protein of Termus Thermophilus Sulfate Thiohydrolase (PDB code: 2WDC; A chain for Sox B domain).The residues show conformational adaptability towards helix, β strand and coil conformations. There are four helical and five βstrand regions in the modeled protein structure (14~24, 63~68, 71~81,114~122 as helical structure and 26~31, 36~40, 46~50, 95~99 and 133~136 as β strand structure). The helical and β strand regions mainly interspersed with coil regions. The structure is presented in Figure 3.

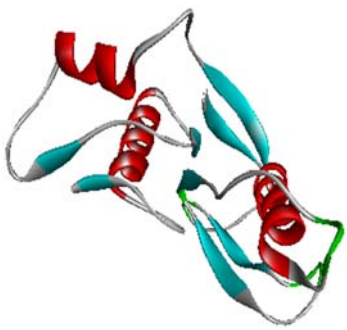

Figure 3 Model structure 5'-nucleotidase, C-terminal domain of SoxB protein from *A.Vinosum*
Note: With distinct secondary structure showing as α-helix, β sheet and random coil

Table 1 Protein-protein side chain-side chain hydrogen bonds

POS	CHAIN	RES	ATOM	POS	CHAIN	RES	ATOM
41	Y	THR	OG1	49	X	GLU	OE1
81	Y	ARG	NH1	50	X	HIS	NE2
81	Y	ARG	NH1	50	X	HIS	NE2
81	Y	ARG	NH2	50	X	HIS	NE2
81	Y	ARG	NH2	50	X	HIS	NE2
81	Y	ARG	NH2	53	X	ASP	OD1
81	Y	ARG	NH2	53	X	ASP	OD1
81	Y	ARG	NH2	53	X	ASP	OD2
81	Y	ARG	NH2	53	X	ASP	OD2
18	Z	LYS	NZ	68	Y	GLU	OE1
18	Z	LYS	NZ	68	Y	GLU	OE2
62	Z	SER	OG	53	X	ASP	OD1

1.4 Interaction of C-terminal domain of SoxB with SoxYZ complex

In order to find the interactions between the C-terminal domain of SoxB with SoxYZ the three dimensional structures of the proteins were docked by the software tool cluspro 2.0. SoxYZ and SoxB domain are found to interact strongly with each other. There are extensive H-bonding interactions involving both the main and the side chains of the two proteins. There are also hydrophobic interactions between two proteins. Apart from this there are protein-protein ionic and aromatic-aromatic interactions. Table 1 represents the extensive protein-protein hydrogen bonding interactions through their side chains between SoxYZ protein complex and 5'-nucleotidase, C-terminal domain of SoxB protein. The complex modelled structure of SoxYZ and 5'-nucleotidase, C-terminal domain of SoxB protein is presented in Figure 4.

Figure 4 Interaction of SoxYZ complex (yellow) and 5'-nucleotidase, C-terminal domain of the SoxB (blue) are shown in the complex

2 Discussion

In this study, an attempt has been made to elucidate the structural basis of the involvements of SoxY, SoxZ and 5'-nucleotidase, C-terminal domain of SoxB in binding and their role in sulphur oxidation. For this analysis the three-dimensional structures of the proteins SoxY, SoxZ, and 5'-nucleotidase, C-terminal domain of SoxB have been built and analyzed. Due to large amino acid residues of SoxB only 5'-nucleotidase, C-terminal domain of SoxB has modelled. Pfam has been used to predict domain position in the SoxB protein and the result is also verified by blast run. Domains are the conserved portion of the protein structure responsible for functionality. However there is no well documented report regarding residue level interactions of SoxB and SoxYZ complex protein. In a nutshell, the results from our study may introduce a new understanding of the three dimensional structures of the SoxYZ protein complex and the C-terminal domain of SoxB. Furthermore, our results may lead an elucidation of the structural basis behind the molecular functions of these proteins. This model provides a rational platform to design experiments for the determination of the contribution of the various amino acid residues in SoxY, SoxZ and SoxB proteins to predict the molecular basis of their interactions.

3 Materials and Method

3.1 Sequence analysis and homology modelling of SoxY, SoxZ and C-terminal domain of SoxB protein

The amino acid sequences of SoxY, SoxZ and SoxB proteins of *A.vino* were obtained from NCBI

nucleotide database (Acc. No. NC_013851). These amino acid sequences were used separately to build homology models by modeller by discovery studio. These amino acid sequences were used to do BLAST against Protein Data Bank to find suitable template for homology modelling. The search result showed the X-ray crystal structure from *Chlorobium Limicola F Thiosulfatophilum* (PDB code: 2NNC A chain for SoxY) and from *Paracoccus Pantotrophus* (PDB code: 2OXG Z chain for SoxZ). The sequences of SoxY and SoxZ share 49% and 36% sequence identity respectively with template sequences. Due to large SoxB protein sequence of only C-terminal domain (5'-nucleotidase, C-terminal domain) had been modelled using discovery studio modeller. Pfam was used to identify conserved domain of this protein. The same was also verified by BLAST.The domain was 146 amino acid residue lengths. For SoxB domain the best template was to be the x-ray crystal structure of Sulfate Thiohydrolase Soxb from *Termus Thermophilus* (PDB code: 2WDC A chain for SoxB domain) with sequence identity 44%. The root-mean-square deviation (RMSD) is used to study the globular protein conformation. The RMSD for each and individual model protein structure was calculated by superimposing separately on each of the crystal templates about their main chain conformation (A chain of 2NNC for SoxY, Z chain of 2OXG for SoxZ and A chain of 2WDC for SoxB 5'-nucleotidase, C-terminal domain). The RMSD for the superimpositions were 0.255 Å for SoxY, 1.117 Å for SoxZ and 0.477Å for 5'-nucleotidase, C-terminal domain SoxB.

The models of the proteins were then energy minimized in two steps. In first step the steepest decent technique was used and in the next step conjugate gradient technique was used to minimize the overall structure of the three models using the Discovery studio software until the structures reached the final RMS gradient of 0.0001. All energy minimization were done using CHARRAM force field and fixing backbone of proteins (Brooks et al., 1983).

3.2 Validation of models

The z score of each and individual model protein are calculated using Prosa webserver. The Z-score showed that predicted model structures were well inside the native structure (Sippl, 1993). Saves server was used to verify the main chain properties of the modelled proteins. No considerable bad contacts or C_α tetrahedron distortions were found. VERIFY3D was used to check amino acid residue profile of the three dimensional models (Eisenberg et al., 1997). The stereo chemical qualities of the models and Ramachandran plots were analysed using PROCHECK web server (Laskowski et al., 1993). In the Ramachandran plot no residues were found to be in disallowed region (Ramachandran and Sashisekharan, 1968).

3.3 Molecular docking simulations

In order to study the interactions between SoxYZ complex protein and 5'-nucleotidase, C-terminal domain SoxB and first the models of the SoxY and SoxZ proteins were docked using the software Cluspro 2.0 (Comeau et al., 2004). Cluspro 2.0 is a fully automated web based program for protein-protein docking (Comeau et al., 2004). The docked structure of SoxY and SoxZ protein was again docked with model structure of 5'-nucleotidase, C-terminal domain of SoxB protein of *A.vino* using Cluspro 2.0 protein-protein docking server. Using advanced option of Cluspro 2.0 the unstructured residues from receptor and ligand was removed. The model 0 was chosen from different displayed model structures because it had the best cluster size among all the possible docked structures, was selected and analysed. The complex structure was energy minimized by fixing backbone of the proteins in complex by the steepest descent technique using CHARRAM force fields (Brooks et al., 1983).

3.4 Calculation of protein-protein interaction

To find out the interactions between the SoxY, SoxZ and SoxB domain, Protein Interaction Calculator (P.I.C) web server was used. P.I.C web server is an online web server in which protein three dimensional structures was provided to calculate various kinds of interactions; such as disulphide bonds, hydrophobic interactions, ionic interactions, hydrogen bonds, aromatic-aromatic interactions, aromatic-sulphur interactions and cation - π interactions within a protein or between proteins in a complex (Tina et al., 2007).

Authors' contributions

SR performed the experiments. AB and SR formulated the work. AB and SR wrote the manuscript.

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References

Meyer B., Imhoff J.F., and Kuever J., 2007, Molecular analysis of the distribution and phylogeny of the *soxB* gene among sulfur-oxidizing bacteria-evolution of the Sox sulfur oxidation enzyme system, Environ. Microbiol., 9(12): 2957-2977
http://dx.doi.org/10.1111/j.1462-2920.2007.01407.x
PMid:17991026

Freidrich CG., Bardischewsky F., Rother D., Quentmeier A., and Fischer J., 2005, Prokaryotic sulfur oxidation, Curr. Opin. Microbiol., 8(3):253-259
http://dx.doi.org/10.1016/j.mib.2005.04.005
PMid:15939347

Bagchi A., 2012, Structural insight into the mode of interactions of *SoxL* from *Allochromatium vinosum* in the global sulfur oxidation cycle, Mol. Biol. Rep., 39(12): 10243-10248
http://dx.doi.org/10.1007/s11033-012-1900-9
PMid:23053932

Welte C., Hafner S., Krätzer C., Quentmeier A., Friedrich G C., and Dahl C., 2009, Interaction between Sox proteins of two physiologically distinct bacteria and a new protein involved in thiosulfate oxidation, FEBS Lett., 583(8): 1281-1286
http://dx.doi.org/10.1016/j.febslet.2009.03.020
PMid:19303410

Comeau S.R., Gatchel D.W., Vajda S., and Camacho C.J., 2004, ClusPro: an automated docking and discrimination method for the prediction of protein complexes, Bioinformatics, 20(1): 45-50
http://dx.doi.org/10.1093/bioinformatics/btg371
PMid:14693807

Brooks B.R., Bruccoleri R.E., Olafson B.D., States D.J., Swaminathan S., and Karplus M., 1983, CHARMM: a program for macromolecular energy, minimization, and dynamics calculations, J. Comp. Chem., 4(2): 187–217
http://dx.doi.org/10.1002/jcc.540040211

Sippl M.J., 1993, Recognition of errors in three-dimensional structures in proteins, Proteins, 17(4): 355–362
http://dx.doi.org/10.1002/prot.340170404
PMid:8108378

Eisenberg D., Luthy R., and Bowie J.U., 1997, VERIFY3D: assessment of protein models with three-dimensional profiles, Methods Enzymol., 277: 396–404
http://dx.doi.org/10.1016/S0076-6879(97)77022-8

Laskowski R.A., MacArthur M.W., Moss D.S, and Thornton J.M., 1993, PROCHECK: a program to check the stereochemistry of protein structures, J. Appl. Cryst., 26: 283–291
http://dx.doi.org/10.1107/S0021889892009944

Ramachandran G.N., and Sashisekharan V., 1968, Conformation of polypeptides and proteins, Adv. Protein Chem., 23: 283-438
http://dx.doi.org/10.1016/S0065-3233(08)60402-7

Tina K.G., Bhadra R., and Srinivasan N., 2007, PIC: Protein Interactions Calculator, Nucleic Acids Research, 35: W473–W476
http://dx.doi.org/10.1093/nar/gkm423
PMid:17584791 PMCid:PMC1933215