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Homology Modeling and Molecular Dynamics Study of the Interactions of SoxY and SoxZ: The Central Player of Biochemical Oxidation of Sulfur Anions in *Pseudaminobacter salicylatoxidans*

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Abstract: Microbial redox reactions of inorganic sulfur compounds, mainly the sulfur anions, are one of the vital reactions responsible for the environmental sulfur balance. These reactions are mediated by phylogenetically diverse prokaryotes, some of which are involved in the extraction of metal ions from their sulfur containing ores. The sulfur oxidizing gene cluster (*sox*) of α-Proteobacteria comprises of at least 15 genes, forming two transcriptional units, viz., *soxSR* and *soxVWXYZABCDEFGH*. SoxY is known to be a sulfur covalently binding protein, which binds sulfur anions to form the first covalently bound sulfur adduct in the novel global sulfur anion oxidation cycle. SoxZ, a sulfur compound chelating protein, binds to SoxY forming a complex to which SoxB, a sulfate thiol-esterase binds and ultimately cleaves the sulfur adduct. We employed homology modeling to construct the three-dimensional structures of the SoxY and SoxZ proteins from *Psedaminobacter salicylatoxidans*. With the help of docking and molecular dynamics studies we have identified the residues of SoxY and SoxZ involved in the interaction. The probable mechanistic details of the interactions of the SoxYZ complex in the binding of sulfur anions are also established. Present study provides a rational basis to illustrate the molecular mechanism of the biochemistry of sulfur anion oxidation reactions by these ecologically important organisms.

Key words: Sulfur oxidation, homology modeling, sulfur anion binding, protein-protein interaction

INTRODUCTION

Microbial redox reactions of sulfur are mainly responsible for cycling of this element in the environment to maintain environmental sulfur balance. Sulfur has a unique range of oxidation states that varies from + 6 to -2 and as a result several important biological processes involving transformations of sulfur from one form to other have been evolved. Sulfur based chemo- or photolithotrophy is one of such processes in which electron transfer from reduced sulfur compounds is used by phylogenetically diverse prokaryotes (Friedrich *et al*., 2005; Acosta *et al*., 2005; Okabe *et al*., 1999). Various sulfur anions such as, sulfide, polysulfide, thiosulfate, polythionates, sulfites as well as elemental sulfur are the different forms of sulfur in the environment (Friedrich *et al*., 2005; Le Faou *et al*., 1990), which serve as the electron donors in the process of respiration, or photosynthesis of the sulfur oxidizing prokaryotes. Only little is understood about the molecular mechanism of this oldest known process.

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Recent studies with both chemo- and photolithotrophic α-Proteobacteria, such as *Paracoccus pantotrophus* (Para), *Rhodovulum sulfidophilum*, revealed that multiple-gene cluster, *shxVW* (*soxVW*) and *soxXYZABCDEFGH*, is associated with metabolism sulfur anions (Appia-Ayme *et al*., 2001, Rother *et al*., 2001). SoxXA, SoxYZ, SoxB and SoxCD are required for sulfur-dependent cytochrome *c* reduction. The eight-electron oxidation of a molecule of thiosulfate is governed by cytochrome *c* complex multienzyme system (TOMES) encoded by *soxXYZABCD* (Rother *et al*., 2001; Friedrich *et al*., 2001).

Pseudaminobacter salicylatoxidans KCT001 (KCT) is a sulfur oxidizing chemolithotrophic α-Proteobacterium (Mukhopadhyaya *et al*., 2000). It possesses the sox gene cluster as in the case of Para (Lahiri *et al*., 2006). Though a considerable progress in the genetics of sulfur lithotrophy is noted, molecular mechanism of regulation of *sox* gene expression has not yet been addressed. In this present study, our aim is to understand the possible mechanistic details of the interactions of SoxY and SoxZ leading to the binding and cleavage of sulfur anions.

SoxY and SoxZ of KCT are proteins with 150 and 109 amino acid residues, respectively. Sequence analysis reveals that SoxY is a sulfur covalently binding protein while SoxZ is a sulfur compound chelating protein. During the sulfur oxidation process SoxY and SoxZ combine with each other to form a complex (SoxYZ complex). The sulfur anion then combines with the free thiol group of a conserved cysteine residue (Cys) of SoxY to form the first covalently bonded sulfur adduct during sulfur anion oxidation by *sox* operon (Quentmeier *et al*., 2003). SoxB, which is a sulfate thiol esterase, then interacts with the SoxYZ complex and subsequently hydrolyzes the protein-bound sulfur anion to form S-thiocysteine (Quentmeier *et al*., 2003). However to date the detailed structural information about the involvements of these proteins in the global sulfur cycle is not available. In the present study our aim is to understand the structural basis of the interaction of SoxY and SoxZ to investigate the molecular mechanism of sulfur anion oxidation via *sox* operon. We describe the three-dimensional structures of SoxY and SoxZ obtained by homology modeling. We have employed molecular docking in order to investigate the favorable binding modes of these homology-modeled proteins. Molecular dynamics simulations have been performed on the proteins in order to properly find out the amino acid residues involved in the protein-protein interaction. Binding interactions of SoxY with SoxZ determined by docking studies have been demonstrated and analyzed to predict the possible molecular mechanism of sulfur anion oxidation. We also identified the possible mode of binding of sulfur anion with SoxY. These studies provide detailed structural information regarding the molecular biochemistry of the binding of SoxY with SoxZ as well as the sulfur anion with SoxY. As this is the first report regarding the structural aspects of the interaction of SoxY and SoxZ in the process of oxidation of sulfur anions via *sox* operon in KCT, present studies are expected to contribute towards the understanding of the molecular details of the biochemical pathway of sulfur anion oxidation by these ecologically important microorganisms.

MATERIALS AND METHODS

Sequence Analysis and Homology Modeling of Monomeric SoxY and SoxZ

The amino acid sequences of SoxY and SoxZ of KCT were obtained from EMBL database (Accession No. AJ404005). These amino acid sequences were used separately to search Brookhaven Protein Data Bank (PDB) (Berman *et al*., 2000) using the software BLAST (Altschul *et al*., 1990) for finding suitable template for homology modeling. Multiple sequence alignment by CLUSTALW (http://www.ebi.ac.uk/clustalw) was used to compare the sequences of SoxY and SoxZ with the sequences of the structures having 25% or above sequence identity as obtained from BLAST search results. The three dimensional models of SoxY and SoxZ were built on X-ray crystal structure of human homologous-pairing protein Dmc1 (pdb code: 1V5W) (Kinebuchi *et al*., 2004) and Soxz protein from *Thermus Thermophilus* Hb8 (pdb code: 1V8H). Homology modeling was performed using Modeller (Šali and Blundell, 1993).

Fig. 1: Superimposition of the α-carbon backbones of SoxY (Black) on 1V5W (Red)

Fig. 2: Superimposition of the α-carbon backbones of SoxZ (Black) on 1V8H (Red)

Modeled structures were then superimposed separately on each of the crystal templates without altering the coordinate system of atomic positions in the respective templates (1V5W for SoxY and 1V8H for SoxZ). The mean r.m.s deviations for the superimpositions were 0.9Å and 0.4Å for SoxY and SoxZ, respectively on their corresponding crystal templates (Fig. 1 and 2, respectively). Short contacts and bad regions were rectified manually by Insight II. The models were then energy minimized fixing the backbones to ensure proper interactions. In the first few rounds of minimization Steepest Descent (SD) method was employed and then conjugate gradient method (CG) was employed with the GROMOS force field (Lindahl *et al*., 2001). The resulting energy minimized structures were validated using VERIFY3D (Eisenberg *et al*., 1997). PROCHECK (Laskowski *et al*., 1993) analysis was performed in order to assess the stereo-chemical qualities of the three dimensional models and Ramachandran plots (Ramachandran and Sashisekha-ran, 1968) were drawn. No residues were found to be present in the disallowed regions of the Ramachandran plot.

Docking of SoxY and SoxZ

To study the interactions involved in the binding of SoxY and SoxZ, the modeled proteins were docked using the software GRAMM (Vakser, 1995). The docking of the proteins was also performed with DOT (Mandel *et al*., 2001) and ZDOCK (Chen *et al*., 2003), using the ClusPro server (Comeau *et al*., 2004) and also with Patchdock server (Schneidman-Duhovny *et al*., 2003) in order to

get a comprehensive result. The docked structures, that yielded the best score were selected and analyzed visually using the software Insight II. The docked structures of the SoxYZ complex, thus obtained, were again subjected to energy minimization, using the GROMOS force fields, initially by SD and then by CG keeping the backbone atoms fixed.

Molecular Dynamics Calculations

The SoxYZ complexes, formed after docking, were solvated by a 10Å water layer. The resulting systems were subsequently energy minimized in two steps. In the first the step, the whole protein complex was kept fixed and in the next round of minimization backbones of the SoxYZ protein complex was fixed to perform rigorous minimization. In the next step of energy minimization, the whole system (i.e., both the protein-protein complex and water) was allowed to move. The minimization process was done initially by SD and then by CG using GROMOS force field. PROCHECK analysis of the complex showed that no residues were in the disallowed regions of the Ramachandran plot. The minimized systems were equilibrated for period of 210 ps with constant volume and temperature (NVT ensemble) through the velocity verlet integrator (Verlet, 1967). The temperature was kept at 300 K, as KCT is a mesophilic bacterium (Friedrich *et al*., 2001). The time step for integration was 1fs. The last 120 ps trajectories were analyzed saving the coordinates at every 0.2 ps interval. All the simulations were performed with GROMOS force field for non-bonded calculations. The stereo-chemical qualities of the final structures were analyzed with PROCHECK.

RESULTS AND DISCUSSION

Description of the Structure of SoxY

The modeled structure is a 150 amino acid residue long protein. The predicted structure is similar to X-ray crystal structure of human homologous-pairing protein Dmc1 (Kinebuchi *et al*., 2004). It starts with a helix (amino acid residues 5 to 18) followed by a short turn region (amino acid residue 19 to 21). Then there is another helix (amino acid residues 32 to 44) adjacent to a three stranded anti-parallel β sheet (amino acid residues 45 to 48, 51 to 59 and 68 to 74). The rest part is made up of β-strands interspersed with helices and turn regions. At the middle of this protein there is a three-stranded anti-parallel β sheet (amino acid residues 88 to 94, 97 to 104, 107 to 111), which is flanked by 2 other short β sheets (amino acid residues 126 to 128 and 134 to 134). The structure is presented in Fig. 3.

Description of the Structure of SoxZ

The model consists of 109 amino acid residues. The predicted structure is similar to the Soxz protein from *Thermus thermophilus*. The protein is made up of a series of anti-parallel β-sheets (amino acid residues 2 to 5, 14 to 20, 43 to 50, 53 to 58, 69 to 75, 80 to 88 and 93 to 100). The overall topology of the protein is a five-stranded anti-parallel β-sheet connected by loops. Figure 4 represents the structure of the modeled protein.

Interaction of SoxY and SoxZ

SoxY and SoxZ are found to interact strongly with each other. The protein-protein interface is found to contain mainly the polar amino acid residues. The interior of the complex is made up of hydrophobic amino acids. The interactions are mainly hydrogen bonding interactions (H-bonding). The side chains of Arg8, Gln103 and Gln104 of SoxZ are involved in H-bonding with the side chains of Arg5 of SoxY. The main chains of Ala65 and Asp79 of SoxZ are found to form H-bond with the side chains of Arg100 and Glu65 of SoxY, respectively. The H-bonding interaction scheme is presented in Table 1. The SoxYZ complex is also stabilized by ionic interactions. The residues of the proteins

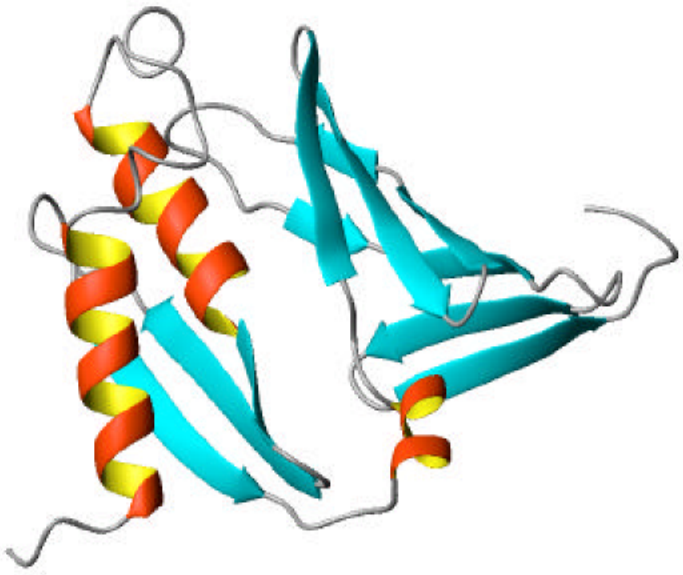

Fig. 3: Ribbon representation of modeled SoxY. α-helices (Red and Yellow) and β-sheets (Cyan) are shown as helices and ribbons, respectively. The rest are shown as loops. The figure was prepared by MOLSCRIPT (Kraulis, 1991)

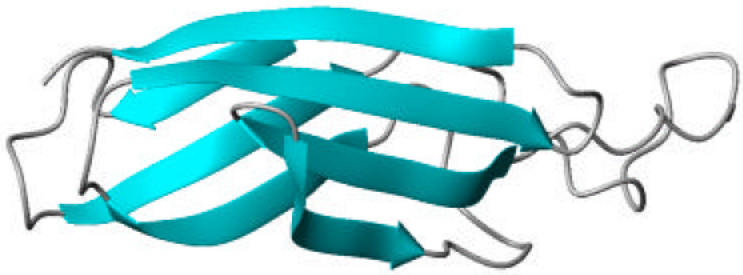

Fig. 4: Ribbon representation of modeled SoxZ. β-sheets (Cyan) are shown as ribbons. The rest are shown as loops (White). The figure was prepared by MOLSCRIPT Kraulis, 1991)

Table 1: Residues of SoxY and SoxZ involved in H-bonding in the SoxYZ complex

SoxY:	Arg5, Gln7, Lys51, Arg81, Glu84, Asn92, Ile124, Ile145
SoxZ:	Ala2, Ala3, Arg8, Glu30, Asn73, Asp96, Gln103, Gln104, Lys105

Table 2: Amino acid residues of SoxY and SoxZ involved in ionic interaction

SoxY:	Asn4, Arg5, Gln7, Arg81, Asn92
SoxZ:	Lys6, Arg8, His29, Glu30, Asn73, Lys89, Tyr100, Glu103, Lys105

involved in the formation of ion-pair are presented in Table 2. SoxY protein of Para contains a sulfate binding signature sequence GGCGG at the C-terminus end of the protein (Friedrich *et al*., 2001). Interestingly amino acid residues from the sulfate-binding motif of SoxY do not take part in the docking interactions.

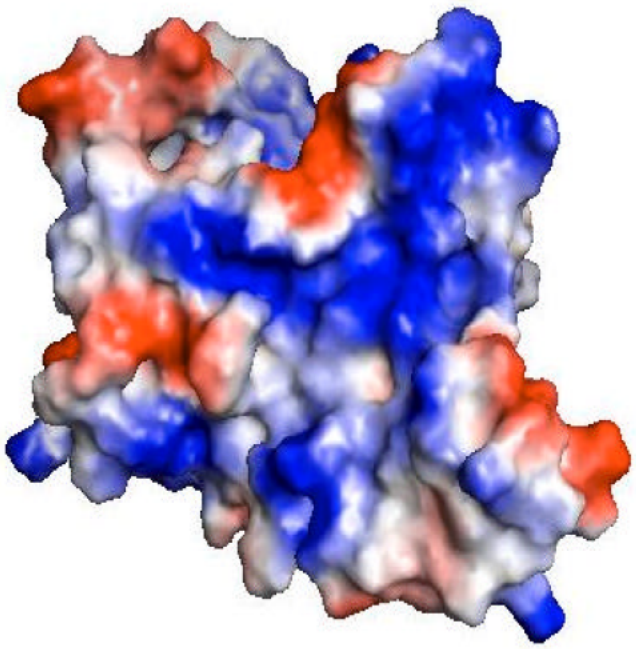

Fig. 5: The SoxY-SoxZ protein complex. The central blue region represents the catalytic positively charged cluster of amino acid residues in the complex, which binds the negatively charged sulfur anions during the sulfur anion oxidation process

When the 3D coordinates of the modeled SoxZ were used to search structural homologs using DALI (Holm and Sander, 1997) the search results showed that the most structurally conserved protein (except the template 1V8H) is an oxidoreductase from *Pyrococcus furious* (pdb code: 1DO6) (Yeh *et al*., 2000) (DALI Z-score = 9, r.m.s.d. = 2 Å over 83 C_α residues). In 1DO6, there is an electrostatically positively charged cluster of amino acid residues, which would serve to guide anions to the active site of the protein (Yeh *et al*., 2000). In the SoxYZ complex, the sulfur anion-binding region of the SoxY is surrounded by the side chains of eight positively charged amino acid residues of SoxZ to create a strong positive environment as observed in 1DO6 (Yeh *et al*., 2000) and therefore helps to lead the sulfur anion to bind strongly to SoxY. Electrostatic potential mapped on to the surface of the SoxY-SoxZ protein complex shows the anion-binding region in it. The blue areas of the molecular surface represent the electrostatically positive regions (Fig. 5). Since the sulfur anion binds covalently to the Sγ atom of the cysteine residue (Cys148) of SoxY and the whole sulfur anion-binding region of the protein is involved in the binding of the anion implying that the sulfur anion binds very strongly to the protein and therefore forms the first stable protein bound sulfur adduct during the oxidation of sulfur anions as proposed by Quentmeier *et al*. (2003).

CONCLUSION

In this study, we have tried to elucidate the structural basis of the involvements of SoxY and SoxZ during the oxidation of sulfur anions via the global sulfur oxidation reaction cycle. We have described the three dimensional structures of the SoxY and SoxZ proteins and established the geometry of the sulfur anion binding region of SoxY. The docking studies with SoxY and SoxZ have allowed us to identify the details of SoxY-SoxZ interactions. The structural basis of the formation of the hetero-

dimeric complex of SoxY-SoxZ has also been demonstrated to predict the biochemical pathway of sulfur anion binding, oxidation and hydrolysis via these proteins in the novel global sulfur oxidation reaction cycle as SoxYZ complex plays the key role in the oxidation of sulfur anions. Since there have been no previous reports regarding the structural biology of these proteins, results from present study will be important for the understanding of the three dimensional structures of SoxY and SoxZ as well as to elucidate the structural basis of their mode of actions. Our model provides a rational framework for designing experiments aimed at determining the contribution of various amino acid residues in these proteins to predict the molecular basis of the interactions both among themselves as well as with various sulfur anions in these biologically as well as ecologically important micro-organisms.

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