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Structural insight into the interactions of SoxV, SoxW and SoxS in the process of transport of reductants during sulfur oxidation by the novel global sulfur oxidation reaction cycle

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Abstract

Microbial redox reactions involving 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 also take part in the extraction of metal ions from their sulfur containing ores. These sulfur oxidizers oxidize inorganic sulfur compounds like sulfide, thiosulfate etc. to produce reductants that are used for carbon dioxide fixation or in respiratory electron transfer chains. The sulfur oxidizing gene cluster (*sox*) of α-Proteobacteria comprises of at least 15 genes, forming two transcriptional units, viz., *soxSR* and *soxVWXYZABCDEFGH*. SoxV is known to be a CcdA homolog involved in the transport of reductants from cytoplasm to periplasm. SoxW and SoxS are periplasmic thioredoxins, which (SoxW) interact with SoxV and thereby help in the redox reactions. We have employed homology modeling to construct the three-dimensional structures of the SoxV, SoxW and SoxS proteins from *Rhodovulum sulfidophilum*. With the help of docking and molecular dynamics simulations we have identified the amino acid residues of these proteins involved in the interaction. The probable biochemical mechanism of the transport of reductants through the interactions of these proteins has also been investigated. Our study provides a rational basis to interpret the molecular mechanism of the biochemistry of sulfur anion oxidation reactions by these ecologically important organisms.

Keywords: Sulfur oxidation; Homology modeling; Molecular dynamics; Protein–protein interaction

1. Introduction

Microbial redox reactions of sulfur are mainly responsible for cycling of this element in the environment. Sulfur has a unique range of oxidation states that varies from +6 to −2 and as a result of which 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 [1]. These microbes oxidize inorganic sulfur compounds like sulfide, thiosulfate, etc. to produce reductants that are used for carbon dioxide fixation or in respiratory electron transfer chains [2]. The transformation of the sulfur compounds from one form to the other is a major component of the biogeochemical sulfur cycle. Besides some of these bacteria (though they may not contain the *sox* gene cluster) also take part in the extraction of metals form their sulfur containing ores [3,4]. Nevertheless, the biochemical mechanism of the bacterial sulfur oxidation process is, in general, still poorly understood.

The sulfur oxidizing gene cluster (*sox*) of α-Proteobacteria comprises of at least 15 genes, which form two transcriptional units, viz., *soxSR* and *soxVWXYZABCDEFGH*. Recent studies with both chemo- and photolithotrophic α-Proteobacteria such as *Paracoccus pantotrophus* (Para) and *Rhodovulum sulfidophilum* (Rsulf) revealed that multiple-gene cluster, *shxVW* (*soxVW*) and *soxXYZABCDEFGH*, is associated with the metabolism sulfur anions [5,6]. 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*. SoxV, SoxW and SoxS of Rsulf are proteins with 245, 187 and 135 amino acid

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residues, respectively. SoxV is a protein with 6 transmembrane channel forming helices. It is structurally related to CcdA of Para, which mediates transport of reductant from the cytoplasm to the periplasm [7]. SoxW is a periplasmic thioredoxin, which takes part in electron transport process [7]. SoxV interacts directly with SoxW to keep the latter in the reduced state [7]. The SoxVW complex is known to be essential for lithotrophic growth of the sulfur oxidizing bacteria [7]. It is also well known that SoxV is both essential for thiosulfate oxidation and maintains SoxW in a reduced state and is able to reduce multiple periplasmic thioredoxin partners [7]. However, cells without SoxW can still perform the sulfur oxidation reactions. In absence of SoxW, SoxS, which has a distinct thioredoxin motif [8], may serve the purpose by interacting with SoxV. However to date the detailed structural information about the involvement of these proteins in global sulfur cycle is not available. In the present study our aim is to understand the structural basis of SoxV, SoxW and SoxS proteins as the mediator of transport of reductants in *sox* operon. We describe the three-dimensional structures of SoxV, SoxW and SoxS obtained by homology modeling. We have found out the molecular basis for the classification of SoxW and SoxS as thioredoxins. We have used molecular docking and molecular dynamics in order to investigate the favorable binding modes of these modeled proteins. Binding sites of SoxV, SoxW and SoxS have been predicted, analyzed and compared to that of previously reported experimental results. These studies provide detailed structural information on the binding of SoxV, SoxW and SoxS, their mode of action in the transport of reductants during the oxidation of sulfur anions. As this is the first report regarding the structural basis of the involvements of SoxV, SoxW and SoxS in the process of biochemical oxidation of sulfur anions, our studies may contribute towards the understanding of the molecular mechanism of sulfur anion oxidation by these ecologically important microbial species.

2. Material and methods

2.1. Sequence analysis and homology modeling of monomeric SoxV, SoxW and SoxS proteins

The amino acid sequences of SoxV, SoxW and SoxS proteins of Rsulf were obtained from Entrez database (Accession Nos. AAF99429, AAF99430 and AAO11779, respectively). These amino acid sequences were used separately to search Brookhaven Protein Data Bank (PDB) [9] using the software BLAST [10] for finding suitable template for homology modeling. The BLAST search result of SoxV revealed it had 44% identity with X-ray crystal structure of 4-(Cytidine 5′-Diphospho)-2-C-Methyl-D-Erythritol Kinase from *Thermus thermophilus* (pdb code: *1UEK*) [11]. The N-terminal part of SoxW had 48% sequence identity with crystal structure of the Disulfide Interchange Protein from (pdb code: *1VRS*) from *E. coli* [12] and the rest part of SoxW exhibited 40% sequence identity with the D-xylose isomerase (pdb code: *1XYC*) of *Streptomyces olivochromogenes* [13]. The BLAST search result for SoxS picked up the crystal structure of thioredoxin-2 (pdb code: *1THX*) from Anabaena [14] with 45% sequence identity. The proteins were modeled separately using the corresponding crystal structures as templates. Homology modeling was performed using the program Modeller [15] that is incorporated in the Homology module of Insight II [Accelrys, San Diego, CA, USA] on a Silicon Graphics Indigo II workstation.

Modeled structures were then superimposed separately on each of the crystal templates without altering the coordinate system of atomic positions in the respective templates (*1UEK* for SoxV, *1VRS* and *1XYC* for the SoxW and *1THX* for SoxS, respectively). The r.m.s. deviations for the superimpositions were 0.9 Å for SoxV on *1UEK* and 0.6 and 0.7 Å for the corresponding regions of SoxW on *1VRS* and *1XYC*, respectively, as well as 0.2 Å for SoxS on *1THX*. The modeled subunits of SoxW were subsequently joined using the program Biopolymer of Insight II to form a complete three-dimensional structure of the protein. The models were energy minimized fixing the backbones to ensure proper interactions. Conjugate gradient (CG) method was employed for minimization with the consistent valence force field (CVFF) [16] using the program DISCOVER until all the structures reached the final derivative of 0.001 kcal/mol.

2.2. Validation of the models

Regarding the main chain properties of the modeled proteins, no considerable bad contacts nor C_α tetrahedron distortion nor hydrogen bond energy problems were found. Moreover, the average *G* factor, the measure of the normality degree of the protein properties, was of −1.04, which is inside the permitted values for homology models. Furthermore, there

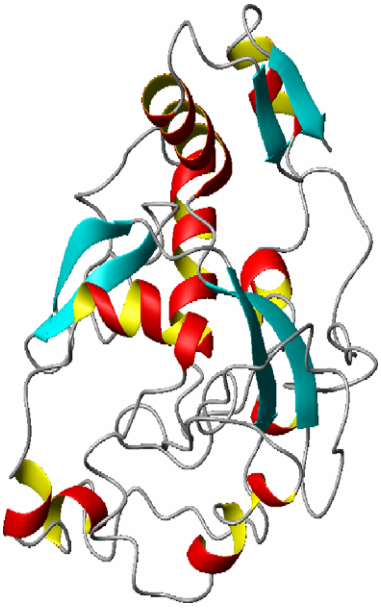

Fig. 1. Ribbon representation of modeled SoxV. α-helices and β-sheets are shown as helices (red and yellow) and ribbons (cyan), respectively. The rest are shown as loops (white). The figure was prepared by MOLSCRIPT [46]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

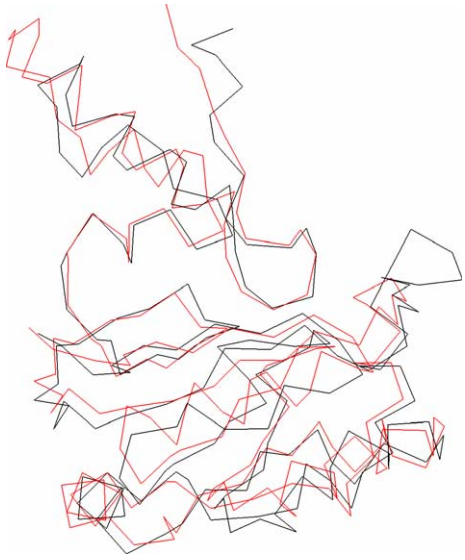

Fig. 2. Superimposition of the α-carbon backbones of SoxV (black) on 1UEK (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were no distortions of the side chain torsion angles found. The *Z*-scores calculated using the software PROSA 2003 [17] showed that the predicted models of the proteins were well inside the range of a typical native structure [18]. The residue profiles of the three dimensional models were further checked by VERIFY3D [19]. PROCHECK [20] analysis was performed in order to assess the stereo-chemical qualities of the three dimensional models and Ramachandran plots [21] were drawn. No residues were found to be present in the disallowed regions of the Ramachandran plot.

2.3. Docking and molecular dynamics calculations

To study the interaction between SoxV and SoxW as well as that between SoxV and SoxS, the models were docked using the software GRAMM [22]. The docking of the proteins was also performed with DOT [23] and ZDOCK [24,25], using the ClusPro server [26,27] and also with Patchdock server [28] 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. Molecular dynamics (MD) simulations were performed on the docked structures to predict the favourable binding interactions. The docked structures were solvated with an average of 2000 simple point charges [29] water molecules. The system was minimized initially keeping the water and the backbones of the proteins fixed. In the next step of minimization, the protein complex was kept fixed and the water molecules were allowed to move. The first few rounds of minimizations were performed by steepest descent (SD) method and then CG method was employed. The minimized system was equilibrated for a period of 10 ps with positional restraints. Then a 120 ps MD run was performed without restraints. Weak coupling of the protein to a solvent bath of constant temperature (300 K) and constant pressure (1 bar) was maintained with a coupling of 1.0 ps. For all energy

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SoxV        ------MLEISYGGA---------A---------LAGLLSFLSPCILPIVPFYLCYMAGI
Rc          MTPTDLLFDAGFLTA---------AA-----TAFLAGVLSFLSPCVLPIVPPYLAYMGGV
Ml          MTGFTEIAAAGPLLV---------ALG----VCMLAGLVSFVSPCVVPLVPGYLSYLAAV
Bs          ------MGDVNYFLT---------FG---------AGFLSFISPCCLPLYPAFLSYITGV
Hp          -----MMFDNTLVNL---------FDTAPLLTSLLAGILTFLSPCVLPLIPAYMSYISQI
Hi          ------MLDQQLL----------------IGTVFLAGLASFLSPCIFPIIPIYFGILS--
Pp          MKPDVLLYNSQHLINNITLYQLNHINIASFSFVFFSGLFTSFSPCLISILPICIMYISG-

SoxV        SMTELRGSDR------IPPGAVRRLVVSAIAFALGVTSIFVLLGMGATALGQGFREWKDE
Rc          TVSDMGAG--------R-PAARGPVLLAALFFVLGLSTVFLMMGLGASALGQALGAWKDT
Ml          VGVSHEETQPGAGVIKTPPAARWRVAGSAVLFVAGFTTVFVLDTVAVLGMTTVLTTHQVL
Bs          SMDDVKTEK---------LLLQKRSLFHTLCFLLGFSVIFIALGYGTSFIGSLFRDYHDA
Hp          SLEDIKDGKAK---------RVS-VFLKSLMFVVGFSLVFLGVGMSMAKLIHSFSFS--W
Hi          -----KGGKK----------------VLNTFLFILGLSLTFVSLGFSFGFLGNILFSN--T
Pp          -----EGQK----------LSQIDKLKNLFFFCLGAISSFTTLGLIATLLAKTYSQLFNG

SoxV        LSYVAALMLFLFGLHFLGILRIPLLYREARIETRA--EPSTLVGAYLMGLAFGFGWTPCV
Rc          LAKVSGLVVMIFGAHFIGVYRIGFLDREMRFDTGD--QGGSALGAYLLGLAFAFGWTPCL
Ml          LQRVGGVLTIVMGLVFVGLL--PALQRQVQFSLR---QLTTVAGAPVLGTVFALGWTPCL
Bs          IRQIGALLIILFGFITLGVFRPEAMMKERRIHFKH--KPSGFLGSVLIGMAFAAGWTPCT
Hp          VNYIAGGIVILFGLHFLGVFRFAFLYKTQSVGLASKSNSMQRFYPFLLGMSFALGWTPCI
Hi          TRIIAGVIVIILGIHQLGIFKIGLLERTKLVEIKTSGKSTA-LEAFVLGLTFSLGWTPCI
Pp          IPVISALVIIYMGFSLLNIVPLSTNNLNTRINNTN-----QNIKMYLSGVGIGLAISSCS

SoxV        GPALAAILMVASGMG--DIGRGGLLLLVYGLAMTLPFVLAAAFARPFLAWMQRNRRHLGH
Rc          GPVLGTIASMAMAEG--TIGRGMGLLSAYAAGLGLPFLIVAAFFPSLGGALAFMRRNMGR
Ml          GPTLSGVITVAAATDGVNVTRGILLVIAYCMGLGIPFVLLASGSAQAVAGLRWLRQYGRA
Bs          GPILAAVITLAGTNP--GSAVP--YMMLYVLGFAVPFLLLSFFIT----KLKWIRKNQLF
Hp          GPIFTSIVIMSAS----KDAYGLILMVVFVMGLAIPFLLVALMLERALLFLKSLKKYNRA
Hi          GPILASVLALSGDEG--SALYGASMMFVYVLGLATPFVLFSFLSDSLLKRAKGLNKHLDK
Pp          TPIFVTLLIWVTSN--HNLFIGLIFILIYSIGYIFPIIIGSLFSSRFLTTASSPFLN-NL

SoxV        VEKAMGAMLVVFAILIATNSVGYLAQVLIDNVPWFTTLG-
Rc          IEKISGLLLWTIGLMMLTGQLSDLSFWLLDTFPALAELG-
Ml          IQVFGGMLLIAIGAALIAGVWDDFVSWLRDAVVSDMRVSI
Bs          IMKAGGVLMIVIGVLLFFNWMSLIIILLSDLFGGFTGF--
Hp          IEIVSGLVLILMGILIMTNSLESLTNFLQK----------
Hi          FKIGGGILIIVMGILLITNNFS-------------------
Pp          WAPFSGTILLSAGTFSLFSSILKY-----------------
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Fig. 3. Sequence alignment of SoxV with well-charaterized CcdA proteins. Conserved amino acid residues are presented in bold face. Rc: *Rhodobacter capsulatus*, Ml: *Mycobacterium leprae*, Bs: *Bacillus subtilis*, Hp: *Helicobacter pylori*, Hi: *Haemophilus influenzae*, Pp: *Porphyra purpurea*.

minimizations and MD simulations GROMACS molecular simulation package [30] was used. All the structures were finally analyzed by PROCHECK [20].

2.4. Calculations for protein–protein interactions

To find out the interactions between the protein complexes (i.e., SoxVW and SoxVS), What If software package [31] as well as the Biopolymer module of Insight II were used. These programs calculate the interactions between two groups by measuring the distance between them.

3. Results and discussion

3.1. Description of the structure of SoxV

SoxV protein is a 245 amino acid residues long monomeric protein. The modeled structure is identical with the X-ray crystal structure of 4-(Cytidine 5′-Diphospho)-2-C-Methyl-D-Erythritol Kinase from *T. thermophilus* (pdb code: *1UEK*) [11] (Figs. 1 and 2). The predicted structure consists of two domains. The N-terminal domain (amino acid residues 1 to 140) is made up of five alpha helices, a short 3_{10} helix, three beta strands and several turns connected by random coil fragments. The C-terminal domain of the protein is made up of four alpha helices and three beta strands along with several turns and bends. The protein has six transmembrane helices. The transmembrane topology was predicted from the amino acid sequence of SoxV by averaging the results of five different predictive algorithms: DAS [32], PHDHTM [33], HMMTOP [34], TMHMM [35] and TMPRED [36].

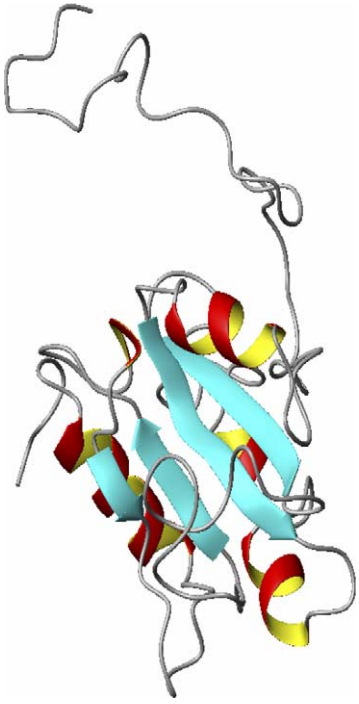

Fig. 4. Ribbon representation of modeled SoxW. α-helices and β-sheets are shown as helices (red and yellow) and ribbons (cyan), respectively. The rest are shown as loops (white). The figure was prepared by MOLSCRIPT [46]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

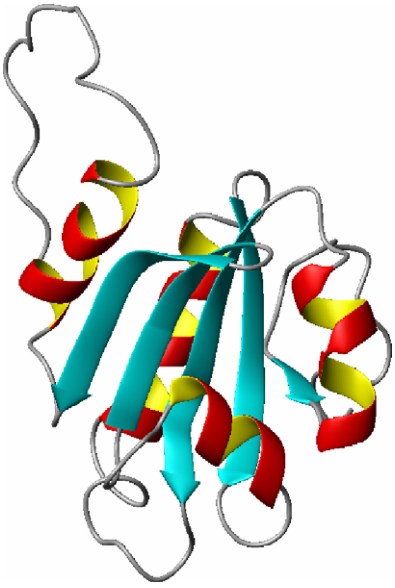

Fig. 5. Ribbon representation of modeled SoxS. α-helices and β-sheets are shown as helices (red and yellow) and ribbons (cyan), respectively. The rest are shown as loops (white). The figure was prepared by MOLSCRIPT [46]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Putative active site geometries of SoxV

SoxV belongs to the CcdA family of proteins, which are involved in transport of reductants. Recently, Page et al. [37] proposed that there are membrane-embedded invariant cysteine residues in these family proteins, which are involved in the shuttling of reducing equivalents. There are also several other conserved residues present in these well-studied CcdA proteins. Alignment of the amino acid sequence (Fig. 3) of SoxV with the well-characterized CcdA proteins revealed that SoxV also has two cysteine residues in the identical positions. The other conserved amino acid residues as observed in the well-studied CcdA proteins are also found to be present in SoxV in identical positions. Moreover, when the 3D coordinates of the modeled SoxV were used to search structural horologes using

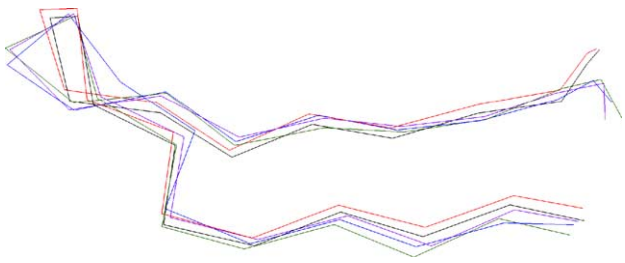

Fig. 6. Superimposition of the α-carbon backbones of the thioredoxin motif of SoxS (black) on 1THX (red), 2TRX (violet), 3TRX (blue) and 1ERV (green). The mode of superimposition is similar for the thioredoxin motif of SoxW; therefore only one is shown for clarity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Residues from SoxV and SoxW involved in H-bonding interaction

SoxV:	Gly8, Ala12, Ile22, Cys21, Leu73, Tyr93, Cys141, Asn200, Arg201, His203, His206, Glu208, Lys209, Ala210, Met211, Gln232, Val233, Asp236
SoxW:	Ala8, Asp44, Ala48, Glu49, Glu51, Asn52, Arg54, Cys64, Gln92, Lys112, Glu113, Gly116, Trp118, Met129, Pro130, Glu131, Glu135

Table 3
Residues from SoxV and SoxS involved in H-bonding interaction

SoxV:	Cys21, Ser44, Asp45, Arg46, Cys141, Asn200, His203, His206, Glu208, Lys209, Gln232, Asp236
SoxS:	Thr2, Met6, Thr7, Gly8, Gly9, Asp30, Arg38, Cys41, Glu60, Arg95, Asp96

DALI [38] the top hits were the proteins, which are the members of transferase family of proteins. These proteins are involved in transport of reductants. This further justifies the inclusion of SoxV in the family of transport proteins, which are involved in the passage of reductants from the cytoplasm to periplasm. It is also observed that these well-studied CcdA proteins interact with an adjacent thioredoxin, located just downstream of the CcdA encoding gene, which are involved in the acceptance of the reducing equivalents [7,37]. In case of *sox* operon, *soxW* (which codes for SoxW protein) which is present just downstream of *soxV* (whose gene product is SoxV) (and SoxS in absence of SoxW) serve the similar purpose. These CcdA family proteins have multiple periplasmic thioredoxin partners, which interact with them in absence of the other [37]. In case of *sox* operon, SoxS may serve the same purpose as the SoxW in its absence.

3.3. Description of the structure of SoxW

SoxW is a 187 amino acid residue long protein. The protein is composed of a four-stranded β-sheet sandwiched between two helices on one side and third on the other. There are two parallel and two anti-parallel β-sheets. The overall topologic arrangement of the secondary structural elements is like β-α-β-α-β-β-α-(Fig. 4). The first helix of the protein (amino acid residues 42 to 48) is connected to the β-sheet by a short coil. Thioredoxin motif of the protein comprises of the first β-strand (amino acid residue 53 to 60) connected to the second helix (amino acid residue 65 to 70). When the 3D coordinates of the modeled SoxW were used to search structural homologs using DALI, the search results showed that the top hits (except the templates *1VRS* and *1XYC*) belong to known thioredoxin proteins.

3.4. Description of the structure of SoxS

SoxS is a 135 amino acid residue long protein. The structure shows a remarkable similarity to the crystal structure of thioredoxin-2 (pdb code: *1THX*) from Anabaena [14]. The protein is made up of five helices and four β-sheets. The central core of the protein consists of a four stranded anti-parallel β-sheet distributed between helices. The thioredoxin motif of the protein starts with a β-sheet (amino acid residue 32 to 40) and ends in a helix (amino acid residue 51 to 61) connected to it by a short turn region (Fig. 5). Searching for structural homologs using DALI resulted in similar thioredoxin proteins as obtained in case of SoxW.

3.5. Comparison with other thioredoxin proteins

Both SoxS and SoxW have strong structural similarities with other known thioredoxins viz., *Anabena* sp. (*1THX*) [14], *E. coli* (*2TRX*) [39], and Human thioredoxins (*3TRX* and *1ERV*) [40,41]. Superimpositions of the thioredoxin motifs of SoxS and SoxW onto the corresponding motifs in *1THX*, *2TRX*, *3TRX* and *1ERV* produced r.m.s. deviations of 0.2, 0.7, 0.6 and 0.9 Å, respectively, for SoxS and 0.3, 0.9, 0.5 and 0.9 Å, respectively, for SoxW. This shows that the overall structures of SoxS and SoxW are similar to these well-characterized thioredoxin family proteins (Fig. 6).

3.6. Putative active site geometries of SoxS and SoxW

The first 20 amino acid residues of both SoxW and SoxS show the typical feature of a signal peptide. This is predicted by the software PrediSi [42]. Amino acid residues from 9 to 20 of SoxW and that of 10 to 19 of SoxS show the presence of a transmembrane domain with the topology of N-terminal inside. This is predicted by DAS Transmembrane Prediction server [31].

The redox active sites of the proteins (Cys64-Ile65-Tyr66-Cys67 for SoxW and Cys41-Ala42-Tyr43-Cys44 for SoxS) are located just in front of the N-terminal of the first helix. This redox active site is solvent accessible from one side of the molecule whereas inaccessible from other side as observed in case of other thioredoxins [43]. In both SoxW and SoxS the active sites are covered by the side chains of mostly hydrophobic amino acid residues (Gln61, Ile65, Tyr66, Met70, Val120 and Leu126 in SoxW and Tyr43, Val45, Leu46, Glu50, Ile51, Leu71 and Tyr106 in SoxS, respectively) which is also a structural characteristic of most known thioredoxins. There are hydrophobic patches on opposite sides of the redox active sites of both SoxW and SoxS as observed in other well-studied thioredoxins. This positioning of the hydrophobic residues is known to play an important role in substrate binding.

Table 2
Residues from SoxV and SoxW involved in ionic interaction

SoxV:	Ser5, Cys21, Tyr32, Arg191, Cys141, Asn200, Arg201, Arg202, His203, His206, Glu208, Lys209, Gln232
SoxW:	Asp25, His29, Asp44, Glu49, Glu51, Asn52, Arg53, Arg54, Cys64, Gln92, Asn94, Lys112, Glu113, Glu131, Glu135, Asp165

Table 4
Residues from SoxV and SoxS involved in ionic interaction

SoxV:	Ser5, Cys21, Tyr32, Cys141, Arg191, Asn200, Arg201, Arg202, His203, His206, Glu208, Lys209, Gln232, Asp236, Asn237
SoxS:	Arg11, Asp30, Asp38, Cys41, Arg49, Glu50, Lys57, Glu60, Arg95, Asp96, Arg102

Table 5
Comparative results of binding interactions of SoxV with SoxW and SoxS $T=300$ K

Complex	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)	ΔG (kcal/mol)
SoxV–SoxW	2.98	51.23	−48.25
SoxV–SoxS	2.27	49.69	−47.42

3.7. Interaction of SoxV with SoxW and SoxS

Both SoxW and SoxS are found to interact strongly with SoxV. The protein–protein interfaces in both the cases are found to contain mainly the polar amino acid residues. The interiors of the complexes are made up of hydrophobic amino acids. The interactions between the proteins are mainly H-bonding interactions. Interestingly the first cysteine residues (Cys64 of SoxW and Cys41 of SoxS) of the thioredoxin motifs of both SoxW and SoxS are involved in H-bond as well as ion-pair formation with the membrane embedded cysteines (Cys21 and Cys141) of SoxV. This would suggest that the first cysteine residue of the thioredoxin motifs of both SoxW and SoxS are strongly interacting with SoxV. Thus it is unlikely that these cysteine residues of the thioredoxin motifs of both the proteins would be able to get oxidized. This observation supports the fact that SoxV would keep SoxW in the reduced rather than oxidized form [7]. The SoxVW and SoxVS complexes are also stabilized by ionic interactions. The residues involved in H-bonding as well as ionic interactions are presented in Tables 1–4. Moreover, the binding interactions of each complex were quantitatively evaluated by calculating the thermodynamic parameters, using the Structural Thermodynamics Calculator (STC) program [44]. The results are presented in Table 5. The calculated free energy change on binding between SoxV and SoxW is comparable to that between SoxV and SoxS complex. These results also point towards the fact that SoxS interacts with SoxV in a similar fashion as SoxW.

The mechanism of the transport of reducing equivalents by the CcdA proteins occurs via the interactions of the membrane bound cysteine and the cysteine residues of the thioredoxin motifs of its partners. For that reason the membrane bound cysteines must be accessible to the cysteines of the thioredoxins [45]. Both the cysteine residues of SoxV are found to interact with the cysteine residues of the redox active sites of both SoxW and SoxS (Tables 1–4) as observed during the interactions between other CcdA and thioredoxins [45]. According to the mechanism of oxidation of sulfur anions by *sox* operon, SoxV interacts with SoxW to keep it reduced. Since the first cysteine (Cys64) of SoxW is bound to SoxV very strongly it is unlikely that there is a disulfide bond between Cys64 and Cys67 of the thioredoxin motif of SoxW as predicted by Appia-Ayme et al. It is also well known that SoxW is dispensable and SoxV has a multiple periplasmic partners [7]. Since SoxS interacts in an analogous way (as SoxW) with SoxV, SoxS may therefore be considered to supplement the role of SoxW.

4. Conclusion

In this study, we have tried to elucidate the structural basis of the involvements of SoxV, SoxW and SoxS in the transport of reductants during oxidation of sulfur anions. We have described the predicted three-dimensional structures of the SoxV, SoxW and SoxS. We have identified the putative active site geometries of SoxV as well as of SoxW and SoxS to classify them as a novel transferase and thioredoxins respectively. The dockings of SoxV with SoxW and SoxS have allowed us to identify the details of SoxV–SoxW and SoxV–SoxS interactions. The structural basis of the formation of SoxV–SoxW and SoxV–SoxS complexes has also been demonstrated to predict the pathway of transport of reductants via these proteins in the novel global sulfur cycle. Results from this study will be important for the understanding of the structures of novel transferase SoxV and thioredoxins SoxW and SoxS. Our model provides a rational framework for designing experiments aimed at determining the contribution of various amino acid residues in the complex formation as well as transport of reductants via these proteins in the novel *sox* operon in *R. sulfidophilum*.

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