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Prediction of possible interaction of SoxK with SoxXA complex in *Allochromatium vinosum* through Protein-protein docking: An *in silico* approach

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ABSTRACT

Three periplasmic Sox proteins encoded by the soxBXAK and soxYZ genes are mainly responsible for thiosulfate oxidation in *Allochromatium vinosum.* Thiosulphate ($S_2O_3^{2-}$) fulfils an important role in the natural sulphur cycle. It is a stable and environmentally abundant sulphur compound of intermediate oxidation state. Thiosulphate-oxidizing sox enzyme can be classified as two types, type one group forms sulphur globules as intermediate for ex *Allochromatium vinosum* and another which does not form sulphur globules as intermediate for ex example Paracoccus pantotrophus. It is well documented that thiosulphate oxidation in *Allochromatium vinosum* is mainly dependent on the presence of three periplasmic Sox proteins encoded by the soxB, soxXAK, and soxYZ genes. So the interactions between SoxXA complexes with soxK are very crucial to understand thiosulphate oxidation in *Allochromatium vinosum*. In the present work, homology modeling and *ab-initio* structure prediction have been used to build the three dimensional structures of SoxA, SoxX and SoxK. With the help of protein-protein docking complex structure of SoxAX is formed, The Cluspro 2.0 protein-protein docking and P.I.C server have been used to predict possible interaction between soxAX and soxK protein. The interactions between the SoxAX complex, and SoxK proteins are mediated mainly through hydrogen bonding, hydrophobic interaction, electrostatic interaction.

Keywords: Homology modeling, Protein-protein interactions, Docking simulations, Environmental sulphur balance, Sox operon, Sulfur oxidation

INTRODUCTION

Microbial redox reactions are Fundamental way to maintain the environmental sulphur balance. Different types of microorganisms are mainly responsible for chemo and photolithotrophic redox reaction of sulphur [1]. Sulfide, thiosulfate, tetrathionate are the major sources of sulphur for these chemo and photolithotrophic redox reaction [2]. The gene cluster called the sox operon are mainly responsible for carrying out the microbial redox reaction of sulphur [3, 8]. The organism *Allochromatium vinosum* (*A.vinosum* or *A.vino*) is a Gram-negative proteobacteria that belongs to the family Chromatiaceae and is an ideal model organism for studying thiosulphate oxidation [9]. SoxAX protein complex plays a vital role in the sulphur oxidation process. The interactions between SoxK and SoxAX protein complex are very important in *A.vino*. However to date detailed structural information regarding the interactions between SoxAX protein complex and SoxK protein have not been fully understood. In the present study, the three dimensional structures of SoxA , SoxX and SoxK from *A.vino* obtained by homology modeling and *ab-initio* structure prediction have been described. Molecular docking simulations have been performed in order to find out the possible modes of binding of SoxAX complex with SoxK. Binding sites of SoxAX complex and SoxK have been predicted and analyzed. These studies provide a detailed structural insight into the plausible molecular mechanism of the involvements of these proteins in the global sulphur oxidation reaction cycle.

MATERIALS AND METHODS

Sequence analysis and homology modelling

The amino acid sequences of SoxA, and SoxX 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 modweb. Modweb is a web server for protein structure modelling using comparative modelling approach. Models were built for each one of the sequence-structure matches using modeller. Modelled residues of SoxX and SoxA were 102 and 260 residue length. The model structures of SoxA and SoxX were based on crystal structures of 1h32A (~30%) and 3oa8B with 30% sequence identity. The structure of SoxX protein were refined by the chiron and followed by koba energy minimization server [10, 11, 12].

The amino acid sequences of SoxK protein of *A.vino* was obtained from NCBI nucleotide database (Acc. No. NC_013851).The protein was modelled using Quark *ab-initio* structure prediction server as there was no suitable template structure for doing Homology modelling. QUARK is a web based computational algorithm for *ab-initio* protein structure prediction based on information about amino acid sequence [13]. The structure of SoxK protein was refined by the chiron and followed by koba energy minimization server [11, 12].

The software PROSA 2003 was used to calculate Z scores [14]. The result showed that the predicted homology models were well inside the range of typical native structures [15]. The residue profiles of the three dimensional models were further checked by VERIFY3D [16]. PROCHECK [17] analyses were performed in order to assess the stereo chemical qualities of the models and Ramachandran plots [18] were drawn. No residues were found to be present in the disallowed regions of the Ramachandran plots.

Molecular docking simulations

In order to study the interactions between SoxAX complex and SoxK proteins at first the models of the SoxA and SoxX proteins were docked to form complex using the software Cluspro 2.0. Cluspro is fully automated web server for protein-protein docking [19]. The two modelled protein structures of SoxA and SoxX were uploaded through the Cluspro2.0 webserver. The docked structure of the SoxAX complex that yielded the best clustering size among all the other possible docked structures was selected and the model of the complex protein was then energy minimized using steepest decent technique by fixing the backbone of two proteins in the complex structure to ensure proper interactions. All energy minimizations were done with CHARMM force fields [20] using the program Discovery studio until the structures reached the final RMS gradient of 0.1. SoxAX complex and SoxK proteins were docked using Cluspro 2.0 and the Complex SoxAXK was energy minimised using same protocol as predicted above.

Calculation of protein–protein interactions

To find out the interactions between the SoxAX, SoxK proteins, PIC web server were used. This web server were designed 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[21].

RESULTS AND DISCUSSION

Description of the structure of SoxA

SoxA is 260 amino acid long protein. We used GORV to predict the secondary structure of SoxA [22]. The algorithm uses a protein sequence as query sequence input and predicts the each amino acid residue into alpha helix ('H'), beta sheet ('E') or random coil ('C') with certain confidence for each residue [22]. GOR-V method predicted the presence of helical and coil regions.There are mainly eleven regions (amino acid residues 22-30, 47-51,58-69,77-89,120-127,144-155,175-179,181-185,221-227,233-237 and 255-265) predicted as helical. The modelled structure is presented in Fig. S1.

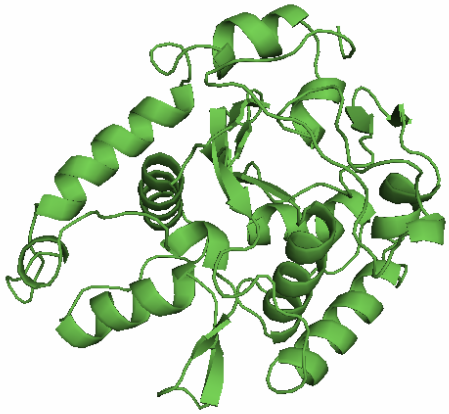

Figure S1. Model structure of SoxA protein from A.Vinosum. With distinct secondary structure showing as alpha helix , beta structure and random coil

Description of the structure of SoxX

The modelled structure of SoxX is a 102 amino acid residue long protein. The secondary structures of whole protein were predicted by GORV [22]. There are mainly four regions (amino acid residues 13-23, 45-50, 88-93, 115-123) predicted as helical and rest of the structure predicted as coil. The modelled structure is presented in Fig. S2.

Description of the structure of SoxK

The modelled structure of SoxX is a 114 amino acid residue long protein. Computational algorithm, GORV [22] was used to define the structural propensities of all the protein residues. There are mainly five regions (amino acid residues 17-52,60-67,77-92,94-101,106-110) predicted as helical and rest of the structure predicted as coil. The modelled structure is presented in Fig. S3

Table 1. Protein-protein Side chain-Side chain hydrogen bonds. Where X represents chain of the SoxA protein, A represents chain of the SoxX protein, and B represents chain of the SoxK protein.

POS	CHAIN	RES	ATOM	POS	CHAIN	RES	ATOM
41	X	GLN	NE2	111	B	ASP	OD1
41	X	GLN	NE2	111	B	ASP	OD1
41	X	GLN	NE2	111	B	ASP	OD2
41	X	GLN	NE2	111	B	ASP	OD2
42	X	ARG	NH1	104	B	ASP	OD1
42	X	ARG	NH1	104	B	ASP	OD1
42	X	ARG	NH1	104	B	ASP	OD2
42	X	ARG	NH1	104	B	ASP	OD2
42	X	ARG	NH2	104	B	ASP	OD2
42	X	ARG	NH2	104	B	ASP	OD2
115	X	ARG	NH1	12	A	ASP	OD2
115	X	ARG	NH2	20	A	GLU	OE2
115	X	ARG	NH2	20	A	GLU	OE2
115	X	ARG	NH2	24	A	ASP	OD1
115	X	ARG	NH2	24	A	ASP	OD1
115	X	ARG	NH2	24	A	ASP	OD2
115	X	ARG	NH2	24	A	ASP	OD2
165	X	GLU	OE1	29	A	ASN	ND2
165	X	GLU	OE1	29	A	ASN	ND2
165	X	GLU	OE2	29	A	ASN	ND2
165	X	GLU	OE2	29	A	ASN	ND2
172	X	ARG	NH1	44	B	GLU	OE1
172	X	ARG	NH1	44	B	GLU	OE1
172	X	ARG	NH1	44	B	GLU	OE2
172	X	ARG	NH1	44	B	GLU	OE2
172	X	ARG	NH2	44	B	GLU	OE1
172	X	ARG	NH2	44	B	GLU	OE1
178	X	GLU	OE1	101	B	GLN	OE1
178	X	GLU	OE1	101	B	GLN	OE1
178	X	GLU	OE1	101	B	GLN	NE2
178	X	GLU	OE1	101	B	GLN	NE2
181	X	LYS	NZ	101	B	GLN	OE1
182	X	ARG	NH1	100	B	GLU	OE1
182	X	ARG	NH1	100	B	GLU	OE1
182	X	ARG	NH1	100	B	GLU	OE2
182	X	ARG	NH1	100	B	GLU	OE2
182	X	ARG	NH2	100	B	GLU	OE1
182	X	ARG	NH2	100	B	GLU	OE1
182	X	ARG	NH2	100	B	GLU	OE2
182	X	ARG	NH2	100	B	GLU	OE2
187	X	ARG	NH1	102	B	ASN	OD1
187	X	ARG	NH1	102	B	ASN	OD1
187	X	ARG	NH2	102	B	ASN	OD1
187	X	ARG	NH2	102	B	ASN	OD1
19	A	ARG	NE	110	X	TYR	OH
19	A	ARG	NH2	110	X	TYR	OH
19	A	ARG	NH2	110	X	TYR	OH
29	A	ASN	ND2	165	X	GLU	OE1
29	A	ASN	ND2	165	X	GLU	OE1
29	A	ASN	ND2	165	X	GLU	OE2
29	A	ASN	ND2	165	X	GLU	OE2
5	B	LYS	NZ	40	A	GLU	OE1
5	B	LYS	NZ	40	A	GLU	OE2
6	B	ARG	NH1	170	X	ASP	OD2
6	B	ARG	NH1	170	X	ASP	OD2
6	B	ARG	NH2	170	X	ASP	OD1
6	B	ARG	NH2	170	X	ASP	OD1
6	B	ARG	NH2	170	X	ASP	OD2
6	B	ARG	NH2	170	X	ASP	OD2
49	B	ARG	NH1	38	X	TYR	OH
49	B	ARG	NH1	38	X	TYR	OH
49	B	ARG	NH2	38	X	TYR	OH
49	B	ARG	NH2	38	X	TYR	OH
49	B	ARG	NE	178	X	GLU	OE1
49	B	ARG	NH2	178	X	GLU	OE1
49	B	ARG	NH2	178	X	GLU	OE1
49	B	ARG	NH2	178	X	GLU	OE2
49	B	ARG	NH2	178	X	GLU	OE2
101	B	GLN	OE1	178	X	GLU	OE1
101	B	GLN	OE1	178	X	GLU	OE1
101	B	GLN	NE2	178	X	GLU	OE1
101	B	GLN	NE2	178	X	GLU	OE1
108	B	TYR	OH	178	X	GLU	OE2
111	B	ASP	OD1	41	X	GLN	NE2
111	B	ASP	OD1	41	X	GLN	NE2

Interaction of SoxAX with SoxK

In order to find the interactions between the proteins the three dimensional coordinates of the proteins were docked by the software tool cluspro. SoxAX and SoxK are found to interact strongly with each other. The protein–protein interface is found to mainly contain the polar amino acid residues. There are also hydrophobic interactions between two proteins. There are extensive H-bonding interactions involving both the main and the side chains of the two protein molecules. Apart from this there are protein-protein ionic and cation–pi interaction. Table1 represents the extensive protein-protein hydrogen bonding interactions through their side chains between SoxAX protein complex and SoxK protein. The modelled structure of SoxAX and SoxK protein is presented in Fig.1

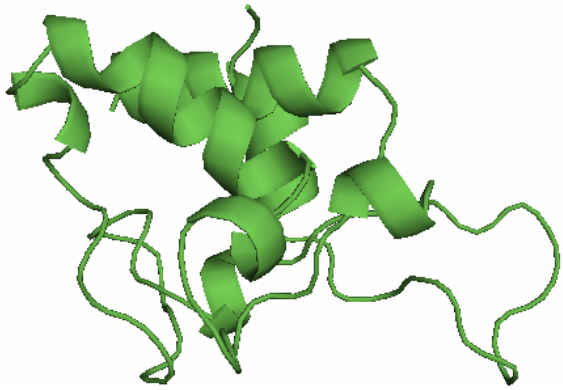

Figure S2. Model structure of SoxX protein from A.Vinosum. With distinct secondary structure showing as alpha helix, and random coil

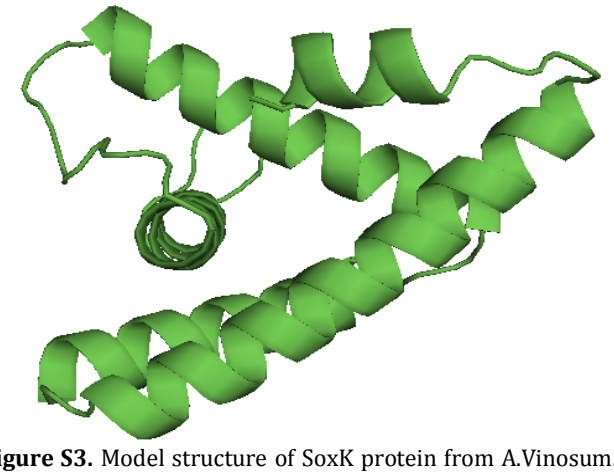

Figure S3. Model structure of SoxK protein from A.Vinosum. With distinct secondary structure showing as alpha helix, and random coil.

In this study, an attempt has been made to elucidate the structural basis of the involvements of SoxAX, SoxK, in binding. For that matter the three-dimensional structures of the proteins SoxA, SoxXand SoxK have been built and analysed. Till date there have been no

previous reports regarding the structural biology of these proteins. In that sense, the results from this study may give a new insight to understand the three dimensional structures of the complex of SoxAX, SoxK as well as to elucidate the structural basis of the molecular functions of these proteins. This model provides a rational framework for designing experiments to determine the contribution of the various amino acid residues in these proteins to predict the molecular basis of their interactions.

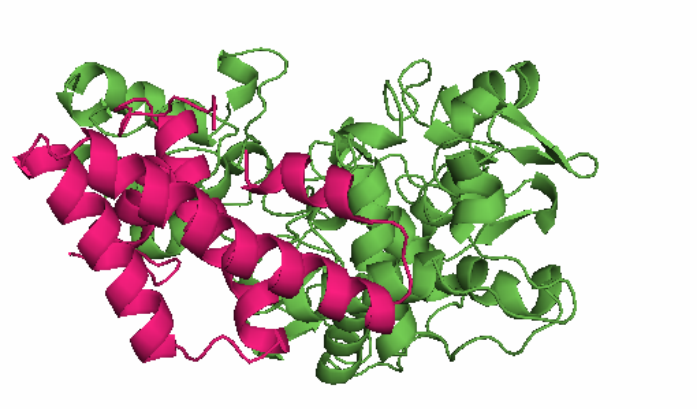

Figure 1. Interaction of SoxAX complex (green) and SoxK (magenta) are shown in the complex

CONCLUSION

Our investigation showed computationally the structural basis of involvements of SoxAX, SoxK interactions in *A vinosum*. Modweb web server has been used to build three dimensional structures of SoxA and SoxX. However soxK protein was modelled using *ab-initio* protein modelling approach as there was no suitable structure for SoxK protein in template based protein modelling approach. The residues of SoxA, SoxX and SoxK showed conformational adaptability towards helix and coil conformations.Interactions of SoxAX complex with SoxK protein were also analysed. SoxA and SoxX are two very important proteins thiosulphate oxidation in A *vinosum.* SoxK protein plays a vital role in case of stabilization of SoxAX complex protein. Based on current work it seems reasonable to suggest that SoxAX complex interact very strongly with SoxK protein mainly through H-bonds. This information will advance our understanding the role of SoxAX and SoxK interaction on thiosulphate oxidation of *A.vinosum.*

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