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Biochemical and Biophysical Research Communications 335 (2005) 609–615
BBRC
www.elsevier.com/locate/ybbrc

A structural study towards the understanding of the interactions of SoxY, SoxZ, and SoxB, leading to the oxidation of sulfur anions via the novel global sulfur oxidizing (*sox*) operon

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Received 4 July 2005

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, which also take part in the extraction of metal ions from their sulfur containing ores. The sulfur oxidizing gene cluster (*sox*) of α-Proteobacteria comprises of at least 16 genes, forming two transcriptional units, viz., *soxSRT* and *soxVWXYZABCDEFGH*. SoxY is known to be a sulfur covalently binding protein, which binds sulfur anions (such as sulfate) to form SoxY-thiocysteine-*S*-sulfate, 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, SoxZ, and SoxB from *Paracoccus pantotrophus*. With the help of docking and molecular dynamics studies we have identified the residues of SoxY, SoxZ, and SoxB involved in the interaction. The probable mechanisms of the binding of SoxY with sulfate as well as the removal of sulfate from the SoxYZ complex are also established. Our study provides a rational basis to illustrate the molecular mechanism of the biochemistry of sulfur anion oxidation reactions by these industrially important organisms.

Keywords: Sulfur oxidation; Homology modeling; Sulfate binding; Protein–protein interaction

Microbial redox reactions of sulfur are mainly responsible for the 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, which also participate in the extraction of metals by leaching the metals from their sulfur containing ores [1–3]. Various sulfur anions such as sulfide, polysulfide, thiosulfate, polythionates, sulfites as well as elemental sulfur are the different forms of sulfur in the environment [4], 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.

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

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0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.07.115

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* [6].

SoxY, SoxZ, and SoxB of Para are proteins with 140, 109, and 564 amino acid residues, respectively. SoxY is a sulfur covalently binding protein while SoxZ is a sulfur compound chelating protein. On the other hand, SoxB is a sulfate thiol esterase containing the signature sequence of 5′-nucleotidase [7,8]. During the sulfur oxidation process, SoxY and SoxZ combine with each other to form a complex (SoxYZ complex). The sulfate ion then combines with the free thiol group of a conserved cysteine residue (Cys138) of SoxY to form SoxY-thiocysteine-*S*-sulfate, the first covalently bonded sulfur adduct during sulfur anion oxidation by *sox* operon [8]. SoxB, which is a sulfate thiol esterase, then interacts with the SoxYZ complex and subsequently hydrolyzes the protein-bound sulfate to form *S*-thiocysteine [8]. However to date, the detailed structural information about the involvement 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, SoxZ, and SoxB to investigate the molecular mechanism of sulfur anion oxidation via *sox* operon. We describe the three-dimensional structures of SoxY, SoxZ, and SoxB 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 as well that of the SoxYZ complex with SoxB determined by docking studies have been demonstrated and analyzed to predict the possible molecular mechanism of sulfur oxidation. We also identified the possible mode of binding of sulfate ion with SoxY. These studies provide a detailed structural information regarding the molecular biochemistry of the binding of SoxY with SoxZ, the SoxYZ complex with SoxB as well as the binding of sulfate with SoxY. As this is the first report regarding the structural aspects of the interaction of SoxY, SoxZ, and SoxB in the process of oxidation of sulfur anions via *sox* operon, our studies are expected to contribute towards the understanding of the molecular details of the biochemical pathway of sulfur anion oxidation by these industrially important microorganisms. Since, these sulfur oxidizing prokaryotes are also involved in metal extraction processes, and SoxY, SoxZ, and SoxB are the central players of this sulfur anion oxidation operon, our study would help to find out a commercially viable way to use these organisms efficiently in the process of extraction of metals from their sulfur containing ores.

Materials and methods

Sequence analysis and homology modeling of monomeric SoxY, SoxZ, and SoxB. The amino acid sequences of SoxY, SoxZ, and SoxB of Para were obtained from Entrez database (Accession Nos. CAB94380, CAB94381, and CAA55824, 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 SoxY showed that it had 43% sequence similarity with the X-ray crystal structure of human homologous-pairing protein Dmc1 (pdb code: 1V5W) [11]. For SoxZ, the result picked up the crystal structure of Soxz protein from *Thermus thermophilus* Hb8 (pdb code: 1V8H) with 40% sequence similarity. The amino acid sequence of SoxB had 42% sequence similarity with the X-ray crystal structure of 5′-nucleotidase from *Escherichia coli* (pdb code: 1USH) [12]. The proteins were modeled separately using the corresponding crystal structures as templates. Homology modeling was performed using the program Homology of Insight II (Accelrys, San Diego, 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 (1V5W for SoxY, 1V8H for SoxZ, and 1USH for SoxB). The mean r.m.s. deviations for the superimpositions were 0.7, 0.4, and 0.7 Å for SoxY, SoxZ, and SoxB, respectively, on their corresponding crystal templates (Figs. 1–3, 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. Conjugate gradient (CG) method was employed for minimization with the consistent valence force field (CVFF) [13] using the program DISCOVER3 of Insight II until all the structures reached the final derivative of 0.001 kcal/mol and were validated using VERIFY3D [14]. PROCHECK [15] analysis was performed in order to assess the stereo-chemical qualities of the three-dimensional models and Ramachandran plots [16] were drawn. No residues were found to be present in the disallowed regions of the Ramachandran plot.

Docking of SoxY with sulfate. SoxY has a sulfate-binding signature sequence at the carboxy terminal end [7]. In order to predict the mode of binding of the sulfate ion with SoxY, these two (SoxY and sulfate ion) are subjected to docking by the software GOLD (Genetic Optimization of Ligand Docking) [17,18]. GOLD ran up to 50 docking procedures. The final docking solution was selected on the basis of the fitness score obtained from the fitness function as implemented in the GOLD program. The complex of the SoxY bound to sulfate was subjected to energy minimization to reduce the bad contacts.

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 [19]. The docking of the proteins was also

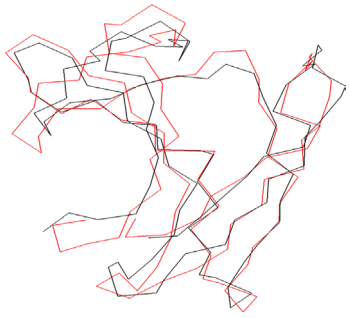

Fig. 1. Superimposition of the α-carbon backbones of SoxY (black) on 1V5W (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)