

Crystal Structure of the Oxidative-Stress Sensor SoxR Bound to DNA

Reactive oxygen species (ROS) provide cellular and genetic damages to aerobic organisms, and cells have thus evolved defense systems against oxidative stress. In *Escherichia coli*, the *soxRS* regulon functions in the protection of cells against oxidative stress [1]. The *soxRS* regulon is induced in two steps: first, SoxR activates transcription of another transcription factor, SoxS in response to oxidative stress such as superoxide, nitric oxide; and then the increased level of the SoxS protein enhances the production of various antioxidant proteins and repair proteins. SoxR forms a homodimer in solution, with each 17-kDa subunit containing a [2Fe-2S] cluster. The [2Fe-2S] cluster is essential for activity of SoxR. In the absence of oxidative stress, the [2Fe-2S] cluster is maintained in reduced states and SoxR is inactive for transcription. Upon oxidative stress, the metal center is oxidized and SoxR is converted to the active form to enhance transcription of the *soxS* gene up to 100-fold. Apo-SoxR and reduced SoxR can bind to DNA with an affinity similar to that of oxidized SoxR, but only oxidized SoxR is able to activate transcription of the *soxS* gene. Therefore, it is suggested that structural changes between the oxidized and reduced forms of SoxR regulate the activity of SoxR.

SoxR belongs to the MerR family of transcriptional activators, which responds to various environmental stresses [2]. MerR family proteins have a homologous N-terminal DNA binding domain and a less conserved C-terminal sensor domain. The target promoter sequences of MerR family proteins have an unusual 19 or 20 bp spacer between the -35 and -10 elements, in contrast to the optimal 17 bp spacer. Hence, the MerR family is assumed to possess a common DNA distortion mechanism for transcriptional activation. To provide deep insights into the transcriptional activation

mechanism by the redox regulation of SoxR, we have determined the crystal structures of SoxR from *E. coli* and its complex with the promoter DNA in the oxidized state at 3.2 Å and 2.8 Å resolution, respectively, using beamlines **BL41XU** and **BL44B2** [3].

The overall structure of SoxR consists of a DNA-binding domain, a dimerization helix ($\alpha 5$) and an Fe-S cluster-binding domain (Fig. 1(a)). The dimerization $\alpha 5$ helix forms an antiparallel coiled coil stabilizing the SoxR dimer. The overall architecture of the SoxR-DNA complex is similar to those of other MerR family proteins such as BmrR and MtaN [4,5]. Upon binding to DNA, the DNA binding domain undergoes an outward rotation of approximately 9° and the Fe-S cluster-binding domain receives an outward rotation of about 6°, resulting in a widening of the distance between the $\alpha 2$ and $\alpha 2'$ helices from 29.3 to 31.5 Å (Fig. 1(b)). The dimerization helix connecting both domains shows an inner helical twist, which leads to a change in the relative positions of the dimerization helices. These conformational changes of SoxR are different from those observed in MtaN [5]. However, the observed movement of MtaN may result from the missing of a sensor domain of MtaN, which probably interacts with the DNA binding domain of the other subunit. Therefore, conformational changes similar to those observed in SoxR are expected to occur in other MerR family proteins during binding to their target promoters.

The [2Fe-2S] cluster of SoxR is coordinated by conserved four cysteine residues (Fig. 2(a)). The Fe-S cluster-binding domain is further stabilized by interactions with the conserved residues of the DNA binding domain from the other subunit (Fig. 2(b)). The remarkable feature of the [2Fe-2S] cluster of SoxR is its asymmetric environment (Fig. 2(a)). The lower

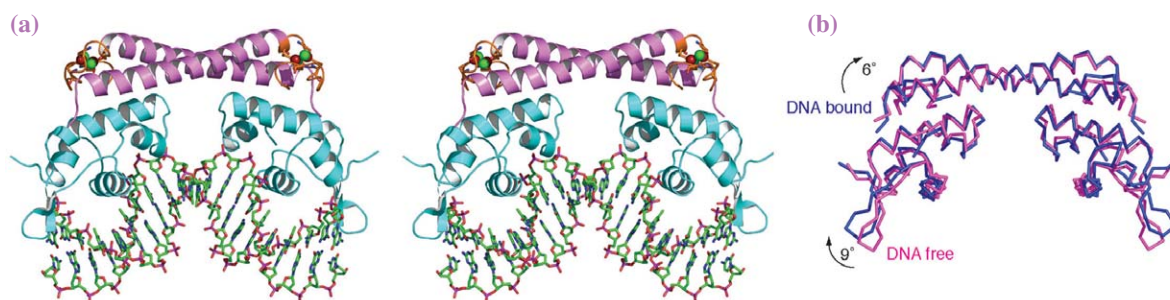


Fig. 1. Overall structure of the SoxR-DNA complex. (a) Stereo view of the overall structure of the SoxR-DNA complex. The SoxR dimer is shown in a ribbon representation and the DNA fragment appears in a stick model. The DNA binding domain, dimerization helix and Fe-S cluster binding domain are shown in light-blue, pink and orange, respectively. The two iron and two sulfur atoms of the [2Fe-2S] cluster are represented with brown and green spheres, respectively. (b) The C α backbone of the DNA-bound (blue) and DNA-free (magenta) forms of SoxR. The two structures are superimposed based on the dimerization helices.

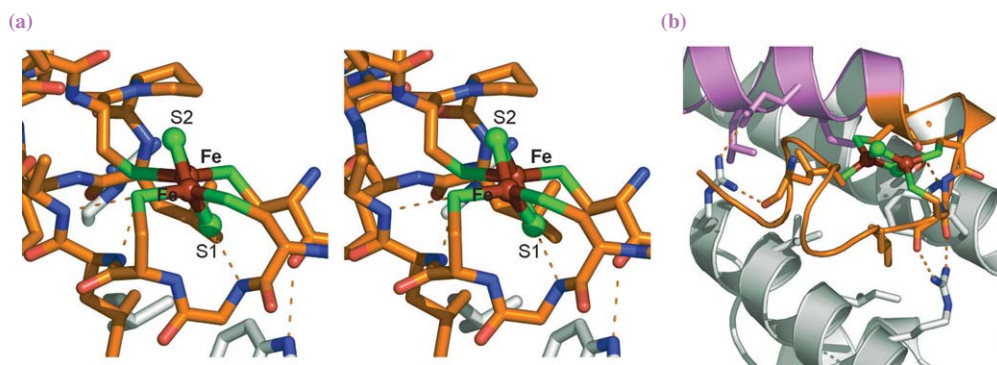


Fig. 2. Asymmetric environment of the [2Fe-2S] cluster of SoxR. (a) Stereo view of the [2Fe-2S] cluster environment in stick representation. Iron and sulfur atoms are indicated by brown and green spheres, respectively. NH-S hydrogen bonds are represented in orange broken lines. (b) Interactions between the Fe-S cluster-binding domain and the DNA-binding domain of the other subunit. The other subunit is shown in white.

sulfur atom (S1) of the cluster is surrounded by three positively charged amide groups of the main chain, while the upper sulfur atom (S2) is not able to interact with any amide groups but with the negatively charged carbonyl oxygen atom. These results suggest that asymmetric charge distribution of the [2Fe-2S] cluster environment causes redox dependent conformational changes of SoxR. Upon reduction, an additional negative charge on the sulfur atoms will attract the amide groups of the main chain and increase the charge repulsion with the oxygen atom, possibly resulting in large conformational changes of SoxR.

The promoter DNA in the SoxR-DNA complex is sharply bent at the middle (Figs. 1(a) and 3(a)). Although the half-site of the structure of the SoxR-DNA complex is similar to those of BmrR and MtaN, the DNA in the SoxR-DNA complex is further bent

approximately 65° at the middle away from the protein, compare to $47\sim 50^\circ$ in BmrR and MtaN (Fig. 3(b)). Consequently, the overall end-to-end length of the 20-bp DNA fragment is further shortened by about 3.4 \AA , which corresponds to the length of 1-bp, compared to those of the activated *bmr* and *mta* promoters. Therefore the DNA structure of SoxR is distorted and unwound by about 3-bp, compared to a B-form DNA. Comparison of the target promoter sequences of the MerR family indicates that the present DNA structure represents an activated conformation of the target promoter with a 20-bp spacer in the MerR family. In the target promoters with a 20-bp spacer, the DNA distortion observed in the SoxR-DNA complex unwinds the promoter by 3 bp and aligns the -35 and -10 elements for productive interactions with RNA polymerase.

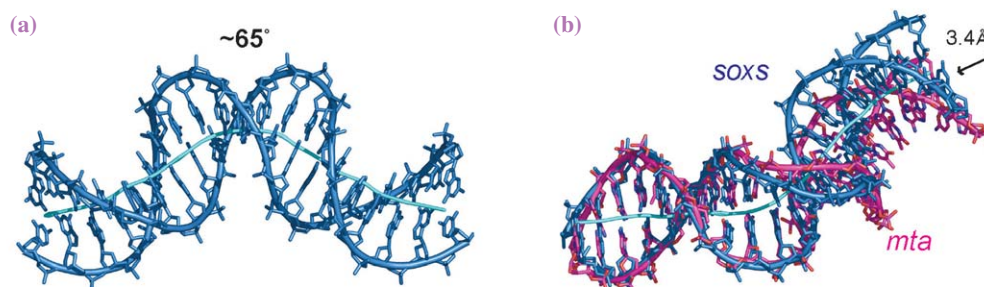


Fig. 3. Activated conformation of target promoter of SoxR. (a) The overall structure of the *soxS* promoter with the global DNA helical axis. (b) Comparison of the 20-bp promoter structures of SoxR (blue) and MtaN (magenta). Two promoter structures are superimposed on each half-site of DNA.

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