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Crystallization and preliminary X-ray crystallographic studies of the oxidative-stress sensor SoxR and its complex with DNA

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SoxR, a member of the MerR family of transcriptional activators, functions as a sensor of oxidative stress. The redox states of the 2Fe–2S cluster of SoxR regulate the activity of SoxR. Here, the crystallization and preliminary crystallographic analysis of SoxR and its complex with DNA are reported. Crystals of SoxR were obtained using PEG 10 000 and glycerol as precipitants. The crystals of SoxR belong to space group $P6_2$ or $P6_4$, with unit-cell parameters $a = b = 80.0$, $c = 88.1$ Å. Crystals of the SoxR–DNA complex were obtained using a 20 bp DNA fragment from a condition containing PEG 10 000 and sodium/potassium tartrate. The crystals of the SoxR–DNA complex belong to space group $P6_122$ or $P6_522$, with unit-cell parameters $a = b = 53.5$, $c = 355.6$ Å. Diffraction data were collected to a maximum resolution of 3.2 and 2.7 Å for SoxR and the SoxR–DNA complex, respectively.

1. Introduction

Oxidative stress has a harmful effect on all aerobic organisms. Bacteria have sophisticated molecular systems to protect cells from oxidative stress by activating various defence genes (Zheng & Storz, 2000; Pomposiello & Demple, 2001). In *Escherichia coli*, the *soxRS* regulon functions in the protection of cells against superoxide and nitric oxide (Tsaneva & Weiss, 1990; Greenberg *et al.*, 1990; Nunoshiba *et al.*, 1993). Induction of the *soxRS* regulon occurs in two stages (Nunoshiba *et al.*, 1992; Wu & Weiss, 1992). SoxR is first activated by oxidative stress and enhances the transcription of *soxS*. The increased level of SoxS, a member of the AraC family, activates the production of various antioxidant proteins (Pomposiello *et al.*, 2001).

SoxR forms a homodimer and each 17 kDa monomer contains a 2Fe–2S cluster (Wu *et al.*, 1995; Hidalgo *et al.*, 1995). The 2Fe–2S cluster plays an important role in the activity of SoxR (Hidalgo & Demple, 1994). In the absence of oxidative stress, the 2Fe–2S cluster is maintained in the reduced state by specific proteins (Hidalgo *et al.*, 1997; Kobayashi & Tagawa, 1999; Koo *et al.*, 2003) and SoxR is inactive. When this metal centre is oxidized by oxidizing agents, SoxR is converted to the active form (Gaudu & Weiss, 1996; Ding *et al.*, 1996). Nitric oxide also activates SoxR by direct nitrosylation of the 2Fe–2S cluster (Ding & Demple, 2000). Apo-SoxR and reduced SoxR can bind to DNA with similar affinity to that of oxidized SoxR, but only oxidized SoxR is able to activate the transcription of the *soxS* gene up to 100-fold (Hidalgo & Demple, 1994; Gaudu & Weiss, 1996). Therefore, it has been assumed that a structural change between the oxidized and reduced forms of SoxR regulates the transcription of the *soxS* gene.

SoxR belongs to the MerR family of transcriptional activators (Amabile-Cuevas & Demple, 1991; Wu & Weiss, 1991), which respond to various environmental stresses. MerR proteins have a homologous N-terminal DNA-binding domain and a less conserved C-terminal sensor domain (Brown *et al.*, 2003). They form a homodimer by forming an antiparallel coiled coil, as shown in the crystal structures of BmrR, MtaN, CueR and ZntR (Heldwein & Brennan, 2001; Changela *et al.*, 2003; Newberry & Brennan, 2004). The target promoter sequence of MerR proteins has 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 members are assumed to possess a common DNA-distortion mechanism for transcriptional activation (Hidalgo & Demple, 1997; Outten *et al.*, 1999; Ansari *et al.*, 1995). The crystal structures of BmrR and MtaN bound to their target promoters have provided evidence of the DNA-distortion mechanism, in which the promoters are bent sharply at the centre bases with base-pair breakage, resulting in the rearrangement of the −35 and −10 elements (Heldwein & Brennan, 2001; Newberry & Brennan, 2004). However, how their activation signals are linked to the DNA distortion and transcriptional activation is not clear.

To provide further insight into the mechanism of transcriptional activation by redox regulation of SoxR, we have purified SoxR with the oxidized 2Fe–2S cluster from *Escherichia coli* and crystallized it in both the DNA-free form and in complex with the *soxS* promoter.

2. Materials and methods

2.1. Expression and purification

The expression plasmid for SoxR (Kobayashi & Tagawa, 1999) was transformed into *E. coli* C41(DE3) and co-expressed with the *isc* operon (Nakamura *et al.*, 1999). 4 ml saturated *E. coli* C41(DE3) culture was inoculated into 400 ml Terrific Broth medium with 50 µg ml^{-1} ampicillin, 10 µg ml^{-1} tetracycline and 0.1 mg ml^{-1} ferric ammonium citrate at 310 K. Expression was induced by adding 0.5 m*M* isopropyl β-D-thiogalactopyranoside (IPTG) at an OD_{600} of 0.6. After addition of IPTG, cells were quickly cooled to 291 K and grown for 24 h.

Purification of SoxR was performed at 277 K under aerobic conditions as described previously (Demple *et al.*, 2002), but incorporating several modifications. The thawed cells were incubated for 60 min in buffer *A* (20 m*M* MOPS pH 7.6, 0.2 *M* KCl, 10 m*M* potassium/sodium tartrate, 10% glycerol and 1 m*M* DTT) with 0.5 mg ml^{-1} egg-white lysozyme and 1 m*M* 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride. The suspension was sonicated on ice and insoluble debris was removed by centrifugation at 30 000*g* for 60 min. The supernatant was applied onto a cation-exchange column (P-11 phosphate cellulose, Whatman) equilibrated with buffer *A*. The column was then washed with four column volumes of a buffer containing 20 m*M* MOPS pH 7.6, 0.35 *M* KCl, 10 m*M* potassium/sodium tartrate, 10% glycerol and 1 m*M* DTT. Proteins were eluted with a linear gradient of 0.35–1.0 *M* KCl. Fractions containing Fe-SoxR were collected and applied onto a gel-filtration column (HiLoad 16/60 Superdex 75 pg, GE Healthcare) equilibrated with buffer *B* (20 m*M* MOPS pH 7.6, 0.2 *M* potassium/sodium tartrate, 1 m*M* DTT). Peak fractions containing the SoxR dimer were collected and concentrated to 10–13 mg ml^{-1} in buffer *B*. The protein concentration was determined using an extinction coefficient of 12.7 mM^{-1} at 417 nm (Wu *et al.*, 1995).

2.2. Crystallization

Crystallization of SoxR was performed by the sitting-drop vapour-diffusion method at 277 K under aerobic conditions. Several commercial screening kits from Hampton Research and Emerald BioSystems were used to determine initial crystallization conditions. Drops were made by mixing 0.5–1 µl protein solution with an equal volume of reservoir solution and were equilibrated against 100 µl reservoir solution. Several palindromic oligonucleotides were purchased from Hokkaido System Science Co. Ltd. They were dissolved in buffer (20 m*M* Tris–HCl pH 8.0, 50 m*M* KCl) at 6 m*M*. The oligonucleotide solutions were heated to 367 K and gradually cooled to room temperature. The palindromic oligonucleotides formed double-stranded DNAs by themselves. Prior to crystallization, protein solution at 360 µ*M* and the oligonucleotide solutions were mixed in a 2:1.05–1.1 molar ratio and incubated for more than 4 h at 277 K. Crystallization of the SoxR–DNA complex was carried out in the same manner as for DNA-free SoxR.

2.3. X-ray diffraction study

X-ray diffraction experiments were performed at the BL41XU and BL44B2 beamlines at SPring-8 with ADSC CCD detectors and at the NW12 beamline at PF-AR with an ADSC CCD detector. All data were processed with the *HKL*-2000 suite (Otwinowski & Minor, 1997).

3. Results and discussion

The purified SoxR was obtained as the active form containing an oxidized 2Fe–2S cluster because the purification was performed under aerobic conditions. SoxR has previously been reported to be stable for only one week at 277 K in a buffer containing >0.2 *M* KCl and 1 m*M* DTT (Wu *et al.*, 1995). After preliminary crystallization experiments, SoxR was shown to be dramatically more stable at 277 K in a condition containing potassium/sodium tartrate (data not shown). After modification of the purification procedure, initial

Figure 1
(*a*) Crystal of SoxR. (*b*) Crystal of the SoxR–DNA complex. (*c*) 15% native polyacrylamide gel of crystals of the SoxR–DNA complex. Crystals of the SoxR–DNA complex were washed, dissolved and loaded onto lanes 1 and 2. Lane 1 was stained with Coomassie Brilliant Blue for the detection of proteins. Lane 2 was stained with ethidium bromide for the detection of DNA fragments.

Table 1
Data collection for SoxR and the SoxR–DNA complex.

Values in parentheses are for the highest resolution shell.

Data	SoxR	SoxR–DNA complex
Space group	$P6_2$ or $P6_4$	$P6_122$ or $P6_522$
Unit-cell parameters		
$a = b$ (Å)	80.01	53.52
c (Å)	88.12	355.58
Wavelength (Å)	1.0000	1.0000
Resolution (Å)	50–3.20 (3.31–3.20)	50–2.70 (2.82–2.70)
Total reflections	50187	100128
Unique reflections	4881	8777
Completeness (%)	90.8 (58.8)	94.3 (64.8)
$I/\sigma(I)$	27.1 (7.2)	43.3 (5.1)
R_{sym}† (%)	5.4 (19.3)	4.5 (25.0)

† $R_{sym} = \sum |I_h - \langle I_h \rangle| / \sum \langle I_h \rangle$, where I_h is the observed intensity and $\langle I_h \rangle$ is the average intensity over symmetry-equivalent measurements.

crystals of oxidized SoxR were obtained from several conditions containing polyethylene glycol (PEG) and salts. After optimization of these crystallization conditions, crystals grew in a few days to dimensions of approximately 0.4 × 0.2 × 0.2 mm by mixing 5 µl protein solution (8 mg ml^{-1} protein in buffer *B* with 10% glycerol) with 5 µl precipitant solution [0.1 *M* Tris–HCl pH 8.6, 2%(*w*/*v*) PEG 10 000, 30%(*v*/*v*) glycerol] and equilibrating against 500 µl precipitant solution (Fig. 1*a*). Prior to data collection, crystals were taken directly from mother liquor and flash-cooled in a liquid-nitrogen stream. The crystals of SoxR diffracted X-rays to beyond 3.0 Å resolution, but the diffraction was highly anisotropic (3.0 × 3.5 Å resolution). The low value of the completeness in the highest resolution shell is a consquence of this anisotropy. The crystals of SoxR belong to space group $P6_2$ or $P6_4$, with unit-cell parameters $a = b = 80.0$, c = 88.1 Å. Assuming the presence of two SoxR molecules in the asymmetric unit, the value of the Matthews coefficient (Matthews, 1968) was 2.5 Å^3 Da^{-1}. The best diffraction data of SoxR were successfully collected at 3.2 Å resolution at the NW12 beamline from crystals soaked overnight in precipitant solution containing 0.1 m*M* $KAu(CN)_2$ (Table 1).

Crystallization of the oxidized SoxR–DNA complex was carried out using 20, 22, 24 and 26 bp oligonucleotides from the *soxS* promoter sequence. Crystals of the SoxR–DNA complex suitable for X-ray diffraction grew using the 20 bp DNA (5′-G**CCTCAAGTTAACTTGAGG**C-3′, where the *soxS* promoter sequence is in bold) from several conditions containing polyethylene glycol and inorganic compounds. After optimization of crystallization conditions followed by microseeding, hexagonal crystals of the SoxR–DNA complex grew in 10 d to typical dimensions of about 0.3 × 0.3 × 0.05 mm (Fig. 1*b*). Drops for microseeding were made by mixing 2 µl protein–DNA solution with 2 µl precipitant solution [50 m*M* Bis-Tris pH 6.6–6.8, 0.3 *M* potassium/sodium tartrate and 15%(*w*/*v*) PEG 10 000] and equilibrating against 100 µl precipitant solution. Gel-electrophoretic analysis indicated that the crystals obtained contain both the protein and the DNA (Fig. 1*c*). For data collection, a cryoprotectant solution [50 m*M* Bis-Tris pH 6.6–6.8, 0.1 *M* potassium/sodium tartrate, 20%(*w*/*v*) PEG 10 000 and 15%(*v*/*v*) glycerol] was added to the drops and the crystals were flash-cooled in a liquid-nitrogen stream. The hexagonal crystals of the SoxR–DNA complex diffracted X-rays to beyond 2.3 Å resolution, but anisotropic diffraction patterns were again observed (2.3 × 3.0 Å resolution). The hexagonal crystals belong to space group $P6_122$ or $P6_522$, with unit-cell parameters $a = b$ = 53.5, c = 355.6 Å. Assuming the presence of one SoxR monomer and half of the DNA fragment in the asymmetric unit, the Matthews coefficient is calculated as 3.3 Å^3 Da^{-1}. Diffraction data of the SoxR–DNA complex were successfully collected at 2.7 Å resolution at the NW12 beamline and are suitable for further structure determination (Table 1).

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