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Crystallographic Study of Oxidative Response Regulation System

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Background

Cellular resistant to excess oxidative stress is triggered by the activation of some specific defense genes. The molecular signals that activate these defense systems have been the objects of considerable interest. In *Escherichia coli*, the *soxRS* regulon mediates an oxidative stress response that protects the cell against superoxide radical or nitric oxide [1, 2]. The soxRS response can be distinguished in two stages. The first stage is that an intracellular signal of oxidative stress converts existing SoxR protein into a potent transcriptional activator of the *soxS* gene. The second stage is that the resulting increase in SoxS levels triggers expression of the following regulon genes.

SoxR protein is a homodimer of 17kDa subunits containing a 2Fe-2S cluster, which is essential for the transcriptional activation of *soxS* promoter [3, 4]. SoxR is composed of a N-terminal DNA biding domain that possess helix-turn-helix motif (residues 1-88), and a C-terminal domain that contains [2Fe-2S] cluster (residues 89-154). In the absence of a superoxide stress, SoxR protein shows its EPR spectroscopic signals characteristic of the reduced state ($[2Fe\text{-}2S]^{+}$), and under the superoxide stress, it become to be transcriptionally active with the oxidized state ($[2Fe\text{-}2S]^{2+}$) [5, 6]. To elucidate the mechanism of the transcriptional activation, three-dimensional structure determination of SoxR protein is necessary.

Results

We started crystallographic studies of the C-terminal [2Fe-2S] cluster domain to elucidate the structural basis of redox regulation in SoxR. Crystals of [2Fe-2S] cluster domain were obtained at room temperature by the sitting-drop vapor-diffusion method using polyethylene glycol 6K as a precipitant and lithium chloride as an additive reagent (Figure 1). The average size of crystals is 0.05mm. X-ray diffraction experiments were performed at BL6A and BL18B with crystal cooled in nitrogen stream at 100K. The crystal belongs to the hexagonal space group $P6_222$ or $P6_422$, with the cell parameters a=b=143Å, c=109Å. Assuming 6 - 10 molecules per asymmetric unit, the Matthews coefficient V_m is calculated to be 3.4 – 2.0 Å^3/Da and the solvent content of the crystal is calculated to be 63 - 40% [7]. Intensity data collection with synchrotron radiation at the BL6A was performed under the cryoconditions of 100K. The X-ray beam was monochromatized to 0.978Å. However we tried to collect intensity data with some crystals, only few diffraction spots were observed. We supposed that we could get only the low resolution data because of the size of crystals. The crystallization condition search to obtain larger crystals is in progress.

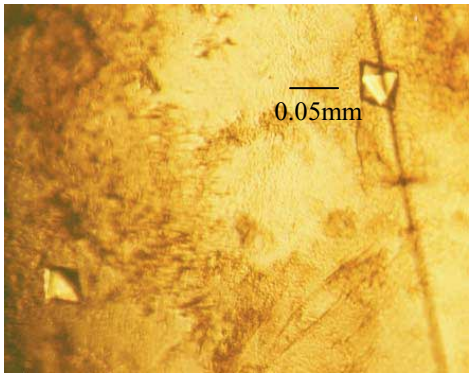

Figure 1

References

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