The X-ray Absorption Spectroscopic Analysis of SoxR Transcriptional Factor from *Escherichia coli*

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SoxR is a transcriptional factor in *E. coli* [1,2] that induces the expression of SoxS to initiate the production of enzymes in response to oxidative stress. The metallic centre of SoxR is an iron-sulfur cluster. In addition to superoxide, SoxR is also sensitive to cellular nitric oxide and is nitrosylated to produce a protein-bound dinitrosyl iron complex with a characteristic EPR signal at g = 2.03. Interestingly, this protein-bound DNIC still exhibits the capability to stimulate the subsequent transcriptional activation and induce the cellular response to diminish the nitrosative stress through the activation of *SoxS.* [3]

In this study, we have cloned and over-produced SoxR proteins that were purified through gel filtration column chromatography. The fundamental properties of the recombinant SoxR protein were verified by the encoded DNA sequence of the inserted oligonucleotide, its molecular weight from one-dimensional SDS/PAGE gel electrophoresis as well as peptide mass fingerprint analysis of the trypsin-digested protein fragments (fragments coverage: 57 %). The iron contents and the redox states of the purified proteins have also been determined to ensure that these properties are consistent with literature data [4,5]. We have further confirmed that exogenous NO and RSNO could interact with the recombinant SoxR to form protein-bound DNIC complexes with the appearance of the anisotropic EPR signal at g_{av} = 2.03 [6, 7].

The iron K-edge X-ray absorption spectra were recorded at beamline 17C of NSRRC. The data were collected at room temperature in the fluorescence yield mode using an energy-resolving 13-element germanium solid-state array dector. No photoreduction was observed in comparison between the first and last spectra for the SoxR samples. Energies were calibrated using an internal Fe foil stardard, assigning the first inflection point to 7111.3 eV.

In Figure 1, the K-edge X-ray absorpotion spectroscopic data indicated the oxidation states of the irons all appeared as Fe(III). The high intensity of 1s→3d transition at ~7113 eV indicated the coordnination number of the iron complex center would be low because of the loss of inversion symmetry of iron site.

The extended X-ray absorption fine structure (EXAFS) of the SoxR protein was resolved by fourier transform and highlighted in Figure 2. The curve fitting analysis of SoxR protein from *E. coli* shown that the coordination environment for the iron centre presumably with four sulfur atoms at 2.27 (3) Å and one iron atom 2.76 (3). The spectroscopic features in Figure 2 has indicated that SoxR from *E. coli* indeed contain a typical oxidized [2Fe-2S] cluster.[8] From these X-ray absorption spectroscopic data, we could surmise the chemical structure of active site of SoxR most likely would appear as the one in the inset of Figure 2. The predition with one SoxR protein containing two iron is consistent with the elemental analysis data via ICP-AES where one protein contained 2.7±0.3 iron elements. [2]

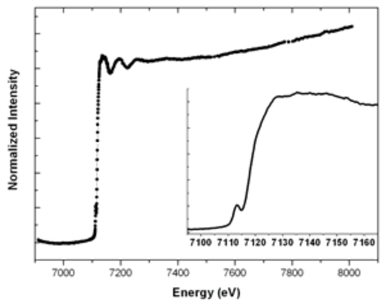

Figure 1. Fe K-edge X-ray absorption spectroscopy. The inset is the X-ray absorption near edge structure (XANES).

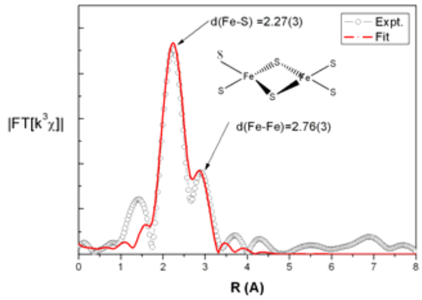

Figure 2. The extended X-ray absorption fine structure (EXAFS) of SoxR protein from *E. coli*.

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