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

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We continued to characterize the iron-sulfur cluster of oxidized and reduced SoxR proteins[1-6] from *E. coli* via X-ray absorption Near Edge Structure (XANES) and Extended X-ray Absorption Fine Structure (EXAFS) methods in order to obtain more consistent data.

The normalized absorption coefficients, μ (E), in XANES region of all three proteins are displayed in Fig. 1. All three spectra presented significant absorption intensity around 7112 eV in pre-edge region of the transition 1s→3d indicate the local geometry of Fe center could be in a noncentrosymmetric environment such as Td symmetry owing to the forbidden transition with the involvement of d-p mixing.

In comparison with the three XANES features, including the pre-edge peak positions and the rising edges, the reduced SoxR does show the reduction happened on the Fe site, which is different from that proposed on the sulfur atoms by Watanabe et al.[1] Based on crystal field theory as well as EPR results[2-3] with one unpaired electron in reduced SoxR but silent in oxidized SoxR,[4] both irons in oxidized SoxR can be assigned as Fe(III) with d^5 at $e^2t_2^3$ configuration,whereas one of irons within the cluster got reduced and converted from $e^2t_2^3$ to $e^3t_2^3$ with Fe(II) in reduced SoxR. In both proteins, the strong antiferromagenetic coupling[2-3] between both Fe sites leads to EPR silent in oxidized SoxR but one unpaired electron in reduced SoxR. As the intensity of the pre-edge peak is proportional to the unoccupied states of the transition 1s→3d, the observed intensity decreased can be rationalized to the less unoccupied states in Fe(II) than that of Fe(III).

The Fourier transformed data in R space indicated that the irons in both oxidized and reduced proteins are bonded by four S with Fe-S distance 2.26(1) Å and 2.28(1) Å, respectively. In combination with the pre-edge intensity feature, a tetrahedral environment of Fe center is proposed. Moreover, there is one nearest Fe found in the distances 2.70(2) Å and 2.71(3) Å for oxidized and reduced proteins, respectively. Therefore, a tetrahedral dimeric Fe-S cluster, [2Fe-2S], is proposed for both oxidized and reduced proteins. Both of the EXAFS fitting results have reached better consistences with the single crystal data with average bond length of Fe-S = 2.23(5) Å and distance of Fe-Fe = 2.699 Å.[7]

Both of the oxidized and reduced SoxR proteins displayed well folded structures with appropriate chirality from their CD spectra at the absorption region from 300 nm to 600 nm. The results are similar to the recent studies on Miner et al[5] and have further revealed that the iron-sulfur clusters are still intact either in oxidized or reduced state. Like most of the other proteins containing iron-sulfur clusters, the redox chemistry may not change too much on the core structures of metal active center.[6-8] (the differences in the distances of Fe-Fe or Fe-S <0.1Å) However, one-electron oxidization or reduction on the core metals may significantly change the electrostatic, hydrogen bonding or dipolar interactive environment of neighboring residues and resulting in severe conformational change within the proteins.[6-8] The ease of solvent exposure or hydration of the metal active site could be crucial to control the redox chemistry of iron-sulfur clusters.[9] SoxR must also lean on similar mechanism to adjust its redox behaviors in the presence or absence of DNA so that it could sense the oxidative damages appearing on the DNA.[10-11]

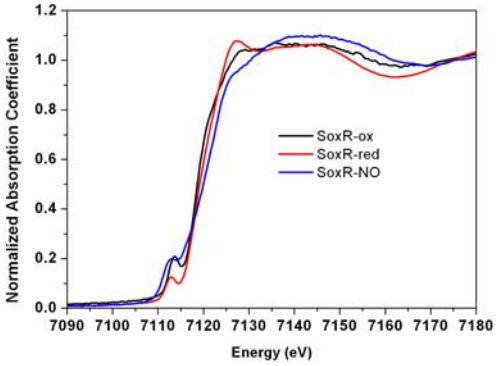

Fig. 1: The normalized x-ray absorption near edge spectra (XANES) at Fe K-edge of oxidized SoxR (black line), reduced SoxR (red line) and nitrosylated SoxR (green line).

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