

The X-ray Absorption Spectroscopic Studies of FNR Transcriptional Factor from *E. coli*

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FNR protein (fumarate and nitrate reduction) is an oxygen sensing transcriptional factor.¹ It exhibits [4Fe-4S]²⁺ cluster in *E. coli* under anaerobic condition and presents as dimeric molecules. In the presence of dioxygen, the protein will become monomer and the metal core will be converted to a [2Fe-2S]²⁺. At this stage, *E. coli* bacteria would undergo aerobic respiratory pathways. FNR protein has also been considered as significant negative regular of *hmp* gene transcription. Hmp protein is a flavohaemoglobin containing proteins and crucial for detoxification of high levels of NO production. In aerobic environment, Hmp proteins behaving like NO oxygenase to convert NO to nitrate²⁻³ whereas the corresponding proteins produce nitrous oxide under anaerobic growth.³⁻⁴ It was believed that the nitrosylated FNR protein would not responsive to the anaerobic growth where the Hmp proteins could be over-produce to dissimilate the NO stress. Therefore, the modulation of FNR mediated by NO might both be structurally and functionally significant for nitrosative stresses.

We carried out the experiments to characterize the nitrosylated iron-sulfur cluster of FNR protein from *E. coli* via determination of its X-ray absorption Near Edge Structure (XANES). The dithionite treated nitrosylated FNR was also studied here. Both of the normalized absorption coefficients, $\mu(E)$, in XANES region are displayed in Fig. 1. By comparison with the standards of iron foil and ferrous chloride, the nitrosylated FNR spectra presented significant absorption intensity around 7113 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.

From the EPR studies (Fig. 2), we can infer that the nitrosylated FNR should exhibit one dinitrosyl iron complex and the rest of the irons most likely behaving as a diamagnetic species. The observed absorption at 7113 eV of nitrosylated FNR indicated that the iron centers may all presented as tetrahedral geometry. After we reduced the species with dithionite, the diamagnetic species would gradually be reduced to anionic Roussin's red ester.⁵⁻⁶ The absorption of edge jump for the nitrosylated FNR is close to the ferrous chloride standard whereas the nitrosylated FNR treated with dithionite is around the edge jump of iron foil. The corresponding near edge data is actually consistent with the previous EPR studies. Consequently, the results here should set up a basis to allow us moving forwards for unraveling the metal core structures of nitrosylated FNR via the techniques of Extended X-ray Absorption Fine Structure (EXAFS) in near future.

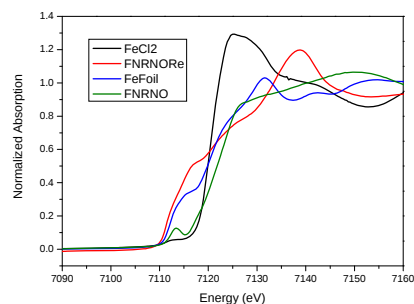


Fig. 1: The normalized x-ray absorption near edge spectra (XANES) at Fe K-edge of nitrosylated FNR (green line), nitrosylated FNR treated with sodium dithionite (red line), iron foil (blue line) and ferrous chloride (black line).

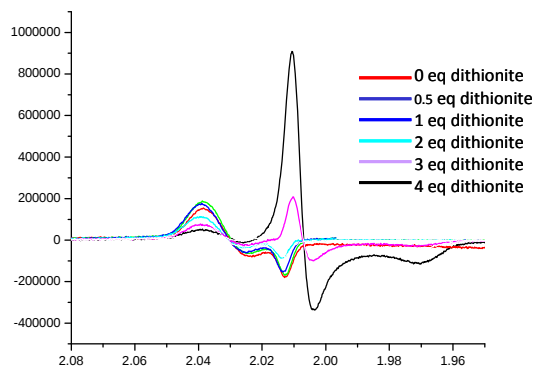


Fig. 2: The EPR studies of nitrosylated FNR protein containing [4Fe-4S]. By varying the addition concentration of dithionite, we can observe the increment of the anionic species of Roussin's red ester.⁵⁻⁶

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