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Experiment Report Form

	Experiment title: Iron trafficking in the Escherichia coli $SufBC_2D$ complex during Fe-S cluster assembly	**Experiment number**: LS-2473
Beamline: BM30B	**Date of experiment**: **from:** 30/03/2016 **to:** 05/04/2016	**Date of report**: 15/02/2017
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Report:

The aim of the experiment was to reveal Fe trafficking pathways during Fe/S cluster biosynthesys in the $SufBC_2D$ protein complex. In order to do so, we applied Fe K-edge XAS to the $SufBC_2D$ complex and to selected subunits at different stages of the Fe/S cluster assembly reaction. Our hypothesis, supported by biochemical assays performed at our home laboratory, was that flavin ($FADH_2$) triggers Fe transportation between different subunits of the Suf complex, delivering the metal to the B subunit where the Fe/S cluster is formed. XAS measurements confirmed our hypothesis, and suggested a complex scenario for Fe/S cluster biosynthesis, including unexpected intermediate steps of the reaction that will be the subject of futur studies.

Methods

Fe K-edge XAS spectra were acquired in the He cryostat of BM30B, at 15 K. Protein solutions were prepared in our home laboratory, incubated with Fe, FADH and/or SSH, then deposited on the sample holder equipped with kapton windows and immediately frozen in LN2, in order to trap the desired reaction steps. 1Fe/protein complex was added, limiting the Fe concetration in the sample to the maximum concetration allowed not to induce denaturation of Suf complexes, i.e. 1.8-2.0 mM. Being Fe extremely diluted, up to 12h integration were necessary to reach 10^6 photon counts after the absorption edge with a 30-elements Ge detector. In order to avoid radiation damage, different spectra of ~45 min each (over 1 keV energy range) were acquired on different spots of the frozen drops.

Results

We could acquire high quality XAS spectra that revealed that both $FADH_2$ and SSH induce reduction from Fe^{3+} to Fe^{2+} in the $SufBC_2D$ complex and a variation of Fe coordination environment, as suggested by the shift towards lower energies and the change in XANES spectral features (Fig.1a).

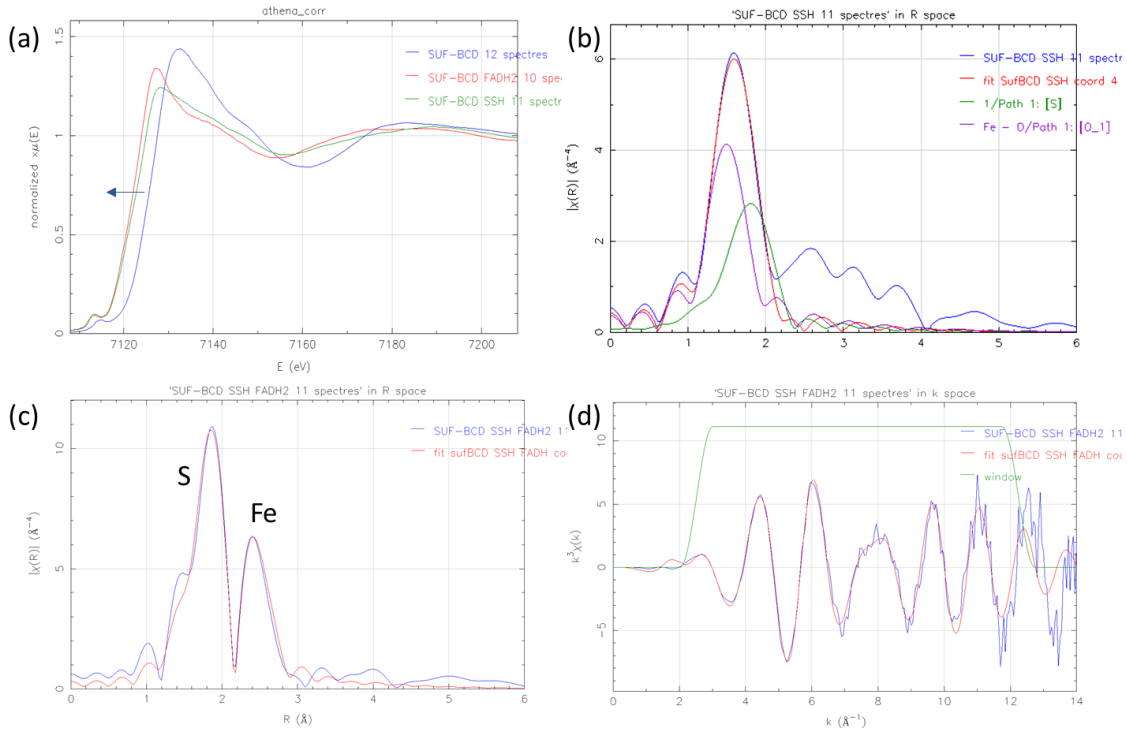

Fig. 1. (a) XANES spectra of the Fe-SufBC$_2$D complex before (blue) and after reaction with FADH$_2$ (red) or SSH (green). (b) FT experimental EXAFS spectrum (blue) of the Fe-SufBC$_2$D complex reacted with SSH, and the best-fitting curve (red) based on a combination of N/O (purple) and S (green) contributions. (c, d) Experimental (blue) and theoretical (red) EXAFS spectra of the Fe-SufBC$_2$D complex reacted with SSH and FADH$_2$, Fourier-Transformed (c) or in the k-space (d).

EXAFS data could be extracted and Fourier-transformed in the range [2.5-10] Å^{-1} for all samples, in the range [2.5-12.5] Å^{-1} for most concentrated samples.

Fourier-transformed data were subjected to first-shell analysis in order to retrieve the number and nature of Fe ligands. First-shell analysis revealed that Fe is mobilized from a 5-coordinated N/O shell to a 4-coordinated environment where S ligands are present (Fig. 1b) upon addition of FADH$_2$ or SSH. The number of N/O and S atoms is not the same in the two 4-coordinated environments, suggesting two different Fe trafficking pathways.

Surprisingly, we found that the reaction with FADH$_2$ and SSH leads to an intermediate Fe/S cluster formation, where two Fe atoms are bridged by S ligands (Fig. 1c). On the basis of first-shell analysis results, a 2Fe2S cluster model with two bridging cysteine residues was built and fitted to experimental EXAFS data, directly in the k-space. This model provides an excellent agreement with the experimental spectrum (Fig.1d), and allowed us to measure first-shell distances with high precision (Fe-S = 2.298±0.007 Å, Fe-Fe= 2.742±0.008 Å).

Perspectives

This XAS experiment revealed novel Fe trafficking pathways during biosynthesis of Fe/S clusters by the Suf proteins, and completed the scenario suggested by biochemical characterization and Mössbauer spectroscopy. The results are the subject of a publication in preparation to be submitted to a high-impact journal.

New synthesis routes were observed, which would deserve deeper investigation: the pre-formation of a Fe/S cluster, its dependence on the reactants stoichiometric ratios and on the multi-steps reaction sequence. Clarifying these issues would bring further insight into the Fe/S assembly mechanism.