ESRF	**Experiment title:** STRUCTURAL CHARACTERIZATION OF THE METAL BINDING SITES OF THE FERRIC UPTAKE REGULATION (FUR) PROTEIN BY X-RAY ABSORPTION SPECTROSCOPY (XANES AND EXAFS)	**Experiment number:** LS 718
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Report:

The aim of the project is to get a deeper insight at the molecular level in the structure and the functioning of the Ferric Uptake Regulation (FUR) protein using x-ray absorption techniques (XANES and EXAFS). This protein controls the intracellular iron concentration in Gram negative bacteria. It is activated by iron binding and operates by blocking a DNA sequence, therefore repressing the synthesis of most of the proteins involved in iron uptake as well as Superoxide Dismutase. Some of these bacteria which are resistant to antibiotics proliferate in hospitals where they are especially active against patients whose immune system is weakened. Other bacteria whose growth is highly dependent on their iron uptake system are involved in plant degenerescence diseases. Iron regulation is essential to these bacteria. Therefore a better understanding of the structure and function of the FUR protein could ultimately lead to the design of specific inactivators able to kill the bacteria.

Very scarce molecular data are available on the protein in the literature and thus a picture of the metal(s) binding site(s) cannot be drawn. We have undertaken a program aimed at elucidating the basic structural features of the FUR protein. This program combines the use of a great variety of spectroscopic, magnetic and structural techniques and takes advantage of the possibility to replace the active metal (Fe^{2+}) by several divalent ions (Mn^{2+}, Co^{2+}, Cu^{2+}) without loosing activity. Recent results [R1, R2] from electronic absorption spectroscopy, magnetic techniques and mass spectrometry have identified two different metal binding sites. The first one binds one Zn^{2+} ion whose environment has been investigated by X-ray absorption spectroscopy [R3], while the second one binds Fe^{2+} (or similar divalent ions) and involves N and 0 ligands probably in an octahedral geometry.

The methodology used to extract precise bond distances and neighbours nature and numbers from EXAFS measurements involves a four-step procedure :

1 - *The* FEFF6 code is used for *ab initio* calculations of the phase and amplitude of the retrodiffusion and mean free path of the photoelectron for every purported absorber-scatterer pair.

2 - Homoleptic derivatives are used to calibrate the other EXAFS parameters (AE and S_0^2) for every purported absorber-scatterer pair.

3 - Validation of these parameters (AE and S_0^2) with heteroleptic derivatives whose structure is known from X-ray diffraction crystallography.

4 - Determination of the bond distances and neighbours nature and numbers of unknown metalloprotein sites from simulation of the EXAFS data using the parameters calculated and validated in steps 1-3 as fixed values.

The zinc K-edge X-ray absorption spectra of the FUR protein have been analyzed in frozen solution to determine details of the zinc coordination. The spectra of the 'apoprotein' (apoFUR) and of the cobalt substituted protein (CoFUR) have been analyzed and compared in order to see the influence of the cobalt incorporation on the geometry of the zinc site. EXAFS analysis gave for both samples (apoFUR and CoFUR) a tetrahedral environment for the zinc atom with two sulfur donor ligands at a distance of 2.3 Å from the zinc and two N/O donor ligands at 2.0 Å. The two sulfur donor ligands are probably two of the four cysteines present in each FUR monomer and could be Cys 92 and Cys 95 which are known from mutagenesis studies to be essential for FUR activity. The distances obtained from our fits were always too short to be compatible with a penta- or hexa-coordination. The typical pattern observed for the Fourier transform of the EXAFS oscillations suggests the presence of at least one imidazole ligand. The XANES of these two forms of the protein are similar but significantly different. This suggests a change of the conformation of the zinc site upon cobalt incorporation. This study has provided the first unambiguous evidence for the presence of a structural zinc site in the FUR protein, *i.e.* a zinc site whose role is to maintain the tertiary structure conformation of the protein.

The interpretation of the x-ray absorption studies of both Mn^{2+} and Co^{2+} FUR derivatives is being completed. The same environment is found for the two ions [R4] with 6 N/O donor ligands at 2.21 A from the Mn^{2+} and at 2.12 Å from the Co^{2+}. Moreover, no sulfur donor ligand has been found in the metal surrounding, and by comparison with model compounds, we can propose-that at least two imidazole ligate the ions.

References

[RI] Michaud-Soret I., Adrait A., Jaquinod M., Forest E., Touati D., Latour J.-M. (1997) FEBS Letters 413, 473-476.
[R2] Michaud-Soret I. *et* al. manuscript in preparation
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[R4] Jacquamet L., Aberdam D., Adrait A., Hazemann J.-L., Latour J.-M., Michaud-Soret I. (1997) Biochemistry, in press