



Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application**:

<http://193.49.43.2:8080/smis/servlet/UserUtils?start>

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.



	Experiment title: Complexation of Co(II) by cyteine, tri- and di-peptides	Experiment number: ME 1297
Beamline: BM29	Date of experiment: from: 18/11/2005 to: 22/11/2005	Date of report: 12/01/2006
Shifts: 12	Local contact(s): P. L. Solari	<i>Received at ESRF:</i>

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Metallic compounds in biological systems have become an important field of investigation on their own due to the biochemical function of most of metals in the metabolic activities of living organisms. Therefore, the knowledge of the speciation of a metallic ion is an essential prerequisite to evaluate its behaviour in different media, to understand the mechanisms of its absorption and transport in living organisms, as well as its toxic or beneficial effects. Essential information on the speciation of a given metallic ion in a metal-bioligand system may be provided by solution studies. However, structures of the different species involved in a speciation pattern remain elusive. Clarification of the binding properties between the metallic centre and the donor atoms involved in the coordination of species arising from aqueous solution may promote our understanding of the chemical behaviour and the biological role of metallic ions.

Cobalt is a radionuclide of interest in the field of nuclear toxicological studies and particularly the ^{60}Co radioisotope, which is encountered in nuclear industry as an activation product of ^{59}Co by thermal neutrons. The existence of soluble, potentially available cobalt species, in biological fluids and tissues is essential to biological activities (benefic or toxic). In case of individual contamination by cobalt, its chemical forms have to be discovered. Consequently, structural studies of cobalt complexes involving biorelevant ligands in solution state represent a great interest regarding the speciation of this radionuclide in the biosphere and its removal from radioactive contaminated individuals. In this field, we have focused our attention on building blocks of proteins, *i. e.* amino acids and small peptides, used in model studies of local metal coordination sites mimicking the naturally occurring metallobiomolecules. Cysteine (Cys), the only naturally occurring thiol-containing amino acid, is a major metal binding site in proteins, giving unique and important properties in enzyme activity and protein structure, especially in folding and defolding mechanisms. N-(2-Mercaptopropionyl)glycine (MPG) is a synthetic sulfhydryl structural analogue of glutathione (GSH), representing the right-hand portion of this latter. Glutathione, an important cysteine-containing tripeptide (γ -Glu-Cys-Gly), is a major intracellular reducing agent and one of the important small biomolecules present in cells of all organisms at millimolar concentrations. It possesses a multitude of physiological functions. MPG

acts as radioprotective agent by scavenging reactive oxygen species produced by irradiation and has the ability to protect against drug-induced toxicity. The cobalt-cys and cobalt-MPG systems have been investigated with various initial conditions. We report here on three complexes in aqueous solution : **Co(II):3Cys**; **Co(III):3Cys** and **Co(II):1MPG**. The preparation of **Co(III):3Cys** and **Co(II):1MPG** is described in a paper accepted for publication [1].

Results

Figure 1 presents the Co K edges of the three solutions. Concerning the cobalt-cysteine system, aerobic and anaerobic conditions have been tested for the metal-to-ligand ratio (M:L) 3. The first solution is air sensitive (dark purple color) and corresponds to **Co(II)(Cys)₃** as shown in Figure 1. The spontaneous oxidation of this solution under aerobic conditions (dark green) leads to the formation of the complex **Co(III)(Cys)₃** described in the literature. Concerning the cobalt-MPG system the M:L 1 has been studied under aerobic conditions and led to the formation of a complex of 1:1 stoichiometry **Co(II)MPG**.

Furthermore, the phenomenological analysis of the XANES spectra indicates that two different Co formal oxidation states are encountered, depending on the nature of the ligand: **Co(III)** for the Cysteinato complex and **Co(II)** for the MPG complex, both under aerobic conditions. In all cases, the cation seats in an octahedral or pseudo octahedral symmetry as shown by the intensity of the pre-edge feature.

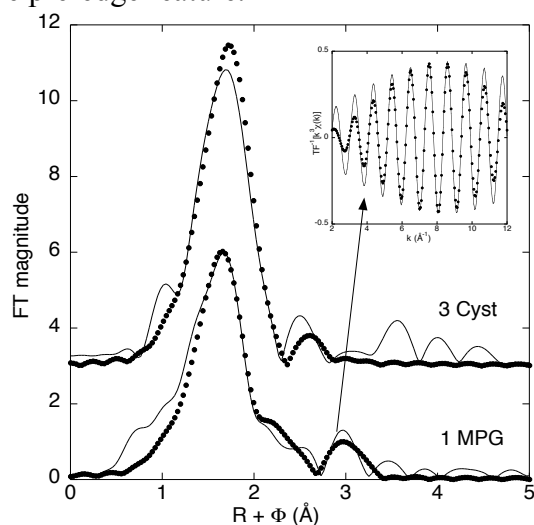
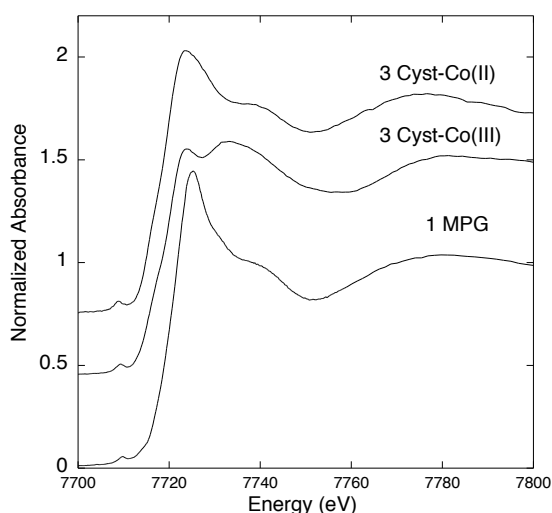


Fig. 1 : Co K edge spectra for solutions **Co(II):3Cys**, **Co(III):3Cys** (corresponding to the oxidation of **Co(II):3Cys**) and **Co(II):1MPG**. Fig. 2 : Co K edge EXAFS spectra of solutions **Co(III):3Cys** and **Co(II):1MPG**

Adjustment of the EXAFS spectra (Figure 2) have been fixed to 6 according to the general the formula: **Co(III)Cys₃** for **Co:3Cys**; and **[Co₂(II)MPG₂L₆]** for **Co:1MPG** where L is assumed to be an exogenous ligand coming from the aqueous solution, *i.e.* H₂O. The coordination sphere in **Co:3Cys** is characterized by 3 N at 2.01 Å and 3 S at 2.24 Å. As expected from the nature of the sulphur bond, the Co-S bond is about 0.2 Å larger that the Co-O/N bond. According to the EXAFS data, **Co:1MPG** is consistent with a dimer, in which each MPG is coordinated to the Co(II) centers through the carboxy and the thio functions, these later having a bridging role between the two Co(II) atoms. The octahedral sphere of the metallic cation is fulfilled by 3 water molecules. The first coordination sphere of Co(II) compares surprisingly well with the cysteine adducts in which Co is at oxidation state (III) : 4 O at 2.03 Å and 2 S at 2.26 Å. The additional Co...Co contribution provides one Co at 3.35 Å [1].

Concerning the peptidic ligand holding an amido function MPG, the thiolato group has been demonstrated to be an anchor for many transition metals, but in neutral medium, it is not effective enough for Co(II) to promote deprotonation of the amido group and subsequent coordination to the cobalt centre, involving its oxidation. On the other hand regarding the amino acid ligand Cys, it has been demonstrated that + II oxidation of cobalt was stabilised under anaerobic conditions, while +III oxidation state was assessed under aerobic conditions.

[1] *New. J. Chem.*, C. Bresson, S. Esnouf, C. Lamouroux, P. L. Solari, C. Den Auwer, accepted for publication.