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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.

ESRF	**Experiment title:** Molecular mechanisms underlying amyloidosis	**Experiment number**: LS-1979
Beamline:	**Date of experiment**: from: 03 October 2001 to: 07 October 2001	**Date of report**:
Shifts:	**Local contact(s)**: Barbara Fayard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Ana Damas, Luis Gales Pinto*

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Report:

The aim of this experiment was to study the possible role of sulfur in the aggregation of transthyretin (TTR) proteins and formation of amyloid fibrils. The design of therapies for the prevention of amyloid formation or the promotion of its dissociation need detailed knowledge of the fibrils molecular structure and a complete view about the factors responsible for protein aggregation.

TTR is a plasma protein implicated in the transport of thyroxine and vitamin A. It is a homotetramer, with an extensive β-sheet structure. Each monomer has 127 amino acids, with one cysteine at position 10 and one methionine at position 13, and forms two four-stranded β-sheets. The four monomers associate through hydrogen bonds and hydrophobic interactions around a well defined channel where is located the thyroxine binding site.

More than 80 TTR different variants are known, most of them are pathogenic and related to amyloidosis. The packing interactions of the protein subunits in TTR fibres, namely the possible role of sulfur-containing amino acids, remain unknown. Based on structural and biochemical analysis of the most frequent amyloidogenic variant, V30M-TTR, it has been proposed that amyloid fibrils could result from association of TTR molecules through disulfide bridges.

Sulfur K-edge X-ray absorption spectroscopy provides an analytical tool for the analysis of biological materials without chemical pre-treatment and consequently without perturbation of the sulfur redox state.

The K-edge spectra of the reference compounds cysteine, cystine, methionine, methionine sulfoxide and anthraquinone-2-sulfonic were recorded (figure 1). The shapes of the spectra are clearly different except for cysteine and methionine that look very similar and the energies of maximum absorption for these compounds are 2.4730, 2.4723, 2.4731, 2.4759 and 2.4809 KeV, respectively.

Soluble and fibrillar protein samples were then investigated. The soluble proteins were used at a concentration 5 to 10 mg/mL. The fibril solutions were left to sediment for a few hours and a fraction of the pellet was used to record the spectra.

Significant differences between the spectra of soluble and fibrillar samples were observed [1]. As an example the spectra of V30M-TTR are shown in figure 2. Comparison with the reference spectra indicates the presence of cysteine and/or methionine, methionine sulfoxide and sulfonated cystein, with a predominance of the more oxidized sulfur forms in the TTR fibrils. To quantify the relative amount of each sulfur form, the protein spectrum was simulated by linear combination of the spectra of the reference samples.

Sulfur oxidation possibly results from a conformational alteration of the protein during or upon polymerization, exposing these residues to the solvent.

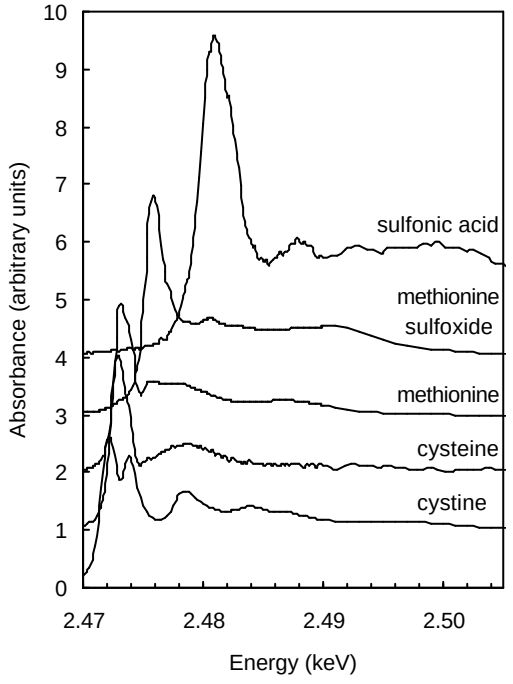

Figure 1. Sulfur K-edge XANES spectra for some reference compounds.

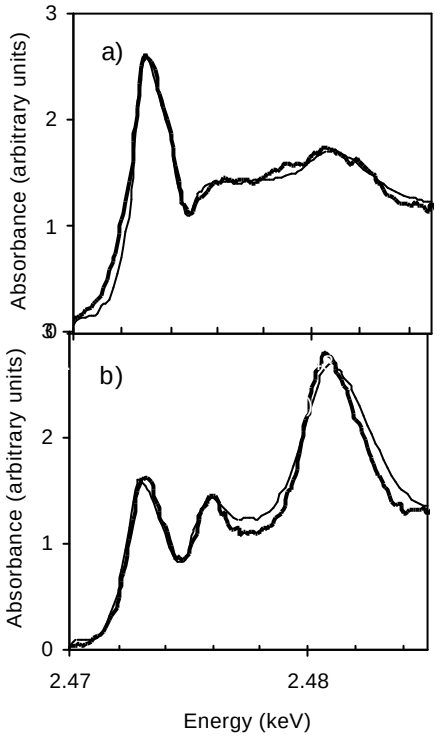

Figure 2. Sulfur K-edge XANES spectra of V30M-TTR (thick lines) and the corresponding simulations (thin lines). a) protein solution and b) fibrillar solution.

1. L. Gales, I. Cardoso, B. Fayard, M.J. Saraiva and A.M. Damas. Sulphur K-edge XANES spectroscopy of transthyretin amyloid fibres. Spectroscopy (2002) in press;

L. Gales, I. Cardoso, B. Fayard, M.J. Saraiva and A.M. Damas. Determination of sulphur oxidation state in the soluble and fibrillar forms of transthyretin by XANES spectroscopy. Presented in First International Conference on Biomedical Sprectroscopy: From Molecules to Men. Cardiff, 7-10 July 2002.