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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:** The role of sulfur containing amino acids in transthyretin fibrillization	**Experiment number**: Sc-1463
Beamline:	**Date of experiment**: from: **06/03/2004 at 8:00** to: **11/03/2004 at 8:00**	**Date of report**: 01/09/2004
Shifts: 12	**Local contact(s)**: Barbara Fayard	*Received at ESRF:*

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Report:

Transthyretin (TTR) is a homotetrameric protein. 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 crystal structure of human transthyretin was initially reported by Blake (1978) and over the years several TTR variants had their 3D structures determined with the aim to identify potential structural alterations responsible for amyloid formation. It was suggested the TTR polymerisation involves the dissociation of the TTR tetramer followed by an assembly mechanism, where two edge strands (C and D) are displaced and the fibril is formed by end-to-end alignment of the non-native monomers. This model is in agreement with the our observations that the protein sulfur containing amino acids become oxidised upon fibrillization, since these residues are in the vicinity of the displaced strand D and probably become more accessible to the solvent.

Several models for TTR amyloid fibrils have been described in the literature. While some researchers proposed that the TTR tetramers would associate through disulfide bridges, others

proposed that dimers or monomers were the building blocks. We addressed this question using constructed TTR variants in which the four monomers, in one case, and the two dimmers, in a different variant, were linked by disulphide bonding.

Sulfur K-edge X-ray absorption spectroscopy was used to confirm unambiguously that the mutated proteins were in fact linked by S-S bonds. Experiments were performed under vacuum and at cryogenic conditions.

The K-edge spectra of the reference compounds cysteine, cystine, methionine, methionine sulfoxide and anthraquinone-2-sulfonic were already in a previous experiment (LS1979). This time, in order to have a more accurate interpretation of the results, we measured the spectra of calibrated mixtures of cystine / methionine. We also measured the spectra of insulin which has 6 cysteines linked by dissulfide bridges, and confirm the presence of S-S bonds in the native and in the fibrillar form. As similar experimental conditions were used with transthyretin and insulin samples, we concluded that S-S bonds were not affected by radiation damage.

One proposed approaches for the stabilization of TTR native structure include the sulfonation of the single cysteine, situated at position 10 of the polypeptide chain, through the addition of sulfite. It was suggested that the sulfonated cystein forms a new hydrogen bond with the main chain nitrogen of Gly57, positioned in the β-strand D, contributing to the inhibition of tetrameric dissociation. We measured the spectra of samples of WT-TTR and L55P-TTR in presence of sulfite and indeed it seems that the characteristic peak of cystine is present. However, the protein concentration in the samples, and therefore the sulfur concentration, was too low to obtain definite conclusions.

Sulfur K-edge X-ray absorption spectroscopy revealed subtle differences between TTR mutants in the native and fibrillar forms. However, the interpretation of the experimental results, namely the detection of cystine, is problematic when cysteine and methionine are present in the samples. We aim to construct TTR mutants without methionine residues to clarify this question.