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ESRF

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:** Synchrotron microprobe studies on trace metal homeostasis in neuromelanin containing dopaminergic neurons during aging	**Experiment number**: MD258
Beamline: ID21	**Date of experiment**: from: 25 August 2006 to: 29 August 2006	**Date of report**:
Shifts: 12	**Local contact(s)**: Murielle Salomé	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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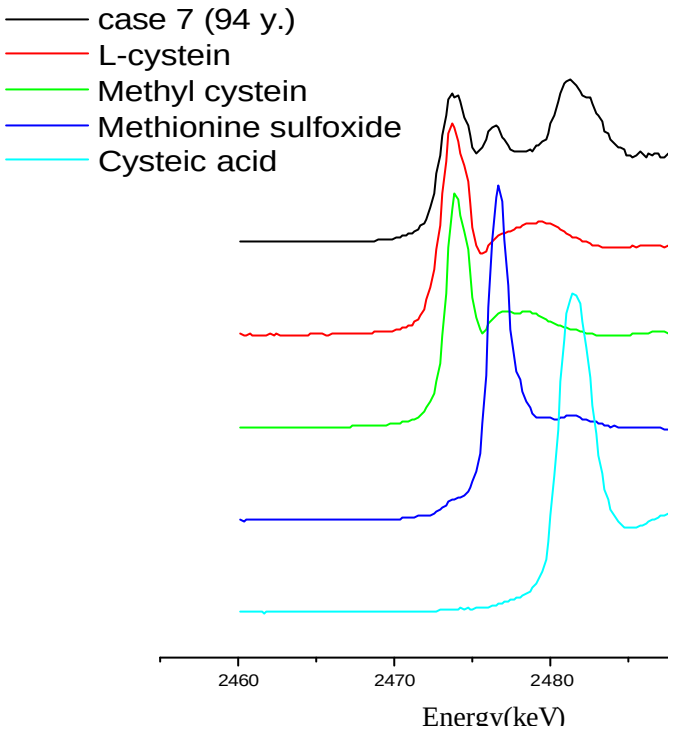
Report:

The physiological function of neuromelanin (NM) is still unknown. It is still unclear why some human dopamine neurons produce an insoluble pigment within their cytoplasm and others do not. Thus, why has not neuromelanin been discarded by natural selection? Why does neuromelanin occur within the highest phylogenetic levels? There is little information regarding the fate of neuromelanin over the lifespan and little is known about neuromelanin's structure (1,2)

Micro-speciation using synchrontron microprobe is intended to bring new information from direct analysis of cellular neuromelanin content, consequently avoid possible contamination from NM chemical extraction methods. NM is known to contain relatively high S concentration ~ 2-2.5 % sulphur (3). The sulphur speciation is of high value considering that uncertainty exist on wether human substantia_nigra neuromelanin contain sulfur aminoacid deriving moieties or not? Most investigation intends to use degradative studies, followed by HPLC, pyrolysis-GC, and MS. Moreover it has been suggested that dopamine-Cysteine-proteins adducts are either phagocytosed into autophagic vesicle to generate the characteristic membrane bound neuromelanin granule, or that dopamine quinone are formed directly within the lysosomal vesicle and then seed the formation of the neuromelanin granule. This experiment try to, following phase of development of the pigment, help in answering whether neuromelanin is auto-oxidatively produced or synthesized under enzymic control

XANES experiment at the sulfur K-edge was carried out on the undulator beamline ID21. Data were obtained with the scanning X-ray microscope in fluorescence mode. The beam was focused down to 0.3 x 0.3 μm^2 using a Fresnel zone-plate. 8 micron thick parrafin section of healthy human brain of increasing age starting from 3 years old up to 20 years old and then older patient of 74 and 94 years. Sections were mounted on 4 micron thick Ultralene foil. The neuromelanin containing neurons were imaged offline using a high resolution visible microscope. The faint pigmentation of the neuron in young brain samples makes the localization of the neurons challenging. 16 sulfur compounds were used as reference materials: glutathione (oxidized and reduced form), cysteine, methyl cysteine, N-acetyl cysteine, cystine, methionine, lipoic acid, lipoic acid reduced, methionine methyl sulfonium chloride, S-adenosyl L-methionine chloride, L-cysteine sulfinic acid, methionine sulphoxide, methionine sulfone, cysteic acid, calcium sulphate. In order to avoid photo reduction effect we experienced during the experiment, we determine the acquisition time necessary to avoid it using fast acquisition of XANES spectra (100 ms per point). The resultant sum spectrum was thus obtained from 15-20 points in NM of various neurons.

Figure 1 :X-ray absoprtion spectroscopy at the sulphur K-edge of neuromelanin contained in doapminergic neuron. Brain section from the substantia nigra of a patient 94 years old. Spectra is compared to some reference compounds used in this study.



Results shows that S present in NM is in the form of cysteine and/or methionine, methionine sulfoxide, and sulfonated cysteine. We performed least-square fitting linear combinations of standard spectra in order to estimate the % and their variation following the pigment development. The results give indication on the importance of sulphur in the pigment formation and it open exciting perspective on the importance of sulphur speciation of NM in neurodegenerative case like Parkinson diseases, Alzheimer, and possibly some difference that could be founds.This also give a basis on S speciation in NM, and it will particularly relevant to perform on diseases samples mapping of NM within neuron at the 3 differents energy features revealed by this work. These results are under preparation for publication.

References

1- Double KL, Zecca L, Costi P, Mauer M, Griesinger C, Ito S, Ben-Shachar D, Bringmann G, Fariello RG, Riederer P, Gerlach M. Structural characteristics of human substantia nigra neuromelanin and synthetic dopamine melanins. J Neurochem. 2000;75(6):2583-9.

2-Zecca L, Tampellini D, Gerlach M, Riederer P, Fariello RG, Sulzer D. Substantia nigra neuromelanin: structure, synthesis, and molecular behaviour Mol Pathol. 2001;54(6):414-8.

3- Double KL, Gerlach M, Schunemann V, Trautwein AX, Zecca L, Gallorini M, Youdim MB, Riederer P, Ben-Shachar D. Iron-binding characteristics of neuromelanin of the human substantia nigra. Biochem Pharmacol. 2003;66(3):489-94.