ESRF	**Experiment title:** Insight into mechanisms involved in silver nanoparticle intracellular dissolution in hepatocytes and its relation with metal and redox homeostasis disruption	**Experiment number**: LS2639
Beamline: BM23	**Date of experiment**: **from:** 16/03/2017 (8 a.m.) **to:** 18/03/2017 (8 a.m.)	**Date of report**: 20/02/2018
Shifts: 6	**Local contact(s)**: Olivier MATHON	*Received at ESRF:*

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Report

Following cellular endocytosis, silver nanoparticles (AgNPs) are dissolved inside cells into Ag(I) species that get coordinated mainly by thiols [1]. However, the cellular processes involved in dissolution are not known, and the cell status in terms of oxidative stress should influence the kinetics of dissolution and therefore of release of Ag(I) that are toxic for the cell. In this context, the aim of the study was to provide an accurate analysis of AgNP dissolution under different redox conditions by the combined use of nanoXRF performed on cell sections and XAS analysis performed on cell pellets. Unfortunately, we only obtained beamtime on BM23 enabling us to aquire only XAS data and thus limiting the overall results obtained.

Methods:

We repeatedly exposed HepG2 cells to citrate- or PVP-coated AgNPs at non toxic concentrations for several days. We compared normal cells exposed to AgNPs with cells simultaneously incubated with BSO, an inhibitor of glutathione (GSH) biosynthesis, thus with impaired redox homeostasis. Treated cells were pelleted, frozen and stored at -80°C until analysis. In order to trap NPs dissolution at a selected incubation time and to avoid beam damage, all the analyses were carried out at 10 K, in the He cryostat. All these samples were analyzed by Ag K-edge XAS. The energy was scanned in the 25.300 – 26.490 keV range, with constant k steps. Slits were opened in order to provide a beam of ~ 1x1 mm^2 at sample, in order to minimize the photon density ($ph/\mu m^2$) and avoid radiation damage. The signal was recorded in fluorescence with a 13-elements Ge detector. The number of scans per sample was chosen depending on Ag concentration (itself depending on the cellular uptake of AgNPs), in such a way that 10^6 total counts after the absorption edge were accumulated. This resulted in good S/N ratio spectra that could be analyzed up to k=14 $Å^{-1}$ (Fig.1).

Results:

The analysis showed that in these conditions of low concentration exposure to AgNPs, the NPs are highly dissolved. Linear combination fitting (LCF) of XANES and EXAFS spectra (Fig. 1a,b) based on reference compounds revealed that between 65 and 80 % of Ag atoms in cells are found as thiol-bonded Ag(I) species, as expected. The extent of dissolution and the mode of Ag(I) coordination are similar whatever the type of AgNP, citrate or PVP and the duration of exposure, 24 or 48 hours. This shows that, at the opposite to high concentration exposure [1], AgNPs are fastly dissolved. In addition, the impact of BSO on AgNP dissolution is very weak (Fig. 1a), with dissolution rates that do not vary within the error, regardless of its presence.

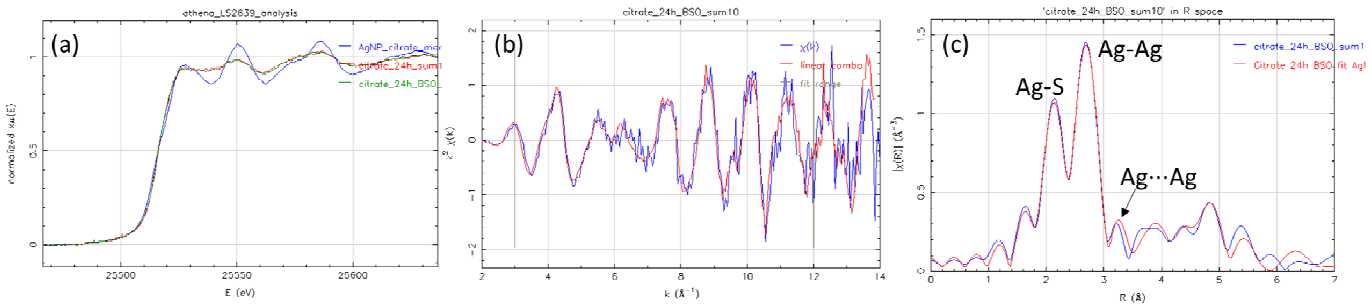

Fig. 1. (a) Experimental Ag K-edge XANES spectra of HepG2 cells exposed to citrate–coated AgNPs (red) or AgNPs and BSO (green) for 24h, and of citrate-coated AgNPs in acellular conditions (blue). (b) Experimental EXAFS spectrum of HepG2 cells exposed to AgNPs and BSO (blue), and best-fitting curve (red) based on linear combination of spectra of AgNPs and Ag-S^- complexes. (c) Fourier-transformed experimental EXAFS spectrum of HepG2 cells exposed to AgNPs and BSO (blue), and best-fitting curve (red) obtained from an *ab initio* model including Ag fcc and AgS_x sites.

In order to fit EXAFS spectra, we used reference compounds showing both AgS_2 and AgS_3 coordination that we extensively characterized in previous studies [2]. This allowed us to reveal that the AgS_x complexes formed *in cellulo* are mainly trigonal. Fourier-Transformed EXAFS spectra were also fitted *ab initio*, with a 2-component starting model taking into account both Ag in AgNPs (in a crystalline fcc structure) and AgS_x sites (Fig. 1c) [3]. Ag-S distances measured in cellulo were 2.51±0.02 Å both in samples with and without BSO, compatible with trigonal AgS_3 coordination. Interestingly, an additional Ag···Agcontribution had to be included in the model (Fig. 1c), corresponding to a non-bonding interaction with an Ag-Ag distance of ~ 3.1 Å.

This experiment brought insight into AgNPs dissolution in cellulo after low-dose exposure. Surprisingly, dissolution and complexation of released Ag(I) ions did not vary in condition of disrupted redox homeostasis. Single-cell nanoXAS experiments are needed to probe Ag speciation in cellulo in a spatially-resolved manner in order to rationalize this phenomenon.

[1] Veronesi, G., Deniaud, A. et al., Nanoscale, 2016, DOI: 10.1039/C6NR04381J.
[2] Veronesi G. et al., Inorg. Chem. 2015, DOI: 10.1021/acs.inorgchem.5b01658.
[3] Veronesi et al., Nanoscale, 2015, DOI: 10.1039/C5NR00353A.