Standard Project

Experimental Report template

Proposal title: Interaction of biological sulfur ligands with silver nanoparticles: influence of nanoparticles properties and exposure history on toxicity		**Proposal number:** 20140958
Beamline: FAME	**Date(s) of experiment:** from: 25/02/2015 to: 03/02/2015	**Date of report:** 04/09/2015
Shifts: 18	**Local contact(s):** Isabelle KIEFFER	*Date of submission:* 09/2014

Objective & expected results (less than 10 lines):

Silver nanoparticles (AgNPs) are produced in a variety of coatings and sizes that might determine their toxicological outcome. Their toxicity has been proposed to be due to the release of Ag^+ ions in cells, and to disruption of Cu^+ homeostasis through complexation of Ag^+ in biological Cu^+ -thiolate sites. The main purpose of this study was to determine the influence of AgNPs' physico-chemical properties and of the exposure scenario on their dissolution, and to predict the Ag species formed *in cellulo*. We previously defined a XAFS analysis protocol that allowed us to measure the extent of dissolution of AgNPs in macrophages[1]: by applying this protocol we expected to disclose the dissolution kinetics in hepatocytes (HepG2) and alveolar epitelial (A549) cell lines as a function of AgNP size and coating, and to measure the interatomic distances in the Ag^+ -complexes formed. This information, crossed with a careful *in vitro* characterization of Ag^+-thiolate complexes, might help in determining which biomolecules chelate the Ag^+ ions released from AgNPs *in cellulo*.

Results and the conclusions of the study (main part):

We adopted a multiscale approach to the problem, which brought us to measure the Ag K-edge XAFS spectra of three families of samples:

a) Molecular approach: characterization of the Ag^+ coordination sphere in biological Cu^+ sites. We characterized therefore the Ag^+ binding site in the human Cu^+ chaperone **Atox1**; Ag^+ was found to bind the Cys of the Cu-binding loop in digonal AgS_2 coordination. This results, together with the characterization of Ag-metallothioneins and Ag-glutathion complexes (data acquired on FAME during the ESRF experiment LS-2331), have been submitted to *Inorganic Chemistry* for publication. [2]

b) Nano-scale approach: determination of the fraction of Ag^+ released from **AgNPs in solution with biological chelators**. Citrate-coated 20nm AgNPs were incubated for 6h or 24h with Metallothionein (MT) or Atox1, or with bio-inspired molecules mimicking the chelating site of these proteins ($\mathbf{P^2}$ for Atox1 [3], $\mathbf{L^1}$ for MT [4]), or with glutathion (GSH), at constant Ag/site ratio. XANES/EXAFS analysis of these solutions allowed us to measure the fraction of Ag^+ ions relased from AgNPs as a function of time: the purpose was to highlight the capability of biological chelators to foster NP dissolution (e.g. by comparing GSH with $\mathbf{P^2}$ and $\mathbf{L^1}$), and the influence of the protein scaffold on this process (e.g. by comparing $\mathbf{P^2}$ with Atox1 and $\mathbf{L^1}$ with MT); the detailed analysis of this series of samples is still ongoing.

c) Cellular approach: determination of AgNPs dissolution and Ag^+ speciation *in cellulo*. We exposed two cell lines (HepG2 hepatocytes and A549 alveolar epitelial cells) to PVP- and citrate-coated AgNPs at sublethal concentration for 6h, 24h, 48h or in chronic mode for 96h (where the same total dose in supplied in 4 daily exposures). The Ag K-edge EXAFS spectra were then interpreted on the basis of a two-components model: Ag^0 in fcc crystal structure (i.e. Ag atoms in undissolved NPs) and an AgS_x component; the model, described in ref. [1], allows for the determination of the fraction of Ag^+ atoms released *in cellulo* and of the Ag-S bond length in the Ag-thiolate complexes formed. We observed a progressive release of Ag^+ ions with incubation time in both cellular models, which is enhanced in chronic with respect to acute exposure (see Figure 1). Dissolution rates are higher for citrate- than for PVP-coated NPs. The fits of the EXAFS region of the spectra based on *ab initio* models provide an excellent agreement with the experimental data (see Fig.2); the measured Ag-S distances suggest the presence of both AgS_2 and AgS_3 species in HepG2 cells, and a large predominance of AgS_3 in A549. These results are merged with subcellular Ag localization obtained through micro- and nano-XRF imaging on beamlines ID21 and ID16B of the ESRF, as well as with biochemical and cell biology assays performed in our home laboratory, and are the subject of two distinct publications in preparation.

Justification and comments about the use of beam time (5 lines max.):

The 18 allocated shifts were fully exploited and all the prepared samples could be measured. Lowest concentration samples (~2 mM) needed ~5 hours integration time each to provide 10^6 total photons above the absorption edge, thanks to the high sensitivity of the 30-elements Ge detector. The He cryostat available on FAME allowed us to performe the experiment at 16K, which was

crucial to assure sample preservation and to reliably assess the speciation of Ag.

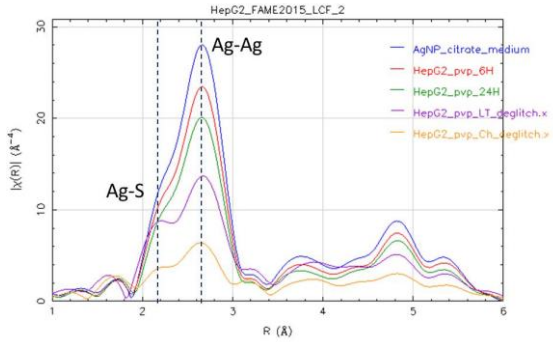

Figure 1: Fourier-transformed experimental Ag K-edge EXAFS spectra of PVP-coated AgNPs diluted in cell culture medium (blue), and of HepG2 cells exposed to PVP-coated AgNPs for 6h (red), 24h (green), 48h (purple) or chronically over 96h (yellow).

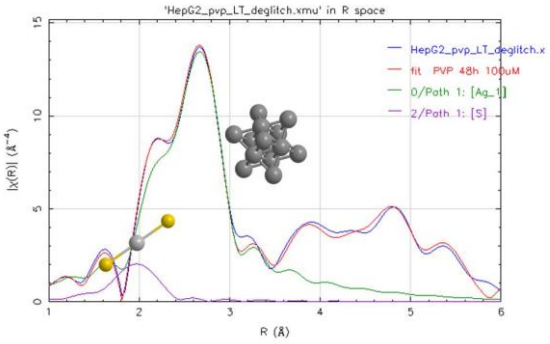

Figure 2: Fourier-transformed experimental Ag K-edge EXAFS spectrum of HepG2 cells exposed to PVP-coated AgNPs for 48h (blue), and its best-fitting curve (red) based on *ab initio* models. Contribution of first-shell Ag-S (purple) and Ag-Ag (green) paths to the final fit.

Publication(s):

[1] Veronesi, G.; Aude-Garcia, C.; Kieffer, I.; Gallon, T.; Delangle, P.; Herlin-Boime, N.; Rabilloud, T. & Carriere, M. Exposure-dependent Ag^+ release from silver nanoparticles and its complexation in AgS_2 sites in primary murine macrophages *Nanoscale*, **2015**, *7*, 7323-7330 (**Based on ESRF experiment LS-2331 on FAME**)

[2] Veronesi, G.; Gallon, T.; Deniaud, A.; Boff, B.; Gateau, C.; Lebrun, C.; Vidaud, C.; Rollin-Genetet, F.; Carrière, M.; Kieffer, I.; Mintz, E.; Delangle, P.; Michaud-Soret, I. XAS investigation of silver(I) coordination in copper(I) biological binding sites. *Inorg Chem*, submitted. (**Based on this experiment and ESRF experiment LS-2331 on FAME**)

References:

[3] Pujol, A. M.; Cuillel, M.; Renaudet, O.; Lebrun, C.; Charbonnier, P.; Cassio, D.; Gateau, C.; Dumy, P.; Mintz, E.; Delangle, P. *J. Am. Chem. Soc.* **2011**, *133*, 286-296.

[4] Pujol, A. M.; Gateau, C.; Lebrun, C.; Delangle, P. *J. Am. Chem. Soc.* **2009**, *131*, 6928-6929.