FAME-BM30B CRG Beam Time - exp 30-02 980 - *Local contact Olivier Proux*

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Experiment report

Introduction

Silver nanoparticles (AgNPs) are frequently used as antimicrobials. While the mechanism(s) by which AgNPs are toxic and unclear, their increasing use raises the concern that release into the environment could lead to environmental toxicity. We characterized the physicochemical behavior, uptake, toxicity (growth inhibition, embryo mortality), and mechanism of toxicity of AgNPs coated with polyvinylpyrrolidone (PVP), gum arabic or citrate coatings to the embryo of *Fundulus heteroclitus* (killifish). Our embryotoxicity results suggest that marine and estuarine systems, which may accidentally receive AgNPs, may be particularly susceptible to the embryotoxic effects of AgNPs (Matson et al. 2010). The aim of this XAS experiment was to determine the speciation of Ag in the embryo and to elucidate the physico-chemical mechanisms of embryotoxicity.

Experimental details

Three sets of samples (prepared at Duke university, USA) were analyzed during this experiment: (A) entire *Fundulus heteroclitus* embryo incubated with AgNPs, (B) their chorions (outer membrane), and (C) the dechorionated embryo.

The fish embryos were incubated during 48h at 28ºC with respectively 5 mg/L of citrate-coated AgNPs, 0.5 mg/L of gum arabic-coated AgNPs, 0.5 mg/L of PVP-coated AgNPs, or $AgNO_3$. All these incubations were done both in pure water and within a saline media (10 mg/mL of Instant ocean® salts). After incubation, the embryos were lightly rinsed with deionized water. Half of them were freeze-dried (set A) and the others were dechorionated (removal of the outer membrane enclosing the embryo). Both the removed chorions (set B) and the dechorionated embryo (set C) were freeze-dried and analyzed. We estimated that the concentrations of silver in our samples are ranged between 1 to 100 ppm (the ICP-MS measurements are in progress).

The dried samples and reference compounds (AgNPs before any interaction with organisms, Ag_2S, AgCl, $C_6H_5Ag_3O_7$, $AgNO_3$, Ag_3PO_4 and Ag_2O standards) were diluted in cellulose and pressed into thin pellets. The pellets were cooled down to a temperature close to that of liquid Helium (around 10 K) during spectra acquisition. This procedure improves spectrum quality by minimizing radiation damages, and decreasing thermal motions of atoms. XAS analysis was done either in the fluorescence mode (using the 30 elements fluorescence detector, due to the low amount of Ag in the samples) or in transmission for the reference compounds. In order to improve the energy resolution in the XANES a Si(220) monochromator was used.

Moreover, a feasibility experiment was performed on a link of the trophic chain of the nematode *C. elegans*. Adult worms (3 days old) were fed with bacteria and PVP- or citrate-coated AgNPs. Previous toxicity experiments have shown that both of them induce a strong decrease of the growth of the *C.elegans* and different mechanisms were suspected (Meyer et al. 2010). XAS at the Ag K-edge could help us to identify them. Two sets of samples were analyzed: *(i)* the bacteria (*Escherichia coli*) incubated with AgNPs and *(ii)* the adult *C. elegans* incubated with the bacteria and the AgNPs simultaneously. Both samples were freeze-dried, pressed into pellets and analyzed as precise above.

Main results

First, reference compounds of silver with different oxidation states and different atoms in the first atomic shell were analyzed (Figure 1 A and B), as well as the three coated AgNPs (Figure 1 C) before any interaction with the embryo. We observed that silver within the synthetic AgNPs (citrate-caoted and gum arabic coated) and the commercialized AgNPs (PVP-coated AgNPs) is mostly Ag^0. Whereas the citrate- and gum arabic-coated AgNPs are very small (7 nm ± 11 and 6 nm ± 2nm), the PVP-coated silver AgNPs are larger 21 nm ± 11 nm. Since XAS gives global information on the oxidation state, we are more sensitive to the surface of the small NPs for which about 30% of the atoms are at the surface compare to the PVP-coated. Consequently we might miss a possible oxidation of the surface of the PVP-coated AgNPs.

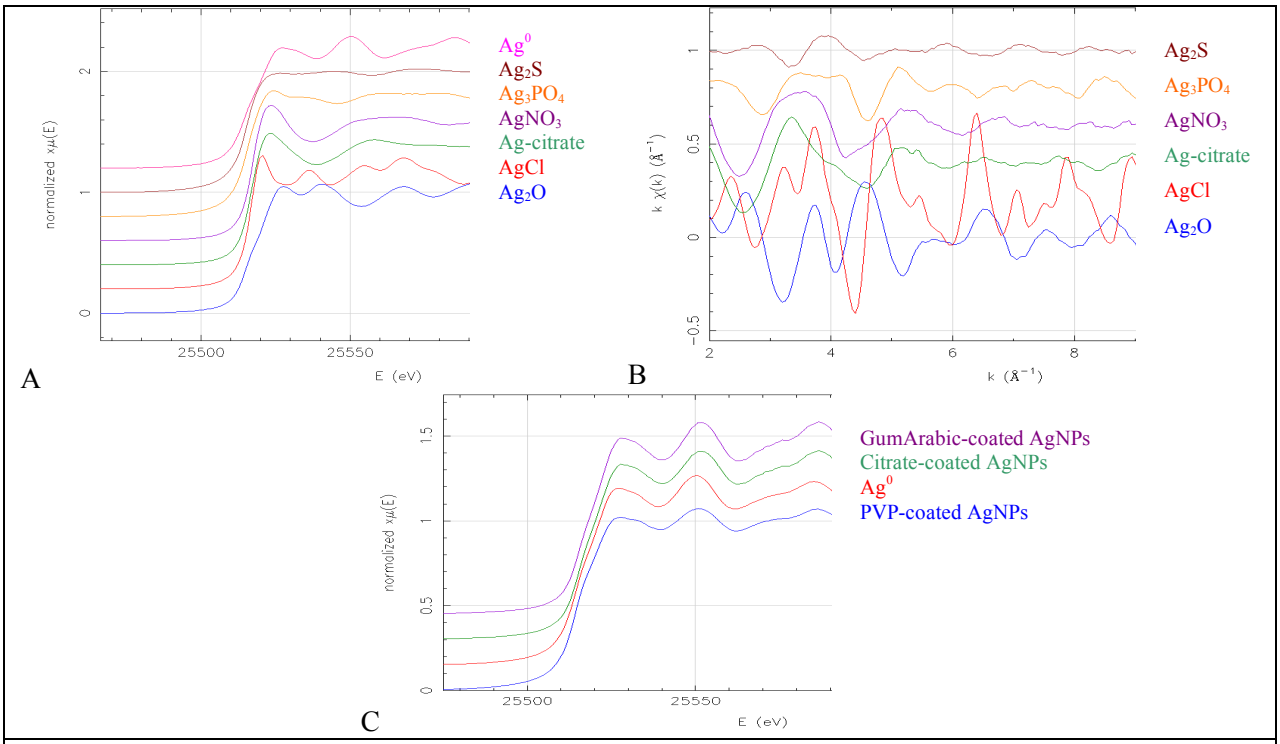

Figure 1. XANES and EXAFS spectra at the silver K-edge of reference compounds (A, B) and the coated silver nanoparticles (C) studied.

Then, we analyzed the outer membrane (chorion) and the dechorionated embryo (Figure 2). The three kinds of AgNPs strongly interact with the chorion, even after the rinsing process whatever the salinity of the media is. Changes in the speciation are observed in all cases, expect for the citrate-coated AgNPs for which silver remains metallic when the incubation is done at high salinity. Using linear combination of EXAFS and XANES spectra, we found that an oxidation/sulfidation occurs. The exact speciation of silver still remains to be determined. Simulation using FEFF and the ifeffit package are in progress.

Interestingly, we observed citrate- and gum arabic-coated AgNPs within the dechorionated embryo whereas we do not detect any silver after incubation with the PVP-caoted AgNPs. Both citrate- and gum arabic-coated AgNPs passed through the chorion membrane whatever the salinity of the media was. However, while they both remain under a metallic form at high salinity, we observed a change in speciation in pure water. Using linear combination of EXAFS and XANES spectra, we found that in pure water an oxidation/sulfidation occurs. The exact speciation of silver has still to be determined.

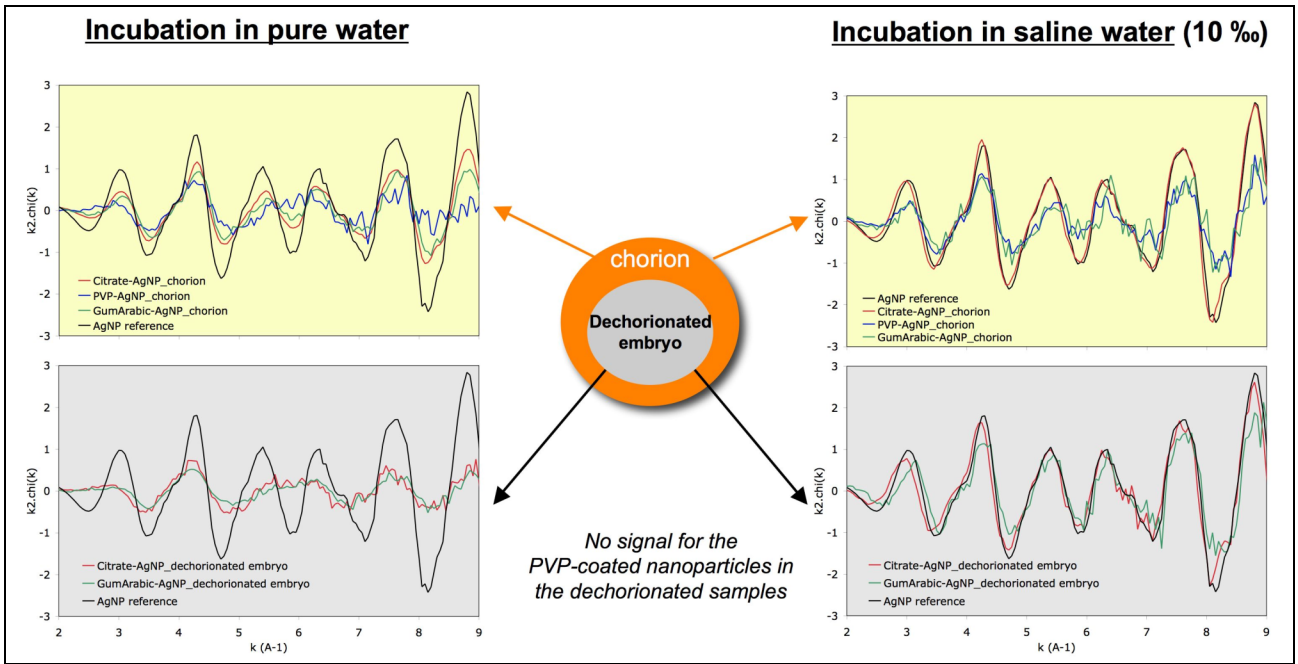

Figure 2. EXAFS spectra at the silver K-edge of silver in the chorion and the dechorionated embryo within two media (pure water, and saline water).

At the end of this experiment, a feasibility test was performed on the nematode *C. elegans*. The aim was to study the speciation of silver after incubation with the worms and the bacteria used to feed them. As shown in figure 3, no change in the speciation of the coated-AgNPs was observed for the PVP-coated and citrate-coated AgNPs.

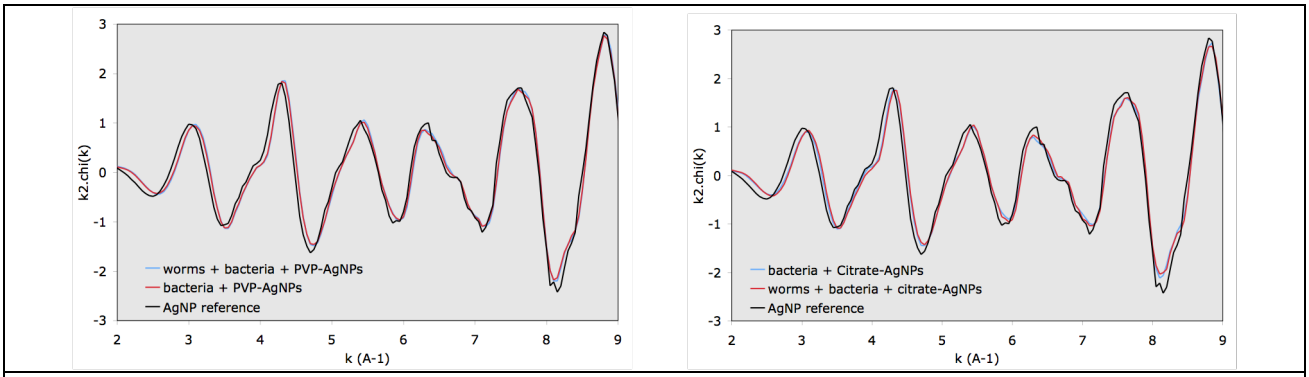

Figure 3. EXAFS spectra at the silver K-edge of silver nanoparticles incubated with worms and the bacteria used to feed them. No change in the speciation of the AgNPs was observed before and after incubation.

References

Meyer, J., C. A. Lord, X. Y. Yang, E. A. Turner, A. R. Badireddy, S. M. Marinakos, A. Chilkoti, M. Wiesner and M. Auffan (2010). "Intracellular uptake and associated toxicity of silver nanoparticles in Caenorhabditis elegans." aquatic toxicology **in press**.

Matson, C. W., (conferencier), M. Auffan , M. R. Wiesner and R. T. Di Giulio (2009 november). "Silver nanoparticle behavior and fish embryotoxicity across a salinity gradient." SETAC **New Orleans, USA**.