	Experiment title: Arsenic toxicity: Cellular mechanisms of arsenic transfer and resistance: effect of Fe magnetic nanoparticles	**Experiment number**: 30 02 740
Beamline: BM30b	**Date of experiment**: from: 29 June 2005 to: 4 Jully 2005	**Date of report**: 26/09/05
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Report:

Aim of the project

Arsenic is a toxic metalloid largely studied worldwide. Its toxicity is known to be related to its oxidation state and speciation. In cellular media, arsenic shows a strong affinity to thiol functions, which play an important role on As biocellular transformations and cancerogenic effects. Serious health effects due to high rates of arsenic in drinking water, amplified the interest in minerals such as iron oxide which can immobilize arsenic species on their surfaces and so decrease their toxicity. Recently, Nano-Maghemites (iron oxide nanoparticles with a diameter <10nm), are studied for biomedical purposes (MRI contrast agents, drug delivery or cell engineering). These nanoparticles are very attractive because approximately 50% of their atoms are at the surface that increases significantly their surface energy, reactivity and affinity with adsorbates.

In that context, our project aimed to assess the synergetic effects of Nano-maghemites and As on their mechanisms of transfer and toxicity for human cells (fibroblasts). Since the interaction between Fe and As is strong and the surface developed by nanoparticles is high, it can be postulated that Nano-maghemites can decrease the transfer of As onto fibroblasts and consequently lower As toxicity. On the other hand Fe nanoparticles can be internalized coated or not with arsenic and induce toxic effects.

The aim of this XAS experiment was (1) to determine whether or not Nano-maghemites can fix As initially present in the extra and/or intracellular media and (2) to study the at the atomic scale the As/Nano-maghemites surface interactions (As adsorption, presence of competitive anions, surface passivation…).ADDINADDIN

Experiment

The experiment was conducted on BM-30b beamline 'FAME' from 29th June to 4th July 2005. During the measurement of the XAS spectra the samples (under liquid or lyophilized forms) were cooled at the liquid Helium temperature (4 K) in a cryostat. This procedure allowed to improve spectrum quality by minimizing radiation damage and by decreasing thermal motions of atoms. Moreover, the use of a cryostat, allowed to keep the As in the same oxidation state during the experiment which is necessary in our experiment.

The sample analysed, contained 27 mg/L of Nano-maghemites coated with DMSA (2,3-dimercaptosuccinic acid) and 30 μM of As_2O_3, which were injected in the fibroblasts media. The coating with DMSA was used to enhance the colloidal stability of Nano-maghemites at physiological conditions. This sample was analysed in fluorescence mode in two times: (1) the cellular part and (2) the nutritive cell solution. Thirteen organic and inorganic As references compounds at different valence and local atomic environment were also analyzed (As_2O_3, As_2O_5, As_2S_3, phenylarsine, arsanilic acid, arsenopyrite…). For each reference, 3 to 6 scans were performed in transmission mode. At the end of the aquisition the signal/noise ratio of the XANES and EXAFS spectra was extremely good.

First results

We studied the impact of the sample freeze drying process on the As speciation. This point is of high importance in order to confirm that no modification of the As speciation could be generated by the sample preparation. Figure 1 shows the EXAFS and the corresponding Fourier Transform of the sample containing cells initially freeze dried or not. No modification of the As oxidation state due to the freeze drying process is observed. Then, in the continuation of the XAS experiment, the samples and references compounds were analyzed under lyophilized form.

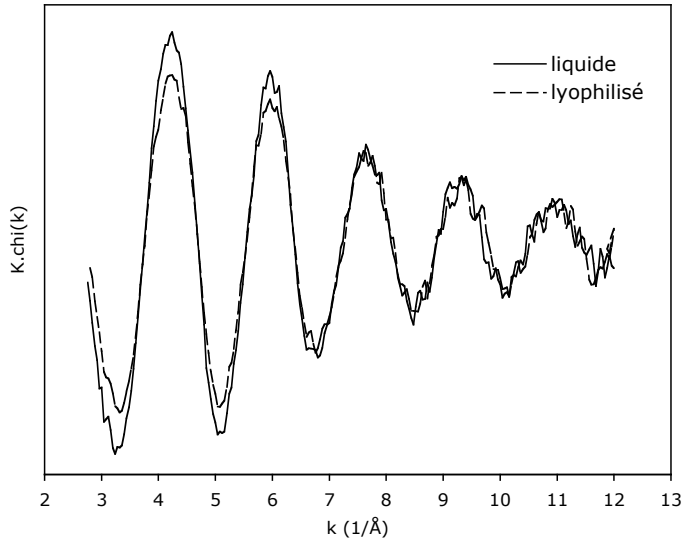

Figure 1 : EXAFS spectra of the fresh and freeze dried cell samples

In order to determine the effects of Nano-maghemites on the As behaviour in contact with fibroblasts, we compared the As oxydation state and local atomic environment in the intra- and extra-cellular medium versus reference compounds (figure 2).

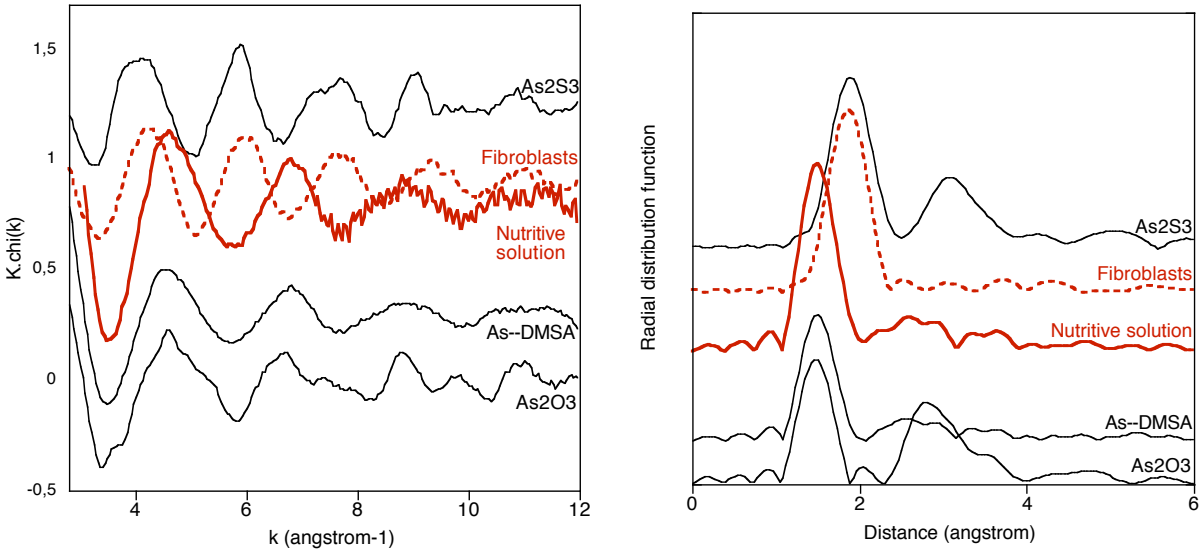

Figure 2 : EXAFS and Fourier Transform of As in the intra- (fibroblasts) and extra-cellular medium (nutritive solution) compared with reference compounds.

The figure 2 shows that the arsenic speciation in the intra- and extra-cellular medium is different:

- In the intracellular part: the speciation of As which is internalized by fibroblasts is similar to As speciation in As_2S_3. Due to these spectra and to the XANES results, we can suppose that an important part of the intra-cellular As is trivalent and link to sulfidric groups (glutathione, cysteine, dithiols from proteins…). It doesn't seem that As is linked to the Nano-maghemites surface but these hypothesis have to be confirmed by the EXAFS modelisations which are in progress.
- In the extracellular solution: there are a lot of similarities between the As speciation in the extra-cellular medium and As which is linked to DMSA coated Nano-maghemites (called "As-DMSA"). The XANES results show that As is trivalent in the extra-cellular medium with oxygen atoms in the first coordination sphere. EXAFS modeling are in progress.

These preliminary results need to be confirm, but it seems that Nano-maghemites are not internalized by cells and that they adsorb As on their surface via DMSA.