ESRF

Experiment title: Toxicité de l'arsenic: mécanismes cellulaires de transfert et de résistance à l'arsenic Arsenic toxicity! : Cellular mechanisms of arsenic transfer and resistance.	**Experiment number**: 30-02-667

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Report:

Introduction

Arsenic is a significant environmental concern worldwide because millions of people are at risk of drinking water contaminated by arsenic. Epidemiological data show that chronic exposure of humans to inorganic arsenic is associated with hepatic injury, peripheral neuropathy, and increased rates of a wide variety of cancers, particularly of the skin, lung, bladder, and liver. Arsenic also produces toxic effects on the female reproductive system, including ovarian dysfunction, aberrant embryo development and lethality, and postnatal growth retardation. Interestingly, it also has proven useful for anti-cancer therapy, although the mechanisms underlying its paradoxical (antineoplastic) effects remain unclear. The predominant form of inorganic arsenic in aqueous environments is arsenate (As(V)) whereas arsenite (As(III)) occurs in anoxic environments. The mode of toxicity is strongly related to the arsenic chemical form. As(III) is known to be more soluble and toxic than As(V). In mammalian cells, As(III) can binds to sulfhydryl groups and impairs the many proteins functions. More over As(III) can interacts with DNA resulting in single-strand DNA breaks (Oremland et al, 2003). Most mammals methylate inorganic arsenic to methylarsonic (MMA) and dimethylarsinic acid (DMA). Compare with inorganic species, the methylated arsenic forms are less toxic and more readily excreted in the urine. The biomethylation of arsenic has long been considered as one major detoxification process. However recent studies have shown that methylated As(V) and As(III) can induce oxidative DNA damage. (Vahter et al, 2000; reviewed in Vahter, 2002).

Carter et al. (2003), concluded their important review on the metabolism of inorganic arsenic oxide by different questions that are still unanswered. One important point concerns the mechanism of As transport in the cell and Carter et al. (2003) indicated that we still have to *"demonstrate the displacement of the arsenic glutathione complex to form the As(III)-lipoate using in vitro system? Can we do it on biological systems?"* They also concluded that '*unfortunately it is not easy to determine an accurate oxidation state for arsenic that reflects its potential reaction*".

The aim of our work was to access the capability of X-ray Absorption Spectroscopy to determine the arsenic speciation in situ and to answer or help answering to some of the latter questions.

Material and methods.

Human fibroblast cells were obtained after removal of skin fragments during plastic surgery operations. Cells were grown in DMEM medium containing 10% of calf serum. Cultures were performed at 37.8°C in a 5% CO_2 atmosphere. Powdered As_2O_3 was dissolved in 1 N NaOH as 1 M solution (As^{III}) then diluted with PBS and added directly to the nutritive medium. A fresh 1M As^{III} solution was prepared for each new experiment. The As concentration in the nutritive medium was 2.10^{-5} $mol.l^{-1}$. Cells were seeded at 1 x 10^5 cells/ml in 96 well micro-plates for 24 hours. In the report 'Nutritive medium' and 'As-fibroblast' refer to the extra-cellular medium and intra-cellular medium respectively.

XAS experiments have been conducted on the XAS CRG BM-30b French beamline (FAME) at the As K edge. The Si(111) monochromator was used. The beam was focused vertically on the sample using the 2nd mirror and horizontally with the second crystal of the monochromator. The spot size was 200*200μm. XAS spectra were recorded in the fluorescence mode with a high purity Ge multichannel fluorescence detector. We have tested various setups to keep arsenic in its initial oxidation state and to prevent any modification due to high X-ray photon flux. In a first set up, human fibroblasts contaminated with arsenic (III) were placed in 1 mm diameter quartz capillary before XAS analysis. Before selling the capillary, a setup was fixed at the entrance of the capillaries to remove air and replaced by N_2. This protocol was repeated four times to prevent the presence of oxygen in the capillaries. A second set up was tested using Borosilicate capillaries (free of As…) sealed after air removed. Both capillaries gave same results. For solid compounds (powder references compounds), samples were pressed and the pellets were placed in a vacuum chamber. Prior to XAS sample analysis we have performed spectra acquired in the continuous scanning mode (Quick-EXAFS) to test the various setups and to ensure that the extremely high photon flux did not induce changes of the oxidation state and speciation of arsenic during measurements and that oxygen was properly remove from the capillaries. Because the scans are acquired rapidly (120 s/scan), any radiation-induced modifications of the redox state of arsenic can be determined by comparing the individual data scans over the first 2 minutes. For all samples no changes were observed. After this step, XAS were collected in a step-by-step mode.

Results:

Comparison between EXAFS spectra of reference compounds and samples suggests that the arsenic atomic environment of both samples resembles the atomic environment of As2S3 (figure 1). In the low k region 'As-fibroblasts' and 'nutritive medium' EXAFS spectra are in better agreement with oscillation features of As2O3. From the Fourier transforms it clearly appears that the second peak of the RDF of the nutritive medium and the As-fibroblasts is centered at the same position with the first peak of As2S3 RDF for which As is surrounded by 3 sulfur atoms at 2.25 Å. Therefore sulfur is present around As for both samples.

Partial EXAFS spectra have been modeled. The analysis of the first coordination sphere (number and distance of atoms surrounding As) indicates that arsenic from the nutritive medium is mainly linked to sulfur atoms (>90%) and 100% of As is in the 3+ form. In the case of the As-fibroblasts results indicate that 56 %±10 of As can be involved in As-(thiol)3 molecules and that 42%±8 of As is in the 5+ form.

From the analysis of the second coordination sphere we have determined the presence of carbon atoms around As. In the case of the nutritive medium the presence of carbon is due to the formation of As-SR_3 complexes. Indeed if one R-SH molecule is linked to As therefore a As-(S)-C linkage exist for which the As-(S)-C interatomic distance is in a 2.85-3.40 Å range. The latter As--C interatomic distance range has been established by the examination of a high number of reference molecules from the CSD database (Cambridge Structural Database (*www.cmbi.kun.nl/cheminf/freeserv.shtml*) for which As-(S)-C linkages exist.

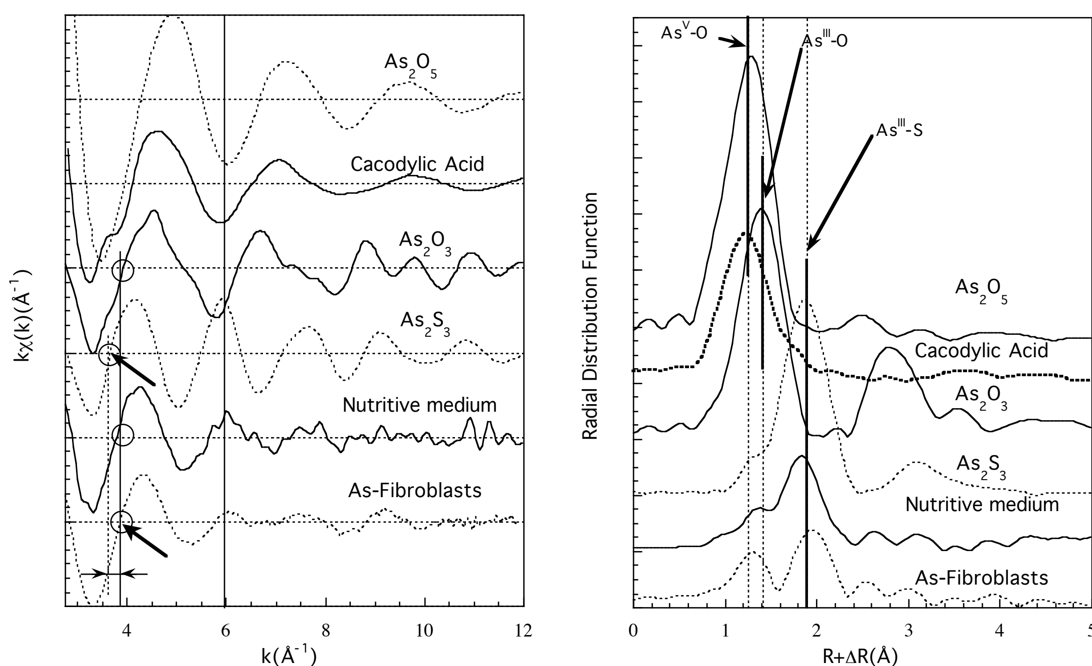

FIGURE 1. (a) Raw EXAFS data and (b) corresponding modulus of the Fourier transforms for As-fibroblasts and nutritive medium and arsenic model compounds.

In the case of the As-Fibroblasts, As-(S)-C distances decreases from 2.97 to 2.87 Å and 3.25 to 3.15 Å for As--C_1 and As--C_2 compared to the extra-cellular media. The decrease of the As-(S)-C interatomic distances can be due to a modification of the As-thiol linkage type. Arsenic can be linked to monothiol or dithiol molecule. The modification of linkage type between monothiol and dithiol has been proven in aqueous solution experiments (Carter et al, 2003). Lipoic acid (dithiol) presents the ability to displace glutathion (monothiol) linked to As. The decrease of the As-(S)-C distance between bidentate and monodentate is certainly linked to the increase of the bond strength and the change of the interatomic angles. The bond strengths are generally substantially larger for the dithiols (bidentate) than for two monothiols (monodentate) (Carter et al, 2003).

Conclusion

Even if the initial arsenic concentration was low (2.10^{-5} $mol.l^{-1}$) the use of a third generation synchrotron X-ray source enables to obtain XAS spectra with a high signal/noise ratio. Results indicate that arsenic remains in the 3+ form in the nutritive medium and that almost 90% of arsenic is linked to thiol molecules from the DMEM medium (cysteine). Only 10% of the initial arsenic is present as arsenite which suggests, if we assume that As-cysteine can not be transported though membrane cell, that only 10% of the initial concentration is toxic toward fibroblast cells. We have also determined that almost 45% of As(III) is oxidized in the intracellular medium. The fraction of As linked to sulfhydryl groups corresponds to approximately 55%. It is interesting to note that the structure of the As-thiol compounds evolves from the nutritive medium to the cell. It seems that the As is complexed to monothiol in the nutritive medium and to dithiol in the cells certainly from proteins. The structure of the As(V) fraction in cells is more difficult to interpret. The presence of MMA and DMA is not proven by XAS.

Literature

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