Selenium reduction by Agrobcaterium tumefaciens

The reduction of selenite (Na_2SeO_3, IV) by strain C58 of *Agrobacterium tumefaciens* [1 , 2] follows two different and independent detoxification pathways, which are not yet characterized at the molecular level. Selenite is either reduced directly to elemental selenium (0) [3] or to dimethyl selenide (CH_3-Se-CH_3, II) and di-methyl-di-selenide (CH_3-Se-Se-CH_3, II) [2]. Metal reduction and oxidation kinetics are accessible to X-ray analyses [4, 5]. We have shown that the combination of μXANES and μXRF analyses allow the efficient monitoring of the oxidation states of Selenium in solution during microbial reduction [4], with a detection limit lower than 1ppm in cultures performed at room pressure in the laboratory.

Performing the same analyses *in situ* requires the direct contact between the live cells and the X-ray beam. X-rays are very damageable to living cells, inducing direct physical damage to organic molecules and structures, although there was no quantitative data available for micro organisms. Preliminary experiments showed that strain C58 does not survive the X-ray irradiation, which would be required to acquire 1 set of XANES or XRF spectra (less than 1 surviving cell in 10^{+8} cells, Oger et al. unpublished). The sterilizing impact of X-ray is however limited to the cylinder defined by the intersection of the X-ray beam and the sample. Therefore, providing the beam size is sufficiently small compared to the experimental volume, it might be possible to study cell driven metabolism and correct for the loss of viability. This hypothesis was tested with strain C58 to evaluate the compatibility, the sensitivity and the efficiency of XANES to study the kinetics of selenite reduction by live prokaryotes *in situ* under controlled pressure and temperature.

Experimental setup

Cells from an overnight culture of *Agrobacterium tumefaciens* strain C58 [6] were washed twice with fresh low salt LB medium (10 g yeast extract, 5 g peptone, 5 g NaCl per

liter), resuspended in the same medium supplemented with 5 ppm sodium selenite (Na_2SeO_3, Sigma-Aldrich™, France) at a final density equivalent to an OD_{600} of 10. The high pressure cell of beamline BM30B was loaded with the bacterila suspension. The temperature inside the high pressure cell was raised to the optimal growth temperature of strain C58, *e.g.* 30°C, after the reference XRF and XANES spectra at t=0 were acquired.

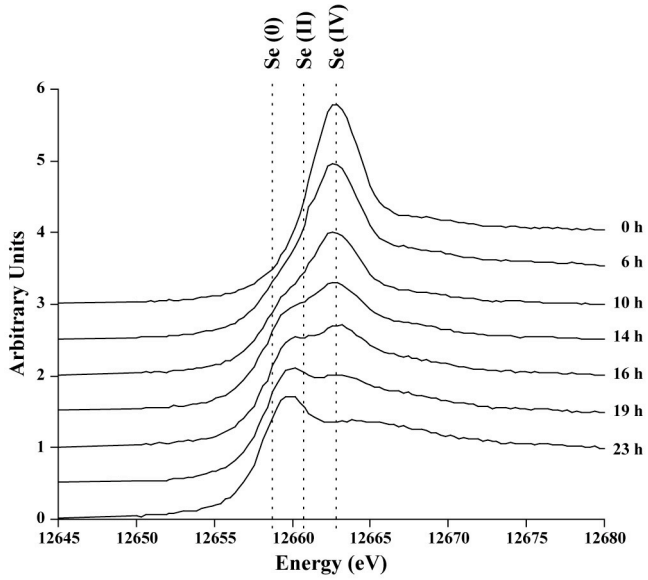
The size of the beam was 150 µm x 300 µm (vert. x hor.), for an incident flux ca. 1-3 10^{10} ph/s. Spectra were acquired for 100s live time. The incident flux, used for normalization was measured using an ionisation chamber placed before the sample. The XANES spectra were acquired in the fluorescence mode for 1s per point (101 points) in the range 12.60-12.78 keV. µXRF and µXANES data were acquired every hour, while maintaining the DAC on the beamline.

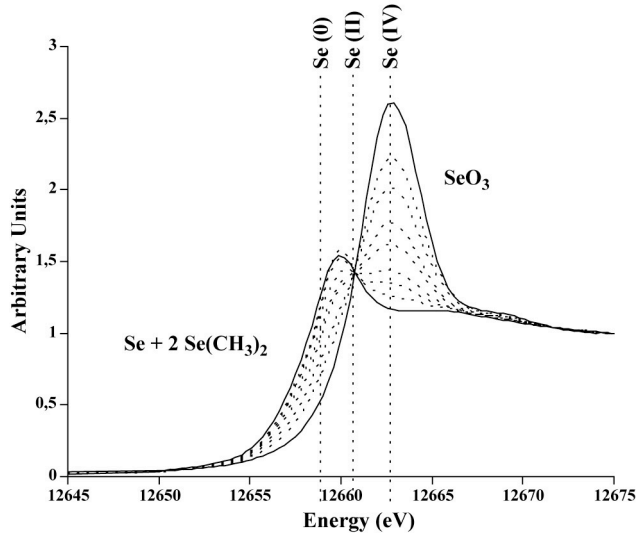
Se concentrations in solution was derived from the transmission spectra. The redox state of Se species in solution was derived from the centroid of the normalised XANES spectra. The relative proportion of each species was determined by fitting the experimental spectrum with a linear combination of individual µXANES spectra obtained for standard Se species [4].

Kinetics of Selenite reduction by live microbes

Figure 1 summarizes the results obtained for an experiment performed at 25 MPa (Oger unpublished results). The shift of the spectrum towards lower energies indicates that selenite is reduced over the 24 hours of the experiment. This reduction is not observed if C58 cells are not added to the medium. However, no reduction of selenite was also observed for an experiment performed at 60MPa (Data not shown). The spectrum obtained at 0h is typical of selenite. Spectra acquired for intermediate incubation times clearly show a gradual replacement of selenium species in solution, with the apparition of a shoulder on the XANES spectra. After 24 hours, the XANES spectrum does not show any contribution of selenite. It

does not however correspond to elemental selenium nor to a methylated selenide species. Linear combinations of 7 different redox species of selenium showed that only two species contributed significantly to the experimental data, elemental selenium and di-methyl selenide (Oger, unpublished). By using a combination of the spectra from selenite, di-methyl selenide and selenium, we can reproduce the experimental data (Figure), and determine that for each molecule of selenium, two molecules of methylated selenide are produced.





These results prove that microbiologically mediated or enzymatic reduction of selenite occurred in situ under pressure on the beamline. This constitutes the first report of the study of microbial metabolism with live bacteria by X-ray spectroscopy on a synchrotron light source.

Litterature Cited

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