In situ kinetic studies of metal metabolism by live prokaryotes under controlled pressure and temperature

Experiment ME 1112
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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 Se (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. We attempted to monitor the reduction of selenium by strain C58 of *Agrobacterium tumefaciens in situ* under controlled P and T in a specially designed low-pressure Diamond Anvil Cell. Live prokaryotes were loaded into the DAC, put under appropriate pressure and incubated on the beamline at their optimal growth temperature (30°C). The oxidation state and concentration of Se was monitored by a combination of μXANES and μXRF.

Performing the *in situ* monitoring of microbial activities requires the direct contact between the live cells and the incident X-ray beam. X-rays are however highly damageable to living cells, inducing direct physical damages to organic molecules and structures, although there is yet no quantitative data available for microorganisms. We showed that the test strain does not whistand the X-ray doses necessary for a single XANES analysis, hence the numerous XANES scans required to monitor its activity over a 24h incubation period [6]. The sterilizing impact of X-rays is however limited to the cylinder defined by the intersection of

the X-ray beam and the bacterila culture. 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 on ESRF-CRG beamline BM30B in June 2005, with strain C58 as a test organism. 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. and an incubation chamber of 3 mm (height) x 5 mm (diameter). Hence the beam to incubator ration was ca. 1/250. Three XANES spectra were acquired every hour to monitor the bacterial activity. Using this setup we could demonstrate that monitoring the activity of live bacteria was possible under controlled pressure and temperature while growing the cells directly in the synchrotron beamline (see report 29437) [7]. The survival of the bacterial cells was only a few percent after the 24h experimentation, which might explain why the reaction under study did not go to completion. It is therefore absolutely necessary to decrease the beam to incubator size ratio in order to improve the survival of the test strain and increase the specific microbial activity.

We used experiment ME1112 to valid this hypothesis. We worked with a low-pressure dedicated DAC [7,8] specially designed to monitor in situ the activity of microbes by different spectroscopic techniques, at a beam to incubator size ration of ca. 1/2500.

Experimental setup

Cells from an overnight culture of *Agrobacterium tumefaciens* strain C58 [9] 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 low-pressure DAC was loaded with the bacterial 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 X-ray beam was 2 µm x 3 µm (h x w). The incubator 500 µm in diameter. 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]. Four pressure conditions were explored (Ambient, 25 MPa, 30 MPa and 45 MPa, 60 MPa).

Results

The reduction of Selenium in the DAC was evidenced for each pressure tested except for the 60 MPa (quantifications under . These results are congruent with our previous observations, in in situ or ex situ experiments, which indicate that strain C58 is not active at 60 MPa. These results valid our hypothesis that the DAC can be used to monitor the Dissimilatory Reduction of Metals by prokaryotes under controlled Pressure and Temperature. In addition, the DAC offers the most versatile setup in terms of Pressure and Temperature, as well as the opportunity to observe the microbial by classical or epifluorescence confocal micoroscopy to check the viability of the cells.

The high energy concentrated in the 2 x 3 μm^2 X-ray beam induces the reduction of selenite into elemental selenium after 15 mn. As a consequence, obtaining meaningful data required that more than one analysis point in the DAC was measured. Instead of a single point for analysis, a 8 x 8 checkboard-like pattern was explored to reduce the impact of the beam on the medium. This does not impact on the kinetics of Se reduction by the bacteria.

After 24h, Selenite is reduced to a mixture of Se (0) and Se (II) species at a ration of 1/2, confirming the results obtained on ESRF beamline BM30B that the kinetics of selenite reduction of strain C58 differ significantly between oxic and anoxic conditions.

This second experiment proves that our setup is the most appropriate to study the dissimilation of metal under controlled pressure and temperature on the synchrotron. The next step id to monitor the activity of mid ocean ridge archaea in the conditions of the deep hydrothermal vent ecosystem (high pressure/high temperature).

Part of this work has been used in the following publications :

Oger, P., I. Daniel, and A. Picard. 2006. Development of a low-pressure diamond anvil cell and analytical tools to monitor microbial activities in situ under controlled P and T. BBA Proteins Proteomics.

Two articles summarizing our high pressure monitoring of Selenium by live prokaryotes by μXANES and μXRF are under preparation.

This work has been/will be presented at the following venues :

1) 8th Symposium on Bacterial Genetics and Ecology, Lyon, 26-29 June 2005 (Poster).

2) Trends in High Pressure Protein Sciences. European COST D30 workshop Satellite meeting of the International Biophysics Congress. September 1st - 3rd , 2005 (Oral)

3) European Conference on X-Ray Spectrometry, Paris, June 19-23, 2006 (Oral)

4) Thermophiles, Brest, September 2006.

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