

Objective and expected results:

One of the most remarkable examples of the phenomenon of biomineralization is the complex-shaped calcitic particles, termed coccoliths, which are produced in vast amounts by algae known as coccolithophores. Each coccolith is built of a set of calcite (CaCO_3) crystals, which exhibit morphologies not observed in geologically-formed calcite. Coccoliths mature intracellularly and are secreted and attached to the cell surface when complete. The pathway of coccolith crystal formation is currently unknown. Deciphering the crystallization pathway is utilized by coccolithophores is an important milestone towards an understanding of coccolith formation at the molecular level.

Results and the conclusions of the study:

During our stay at ID26 at the ESRF, we recorded near-edge X-ray fine absorption spectra (XANES) of shock-frozen (i) coccolith bearing cells, (ii) coccolith-free cells, (iii) coccolith-free cells induced to form coccoliths, and (iv) calcium reference samples at the calcium K-edge. As there was no established protocol in the preparation of coccolithophores for XAS, our initial focus was to establish a strategy for sample preparation. Signal-to-noise ratios for all algae samples were sufficiently high to have all features in the XANES spectrum clearly observable, however use of Kapton film potentially added calcium signal to the background. We also tested the sample sensitivity to beam damage, and found that the high brightness could, after 3-5 measurements, alter the signal intensity so use of a macro to mitigate beam damage was used.

A further important parameter for sample preparation is the washing of cells to remove medium remnants that potentially interfere with the measurement (e.g. sulfur, magnesium, chloride) and protecting cells from freeze damage. To find a suitable solution, we tested washing the cells and freezing them in solution or filtering them and shock-freezing the polycarbonate filters. These tests led to protocol that is neutral to the cells as well to the calcium reference samples. The X-ray absorption spectra of coccolith-free cells and cells induced to form coccoliths at ID26 were of high quality and allow for the development of a preliminary model regarding the calcium pools inside the cells. This was not necessarily to be expected as calcium in the Kapton foils posed a problem for extra-experimental sources of calcium signal in the XANES spectra. After ~10 min since CaCl_2 had been added to these cells, to induce coccolith formation, signal intensity (χ_μ) at around 4060 eV increased notably suggesting that cells started to form calcite (figure 1). Linear combination fitting of the sample data using the calcium reference samples showed that intracellular free calcium is decreasing over time while calcite is increasing (figure 2). Most interestingly, linear combination fitting with free calcium and calcite only resulted in bad fits suggesting that a third calcium phase is present in the cells.

Justification and comments about the use of beam time:

The measurements we did at ID26 have provided promising first information regarding the crystallization pathway of coccolithophores. Time at ID26 demonstrated that it is a beamline well suited for our scientific questions and that high quality data can be obtained from our algae samples even when containing very low amounts of calcium. Cryo-stage and sample transfer were optimal for our needs, and the high brightness allows for collection of data quickly guaranteeing that samples could be tested under many different conditions.

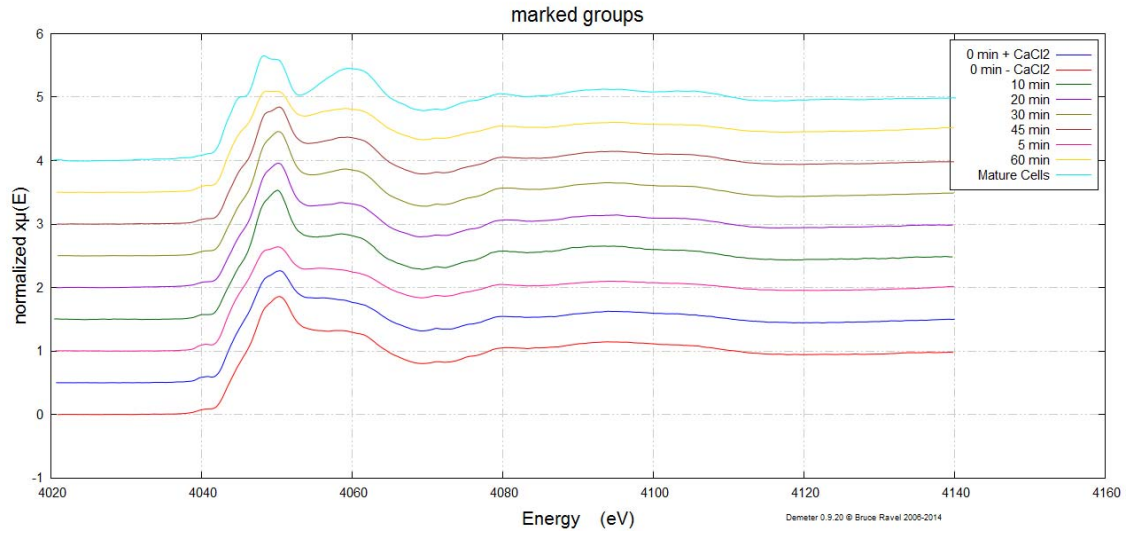


Figure 1 : XANES spectra of coccolith formation after calcium addition. After 10 min. calcitic peak strengthens considerably.

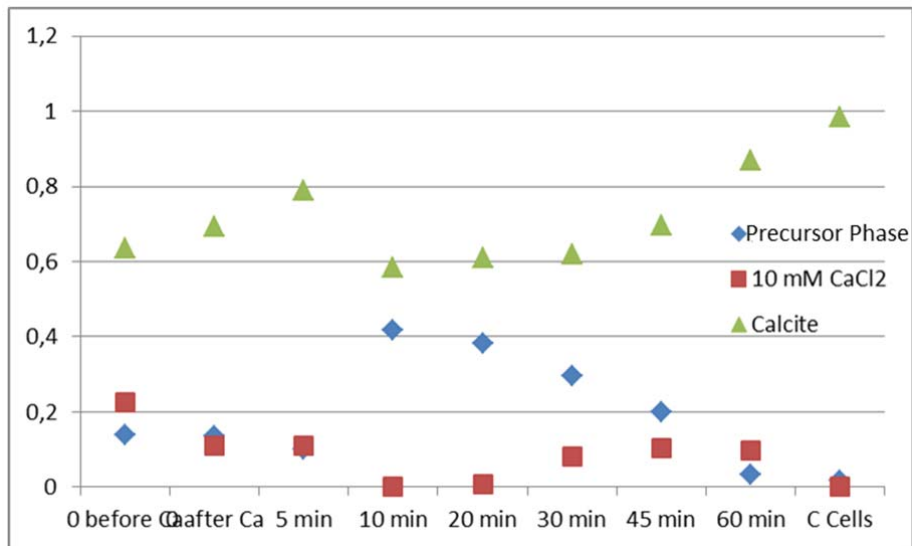


Figure 2 : Relative amounts of the three possible calcium states that are observed during coccolith formation, acquired from linear combination fitting (LCF) of XANES spectra. Preliminary LCF does not fit early time points well (explaining the high amounts of calcite).