3-d chemical imaging with STXM tomography

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Abstract. Three dimensional (3-d) chemical mapping using angle scan tomography in a soft X-ray scanning transmission X-ray microscope (STXM) has been applied to quantitative chemical mapping in three dimensions of calcium carbonate biomineralization by planktonic freshwater cyanobacteria of the strain *Synechococcus leopoliensis* PCC 7942. Aspects of making and mounting grid sections, acquiring multi-energy, multi-angle sequences, and data analysis are discussed.

1. Introduction

Previous tomography studies using full field soft X-ray microscopy [1,2] or scanning transmission X-ray microscopy on plunge frozen specimens (cryo-STXM) [3] have been performed at only a single photon energy, which provides mainly density/thickness contrast with limited and potentially ambiguous chemical information. In contrast, when tomograms are acquired with images at multiple photon energies at each angle [4-7], it is possible to provide spectrally verifiable, quantitative 3-d chemical mapping through analysis of the species-specific, near edge X-ray absorption spectrum (NEXAFS) of each voxel. We have recently extended the types of sample mounting that we are able to use to include ~120 μm wide strips of 400 mesh Cu transmission electron microscopy grids. Although these supports are delicate and challenging to mount and align, since the sample is in a dry state, they do allow access to the full photon energy range of STXM, in contrast to pulled glass capillaries which, to date, have only been used successfully in the region just below the O 1s threshold [4-7]. Here we describe recent studies with C 1s and Ca 2p NEXAFS of calcium carbonate biomineralization on the surface of planktonic freshwater cyanobacteria of the strain *Synechococcus leopoliensis* PCC 7942. This work is an extension to 3-d mapping of an extensive study of the same system using STXM without tomography [8] and should be viewed as a preliminary report rather than the final exposition of the data.

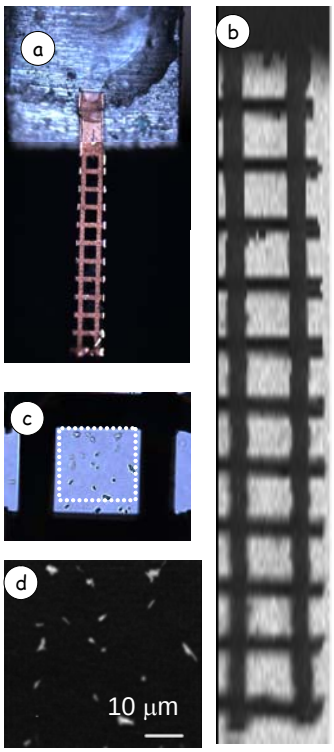

Figure 1 (a,c) Optical image; (b) STXM image of grid-section attached to the tomo-rotator. (d) STXM OD image (352.6 eV) of one grid square.

2. Experimental

The miniature stepping motor, alignment system, as well as the acquisition and analysis procedures have been described in detail

elsewhere [6,9]. Figure 1 displays optical and STXM images of a grid section mounted on the rotation holder. In the STXM image Fig. 1b, the lower part of the grid section is slightly out of focus since the section was not perfectly flat after sample mounting. The width is sufficiently narrow that full 360° rotation is possible, even with the standard 290 μm sample-OSA spacing used at the C 1s edge. However the combination of excessive absorption, foreshortening, and ultimately masking of the view by the grid bars limit data collection to -70 and +70° (where 0° corresponds to having the sample plane orthogonal to the X-ray propagation axis) even for samples near the centre of the grid square. For these initial studies we emphasized collection of more data in the energy domain and thus the angle spacing was only every 3 or 5°, which, along with the incomplete coverage of the angle domain and thus missing wedge effect [10], might lead to artifacts in the tomographic reconstructions.

The cyanobacteria were cultured in the nutrient-poor medium Z/10 and incubated 12 hours in a solution of $NaHCO_3/CaCl_2$ which was ~6 times saturated with respect to calcite [8]. Lacey carbon coated 400 mesh Cu grids were dipped into the cell suspension and the water was sucked away using filter paper. The grid was air dried under an optical microscope. A stripe of grid squares containing multiple cells was chosen and sectioned using a fresh scalpel blade. The grid stripe was then aligned and glued to the rod of the rotation stage (Fig. 1a) using a micromanipulator.

3. Results

Figure 2 presents the results of quantitative chemical mapping of a single *S. leopoliensis* cell at the C 1s (upper) and Ca 2p (lower) edges. The results are in agreement with other work [8]. While these results are based on measurements with an extensive sampling of the spectral space (88 C 1s energies and 89 Ca 2p energies), when a sub-set of these images is carefully chosen so as to select energies with good contrast among the species, then similar results can be obtained. Based on such explorations a reduced set of 5 C 1s and 6 Ca 2p

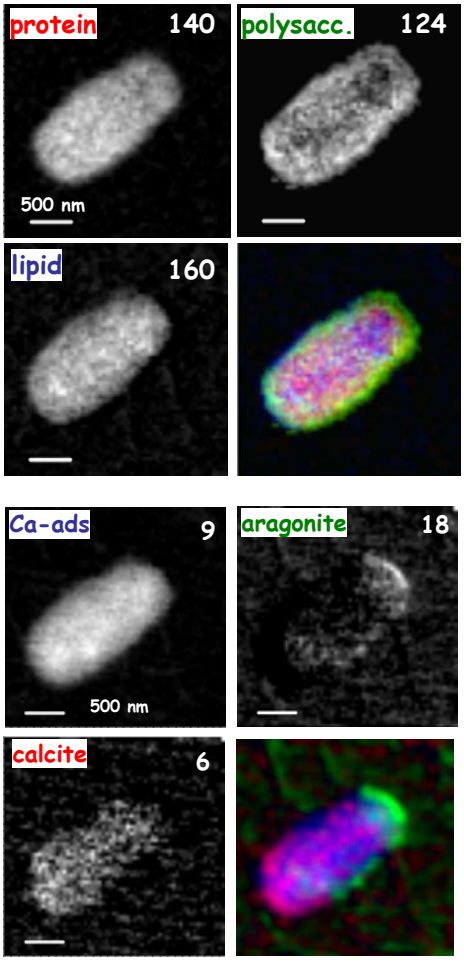

Figure 2. Component chemical maps and color-coded composite map for a single projection of one *Synechococcus leopoliensis PCC 7942* bacterium. (upper) **C 1s** results highlighting the biological components, and (lower) **Ca 2p** results for the Ca-adsorbed, aragonite and calcite components. Bold numbers indicate maximum thickness in nm for pure white (black = 0).

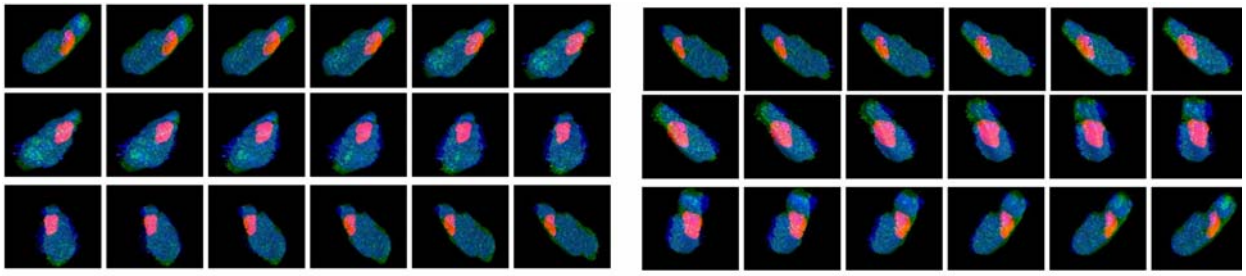

Figure 3 Projection views (after alignment) of the net protein ($OD_{288.2}$- $OD_{280.0}$, green) and Ca 2p signal ($OD_{352.6}$ – $OD_{350.2}$). The Ca 2p signal is displayed in 2 ranges with segmentation based on optical density per thickness (blue ~ $2.5 \cdot 10^{-3}$OD / nm (~12%) Ca adsorbed to extracellular polymers, red ~ $4.4 \cdot 10^{-3}$OD / nm (~ 22%) aragonite, assuming that the red volume contains solely aragonite and organic carbon, and the blue volume contains solely the adsorbed Ca species and organic carbon.)

energies was chosen, and then simultaneous C 1s and Ca 2p 11-energy image sequences were measured at 46 angles between -70 and +70°.

Figure 3 presents some of the results in the form of 36 projections in which the Ca signal ($OD_{352.6} - OD_{350.2}$, total Ca, shown in blue) is combined with the 'biological signal' ($OD_{288.2} - OD_{280.0}$, which is dominated by protein, shown in green). Further analysis to extract detailed speciation (protein, polysaccharide and lipid from C1s; adsorbed-Ca, aragonite and calcite from Ca 2p) is in progress and will be reported elsewhere.

Figure 4 is a rendering of the net Ca signal determined as the difference of the signal at 350.2 eV (no Ca), and 352.6 eV (max of the $Ca_{3/2} \rightarrow 3d$ signal, which is common to all species). There is a rather uniform layer of Ca, which the detailed speciation studies indicate is dominated by the adsorbed-Ca component. In addition there is a 'hot spot' (red) which is most likely the start of an aragonite deposit.

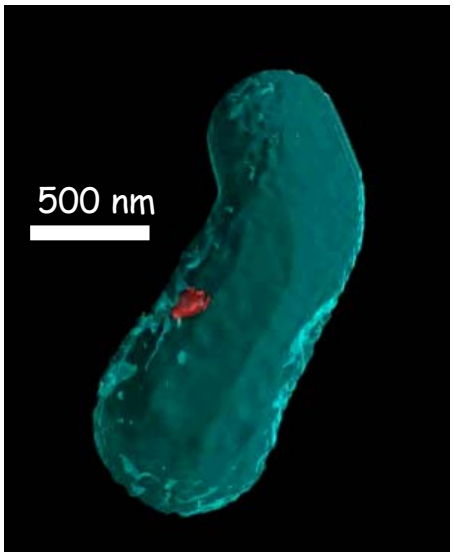

Figure 4 Rendering of the Ca distribution from a single *S. leopoliensis* bacterium, derived from the $OD_{352.6} - OD_{350.2}$ signal. A region of higher Ca signal level is identified by the red coloured voxels.

S. leopoliensis cells are known to facilitate the precipitation of calcite on their surface. Other Ca 2p spectromicroscopic studies [8] have identified $CaCO_3$ with aragonite-like short range order to be a precursor phase of calcite precipitation. 3d-chemical mapping will now allow us to further investigate and possibly determine if the $CaCO_3$ phases originate from the cell membrane, or if extracellular polymers act as a template for mineral nucleation.

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