Nitrogen mapping of the filamentous and heterocystous cyanobacterium *Anabaena* sp. PCC 7120 cells by soft X-ray microscopy

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1. Introduction

The natural and anthropogenic conversion of unreactive N_2 to more reactive nitrogen compounds is one of the main issues for ecosystem and food production [1]. Although the Haber–Bosch process chemically produces ammonia and fills the industrial demand, a biological nitrogen fixation process is expected to displace the industrial nitrogen production in terms of a sustainable and environment-friendly, low-carbon society. The filamentous cyanobacterium *Anabaena* sp. PCC 7120 is one of its promising candidates [2]. It forms a filament of "vegetative cells" responsible for oxygenic photosynthesis; when starved for reactive nitrogen compounds, it differentiates a specialized cell for nitrogen fixation, called "heterocyst", out of about ten vegetative cells. The heterocyst differentiation is believed to be triggered by a high carbon-to-nitrogen ratio (C/N ratio) of the vegetative cell. However, the C/N ratio change in the single differentiating cell had never been observed so far. In this study we observed nitrogen distribution in the heterocysts and vegetative cells of *Anabaena* sp. PCC 7120 by soft X-ray microscopy at the wavelength shorter and longer than the nitrogen *K-absorption edge*.

2. Experiment

The *Anabaena* sp. PCC 7120 cells grown on a nitrogen-depleted agar plate were suspended in pure water and tightly packed between two silicon nitride membranes. The absolute positions of each heterocyst were determined by optical and fluorescence microscopy. The soft X-ray microscopic observation was performed at the wavelengths (λ) of 2.98 and 3.11

nm at BL12 end station in the Ritsumeikan SR Center. The specification of the soft X-ray microscope setup was described in detail elsewhere [3].

3. Results and Discussion

Figure 1 shows the resultant absorbance images of each irradiated wavelength; the heterocysts, enclosed in red circles in the panel (a), appeared to have higher absorbance than the surrounding vegetable cells. The statistical analysis provided histograms of element-specific absorbance of 146 vegetative cells and 54 heterocysts, as shown in Figure 2.

The mean values of absorbance of vegetative cell and heterocyst were 0.53 and 0.73 at λ = 3.11 nm, respectively, while those were 0.44 and 0.51 at λ = 2.98 nm. The unexpected absorbance decrease at λ = 2.98 nm, which is shorter than the nitrogen K-edge, could be due to an unwanted structured absorption profile such as double excitation in cellular proteins. Nevertheless, the absorbance difference between vegetable cells and heterocysts at each wavelength would be a direct evidence for global change of elemental composition through the heterocyst differentiation.

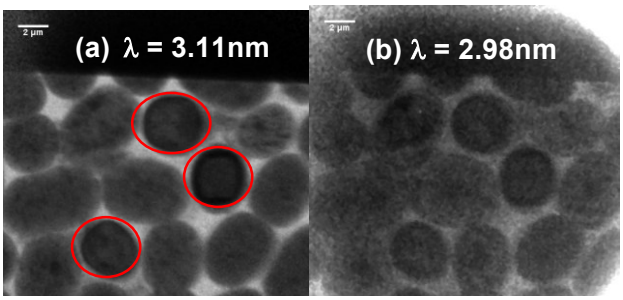

Figure 1:
Soft X-ray microscopic images of cells of *Anabaena* sp. PCC 7120 at λ = 3.11 nm (a) and 2.98 nm (b)

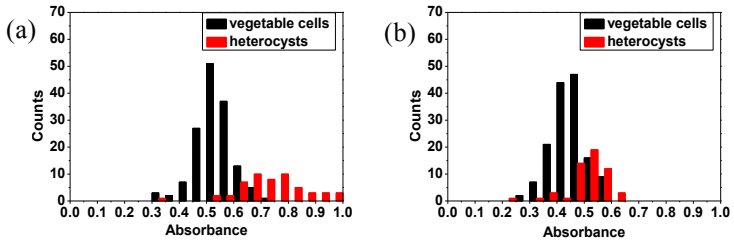

Figure 2:
Histograms of the absorbance of each vegetable cell (black bars) and heterocyst (red bars) at λ = 3.11 nm (a) and 2.98 nm (b)

References

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