Direct observation of nitrogen fixation in filamentous cyanobacteria by using soft X-ray microscopy

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1. Introduction

In nitrogen fixation process, nitrogenase is a useful enzyme to convert nitrogen in air to amino acids. Nitrogenase can survive in anoxic condition, otherwise it become inactive irreversibly and easily due to degradation of its active center by oxygen. So there is controversial debates about nitrogen fixation especially in oxygenic photosynthetic organisms. The cyanobacterium *Anabaena* sp. PCC 7120 is an oxygenic photosynthetic prokaryote that performs nitrogen fixation[1]. It forms a filament consisting of two different types of cells, so-called “vegetative cell” and “heterocyst”, at a ratio of 10:1. The vegetative cells are responsible for oxygenic photosynthesis, and the heterocysts are specialized for nitrogen fixation. Fixed nitrogen in the heterocyst is supplied to the vegetative cells as amino acids while vegetative cells supply the photosynthetic products, such as sucrose, to the neighbor heterocyst. Differentiation of heterocyst is believed to be triggered by the carbon-nitrogen ratio (C/N ratio) in the cell; however, the difference of the C/N ratios of individual cells have never been examined directly. In this study we tried to observe the heterocyst and vegetative cells of *Anabaena* sp. PCC 7120 by using soft X-ray microscopy and directly map nitrogen atoms in each cell by observing at the wavelength shorter and longer than the nitrogen *K-absorption edge*.

2. Experiment

The *Anabaena* sp. PCC 7120 cells cultured on a nitrogen-depleted plate were suspended in pure water and packed into a glass capillary. The position of each heterocyst in the capillary was identified by optical microscopy (OM) and fluorescence microscopy (FM) before the

soft X-ray microscopic observation. The soft X-ray microscope at BL12 end station in Ritsumeikan SR Center was operated at the wavelengths (λ) of 2.8 and 3.1 nm. The specification of soft X-ray microscope setup is described in detail elsewhere[2].

3. Results and Discussion

Figure 1 shows the OM and FM images of the *Anabaena* sp. PCC 7120 filament in a glass capillary. It is clear that there is a non-fluorescent cell in the line of red fluorescent cells. The non-fluorescent cell is the heterocyst (the cell in the circle in fig.1 (b)) because it lacks photosystem 2 that is essential for oxygenic photosynthesis and has auto-fluorescent chlorophyll pigments.

Figure 2 shows the resultant images of the soft X-ray microscopic observations. At the wavelength longer than the N K-edge (λ=3.1 nm), there is no significant difference in the transmittance between the heterocyst (0.10) and neighbor vegetable cells (0.11). Meanwhile, at the wavelength shorter than the N K-edge (λ=2.8 nm), the transmittance of the vegetable cells (0.17) is lower than the heterocyst (0.20). This estimation is contrary to the expectation that heterocysts have a higher nitrogen content due to nitrogen fixation. Further measurements and analysis are needed for solving the contradiction.

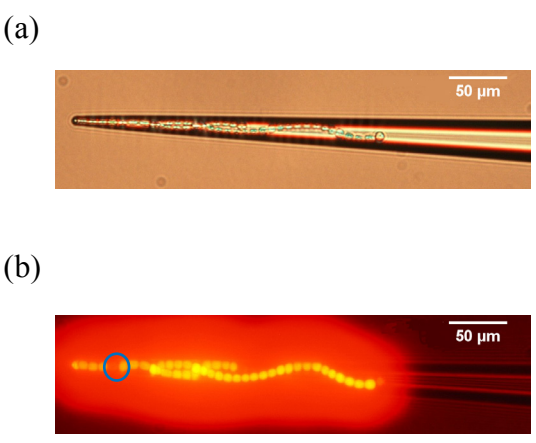

Fig1.
Microscopic image of cyanobacteria by using OM (a) and FM (b)

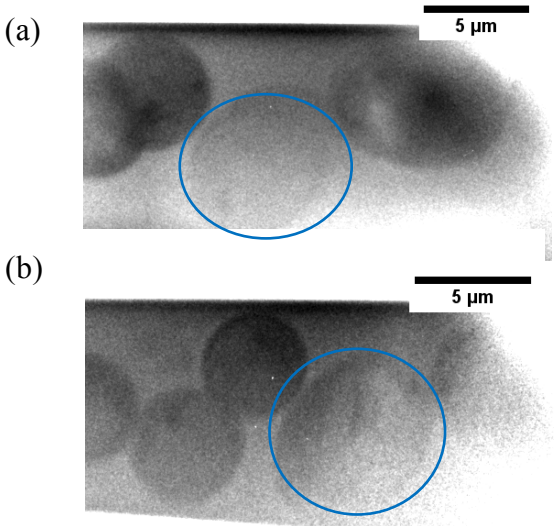

Fig2.
Microscopic image of cyanobacteria by using soft X-ray microscopy with λ=2.8nm (a) and λ=3.1nm(b).
Circles corresponding to the heterocysts.

References

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