Anabaena cell ageing monitored with confocal fluorescence spectroscopy

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Cyanobacteria use a sophisticated system of pigments to collect light energy across the visible spectrum for photosynthesis. The pigments are assembled in structures called phycobilisomes, composed of phycoerythrocyanin, phycocyanin and allophycocyanin, which absorb energy and transfer it to chlorophyll in photosystem II reaction centres. All of the components of this system are fluorescent, allowing sensitive measurements of energy transfer using single cell confocal fluorescence microscopy. The native pigments can be interrogated without the use of reporters. Here, we use confocal fluorescence microscopy to monitor changes in the efficiency of energy transfer as single cells age, between the time they are born at cell division until they are ready to divide again. Alteration of fluorescence was demonstrated to change with the age of the cyanobacterial cell.

INTRODUCTION

We have been interested in investigating bacterial ageing using Anabaena sp. PCC 7120 as a model. A first goal of this project was to identify the ageing state of an individual Anabaena cell: could we determine whether it was young or old? The cell right after division is here defined as young, while the cell just before division is defined as old. During the cycle from one division to the next, the Anabaena cell ages. During this cycle, some of the proteins in an Anabaena sp. PCC 7120 cell undergo changes. Some of these proteins are fluorescent, particularly the ones associated with the photosynthetic apparatus (Gantt, 1981; Glazer, 1985). Could changes in fluorescence provide us with an approach to identify cell age? Such fluorescence spectra would have to be acquired at room temperature, a normal condition for cyanobacterial growth. This is the reason we grew and monitored Anabaena sp. PCC 7120 cells in gel micro-channels, which exhibit convincing advantages compared to an open well. The channels enable filament growth in 1D and facilitate unconfined use of differential interference contrast (DIC) microscopy. The entire growth area can be visualized with the DIC technique (Inoué & Spring, 1997). Here, we report such tracking fluorescence spectra during the ageing of Anabaena sp. PCC 7120 cells in the micro-channels, and the application of these spectra to identify the cell age.

METHODS

A BG11+ (0.75 %) agarose slab gel surface was patterned by a template (provided by Professor Douglas Weibel, University of Wisconsin, Madison, WI, USA) to make parallel channels with the depth 2.5 μm and the width 10 μm. The thickness of the gel was controlled to match the working distance of the microscope lens, in our case, less than 280 μm above the coverslip-bottomed 35 mm Petri dish. Anabaena sp. PCC 7120 cultures were grown in BG11+ medium at room light (−9 μE m−2 s−1) and temperature (~24 °C). The Anabaena sp. PCC 7120 filamentous in BG11+ medium were directly pipetted on the gel surface, then gently washed twice with BG11+ solution and dried in a hood for about 1 h. Some filaments stayed in the channels as shown in Fig. 1(a). The dish with filaments in the channels was sealed with Nesofilms to retain moisture for the growth of Anabaena sp. PCC 7120 on the gel surface.

The coverslip-bottomed dish was directly mounted on the microscope stage. The fluorescence spectra were acquired by a Leica SP5 tandem scanner spectral 2-photon confocal microscope with a Leica 63 × NA 1.3 glycerol lens. The fluorescence spectra were measured (one-photon mode) at a pinhole value of 1.3 AU (arbitrary unit) with 4.5 micron focus thickness along the Z-axis (more than one cell thickness). Samples were excited with a solid-state laser at 561 nm and the fluorescence emission spectra were collected from 615 to 740 nm in 5.0 nm steps. Each sample was scanned 16 times and the mean provided the fluorescence spectra presented. The fluorescence spectra associated with the ageing of individual Anabaena cells was continuously monitored hourly for 16 h. From these images, we can identify when and which individual cell divides, as demonstrated in

Abbreviations: DIC, differential interference contrast; PSII, photosystem II.
Anabaena cell ageing

RESULTS

From the image tracking as shown in Fig. 2(a), cells in the targeted filament can be assigned to three groups during the 16 h observation. In group 1 the individual cells divided once: cell 1 divided after 6 h, and cells 13, 14 and 15 divided after 4 h. Cells belonging to group 2 divided twice: cell 12 divided into cell 12a and cell 12b after 2 h, and cell 12b divided again into cell 12ba and cell 12bb after 14 h. Cells in the last group were not observed to divide: cells 5, 7, 8 and 18 as shown in Table 1.

The cells just before division were grouped as 'old': such as cell 12 at hour 1; cells 13, 14 and 15 at hour 3; cell 1 at hour 5, and so on. The cells just after division were grouped as 'young': such as cell 12 at hour 2; cells 13, 14 and 15 at hour 4; cell 1 at hour 6, and so on. The difference between the young and the old in the averaged fluorescence spectra is shown in Fig. 3 by subtracting the fluorescence spectrum of the young cells from the fluorescence spectrum of the old cells. The young cell has a slightly higher emission intensity than the old cell from 615 to 625 nm, while the old cell has a higher intensity from 665 to 695 nm. To avoid environmental effects such as temperature, moisture and power supply on the fluorescence spectra during this 16 h observation, the normalized fluorescence intensity was used and the ratio of the averaged intensity from 665 to 695 nm to the averaged intensity from 615 nm to 625 nm per cell was recorded to maximize the difference between the young cell and the old. Ratio maps were created by dividing the mean of the 5 nm spectral images collected between 665 to 695 nm by the mean between 615 to 625 nm using Image J. The variation in ratio values was accentuated by using a pseudo-colour ‘fire’ lookup table (Fig. 2b) and a colour-plus-height map (‘surface plot’; Fig. 2c) (Marques, 2011; Russ, 2011; Fu & Caulfield, 2007). As shown in Fig. 2(b), these ratios decreased during cell division, increased gradually after cell division, and after reaching a maximum again decreased very slowly before the next cell division. We will refer to such ratios as the Ratio, below.

Fig. 4 shows the Ratio from individual cells during the 16 h observation. Three cells were chosen for comparison. Cell 5 in Fig. 4(a) did not divide during the 16 h. For this cell, the Ratio gradually increases, but without any later decrease. On the contrary, cell 2 in Fig. 4(b) has a Ratio that decreases gradually until hour 8 and then increases. Cell 2 divided at hour 8. The third example, cell 12, divided at hour 2 and again at hour 14. For this cell, the Ratio was observed to decrease from hour 1 to hour 2, when it divided. Both daughters then increased from hour 2 to hour 7, then decreased until hour 16 for cell 12a, and until hour 14 for cell 12b, which divided again at that time.

Based on these observations, the fluorescence spectra of the Anabaena filament can be explored to identify the age of each cell. A young cell after division shows the Ratio to increase gradually. The Ratio is stable after the cell reaches the mature state and then decreases gradually until the next division.

This model is supported by observations from another filament, in which there were 12 cells initially, dividing into 19 cells during the first 7 h, and then dividing only once during the next 8 h. We averaged the fluorescence spectra of all cells in this filament hourly. The Ratio variation with time is shown in Fig. 5. Since most divisions (seven) happened during the first 7 h, the whole filament was relatively young and the Ratio increased gradually. After hour 8, only one division occurred during the next 8 h. The filament was considered mature and relatively old; the Ratio increased to a maximum value, and then began to decrease.

DISCUSSION

After cell division, the young cell does not have a mature phyobilisome system. Energy transfer from phycoerythrocyanin—phycocyanin—allophycocyanin—photosystem II (PSII) is not fully efficient (Glazer, 1982;...

Fig. 1. (a) Bright-field image of Anabaena PCC 7120 filaments developed in micro channels. (b) DIC growth images of an individual Anabaena filament in the channel. (c) The tracking labels of individual cells. Magnifications are: (a) ×200; (b) ×500; (c) ×1800.
Fig. 2. (a) DIC growth images of an individual *Anabaena* PCC 7120 filament in the channel from hour 0 to hour 16. (b) The Ratio of the averaged intensity from 665 to 695 nm to the averaged intensity from 615 to 625 nm versus time for individual cells in the filament. Ratio values are pseudo-coloured with the calibration as shown in the key (top right). (c) 3D surface plot using colour and height to show the variance of the Ratio values shown in (b).

Table 1. Lineage of individual cells

The time for the individual cell to divide is indicated. For example, cell 1 divided into cell 1a and cell 1b at hour 6, and cell 14 divided into cell 14a and cell 14b at hour 4.

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Kumazaki et al., 2013). Thus, more energy stays below 660 nm and the energy transferred to PSII is relatively low. Then the energy transfer system is gradually improved, with more energy transferred to PSII. In the associated fluorescence, more intensity is emitted beyond 660 nm that makes the Ratio mentioned above increase. The Ratio reaches a maximum and is stable around that value for some time after the cell matures. After that, the cell ages and is ready to divide. Some proteins involved in the energy transfer system may be impaired and the energy transfer is reduced again. As a result, the Ratio gradually decreases.

Such cell improvement in energy transfer provides an approach to identify the cell age. Generally, the fluorescence spectra will be acquired from an individual cell twice within 1 h. That cell will belong to the ‘young’ type if the Ratio increases; the cell will belong to the mature type if the Ratio is stable; and the cell will belong to the ‘old’ type if the Ratio decreases. For example, comparing the fluorescence spectra at hours 7 and 8, we could assign at hour 8 cells 3, 6, 7, 9, 13, 14, 16 and 17 as young, and cells 2, 4, 5, 8, 10, 11, 12, 13, 15 and 18 as old or mature. It is not possible to identify the cell age by a single fluorescence spectrum.

We appreciate that our assignment of the fluorescence changes with cell age to changes in the efficiency of energy transfer from phycobilisomes to chlorophyll in reaction centres is one of several possibilities. The ratio of phycobilisomes to reaction centres might change with age; the bilisomes themselves might gain or lose phycoerythrocyanin with age; or the ratio of PSI to PSII might change with age. Further work is needed to test these possibilities.
ACKNOWLEDGEMENTS

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REFERENCES


Fig. 5. (a) DIC growth images of an individual Anabaena filament. (b) The Ratio of the averaged fluorescence emission intensity from 665 to 695 nm to the averaged intensity from 615 to 625 nm as a function of time. The fluorescence intensity was acquired hourly from the averaged value of all the cells in the filament.

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