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Acclimation of a mesophilic cyanobacterium, *Synechocystis* sp. PCC 6803 to the growth temperature

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Introduction

Photosynthetic organisms usually experience large changes in environmental factors, such as temperature [Falk et al. 1996], light intensity or light quality [Pearcy et al. 1996]. Temperature is one of the most important factors, which control the enzymatic reaction including photosynthesis. Murata and his co-workers have investigated the acclimation mechanisms of mesophilic cyanobacteria to environmental temperatures and reported relationship between desaturation of fatty acid and acclimation to the temperature [e.g. Los et al. 1997]. They showed that turn over of the damaged D1 protein of photosystem II (PS II) become inhibited in the chilly temperatures when desaturation enzymes were destroyed [Kanervo et al. 1997]. However, the acclimation process to high temperature in cyanobacteria is not well understood. We have reported the responses of photosynthetic systems in a thermophilic cyanobacterium to high temperature stress [Inoue et al. 2000]. In that report, it was shown that the PS II was most sensitive to high temperatures. In a more recent paper, we showed that the mesophilic cyanobacterium *Synechocystis* sp. PCC 6803 regulated both the initial photochemical activity of PS II and the oxygen-evolving activity so as to acclimate to high temperatures [Inoue et al. 2001]. It seems that this cyanobacterium acclimates to the surrounding temperature to maintain a certain rate of the PS II activity irrespective of the growth temperature.

In this work, we tried to find out weather the PS II activity is the most important factor which defines the growth at non-suitable temperatures. *Synechocystis* 6803 has an advantage for these investigation because it can grow not only photoautotrophically but also photomixotrophically in the presence of glucose and an inhibitor of PS II.

Materials and methods

A mesophilic cyanobacterium, *Synechocystis* 6803 was grown at various temperatures in BG11 medium [Rippka et al. 1979] supplemented with 10 mM TES-NaOH (pH 8.0) and bubbled with air, containing 5% CO_2. Where indicted, 5 mM glucose and 10 μM atrazine were added to the culture medium. Continuous white light was provided at about 50 μmole photons·m^{-2}·sec^{-1} PAR. Growth of the cells was monitored by turbidity at 750 nm using a Multipurpose Spectrophotometer, model MPS-2000 (Shimadzu, Japan).

Fluorescence spectra at 77K were recorded using a laboratory-constructed fluorometer. Blue exciting light was supplied from a 100 W halogen lamp, which was passed through a Corning 4-96 band-pass filter.

The rate of oxygen evolution was determined with a Clark-type oxygen electrode. The saturating actinic light from a 100 W halogen lamp was passed through a HOYA HA-50 and a Toshiba Y-50 filter. The BG11 medium was used as a reaction mixture. Cell suspension corresponding to 10 μg Chl /mL was used.

The chlorophyll concentration was determined after MacKinney [1940].

Results

Growth rates of a mesophilic cyanobacterium, *Synechocystis* 6803, were highly dependent on the surrounding temperature (Figure 1). Below 15°C, the cells could not grow. It showed the highest growth at around 30°C, but the growth rate was rather constant in a temperature range from 25 to 40°C. Above 43°C, the growth rate dropped sharply and no growth was observed at temperatures higher than 45°C. Fluorescence spectra at 77K showed an increase in the ratio of PS II to PS I at both low (20°C) and high (43°C) temperatures (data not shown, but see Figure 3). Furthermore, the PS II activity was very sensitive to photoinhibition in the cells grown under low or high temperatures (data not shown).

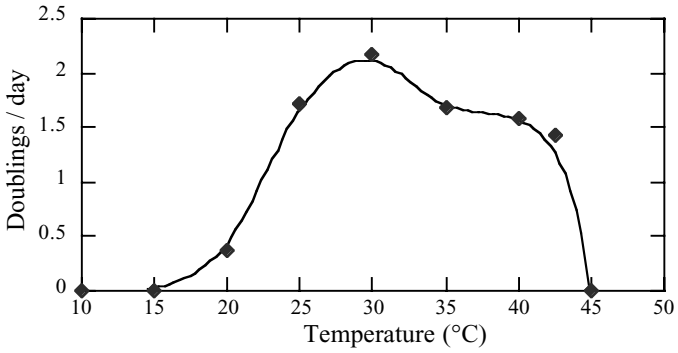

Fig 1. Doubling times of *Synechocystis* 6803 grown at various temperature

To examine the contribution of PS II to the restriction of growth in high or low temperatures, cells were cultivated in the presence or absence of glucose plus atrazine, and the growth rates were compared (Figure 2). Glucose functions as an electron donor to PS I when the electron flow from PS II was inhibited by atrazine. In the presence of glucose/atrazine, growth of the cells was distinctly accelerated at temperatures lower than 22.5°C. This clearly shows that the growth at those temperatures was determined by the stability of PS II.

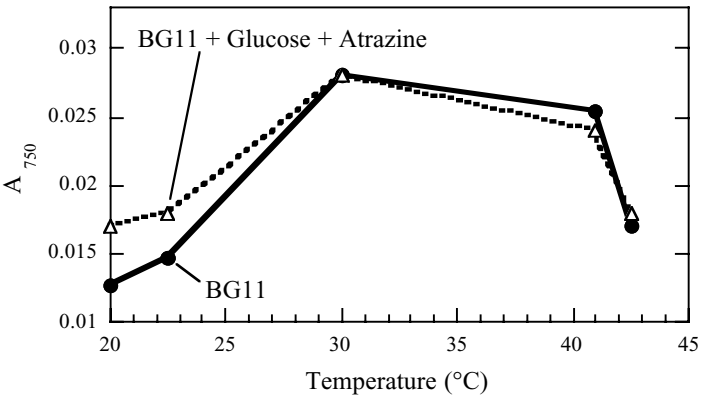

Figure 2. Growth of *Synechocystis* 6803 in the presence (open triangle) or absence (closed circle) of 5 mM glucose plus 10 μM atrazine. Turbidity at 750 nm was measured 24 h after the start of cultivation.

Actually, fluorescence spectra at 77K of cells grown at 22.5°C showed that the energy transfer from phycocyanin to PS II was not decoupled in the presence of glucose/atrazine

(Figure 3). The large fluorescence peak at 655 nm (phycocyanin) in the absence of glucose/atrazine suggests the presence of larger portion of impaired PS II probably due to slow recovery of photoinhibited PS II complexes. By contrast, the growth rates were almost the same at high temperatures (between 30 and 43°C) irrespective of the presence or absence of glucose/atrazine (Figure 2).

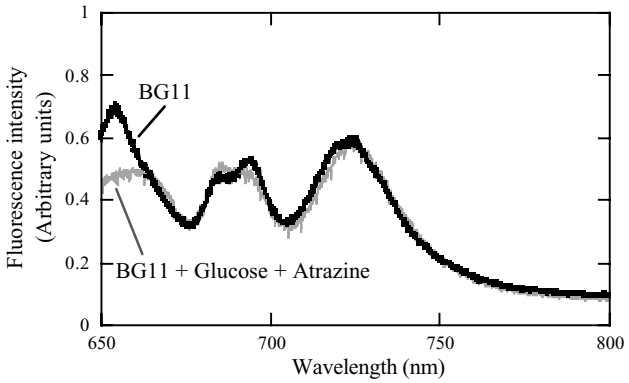

Figure 3. Fluorescence spectra at 77K from the cells grown at 22.5°C in the presence or absence of glucose/atrazine.

Discussion

Growth of *Synechocystis* 6803 cells at high or low temperatures increased the amount of PS II relative to that of PS I (for low temperatures, see Figure 3). This phenomenon can be considered as a compensatory mechanism of the cells for the decrease in the activity of PS II. Figure 2 shows that, actually, the growth of the cells was restricted by the stability of PS II at low temperatures. These data are in agreement with those of Murata's group [Kanervo et al 1997, Los et al 1997].

On the contrary, the growth of the cells at high temperatures was not increased by the addition of glucose/atrazine (Figure 2). This result can be explained if we assume that the thermostability of the reaction other than PS II determines the growth of the cells. In fact, effects of high temperatures are different from those of low temperatures. The cells, which had been cultivated at temperatures below 15°C for several days, could grow normally when the temperature was up-shifted to 30°C. However, the cells cultivated at temperatures higher than 45°C could not grow any more even when the growth temperature was lowered to 30°C (data not shown). These results suggest that high temperatures, but not low temperatures, have lethal or irreversible effects on the cells.

As a working hypothesis, however, we propose that permeability of thylakoid membranes has a critical role for both the PS II activity and viability of the cells, and determines their growth and the thermostability of the PS II activity. In a thermophilic cyanobacterium, we showed that the viability of the cells was determined by the permeability of the cytoplasmic membranes [Inoue et al. 2000]. A close relationship between the threshold temperature of the permeability of thylakoid membrane and the thermotolerance of the PS II activity has also been shown [Satoh et al. unpublished data], and we showed a close relationship between the PS II activity and the cell growth [Inoue et al 2001]. Experiments to assess the relationship between the two are in progress.

Acknowledgments

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