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Acclimation to the Growth Temperature and the High-Temperature Effects on Photosystem II and Plasma Membranes in a Mesophilic Cyanobacterium, *Synechocystis* sp. PCC6803

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High-temperature effects on Photosystem II and plasma membranes, temperature dependence of growth, and acclimation to the growth temperature were studied in a mesophilic cyanobacterium, *Synechocystis* sp. PCC6803. The following results were obtained. (1) Small but distinct temperature acclimation of the PSII reaction center activity was shown for the first time when the activity was measured at inhibitory high temperatures. However, the reaction center activity showed no apparent acclimation when it was measured at growth temperatures after heat stress. (2) Oxygen-evolving activity and the permeability of plasma membranes showed higher resistance to heat when PCC6803 cells were grown at higher temperatures. (3) Acclimation of photosynthesis to the growth temperature seemed to occur so as to maintain photosynthesis activity not at a maximum level but in a certain range at the growth temperatures. (4) Neither sensitivity to high-temperature-induced dissociation of phycobilisomes from the PSII reaction center complexes nor degradation of phycocyanin were altered by changes in the environmental temperature. (5) A close relationship between the viability of cells and the structural changes of plasma membranes (but not the inactivation of photosynthesis) was observed. The denaturation process of PSII complexes and the relationship between the temperature dependence of the growth of *Synechocystis* PCC6803 cells and that of the photosynthetic activity are also discussed.

Key words: Cell membrane — Heat stress — High-temperature stress — O_2 evolution — Photosystem II — *Synechocystis* sp. PCC6803.

Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCIP, 2,6-dichlorophenolindophenol; DPC, 1,5-diphenylcarbazide; Fo, minimum level of Chl fluorescence; Fm, maximum level of Chl fluorescence; Fv, variable part of Chl fluorescence (Fv=Fm–Fo); HSP, heat shock protein; LHCII, light-harvesting Chl *a/b* protein complexes of PSII; PBS, phycobilisome(s); Q_A and Q_B, primary and secondary quinine electron acceptors of PSII; TQ, toluquinone (methyl- *p*-benzoquinone).

Introduction

It is well known that high temperature inhibits the oxygen-evolving activity of PSII (Katoh and San Pietro 1967). At temperatures slightly higher than the growth temperatures (e.g. at around 40°C in the case of spinach), the manganese-stabilizing 33 kDa protein is dissociated from the PSII reaction center complex, resulting in the release of manganese atoms and the deactivation of oxygen evolution (Enami et al. 1994, Yamane et al. 1998). At higher temperatures (at around 60°C in spinach) the primary charge separation reaction of PSII becomes inhibited (Döring et al. 1969, Klimov and Krasnovskii 1981, Yamane et al. 1998). At chilling temperatures, on the other hand, the repair cycle of a damaged D1 protein of a photoinhibited PSII reaction center complex has been shown to be suppressed in cyanobacteria (Gombos et al. 1994, Kanervo et al. 1997). Accordingly, light becomes quite harmful to PSII at both high and low temperatures.

It is also well known that oxygenic photosynthetic organisms acclimate to changes in the environmental temperature, although there are high- and low-temperature limits for this phenomenon (Berry and Björkman 1980). From the results mentioned above it seems reasonable to assume that the temperature sensitivity of PSII determines both the high- and low-temperature growth limits of photosynthetic organisms, and that acclimation of their growth can be attributed to the change in the sensitivity of PSII to high or low temperatures. To our best knowledge, however, there has been no report that clearly proves this idea.

In cyanobacteria, acclimation to low temperatures takes place in parallel with an increase in the desaturation level of membrane fatty acids (Los et al. 1993). It is supposed that the fluidity of the thylakoid membranes is controlled so as to maintain the membranes in the optimal condition for the damaged D1 protein to be digested and then to be replaced by newly synthesized protein (Kanervo et al. 1997, Sippola et al. 1998). An increase in the desaturation level of fatty acids in the thylakoid galactolipids increases the resistance of the cyanobacterial cells to low temperatures (Wada et al. 1994).

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When the ambient temperature is elevated, photosynthetic organisms should decrease the fluidity of membranes, but the relationship between the fluidity change and the high-temperature acclimation of the oxygen-evolving system is not clear (Wada et al. 1994, Nishiyama et al. 1999). At first, a decrease in the desaturation level of fatty acids was thought to increase the resistance to heat stress (Pearcy 1978, Raison et al. 1982). Later, deletion of Δ^{12}-desaturase (Gombos et al. 1991) or an increase in the unsaturation of membrane lipids (Wada et al. 1994) was shown to have no effect on heat tolerance in cyanobacteria. However, Arabidopsis (Hugly et al. 1989, Kunst et al. 1989) and *Chlamydomonas* (Sato et al. 1996) mutants, which have reduced content of highly unsaturated fatty acids in chloroplasts, have been reported to have enhanced thermal stability of PSII.

Although the mechanism mentioned above is controversial, acclimation phenomena of oxygen evolution to high temperatures in cyanobacteria are well known. Using deletion mutants, Nishiyama et al. (1994) and Nishiyama et al. (1997) suggested that Cyt c550 (PsbV) or the 12 kDa protein (PsbU) is related to the acclimation phenomenon. Conversely, the activity of the reaction center of PSII was shown not to acclimate to high temperatures (Nishiyama et al. 1993).

Heat-shock proteins (HSPs) are induced in cyanobacterial cells by certain high-temperature treatment: about 10°C higher than their usual growth temperatures (Nakamoto et al. 2000, Tanaka and Nakamoto 1999). Furthermore, there have been several reports that HSPs, especially low-molecular-weight HSPs, have protective effects for the oxygen-evolving activity and viability of the cells against heat stress (Lee et al. 1998, Nakamoto et al. 2000). However, acclimation to the growth temperature occurs at any growth temperature where HSPs are not expected to be induced.

It should be pointed out that, in most work on temperature acclimation of photosynthesis, activities in the cells were measured at their growth temperatures after high-temperature treatments, and that photoautotrophic growth of the treated cells was used as the measure of acclimation to high temperatures. In the former case, reversible effects are ignored, and in the latter case, all other fatal effects of the heat stress can be mixed with the damage in photosynthesis.

In this paper we show the relationship between the temperature dependence of growth, temperature dependence of photosynthetic activity (especially that of PSII activity), and acclimation to high temperatures in a mesophilic cyanobacterium, *Synechocystis* sp. PCC6803. Effects of heat stress were measured both at high temperatures and at growth temperatures after the high-temperature treatments. We discuss the high-temperature-induced degradation process of PS II complexes and the relationship between the growth and the remaining photosynthetic activity in mesophilic cyanobacteria. We show that effects of high temperatures on photosynthetic activities are not directly related to the viability of cells.

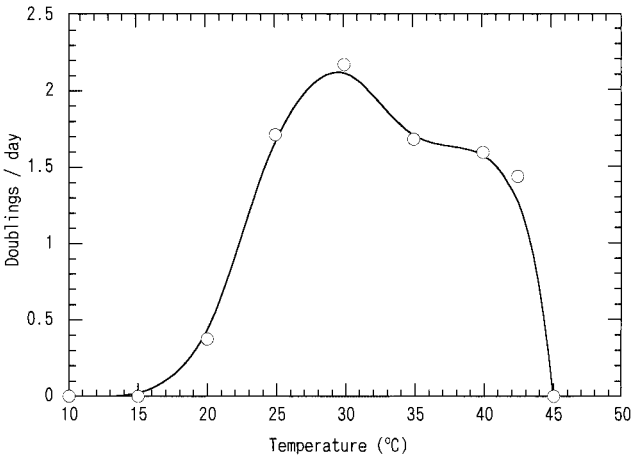

Fig. 1 Temperature dependence of photoautotrophic growth of *Synechocystis* sp. PCC6803. For details, see Materials and Methods.

Results

Temperature dependence of the photoautotrophic growth of Synechocystis PCC6803

The growth of PCC6803 under usual light conditions (60 μE cm^{-2} s^{-1}) has quite characteristic temperature dependence (Fig. 1). Maximum growth was obtained at 30°C, but this cyanobacterium grew at a quite similar rate in a temperature range from 25 to 40°C. Below 25°C and above 40°C, the cells seemed to suffer from low- and high-temperature stress, respectively, and they stopped growing at 15 or 45°C. This temperature dependence of growth was quite different from that in thermophilic cyanobacteria, *Thermosynechococcus elongatus* (Yamaoka et al. 1978) and *T. vulcanus* (Inoue et al. 2000). The latter showed a clear peak at around 55°C, and it dropped gradually at the lower side and sharply at the higher side of the peak temperature.

Temperature dependence of photosynthesis and the PSII activity in PCC6803 cells grown at different temperatures

Temperature dependence of oxygen evolution in the presence of bicarbonate is shown in Fig. 2. The rate of CO_2 fixation at 25°C in the cells grown at this temperature was about 140 μmol O_2 (mg Chl)$^{-1}$ h^{-1}, and the rate increased with elevation of the measurement temperature, until it reached a maximum at around 42°C. The maximum activity was around 400 μmol O_2 (mg Chl)$^{-1}$ h^{-1}: almost three times larger than measured at 25°C. On the other hand, the rate of oxygen evolution in the cells grown at 35°C was much lower than that in the cells grown at 25°C at any temperature tested, except at around 52°C. The rate at 35°C was around 150 μmol O_2 (mg Chl)$^{-1}$ h^{-1} and reached a maximum at around 44°C. The rate at the peak temperature (230 μmol O_2 (mg Chl)$^{-1}$ h^{-1}) was almost a half of that of the cells grown at 25°C. At measurement temperatures

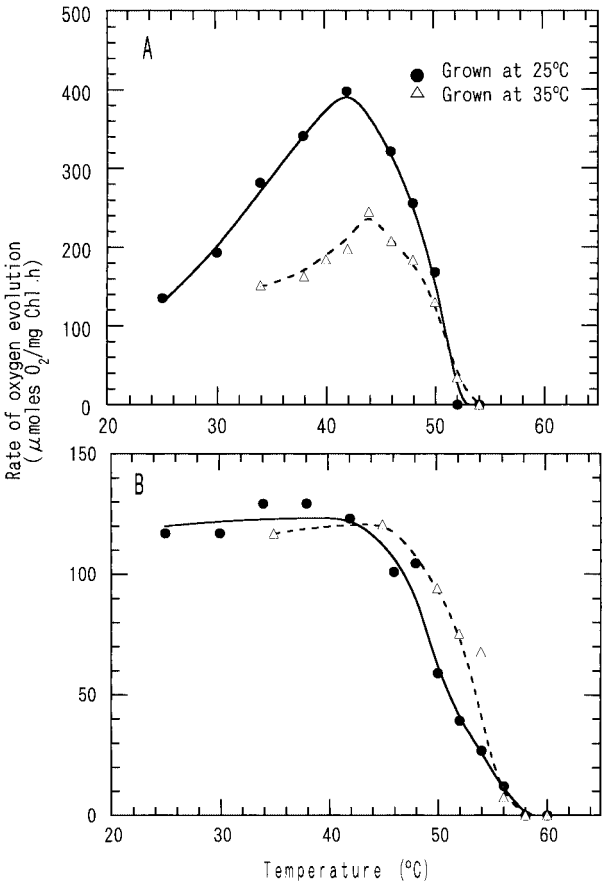

Fig. 2 Effects of high temperatures on photosynthesis in PCC6803. (A) Rates of oxygen evolution in the presence of 5 mM $NaHCO_3$ were measured immediately after 5 min of incubation at the indicated temperatures or (B) at the growth temperatures after the treatments at temperatures indicated in the figure in 25°C-grown cells (circles) and 35°C-grown cells (triangles). For other conditions, see Materials and Methods.

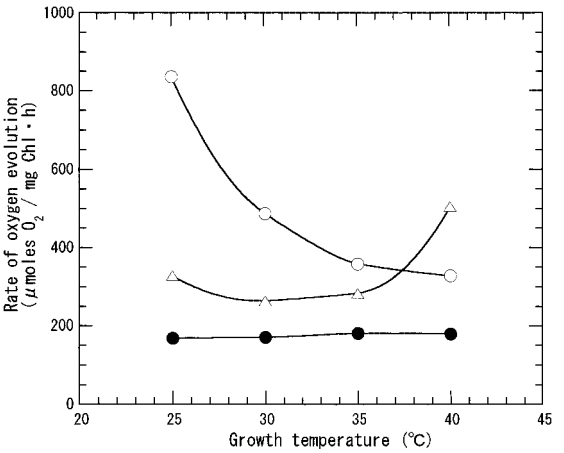

Fig. 3 Effects of growth temperatures on the rate of photosynthesis at the growth temperatures (solid circles) or at 43°C (open circles) or on the PSII activity (triangles) in PCC6803 cells. Photosynthesis was measured by oxygen evolution in the presence of 5 mM $NaHCO_3$, and the PSII activity in the presence of 1 mM TQ, 1 mM ferricyanide, and 3 μM DBMIB. For other conditions, see Materials and Methods.

higher than 44°C, the activity of the cells grown at 35°C decreased in a similar way as in the cells grown at 25°C. There was no activity in the cells grown at 25°C but a small activity in the cells grown at 35°C when measured at 52°C, showing somewhat increased resistance to heat in the cells grown at 35°C.

The quite interesting point is that the oxygen-evolving activity measured at 35°C of the cells grown at 35°C was almost the same as that in the cells grown at 25°C and measured at 25°C. In other words, cells show almost the same oxygen evolution rate when measured at their growth temperature. In another set of experiments, the photosynthetic rate and electron transport activity through PSII were compared in cells grown at different temperatures (Fig. 3). The rates of photosynthesis were almost the same when they were measured at the growth temperatures (solid circles). Because these cells showed almost the same PSII activity (triangles), the constant rate of photosynthesis seems to be due to PSII except in the cells grown at 40°C, in which the PSII activity was markedly higher than the photosynthesis activity. As can be expected from the data shown in Fig. 2A, the maximum photosynthetic rate, which measured at 43°C, decreased with an increase in the cultivating temperature. These characteristics were quite different from those of a thermophilic cyanobacterium, *T. elongatus*. In this thermophilic cyanobacterium, the temperature dependence of photosynthesis was little changed by its growth temperature (Yamaoka et al. 1978).

When the rate of oxygen evolution was measured at the growth temperature after a 5-min high-temperature treatment, the cells grown at 35°C became resistant to high temperatures (Fig. 2B). The temperature at which the inhibition became recognizable or the temperature at which the oxygen evolution was inhibited by 50%, shifted about 2°C upward in the cells grown at 35°C compared with the cells grown at 25°C.

Fundamentally the same results were obtained when oxygen evolution mediated solely by PSII was measured (Fig. 4). In the presence of DBMIB, which inhibits electron flow through the cytochrome b_6/f complex, and toluquinone (TQ) as an electron acceptor, high rates of oxygen evolution were observed. The maximum temperature clearly shifted with a change in the growth temperature. But, as shown in Fig. 3, the activities at their growth temperatures were almost the same (Fig. 4A). When the PSII activity was measured at the growth temperatures after high-temperature treatments (Fig. 4B), similar results as in Fig. 2B were obtained. It is clear that inhibition by, and acclimation to, high temperatures of photosynthesis can be attributed to those of PSII activity.

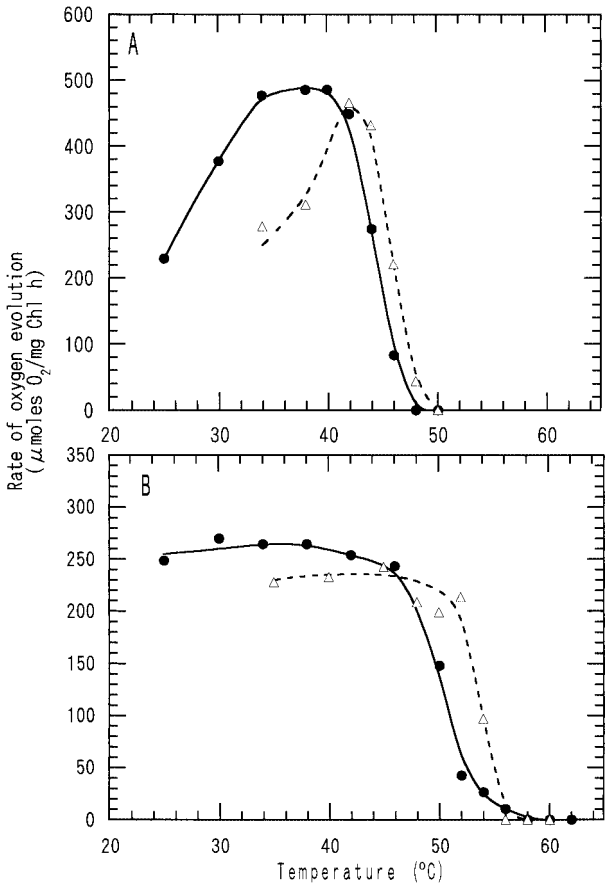

Fig. 4 Effects of high temperatures on the PSII activity in PCC6803 cells. (A) Rates of oxygen evolution in the presence of 1 mM TQ, 1 mM ferricyanide and 3 μM DBMIB were measured immediately after 5 min of incubation at the indicated temperatures or (B) at the growth temperatures after the treatments at temperatures indicated in the figure in 25°C-grown cells (circles) and 35°C-grown cells (triangles). For other conditions, see Materials and Methods.

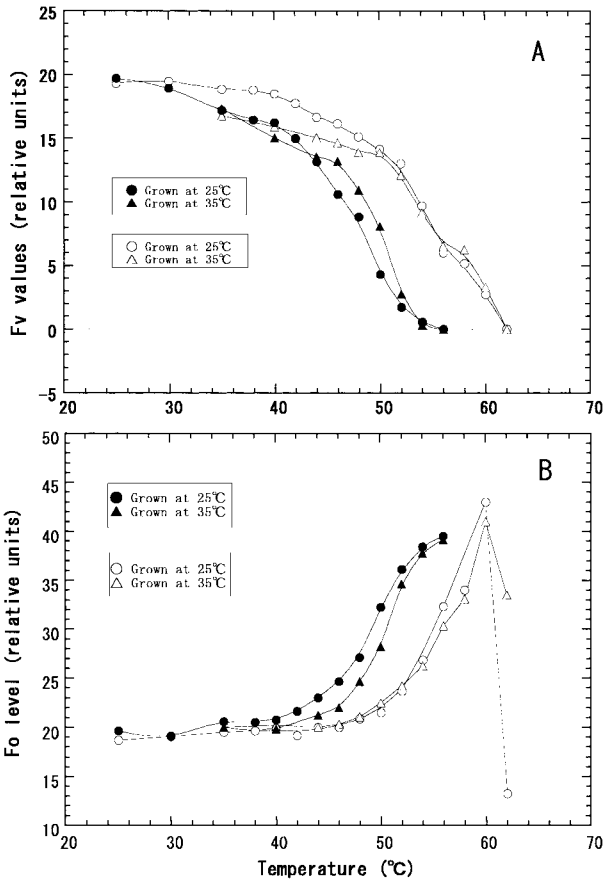

Fig. 5 Effects of high-temperature treatments on the Fv values (A) of Fo levels (B) in the presence of NH_2OH and DCMU in PCC6803 cells. Fluorescence Fv values and Fo levels were measured immediately after 5 min of incubation at the indicated temperatures (closed symbols) or at the growth temperatures after the treatments at the indicated temperatures (open symbols) in 25°C-grown (circles) and 35°C-grown (triangles) cells in the presence of 0.1 mM NH_2OH and 10 μM DCMU. For other conditions, see Materials and Methods.

Temperature dependence of the reaction center activity of PSII

The Fv values in the presence of NH_2OH and DCMU (Fig. 5A) were found to reflect the PSII reaction center activity, that is, the electron flow from P680, the reaction center Chl dimmer of PSII, to Q_A (Yamane et al. 1998). Although the difference between the cells grown at 25°C and those grown at 35°C was small, it was clearly and constantly observed that the Fv value in the cells grown at 35°C showed higher heat tolerance than that in the cells grown at 25°C when the values were measured at the treatment temperatures (Fig. 5A, closed symbols). This suggests that the PSII reaction center activity also becomes tolerant to heat when the cells are grown at higher temperatures. However, when the Fv value was measured at the growth temperature after the heat treatment, the difference became almost negligible in cells treated at a temperature higher than 50°C (Fig. 5A, open symbols).

To examine if the Fv value in the presence of DCMU and NH_2OH really reflects the reaction center activity of PSII in cyanobacteria, we compared the Fv value with the DCIP photoreduction activity with DPC as an electron donor in thylakoids isolated from PCC6803 (Fig. 6). Both the Fv value and the PSII reaction center activity decreased by high-temperature treatment almost in the same way, supporting the notion that the Fv value in the presence of DCMU and NH_2OH reflects the reaction center activity of PSII. The higher thermostability of the PSII reaction center activity in isolated thylakoids (Fig. 6) than in cells (Fig. 5A) may come from the difference in the media surrounding the thylakoids.

Temperature dependence of a fluorescence parameter, Fo

High-temperature-induced increases in minimum fluorescence, Fo, has been shown to reflect deactivation of the PSII reaction center and dissociation of phycobilisomes (PBS) from

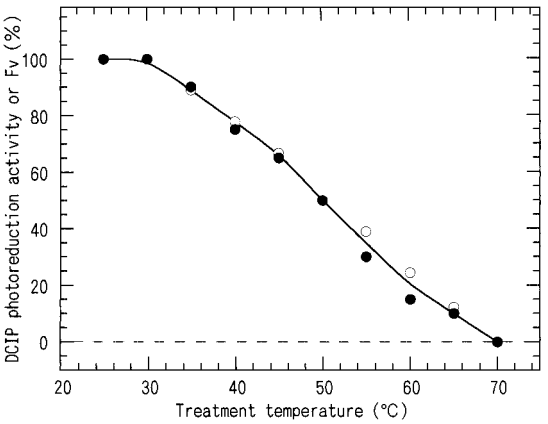

Fig. 6 Effects of high-temperature treatments on the DCIP photoreduction with DPC as an electron donor and on the Fv values in the presence of NH_2OH and DCMU in PCC6803 thylakoids. Thylakoid membranes from 25°C-grown PCC6803 cells were treated at the indicated temperatures for 5 min and then further treated at 25°C for another 5 min, and the reaction center activities (open circles) and the Fv values (closed circles) were measured at 25°C. The reaction mixture contained 50 mM tricine-NaOH (pH 7.5), 10 mM $CaCl_2$, 0.6 M sucrose, 1.0 M betaine, 1 mM DPC, and 50 μM DCIP. The activity of DCIP photoreduction and the Fv values normalized by Fo at 100% were 50 μmol DCIP reduced (mg Chl)$^{-1}$ h^{-1} and 0.14, respectively. For other conditions, see Materials and Methods.

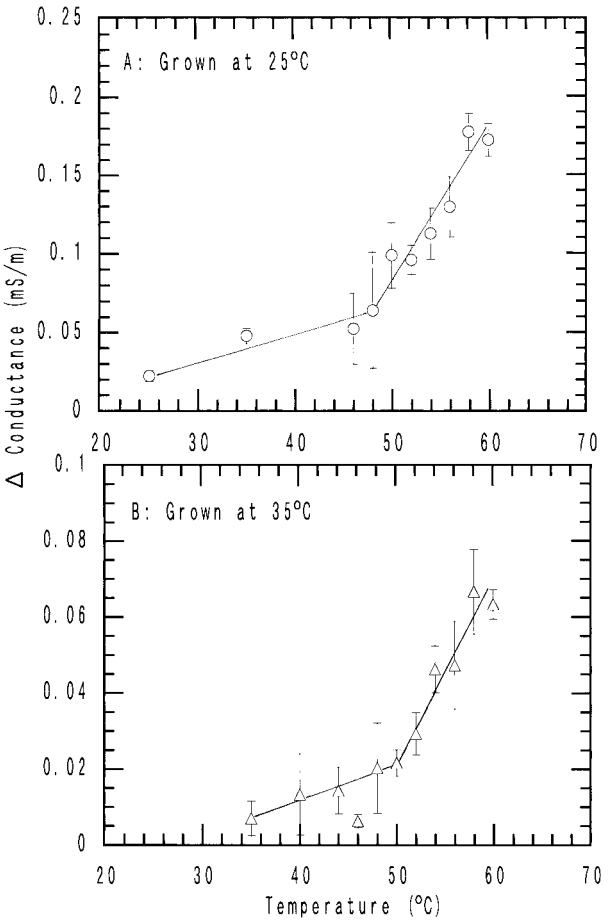

Fig. 7 Effects of high-temperature treatments on electrolyte leakage in 25°C- and 35°C-grown PCC6803 cells. Electrolyte leakage was estimated from an increase in the conductance. Cells were treated at the indicated temperatures for 5 min, further incubated at their growth temperatures (25°C for A and 35°C for B) for 5 min, and then the conductance was measured at each temperature. Data are average of three different experiments ± S.D. For other conditions, see Material and Methods.

the PSII reaction center complexes in cyanobacteria (Inoue et al. 2000). In accordance with the data shown in Fig. 5A by closed symbols, the Fo level showed higher heat resistance in the cells grown at 35°C than in the cells grown at 25°C when it was measured at the treatment temperature (Fig. 5B, closed symbols). However, when the Fo level was measured at the growth temperature after the high-temperature treatments (open symbols), there was almost no difference between the cells grown at 25°C and those grown at 35°C. Because detachment of PBS from the PSII reaction center complex is irreversible (data not shown, but see Inoue et al. 2000), no difference in the Fo level measured at the growth temperatures suggests that the binding affinity of PBS to PSII reaction center complexes is not acclimated to high temperature. The rapid decrease in fluorescence at a temperature over 60°C is due to denaturation of phycobiliproteins and the PSII complexes (Inoue et al. 2000). A precise measurement of high-temperature-induced denaturation of phycocyanin (absorbance decrease at 620 nm) showed no difference between the cells grown at 25°C and those grown at 35°C (data not shown).

Permeability of plasma membranes of PCC6803 cells

High temperatures cause structural changes of plasma membranes, resulting in increased leakage of various ions through the membranes (Inoue et al. 2000). Cells grown at 25°C and 35°C were examined for high-temperature-induced electrolyte leakage in order to check acclimation of the cell membranes to the growth temperature (Fig. 7). The conductance increases as the pre-treatment temperature increases; but at a certain temperature, the slope of the increase becomes sharper, showing that the cell membranes become leaky at that temperature. The break point for cells grown at 25°C was 48°C (Fig. 7A), while that for cells grown at 35°C was 50°C (Fig. 7B), indicating a slight increase in the resistance of cell membranes to heat.

Viability of PCC6803 cells after high-temperature treatments

As Inoue et al. (2000) reported previously using thermophilic *T. vulcanus* cells, the high-temperature-induced damage in the plasma membranes, indicated by increased leakage of the electrolyte, is critical for survival of the cells at high temperatures. Figure 8A and B show growth curves for cells

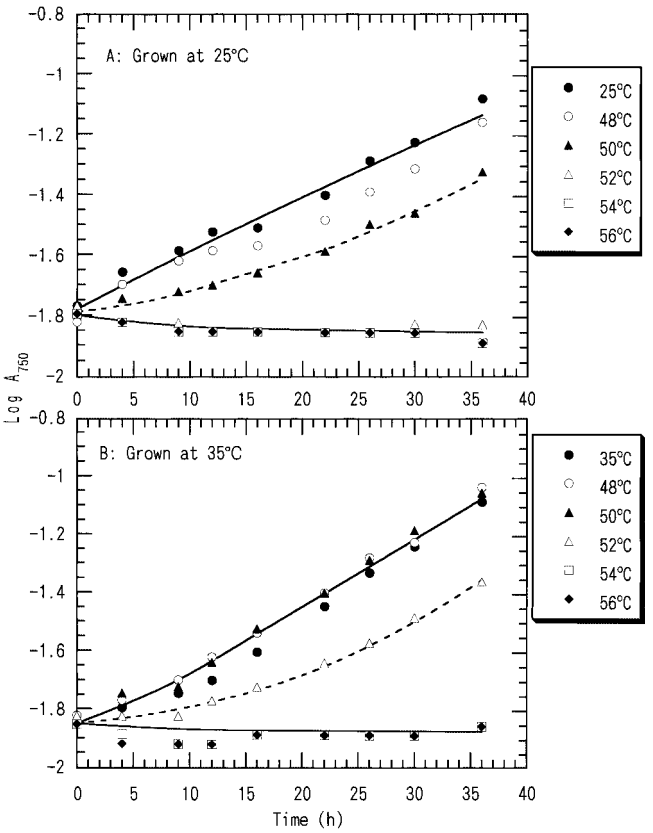

Fig. 8 Growth curves of PCC6803 after high-temperature treatments. After treatments at the indicated temperatures for 5 min, the cells grown at 25°C (A) or 35°C (B) were cultivated in the culture medium at 25°C. For other conditions, see Materials and Methods.

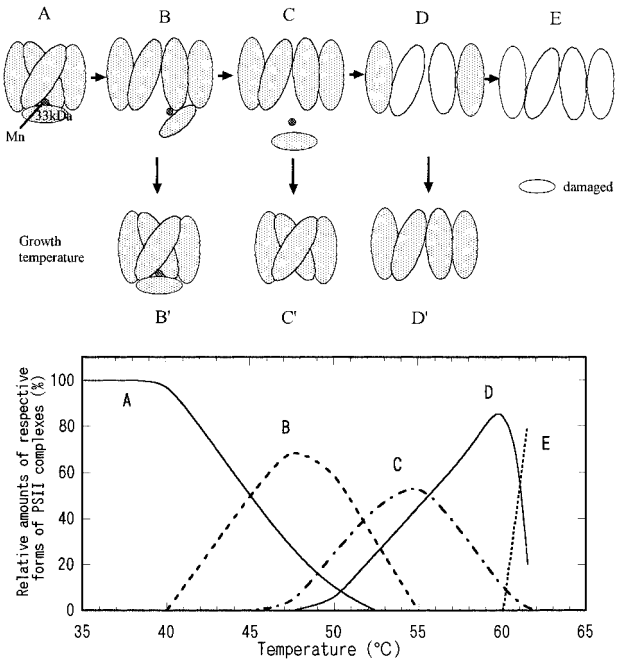

Fig. 9 A schematic model of the high-temperature-induced denaturation process of the PSII complex in mesophilic cyanobacteria (upper panel). PSII complex denaturates by heat through three intermediate forms. Forms B′, C′ and D′ are forms at the growth temperature after high-temperature stress. Form A, a native form. In form B, the PSII complex has no oxygen-evolving and no reaction center activities, but the both activities are recovered when the cells were cooled down to the growth temperature (form B′). In form C, the PSII complex has no manganese cluster and no reaction center activity, but it recovers the reaction center activity without recovery of oxygen evolution on cooling to the growth temperature (form C′). In form D, the PSII complex becomes a less-fluorescent form, but it returned to a fluorescent but not active form (form D′) after incubation at the growth temperature. Form E, a completely denatured and less-fluorescent form. Temperature dependence of transfer to forms B+C, D, and E (lower panel) in cells grown at 25°C can be drawn by the Fo increase at high temperatures (Fig. 5B, closed circles), the Fo increase at 25°C (Fig. 5B, open circles), and the Fo decrease at 25°C at temperatures over 60°C (Fig. 5B, open circles), respectively. Conversion to form C, which corresponds to degradation of form B, can be calculated from the decrease of oxygen evolution at 25°C (Fig. 4B, closed circles).

grown at 25°C and 35°C after a 5-min treatment of the cells at various temperatures. Treatment of the cells grown at 25°C at temperatures up to 48°C for 5 min had almost no effect on their growth; but a treatment at 52°C (only 4°C higher) completely inhibited growth. A treatment at 50°C caused a marked time lag for the growth of cells grown at 25°C, while there was no effect for cells grown at 35°C. It is evident that the critical temperature shifted from 50 to 52°C when the growth temperature of PCC6803 cells was shifted from 25 to 35°C.

Discussion

Acclimation to the growth temperature in a mesophilic cyanobacterium, PCC6803, seems to occur in a manner to maintain the photosynthetic rate in a certain range of around 120–200 μmol O_2 (mg Chl)$^{-1}$ h^{-1} (or the PSII activity, around 200–300 μmol O_2 (mg Chl)$^{-1}$ h^{-1} except in the cells grown at 40°C). In Fig. 3 we did not show the data for cells grown at temperatures lower than 25°C or higher than 40°C because the PSI/PSII ratio and binding of PBS to PSII had been modified at these growth temperatures (data not shown). Changes in the fluidity of thylakoid membranes must play a crucial role in maintaining the rate of electron flow in a certain range (Los et al. 1993). This is because the rate-limiting step of the photosynthetic electron transport chain is on the site of plastoquinone movement in thylakoids, which is highly temperature-dependent (Hirano et al. 1981). However, the physiological importance of and mechanisms that regulate the electron transfer rate have yet to be clarified. As for the physiological meaning, we suppose that the regulation of electron flow is to avoid formation of active oxygen species over its capacity to scavenge them because mechanisms to quench excess light energy such as xanthophyll cycles are not developed in cyanobacteria (Demmig-Adams 1990). Another possibility is that a highly

reduced state in the cell disorders metabolism, and that there might be a mechanism to keep a certain redox level inside the cell by changing the activity of photosynthesis. The original strain of PCC6803 cannot grow with glucose under light (Williams 1988); light seems to increase greatly the reduction level inside the cells.

PCC6803 has a quite different temperature dependence of growth compared to thermophilic cyanobacteria, *Thermosynechococcus elongatus* and *T. vulcanus* (Yamaoka et al. 1978, Inoue et al. 2000). From the sequence of the 16 S rRNA, a thermophilic *Synechococcus elongatus* was found to be a quite different species from a mesophilic *S. elongatus*. So, accordingly, Katoh et al. (2001) proposed a new genus name, *Thermosynechococcus*, for the thermophilic ones. The temperature dependence of growth in these thermophilic cyanobacteria is almost the same as the temperature dependence of photosynthesis, but, in PCC6803, they are quite different (Fig. 1, 2). The growth rates at 25°C, 30°C, and 35°C were nearly the same (Fig. 1), which is consistent with the rate of photosynthesis in cells grown at these temperatures (Fig. 3). The temperature limitation of growth at high temperatures as well as at low temperatures seems to be due to limitation of PSII activity. When the oxygen-evolving activity was measured at the treatment temperature, the peak temperature shifted from 42°C to 44°C when the growth temperature was increased from 25°C to 35°C (Fig. 2A). When the cells were grown at 42.5°C, the peak temperature of the PSII activity was also at 42.5°C (data not shown). Therefore, it is reasonable to assume that photoautotrophic growth of the cells at temperatures higher than 42.5°C is limited by PSII activity.

When cells were grown at higher temperatures (in the experiments shown here, 35°C compared to 25°C), both oxygen evolution and reaction center activity of PSII became more tolerant to heat. An interesting point is that, for reaction center activity, slight but clear resistance to heat became negligible when the activity was measured at the growth temperature after high-temperature treatment (Fig. 5). This might be the reason why PSII reaction center activity has not been reported to acclimate to high temperatures in cyanobacteria (Nishiyama et al. 1993). On the contrary, oxygen evolution reaction in the cells grown at 35°C showed higher and clearer heat resistance than the cells grown at 25°C when the activity was measured at their growth temperatures after heat treatments (Fig. 2, 4). Another interesting point is that the high-temperature sensitivity of oxygen evolution (Fig. 2A) was almost the same as that of the PSII reaction center activity (Fig. 5).

We propose the following high-temperature-induced deactivation process of PSII complexes in mesophilic cyanobacteria (Fig. 9). There are at least three intermediate forms of the PSII reaction center complex between a native form (form A) and a completely denatured less-fluorescent form (form E). The PSII complex in form B has no oxygen-evolving and no reaction center activities, but it recovers both activities when the cells are cooled to the growth temperature (form B′). In form C, the PSII complex may lose the manganese cluster (Yamane et al. 1998), and so the reaction center activity recovers with no recovery of oxygen evolution on cooling to the growth temperature (form C′). The PSII complex in form D is less-fluorescent (Inoue et al. 2000), and it returned to form D′ after incubation at the growth temperature, which is fluorescent as the native form but has no reaction center activity (Yamane et al. 1998). The data shown in Fig. 2, 4, and 5 clearly show that only the formation and degradation steps of form B acclimate to the growth temperature.

Heat-induced death of the cells was a phenomenon different from the inhibition of photosynthesis. The cells grown at 25°C and then treated at 52°C for 5 min could not grow at 25°C (Fig. 7), but they still showed some photosynthetic activity at 25°C (Fig. 2B) after 5 min of incubation. Similar phenomena can be observed in the cells grown at 35°C. Although treatment at 50°C had some inhibitory effects on photosynthesis in these cells, the treatment had no effect on their growth.

It is beyond doubt that photoautotrophic growth of cyanobacteria depends on their photosynthetic activities, but care should be taken when the cell membranes have less tolerance than photosynthesis, as in the case shown here. Fig. 7 and 8 clearly show that viability of PCC6803 cells and their acclimation to the growth temperature, respectively, are related to the semipermeability and acclimation to high temperatures of the plasma membranes. The heat sensitivity of photosynthesis seems not to be directly related to the viability, but seems to be related to the high-temperature growth limit of the cells in PCC6803.

Materials and Methods

A mesophilic cyanobacterium, *Synechocystis* sp. PCC6803 (glucose tolerant), was grown at various temperatures in a BG11 medium supplemented with 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES)-NaOH buffer (pH 8.0) using a cultivator FD 403 (Fujimoto Rika, Tokyo, Japan). Cells were continuously illuminated with an incandescent lamp (60 μE m^{-2} s^{-1} photosynthetically active radiation) and bubbled with air containing 5% CO_2. Numbers of cells were monitored by turbidity of the culture medium at 750 nm.

The cells were placed in the dark for about 30 min at their growth temperatures and were treated at high temperatures for 5 min; and then oxygen-evolving activities, fluorescence intensities, or their absorption spectra were immediately measured at the treatment temperatures. In other sets of experiments, the cells were cooled to their growth temperatures after the high-temperature treatments and incubated for another 5 min; and then oxygen evolution, Chl *a* fluorescence, or their absorption spectra were observed at the growth temperatures. Cell suspensions corresponding to 10–20 μg Chl ml^{-1} were used for all measurements.

The rate of oxygen evolution was determined with a Clark-type oxygen electrode. The saturating actinic light from a 12 V, 100 W halogen lamp was passed through a Toshiba Y-50 filter. The reaction mixture contained BG11 and 5 mM $NaHCO_3$ for CO_2 fixation measurements, or BG11, 1 mM methyl-*p*-benzoquinone (toluquinone, TQ), 3 μM 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB),

and 1 mM potassium ferricyanide for measuring PSII activity.

Chl fluorescence was measured with a pulse amplitude modulating fluorometer (PAM 101–103, Walz, Germany) as reported previously (Yamane et al. 1997). PSII reaction center activities were measured by the photoreduction of 2,6-dichlorophenolindophenol (DCIP) with 1,5-diphenylcarbazide (DPC) as an electron donor with a Hitachi 356 dual-wavelength spectrophotometer (Hitachi, Japan), or by fluorescence increases in the presence of 0.1 mM NH_2OH and 10 μM DCMU (Yamane et al. 1998). For detection of photoreduction of DCIP, light-induced absorbance decreases at 580 nm were measured. The actinic light from a 12 V, 100 W halogen lamp was passed through a 5-cm water layer and a Toshiba R65 filter, and the photomultiplier was protected by a Corning 4–96 filter. The reaction medium contained 50 mM tricine-NaOH (pH 7.5), 10 mM $CaCl_2$, 0.6 M sucrose, 1.0 M betaine, 1 mM DPC, and 50 μM DCIP.

Thylakoid membranes were prepared as reported by Mamedov et al. (1991).

Turbidity at 750 nm and absorption spectra were measured with a Multipurpose Spectrophotometer model MPS-2000 (Shimadzu, Kyoto, Japan) equipped with a constant-temperature cell holder (P/N 204–0803).

For measuring 77 K fluorescence emission spectra, cells in a brass cuvette were incubated at high temperatures for 5 min and quickly dipped into liquid nitrogen. The spectra were measured by a laboratory-constructed fluorescence spectrophotometer as mentioned previously (Yamane et al. 1997). Exciting light from a 12 V, 100 W halogen lamp was passed through a Corning 4–96 filter.

Permeability of the cell membranes was measured by detecting electrolyte leakage from the cells during high-temperature treatments. Cells were sedimented by centrifugation at 5,000×*g* for 5 min and were then suspended in distilled water, with this process repeated twice. The electric conductivity of the cell suspension after 5-min high-temperature treatments was recorded at 25°C or at 35°C by a conductivity meter (CM-14P, Toa, Japan) equipped with a conductivity cell (CVP-101P).

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References

Berry, J. and Björkman, O. (1980) Photosynthetic response and adaptation to temperature in higher plants. *Annu. Rev. Plant Physiol.* 31: 491–543.

Demmig-Adams, B. (1990) Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. *Biochim. Biophys. Acta* 1020: 1–24.

Döring, G., Renger, G., Vater, J. and Witt, H.T. (1969) Properties of the photoactive chlorophyll-a II in photosynthesis. *Z. Naturforsch.* 24b: 1139–1143.

Enami, I., Kitamura, M., Tomo, T., Isokawa, Y., Ohta, H. and Katoh, S. (1994) Is the primary cause of thermal inactivation of oxygen evolution in spinach PSII membranes release of the extrinsic 33 kDa protein or of Mn? *Biochim. Biophys. Acta* 1186: 52–58.

Gombos, Z., Wada, H. and Murata, N. (1991) Direct evaluation of effects of fatty-acid unsaturation on the thermal properties of photosynthetic activities, as studied by mutation and transformation of *Synechocystis* PCC6803. *Plant Cell Physiol.* 32: 205–211.

Gombos, Z., Wada, H. and Murata, N. (1994) The recovery of photosynthesis from low-temperature photoinhibition is accelerated by the unsaturation of membrane lipids: A mechanism of chilling tolerance. *Proc. Natl. Acad. Sci. USA* 91: 8787–8791.

Hirano, M., Satoh, K. and Katoh, S. (1981) The effect on photosynthetic electron transport of temperature-dependent changes in the fluidity of the thylakoid membrane in a thermophilic blue-green alga. *Biochim. Biophys. Acta* 635: 476–487.

Hugly, S., Kunst, L., Browse, J. and Somerville, C. (1989) Enhanced thermal tolerance of photosynthesis and altered chloroplast ultrastructure in a mutant of *Arabidopsis* deficient in lipid desaturation. *Plant Physiol.* 90: 1134–1142.

Inoue, N., Emi, T., Yamane, Y., Kashino, Y., Koike, H. and Satoh, K. (2000) Effects of High-temperature treatments on a thermophilic cyanobacterium *Synechococcus vulcanus. Plant Cell Physiol.* 41: 515–522.

Kanervo, E., Tasaka, Y., Murata, N. and Aro, E.M. (1997) Membrane lipid unsaturation modulates processing of the photosystem II reaction-center protein D1 at low temperatures. *Plant Physiol.* 114: 841–849.

Katoh, H., Itoh, S., Shen, J.-R. and Ikeuchi, M. (2001) Functional analysis of *psbV* and a novel c-type cytochrome gene *psbV2* of the thermophilic cyanobacterium *Thermosynechococcus elongatus* strain BP-1. *Plant Cell Physiol.* In press.

Katoh, S. and San Pietro, A. (1967) Ascorbate-supported NADP photoreduction by heated *Euglena* chloroplasts. *Arch. Biochem. Biophys.* 122: 144–152.

Klimov, V.V. and Krasnovskii, A.A. (1981) Pheophytin as the primary electron acceptor in Photosystem II reaction centers. *Photosynthetica* 15: 592–609.

Kunst, L., Browse, J. and Somerville, C. (1989) Altered chloroplast structure and function in a mutant of Arabidopsis deficient in plastid glycerol-3-phosphate acyltransferase activity. *Plant Physiol.* 90: 846–853.

Lee, S., Prochaska, D.J., Fang, F. and Barnum, S.R. (1998) A 16.6-kirodalton protein in the cyanobacterium *Synechocystis* sp. PCC 6803 plays a role in the heat shock response. *Curr. Microbiol.* 37: 403–407.

Los, D., Horvath, I., Vigh, L. and Murata, N. (1993) The temperature-dependent expression of the desaturase gene desA in *Synechocystis* PCC6803. *FEBS Lett.* 318: 57–60.

Mamedov, M.D., Hayashi, H., Wada, H., Mohanty, P., Papageorgiou, G.C. and Murata, N. (1991) Glycinebetaine enhances and stabilizes the evolution of oxygen and the synthesis of ATP by cyanobacterial thylakoid membranes. *FEBS Lett.* 294: 271–274.

Nakamoto, H., Suzuki, N. and Roy, S.K. (2000) Constitutive expression of a small heat-shock protein confers cellular thermotolerance and thermal protection to the photosynthetic apparatus in cyanobacteria. *FEBS Lett.* 483: 169–174.

Nishiyama, Y., Hayashi, H., Watanabe, T. and Murata, N. (1994) Photosynthetic oxygen evolution is stabilized by cytochrome c550 against heat inactivation in *Synechococcus* sp. PCC 7002. *Plant Physiol.* 105: 1313–1319.

Nishiyama, Y., Kovacs, E., Lee, C.B., Hayashi, H., Watanabe, T. and Murata, N. (1993) Photosynthetic adaptation to high temperature associated with thylakoid membranes of *Synechococcus* PCC7002. *Plant Cell Physiol.* 34: 337–343.

Nishiyama, Y., Los, D.A., Hayashi, H. and Murata, N. (1997) Thermal protection of the oxygen-evolving machinery by PsbU, an extrinsic protein of photosystem II, in *Synechococcus* species PCC 7002. *Plant Physiol.* 115: 1473–1480.

Nishiyama, Y., Los, D.A. and Murata, N. (1999) PsbU, a protein associated with photosystem II, is required for acquisition of cellular thermotolerance in *Synechococcus* species PCC 7002. *Plant Physiol.* 120: 1–8.

Pearcy, R.W. (1978) Effects of growth temperature on the fatty acid composition of the leaf lipids in *Atriplex lentiformis* (Tor) Wats. *Plant Physiol.* 61: 484–486.

Raison, J.K., Roberts, J.K.M. and Berry, J.A. (1982) Correlation between the thermal stability of chloroplasts (thylakoid) membranes and the composition and fluidity of their lipids upon acclimation of the higher plants, *Nerium oleander*, to growth temperature. *Biochim. Biophys. Acta* 688: 218–228.

Sato, N., Sonoike, K., Kawaguchi, A. and Tuzuki, M. (1996) Contribution of lowered unsaturation levels of chloroplast lipids to high temperature tolerance of photosynthesis in *Chlamydomonas reinhardtii*. *J. Photochem. Photobiol.* 36: 333–337.

Sippola, K., Kanervo, E., Murata, N. and Aro, E.M. (1998) A genetically engineered increase in fatty acid unsaturation in *Synechococcus* sp. PCC 7942 allows exchange of D1 protein forms and sustenance of photosystem II activity at low temperature. *Eur. J. Biochem.* 251: 641–648.

Tanaka, N. and Nakamoto, H. (1999) HtpG is essential for the thermal stress management in cyanobacteria. *FEBS Lett.* 458: 117–123.

Wada, H., Gombos, Z. and Murata, N. (1994) Contribution of membrane lipids to the ability of the photosynthetic machinery to tolerate temperature stress. *Proc. Natl. Acad. Sci. USA* 91: 4273–4277.

Williams, J.G.K. (1988) Construction of specific mutations in photosystem II

photosynthetic reaction center by genetic engineering methods in *Synechocystis* 6803. *Methods Enzymol.* 167: 766–778.

Yamane, Y., Kashino, Y., Koike, H. and Satoh, K. (1997) Increases in the fluorescence Fo level and reversible inhibition of photosystem II reaction center by high-temperature treatment in higher plants. *Photosynth. Res.* 52: 57–64.

Yamane, Y., Kashino, Y., Koike, H. and Satoh, K. (1998) Effects of high temperatures on the photosynthetic systems in spinach: Oxygen-evolving activities, fluorescence characteristics and the Denaturation process. *Photosynth. Res.* 57: 51–59.

Yamaoka, T., Satoh, K. and Katoh, S. (1978) Photosynthetic activities of a thermophilic blue-green alga. *Plant Cell Physiol.* 19: 943–954.

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