SigC sigma factor is involved in acclimation to low inorganic carbon at high temperature in Synechocystis sp. PCC 6803

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Inactivation of the sigC gene (sll0184), encoding the group 2 sigma factor SigC, leads to a heat-sensitive phenotype of Synechocystis sp. PCC 6803. Cells of the ΔsigC strain grew poorly at 43 °C at pH 7.5 under ambient CO₂ conditions. Addition of inorganic carbon in the form of 3% CO₂ or use of an alkaline growth medium (pH 8.3) restored the growth of the ΔsigC strain at 43 °C. These treatments compensate for the low concentration of inorganic carbon at high temperature. However, addition of organic carbon as glucose, pyruvate, succinate or 2-oxoglutarate did not restore growth of the ΔsigC strain at 43 °C. In the control strain, the amount of the SigC factor diminished after prolonged incubation at 43 °C if the pH of the growth medium was 7.5 or 6.7. Under alkaline conditions, the amount of the SigC factor remained constant at 43 °C and cells of the control strain grew better than at pH 7.5 or pH 6.7. The pH dependence of high-temperature growth was associated with changes in photosynthetic activity, indicating that the SigC factor is involved in adjustment of photosynthesis according to the amount of available inorganic carbon. Our results indicate that acclimation to low inorganic carbon is a part of acclimation to prolonged high temperature and that the SigC factor has a central role in this acclimation.

INTRODUCTION

Cyanobacteria are a group of evolutionarily ancient eubacteria that perform oxygenic photosynthesis, like higher plants and algae. According to the endosymbiosis theory, cyanobacteria are progenitors of plant chloroplasts. The ability of cyanobacteria to survive under a range of different environmental stress conditions makes them valuable model organisms for studies of molecular mechanisms of acclimation. In particular, the unicellular cyanobacterium Synechocystis sp. PCC 6803 (hereafter referred to as Synechocystis) has been extensively used in gene expression studies under a variety of different stress conditions (Huang et al., 2002; Suzuki et al., 2005; Osanai et al., 2006; Singh et al., 2006; Slabas et al., 2006; Tuominen et al., 2008). These and other studies have demonstrated that acclimation to changing environmental conditions requires changes in gene expression over a wide range of different functions. The sigma (σ) subunit of RNA polymerase holoenzyme is a key determinant of promoter recognition and transcription initiation in eubacteria, and replacement of a σ factor in RNA polymerase holoenzyme with another one switches the transcription pattern (Wösten, 1998).

The genome of Synechocystis contains nine genes encoding σ factors (Kaneko et al., 1996). The primary σ factor SigA is essential for cell viability (Imamura et al., 2003). The sigB, sigC, sigD and sigE genes encode group 2 σ factors that closely resemble the SigA factor but are not essential for cell viability (Pollari et al., 2008). Recent studies show that group 2 σ factors are important for acclimation of cyanobacteria to suboptimal conditions (Muro-Pastor et al., 2001a; Imamura et al., 2003; Osanai et al., 2005, 2006; Singh et al., 2006; Tuominen et al., 2003, 2006, 2008; Foster et al., 2007; Pollari et al., 2008, 2009). The sigF, sigG, sigH and sigI genes of Synechocystis encode alternative σ factors. The SigF factor is important for pilus formation and cell motility (Blaya et al., 1999; Asayama & Imamura, 2008), and sigG is known to be an essential gene (Matsui et al., 2007), but otherwise the physiological roles of the alternative σ factors remain to be elucidated.

The temperature optimum of Synechocystis is 30–32 °C, but the cells can grow at temperatures ranging from 15 to 43 °C (Inoue et al., 2001). For short periods, Synechocystis cells can tolerate temperatures up to 50 °C (Inoue et al.,...
Heat acclimation of *Synechocystis* sp. PCC 6803

2001), and exposure to non-lethal high temperature improves the overall thermotolerance of *Synechocystis* (Nishiyama et al., 1999; Lee et al., 2000). Photosynthesis is a heat-sensitive process, with the oxygen-evolving complex of photosystem II (PSII) being regarded as the most heat-sensitive component (Mamedov et al., 1993).

The processes of acclimation to high temperature have only been partly resolved in *Synechocystis*. An immediate response to high temperature is the transient production of heat-shock proteins. The histidine kinase Hik34 is a component of a negative regulatory pathway of some heat-shock genes (Suzuki et al., 2005; Slabas et al., 2006), and the CIRCE/HrcA system is involved in the regulation of the *groEL1* operon and the *groEL2* gene encoding heat-shock proteins (Nakamoto et al., 2003; Singh et al., 2006). The SigC factor has a crucial role in heat-shock responses and acquired thermal tolerance in *Synechocystis*, as it positively regulates the expression of heat-shock genes (Singh et al., 2006; Tuominen et al., 2006).

We recently noticed that another group 2 σ factor, the SigC factor, is also involved in high-temperature responses (Tuominen et al., 2008). However, the expression of heat-shock genes was not altered in the ΔsigC strain. In this study, we further investigated heat acclimation in *Synechocystis*. One consequence of high temperature is that less inorganic carbon is available because the solubility of CO₂ decreases with increasing temperature. Our results suggest that the SigC factor is required for adjustment of photosynthesis under high-temperature conditions.

**METHODS**

**Bacterial strains.** The glucose-tolerant strain *Synechocystis* sp. PCC 6803 was used as the control strain (CS) and the construction of the ΔsigC strain has been described previously (Tuominen et al., 2008). The BG-11 plates for the ΔsigC strain were supplemented with 50 μg kanamycin ml⁻¹, but no antibiotics were added to liquid cultures.

**Growth measurements.** *Synechocystis* cells were grown in BG-11 medium supplemented with 20 mM HEPES-NaOH, pH 7.5, at 32 °C under the continuous photosynthetic photon flux density (PPFD) of 40 μmol m⁻² s⁻¹. The cultures were shaken at 90 r.p.m. in a growth chamber at ambient air conditions. Growth was monitored by measuring OD₇₅₀ with a spectrophotometer (Lambda Bio, Perkin Elmer). Samples were diluted with BG-11 before measurements so that the OD₇₅₀ did not exceed 0.4, and the dilutions were accounted for in the calculation of results. One OD₇₅₀ unit corresponds to 4 × 10⁸ cells ml⁻¹ in both strains.

For CO₂ treatments, OD₇₅₀ was set to 0.1, and 30 ml cell cultures were stirred in 100 ml Erlenmeyer flasks inside a plexiglass box under the PPFD of 40 μmol m⁻² s⁻¹ at 32 °C. The box was filled under slight overpressure with a gas mixture containing, like air, 79% N₂ and 21% O₂, but only 30 p.p.m. CO₂ (AGA). In control experiments, the box was filled with normal air (~300 p.p.m. CO₂).

At the start of the heat experiments, OD₇₅₀ was set to 0.14 and growth was monitored at 43 °C under the PPFD of 35 μmol m⁻² s⁻¹. The pH of BG-11 medium was set to 6.7, 7.5 or 8.3 with 20 mM HEPES-NaOH, as indicated. The pH of the growth medium was measured after the growth period and was found to remain constant. In some experiments, 5 mM glucose, 0.1 mM cyclic adenosine 3',5'-monophosphate (cAMP), 20 mM 2-oxoglutarate, 20 mM pyruvate or 20 mM succinate was added, as indicated. To follow the recovery of the cells from high-temperature treatments, cell cultures were transferred to standard growth conditions.

**Determination of cAMP content.** Cells [10 μg ml⁻¹ chlorophyll a (chl a)] were heat-treated at 43 °C for 0, 1, 24 or 72 h. cAMP was isolated as described by Terauchi & Ohmori (1999), and cAMP concentrations were measured using a cAMP Biotrak enzyme immunoassay system (Amersham).

**Determination of photosynthetic activity in vivo.** The OD₇₅₀ of the cell culture was set to 0.14 and cells were incubated at 43 °C under a PPFD of 35 μmol m⁻² s⁻¹. The pH of the growth medium was set to 6.7, 7.5 or 8.3 with 20 mM HEPES-NaOH, as indicated. Aliquots of 1 ml were withdrawn after 1, 13, 16, 18, 44, 56, 75 or 95 h of incubation at 43 °C, the light-saturated rate of oxygen evolution was measured with an oxygen electrode (Hansatech) at 43 °C, and the OD₇₅₀ of each sample was measured. Photosynthetic activity was then calculated as μmol O₂ produced h⁻¹ (ml cell culture)⁻¹ at OD₇₅₀=1. As a control, the light-saturated rate of oxygen evolution at 32 °C was determined for cells grown under standard growth conditions at 32 °C.

Decay of chl a fluorescence yield after a single-turnover flash was measured with an FL 20 fluorometer (P.S. Instruments) in the absence and presence of 10 μM 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU). The 1 ml samples (containing 4.4 μg chl a) were measured at 32 °C (samples from growth conditions) or at 43 °C (samples from cultures treated at 43 °C for 1 or 24 h). The samples were first dark-incubated for 5 min. Each fluorescence relaxation curve started with measurement of the initial fluorescence level with low-intensity probe flashes, then a strong flash was fired and fluorescence intensity was probed with weak probe flashes. The time delay between the probe flashes increased exponentially in the course of each curve. In measurements done in the absence of DCMU, the first probe flash was fired 150 μs after the strong flash and eight probe flashes were fired per decade of time unit. Only two probe flashes per decade were used in the presence of DCMU.

The fluorescence curves were processed by subtracting initial fluorescence and normalizing the curves by dividing by the value obtained with the first probe flash after the strong flash. Analysis of the decay of chl a fluorescence yield after the strong flash was done using four first-order component reactions. Two fast components represent populations of PSII in which the primary quinone (QA) is oxidized by electron transfer to the secondary quinone (QB) or QA (submillisecond component), and PSII with an empty QA site (milliseconds component) (see Tyyystjärvi & Vass, 2004). Two slow components represent inactive PSII centres, one component tentatively representing PSII centres that oxidize QA via the recombination reactions S₂/Q₅ → S₂/Q₅ (1–5 s component), another component representing centres undergoing very slow oxidation of QA (component with ~20 s half-time).

**Western blotting.** Changes in the amount of the SigC factor were followed for 72 h at 43 °C in the cell cultures grown in BG-11 medium buffered with 20 mM HEPES at pH 6.7, 7.5 or 8.3. After the treatments, cells were rapidly cooled and collected by centrifugation at 10,000 g for 5 min at 4 °C. The cell pellets were washed with ice-cold STNE buffer (0.4 M sucrose, 10 mM Tris/HCl, pH 8.0, 10 mM NaCl, 20 mM sodium EDTA), and resuspended in 75 μl STNE buffer. One-third volume of acid-washed glass beads (Sigma) was added and cells were broken by vortexing three times for 2 min. Glass beads and unbroken cells were removed by centrifugation at 4000 g for 4 min at 4 °C. Membranes were collected by centrifugation at 19,500 g for 15 min at 4 °C. The supernatant was collected and recentrifuged for 15 min at 19,500 g at 4 °C to get the soluble protein fraction. The concentrations of proteins were measured with the Lowry protein

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assay (Bio-Rad). Proteins (50 μg) were separated by using 10% NEXT GEL SDS-PAGE according to the manufacturer’s instructions (Amresco). After electrophoresis, separated proteins were transferred to Immobilon-P membranes (Millipore). A SigC specific antibody was raised against amino acids 392–404 of *Synechocystis* SigC protein (Innovagen), and the CDP Star chemiluminescence kit (New England Biolabs) was used for detection in Western blotting. The SigC antibody was found to recognize a protein of ~51 kDa and another one at 60 kDa. The 51 kDa protein corresponded to the expected molecular mass of SigC, and was found to be missing from the ΔsigC strain. The 60 kDa band, on the other hand, was too big to be SigC, and was found in the ΔsigC samples. These data indicate that the 51 kDa band is SigC, while the 60 kDa band is non-specific.

**RESULTS AND DISCUSSION**

**Addition of organic carbon sources does not restore the growth of the ΔsigC strain at 43 °C**

We have previously shown that the ΔsigC strain is barely able to grow at 43 °C under normal air (Tuominen *et al.*, 2008). The equilibrium concentration of CO₂ at 43 °C can be estimated to be 76% of the concentration at 32 °C (calculated by using the temperature dependency of Henry’s law for CO₂). Thus, an inevitable consequence of high temperature is that less inorganic carbon is available in the growth medium. Interestingly, addition of inorganic carbon in the form of 3% CO₂ to the growth chamber partially restores the growth of the ΔsigC strain at 43 °C (Tuominen *et al.*, 2008).

At the standard growth temperature, 32 °C, addition of the organic carbon sources 5 mM glucose (Pollari *et al.*, 2008) or 20 mM pyruvate (Fig. 1a) enhanced growth of both the control and the ΔsigC strains. However, addition of glucose or pyruvate did not enhance growth at 43 °C (Fig. 1b). After 1 day of treatment at 43 °C, both strains recovered well if cell cultures were transferred to standard growth conditions (Fig. 1c). The addition of pyruvate enhanced growth during the recovery period in both strains (Fig. 1c).

**Fig. 1.** Effects of organic carbon sources on the growth of *Synechocystis* PCC 6803 control (CS) and ΔsigC strains at 32 and 43 °C. (a) The OD₇₃₀ of the cell cultures was set to 0.14 and the control (circles) and ΔsigC (triangles) cells were grown in BG-11 medium, pH 7.5, without (white symbols) or with 20 mM pyruvate (black symbols) at 32 °C. (b) The control (circles) and ΔsigC (triangles) cells were grown at 43 °C in BG-11 medium, pH 7.5, without additions (white symbols), with 20 mM pyruvate (black symbols) or with 5 mM glucose (grey symbols). (c) The control (circles) and ΔsigC (triangles) cells were first grown at 43 °C for 1 day and thereafter the cultures were transferred to standard growth conditions at 32 °C. Experiments were done with (black symbols) or without (white symbols) addition of 20 mM pyruvate. (d) The control (circles) and ΔsigC (triangles) cells were grown at 43 °C in BG-11 medium, pH 7.5, without additions (white symbols), with 20 mM succinate (black symbols) or with 20 mM 2-oxoglutarate (grey symbols). Each growth curve represents the mean of at least three independent experiments and error bars denote SEM.
To further test the role of low inorganic carbon, we grew the ΔsigC and control strains in low-CO₂ conditions at 32 °C. When the CO₂ concentration was lowered to 30 p.p.m., which corresponds to approximately 10% of the atmospheric concentration, cells grew more slowly than under air-level CO₂; at the beginning of the experiments the doubling time was 10.5 h for both strains under air level CO₂, and under low CO₂ the doubling time was 21 h for the control strain and 22 h for the ΔsigC strain (Fig. 2a). The ΔsigC strain grew almost as well as the control strain at the beginning of the low CO₂ experiment, but after 3 days the doubling time was 95 h for the control strain and twice as long, 197 h, for the ΔsigC strain. Transfer of cells to air-level CO₂ after 6 days in low CO₂ reactivated growth, the doubling time being about 35 h in both strains (Fig. 2b).

Transfer of Synechocystis cells from high-CO₂ conditions (3%) to normal air induces expression of many genes involved in carbon-concentrating mechanisms (Wang et al., 2004). In accordance with the lower availability of inorganic carbon at high temperatures, many of these genes are activated when cells are transferred from 32 to 43 °C (Tuominen et al., 2008). Cyanobacteria can actively take up both CO₂ and HCO₃⁻ using distinct inorganic carbon acquisition systems, some of which function constitutively and some of which are upregulated at low carbon concentrations (Ogawa & Kaplan, 2003). Inactivation of the two CO₂ uptake systems and three HCO₃⁻ uptake systems in the Δ5 mutant strain of Synechocystis results in a strain that does not show CO₂ or HCO₃⁻ uptake activity, does not grow under normal air, but grows in high-CO₂ conditions (Xu et al., 2008). However, deficiencies in the carbon-concentrating mechanisms are unlikely to explain the poor growth of the ΔsigC strain at 43 °C, as DNA microarray analyses have shown that the mRNAs for all known proteins of carbon-concentrating mechanisms are at least as abundant in the ΔsigC strain as in the control strain (Tuominen et al., 2008).

The Synechocystis mutant strain Δ0776, which contains an inactivated spkD gene encoding a serine/threonine kinase, can grow under normal air only if the growth medium is supplemented with HCO₃⁻ (Laurent et al., 2008). Uptake of inorganic carbon, however, functions in the Δ0776 strain as well as in the control strain. It has been shown that the Δ0776 strain contains low amounts of intermediates of the tricarboxylic acid (TCA) cycle, and the growth of the Δ0776 strain is restored if TCA cycle intermediates are added but not if other organic carbon sources such as pyruvate or glucose are added (Laurent et al., 2008). Growth of the ΔsigC strain at 43 °C, in turn, was not affected by addition of TCA cycle intermediates, 20 mM succinate or 20 mM 2-oxoglutarate (Fig. 1d). The finding that addition of any organic carbon source did not restore the growth of the ΔsigC strain at 43 °C, although supplementing the air in the growth chamber with 3% CO₂ partially did (Tuominen et al., 2008), suggests that the slow growth of the ΔsigC strain at high temperature is due to deficiencies in photosynthesis, although the reason is more complicated than just slow production of carbon skeletons.

**Fig. 2.** Growth of control (CS) and ΔsigC strains under low CO₂. (a) The control (circles) and ΔsigC (triangles) cells were grown under the PPFD of 40 µmol m⁻² s⁻¹ at 32 °C under normal air (open symbols) or air containing only 30 p.p.m. CO₂ (filled symbols). (b) The control (circles) and ΔsigC (triangles) cells were first grown for 6 days under low-CO₂ conditions, and thereafter cells were transferred to standard conditions for 4 days.

**Growth at 43 °C is dependent on pH**

The pH of the growth medium remarkably affects the form and amount of available inorganic carbon. We used BG-11 medium buffered to pH 6.6, 7.5 or 8.3. By using the dissociation constant of HCO₃⁻ of 4.3 × 10⁻² mol l⁻¹, the equilibrium carbon concentrations (CO₂ plus HCO₃⁻) of the growth medium at pH 6.7 and pH 8.3 were calculated to be 0.2 and six times the carbon concentration at pH 7.5, respectively. At alkaline pH, most carbon is in the form of HCO₃⁻, while at pH 6.7, 46% of the dissolved carbon is CO₂.

In our standard growth medium at pH 7.5, ΔsigC cells hardly grew at all at 43 °C, but cells of the control strain...
grew quite well for up to 3 days (Fig. 3a). In the control strain, the amount of SigC diminished on the third day of incubation (Fig. 4). At pH 6.7, the control strain grew slowly (Fig. 3b) and only a trace amount of SigC protein was left after 3 days at 43 °C. These results might indicate that the SigC factor is important for growth when the amount of inorganic carbon, particularly HCO₃⁻, is low. Under alkaline conditions, where more inorganic carbon is available, both the control and the ΔsigC strains grew well (Fig. 3c). Similarly, the ΔsigC strain can grow at 43 °C if the CO₂ concentration inside the growth chamber is high (Tuominen et al., 2008).

The ΔsigC strain contains a low amount of cAMP

The secondary messenger molecule cAMP is a possible player in the same signalling cascade as the SigC factor, as it was earlier shown that bicarbonate regulates the activity of the adenyl cyclase which generates cAMP in Synechocystis (Masuda & Ono, 2005). The importance of cAMP for cell motility on agar plates is well characterized in Synechocystis. Inactivation of the adenylate cyclase gene cya1 (Terauchi & Ohmori, 1999) or a cAMP receptor gene sycrp1 (Yoshimura et al., 2002) has been reported to lead to a non-motile phenotype. More recently, three phases were resolved in cell motility and it was reported that the third phase is affected in cya1 and sycrp1 mutants (Bhaya et al., 2006). Those authors also showed that the motility of the cya1 mutant is restored with the addition of 0.1 mM cAMP. Computational prediction of cAMP receptor protein binding sites suggests that cAMP is involved in the regulation of other cellular processes as well, as putative target genes include photosynthesis, carbon metabolism, transporter and transcription factor genes (Xu & Su, 2009).

We followed changes in cAMP content at 43 °C in the control and ΔsigC strains. In the ΔsigC strain, the amount of cAMP was lower under standard conditions and after 1, 24 and 72 h treatments at 43 °C (Fig. 5a). This observation suggests that the SigC factor is involved in the regulation of the cAMP content of the cells. In the control strain, in turn, the amount of cAMP decreased with the incubation time at 43 °C, and after 72 h incubation, the amount of cAMP had decreased to 13% of that measured under standard growth conditions. However, addition of 0.1 mM cAMP only slightly activated growth of the ΔsigC strain at 43 °C (Fig. 5b).

Photosynthetic activity of the control and ΔsigC strains depends on the pH of the growth medium at 43 °C

After 1 h at 43 °C, the photosynthetic activity was approximately three times higher than that measured at 32 °C in both the control and ΔsigC strains, whether the pH was 6.7, 7.5 or 8.3 (Fig. 6). The measured triple activity of photosynthesis at the beginning of 43 °C treatment is similar to that reported by Inoue et al. (2001), who found that the photosynthetic activity doubled when the cells were shifted from 32 to 42 °C and the activity was measured after 5 min.

Analysis of the decay of chl a fluorescence yield after a single-turnover flash indicated that after 1 h treatment at
43 °C, electron transfer from PSII to the plastoquinone pool was slower than under standard growth conditions. The two fast components reflecting forward electron transport of PSII showed lower amplitudes and slightly longer half times in cells treated for 1 h at 43 °C than in cells grown at 32 °C (Table 1). Thus, changes in the function of PSII do not explain the upregulation of photosynthesis during 1 h incubation at 43 °C. The increase in light-saturated photosynthetic activity is most probably related to more efficient function of the Calvin–Benson cycle at higher temperature.

The photosynthetic activity of the control strain further increased during prolonged growth in BG-11 medium, pH 7.5, at 43 °C, and maximal photosynthetic activity was measured after 18 h of high-temperature treatment (Fig. 6a). Thereafter, the light-saturated rate of oxygen evolution of the control strain diminished gradually (Fig. 6a). Unlike in the control strain, the light-saturated rate of oxygen evolution of the ΔsigC strain did not further increase after the first hour at 43 °C; furthermore, the gradual decrease occurred more rapidly, and in the last sample taken after 95 h at 43 °C, no oxygen evolution was detected in the ΔsigC strain (Fig. 6a).

When cells were grown in BG-11 medium at pH 6.7 at 43 °C, the light-saturated photosynthetic activity of both the control and the ΔsigC strains gradually decreased after 1 h of high-temperature treatment. The loss of photosynthetic activity was faster in the ΔsigC strain than in the control strain (Fig. 6b). At pH 8.3, in contrast, the light-saturated photosynthetic activity of both strains remained high throughout the experiment (Fig. 6c). This finding is consistent with the result that both strains were able to grow at 43 °C if the pH was 8.3.

The oxygen-evolving complex of PSII has been shown to be heat sensitive in cyanobacteria (Mamedov et al., 1993). In order to test for known symptoms of damage to the oxygen-evolving complex, we measured the relaxation of fluorescence yield after a single-turnover flash in the presence of the herbicide DCMU. For these experiments, the control and ΔsigC strains were incubated at pH 7.5 for

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**Fig. 4.** Amount of the SigC protein. The control (CS) and ΔSigC strains were grown in BG-11 medium, pH 7.5, under standard conditions for 72 h (leftmost panel). In the three other panels, CS cells were grown at 43 °C in BG-11 medium buffered with 20 mM HEPES at pH 7.5, pH 6.7 or pH 8.3 for 24, 48 or 72 h prior to protein isolation, as indicated. Proteins were separated by SDS-PAGE, and the amount of SigC protein was determined by Western blotting using a SigC protein specific antibody. The protein standard was MagicMarkXP protein standard (Invitrogen).

**Fig. 5.** Secondary messenger cAMP in the control and ΔsigC strains. (a) Control (black bars) and ΔsigC (grey bars) cells were treated at standard growth conditions (32 °C), and at 43 °C for 1, 24 and 72 h. Each bar represents the mean of three independent experiments and error bars denote SEM. (b) The OD<sub>730</sub> of the cell cultures was set to 0.14 and the control (CS, circles) and ΔsigC (triangles) cells were grown at 43 °C in BG-11 medium without (open symbols) or with 0.1 mM cAMP (filled symbols). Each growth curve represents the mean of three independent experiments and error bars denote SEM.
1 or 24 h at 43 °C. If the oxygen-evolving complex is damaged, these measurements are expected to reveal a rapid recombination reaction between Q_A and oxidized tyrosine D of PSII (Vass et al., 1999). Signs of such a reaction were not seen in the control strain after 1 or 24 h incubation at 43 °C (Fig. 7). However, the ΔsigC strain showed faster initial relaxation of fluorescence yield in the presence of DCMU after 24 h treatment at 43 °C (Fig. 7). The signal to noise ratio was low in these data because the amplitude of variable fluorescence was very low after prolonged heat treatment.

Although changes in the activity of the oxygen-evolving complex were not obvious in the control strain, analysis of fluorescence relaxation in the absence of DCMU revealed that moderate heat treatments caused changes in PSII function. The percentage of inactive PSII centres increased from 24% to 43–47% in the control strain and from 34% to 49–51% in the ΔsigC strain; this increase occurred at the expense of PSII centres that take part in photosynthetic electron transfer from Q_A to Q_B (Table 1). Interestingly, the time constant of the millisecond component of fluorescence relaxation, associated with PSII centres in which the rate of electron transfer from Q_A to Q_B is limited by the binding of plastoquinone to the Q_B pocket of PSII, increased substantially during prolonged high-temperature treatment in both strains (Table 1). These data suggest that moderate heat treatment inactivates the binding of plastoquinone to the Q_B pocket in a fraction of PSII centres.

Photosynthesis has been reported to be pH dependent in some *Synechocystis* mutants. A mutant lacking proteins of the oxygen-evolving complex of PSII is able to grow at alkaline pH but not at neutral or acidic pH (Eaton-Rye et al., 2003). Furthermore, *Synechocystis* strains ΔNdhB, with a non-functional NAD(P)H dehydrogenase complex, and ΔNdhD3/NdhD4, with an inactivated CO₂ uptake system, grow at pH 8.3, but not at pH 7.5 (Zhang et al., 2004).

![Fig. 6. Changes in the photosynthetic activity of the control and ΔsigC strains during growth at 43 °C. The control (black columns) and ΔsigC (grey columns) cells were grown at 43 °C in BG-11 medium buffered with 20 mM HEPES, pH 7.5 (a), pH 6.7 (b) or pH 8.3 (c). The light-saturated oxygen evolution activity, measured with an oxygen electrode, was calculated as μmol O₂ produced h⁻¹ (ml cell culture⁻¹) at OD₇₃₀=1. The 0 h measurement was done at 32 °C. Each bar represents the mean of at least three independent experiments and error bars denote SEM.](image)

![Fig. 7. Relaxation of chl a fluorescence yield after a single-turnover flash in the presence of 10 μM DCMU in the control (black) and ΔsigC (blue) strains. The samples were taken from standard growth conditions (solid line), or after 1 h (dashed line) or 24 h (dotted line) treatments at 43 °C.](image)
SigC factor is required for survival under unfavourable conditions

It has been shown that the SigC factor in Synechocystis and its homologue SigE factor in Synechococcus sp. PCC 7002 contribute to gene expression and survival of the cells in the stationary phase (Gruber & Bryant, 1998; Asayama et al., 2004; Imamura et al., 2006). Inactivation of the sigC gene has been shown to reduce the transcription of the central nitrogen regulatory gene glnB in stationary phase but not during exponential growth (Asayama et al., 2004). Our results indicate that Synechocystis cells enter stationary phase rapidly at high temperature. However, unlike under standard growth conditions, nitrogen starvation is unlikely to be a central player in induction of the stationary phase at high temperature. Cessation of growth at high temperature occurs so rapidly that nitrogen depletion of the growth medium has not yet occurred, and addition of 2-oxoglutarate, which acts as a sensor molecule for nitrogen status (Muro-Pastor et al., 2001b), did not restore growth at 43 °C. Our results point to the involvement of the SigC factor in adjusting photosynthesis at high temperature. Addition of an organic carbon source does not restore the growth of the ΔsigC strain at high temperatures, although high CO₂ or elevated bicarbonate concentrations occurring at high pH do restore growth, suggesting that photosynthesis as such is important. In addition to linear electron transfer and production of carbon skeletons, photosynthetic electron transfer components are involved in cyclic electron flow around photosystem I (PSI). In cyanobacteria, few different routes for cyclic electron flow have been suggested (Mi et al., 1992; Yeremenko et al., 2005). Cyclic electron flow has turned out to be important under many different stress conditions, including low CO₂ and high salt. As the SigC factor seems to be important under many of the same conditions, including low inorganic carbon, as was shown in the present study, or under high salt (Pollari et al., 2008), it might have a regulatory role in cyclic electron flow. This possible connection can be studied in detail after the cyclic electron transfer routes have first been elucidated in detail.

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REFERENCES


**Table 1.** Flash-induced increase and subsequent decay of chl a fluorescence yield in the control (CS) and ΔsigC strains, measured from cells grown under standard conditions and after the cells were treated for 1 or 24 h at 43 °C

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>( \frac{A}{C_0} )Q( \Delta )2B/Q( \Delta )2A (t( \Delta )) (s) (%)</th>
<th>( \frac{A}{C_0} )Q( \omega )11/2Q( \omega )1 (t( \omega )) (s) (%)</th>
<th>Q( \omega )1/2,1/Q( \omega )1 (inactive) (t( \omega )) (s) (%)</th>
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</tr>
<tr>
<td>ΔsigC</td>
<td>24 h at 43 °C</td>
<td>350 28</td>
<td>46 23</td>
<td>1.4 35</td>
</tr>
</tbody>
</table>

Four first-order component reactions were separated, representing populations of PSI in which Q\( \Delta \)A is oxidized by electron transfer to Q\( \Delta \)B or Q\( \Delta \)S (microsecond component), PSI with an empty QB site (millisecond component) and two types of inactive PSI centres, one tentatively oxidizing Q\( \Delta \)A via the recombination reactions S\( \omega \), QB/Q\( \omega \)11/2Q\( \omega \)1 (1–5 s component), another undergoing very slow oxidation of Q\( \Delta \)A, possibly representing inactive PSI in states S\( \omega \), QB/Q\( \omega \)11/2Q\( \omega \)1 (component with ~20 s half-time). The amplitude of the relaxation component is shown as a percentage (%).


Edited by: A. Wilde