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 (*slr0184*), encoding the group 2 sigma factor SigC, leads to a heat-sensitive phenotype of *Synechocystis* sp. PCC 6803. Cells of the Δ sigC 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 Δ sigC 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 Δ sigC 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.

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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 (Bhaya *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.*,

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Abbreviations: chl *a*, chlorophyll *a*; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; PPFD, photosynthetic photon flux density; PSII, photo-system II.

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 *groESL1* operon and the *groEL2* gene encoding heat-shock proteins (Nakamoto *et al.*, 2003; Singh *et al.*, 2006). The SigB 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 Δ sigC 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 Δ sigC strain has been described previously (Tuominen *et al.*, 2008). The BG-11 plates for the Δ sigC strain were supplemented with 50 μ g 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 μ mol 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 OD₇₃₀ with a spectrophotometer (Lambda Bio, Perkin Elmer). Samples were diluted with BG-11 before measurements so that the OD₇₃₀ did not exceed 0.4, and the dilutions were accounted for in the calculation of results. One OD₇₃₀ unit corresponds to 4×10^6 cells ml⁻¹ in both strains.

For CO₂ treatments, OD₇₃₀ 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 μ mol 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, OD₇₃₀ was set to 0.14 and growth was monitored at 43 °C under the PPFD of 35 μ mol 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 μ g 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 OD₇₃₀ of the cell culture was set to 0.14 and cells were incubated at 43 °C under a PPFD of 35 μ mol 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 OD₇₃₀ of each sample was measured. Photosynthetic activity was then calculated as μ mol O₂ produced h⁻¹ (ml cell culture)⁻¹ at OD₇₃₀=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 200 fluorometer (P.S. Instruments) in the absence and presence of 10 μ M 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU). The 1 ml samples (containing 4.4 μ g 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 μ s 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 (Q_A⁻) is oxidized by electron transfer to the secondary quinone (Q_B) or Q_B⁻ (submillisecond component), and PSII with an empty Q_B site (millisecond component) (see Tyystjärvi & Vass, 2004). Two slow components represent inactive PSII centres, one component tentatively representing PSII centres that oxidize Q_A via the recombination reactions S_{2/3}Q_A⁻→S_{1/2}Q_A (1–5 s component), another component representing centres undergoing very slow oxidation of Q_A⁻ (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 μ l 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

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 Δ sigC strain. The 60 kDa band, on the other hand, was too big to be SigC, and was found in the Δ sigC 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 Δ sigC strain at 43 °C

We have previously shown that the Δ sigC 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 Δ sigC 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 Δ sigC 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 Δ sigC strains at 32 and 43 °C. (a) The OD₇₃₀ of the cell cultures was set to 0.14 and the control (circles) and Δ sigC (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 Δ sigC (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 Δ sigC (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 Δ sigC (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 $\Delta sigC$ and control strains in low- CO_2 conditions at 32 °C. When the CO_2 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 CO_2 ; at the beginning of the experiments the doubling time was 10.5 h for both strains under air level CO_2 , and under low CO_2 the doubling time was 21 h for the control strain and 22 h for the $\Delta sigC$ strain (Fig. 2a). The $\Delta sigC$ strain grew almost as well as the control strain at the beginning of the low CO_2 experiment, but after 3 days the doubling time was 95 h for the control strain and twice as long, 197 h, for the $\Delta sigC$ strain. Transfer of cells to air-level CO_2 after 6 days in low CO_2 reactivated growth, the doubling time being about 35 h in both strains (Fig. 2b).

Transfer of *Synechocystis* cells from high- CO_2 conditions (3 %) to normal air induces expression of many genes

Fig. 2. Growth of control (CS) and $\Delta sigC$ strains under low CO_2 . (a) The control (circles) and $\Delta sigC$ (triangles) cells were grown under the PPFD of $40 \mu mol m^{-2} s^{-1}$ at 32 °C under normal air (open symbols) or air containing only 30 p.p.m. CO_2 (filled symbols). (b) The control (circles) and $\Delta sigC$ (triangles) cells were first grown for 6 days under low- CO_2 conditions, and thereafter cells were transferred to standard conditions for 4 days.

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 CO_2 and HCO_3^- 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 CO_2 uptake systems and three HCO_3^- uptake systems in the $\Delta 5$ mutant strain of *Synechocystis* results in a strain that does not show CO_2 or HCO_3^- uptake activity, does not grow under normal air, but grows in high- CO_2 conditions (Xu *et al.*, 2008). However, deficiencies in the carbon-concentrating mechanisms are unlikely to explain the poor growth of the $\Delta sigC$ 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 $\Delta sigC$ strain as in the control strain (Tuominen *et al.*, 2008).

The *Synechocystis* mutant strain $\Delta 0776$, which contains an inactivated *spkD* gene encoding a serine/threonine kinase, can grow under normal air only if the growth medium is supplemented with HCO_3^- (Laurent *et al.*, 2008). Uptake of inorganic carbon, however, functions in the $\Delta 0776$ strain as well as in the control strain. It has been shown that the $\Delta 0776$ strain contains low amounts of intermediates of the tricarboxylic acid (TCA) cycle, and the growth of the $\Delta 0776$ 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 $\Delta sigC$ 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 $\Delta sigC$ strain at 43 °C, although supplementing the air in the growth chamber with 3 % CO_2 partially did (Tuominen *et al.*, 2008), suggests that the slow growth of the $\Delta sigC$ strain at high temperature is due to deficiencies in photosynthesis, although the reason is more complicated than just slow production of carbon skeletons.

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 HCO_3^- of $4.3 \times 10^{-7} mol l^{-1}$, the equilibrium carbon concentrations (CO_2 plus HCO_3^-) 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 HCO_3^- , while at pH 6.7, 46 % of the dissolved carbon is CO_2 .

In our standard growth medium at pH 7.5, $\Delta sigC$ 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

Fig. 3. Growth of the control (CS) and $\Delta sigC$ strains at 43 °C in BG-11 medium, pH 7.5 (a), pH 6.7 (b) and pH 8.3 (c). The OD₇₃₀ of the cell cultures was set to 0.14, and the control (circles) and $\Delta sigC$ (triangles) cells were grown at 43 °C. The BG-11 medium was buffered with 20 mM HEPES-NaOH. Each growth curve represents the mean of five independent experiments and error bars denote SEM.

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 HCO_3^- , is low. Under alkaline conditions, where more inorganic carbon is available, both the control and the $\Delta sigC$ strains grew well (Fig. 3c). Similarly, the $\Delta sigC$ strain can grow at 43 °C if the CO₂ concentration inside the growth chamber is high (Tuominen *et al.*, 2008).

The $\Delta sigC$ 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 $\Delta sigC$ strains. In the $\Delta sigC$ strain, the amount of cAMP was low 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 $\Delta sigC$ strain at 43 °C (Fig. 5b).

Photosynthetic activity of the control and $\Delta sigC$ 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 $\Delta sigC$ 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

Fig. 4. Amount of the SigC protein. The control (CS) and Δ SigC 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 Δ SigC strains. (a) Control (black bars) and Δ SigC (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 OD₇₃₀ of the cell cultures was set to 0.14 and the control (CS, circles) and Δ SigC (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.

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 Δ SigC 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 Δ SigC 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 Δ SigC strains gradually decreased after 1 h of high-temperature treatment. The loss of photosynthetic activity was faster in the Δ SigC 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 Δ SigC strains were incubated at pH 7.5 for

Fig. 6. Changes in the photosynthetic activity of the control and Δ sigC strains during growth at 43 °C. The control (black columns) and Δ sigC (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 $\mu\text{mol O}_2$ 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.

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 Δ sigC 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 Δ sigC 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 Δ NdhB, with a non-functional NAD(P)H dehydrogenase complex, and Δ NdhD3/NdhD4, with an inactivated CO₂ uptake system, grow at pH 8.3, but not at pH 7.5 (Zhang *et al.*, 2004).

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 Δ sigC (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.

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

Four first-order component reactions were separated, representing populations of PSII in which Q_A^- is oxidized by electron transfer to Q_B or Q_B^- (microsecond component), PSII with an empty Q_B site (millisecond component) and two types of inactive PSII centres, one tentatively oxidizing Q_A via the recombination reactions $S_{2/3}Q_A^- \rightarrow S_{1/2}Q_A$ (1–5 s component), another undergoing very slow oxidation of Q_A^- , possibly representing inactive PSII in states $S_{1/0}Q_A^-$ (component with ~20 s half-time). The amplitude of the relaxation component is shown as a percentage (%).

Strain	Treatment	$Q_A^- Q_B \rightarrow Q_A Q_B^-$		$Q_A^- [] \rightarrow Q_A Q_B^-$		$Q_A^- S_{2/3} \rightarrow Q_A S_{1/2}$		$Q_A^- \rightarrow Q_A$ (inactive)	
		$t_{1/2}$ (μ s)	(%)	$t_{1/2}$ (ms)	(%)	$t_{1/2}$ (s)	(%)	$t_{1/2}$ (s)	(%)
CS	32 °C	350	52	11	24	4.6	13	23	11
CS	1 h at 43 °C	350	34	13	19	1.5	28	23	19
CS	24 h at 43 °C	400	36	120	21	1.3	27	23	16
Δ sigC	32 °C	340	41	10	25	1.5	16	23	18
Δ sigC	1 h at 43 °C	350	31	14	18	1.3	33	17	18
Δ sigC	24 h at 43 °C	350	28	46	23	1.4	35	23	14

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 Δ sigC 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; Yermenko *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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