The SigB σ Factor Regulates Multiple Salt Acclimation Responses of the Cyanobacterium *Synechocystis* sp. PCC 6803[^W]

Hanna-Leena Nikkinen[^2], Kaisa Hakkila[^2], Liisa Gunnelius, Tuomas Huokko, Maija Pollari[^3], and Taina Tyystjärvi[^*]

Department of Biochemistry and Food Chemistry, Molecular Plant Biology, University of Turku, FIN–20014 Turku, Finland

Changing of principal σ factor in RNA polymerase holoenzyme to a group 2 σ factor redirects transcription when cyanobacteria acclimate to suboptimal environmental conditions. The group 2 sigma factor SigB was found to be important for the growth of the cyanobacterium *Synechocystis* sp. PCC 6803 in high-salt (0.7 M NaCl) stress but not in mild heat stress at 43°C although the expression of the sigB gene was similarly highly, but only transiently up-regulated at both conditions. The SigB factor was found to regulate many salt acclimation processes. The amount of glucosylglycerol-phosphate synthase, a key enzyme in the production of the compatible solute glucosylglycerol, was lower in the inactivation strain than in the control strain. Addition of the compatible solute trehalose almost completely restored the growth of the sigB strain at 0.7 M NaCl. High-salt conditions lowered the chlorophyll and phycobilin contents of the cells while protective carotenoid pigments, especially zeaxanthin and myxoxanthophyll, were up-regulated in the control strain. These carotenoids were up-regulated in the ΔsigCDE strain (SigB is the only functional group 2 σ factor) and down-regulated in the ΔsigB strain under standard conditions. In addition, the HspA heat shock protein was less abundant and more abundant in the ΔsigB and ΔsigCDE strains, respectively, than in the control strain in high-salt conditions. Some cellular responses are common to heat and salt stresses, but pretreatment with mild heat did not protect cells against salt shock although protection against heat shock was evident.

Different cyanobacterial species have adapted to ecological niches varying from fresh water to hypersaline environments. Research of salt acclimation is important because large-scale cultivation of cyanobacteria, for example for bioenergy production, would best occur in brackish water or seawater, as fresh water supplies of the Earth are limited. Salt acclimation processes in cyanobacteria are already fairly well known (for recent review, see Hagemann, 2011) but the sensing and transmission of salt signals are not yet well understood.

Salt stress acclimation and signaling processes are best characterized in a model cyanobacterium *Synechocystis* sp. strain PCC 6803 (hereafter *Synechocystis*). *Synechocystis* is a unicellular, nontoxic, nonnitrogen-fixing freshwater cyanobacterium that is moderately halotolerant. Salt acclimation in cyanobacteria occurs in five phases (Hagemann, 2011). A salt shock causes rapid shrinking of the cells due to loss of water (Blumwald et al., 1983) via aquaporins (Shapiguzov et al., 2005). In the second phase, ions including Na⁺ and Cl⁻ passively enter the cells and water flows back to the cells (Reed et al., 1985) but cellular processes including photosynthesis (Allakhverdiev et al., 2000) and gene expression (Hagemann et al., 1994; Fulda et al., 2006) remain slow due to high ion (especially Na⁺) content of the cells. The third phase is characterized by an exchange of Na⁺ to K⁺ that allows the reactivation of photosynthesis, and the beginning of synthesis of compatible solutes (Reed et al., 1985). In *Synechocystis*, as in many other moderately halotolerant species, the main compatible solute is glucosylglycerol (Hagemann, 2011). Salt addition activates an inactive form of glucosylglycerol-phosphate synthase, and also enhances the transcription of the *ggpS* gene encoding glucosylglycerol-phosphate synthase (Marin et al., 2002; Stirnberg et al., 2007; Hagemann, 2011; Novak et al., 2011). In the fourth phase, typically occurring 2 to 12 h after the onset of salt stress, compatible solutes accumulate to such high concentrations that they are mainly responsible for the maintenance of the osmotic potential. In this phase, also the gene expression pattern changes (Hagemann et al., 1994; Marin et al., 2004; Fulda et al., 2006) to finally allow full acclimation to high-salt conditions, which is the fifth phase.

[^W]: The online version of this article contains Web-only data. www.plantphysiol.org/cgi/doi/10.1104/pp.111.190058

[^1]: This work was supported by the Academy of Finland and Finnish Graduate School for Plant Biology.

[^2]: These authors contributed equally to the article.

[^3]: Present address: VTT Technical Research Centre of Finland, FI–02044 Espoo, Finland.

[^*]: Corresponding author; e-mail taitty@utu.fi.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Taina Tyystjärvi (taitty@utu.fi).

[^1]: The online version of this article contains Web-only data. www.plantphysiol.org/cgi/doi/10.1104/pp.111.190058
The actual mechanism of salt sensing is still unclear. In *Synechocystis*, transcriptome analyses with His kinase/response regulator mutants have revealed that at least Hik33/Rre31, Hik34/Rre1, Hik2/Rre1, Hik16/Hik41/Rre17, and Hik10/Rre3 participate, but any of these regulators could be inactivated without great impact on growth in salt stress conditions, suggesting functional redundancy (Shoumskaya et al., 2005). Furthermore, these regulators are involved in other stresses like osmotic, oxidative, or heat stress (Paithoonrangsarid et al., 2004; Shoumskaya et al., 2005; Suzuki et al., 2005; Kanesaki et al., 2007).

The microarray technique has revealed that several hundred genes are up- or down-regulated during salt acclimation, many of them encoding proteins with unknown function (Kanesaki et al., 2002; Marin et al., 2004; Fulda et al., 2006). Highly activated genes include genes encoding enzymes involved in osmolyte synthesis and transport, ion transporters, and common stress proteins like heat shock proteins and Hlips (Marin et al., 2004). The actual regulatory mechanisms remain to be solved. In cyanobacteria, the central role of group 2 σ factors in acclimation to many suboptimal environmental conditions has been noticed (for review, see Osanai et al., 2008). Many σ subunits of the RNA polymerase holoenzyme might be involved in adjustments of gene expression during salt acclimation. Inactivation of the group 2 σ factor SigB leads to a salt-sensitive phenotype, and also the ΔsigC and ΔsigE strains grow slowly after addition of 0.7 M NaCl (Pollari et al., 2008). Furthermore, inactivation of the group 3 σ factor SigF delayed the activation of the *ggps* gene and the accumulation of glucosylglycerol (Marin et al., 2002). A specific repressor protein, GppR, that suppresses the expression of the *ggpS* gene under low-salt conditions was recently discovered (Klahn et al., 2010).

In this study, we focused on the role of the SigB factor in salt acclimation processes by comparing the ΔsigB and ΔsigCDE strains (SigB is the only functional group 2 σ factor). The sigB gene is rapidly but only transiently induced in many stress conditions including high salt and hyperosmotic stress (Kanesaki et al., 2002), heat stress (Marin et al., 2004; Tuominen et al., 2006; Singh et al., 2006), and hydrogen-peroxide-induced oxidative stress (Kanesaki et al., 2007). The expression of SigB depends on light (Imamura et al., 2003; Tuominen et al., 2003), and the SigB factor is important for high-light tolerance of *Synechocystis*, too (Pollari et al., 2011). We show that the SigB factor regulates many cellular processes involved in salt acclimation, including the production of the HspA heat shock protein and the GgpS enzyme as well as the carotenoid content of the cell. Furthermore, our results show that although partly the same regulatory factors are involved in heat and salt stresses, pretreatment with mild heat stress does not protect cells against salt shock although protection against heat shock is evident.
RESULTS AND DISCUSSION

The SigB Factor Is Crucial for Acclimation to Mild Salt Stress But Not for Acclimation to Mild Heat Stress

The expression of the sigB gene is rapidly but only transiently up-regulated both at high temperature (43°C) and in high salt (0.7 M NaCl) in the Glc-tolerant control strain of the cyanobacterium *Synechocystis* sp. PCC 6803 (Fig. 1A). The temperature optimum of *Synechocystis* is 30°C to 32°C, but it can grow for a few days at 43°C (Inoue et al., 2001; Tuominen et al., 2006; Gunnelius et al., 2010). In mild heat stress at 43°C, both the ΔsigB and ΔsigCDE strains grew as well as the control strain (Fig. 1B). The form of all growth curves was typical to mild heat stress conditions, as cells grew well for 3 d but thereafter growth was arrested. If incubation at 43°C was further continued, cultivations completely bleached within next few days (data not shown). According to these results, the SigB factor is not particularly necessary for long-term acclimation to mild heat stress.

When the cells were subjected to high salt stress (0.7 M NaCl), growth of the control strain slowed down, duplication times of control strain were approximately 12.5 h in standard BG-11 medium (Rippka et al., 1979) and 22 h in BG-11 medium supplemented with 0.7 M NaCl, respectively. No growth arrest or bleaching was observed if cultivation in high-salt conditions was continued (data not shown). The ΔsigB strain grew more slowly in high-salt conditions than the control strain; the doubling time of the ΔsigB was 50 h at the beginning of the salt stress, and 26 h 2 d after the onset of the salt stress. In contrast, the ΔsigCDE strain grew as well as the control strain (Fig. 1C). These results suggest that the SigB factor is important for efficient acclimation to high salt stress although the cells can slowly acclimate to high-salt conditions even without the SigB factor. In nature, the capacity to acclimate rapidly to suboptimal conditions is most probably highly important, as it guarantees the best-possible premise in competition.

Salt-Induced Changes in Protein Synthesis Are Fairly Similar in the Control and ΔsigB Strains

To follow changes in translational activity during acclimation to high-salt conditions, the control and ΔsigB cells were labeled with [35S]-Met for 30 min directly after adding 0.7 M NaCl, and also after 1 or 4 d of preacclimation to high-salt conditions (Fig. 2A). Addition of 0.7 M NaCl drastically decreased translation, and only a few newly synthesized proteins were detected after the first 30 min in salt stress in the control and ΔsigB strains. The overall translation activity was partially restored after 1 d, and full activation was observed after 4 d of high-salt treatment. The pattern of newly synthesized proteins was quite different in the presence of 0.7 M NaCl compared to that detected under standard conditions (Fig. 2A). Salt-induced translational changes resembling those observed here have been reported earlier (Hagemann et al., 1994; Fulda et al., 2006). The protein patterns closely resemble each other in the control and ΔsigB strains during salt acclimation, but the overall translational activity was slightly more depressed in the ΔsigB strain than in the control strain in the beginning of the salt stress.

Similarly, the light-saturated oxygen-evolving activity was measured from the control (black bars) and ΔsigB (gray bars) strains with oxygen electrode using 0.7 mM 2,6-dichloro-p-benzoquinone as an electron acceptor. Before measurements, cells were grown for 2 d in standard BG-11 medium (0) or 2, 3, and 4 d in the BG-11 medium supplemented with 0.7 M NaCl. PSII activities are expressed as a percentage of the activity measured in the standard BG-11-grown cells. One milliliter of the control and ΔsigB strain cell culture (Δ750 = 1) produced 2.75 ± 0.30 and 2.85 ± 0.09 μmol O₂ h⁻¹, respectively.
fast in high-salt conditions but the repair of damaged PSII centers was severely impaired (Allakhverdiev et al., 2002). In our experiments, the recovery of PSII activity after long acclimation to high-salt conditions might be related to the recovery of the translation. We have previously shown that SigB and SigD or factors are important for the normal up-regulation of the psbA genes in high-light conditions (Pollari et al., 2009) and here we found that PSII recovery was not as efficient in the ΔsigB strain as in the control strain in high-salt conditions.

**SigB Is Required for Normal Production of the HspA and GgpS Proteins**

We selected two proteins, GgpS and HspA, for more detailed analyses. The GgpS enzyme is known to be crucial for acclimation to high salt, as a ggpS gene inactivation strain is characterized by inability of the cells to divide normally in high salt although cells grow normally in standard BG-11 medium (Ferjani et al., 2003). The HspA heat shock protein was selected for analysis because the expression of the hspA gene is highly up-regulated in high-salt conditions (Kanesaki et al., 2002). An hspA gene inactivation strain shows various defects in salt acclimation (Asadulghani et al., 2004) and the SigB factor is known to regulate the expression of the hspA gene in high temperature (Tuominen et al., 2006).

In the control strain, the amount of the HspA protein was below the detection limit under our standard conditions and in the beginning of the high-salt treatment but accumulated after 6 h and particularly 24 h after addition of 0.7 M NaCl (Fig. 3A). Less HspA protein was detected in the ΔsigB strain than in the control strain although high-salt-induced accumulation of the HspA protein was evident also in the ΔsigB strain (Fig. 3B). On the contrary, a higher amount of HspA protein accumulated in the ΔsigCDE strain than in the control strain upon salt treatment (Fig. 3B). Northern-blot analyses revealed that the differences in the HspA protein levels in the mutant strains were due to lower and higher amounts hspA mRNAs in the ΔsigB and sigCDE strains, respectively (Fig. 3E).

The GgpS protein was quite abundant in our standard conditions and further accumulation was only seen 24 h after the addition of 0.7 m NaCl (Fig. 3A). Less HspA protein was detected in the ΔsigB strain than in the control strain although high-salt-induced accumulation of the HspA protein was evident also in the ΔsigB strain (Fig. 3B). On the contrary, a higher amount of HspA protein accumulated in the ΔsigCDE strain than in the control strain upon salt treatment (Fig. 3B).

Northern-blot analyses revealed that the differences in the HspA protein levels in the mutant strains were due to lower and higher amounts hspA mRNAs in the ΔsigB and sigCDE strains, respectively (Fig. 3E).

The GgpS protein was quite abundant in our standard conditions and further accumulation was only seen 24 h after the addition of 0.7 m NaCl (Fig. 3C). The amount of the GgpS protein in standard growth conditions was approximately 75% lower in the ΔsigB strain than in the control strain, and remained low during the first hours of salt treatments as well (Fig. 3D). However, after 24-h salt treatment the GgpS protein content was quite similar in all strains. Northern-blot analyses revealed that the differences in the HspA protein levels in the mutant strains were due to lower and higher amounts hspA mRNAs in the ΔsigB and sigCDE strains, respectively (Fig. 3E).
and ΔsigCDE strains, and the highest ggpS mRNA levels were detected 6 h after onset of salt stress. In the ΔsigB strain, however, accumulation of the ggpS mRNA started only 6 h after the salt addition, and highest amount of ggpS mRNA was measured in the 24-h sample (Fig. 3E). Thus, inactivation of the SigB σ factor does not totally prevent salt-induced activation of the ggpS gene but seriously retards the activation process.

A low amount of the GgpS enzyme in the ΔsigB strain might lead to low production of the main compatible solute glucosylglycerol. *Synechocystis* cells are able to uptake externally added compatible solutes glucosylglycerol or trehalose, and addition of these compatible solutes to growth medium allows the growth of a *Synechocystis* mutant that does not synthesize glucosylglycerol in high-salt conditions (Mikkat et al., 1997). Addition of 1 mM trehalose (we used trehalose because it is commercially available unlike glucosylglycerol) almost completely restored the growth of the ΔsigB strain in the high-salt conditions (Fig. 4). Taken together, the results can be interpreted as follows. The low amount of the GgpS protein in the beginning of the salt treatment leads to low content of the compatible solute glucosylglycerol in the ΔsigB strain. That, in turn, disturbs the cell division (observed as slow growth) of the ΔsigB cells. However, after the first day in high-salt conditions, the GgpS protein accumulates also in the ΔsigB strain and enables faster growth of the ΔsigB strain (doubling times were 22 and 24 h for the control strain and 50 and 26 h for the ΔsigB strains just after the onset of salt stress and after couple of days acclimation to high-salt stress, respectively).

**SigB Factor Regulates the Carotenoid Content of the Cells**

High-salt cultures tend to be slightly yellowish compared to cultures in standard BG-11 medium, and cultures supplemented with both 0.7 mM NaCl and 1 mM trehalose were clearly yellowish. In standard BG-11 medium, addition of 1 mM trehalose did not affect the growth or color of the cell cultures. The chlorophyll (Chl) *a* content of the high-salt-grown control and ΔsigB cells was 8% lower than in standard BG-11 medium but similar decrease was not detected in the cells of the ΔsigCDE strains (Fig. 5). When trehalose was added to high-salt cultures, the ΔsigB and ΔsigCDE strains lost slightly more Chl *a* than the control strain (Fig. 5).

The pigment changes were further analyzed by measuring in vivo whole-cell absorption spectra from cells grown in BG-11 medium supplemented with 0.7 mM NaCl with and without 1 mM trehalose for 3 d. A representative spectrum of each treatment is shown in Supplemental Figure S1. In all strains, the ratio of the phycobilin absorption peak at 625 nm to the Chl *a* peak at 678 nm diminished in salt-stressed cells compared to that measured from cells grown in standard BG-11 medium (Fig. 6). When cell cultures were supplemented both with salt and trehalose, the phycobilin to Chl *a* ratio was 5% to 10% lower than under standard conditions (Fig. 6).

Salt-induced changes in the carotenoid content were measured from the in vivo absorption spectra at 485 nm. Under standard growth conditions, the carotenoid to Chl *a* ratio was 10% lower in the ΔsigB strain and almost 20% higher in the ΔsigCDE strain than in the control strain. Since the Chl *a* content of all three strains is identical in standard conditions (Fig. 5), these results indicate that the SigB factor regulates the carotenoid content of the cells. Salt stress alone, and especially the presence of externally added trehalose, substantially increased the carotenoid to Chl *a* ratio in the control and ΔsigB strains while the already high carotenoid to Chl *a* ratio of the ΔsigCDE strain increased only slightly in high salt stress and by 10% when high-salt samples were supplemented with tre-
halose (Fig. 6). Accumulation of carotenoids in the presence of trehalose could protect cells against salt-induced production of reactive oxygen species (ROS), and thus protect the cells against oxidative damage.

HPLC analyses revealed that the high carotenoid content of the \( \Delta \text{sigCDE} \) strain in standard growth conditions is due to high levels of myxoxanthophyll and zeaxanthin (130% and 192% of that measured in the control strain, respectively) while echinenone and \( \beta \)-carotene contents were similar in the \( \Delta \text{sigCDE} \) and control strains (Fig. 7). The \( \Delta \text{sigB} \) strain, in turn, contained only 67% of myxoxanthophyll and 81% of zeaxanthin of those measured from the control strain in standard growth conditions (Fig. 7).

**Figure 6.** The ratio of phycobilins or carotenoids to Chl \( \alpha \) in salt-stressed control, \( \Delta \text{sigB} \), and \( \Delta \text{sigCDE} \) cells. The control (A), \( \Delta \text{sigB} \) (B), and \( \Delta \text{sigCDE} \) (C) cells were grown for 3 d in BG-11 medium, in BG-11 medium supplemented with 0.7 M NaCl, or with 0.7 M NaCl and 1 mM trehalose. The carotenoid peak at 485 nm, phycobilin peak at 625 nm, and Chl \( \alpha \) peak at 678 nm were measured from in vivo absorption spectra, and the ratios of phycobilin to Chl \( \alpha \) (black bars) and carotenoids to Chl \( \alpha \) (light-gray bars) were calculated. Each bar represents the mean of three independent biological replicates, and the error bars denote SE.

**Figure 7.** Carotenoid contents of the control, \( \Delta \text{sigB} \), and \( \Delta \text{sigCDE} \) strains in the standard and high-salt conditions. Cells were grown for 3 d in BG-11 medium (black bars), in BG-11 medium supplemented with 0.7 M NaCl (light-gray bars), or with 0.7 M NaCl and 1 mM trehalose (gray bars). Pigments were extracted with methanol and analyzed with HPLC. Relative amounts of myxoxanthophyll (A), zeaxanthin (B), echinenone (C), and \( \beta \)-carotene (D) were calculated on Chl \( \alpha \) basis. Each bar represents the mean of three independent biological replicates, and the error bars denote SE.
Addition of 0.7 M NaCl highly increased accumulation of myxoxanthophyll (150% of that measured in standard conditions) and doubled the zeaxanthin content of the cells in the control and ΔsigB strains. The amount of echinenone was quite similar in all strains, and only in the presence of trehalose some salt-induced increase in echinenone content was detected in the control and ΔsigB strains.

Carotenoids are efficient nonenzymatic scavengers of ROS. Construction of a carotenoidless double-mutant ΔcrtH/B was possible under light-activated heterotrophic growth conditions but this mutant is not viable in the light (Sozer et al., 2010). Mutants without zeaxanthin or echinenone and especially the double mutant defective of both zeaxanthin and echinenone are sensitive to photooxidative damage (Schäfer et al., 2005). Salt stress has been suggested to induce ROS production, and both photosynthesis and translation are known to be ROS sensitive (Nishiyama et al., 2004, 2006; Kojima et al., 2007). Enhanced accumulation of carotenoids in salt stress might actually be important for reactivation of photosynthesis and translation during salt acclimation.

Instead, the β-carotene content of the ΔsigCDE strain increased. In the presence of 1 mM trehalose, even higher amounts of myxoxanthophyll and zeaxanthin accumulated in high-salt conditions, especially in the control and ΔsigB strains. The amount of echinenone was quite similar in all strains, and only in the presence of trehalose some salt-induced increase in echinenone content was detected in the control and ΔsigB strains.

Figure 8. Survival rates of the control (CS), ΔsigB, and ΔsigCDE strains in high temperature and salt stresses. In the control samples, 68 ± 3, 64 ± 5, and 63 ± 4 colonies were counted in the CS, ΔsigB, and ΔsigCDE strains, respectively. The survival rates were calculated after 1-h treatment with mild heat stress conditions (43°C), after a 15-min heat shock at 48°C without (HS) and with a 1-h pretreatment in mild high-temperature conditions (43°C + HS), and after a 2-h treatment with 1 M NaCl without (salt) and with a 1-h pretreatment in mild high-temperature conditions (43°C + salt). Each bar represents the mean of three or four independent biological replicates, and the error bars denote se.

Mild Heat Pretreatment Protects against Heat Shock But Not against Salt Shock

Although the SigB factor is not important for long-term acclimation to mild heat stress (Fig. 1), previous studies have revealed that the SigB factor is important for survival in severe heat shock and also for acquired thermotolerance, most probably because SigB is required for normal up-regulation of the hspA gene (Tuominen et al., 2006). Since SigB and HspA are up-regulated by mild heat treatment (Tuominen et al., 2006), we tested if a mild heat treatment could improve not only heat tolerance but also salt tolerance of the cells, as HspA has been suggested to play a role in
high-salt tolerance of the cells (Asadulghani et al., 2004).

One-hour incubation at 43°C was used as a mild heat treatment. This mild heat treatment induced the expression of the sigB and hspA genes but did not affect survival rates of the control, ΔsigB, or ΔsigCDE strains (Fig. 8). For a severe heat shock, cells were treated at 48°C for 15 min. After a 15-min heat shock, approximately 25% of cells of the control strain survived while survival rates of the ΔsigB and ΔsigCDE strains were only 10% (Fig. 8). When the severe heat shock was applied after 1-h pretreatment at 43°C, 100% survival was reached in the control and ΔsigCDE strains, indicating that these strains acquired full thermotolerance (Fig. 8). In contrast, the survival rate of the ΔsigB strain cells was only 77% (Fig. 8), indicating the SigB factor is required for full thermotolerance.

We also tested if the heat pretreatment would improve survival during salt shock. Approximately 55% of the control strain cells survived a 2-h treatment with 1 M NaCl. A similar survival rate was measured for the ΔsigCDE strain while only 30% of ΔsigB cells survived, indicating that SigB factor is not only important for acclimation to long mild salt stress but also to survival during sudden extreme salt stress (Fig. 8). Pretreatment with mild heat for 1 h did not induce salt resistance in any of the studied strains. In contrast, all strains were slightly more sensitive to 1 M salt stress than without pretreatment (Fig. 8). The central role of compatible solutes for high-salt tolerance might be the reason why pretreatment with heat doesn’t induce salt tolerance, as heat pretreatment induces production of heat shock proteins but not production of compatible solutes.

CONCLUSION

Many His kinases (Shoumskaya et al., 2005), three group 2 σ factors SigB, SigC, and SigE (Pollari et al., 2008), two group 3 σ factors (Huckauf et al., 2000; Marin et al., 2002), and a transcriptional repressor protein, GppR (Klähn et al., 2010) have been identified as potential regulators of salt acclimation of *Synechocystis* cells. Figure 9 summarizes salt acclimation processes that are at least partly regulated by the SigB factor. The SigB factor is highly but only transiently expressed in high salt, and the effects of SigB are mainly seen in the beginning of salt stress. The slow growth of the ΔsigB strain in high-salt conditions is due to small defects in many processes. Because the addition of the compatible solute trehalose almost completely restored the growth of the ΔsigB strain at high-salt stress, it might be that a low amount of the GppS enzyme, especially in the very beginning of the salt stress, is the main reason why ΔsigB cells acclimate only very slowly to high-salt stress. In addition, the SigB factor is an important regulator of the HspA heat shock protein, as low and high amounts of HspA protein were detected in the ΔsigB and ΔsigCDE strains, respectively. Carotenoids, especially zeaxanthin and myxoxanthophyll, accumulate to high levels in salt stress, and they might have a central role in protecting photosynthesis and translation against oxidative damage. The SigB factor is involved in regulation of carotenoid content as the amounts of myxoxanthophyll and zeaxanthin are approximately 30% and 20% lower, respectively, in the ΔsigB strain than in the control strain in standard conditions. However, these carotenoids are up-regulated in high-salt conditions also in the ΔsigB strain (although especially myxoxanthophyll content remained lower in the ΔsigB strain than in the control strain), and thus possible carotenoid-related ROS-scavenging problems in the ΔsigB strain might be seen mainly in early phases of salt stress.

MATERIALS AND METHODS

Strains and Growth Conditions

The Glc-tolerant strain of *Synechocystis* sp. PCC 6803 (Williams, 1988) was used as a control strain. The constructions of the single-mutant ΔsigB and the triple-mutant ΔsigCDE strains have been described earlier (Tuominen et al., 2006; Pollari et al., 2011). Cells were grown in BG-11 medium supplemented with 20 mM HEPES-NaOH, pH 7.5, under the continuous photosynthetic photon flux density (PPFD) of 40 μmol m⁻² s⁻¹ at 32°C in an ambient CO₂. These conditions are referred to as the standard growth conditions. The BG-11 agar plates for the ΔsigB strain were supplemented with kanamycin (50 μg mL⁻¹), and those for the ΔsigCDE strain with kanamycin (50 μg mL⁻¹), spectinomycin (20 μg mL⁻¹), streptomycin (10 μg mL⁻¹), and chloramphenicol (5 μg mL⁻¹). For the experiments, all strains were grown without antibiotics in liquid BG-11 medium.

For growth measurements the A₅₇₀ of liquid cultures was set to 0.1 (30-mL cell culture in a 100-mL Erlenmeyer flask), and A₅₇₀ was measured every 24 h.

Samples of dense cultures were diluted so that A₅₇₀ did not exceed 0.3, and the dilutions were taken into account when the final results were calculated. The cells were grown in BG-11 medium supplemented with 0.7 M NaCl with or without 1 mM trehalose, as indicated, under the continuous PPFD of 40 μmol m⁻² s⁻¹ at 32°C or in standard BG-11 medium at 4°C under the continuous PPFD of 40 μmol m⁻² s⁻¹.

Light-Saturated PSII Activity

Light-saturated PSII activity in vivo was measured from 1 mL of cell suspension (A₅₇₀ = 1) in BG-11 medium under saturating light (2,000 μmol photons m⁻² s⁻¹) with a Clark-type oxygen electrode (Hansatech) at 32°C. BG-11 medium was supplemented with an electron acceptor, 0.7 mM 2,6-dichlorophenoxybenzoquinone, and 0.7 mM ferricyanide was added to keep the quinone in an oxidized form.

In Vivo Absorption Spectra

In vivo absorption spectra were measured with a UV-3000 spectrophotometer (Shimadzu) from 400 to 800 nm.

Chl a Content and HPLC Analyses of Carotenoids

Chl a was extracted with methanol and Chl a content was measured as described previously (Mackinney, 1941). For HPLC analysis, the harvested cells pellets (A₅₇₀ = 1; 2 mL) were frozen. The pellets were washed two times with BG-11 medium. Pigments were extracted with 200 μL of 100% methanol. Extracts were centrifuged twice at 12,000g for 5 min at +4°C and filtered through Whatman Puradisc FP 13 0.2 μm RC. Pigments were separated by HPLC according to Lehtimäki et al. (2011) with a reverse-phase C18 endcapped column, 5 μm particle size (LiChroCART 125-4, Hewlet Packard), using a series 1100 HPLC device with diode array detector (Agilent Technologies).
Myxoxanthophyll, zeaxanthin, echinenone, and β-carotene were detected at 490 nm and Chl a at 663 nm. Pigment standards were purchased from DHI Lab Products.

Northern-Blot Analysis

The cell culture was supplemented with 0.7 mM NaCl and samples (Ar50 = 1; 15 mL) were withdrawn after 0 min, 15 min, 1 h, and 6 h of incubation under standard growth conditions, or cells were incubated in standard BG-11 medium at 43°C for 0 min, 15 min, 1 h, and 6 h. Total RNA isolation and northern hybridizations were performed as described earlier (Tyyystjäri et al., 2000). Seven micrograms (Fig. 1) or 5 µg (Fig. 3) of RNA was loaded in each well. Equal loading of the gels was confirmed by methylene blue staining. Gene-specific probes were amplified by PCR using primers 5'-ATGCTAAAGTGACAGTTATAT-3' and 5'-GCTCTCATTCTTTCCGTTT-3' for sigB, 5'-GCTCTCATTCTTTCCGTTT-3' for hspA, and 5'-TATCCTCTCGATCTTCTTAC-3' and 5'-CACGGGAAACCAATCTGACCC-3' for ggsS. The probes were labeled using the Prime-a-gene labeling system (Promega) and α-32P dCTP 10 mCi/mL (Perkin Elmer) according to manufacturer's instructions.

35S Met Labeling and Immunological Detection of the HspA and GgpS Proteins

The control and ΔsigB strains were grown under standard conditions. The cells (Ar50 = 1, 15 mL) were pulse labeled for 30 min by adding 5 µL of radioactive Met [35S] L-Met (185 MBq, Perkin Elmer) under standard conditions, directly after addition of 0.7 mM NaCl, and after growing the cells for 24 h or 6 h in BG-11 medium supplemented with 0.7 mM NaCl before the labeling. After the labeling, cold L-Met (0.4 mg mL−1) was added, samples were rapidly cooled, and cells were collected by centrifugation at 4,000 g for 5 min at 4°C. The cell pellets were washed with ice-cold STNE buffer (0.4 M Suc, 10 mM Tris-HCl, pH 8.0, 10 mM NaCl, 20 mM Na-EDTA), and resuspended in 150 µL of STNE buffer. One-third volume of acid-washed glass beads (Sigma) was added and cells were broken by vortexing three times for 2 min at 4°C, and glass beads and unbroken cells were removed by centrifugation at 4,000 g for 4 min at 4°C. The centrifugation was repeated and the collected supernatant was used as a total protein sample. Polypeptides were solubilized for 5 min at 70°C and samples containing 20 µg of proteins were loaded and separated by 10% NEXT GEL SDS-PAGE (Amresco) according to the manufacturer's instructions. The proteins were transferred onto an Immobilon membrane (Millipore) and equal loading was confirmed by staining the membranes with 0.1% PonceauS solution. Radioactive proteins were visualized with autoradiography.

For immunological detection of the HspA and GgpS proteins, total proteins were isolated after 0-min, 30-min, 2-h, and 6-h, and 24-h treatments with BG-11 medium supplemented with 0.7 mM NaCl. Proteins were separated with SDS-PAGE and transferred to membrane as described above. For the HspA protein detection, samples containing 50 µg total proteins were loaded in each well, and a commercial HspA protein antibody (AS08286, Agrisera) was used. For detection of the GgpS protein, samples containing 15 µg total proteins were loaded in each well. The GgpS antibody was a generous gift from Prof. Martin Hagemann. The goat anti-rabbit IgG (H+L) alkaline phosphatase conjugate (Zymed) and the CDP star chemiluminescence kit (New England Biolabs) were used for detection in western blotting. After the immunodetection, the membranes were stained for 1 min with 0.1% Coomassie Brilliant Blue in 40% methanol and 10% acetic acid, and then destained with 15% methanol and 5% acetic acid to verify equal loading of the gels. Three independent biological replicates of western blots were performed. The results were very similar, and representative western blots are shown in Figure 3.

Viability Assays

For mild high-temperature stress, the cells (Ar50 = 0.8) were treated for 1 h at 43°C at the PPFD of 40 µmol m−2 s−1. For heat shock, the cell suspensions (Ar50 = 0.8) were treated at 48°C under dim light for 15 min and with and without a 1-h pretreatment at 43°C at the PPFD of 40 µmol m−2 s−1. For salt-stress treatments, cells (Ar50 = 2.0) were first incubated at 32°C or 43°C for 1 h, as indicated, and then NaCl was added to a final concentration of 1 M and Ar50 was set to 0.8, and the cells were further incubated 2 h in standard growth conditions. For experiments with pretreatment in mild salt stress combined with heat shock, cells (Ar50 = 2.0) were first incubated in 0.5 mM NaCl for 1 h in standard growth conditions. Salt concentration was diluted to 0.2 mM NaCl by addition of liquid BG-11, resulting in Ar50 of 0.8. Cells were then subjected to 48°C for 15 min.

To measure the survival rate, the cell suspensions were serially diluted with BG-11 medium and a 10-µL aliquot from each dilution was spotted onto BG-11 plates, and the cells were then grown in standard conditions for 6 d. Survival rates were calculated by dividing the number of colonies obtained after the treatments by the number of colonies obtained from a control sample taken from the same strain before any treatment.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. In vivo absorption spectra of the control (CS), ΔsigB, and ΔsigCDE strains grown in standard BG-11 medium (A–C), or in BG-11 medium supplemented with 0.7 mM NaCl (D–F), or with 0.7 mM NaCl and 1 mol trehalose (H–J) for 3 d.

ACKNOWLEDGMENTS

We thank Dr. Mikko Keränen for help with HPLC measurements, and Drs. Esa Tyyystjäri and Paula Mulo for helpful discussions. The GgpS antibody was kindly provided by Prof. Martin Hagemann.

Received October 26, 2011; accepted November 16, 2011; published November 17, 2011.

LITERATURE CITED


Salt Acclimation of Synechocystis

Acclimation to the growth temperature and the high-temperature effects on photosystem II and plasma membranes in a mesophilic cyanobacterium, Synechocystis sp. PCC6803. Plant Cell Physiol 42: 1140–1148


523


Downloaded from on July 31, 2017 - Published by www.plantphysiol.org
Copyright © 2012 American Society of Plant Biologists. All rights reserved.