Low-Oxygen Induction of Normally Cryptic *psbA* Genes in Cyanobacteria†

Tina C. Summerfield,†,‡ Jörg Toepel, and Louis A. Sherman*

Department of Biological Sciences, Purdue University, 201 South University Street, Hansen Hall, West Lafayette, Indiana 47907

Received October 7, 2008; Revised Manuscript Received October 27, 2008

ABSTRACT: Microarray analysis indicated low-O_2 conditions resulted in upregulation of *psbA1*, the normally low-abundance transcript that encodes the D1′ protein of photosystem II in *Synechocystis* sp. PCC 6803. Using a Δ*psbA2:*Δ*psbA3* strain, we show the *psbA1* transcript is translated and the resultant D1′ is inserted into functional PSII complexes. Two other cyanobacterial strains have *psbA* genes that were induced by low oxygen. In two of the three strains examined, *psbA* was part of an upregulated gene cluster including an alternative Rieske iron−sulfur protein. We conclude this cluster may represent an important adaptation to changing O_2 levels that cyanobacteria experience.

Cyanobacteria perform oxygenic photosynthesis; however, many strains grow heterotrophically, and some possess anaerobic growth capabilities (*1*). In habitats such as hot springs, soils (e.g., rice paddies or estuarine mud), and eutrophic lakes, cyanobacteria experience anaerobiosis, often accompanied by elevated levels of H_2S (*2*). Under such conditions, cyanobacteria are capable of anoxygenic photosynthesis with sulfide as the electron donor (*3*). Response to anaerobiosis differs among strains, including photosynthesis, that is, simultaneously oxygenic and anoxygenic, either oxygenic or anoxygenic, or neither (*1*).

Because cyanobacteria are able to produce ethanol and H_2 under fermentative conditions, a decreased level of O_2 may play an important role in this energy production. In addition, the hydrogenase and nitrogenase enzymes that produce H_2 are O_2 sensitive (*4, 5*). In *Synechocystis* sp. strain PCC 6803 (hereafter *Synechocystis*), the bidirectional hydrogenase shows maximal activity in the dark, although this strain is capable of light-driven H_2 production (*6*). However, no detailed study of transcriptional response to low O_2 has been performed in any cyanobacterium. We report the use of DNA microarrays in examining gene expression in *Synechocystis* incubated in light and bubbled with 99.9% N_2 and 0.1% CO_2 (see the Supporting Information).

RNA was harvested from cells grown aerobically ($t = 0$) and then bubbled with 99.9% N_2 and 0.1% CO_2, to produce low-O_2 conditions, for 1, 2, and 6 h. Microarrays were used to compare gene expression between aerobically grown and N_2/CO_2-bubbled cells. Approximately 9, 15, and 16% of the 3165 genes were differentially expressed after 1, 2, and 6 h under low-O_2 conditions, respectively, using the criteria in Table 1. At all three time points, >60% of differentially expressed genes were downregulated (Table 1). This included genes encoding proteins with functions in photosynthesis and respiration (e.g., ATP synthase and phycobilisome components) and in translation (e.g., many ribosomal proteins). These responses were consistent with bleaching of *Synechocystis* observed during extended incubation under low-O_2 conditions (data not shown). Details of genes that were differentially transcribed under low-O_2 conditions are provided in Table S1.

Table 1: Functional Categories of Genes Differentially Regulated under Low-O_2 Conditions Compared to Aerobic Conditions in *Synechocystis* sp. PCC 6803

general pathway	total no. of genes	differentially regulated[a] genes under low-O_2 vs aerobic conditions (Up)[b] t_1/t_0	t_2/t_0	t_6/t_0
amino acid biosynthesis	97	8 (2)	15 (4)	16 (5)
biosynthesis of cofactors, prosthetic groups, carriers	124	14 (6)	21 (9)	23 (9)
cell envelope	67	3 (0)	6 (0)	12 (1)
cellular processes	76	10 (1)	11 (2)	17 (3)
central intermediary metabolism	31	4 (1)	5 (1)	4 (1)
DNA replication, repair, restriction, recombination	60	1 (0)	4 (1)	2 (0)
energy metabolism	132	9 (2)	32 (12)	24 (10)
hypothetical	1076	74 (38)	121 (55)	135 (48)
other categories	306	26 (13)	38 (17)	44 (19)
photosynthesis and respiration	141	27 (2)	40 (6)	41 (5)
purines, pyrimidines, nucleosides, nucleotides	41	4 (0)	5 (0)	7 (0)
regulatory functions	146	14 (3)	27 (8)	26 (6)
transcription	30	7 (1)	9 (2)	8 (1)
translation	168	52 (3)	58 (7)	58 (6)
transport and binding proteins	196	13 (8)	31 (19)	34 (20)
unknown	474	34 (19)	55 (29)	53 (25)
total	3165[c]	300 (99)	478 (172)	504 (159)

[a] Genes are considered differentially regulated when FDR = 0.5 and the change is >1.5-fold. [b] Number of genes upregulated in a functional category. [c] Number of genes based on Kazusa annotation prior to 2002.

More than half of the upregulated genes encoded hypothetical or unknown proteins (Table 1). However, five gene clusters were strongly upregulated under low-O_2 conditions: slr1181−slr1185 that contained *psbA*1 and *petC2* [encoding alternative isoforms of the photosytem II (PSII) protein, D1, and Rieske iron−sulfur center protein, respectively] (*7*), sll1220−sll1126 (the *hox* genes encoding the bidirectional hydrogenase), sll0217−sll0221 (including genes encoding flavoproteins Flv2 and Flv4), sll0789 and sll0790 (encoding

† This work was supported by Grant DE-FG02-99ER20342 from the Department of Energy, and by the EMSL Scientific Grand Challenge project at the W. R. Wiley Environmental Molecular Science Laboratory, sponsored by the DOE, located at Pacific Northwest National Laboratory and operated by Battelle.

‡ Present address: Department of Biochemistry, University of Otago, PO Box 56, Dunedin, New Zealand.

* To whom correspondence should be addressed. Phone: (765) 494-0560. Fax: (765) 496-1496. E-mail: lsherman@purdue.edu.

10.1021/bi8018916 CCC: $40.75 © 2008 American Chemical Society
Published on Web 11/11/2008

a two-component sensor Hik31 and response regulator), and sll1875 and sll1876 encoding proteins involved in pigment synthesis.

The increased level of expression of *psbA1* under low-O_2 conditions was unexpected, as upregulation of this very low-abundance transcript has not been observed, although numerous conditions have been examined (*8, 9*). Many cyanobacterial genomes contain *psbA* gene families with two to six copies encoding one to three different D1 protein sequences. Multiple copies of *psbA* respond to specific stresses, mostly involved with light, e.g., high light or UV-B (*8*−*13*). Such conditions are associated with an increased amount of damage to PSII and repair requiring the removal and replacement of inactive D1. The *Synechocystis psbA2* and *psbA3* genes encode the same D1 protein; *psbA2* is constitutively expressed, and the high-light induction of *psbA3* increases the availability of D1. The *psbA1* gene encodes a protein, D1′, differing from D1 by 54 amino acids; the presence of D1′ has not been shown under normal growth conditions. Despite the numerous amino acid differences, under control of the *psbA2* promoter the *psbA1* gene produced D1′ that enabled photoautotrophic growth in a Δ*psbA2*:Δ*psbA3* strain (*8*). Our objectives were to investigate whether the *psbA1* transcript, under the control of its native promoter, is translated into D1′ that is incorporated into functional PSII centers under low-O_2 conditions. A second objective was to examine whether the low-O_2 induction of *psbA* was specific to *Synechocystis* or was part of a wider response found in other cyanobacteria. On the basis of these findings, we discuss the possible role of the alternate D1.

We determined whether the *psbA1* transcripts were translated into protein and if the protein was inserted into productive PSII complexes. Since *psbA* transcripts are abundant under normal growth conditions, ~95% *psbA2* and ~5% *psbA3* (*14*), we utilized a mutant in which both of these genes were knocked out (Δ*psbA2*:Δ*psbA3*). Thus, any PSII activity would be due to the insertion of the *psbA1* gene product (D1′). As expected, semiquantitative RT-PCR showed that the wild type transcribed *psbA2/3* under aerobic conditions, whereas no *psbA2/3* transcripts were detected in the Δ*psbA2*:Δ*psbA3* strain (Figure 1A). The *psbA1* transcript was not detected in the wild-type or mutant strain under aerobic conditions. An increasingly strong signal was observed in the Δ*psbA2*:Δ*psbA3* strain over the 10 h under low-O_2 conditions (Figure 1A). Western blot analysis detected D1′ protein in the mutant after 5 h under low-O_2 conditions (Figure 1B). The 77 K fluorescence spectra of the mutant strain indicated intact PSII complexes were present under low-O_2 conditions (Figure 1C). After excitation with 435 nm light, we observed a fluorescence peak at 695 nm in the wild type, corresponding to excitation of Chl a associated with CP47 and indicative of assembled PSII centers. This 695 nm peak was absent in the mutant grown aerobically, but present under low-O_2 conditions, with an increased peak size at 10 h compared to that at 5 h. D1′ restored photosynthetic capacity for the Δ*psbA2*:Δ*psbA3* mutant, as determined by measuring O_2 evolution using a Clark-type O_2 electrode (Figure 1D). The O_2 evolution rate in the mutant was close to that of the wild type at 5 h under low-O_2 conditions, and this rate was maintained at 10 h. We conclude that the *psbA1* gene was transcribed and translated and that D1′ was inserted to produce functional PSII complexes.

We next wanted to determine if similar low-O_2 induction occurred in other cyanobacterial strains. We examined a

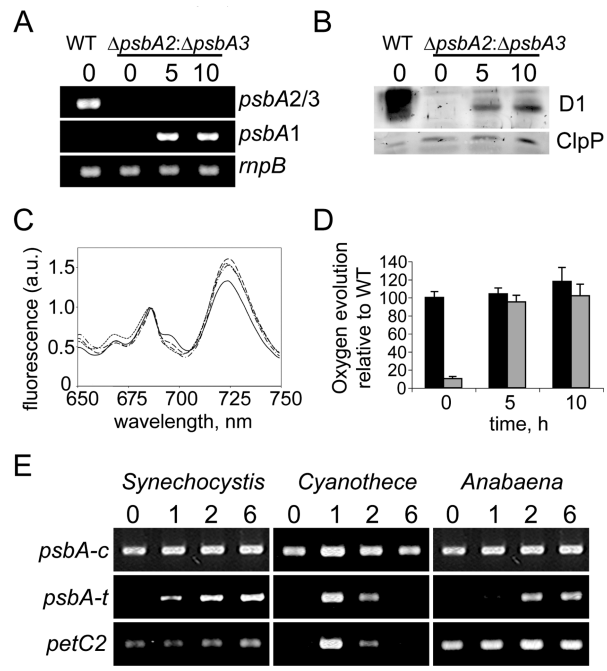

FIGURE 1: Increased abundance of *psbA* transcript and D1 protein under low-O_2 conditions. (A−D) *Synechocystis* sp. PCC 6803 wild type and a Δ*psbA2*:Δ*psbA3* mutant. (A) Semiquantitative RT-PCR using primers for *psbA2/A3*, *psbA1*, and *rnpB*. (B) Western blot using an antibody to D1 and ClpP. In panels A and B, the number above each lane represents time (in hours) under low-O_2 conditions. (C) Fluorescence at 77 K after excitation at 435 nm for WT cells grown in air (—) and the Δ*psbA2*:Δ*psbA3* mutant after growth in air (•••), and after low-O_2 induction for 5 (−•−) and 10 h (---). (D) O_2 evolution for wild-type cells (black bars) and the Δ*psbA2*:Δ*psbA3* mutant (gray bars) under aerobic (0 h) and low-O_2 conditions (5 and 10 h). O_2 evolution was normalized to that of the wild type at 0 h [423 μmol of O_2 evolved (mg of Chl)$^{-1}$ h^{-1}]. (E) Transcript levels of *psbA* and *petC2* determined by semiquantitative RT-PCR for cells grown in air (0 h) and then bubbled with 99.9% N_2 and 0.1% CO_2 for 1, 2, and 6 h. *psbA*-c represents the constitutively expressed gene for each strain: *Synechocystis* (*psbA2*, *psbA3*), *Cyanothece* (*psbA1*, *psbA5*), and *Anabaena* (*psbA1*). *psbA*-t represents the low-O_2 induced genes: *Synechocystis* (*psbA1*), *Cyanothece* (*psbA2*), and *Anabaena* (*psbA0*). *petC* gene in *Anabaena* (*all4511*).

unicellular, N_2-fixing strain *Cyanothece* sp. ATCC 51142 (hereafter *Cyanothece*) (*15*), a filamentous, heterocystous strain *Anabaena* sp. PCC 7120 (hereafter *Anabaena*), and the ancient and divergent strain *Gloeobacter violaceus* sp. PCC 7421 (hereafter *Gloeobacter*) (*13*). Semiquantiative RT-PCR results for *Cyanothece* and *Anabaena psbA* genes are compared to those for *Synechocystis* in Figure 1E. For the sake of simplicity, for each strain we compared *psbA*-c, a constitutive and highly expressed gene, to a gene that is transcribed in only trace amounts under aerobic conditions, *psbA*-t. In both of these strains, the *psbA*-t transcript was induced under low-O_2 conditions. Both strains have five copies of *psbA*, but only one or two of these are highly transcribed under normal aerobic conditions. *Cyanothece* resembles *Synechocystis* in that it has a *psbA* gene clustered near *petC2*. In *Cyanothece*, levels of both transcripts were strongly increased by 1 h under low-O_2 conditions, followed by a fairly rapid decline. Transcript abundance of the other two copies of *psbA* in *Cyanothece* was altered by <2-fold under low-O_2 conditions compared to normal growth conditions (data not shown). The low-O_2-induced expression of *psbA*-t of *Anabaena* showed kinetics more similar to those in *Synechocystis* (Figure 1E). *Anabaena psbAIII* and *psbAIV* transcripts were more abundant under low-O_2 conditions

(Figure S1A); this may represent a general stress response as the levels of *psbAIV* and, to a lesser extent, *psbAIII* transcripts were increased by UV-B treatment (*12*). In *Anabaena*, there was no *petC* gene close to *psbA*-t and none of the four *petC* genes showed induction similar to that of *Synechocystis* or *Cyanothece* (Figure S1B). In low-O_2-incubated *Gloeobacter*, we saw no specific gene induction of the five copies of *psbA* (including two genes expressed at trace levels under normal aerobic conditions) or the single copy of *petC* (Figure S1C,D). The abundance of *psbA* transcripts *gll3144* and *glr1706* decreased under low-O_2 conditions. High light and UV-B exposure decreased the level of the *glr1706* transcript; however, the level of the *gll3144* transcript increased (*13*).

The low-O_2-induced *psbA* expression may increase the D1 availability in a manner similar to that of the light stress-induced expression of *psbA* genes observed in many cyanobacterial strains (*8*−*13*). On the basis of our microarray data, under low-O_2 conditions, the *psbA* mRNA pool in the wild type increased and *psbA1* transcripts were ∼15% of the total, whereas light stress-induced *psbA* transcripts may represent almost 90% of *psbA* transcripts (*12*). However, in a Δ*psbA2*:Δ*psbA3* strain, the sole presence of the *psbA1* gene product (D1′) during the low-O_2 incubation was sufficient to maintain O_2 evolution rates similar to those of the wild type. The upregulation of *psbA* under low-O_2 conditions may help maintain the aerobic cellular environment; the importance of this varies between strains as cyanobacteria differ in their sensitivity to O_2 levels (*1, 3, 16*). Furthermore, low-O_2 conditions are often associated with an increased level of H_2S that can damage PSII; however, oxygen inactivates H_2S (*3*). Increasing D1′ levels could accelerate replacement of damaged D1, both increasing the rate of oxygenic photosynthesis and limiting further damage to PSII. Interestingly, the genomes of *Synechocystis*, *Anabaena*, and *Cyanothece*, but not *Gloeobacter*, contain sulfide quinone reductase-like genes (sqr) (*17*). The SQR protein transfers electrons from H_2S to the quinone pool and is present in many anoxygenic phototrophs, as well as *Aphanothece halophytica*, where it may have a role in detoxification of sulfide (*3*). Alternately, low-O_2 conditions on Earth, when oxygenic photosynthesis arose, raises the possibility that these low-O_2-induced *psbA* genes represent ancient genes from which the highly expressed copies diverged.

Amino acid differences between *psbA1*-encoded D1′ compared to *psbA2*- and *psbA3*-encoded D1 exist throughout the protein, but more are found at the N- and C-termini (*8*). However, the C-terminal cleavage site is present in all three low-O_2-induced D1 proteins. Furthermore, the N-terminal variations between D1′ and D1 in *Synechocystis* are not observed in the low-O_2-expressed D1 of *Cyanothece* or *Anabaena*. Residues identified from the *Thermosynechococcus elongatus* crystal structure as interacting with manganese, calcium, chlorophyll, pheophytin, or the secondary plastoquinone (Q_B) were the same in the D1 and D1′ protein (*18*). Additionally, studies on mutant strains expressing D1′ under normal growth conditions revealed only minor changes in the cycling of S-states or electron transfer through the plastoquinone electron acceptors compared to the wild type (*8, 11, 19*). Therefore, few clues regarding the different function or properties of D1′ can be derived from sequence comparison and characterization under aerobic conditions. Further work is required to investigate the function of D1′ under anaerobic conditions. In addition, it is unclear whether the D1 proteins encoded by the low-O_2-induced genes of *Cyanothece* and *Anabaena* have properties similar to those of D1′. Phylogenetic analyses were inconclusive in determining whether *Synechocystis psbA1* shares a common origin with the monophyletic group containing the *Cyanothece* and *Anabaena* low-O_2-induced *psbA* genes (Figures S2 and S3). The fact that low-O_2 regulation of a *psbA* gene is similar in both unicellular and heterocystous, diazotrophic cyanobacteria provides an important calibration for the evolution of heterocyst formation (*20*).

ACKNOWLEDGMENT

We thank R. Winter and J. Eaton-Rye for the gift of the Δ*psbA2*:Δ*psbA3* strain.

SUPPORTING INFORMATION AVAILABLE

Detailed methodology, microarray data, additional RT-PCR, and phylogenetic tables. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Stal, L. J. (1995) *New Phytol. 131*, 1–32.
2. Stewart, W. D. P., and Pearson, H. W. (1970) *Proc. R. Soc. London, Ser. B 175*, 293–311.
3. Padan, E., and Cohen, Y. (1982) in *The Biology of Cyanobacteria* (Carr, N. G., and Whitton, B. A., Eds.) pp 215−235, Blackwell Scientific Publications, Oxford, U.K.
4. Stal, L. J., and Moezelaar, R. (1997) *FEMS Microbiol. Rev. 21*, 179–211.
5. Ghirardi, M. L., Posewitz, M. C., Maness, P. C., Dubini, A., Yu, J., and Seibert, M. (2007) *Annu. Rev. Plant Biol. 58*, 71–91.
6. Cournac, L., Guedeney, G., Peltier, G., and Vignais, P. M. (2004) *J. Bacteriol. 186*, 1737–1746.
7. Schneider, D., Berry, S., Volkmer, T., Seidler, A., and Rögner, M. (2004) *J. Biol. Chem. 279*, 39383–39388.
8. Salih, G. F., and Jansson, C. (1997) *Plant Cell 9*, 869–878.
9. Campbell, D., Clarke, A. K., Gustafsson, P., and Öquist, G. (1999) *Physiol. Plant. 105*, 746–755.
10. Schaefer, M. R., and Golden, S. S. (1989) *J. Bacteriol. 171*, 3973–3981.
11. Sicora, C., Wiklund, R., Jansson, C., and Vass, I. (2004) *Phys. Chem. Chem. Phys. 6*, 4832–4837.
12. Sicora, C. I., Appleton, S. E., Brown, C. M., Chung, J., Chandler, J., Cockshutt, A. M., Vass, I., and Campbell, D. A. (2006) *Biochim. Biophys. Acta 1757*, 47–56.
13. Sicora, C. I., Brown, C. M., Cheregi, O., Vass, I., and Campbell, D. A. (2008) *Biochim. Biophys. Acta 1777*, 130–139.
14. Mohammed, A., Eriksson, J., Osiewacz, H. D., and Jansson, C. (1993) *Mol. Gen. Genet. 238*, 161–168.
15. Reddy, K. J., Haskell, J. B., Sherman, D. M., and Sherman, L. A. (1993) *J. Bacteriol. 175*, 1284–1292.
16. Camacho, A., Garcia-Pichel, F., Vicente, E., and Castenholz, R. W. (1996) *FEMS Microbiol. Ecol. 21*, 293–301.
17. Bronstein, M., Schütz, M., Hauska, G., Padan, E., and Shahak, Y. (2000) *J. Bacteriol. 182*, 3336–3344.
18. Müh, F., Renger, T., and Zouni, A. (2008) *Plant Physiol. Biochem. 46*, 238–264.
19. Funk, C., Wiklund, R., Schröder, W. P., and Jansson, C. (2001) *FEBS Lett. 505*, 113–117.
20. Tomitani, A., Knoll, A. H., Cavanaugh, C. M., and Ohno, T. (2006) *Proc. Natl. Acad. Sci. U.S.A. 103*, 5442–5447.

BI8018916