JOURNAL OF BACTERIOLOGY, Apr. 2006, p. 2730–2734 Vol. 188, No. 7
0021-9193/06/$08.00+0 doi:10.1128/JB.188.7.2730–2734.2006

P_{II}-Regulated Arginine Synthesis Controls Accumulation of Cyanophycin in *Synechocystis* sp. Strain PCC 6803

Mani Maheswaran,[1] Karl Ziegler,[2] Wolfgang Lockau,[2] Martin Hagemann,[3] and Karl Forchhammer[1]*

Institut für Mikrobiologie und Molekularbiologie, Justus-Liebig Universität Giessen, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany[1]; Institut für Biologie, Humboldt-Universität zu Berlin, Chausseestr. 117, D-10115 Berlin, Germany[2]; and Abteilung Pflanzenphysiologie, Universität Rostock, Albert-Einstein-Str. 3, D-18051 Rostock, Germany[3]

Received 1 December 2005/Accepted 20 January 2006

Cyanophycin (multi-L-arginyl-poly-L-aspartic acid) is a nitrogen storage polymer found in most cyanobacteria and some heterotrophic bacteria. The cyanobacterium *Synechocystis* sp. strain PCC 6803 accumulates cyanophycin following a transition from nitrogen-limited to nitrogen-excess conditions. Here we show that the accumulation of cyanophycin depends on the activation of the key enzyme of arginine biosynthesis, *N*-acetyl-L-glutamate kinase, by signal transduction protein P_{II}.

Cyanophycin (multi-L-arginyl-poly-L-aspartic acid) is a nitrogen-rich reserve polymer present in most cyanobacteria (reviewed in references 4, 5, 34, and 43) as well as in some heterotrophic bacteria (27, 49). It consists of a poly-α-aspartic acid backbone, with arginine linked to the β-carboxyl group of almost every aspartyl residue via isopeptide bonds (44). Cyanophycin is synthesized by a single enzyme, cyanophycin synthetase, from aspartate and arginine in an ATP-dependent reaction using a still-unidentified primer (1, 2, 8, 17, 42, 48). The amount of cyanophycin in cyanobacteria varies considerably with growth conditions. Its content is usually less than 1% of dry weight in rapidly growing cultures but is high (up to 18%) in stationary-phase cultures and under conditions of unbalanced growth such as sulfate or phosphate limitation (6, 30, 40, 45). When nitrogen-starved cyanobacterial cultures were provided with combined nitrogen sources, a rapid but transient accumulation of cyanophycin occurred (3). The cyanophycin contents of *Anabaena cylindrica* and *Synechocystis* sp. strain PCC 6803 increased severalfold when translation was inhibited by chloramphenicol (6, 41), indicating that rapid synthesis of the polymer did not depend on de novo synthesis of cyanophycin synthetase and that consumption of amino acids by protein synthesis may compete with the accumulation of cyanophycin. Furthermore, no correlation was found between the extractable activity of cyanophycin synthetase and the rate of polymer accumulation (31). These and several similar studies could not, so far, elucidate the mechanism(s) by which cyanophycin accumulation is regulated. Recently, it was shown that the genes for cyanophycin metabolism are under nitrogen control in the diazotrophic strain *Anabaena* sp. strain PCC 7120 (35). Furthermore, an involvement of the signal transduction protein P_{II} in the control of cyanophycin synthesis was suggested (19, 29) (see below).

The cyanobacterial P_{II} protein is a member of the large family of P_{II} signal transduction proteins, which play pervasive roles in nitrogen control in bacteria, plants, and some archaea (for recent reviews, see references 7 and 12). Similar to its *Escherichia coli* counterpart, P_{II} from the cyanobacterium *Synechococcus elongatus* PCC 7942 binds ATP and 2-oxoglutarate in a synergistic manner (13, 24). In the presence of increased 2-oxoglutarate levels, corresponding to nitrogen-limited conditions, P_{II} is phosphorylated at seryl residue 49 (14). Dephosphorylation of P_{II}-P in *Synechocystis* sp. strain PCC 6803 is catalyzed by PphA, a phosphatase of the PP2C family (23), under conditions of low 2-oxoglutarate levels (39). Recently, the first molecular target of P_{II} was identified in *S. elongatus*: *N*-acetyl-L-glutamate kinase (NAGK), which catalyzes the committed step in the cyclic arginine synthesis pathway (11). Its activity is strongly enhanced by complex formation with the nonphosphorylated form of P_{II}, signaling nitrogen-excess conditions (19). Furthermore, the effector molecules 2-oxoglutarate, ATP, and ADP as well as Ca^{2+} modulate NAGK-P_{II} complex formation (32). The NAGK-P_{II} interaction seems to be universally conserved in oxygenic phototrophs, including higher plants (10, 46). Based on the key function of NAGK in arginine synthesis, we hypothesized that P_{II} activation of arginine synthesis might play a role in the accumulation of cyanophycin under nitrogen-excess conditions (19). However, *S. elongatus* is one of a few cyanobacteria not able to synthesize cyanophycin, precluding investigation of this issue.

A mutant of the P_{II} phosphatase PphA homologue in the filamentous cyanobacterium *Anabaena* sp. strain PCC 7120 displays increased P_{II} phosphorylation levels in heterocysts (28) and is impaired in formation of cyanophycin polar bodies. By contrast, cyanophycin accumulated in vegetative cells, implying that P_{II} might be involved in controlling cyanophycin distribution along the filament. However, further studies of P_{II} function in *Anabaena* are impeded by the lack of P_{II}-null mutants in the *Nostocales* group (18). The present study was conducted to clarify the role of P_{II} in cyanophycin accumulation. The strain *Synechocystis* PCC 6803 was used since it produces cyanophycin (1, 15) and mutants in the P_{II} signaling system are available. In particular, we used a P_{II}-null mutant (22) and a PphA-deficient mutant (23), which exhibits significantly delayed P_{II} dephosphorylation upon ammonium addition (25), to study the correlation between P_{II} phosphorylation

* Corresponding author. Mailing address: Institut für Mikrobiologie und Molekularbiologie, Justus-Liebig Universität Giessen, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany. Phone: 0049-641-9935545. Fax: 0049-641-9935549. E-mail: Karl.Forchhammer@mikro.bio.uni-giessen.de.

status, NAGK activity, and cyanophycin accumulation following nitrogen-excess treatments.

The transformable wild-type *Synechocystis* sp. strain PCC 6803 (15) and the isogenic *Synechocystis* mutants MPphA (PphA deficient; *pphA*::*kan* [23]) and ΔP_{II} (P_{II} deficient; *glnB*::*spc* [22]) were routinely grown in liquid BG11 medium (38) supplemented with 5 mM $NaHCO_3$. The MPphA strain was maintained with kanamycin (50 μg ml^{-1}) selection and the ΔP_{II}-strain with spectinomycin selection (35 μg ml^{-1}). In the first set of experiments, wild-type cells of *Synechocystis* sp. strain PCC 6803 and the mutants ΔP_{II} and MPphA were shifted from nitrogen-poor to nitrogen-excess conditions. Nitrogen-limited cultures were prepared by harvesting cells from 2 ml of nitrate-replete stock culture and resuspending them in 100 ml of modified BG11 medium (low-N BG11) containing 1 mM of nitrate. These cultures were grown in triple-baffled flasks with shaking at 30°C, under continuous illumination of 50 μmol photons m^{-2} s^{-1} from white fluorescent lamps. When an optical density at 750 nm of 0.8 to 1.0 was reached (after approximately 4 days for the wild-type and MPphA strains and 5 days for the ΔP_{II} strain), cells started to get slightly bleached due to consumption of nitrate. After the time zero aliquots were taken ammonium chloride was added to a final concentration of 5 mM (nitrogen excess), and aliquots of the culture were harvested in the course of time. From these samples, the accumulation of cyanophycin, activity of NAGK, and phosphorylation status of P_{II} were analyzed. Furthermore, at selected time points, the amount of cellular arginine was determined. Cyanophycin was extracted (40) from 10-ml samples and enzymatically hydrolyzed by recombinant cyanophycinase (37) and recombinant isoaspartyl dipeptidase from *Synechocystis* sp. strain PCC 6803 (20) to arginine and aspartic acid. The mass of the polymer was calculated from the liberated aspartic acid, quantified enzymatically (33). Values were reproducible within ±5%. For the determination of NAGK activity and the phosphorylation status of P_{II}, cell extracts of the samples were prepared using a RiboLyser (Hybaid) as described previously (19) and protein concentration was estimated using the Bradford assay (9). One hundred micrograms of extract protein was used for a NAGK assay, and 5 μg of protein was used for P_{II} phosphorylation state analysis. To measure the intracellular arginine level, cells from 4 ml of culture were harvested, suspended in 1 ml of 80% ethanol, and incubated for 3 h at 65°C. Following centrifugation, the supernatant was dried and the arginine content was determined by high-pressure liquid chromatography according to reference 16.

As shown in Fig. 1A, wild-type cells rapidly accumulated cyanophycin following ammonium treatment. By contrast, cyanophycin accumulation was completely absent in the P_{II}-deficient mutant. The PphA-deficient strain showed an intermediate phenotype, having a delayed accumulation of cyanophycin compared to the wild type. Determination of NAGK activity revealed that, following ammonium upshift, wild-type cells rapidly increased the activity of this enzyme. By contrast, the P_{II}-deficient mutant was unable to increase NAGK activity, the same result observed previously in P_{II}-deficient cells of *S. elongatus* sp. strain PCC 7942 (19). The PphA-deficient cells showed a delayed increase of NAGK activity, compared to the wild type. Quantification of intracellular arginine following ammonium upshift revealed that the arginine level in wild-type

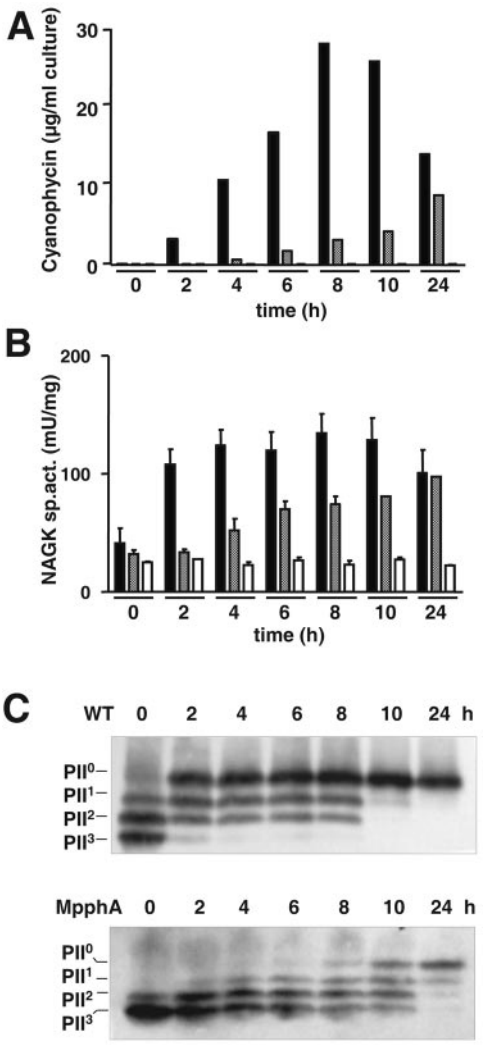

FIG. 1. Analysis of cyanophycin accumulation (A) and NAGK activity (B) in cell extracts prepared from *Synechocystis* sp. strain PCC 6803 cells, which were grown under nitrate-limiting conditions and shifted to 5 mM ammonium chloride. Immediately before the shift (time zero) and after different time points, as indicated, aliquots were removed and extracts were prepared. The results from the *Synechocystis* wild-type strain (black bars), the MPphA strain (gray bars), and the ΔP_{II} strain (open bars) are shown. Note that no cyanophycin was detectable in ΔP_{II} cells. (C) Determination of the phosphorylation state of P_{II} in the extracts of *Synechocystis* wild-type (top) and MPphA (bottom) cells that were analyzed as for panels A and B. The phosphorylation status of P_{II} is shown on the left, with the superscripts indicating the numbers of phosphorylated subunits.

cells increased immediately upon N upshift whereas it remained low in the P_{II}-deficient mutant (Fig. 2). In the PphA mutant, increased arginine levels could be observed only in the last sample, which showed accumulated cyanophycin.

The activation state of NAGK, cyanophycin accumulation, and intracellular arginine concentration strongly correlated with the phosphorylation state of P_{II} (Fig. 1C). Previously, we demonstrated that, in *Synechococcus* sp. strain PCC 7942, the nonphosphorylated form of P_{II} strongly activates NAGK activity (19). Similarly, in the *Synechocystis* sp. strain PCC 6803 wild-type cells, dephosphorylation of P_{II} correlates with an

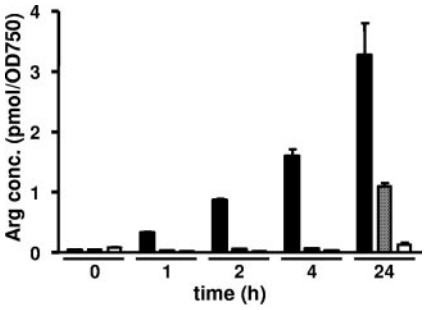

FIG. 2. Quantification of arginine in ethanolic extracts from *Synechocystis* sp. strain PCC 6803 cells which had been grown under nitrate-limiting conditions and shifted to 5 mM ammonium chloride at time zero. The concentration of arginine is given as pmol Arg extracted per unit of optical density at 750 nm (OD_{750}) of cells. The results from the *Synechocystis* wild-type strain (black bars), the MPphA strain (gray bars), and the ΔP_{II} strain (open bars) are shown.

increase in NAGK activity. The increased NAGK activity is accompanied by increased intracellular arginine and cyanophycin concentrations. In contrast, the delay in P_{II} dephosphorylation in the MPphA strain correlated with delayed NAGK activation and cyanophycin and arginine accumulation. Immunoblot analysis using NAGK-specific antibodies revealed that the amount of NAGK protein did not significantly change during the time course of the experiment (data not shown).

The above results strongly suggested that P_{II}-mediated NAGK activation is responsible for increased arginine synthesis, which then leads to cyanophycin accumulation. To verify independently that impaired cyanophycin synthesis in P_{II}-deficient cells is indeed due to limiting arginine levels and not caused by impaired cyanophycin synthetase activity (26), ammonium upshift experiments in the presence of 5 mM arginine were carried out with wild-type and P_{II}-deficient cells (Fig. 3). *Synechocystis* has a highly active arginine transport system (28), resulting in a rapid uptake of externally added arginine. As shown in Fig. 3A, the P_{II}-deficient mutant, despite low NAGK activity, was now able to accumulate cyanophycin, although to a lesser extent than the wild type (Fig. 3B). The difference between wild type and mutant may be due to the lack of internally synthesized arginine in the ΔP_{II} strain or may indicate an additional requirement for P_{II} in cyanophycin synthesis. The recently discovered P_{II} receptor PamA in *Synechocystis* may be considered in this context (47). In any case, cyanophycin accumulation can be restored in the P_{II}-deficient mutant by bypassing the impaired NAGK activity through external addition of arginine, implying that cyanophycin synthesis in the ΔP_{II} strain was limited by the availability of arginine.

Arginine has a dual role in cyanobacteria, first as an amino acid for protein synthesis and second as a nitrogen buffer, storing excess nitrogen in the form of cyanophycin and making it easily available through efficient arginine metabolism (21, 36). P_{II} controls the committed step in arginine synthesis as it activates NAGK activity by complex formation. In addition to increasing NAGK catalytic activity, complex formation with P_{II} also causes a dramatic reduction in arginine feedback inhibition. Whereas free NAGK was almost completely inhibited by arginine concentrations above 50 μM, the P_{II}-complexed NAGK was barely inhibited (32). Therefore, under physiological conditions of P_{II}-NAGK complex formation, efficient arginine synthesis occurs in the presence of appreciably higher levels of arginine compared to conditions favoring P_{II}-NAGK complex dissociation. Complex formation occurs with nonphosphorylated P_{II} at low levels of 2-oxoglutarate, corresponding to nitrogen-rich conditions. Under nitrogen-poor conditions, however, complex formation is impaired, since P_{II} is phosphorylated and the 2-oxoglutarate concentrations are high (19, 32). Cyanophycin synthetase in *Synechocystis* sp. strain PCC 6803 has a K_m for arginine of 49 μM (2), a concentration which is already inhibitory for free NAGK but not for NAGK in complex with P_{II}. Therefore, under nitrogen-poor conditions, cyanophycin cannot be formed. Instead, arginine levels should be just sufficient to meet the requirement for protein synthesis, since the K_m values of aminoacyl-tRNA synthetases for their cognate amino acids are usually in the micromolar range. Under conditions of nitrogen excess, however, the NAGK-P_{II} complex is formed and arginine synthesis is stimulated, allowing cyanophycin synthesis to occur. The other substrates of cyanophycin synthetase, aspartate and ATP (exhibiting K_m values for Asp of 0.45 mM and for ATP of 0.2 mM [2]), do not seem to limit the reaction in the P_{II}-deficient mutant, since arginine addition alone was sufficient to restore cyanophycin synthesis. The regulation of NAGK activity by P_{II} in response to the nitrogen status thus provides the mechanistic

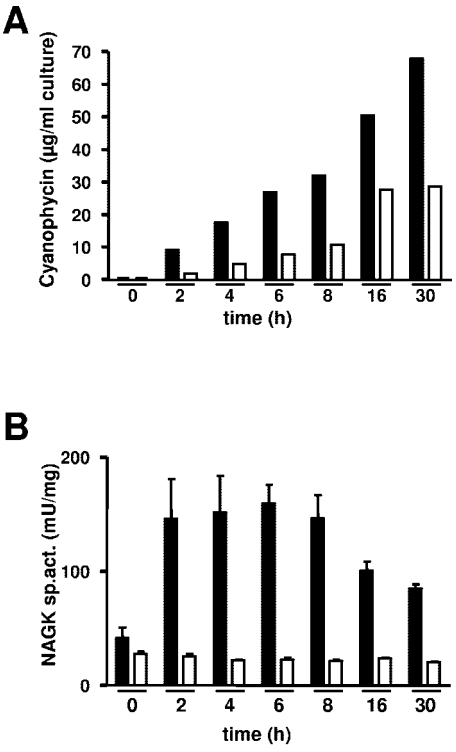

FIG. 3. Analysis of cyanophycin accumulation (A) and NAGK activity (B) in cell extracts from *Synechocystis* sp. strain PCC 6803 cells, which had been grown under nitrate-limiting conditions and shifted to 5 mM ammonium chloride with 5 mM arginine. Immediately before the shift (time zero) and after different time points, as indicated, aliquots were removed and extracts were prepared. The results from the *Synechocystis* wild-type strain are shown by the black bars and those from the ΔP_{II} strain by open bars.

basis for the dual role of arginine: in the nonactivated state, NAGK activity is sufficient to provide arginine for the purpose of protein synthesis; in the P_{II}-activated state, excess nitrogen can be stored in the form of cyanophycin.

We thank S. Bedu (Marseille) for the ΔP_{II} strain used in this study. This work was supported by grants from the DFG (Fo195/4 and Lo286/6–2). M.M. was supported by the Graduiertenkolleg 370 at the University of Giessen.

REFERENCES

1. **Aboulmagd, E., F. B. Oppermann-Sanio, and A. Steinbüchel.** 2000. Molecular characterization of the cyanophycin synthetase from *Synechocystis* sp. strain PCC 6803. Arch. Microbiol. **174:**297–306.
2. **Aboulmagd, E., F. B. Oppermann-Sanio, and A. Steinbüchel.** 2001. Purification of *Synechocystis* sp. strain PCC 6308 cyanophycin synthetase and its characterization with respect to substrate and primer specificity. Appl. Environ. Microbiol. **67:**2176–2182.
3. **Allen, M. M., and F. Hutchinson.** 1980. Nitrogen limitation in the cyanobacterium *Aphanocapsa* 6308. Arch. Microbiol. **128:**1–7.
4. **Allen, M. M.** 1984. Cyanobacterial cell inclusions. Annu. Rev. Microbiol. **38:**1–25.
5. **Allen, M. M.** 1988. Inclusions: cyanophycin. Methods Enzymol. **167:**207–213.
6. **Allen, M. M., F. Hutchinson, and P. M. Weathers.** 1980. Cyanophycin granule polypeptide formation and degradation in the cyanobacterium *Aphanocapsa* 6308. J. Bacteriol. **141:**687–693.
7. **Arcondeguy, T., R. Jack, and M. Merrick.** 2001. P_{II} signal transduction proteins, pivotal players in microbial nitrogen control. Microbiol. Mol. Biol. Rev. **65:**80–105.
8. **Berg, H., K. Ziegler, K. Piotukh, K. Baier, W. Lockau, and R. Volkmer-Engert.** 2000. Biosynthesis of the cyanobacterial reserve polymer multi-L-arginyl-poly-L-aspartic acid (cyanophycin): mechanism of the cyanophycin synthetase reaction studied with synthetic primers. Eur. J. Biochem. **267:** 5561–5570.
9. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
10. **Burillo, S., I. Luque, I. Fuentes, and A. Contreras.** 2004. Interaction between the nitrogen signal transduction protein P_{II} and *N*-acetyl glutamate kinase in organisms that perform oxygenic photosynthesis. J. Bacteriol. **186:**3346–3354.
11. **Caldovich, L., and M. Tuchman.** 2003. N-acetylglutamate and its changing role through evolution. Biochem. J. **372:**279–290.
12. **Forchhammer, K.** 2004. Global carbon/nitrogen control by PII signal transduction in cyanobacteria: from signals to targets. FEMS Microbiol. Rev. **28:**319–333.
13. **Forchhammer, K., and A. Hedler.** 1997. Phosphoprotein PII from cyanobacteria. Eur. J. Biochem. **244:**869–875.
14. **Forchhammer, K., and N. Tandeau de Marsac.** 1995. Phosphorylation of the P_{II} protein (*glnB* gene product) in the cyanobacterium Synechococcus sp. strain PCC 7942. J. Bacteriol. **177:**5812–5817.
15. **Grigorieva, G., and S. Shestakov.** 1982. Transformation in the cyanobacterium *Synechocystis* sp. PCC 6803. FEMS Microbiol. Lett. **13:**367–370.
16. **Hagemann, M., J. Vinnemeier, I. Oberpichler, R. Boldt, and H. Bauwe.** 2005. The glycine decarboxylase complex is not essential for the cyanobacterium *Synechocystis* sp. strain PCC 6803. Plant Biol. **7:**15–22.
17. **Hai, T., F. B. Oppermann-Sanio, and A. Steinbüchel.** 1999. Purification and characterization of cynophycin and cyanphycin synthetase from the thermophilic *Synechococcus* sp. MA19. FEMS Microbiol. Lett. **181:**229–236.
18. **Hanson, T. E., K. Forchhammer, N. Tandeau de Marsac, and J. C. Meeks.** 1998. Characterization of the *glnB* gene product of *Nostoc punctiforme* strain ATCC 29133: *glnB* or the PII protein may be essential. Microbiology **144:** 1537–1547.
19. **Heinrich, A., M. Maheswaran, U. Ruppert, and K. Forchhammer.** 2004. The *Synechococcus elongatus* PII signal transduction protein controls arginine synthesis by complex formation with N-acetyl-L-glutamate kinase. Mol. Microbiol. **52:**1303–1314.
20. **Hejazi, M., K. Piotukh, J. Mattow, R. Deutzmann, and W. Lockau.** 2002. Isoaspartyl dipeptidase activity of plant-type asparaginases. Biochem. J. **364:** 129–136.
21. **Herrero, A.** 2004. New targets of the PII signal transduction protein identified in cyanobacteria. Mol. Microbiol. **52:**1225–1228.
22. **Hisbergues, M., R. Jeanjean, F. Joset, N. Tandeau de Marsac, and S. Bedu.** 1999. Protein PII regulates both inorganic carbon and nitrate uptake and is modified by a redox signal in *Synechocystis* PCC 6803. FEBS Lett. **463:**216–220.
23. **Irmler, A., and K. Forchhammer.** 2001. A PP2C-type phosphatase dephosphorylates the PII signalling protein in the cyanobacterium *Synechocystis* PCC 6803. Proc. Natl. Acad. Sci. USA **98:**12978–12983.
24. **Kamberov, E. S., M. A. Atkinson, and A. J. Ninfa.** 1995. The *Escherichia coli* signal transduction protein is activated upon binding 2-ketoglutarate and ATP. J. Biol. Chem. **270:**17797–17807.
25. **Kloft, N., G. Rasch, and K. Forchhammer.** 2005. Protein phosphatase PphA from *Synechocystis* sp. PCC 6803: the physiological framework of PII-P dephosphorylation. Microbiology **151:**1275–1283.
26. **Krehenbrink, M., and A. Steinbüchel.** 2004. Partial purification and characterization of a non-cyanobacterial cyanophycin synthetase from *Acinetobacter calcoaceticus* strain ADP1 with regard to substrate specificity, substrate affinity and binding to cyanophycin. Microbiology **150:**2599–2608.
27. **Krehenbrink, M., F. B. Oppermann-Sanio, and A. Steinbüchel.** 2002. Evaluation of non-cyanobacterial genome sequences for occurrence of genes encoding protein homologous to cyanophycin synthetase and cloning of an active cyanophycin synthetase from *Acinetobacter* sp. strain DSM 587. Arch. Microbiol. **177:**371–380.
28. **Labarre, J., P. Thuriaux, and F. Chauvat.** 1987. Genetic analysis of amino acid transport in the facultatively heterotrophic cyanobacterium *Synechocystis* sp. strain 6803. J. Bacteriol. **169:**4668–4673.
29. **Laurent, S., K. Forchhammer, L. Gonzalez, T. Heulin, C. C. Zhang, and S. Bedu.** 2004. Cell-type specific modification of PII is involved in the regulation of nitrogen metabolism in the cyanobacterium *Anabaena* PCC 7120. FEBS Lett. **576:**261–265.
30. **Lawry, N. H., and R. D. Simon.** 1982. The normal and induced occurrence of cyanophycin inclusion bodies in several blue-green algae. J. Phycol. **18:**391–399.
31. **Mackerras, A. H., N. M. Dechazal, and G. D. Smith.** 1990. Transient accumulations of cyanophycin in *Anabaena cylindrica* and *Synechocystis* 6308. J. Gen. Microbiol. **136:**2057–2065.
32. **Maheswaran, M., C. Urbanke, and K. Forchhammer.** 2004. Complex formation and catalytic activation by the PII signaling protein of N-acetyl-L-glutamate kinase from *Synechococcus elongatus* strain PCC 7942. J. Biol. Chem. **279:**55202–55210.
33. **Möllering, H.** 1985. Laspartate and L-arginine, p. 350–357. *In* H. U. Bergmeyer, J. Bergmeyer, and M. Grassl (ed.), Methods of enzymatic analysis, 3rd ed., vol. VIII. VCH Verlagsgesellschaft, Weinheim, Germany.
34. **Oppermann-Sanio, F. B., and A. Steinbüchel.** 2002. Occurrence, functions and biosynthesis of polyamides in microorganisms and biotechnological production. Naturwissenschaften **89:**11–22.
35. **Picossi, S., A. Valladares, E. Flores, and A. Herrero.** 2004. Nitrogen-regulated genes for the metabolism of cyanophycin, a bacterial nitrogen reserve polymer. J. Biol. Chem. **279:**11582–11592.
36. **Quintero, M. J., A. M. Muro-Pastor, A. Herrero, and E. Flores.** 2000. Arginine catabolism in the cyanobacterium *Synechocystis* sp. strain PCC 6803 involves the urea cycle and arginase pathway. J. Bacteriol. **182:**1008–1015.
37. **Richter, R., M. Hejazi, R. Kraft, K. Ziegler, and W. Lockau.** 1999. Cyanophycinase, a peptidase degrading the cyanobacterial reserve material multi-L-arginyl-poly-L-aspartic acid (cyanophycin): molecular cloning of the gene of *Synechocystis* sp. PCC 6803, expression in *Escherichia coli*, and biochemical characterization of the purified enzyme. Eur. J. Biochem. **263:** 163–169.
38. **Rippka, R.** 1988. Isolation and purification of cyanobacteria. Methods Enzymol. **167:**3–27.
39. **Ruppert, U., A. Irmler, N. Kloft, and K. Forchhammer.** 2002. The novel protein phosphatase PphA from *Synechocystis* PCC 6803 controls dephosphorylation of the signalling protein PII. Mol. Microbiol. **44:**855–864.
40. **Simon, R. D.** 1973. Measurement of the cyanophycin granule polypeptide contained in the blue-green alga *Anabaena cylindrica*. J. Bacteriol. **114:**1213–1216.
41. **Simon, R. D.** 1973. The effect of chloramphenicol on the production of cyanophycin granule polypeptide in the blue green alga *Anabaena cylindrica*. Arch. Microbiol. **92:**115–122.
42. **Simon, R. D.** 1976. The biosynthesis of multi-L-arginyl-poly(L-aspartic acid) in the filamentous cyanobacterium *Anabaena cylindrica*. Biochim. Biophys. Acta **422:**407–418.
43. **Simon, R. D.** 1987. Inclusion bodies in the cyanobacteria: cyanophycin, polyphosphate, polyhedral bodies, p. 199–225. *In* P. Fay and C. van Baalen (ed.), The cyanobacteria. Elsevier, Amsterdam, The Netherlands.
44. **Simon, R. D., and P. Weathers.** 1976. Determination of the structure of the novel polypeptide containing aspartic acid and arginine which is found in cyanobacteria. Biochim. Biophys. Acta **420:**165–176.
45. **Stephan, D. P., H. G. Ruppel, and E. K. Pistorius.** 2000. Interrelation between cyanophycin synthesis, L-arginine catabolism and photosynthesis in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Z. Naturforsch. **55c:** 927–942.
46. **Sugiyama, K., T. Hayakawa, T. Kudo, T. Ito, and T. Yamaya.** 2004. Interaction of N-acetylglutamate kinase with a PII-like protein in rice. Plant Cell Physiol. **45:**1768–1778.

47. **Takashi, O., S. Sato, S. Tabata, and K. Tanaka.** 2005. Identification of PamA as a PII-binding membrane protein important in nitrogen-related and sugar-catabolic gene expression in *Synechocystis* sp. PCC 6803. J. Biol. Chem. **280:**34684–34690.
48. **Ziegler, K., A. Diener, C. Herpin, R. Richter, R. Deutzmann, and W. Lockau.** 1998. Molecular characterization of cyanophycin synthetase, the enzyme catalyzing the biosynthesis of the cyanobacterial reserve material multi-L-arginyl-poly-L-aspartate (cyanophycin). Eur. J. Biochem. **254:**154–159.
49. **Ziegler, K., R. Deutzmann, and W. Lockau.** 2002. Cyanophycin synthetase-like enzymes of non-cyanobacterial eubacteria: characterization of the polymer produced by a recombinant synthetase of *Desulfitobacterium hafniense*. Z. Naturforsch. **57c:**522–529.