The Unique Cyanobacterial Protein OpcA Is an Allosteric Effector of Glucose-6-phosphate Dehydrogenase in Nostoc punctiforme ATCC 29133*

Received for publication, November 20, 2000, and in revised form, December 11, 2000
Published, JBC Papers in Press, January 4, 2001, DOI 10.1074/jbc.M010472200

Kari D. Hagen and John C. Meeks‡

From the Section of Microbiology, Division of Biological Sciences, University of California, Davis, California 95616

Glucose-6-phosphate dehydrogenase (G6PD), encoded by zuf, is essential for nitrogen fixation and dark heterotrophic growth of the cyanobacterium Nostoc punctiforme ATCC 29133. In N. punctiforme, zuf is part of a four-gene operon transcribed in the order fbp-tal-zuf-opcA. Genetic analyses indicated that opcA is required for G6PD activity. To define the role of opcA, the synthesis, aggregation state, and activity of G6PD in N. punctiforme strains expressing different amounts of G6PD and/or OpcA were examined. A single tetrameric form of G6PD was consistently observed for all strains, as well as for recombinant N. punctiforme His-G6PD purified from Escherichia coli, regardless of the quantity of OpcA present. However, His-G6PD and the G6PD of strain UCD 351, which lacks OpcA, had low affinities for glucose 6-phosphate (G6P) substrate (Km(app) = 65 and 85 mM, respectively) relative to wild-type N. punctiforme G6PD (Km(app) = 0.5 mM). Near wild-type affinities for G6P were observed for these enzymes when saturating amounts of His-OpcA- or OpcA-containing extract were added. Kinetic studies were consistent with OpcA acting as an allosteric activator of G6PD. A role in redox modulation of G6PD activity was also indicated, because thioredoxin-mediated inactivation and reactivation of His-G6PD occurred only when His-OpcA was present.

Cyanobacteria are members of a phylogenetically cohesive group of prokaryotes that perform oxygenic photosynthesis and assimilate CO2 via the reductive pentose phosphate (Calvin) cycle. Excess photosynthate produced in the light is stored as glycogen, which is catabolized to provide maintenance energy during periods of darkness. Although most cyanobacteria are restricted to this photoautotrophic mode of growth, some, such as the filamentous heterocyst-forming isolate Nostoc punctiforme ATCC 29133, exhibit greater nutritional versatility. When provided with exogenous sugars such as fructose or glucose, N. punctiforme is capable of both phototrophic and heterotrophic growth conditions.

The oxidative pentose phosphate (OPP) cycle is assumed to be the major route for carbon catabolism in cyanobacteria, both in vegetative cells for the breakdown of glycolgen or exogenously supplied sugars, and in nitrogen-fixing heterocysts, where it provides a reductant (NADPH) required by nitrogenase (1–3). Regulation of the activity of glucose-6-phosphate dehydrogenase (G6PD; E.C. 1.1.1.49), the enzyme controlling the entry of carbon into the cycle, is complex and poorly understood. Metabolites such as NADPH, ATP, glucose 6-phosphate (G6P), glutamine, and ribulose bisphosphate have been implicated in its regulation (4, 5) as has thioredoxin, which may reductively inactivate and oxidatively reactivate the enzyme in the light and dark, respectively, to prevent futile cycling, which would occur if the Calvin and OPP cycles operated simultaneously (5–8). G6PD has been purified from the filamentous, heterocyst-forming cyanobacteria Anabaena variabilis (5) and Anabaena sp. strain PCC 7120 (Anabaena 7120) (4, 8). Although the A. variabilis enzyme is a 250-kDa tetramer (5), the Anabaena 7120 enzyme is found in multiple aggregation states. Three forms (M1, M2, and M3; 120, 240, and 345 kDa, respectively) with different catalytic activities were initially reported for Anabaena 7120 G6PD (4); the 240-kDa M2 form was by far the most prevalent, but the larger M3 form appeared to be more active. More recently, the Anabaena 7120 enzyme was reported to exist in two aggregation states (250 and 750 kDa) (8). The equilibrium between the different forms of the Anabaena 7120 enzyme depends upon pH, the concentration of the enzyme in solution, and the presence or absence of substrates or other effectors. Multiple reactive bands have also been observed in extracts from Anabaena 7120 and some other cyanobacterial strains after native polyacrylamide gel electrophoresis (PAGE) and staining of the gels for G6PD activity (9). The sizes, numbers, and intensities of the bands vary with the strain and with growth conditions.

A genetic analysis of the physiological role of G6PD and the OPP cycle in N. punctiforme was recently performed (10). In N. punctiforme, zuf, the gene encoding G6PD, is part of a four-gene operon fbp-tal-zuf-opcA with multiple transcriptional start points and complex transcriptional regulation (11). Insertional inactivation of zuf resulted in essentially complete loss of G6PD activity and rendered the zuf mutant incapable of nitrogen fixation or dark heterotrophic growth, indicating that G6PD is required for both of these processes (10). Inactivation of opcA gave rise to a mutant with the same phenotype. The opcA mutant was fully complemented by opcA supplied in trans 7942; MOPS, 3-(N-morpholino)propanesulfonic acid; Ap, ampicillin; Nm, neomycin; Chl a, chlorophyll a; PCR, polymerase chain reaction; His, histidine fusion tag; MBP, maltose binding protein fusion tag; DTT, dithiothreitol; STT, sodium tetrathionate.

*This work was supported by the U. S. National Science Foundation (Grant MCB 96-04270). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Section of Microbiology, University of California, Davis, One Shields Ave., Davis, CA 95616. Tel.: 530-752-3346; Fax: 530-752-9014; E-mail: jcmeeks@ucdavis.edu.

The abbreviations used are: OPP, oxidative pentose phosphate; G6PD, glucose-6-phosphate dehydrogenase; G6P, glucose 6-phosphate; Anabaena 7120, Anabaena sp. strain PCC 7120; PAGE, polyacrylamide gel electrophoresis; Synechococcus 7942, Synechococcus sp. strain PCC 7942.

This paper is available on line at http://www.jbc.org
described previously (10, 14). Strain UCD 482 was constructed by introducing pSCR121 into wild-type *N. punctiforme* via electroporation as described (10). The construction of strains UCD 341, 348, 351, and 364 is described elsewhere (10, 17).

**Cupin-Related G6PD and OpcA proteins** were prepared using the pET system of Novagen and the pMAL protein fusion and purification system of New England Biolabs. DNA fragments containing the *N. punctiforme* *zuf* or *opcA* gene were obtained using the polymerase chain reaction (PCR) with pSCR115 as a template and the following primers: 5'-ATGGTTAATTGCTGGAAATTCTCTTG-3' (His-G6PD forward) and 5'-GCTCTAGAGTTGCGGACGACGGCATCT-3' (EcoRV reverse), or 5'-CCGCCAACAAATCCTGCTAAATAAAC-3' (MBP-OpcA forward) and 5'-GCTCTAGAGTCGTTAAGCTTAAACCCG-3' (MBP-OpcA reverse) or 5'-CCGGCTCTGAGTTAGTGTCAATAGTTAAC-3' (His-OpcA reverse). PCR amplifications were performed in a PerkinElmer Cetus thermal cycler with AmpliTaq polymerase. The 1.5-kb PCR product containing *zuf* was initially cloned into the EcoRV site of pBluescript II KS+ (+). This fragment was removed from pBluescript II KS+ (+) with *SacI* and *PstI* and was inserted into the *SacI* and *PstI* sites of pMAL-c2. A 1.4-kb PCR fragment containing *opcA* was digested with *XhoI* and inserted into the *EcoRI* and *XhoI* sites of pET-28a+ (+) to generate pSCR352, which was used to produce His-OpcA. MBP-OpcA was soluble when expressed in *E. coli* DH5α-MCR, whereas a portion of each of the His-tagged proteins was present in inclusion bodies when expressed in *E. coli* BL21(DE3). In each case, the soluble protein was purified by affinity chromatography according to the kit manufacturers’ instructions, with phenylmethylsulfonyl fluoride (1 mM) added to all buffers used during purification. Purified proteins were stored at 4 °C at concentrations of 0.5–1.5 mg of protein/ml. The His-tagged proteins tended to precipitate out of solution; precipitation occurred more readily when protein concentrations were higher. His-G6PD retained activity for 2–3 weeks when stored at 4 °C in elution buffer (50 mM Tris-HCl, 500 mM NaCl, 1 mM imidazole, pH 7.8). His-OpcA rapidly lost its ability to activate G6PD when it was stored in elution buffer; consequently, it was dialyzed in several changes of 50 mM Tris maleate buffer (pH 6.5) containing 10 mM MgCl₂ prior to storage at 4 °C for 2–3 weeks. Neither protein was stable at ~20 °C when stored in buffers containing 0–50% glycerol nor under any condition if diluted to less than 25% of their original activity during purification and storage. Results have been reported previously for G6PD (4, 8, 18, 19). **PAGE and Immunoblots—** SDS and native PAGE and Coomassie Blue staining were performed according to standard protocols (20) using either the Bio-Rad Mini-Protein II system or the larger Bio-Rad Protein system. Rabbit polyclonal antisera were raised against purified His-G6PD or MBP-OpcA by standard techniques (21) and were used at a dilution of 1:400. Immunoblotting was performed as previously (22). Bound primary antibody was detected either with horse-radish peroxidase-conjugated goat α-rabbit secondary antibody and a 4-iodophenol/Luminol chemiluminescent protocol (22) or with alkaline phosphatase-conjugated goat α-rabbit secondary antibody (1:50,000 dilution) and Amersham Pharmacia Biotech ECF substrate. When the latter detection method was used, detection and quantitation of chemiluminescent bands was performed using a Storm PhosphorImager with ImageQuant software (Molecular Dynamics). Immunoprecipitations were performed according to standard methods (21) with 100 μl of *N. punctiforme* extract (10–20 mg of protein/ml) and 5 μl of α-G6PD or α-OpcA antiserum or preimmune serum. After 1 h on ice, 100 μl of 10% (v/v) protein A-Sepharose CL-4B beads was added, and the mixture was incubated 1 h at 4 °C with gentle rocking. The beads were washed four times with ice-cold lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40). Electrophoresis of the immune complexes was performed using 10% polyacrylamide SDS-PAGE gels with β-mercaptoethanol omitted from the sample buffer. Immunoprecipitated proteins were detected by Western blotting.

The purified His-G6PD and G6PDs in cyanobacterial extracts were determined by Ferguson analysis using 5, 6, and 7% acrylamide native PAGE gels. Standards used for native PAGE were obtained from Sigma Chemical Co. and included the following: bovine serum albumin (66 and 123 kDa), β-amylase (200 kDa), aprotinin (450 kDa), urease (545 kDa), and thyroglobulin (869 kDa). The gels were stained with Coomassie Blue or G6PD activity stain (4) to identify standards and unknowns, respectively. Average RF values for each

**Experimental Procedures**

**Strains, Culture Conditions, and Cell Lysis—** Plasmids and bacterial strains used in this study are shown in Table I. *N. punctiforme* 29133 and *Anabaena* 7120 (available from the National Center for Biotechnology Information on the Web) were used according to the manufacturers’ instructions. Large scale cultures were grown in Escherichia coli *DH5α* (TII) and *E. coli* DH5α (MCR). 

**Plasmid Construction and Protein Purification—** Routine DNA manipulations in *Escherichia coli* strain DH5α-MCR were performed according to standard procedures (18). DNA-modifying enzymes were purchased from New England Biolabs and Life Technologies, Inc. and were used according to the manufacturers’ instructions. Large scale plasmid preparations were obtained using a commercial kit (Qiagen). *N. punctiforme* strain UCD 482 (zuf::pSCR115) was constructed by digesting pSCR115 with *PstI* to remove a 0.7-kb fragment from the *zuf* gene. This fragment was replaced with a 1.1-kb-blunted XbaI fragment from pRL44 containing *PmaA* (encoding Nm resistance) to generate pSCR46, which was digested with *DraI* and *XbaI*. The 6.7-kb fragment containing the *nptI*-interrupted *zuf* gene was ligated to the sacB-containing conjugatable vector pRL21, which had been digested with *EcoRI* and *XbaI*. In the resulting plasmid, pSCR347, *PmaA* is in the same orientation as *zuf* and transcription of *opcA* occurs. Introduction of pSCR347 into wild-type *N. punctiforme* by conjugation, selection for *Nm*- and sucrose-resistant exconjugants, and confirmation of the insertional inactivation by Southern blotting was performed as
protein at each gel concentration were determined by measuring the distances from the center of the bands to the dye front on duplicate gels.

**G6PD Activity Assay**—G6PD activity was assayed by a modification of the method of Schaeffer and Stanier (4), in which the standard assay concentrations were varied for the determination of kinetic parameters. The reaction was initiated by the addition of cell extract or purified protein, and the reduction of NADP at room temperature was followed by monitoring the increase in absorbance at 340 nm, typically for 1 min. To ensure that initial velocities were measured, all assays were determined to be in the linear range with respect to protein concentration, time, and substrate utilization. With crude cell extracts, in which the enzyme concentration varied, at least three total protein concentrations were surveyed for linearity before extended assays were done at the selected protein concentration. For purified His-G6PD, activity in standard assays was linear between at least 0.2 and 2.0 $\mu$g of protein and 0.5 $\mu$g was typically used. The quantity of G6P and NADP substrate consumed was calculated at all substrate, protein, and effector concentrations used. For determinations of $K_{\text{app}}$ for NADP, less than 5% of the initial concentrations of NADP or G6P were consumed and for determinations of $K_{\text{app}}$ for G6P, the amounts of G6P and NADP consumed were always less than 2% of the initial concentrations. In all experiments, at all concentrations of protein, substrates, and effector, changes in $K_{\text{m}}$ of G6PD—thioredoxin and the chemical oxidants and reductants used in redox modulation experiments were obtained from Sigma Chemical Co.

**RESULTS**

**Growth Condition-dependent Activity and Aggregation State of G6PD**—To determine whether growth conditions affected the activity or aggregation state of N. punctiforme G6PD, cells were cultured with ammonium in the light (photoautotrophic conditions), without ammonium in the light (photoautotrophic conditions), or in the dark in the absence of ammonium and presence of 50 mM fructose (heterotrophic, conditions). The activity or aggregation state of G6PD—thioredoxin was determined by measuring the distances from the center of the bands to the dye front on duplicate gels.

### TABLE I

| Plasmids or strain | Relevant characteristics* | Source or reference |
|--------------------|---------------------------|---------------------|
| pBluescript II KS(+) | Expression vector for generation of maltose binding protein fusion proteins; Ap′ | Stratagene |
| pMAL-c2 | Expression vector for generation of His-tagged fusion proteins; Km′ | New England BioLabs |
| pET-28a(+) | Conjugatable nonreplicating cyanobacterial vector containing secB; Em′, Cm′ | Novagen |
| pRL448 | Source of P$_{psbA}$ptII cassette; Ap′, Km′ | (29) |
| pSCR115 | 10-kb PstI fragment containing the N. punctiforme fbp-tal-zwf-opca genes in the PstI site plasmidpBLuescript II KS(+) | (10) |
| pSCR119 | Multicopy replicating vector for use in N. punctiforme and E. coli; Nm′ | (10) |
| pSCR121 | Blunted 1.7-kb SpeI-XbaI fragment from pSCR115 containing only opca in the Smal site of pSCR202; opca transcription appears to be driven by a promoter generated fortuitously during cloning; Ap′ | (10) |
| pSCR122 | 3.0-kb SphI-BamHI fragment from pSCR132 containing zwf and part of opca in the SphI-BamHI sites of pSCR202; Ap′ | (10) |
| pSCR123 | 3.7-kb KpnI-XbaI fragment containing complete zwf and opca in the KpnI-XbaI sites of pSCR202; Ap′ | (10) |
| pSCR132 | 3.0-kb SstI-XhoI fragment containing zwf and part of opca in the SstI-SalI sites of pSCR119; Nm′ | (17) |
| pSCR195 | 1.6-kb SalI-PstI fragment containing opca preceded by the SalI-partial EcoRV sites of the pBluescript II KS(+) MCS in the SstI-PstI sites of pMAL-c2; Ap′ | This study |
| pSCR197 | 1.6-kb BamHI-HindIII fragment containing zwf preceded by the BamHI-partial EcoRV sites of the pBluescript II KS(+) MCS in the SstI-PstI sites of pET-28a(+) | This study |
| pSCR202 | SphI-HindIII fragment containing zwf and part of opca in the PstI-SalI sites of pSCR119; Nm′ | (10) |
| pSCR346 | SphI-HindIII fragment containing zwf and part of opca in the SstI-SalI sites of pSCR119; Nm′ | (10) |
| pSCR347 | 6.7-kb DraI-XhoI fragment from pSCR346 in the Eco136II-XhoI sites of pRL271; Nm′, Cm′, Em′ | This study |
| pSCR352 | 1.4-kb XhoI-digested PCR fragment containing opca in the Eco136II-XhoI sites of pET-28a(+) | This study |
| **E. coli strains** | | |
| DH5αMCR | Methylation-dependent restriction defective derivative of strain DH5α; used for cloning and for expression of MBP-OpcA | Life Technologies |
| BL21(DE3) | Strain BL21 with ΔDE3 prophage carrying T7 RNA polymerase; used for expression of His-tagged fusion proteins | Novagen |
| **N. punctiforme strains** | | |
| ATCC 29133 | Wild-type N. punctiforme | (30) |
| ATCC 29133 with zwf; pMAL-c2 | | |
| UCD 341 | ATCC 29133 with zwf; pMAL-c2; opca not transcribed; Nm′ | (10) |
| UCD 348 | UCD 341 + pSCR123; Nm′, Ap′ | (10) |
| UCD 351 | UCD 341 + pSCR122; Nm′, Ap′ | (10) |
| UCD 364 | ATCC 29133 + pSCR124; Nm′ | (17) |
| UCD 466 | UCD 341 with P$_{psbA}$ptII oriented so that transcription continues into opca; Nm′ | This study |
| UCD 482 | ATCC 29133 + pSCR121; Ap′ | This study |
| **Other cyanobacterial strains** | | |
| Anabaena 7120 | | |
| Anabaena sp. strain PCC 7120 | | (30) |

*Abbreviations for antibiotic resistance markers are Ap′, ampicillin; Km′, kanamycin; Em′, erythromycin; Cm′, chloramphenicol; Nm′, neomycin.
Fig. 1. Western blot analysis of the aggregation states and quantities of G6PD and OpcA in cell extracts prepared from N. punctiforme cultures grown under different conditions. Cell extracts were prepared from cultures grown photoautotrophically with ammonium (lanes 1 and 4), photoautotrophically with dinitrogen (lanes 2 and 5), or heterotrophically in the dark with glucose and dinitrogen (lanes 3 and 6) as described under "Experimental Procedures." Aliquots of the cell extracts containing 70 μg of total protein were loaded on a 10% acrylamide gel, and the proteins were separated by native PAGE. Lanes 1–3 of the blot prepared from this gel were incubated with α-G6PD antibody, whereas lanes 4–6 were incubated with α-OpcA antibody. The G6PD activities of the extracts in lanes 1, 2, and 3 were 0.035, 0.055, and 0.265 μmol of NADP reduced·min⁻¹·mg of protein⁻¹, respectively. The positions of G6PD, OpcA, and the native PAGE protein standards are indicated.

Molecular Mass and Monomer Composition of Native G6PD—N. punctiforme G6PD consistently migrated as a single band with a molecular mass that appeared to be between 200 and 545 kDa based on the positions of native PAGE standards, whereas OpcA migrated as a single band near the 66-kDa marker (Fig. 1). Because the predicted molecular masses of the N. punctiforme zwf and opcA gene products are 58 and 51 kDa, respectively, these results implied that the G6PD band was an oligomer and the OpcA band was a monomer. G6PD and OpcA from N. punctiforme extracts were never observed to comigrate on native PAGE gels (e.g., compare lanes 1–3 with lanes 4–6 in Fig. 1). To confirm the implication that OpcA was not associated with the G6PD oligomer in N. punctiforme cell extracts, immunoprecipitation experiments were performed using either α-G6PD or α-OpcA antibodies. Each protein was immunoprecipitated with its respective antibody, but the two proteins did not coimmunoprecipitate, and neither cross-immunoprecipitated nor immunoprecipitation with pre-immune serum occurred (data not shown).

Ferguson analysis was used to more precisely determine the molecular mass of the G6PD oligomer. Samples containing N. punctiforme cell extract, Anabaena 7120 cell extract (control), or N. punctiforme His-G6PD purified from E. coli were loaded on a series of native PAGE gels (5, 6, and 7% acrylamide) along with native PAGE protein standards. After electrophoresis, the proteins were visualized with Coomassie Blue (standards) or G6PD activity stain (samples containing G6PD). A single strong band that appeared within 15 min of G6PD activity staining was observed for His-G6PD and in N. punctiforme and Anabaena 7120 extracts (Fig. 2). A faint lower molecular mass band was occasionally detected in Anabaena 7120 extract after several hours of staining; no additional bands were observed with His-G6PD or N. punctiforme extracts after prolonged staining, nor were additional bands detected with extracts from cells grown with glucose or fructose (data not shown). RF values were calculated for each protein, and the molecular masses of the cyanobacterial G6PDs were determined as shown in Fig. 3 (A and B). The molecular masses of the G6PDs in N. punctiforme and Anabaena 7120 cell extracts were 200 and 263 kDa, respectively, whereas the molecular mass of His-G6PD was 229 kDa. There was no change in the migration of His-G6PD upon the addition of His-OpcA, nor was the aggregation state of His-G6PD altered by increasing its concentration in solution to 2.5 mg/ml, by changing the buffer pH from 7.9 to 6.5 or by incubating it with G6P or NADP (data not shown).

Effect of the Stoichiometry Between OpcA and G6PD on G6PD Activity and Aggregation State—To determine whether differences in the amount of OpcA present in the cell affected the quantity, aggregation state, or activity of N. punctiforme G6PD, G6PD assays and quantitative Western blots were performed with extracts from several strains that produced amounts of OpcA and G6PD that differed from those normally found in wild-type N. punctiforme. The results are summarized in Table II. Wild-type N. punctiforme extract typically contained 1 or 2 mg of OpcA and G6PD monomers per g of cell protein when the cells were cultured with ammonium or dinitrogen, respectively. The aggregation state of G6PD did not change when the stoichiometry between OpcA and G6PD was altered (Table II); in all strains that carried one or more intact copies of the zwf gene, G6PD monomers were synthesized and assembled into a single aggregation state that corresponded to the 200-kDa form found in wild-type N. punctiforme. The G6PD activity varied, however. In strain UCD 351, which produced 15 times more G6PD than the wild-type, but lacked OpcA, G6PD activity was nearly 9-fold lower than in wild-type. Activity was at baseline levels in strains UCD 466 and UCD 341, which lacked G6PD but differed in their OpcA content. In strains that produced both G6PD and OpcA, G6PD activity depended on the total quantity of G6PD present and on the stoichiometry between OpcA and G6PD: In strain UCD 482, which synthesized normal amounts of G6PD in the presence of elevated amounts of OpcA, G6PD activity was similarly to that of wild-type. Near-wild-type G6PD activity was also observed for strain UCD 364, which overproduced G6PD in the presence of wild-type amounts of OpcA. A large (23-fold) increase in G6PD activity was observed only for strain UCD 348, which overproduced both OpcA and G6PD.
The logarithm of the retardation coefficient for each protein standard was next plotted against the logarithm of its molecular mass. Triangles were determined by measuring the distances from the center of the bands to the dye front on duplicate gels. Retardation coefficients for each standard and unknown protein were then determined by plotting $100 \log (R_F \times 100)$ as a function of the percent gel concentration. The negative slope of each line is the retardation coefficient. Standards: open triangles, bovine serum albumin monomer (66 kDa); inverted open triangles, bovine serum albumin dimer (132 kDa); open circles, $\beta$-amylase (200 kDa); open squares, apoferritin (450 kDa); open diamonds (observed by other data points), urease hexamer (545 kDa); crosses, thyroglobulin (669 kDa). Unknowns: closed squares, N. punctiforme extract G6PD; closed circles, N. punctiforme His-G6PD; closed triangles (some data points are obscured by other data points), Anabaena 7120 extract G6PD. B, the logarithm of the retardation coefficient for each protein standard was next plotted against the logarithm of its molecular mass. The resulting standard curve (closed diamonds) was used to determine the molecular masses of the cyanobacterial G6PDs. Solid line, N. punctiforme extract G6PD, 200 kDa; dotted line, N. punctiforme His-G6PD, 229 kDa; dashed line, Anabaena 7120 extract G6PD, 263 kDa.

### Table II

| Strain     | Characteristics                      | G6PD protein | OpCA protein | G6PD activity | OpCA aggregation state |
|------------|-------------------------------------|--------------|--------------|---------------|------------------------|
| ATCC 29133 | Wild-type N. punctiforme             | 1            | 1            | 0.0459 ± 0.0037 (14) | 200 kDa               |
| UCD 341    | ATCC 29133 with zwf: P$_{pAB}_{pntII}$; opCA not transcribed | None         | None         | 0.0022 ± 0.0003 (3) | No G6PD               |
| UCD 364    | ATCC 29133 + pSCR132 (zwf)          | 14           | 1            | 0.0347 ± 0.0053 (3) | 200 kDa               |
| UCD 438    | ATCC 29133 + pSCR121 (opCA)         | 1            | 5            | 0.0541 ± 0.0003 (2) | 200 kDa               |
| UCD 341    | UCD 341 + pSCR123 (zwf-opCA)        | 20           | 20           | 1.034 ± 0.186 (3)  | 200 kDa               |
| UCD 351    | UCD 341 + pSCR122 (zwf)             | 15           | None         | 0.0050 ± 0.0006 (7) | 200 kDa               |
| UCD 466    | ATCC 29133 with zwf: P$_{pAB}_{pntII}$; opCA transcribed from P$_{pAB}_{pntII}$ | None         | 150          | 0.0026 ± 0.0005 (6) | No G6PD               |

The experiments described above implied that G6PD synthesized in the absence of OpCA was assembled into its native aggregation state but was still relatively inactive. To determine whether the activity of this inactive G6PD could be restored by the addition of OpCA, extract from strain UCD 351 (G6PD overproduced, no OpCA) was mixed with extract from strain UCD 466 (no G6PD, OpCA overproduced). In the experiment shown in Fig. 4, the amount of strain UCD 351 extract was held constant, and increasing amounts of strain UCD 466 extract were added until no further change in G6PD activity was observed. The G6PD activity of the strain UCD 351 extract increased nearly 20-fold, from 0.007 $\mu$mol-min$^{-1}$-mg of protein$^{-1}$ in the absence of strain UCD 466 extract to 0.127 $\mu$mol-min$^{-1}$-mg of protein$^{-1}$ when strain UCD 466 extract was saturating (Fig. 4 and Table III). The increase in G6PD activity upon the addition of strain UCD 466 extract occurred within 30 s after the mixing of the extracts and remained constant after incubation of the mixture on ice for more than 1 h. There was no increase in G6PD activity when strain UCD 341 extract (no G6PD or OpCA) was added to strain UCD 351 extract, nor was there any change in the G6PD activity of wild-type N. punctiforme extract when extract from strain UCD 466 was added (data not shown). Quantitative Western blotting indicated that the strain UCD 351 extract used in the experiment shown in Fig. 4 contained 10.3 ng of G6PD/μg of total protein, whereas the strain UCD 466 extract contained 179 ng of OpCA/μg of total protein. Therefore, the concentration of G6PD in the assay was 13.0 nM, and the concentration of OpCA was varied between 0 and 360 nM. When the data were fitted to the Hill equation and plotted (Fig. 4, inset), the OpCA concentration was determined to be 11.0 nM. Additional experiments performed with G6PD and OpCA from different sources are summarized in Table III. Although the concentrations of UCD 351 G6PD and His-G6PD used in these experiments were similar, the concentration of OpCA required for half-maximal activation was lower for UCD 351 extract G6PD than for His-G6PD.

α-OpCA antibodies were used to examine the interaction between G6PD and OpCA. Aliquots (40 μl) of α-OpCA or preimmune serum were added either to His-OpCA (4.4 μg, 80 pmol)
alone or to a mixture containing an identical amount of His-OpcA plus His-G6PD (0.5 μg, 8 pmol). The samples were incubated on ice for 1 h. The serum-treated His-OpcA samples were then mixed with His-G6PD (0.5 μg, 8 pmol) to test their ability to enhance G6PD activity, whereas the serum-treated samples containing the mixture of His-OpcA and His-G6PD were assayed directly for G6PD activity. His-OpcA that had been incubated with α-OpcA retained only 3% of its initial ability to activate G6PD; no loss in the ability to activate G6PD was observed when His-OpcA was incubated with preimmune serum. Inactivation of His-OpcA also occurred when it was mixed with His-G6PD prior to the addition of α-OpcA; only 15% of the initial activity of the His-G6PD plus His-OpcA protein mixture remained after incubation with α-OpcA, whereas 87% of the initial activity of the mixture remained after incubation with preimmune serum.

Kinetic Constants of G6PD Measured in the Absence and Presence of OpcA—Additional experiments were performed with cell extracts and the purified proteins to determine the nature of the defect in N. punctiforme G6PD synthesized in the absence of OpcA. A comparison of kinetic parameters revealed that the affinity of G6PD for G6P substrate was ~30-fold lower when OpcA was absent. When the amount of cell extract was held constant and the concentration of G6P was varied, a sigmoidal rate versus substrate concentration curve was obtained with strain UCD 351 extract, whereas a hyperbolic curve was obtained with wild-type N. punctiforme extract (Fig. 5A). A sigmoidal curve was also observed with His-G6PD (Fig. 5B). The apparent $K_m$ for G6P ($K_m^{app}$) was determined to be 0.5 ± 0.1 mM for wild-type N. punctiforme G6PD, 85 mM for strain UCD 351 G6PD, and 65 ± 10 mM for His-G6PD (Table IV). Near-wild-type affinities for substrate were restored to His-G6PD and strain UCD 351 G6PD when saturating amounts of His-OpcA were added to the assay (Fig. 5B and Table IV). As illustrated in Fig. 5B, the shift in apparent $K_m$ was accompanied by a change in $V_{max}$. The kinetics of NADP cofactor binding were also examined (Table IV). At saturating G6PD concentrations, NADP binding curves were nearly hyperbolic. In the absence of OpcA, the apparent $K_m$ for NADP was 48 μM for strain UCD 351 extract G6PD and 49 ± 4.2 μM for His-G6PD. Upon the addition of His-OpcA, a 2-fold reduction in the apparent $K_m$ for NADP occurred and $K_m^{(app)}$ values nearly identical to those of wild-type N. punctiforme extract were obtained.

**Effect of Redox Modulators on OpcA and G6PD Activity**—The activity of cyanobacterial G6PD is reported to depend upon its oxidation-reduction state; therefore, the potential for redox regulation of His-G6PD was examined. First, chemical oxidizing or reducing agents were added to His-G6PD samples to a final concentration of 5 or 50 mM. The samples were incubated 30 min on ice and assayed for G6PD activity. Neither the oxidizing agents sodium tetrathionate (STT), diamide, hydrogen peroxide, nor oxidized glutathione nor the reducing agents dithiothreitol (DTT) or reduced glutathione affected the activity of His-G6PD that had not been previously activated by His-OpcA; however, when DTT was added at high concentration (50 mM) to a mixture containing both His-G6PD and His-OpcA, the G6PD activity of the mixture declined 77% after 5 min and 96% after 30 min. No activation of His-G6PD occurred when His-OpcA was incubated with 50 mM DTT for 30 min prior to the mixing of the two proteins.

To determine whether similar results could be obtained with thioredoxin, *Spirulina* thioredoxin that had been reduced or oxidized with 2 mM DTT or 2 mM STT, respectively, was added to His-G6PD or His-OpcA. Like the chemical-oxidizing and -reducing agents, reduced or oxidized thioredoxin had no effect on the activity of His-G6PD when it was added in the absence of His-OpcA (data not shown). However, when reduced thioredoxin was added to His-OpcA, His-G6PD added subsequently to the His-OpcA plus reduced thioredoxin mixture was no longer activated (Fig. 6). The G6PD-activating ability of His-OpcA was restored when STT was added to the His-OpcA/thioredoxin mixture at a concentration that was twice the DTT concentration (Fig. 6), and this reduction/oxidation cycle of inactivation/activation could be repeated in the same sample. When thioredoxin was omitted from the His-OpcA/thioredoxin mixture, the low concentrations of DTT and STT appeared to have little effect, and activation of His-G6PD by His-OpcA was always observed (Fig. 6).

**DISCUSSION**

The opcA gene was previously shown to be required for G6PD activity in *N. punctiforme* (10) and *Synechococcus* 7942 (9), and it has been proposed that OpcA is involved in the assembly of active G6PD oligomers (9). Using *N. punctiforme* strains that lack OpcA or express amounts of G6PD and OpcA that differ from wild-type, we have shown that the assembly of G6PD into its native tetrameric form occurs in *N. punctiforme* even in the absence of OpcA or when the stoichiometry between OpcA and G6PD is greatly altered. Although *N. punctiforme* G6PD is assembled correctly in the absence of OpcA, it is essentially inactive, because it has a very low affinity for G6P substrate. Our kinetic studies with G6PD and OpcA indicate that OpcA may function as a positive allosteric effector that shifts the $K_m$ for G6P substrate to a physiologically relevant value. OpcA may also be involved in redox regulation of G6PD activity, because redox modulators affected G6PD activity only when OpcA was present.

During nitrogen fixation and dark heterotrophic growth, the cellular demand for reducing power is high, and increased transcription of the *zuf* and *opcA* genes of *N. punctiforme* has been documented (11). In extracts from *N. punctiforme* grown under these conditions, G6PD activity was elevated above the levels detected in cells grown photoautotrophically with ammonium. Although there was increased synthesis of G6PD and OpcA, no change in the aggregation state of G6PD was detected (Fig. 1). Thus, rather than producing more highly aggregated and catalytically more active forms of G6PD when carbon flow through the OPP path-
The G6PD activity of strain UCD 351 extract (70 μg of total protein; 13 pmol of G6PD) or His-G6PD (1.0 μg; 16 pmol) was first determined in the absence of added OpcA using standard assay conditions (2 mM NADP and 5 mM G6P) and is reported in the "No Added OpcA" column. The G6PD concentration was then held constant at 13 nM for UCD 351 G6PD and 16 nM for His-G6PD, and increasing amounts of His-OpcA or strain UCD 466 extract were added until G6PD activity no longer increased and OpcA became saturating. The maximum G6PD activity achieved is shown in the "+ OpcA" column. The units of G6PD activity are μmol of NADP reduced · min⁻¹ · mg UCD 351 extract or His-G6PD⁻¹, and activity is reported as mean ± S.E. with the number of trials given in parentheses. Values of n, the Hill coefficient, and Sₐ₀,₅, the concentration of OpcA required for half-maximal activation of G6PD, were determined by fitting the data to the Hill equation.

### TABLE III

| Source of G6PD | Source of OpcA | G6PD activity |
|----------------|----------------|---------------|
| Strain UCD 351 extract | Strain UCD 466 extract | 0.007, 0.127 (1) |
| Strain UCD 351 extract | His-OpcA | 0.007, 0.065 (1) |
| His-G6PD | Strain UCD 466 extract | 0.482, 0.862 (1) |
| His-G6PD | His-OpcA | 1.3 ± 0.7, 57.1 ± 28.3 (3) |

### TABLE IV

| [G6P] | [NADP] |
|-------|--------|
| 0.5 ± 0.1 | 1.0 ± 0.1 (2) |
| 85 | 1.7 (1) |
| 3.2 | 0.9 (1) |
| 65 ± 10 | 2.1 ± 0.3 (4) |
| 1.9 ± 0.2 | 0.9 ± 0.1 (2) |

### FIG. 5

The affinity of _N. punctiforme_ G6PD for G6P substrate was increased in the presence of OpcA. A, activity versus G6P substrate concentration curves for G6PD in strain UCD 351 extract (open circles) or wild-type _N. punctiforme_ extract (closed circles). Strain UCD 351 overproduces G6PD but lacks OpcA, whereas wild-type _N. punctiforme_ contains approximately equal amounts of both proteins. Here the amount of each extract was held constant at 70 μg of total protein, NADP was held constant at 2 mM, and the G6P concentration was varied. Because strain UCD 351 contains more G6PD per unit total protein than wild-type _N. punctiforme_, G6PD activity for each strain is expressed as a percentage of the maximum activity obtained for that strain. B, activity versus G6P substrate concentration curves for purified His-G6PD in the absence (open squares) or presence (closed squares) of saturating His-OpcA. His-G6PD (0.5 μg; 8 pmol) was assayed alone or was mixed with His-OpcA (4.4 μg; 80 pmol). The NADP concentration was held constant at 2 mM. Kinetic parameters were determined by fitting the data to the Hill equation and are Kₐ₀ = 80.4 μM·min⁻¹·mg His-G6PD, Sₐ₀ = 58.9 μM, and n = 2.4 for His-G6PD in the absence of His-OpcA and Vₚₐₐₜₜ = 77.7 μmol·min⁻¹·mg His-G6PD, Sₐ₀ = 1.8 μM, and n = 0.9 for His-G6PD in the presence of His-OpcA. Inset shows detail of the region between 0 and 10 mM G6P.

### TABLE V

| Source of G6PD | Source of OpcA | G6PD activity |
|----------------|----------------|---------------|
| Strain UCD 351 extract | Strain UCD 466 extract | 0.007, 0.127 (1) |
| Strain UCD 351 extract | His-OpcA | 0.007, 0.065 (1) |
| His-G6PD | Strain UCD 466 extract | 0.482, 0.862 (1) |
| His-G6PD | His-OpcA | 1.3 ± 0.7, 57.1 ± 28.3 (3) |

### FIG. 3B

The value of 263 kDa we obtained for the enzyme in _N. punctiforme_ cell extracts is in good agreement with the 240-kDa value obtained by Schaeffer and Stanier (4) for the predomi-nant M2 form of the enzyme, as well as with the 250-kDa value obtained from native PAGE gels stained for G6PD activity (9). Ferguson analysis in our laboratory revealed that the molecular masses of the G6PD in _N. punctiforme_ and _Anabaena_ 7120 cell extracts were 200 and 263 kDa respectively, whereas _N. punctiforme_ His-G6PD synthesized in and purified from _E. coli_ was 229 kDa (Fig. 3B). The value of 263 kDa we obtained for the enzyme in _Anabaena_ 7120 extract is in good agreement with the 240-kDa value obtained by Schaeffer and Stanier (4) for the predomi-nant M₂ form of the enzyme, as well as with the 250-kDa value obtained for the smaller of the two forms observed by Gleason.
lacking OpcA (see Fig. 5 in Ref. 9). In light of the results presented here, this increase in staining intensity is most likely to have been due to the activation of previously assembled, catalytically inactive tetrameric G6PD by OpcA rather than to OpcA-mediated assembly of inactive G6PD monomers into catalytically active oligomers, as was proposed (9).

OpcA was clearly required for the optimal catalytic activity of N. punctiforme G6PD, and its effect was to increase the affinity of G6PD for the G6P substrate ~30-fold to a physiologically relevant value (Table IV). OpcA also affected NADP cofactor binding, because a 2-fold increase in the affinity for NADP occurred for strain UCD 351 G6PD or His-G6PD in the presence of saturating His-OpcA. G6PD activity assays of extracts from strains that overproduced G6PD (Table II, strains UCD 351, UCD 364, and UCD 348) showed that, when G6PD was present in excess, it was the amount of OpcA that determined G6PD activity. As illustrated in Fig. 4 and Table III, the G6PD in strain UCD 351 was activated by the addition of OpcA from strain UCD 466 extract. Both strain UCD 466 extract and purified His-OpcA were able to activate strain UCD 351 G6PD and His-G6PD (Table III), indicating that it was OpcA that was responsible for the activation of G6PD and not some other component in N. punctiforme extract whose activity depended on the presence of OpcA. For strain UCD 351 G6PD, the OpcA concentration required for half-maximal activation of G6PD was much lower than that required for half-maximal activation of His-G6PD (Table III). The His-G6PD tetramer may have interacted less effectively with OpcA than the native G6PD tetramer if the His tag made it less accessible to OpcA.

The affinity of cyanobacterial G6PD for G6P in vitro is reported to vary with the pH, with the concentration of the enzyme in solution, and with its redox state and aggregation state (4, 5, 8, 23). We were able to alter the affinity of purified His-G6PD for substrate only by adding OpcA, but the shift in apparent K_m that occurred after OpcA was added (Fig. 5B and Table IV) resembles the shift that others have observed when dilute purified cyanobacterial G6PD was concentrated in solution (4, 8). Most recently, for example, Gleason (8) reported that G6PD purified from Anabaena 7120 cultured with nitrate was in a “high activity” form when concentrated in solution (≥2 mg/ml) and in a “low activity” form when it was diluted. Both forms of the enzyme exhibited sigmoidal kinetics, but the apparent K_m (S_0.5) for G6P at pH 7 to 8 was 2.2–7.2 mM for the concentrated enzyme and 49–54 mM for the dilute enzyme. OpcA was most likely absent from this purified Anabaena 7120 G6PD preparation: A typical specific activity of 2–6 μmol·min⁻¹·mg of protein⁻¹ was reported for the Anabaena 7120 enzyme, when it was assayed at 20 mM G6P (8). This value is slightly higher than the average specific activity of 0.9 ± 0.1 μmol·min⁻¹·mg of protein⁻¹ we typically obtained for purified His-G6PD preparations at 5 mM G6P, and is comparable to the specific activity of 8.0 μmol·min⁻¹·mg of protein⁻¹ we observed for His-G6PD at 20 mM G6P (Fig. 5B). It is more than an order of magnitude lower than the highest specific activity (86 μmol·min⁻¹·mg His-G6PD protein⁻¹ at 5 mM G6P) we obtained for the OpcA-activated enzyme (Table III).

From Western blots, microscopic measurements, and protein and Chl a assays we have determined that the average wild-type N. punctiforme cell is 2.5 μm x 4 μm and contains ~1 mg of G6PD monomer and 20 mg of Chl a per g of total protein when cultured with ammonium. Assuming the cell is a cylinder, we calculate a cell volume of 2 × 10⁻¹¹ ml, and if a cell contains 1.75 × 10⁻¹³ g of Chl a (14), we estimate that the cellular G6PD concentration is about 0.4 mg/ml. If G6PD is present at a similar concentration in Anabaena 7120 cultured with nitrate, at 2 mg/ml or greater, purified “high activity” G6PD could be as...
much as 5 times more concentrated in vitro than it is in the cell. Purified cyanobacterial G6PD has the unusual in vitro properties of inactivation and precipitation/activation in dilute and concentrated suspension, respectively (see “Experimental Procedures” and ref. 8). We speculate that nonphysiologically high concentrations of the enzyme, or of effectors such as G6P or NADP, and physicochemical factors such as low pH, might stabilize purified G6PD in the absence of OpcA, keeping it in an activated or partially activated conformation that resembles the conformation that is characteristically found in the cell when OpcA is present.

To prevent simultaneous operation of the oxidative and reductive pentose phosphate cycles, cyanobacterial and chloroplast G6PDs are active in the dark when they are in an oxidized state and are inactive in the light when they are in a reduced state, whereas the converse is true of key enzymes of the reductive pentose phosphate cycle (6, 24). The inactivation of G6PD is mediated by thioredoxin (5, 7, 8, 25) and occurs via covalent redox modification of specific regulatory cysteine residues (19). The location of the conserved cysteine residues differs between the cyanobacterial and chloroplast enzymes (19, 26), and plants appear to lack any protein with similarity to cyanobacterial OpcA (BLAST search) (12), therefore, regulation of the two enzymes may be quite different. Because the oxidized and reduced forms of G6PD have been shown to differ in their affinities for G6P (19, 23, 27), we initially considered the possibility that strain UCD 351 G6PD and His-G6PD were in a reduced and inactive form and that the shift in apparent $K_m$ we observed was due to oxidative reactivation of the enzyme upon the addition of OpcA. Two lines of evidence now make this hypothesis unlikely: First, we were unable to oxidatively activate His-G6PD either with oxidized thioredoxin or with chemical oxidants such as STT, diamide, oxidized glutathione, and hydrogen peroxide, which have previously been reported to activate G6PD (7, 27); thus, oxidation by OpcA also seems unlikely. Second, the experiments with α-OpcA antibody indicated that repeated interactions with His-OpcA were necessary to keep His-G6PD in an activated state. If activation required only a single interaction between the two proteins, as would be expected if OpcA activated G6PD by oxidation, then addition of α-OpcA after activation had occurred should have had no effect on G6PD activity. The decline in G6PD activity we observed after the addition of α-OpcA to the His-G6PD plus His-OpcA mixture implies that further interaction between the two proteins was required to keep the G6PD in an activated state and that binding of α-OpcA to His-OpcA either physically prevented this interaction or rendered the His-OpcA inactive.

When His-OpcA was incubated with reduced thioredoxin before it was mixed with His-G6PD, no activation of His-G6PD occurred (Fig. 6); activation of His-G6PD by His-OpcA occurred only after excess sodium tetradecanoate was added to the His-OpcA plus reduced thioredoxin mixture. There are two possible explanations for these results: The first is that the His-OpcA was unaffected by reduced thioredoxin, but once His-G6PD had been activated by His-OpcA, it immediately became sensitive to redox modulation by thioredoxin; the second is that His-OpcA itself was reversibly inactivated by thioredoxin and could not activate His-G6PD when it was in a reduced state. Because the activation of His-G6PD by His-OpcA occurred very rapidly, we could not distinguish between these two possibilities by catalytic assays. Like other cyanobacterial G6PDs, $N. punctiforme$ G6PD contains cysteine residues in conserved positions that may be involved in redox modulation of enzyme activity (26). The $N. punctiforme$ OpcA sequence also contains cysteine residues; of the nine present, those at positions 183, 195, 396, 401, and 407 are conserved in five other cyanobacterial sequences with homology to OpcA and therefore appear to be the most likely targets for thioredoxin-mediated redox modulation of OpcA activity, if it does occur. (The $N. punctiforme$, Synechococcus 7942, and Synechocystis 6803 OpcA sequences are available on the National Center for Biotechnology Information web site; the Prochlorococcus marinus and marine Synechococcus sp. strain WH8102 OpcA sequences are both available at the U. S. Department of Energy Joint Genome Institute web site; and the Anabaena 7120 OpcA sequence is available at the Kazusa DNA Research Institute web site.) Detailed kinetic studies and site-directed mutagenesis of the cysteine residues in G6PD and OpcA should allow us to determine whether reduced thioredoxin inactivates one or both of these proteins.

The physiological role of OpcA and the exact nature of its interaction with G6PD remain to be determined. $N. punctiforme$ strains lacking OpcA have low levels of G6PD activity and exhibit defects in nitrogen fixation and dark heterotrophic growth. The kinetic data presented here are most consistent with a role for OpcA as an allosteric activator of cyanobacterial G6PD that shifts the equilibrium between the T- and R-states of the enzyme toward the higher affinity R-state. We have also shown, however, that, for wild-type $N. punctiforme$ cells, changes in growth conditions did not lead to disproportionate changes in the amounts of OpcA and G6PD protein. If OpcA is an allosteric effector of G6PD, it is unclear how it could function effectively if its concentration in the cell does not change significantly under conditions where regulation of enzyme activity is required. One possibility is that OpcA itself is modified in some manner, such that all or a portion of it is unable to interact with G6PD under conditions when low enzyme activity is desirable. The redox modulation experiments we have performed are consistent with the idea that OpcA is reductively inactivated by thioredoxin, although other forms of protein modification can also be considered.

Acknowledgments—We thank J. L. Ingraham and I. H. Segel for helpful advice and discussions and E. L. Campbell and F. C. Wong for critical reading of the manuscript.

Note Added in Proof—The recent deposition of the Corynebacterium glutamicum oqch and zwf sequences in the GenBank® database has brought to our attention the work of Moritz et al. (Moritz, B., Striegel, K., de Graaf, A. A., and Sahl, H. (2000) Eur. J. Biochem. 267, 3442–3452), who detected the OpcA protein in purified G6PD preparations from $C. glutamicum$. Outside of the division Cyanobacteria, sequences with similarity to OpcA have been identified only in members of the genera Corynebacterium, Streptomyces, Mycobacterium, and Deinococcus. Like the putative OpcA protein sequences from the other Gram-positive and the one Deinococcus group eubacteria, the $C. glutamicum$ sequence has limited similarity (32%) to the $N. punctiforme$ OpcA sequence and lacks the N-terminal and C-terminal amino acid sequences that are consistently present in the cyanobacterial OpcA proteins. Despite these differences, the copurification of $C. glutamicum$ G6PD and OpcA implies that these smaller OpcA proteins may play a role in the activation of G6PD similar to that of the $N. punctiforme$ OpcA.

REFERENCES

1. Winkenbach, F., and Wolk, C. P. (1973) Plant Physiol. 52, 480–483
2. Apte, S. K., Rowell, P., and Stewart, W. D. P. (1978) Proc. Roy Soc. Lond. B Biol. Sci. 200, 1–25
3. Bothe, H., and Neuer, G. (1988) Methods Enzymol. 176, 496–501
4. Schaffer, F., and Stanier, R. Y. (1978) Arch. Microbiol. 116, 9–19
5. Cossar, J. D., Rowell, P., and Stewart, W. D. P. (1984) J. Gen. Microbiol. 130, 991–998
6. Buchanan, B. B. (1980) Ann. Rev. Plant Physiol. 31, 341–374
7. Udvardy, J., Burbely, G., Juhász, A., and Farkas, G. L. (1984) J. Bacteriol. 157, 681–683
8. Gleason, F. (1996) Arch. Biochem. Biophys. 334, 277–283
9. Sundaram, S., Karakaya, H., Scanlan, D. J., and Mann, N. H. (1998) Microbiology 144, 1549–1556
10. Summers, M. L., Wallis, J. G., Campbell, E. L., and Meeks, J. C. (1995) J. Bacteriol. 177, 6184–6194
11. Summers, M. L., and Meeks, J. C. (1996) Mol. Microbiol. 22, 473–480
12. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 3389–3402
OpcA Activates Glucose-6-phosphate Dehydrogenase in Nostoc

13. Newman, J., Karakaya, H., Scanlan, D. J., and Mann, N. H. (1995) FEMS Lett. 133, 187–193
14. Cohen, M. F., Wallis, J. G., Campbell, E. L., and Meeks, J. C. (1994) Microbiology 140, 3233–3240
15. Campbell, E. L., and Meeks, J. C. (1992) J. Gen. Microbiol. 138, 473–480
16. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Summers, M. L. (1995) The Role and Regulation of Glucose-6-Phosphate Dehydrogenase in Diazotrophic and Dark Heterotrophic Growth of the Cyanobacterium Nostoc sp. strain ATCC 29133, Ph.D. dissertation, University of California, Davis
18. Levy, H. R. (1979) in Advances in Enzymology (Meister, A., ed) pp. 97–192, John Wiley & Sons, NY
19. Wenderoth, I., Scheibe, R., and von Schaewen, A. (1997) J. Biol. Chem. 272, 26985–26990
20. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994) Current Protocols in Molecular Biology, John Wiley & Sons, NY
21. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
22. Hanson, T. E., Forchhammer, K., Tandeau de Marsac, N., and Meeks, J. C. (1990) Microbiology 144, 1537–1547
23. Udvardy, J., Juhász, A., and Parkas, G. L. (1983) FEBS Lett. 152, 97–100
24. Buchanan, B. B. (1991) Arch. Biochem. Biophys. 288, 1–9
25. Scheibe, R. (1990) Bot. Acta 103, 327–334
26. Wenderoth, I., Hauschild, R., Lange, C., Pietersma, M., Scheibe, R., and von Schaewen, A. (1999) Plant Mol. Biol. 40, 487–494
27. Scheibe, R., Geissler, A., and Fickenscher, K. (1989) Arch. Biochem. Biophys. 274, 290–297
28. Black, T. A., Cai, Y., and Wolk, C. P. (1993) Mol. Microbiol. 9, 77–84
29. Elhai, J., and Wolk, C. P. (1988) Gene 68, 119–138
30. Rippka, R., and Herdman, M. (1992) Pasture Culture Collection of Cyanobacterial Strains in Axenic Culture, Institut Pasteur, Paris