Recruitment of a Foreign Quinone into the A₁ Site of Photosystem I

I. GENETIC AND PHYSIOLOGICAL CHARACTERIZATION OF PHYLOQUINONE BIOSYNTHETIC PATHWAY MUTANTS IN SYNECHOCYSTIS SP. PCC 6803*

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Genes encoding enzymes of the biosynthetic pathway leading to phyloquinone, the secondary electron acceptor of photosystem (PS) I, were identified in Synechocystis sp. PCC 6803 by comparison with genes encoding enzymes of the menaquinone biosynthetic pathway in Escherichia coli. Targeted inactivation of the menA and menB genes, which code for phytyl transferase and 1,4-dihydroxy-2-naphthoate synthase, respectively, prevented the synthesis of phyloquinone, thereby confirming the participation of these two gene products in the biosynthetic pathway. The menA and menB mutants grow photoautotrophically under low light conditions (20 μE m⁻² s⁻¹), with doubling times twice that of the wild type, but they are unable to grow under high light conditions (120 μE m⁻² s⁻¹). The menA and menB mutants grow photoheterotrophically on media supplemented with glucose under low light conditions, with doubling times similar to that of the wild type, but they are unable to grow under high light conditions unless atrazine is present to inhibit PS II activity. The level of active PS II per cell in the menA and menB mutant strains is identical to that of the wild type, but the level of active PS I is about 50–60% that of the wild type as assayed by low temperature fluorescence, P700 photoactivity, and electron transfer rates. PS I complexes isolated from the menA and menB mutant strains contain the full complement of polypeptides, show photoreduction of F₄₅₀ and F₅₅₃ at 15 K, and support 82–84% of the wild type rate of electron transfer from cytochrome c₅₅₃ to flavodoxin. HPLC analyses show high levels of plastoquinone-9 in PS I complexes from the menA and menB mutants but not from the wild type. We propose that in the absence of phyloquinone, PS I recruits plastoquinone-9 into the A₁ site, where it functions as an efficient cofactor in electron transfer from A₀ to the iron-sulfur clusters.

All well characterized photosynthetic reaction centers are known to contain a bound quinone molecule that participates in the early stages of photochemical charge separation and stabilization (1–3). Type II reaction centers, such as photosystem (PS)II or those present in the purple nonsulfur bacteria, contain a bound benzoquinone or naphthoquinone as the secondary electron acceptor. Type I reaction centers, such as PS I of cyanobacteria and green plants, contain a bound menaquinone, usually phyloquinone (vitamin K₇, 2-methyl-3-phytyl-1,4-naphthoquinone), or less commonly, 5’-monohydroxyphyloquinone, as the secondary electron acceptor (4). (Whether green sulfur bacteria and heliobacteria, which have a PS I-like reaction center, contain a similar bound quinone is still under active investigation.) Two molecules of phyloquinone can be extracted per molecule of P700 from isolated PS I complexes (5–9); however, only one molecule of phyloquinone is considered to participate as an intermediate in electron transfer from A₀ to F₇₃₅ (5, 6, 10).

One strategy to disallow A₁ function is to inactivate genes that code for enzymes involved in the proposed pathway of phyloquinone biosynthesis. Many prokaryotes, as well as chloroplasts, contain the metabolic pathway for phyloquinone (vitamin K₇) biosynthesis. In several bacteria, vitamin K₇ (menaquinone) is used during fumarate reduction in anaerobic respiration (11). The genes encoding the enzymes involved in the conversion of chorismate to menaquinone have been cloned in Escherichia coli (12–14) and Bacillus subtilis (15, 16). Although the route of phyloquinone biosynthesis has not been described in cyanobacteria, the pathway is likely to be similar to the pathway of menaquinone biosynthesis in other bacteria. Menaquinone differs from phyloquinone by the presence of a partly unsaturated, predominantly C-40 side chain rather than a mostly saturated, C-20 phytol side chain. With this exception, the synthesis of the naphthalene nucleus in phyloquinone and menaquinone is expected to include similar steps (Fig. 1). The genome data base for Synechocystis sp. PCC 6803 (17) contains homologs for several genes that encode enzymes for menaquinone biosynthesis: menF (entC) (isochorismate synthase), menD (2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase), menE (O-succinylbenzoic acid-CoA ligase), menB (dihydroxynaphthoate synthase) and menA (identified as “menaquinone biosynthesis protein,” but probably a phytol transferase). Possible homologs of menC and ORF241 (the}

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¶¶ The abbreviations used are: PS, photosystem; EPR, electron paramagnetic resonance; HPLC, high performance liquid chromatography; MS, mass spectroscopy; PCR, polymerase chain reaction; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)methyl]glycine; bp, base pair(s); kb, kilobase; Chl, chlorophyll; Vis, visual light.

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DHNA thioesterase) have also been identified in our data base searches. We propose that menF/EntC, menD, menE, and menB of Synechocystis sp. PCC 6803 are involved in 1,4-dihydroxy-2-naphthoate synthesis, whereas the product of a menA homologue catalyzes the addition of phytol chain. The product of the phytol transferase, 2-phytly-1,4-naphthoquinone, requires a methylation step to become phylloquinone. The gene originally identified as gerC2 (sll1653) in the Synechocystis sp. PCC 6803 data base probably codes for the 2-phytly-1,4-naphthoquinone methyl transferase enzyme that catalyzes this reaction.

There is no known function for menaquinone in cyanobacteria except to provide a precursor for phylloquinone biosynthesis. PS II and respiration require plastoquinone-9, which is a benzoquinone derivative that is synthesized by an independent pathway (18). There are two expected consequences of an interruption in the menaquinone pathway: 1) the participation of men genes in the biosynthetic pathway of phylloquinone in cyanobacteria would be confirmed; and 2) the A₁ site should be empty, thereby allowing a test of the requirement of phylloqui-

none in electron transfer from A₁ to F₉-X. We initially generated mutants in which the menA and menB genes in Synechocystis sp. PCC 6803 have been inactivated by targeted mutagenesis. These two mutations were selected because they would additionally allow us to determine whether the phytol chain is essential for function. If the phytol chain is dispensable, then the menA deletion mutant may allow the head group 1,4-dihydroxy-2-naphthoate to be incorporated into the A₁ site. In this paper, we describe the construction, as well as the genetic and functional characterization, of menA and menB mutant strains of Synechocystis sp. PCC 6803. We propose that in the absence of phylloquinone, the A₁ site does not remain empty. Instead, we suggest that PS I recruits phylloquinone-9 into the A₁ site, and this quinone supports high efficiency electron transfer from A₈ to the iron-sulfur clusters.

Fig. 1. Proposed biosynthetic pathway of phylloquinone biosynthesis in Synechocystis sp. PCC 6803. The gene products responsible for the biosynthesis of menaquinone were initially described in E. coli (see Ref. 14). The homologs of these genes that have been identified in the genome sequence of Synechocystis sp. PCC 6803 are indicated. SAM, S-adenosyl methionine; SAH, S-adenosyl homocysteine.

EXPERIMENTAL PROCEDURES

Phylloquinone Biosynthesis Mutants in Cyanobacteria
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Electron Paramagnetic Resonance (EPR) Spectroscopy of Fe₃ and Fe₄—EPR studies were performed using a Bruker ECS-106 X-band spectrometer and a standard-mode resonator (ST 8615) equipped with a slotted port for light entry. Cryogenic temperatures were maintained with a liquid helium cryostat and an ITC-4 temperature controller (Oxford Instruments, Oxford, UK). The microwave frequency was calibrated with a Hewlett-Packard 5340A frequency counter, and the magnetic field was calibrated using α,α′-diphenyl-β-picryl hydradrazyl as the standard. Sample temperatures were monitored by a calibrated thermocouple located 3 mm beneath the bottom of the quartz sample tube and referenced to liquid N₂. Samples were illuminated with a 150 W xenon arc source (Oriel, Stratford, CT). The EPR spectra of PS I preparations from the wild type were measured through 3 cm of water and a heat-absorbing color filter to remove the near-infrared light. Samples used for EPR measurements contained 1 mg Chl ml⁻¹, 1 mM sodium ascorbate, 4 mM 2,6-dichlorophenol-indophenol in 50 mM Tris, pH 8.3.

Analysis of Phylloquinone Using HPLC-UV/Vis and Mass Spectrometry—Membranes containing 0.1 mg of chlorophyll were centrifuged at 1000 × g for 60 min, and the supernatant was removed. The membrane pigments were sequentially extracted with 1 ml of methanol, 1 ml of 1:1 (v/v) methanol:acetone, and 1 ml of acetone, and the three extracts were combined. The resulting solution was concentrated by vacuum to 2 ml in the dark to approximately 0.8 mg of Chl ml⁻¹. Chromatography with UV/Vis detection was performed on an ISCO dual pump HPLC system (Lincoln, NE). The pumps were operated by ISCO ChroPack version 2.4.4 software. UV/Vis detection was performed with an ISCO V4 absorbance detector set at 255 nm, and data collection and processing were done using JCL6000 version 26 software (Jones Chromatography Limited, Mid-Glamorgan, UK). HPLC separations were also monitored with photodiode array UV-Visible detection using a Hewlett-Packard (Palo Alto, CA) model 1100 quaternary pump and model G1316A photodiode array detector. Sample injections (20 μl) were made on a 4.6 mm × 25-cm Ultrasphere C₁₈ column (4.6 × 250 mm) with 5 μm packing (Beckman Instruments, Palo Alto, CA), using gradient elution (solvent A, methanol; solvent B, isopropanol; 100% A from 0 to 10 min; 3% A→97% B at 30 min, hold until 40 min) at 0.5 ml min⁻¹. A solution of phylloquinone (40 μM) was prepared in absolute ethanol and kept at −20°C as a standard for calibration. Extracts were also analyzed by LC/MS using a Perseptive Biosystems Mariner time-of-flight mass spectrometer using electrospray ionization in negative mode with a needle potential of −3500 V and a nozzle potential of −80 V. A postcolumn flow splitter delivered column eluent to the electrospray ion source at 10 μl min⁻¹. Gas chromatography/MS analyses were performed using a Hewlett-Packard 5972A mass spectrometer coupled to a Hewlett-Packard 5890 gas chromatograph. Splitless injections of 1.0 μl were made onto a 30-m DB-5 column (J & W Scientific, Folsom, CA) using helium (35 cm s⁻¹) as the carrier gas. The column was programmed from 100 to 300°C at a rate of 6°C per min. Data were acquired in both full scanning mode and using selected ion monitoring of m/z = 450 for trace detection of phylloquinone.

RESULTS

Analysis of the Genotype of the menA and menB Mutant Strains—The genotypes of the menA and menB mutant strains were confirmed by Southern blot hybridization analyses and by PCR amplification of the appropriate genomic loci. The left panel of Fig. 2A shows restriction maps of the genomic regions surrounding the menA gene in the wild type and mutant strains. A 440-bp fragment in the menA gene was deleted and replaced by a 1.3-kb kanamycin resistance cartridge encoding the aphII gene. Using primers flanking the coding sequence (Fig. 2A, small arrows), PCR amplification of the menA locus of the wild type produced the expected fragment of 980 bp (Fig. 2A, right panel). PCR amplification of the menA locus of the mutant strain produced the expected 1.9-kb fragment (Fig. 2A, right panel). Because no amplification of the 980-bp fragment occurred when DNA from the mutant strain was used as a template, this result indicates that the wild type and mutant menA alleles had fully segregated. Southern blot hybridization analyses were also performed, and these experiments confirmed that the menA gene was interrupted as expected and that the menA mutant strain was homozygous (data not shown).

Insertional inactivation of the menB gene was also verified.

FIG. 2. Construction and verification of the menA and menB mutant strains of *Synechocystis* sp. PCC 6803. A, inactivation of the menA gene. Left panel, restriction maps of the genomic regions surrounding the menA gene in the wild type and mutant strain. The small arrows indicate the position of the PCR primers used to amplify the menA coding sequence. Right panel, electrophoretic analysis of the DNA fragments amplified from the genomic DNA of the wild type and menA mutant strains. B, inactivation of the menB gene. Left panel, restriction maps of the genomic regions surrounding the menB gene in the wild type and mutant strains. The small arrows indicate the position of the PCR primers used to amplify the menB coding sequence. Right panel, electrophoretic analysis of the DNA fragments amplified from the genomic DNA of the wild type and menB mutant strains.
by both Southern blot hybridization analysis and by PCR amplification of the menB locus from the mutant strain. As shown in the left panel of Fig. 2B, most of the menB gene was deleted and replaced with a 2-kb spectinomycin resistance cartridge. Using primers flanking the coding sequence (Fig. 2B, small arrows), PCR amplification of the menB locus of the wild type produced the expected fragment of 920 bp (Fig. 2B, right panel). However, PCR amplification of this locus in the mutant strain produced a 2.2-kb DNA fragment (Fig. 2B, right panel), and no 930-bp fragment was observed. These results indicate that the menB mutant is homozygous and that full segregation of alleles had occurred. The PCR amplification results were confirmed by Southern blot hybridization analyses, which demonstrated that the menB mutant strain was homozygous and that the menB gene had been insertionally inactivated as shown in the left panel of Fig. 2B.

**Analysis of the Phenotype of the menA and menB Mutant Strains**—We focused initially on the phenotypic analysis of whole cells of the menA and menB mutant strains. Photomixotrophic growth rates of the menA and menB mutants were measured in cells grown in BG11 plus 5 mM glucose under reduced light intensity; units are with reference to absorbance of the whole cells at 730 nm.

**Physiological characteristics of the Synechocystis sp. PCC 6803 wild type and the menA and menB strains**

| Strain               | Wild type | menA       | menB       |
|----------------------|-----------|------------|------------|
| Photoautotrophic growth |          |            |            |
| doubling time (h)    | 34 ± 1.2  | 73 ± 3.4   | 71 ± 2.8   |
| Low light            | 16 ± 1.4  | NMa        | NM         |
| High light           |           |            |            |
| Photomixotrophic growth |        |            |            |
| doubling time (h)    | 17 ± 1.1  | 22 ± 1.8   | 24 ± 2.3   |
| Low light            | 12 ± 0.8  | NM         | NM         |
| High light           |           |            |            |
| Phototrophic growth  |          |            |            |
| doubling time (h)    | 22 ± 1.6  | 25 ± 2.1   | 26 ± 1.8   |
| Low light            | 24 ± 2.2  | 29 ± 2.6   | 31 ± 3.2   |
| High light           |           |            |            |
| Oxygen evolutionb    | (µmol O₂/A₇₃₀ nl) |          |
| Whole chain          | 1260 ± 76 | 787 ± 49   | 718 ± 57   |
| PS II-mediated       | 1450 ± 84 | 1408 ± 106 | 1387 ± 93  |
| Chlorophyll contentb | (µg Chl/A₇₂₀ ml) |          |
|                      | 4.21 ± 0.18 | 3.36 ± 0.23 | 3.43 ± 0.32 |

a NM, not measurable.
b Cells were grown in BG11 plus 5 mM glucose under reduced light intensity.

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menB mutants. In the menA and menB mutant cells, the PS II-chlorophyll fluorescence emission at 685 and 695 nm (30) shows no obvious differences in intensity from the wild type cells. However, the PS I-chlorophyll fluorescence emission at 721 nm was reduced in intensity in the two mutant strains relative to the wild type (Fig. 3). This result indicates that cells of the two mutant strains contain the same amount of PS II per cell as the wild type, but less PS I per cell than the wild type.

The absolute PS I content of whole cells can be determined by the light-induced absorbance increase at 832 nm due to the oxidation of P700 (27, 31). On the basis of equal cell numbers, the light-induced absorbance increase at 832 nm due to the cell as the wild type, but less PS I per cell than the wild type. Of the two mutant strains contain the same amount of PS II per relative to the wild type (Fig. 3). This result indicates that cells

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**FIG. 3.** Fluorescence emission spectra at 77 K of whole cells from *Synechocystis* sp. PCC 6803 wild type and the menA and menB mutant strains. Spectra were recorded at the same cell density (1.0 A_{680} nm^{-1}). Each spectrum was the average of five measurements. The excitation wavelength was 440 nm, which excites mostly chlorophyll. PS II and its accessory pigments exhibit emission maxima at 685 and 695 nm; PS I has a maximum emission at 721 nm.

**FIG. 4.** HPLC profiles of pigment extracts from lyophilized PS I complexes of the wild type strain of *Synechocystis* sp. PCC 6803. The pigments were separated on a 5.0 µm Ultrasphere C_{18} reverse phase column. The detection wavelength was 270 nm. The extract from the wild type shows a peak that co-elutes with authentic phylloquinone at 29.7 min. Top inset, UV/Vis spectrum of authentic phylloquinone. Bottom inset, near-UV/Vis spectrum of HPLC peak that elutes at 29.7 min.
FIG. 5. HPLC profiles analysis of pigment extracts from lyophilized PS I complexes of the *menB* mutant strain of *Synechocystis* sp. PCC 6803. The pigments were separated on a 5-μm Ultrasphere C18 reverse phase column. The peak at 37.2 min in the wild type co-elutes with β-carotene, and shows a spectrum in the visible identical to β-carotene. The peak at 37.2 min in the *menB* mutant shows an additional UV-absorbing component (bottom inset) that co-elutes with plastoquinone-9 and that shows a UV spectrum similar to plastoquinone-9 (top inset) and an *m/z* of 748 (not shown). The LC/MS analysis of the *menA* mutant was similar.

**Strains**—The idea that a foreign quinone might be present in the A1 site first came about when we discovered that a quinone-like EPR signal is present in whole cells of the *menA* and *menB* mutants (see the accompanying paper (33)). To determine the identity of this quinone, solvent extracts of PS I trimers from the *menA* and *menB* mutants were analyzed by HPLC using photodiode array UV-visible detection. The search was initially complicated by the absence of new peaks in chromatograms (λ = 270 nm) from the *menA* and *menB* mutants when compared with the wild type (Fig. 5). We therefore sought evidence of a new component coeluting with another pigment by comparing the UV-visible spectra of peaks in chromatograms of the *menA* and *menB* mutants with the corresponding peaks for the wild type. The only significant difference was in a component that coeluted with β-carotene at 37 min. The difference spectrum of the components eluting at 37 min showed a strong absorbance near 254 nm that was lacking in the wild type (Fig. 5, bottom inset). This is the spectral region in which the biologically occurring benzoquinones, ubiquinones, and naphthoquinones absorb strongly but in which β-carotene has relatively weak absorbance.

We noted that the UV spectrum of the coeluting component was similar to plastoquinone-9, a quinone that is present at a 10-fold higher concentration than phylloquinone in thylakoid membranes (32). Indeed, we found that authentic plastoquinone-9 co-elutes with, and has a UV spectrum that matches, the peak at 37 min (Fig. 5, top inset). Sensitive selected-ion-monitoring analyses of the HPLC eluate at the mass of plastoquinone-9 (*m/z* 748) showed a peak at this retention time. We consistently found levels of plastoquinone-9 in trimeric PS I complexes from the wild type and the *menA* and *menB* mutants in amounts similar to that of the wild type. It should be noted that the quantum yield cannot be determined in these studies because multiple turnovers of the PS I complex occur during continuous illumination. Nevertheless, the quantitative reduction of F₅₇₀ and F₇₃₀ does indicate that the entire population of mutant PS I complexes is competent in electron transport.

**Low Temperature Reduction of F₅₇₀ and F₇₃₀**—The ability of PS I trimers from the *menA* and *menB* mutants to transfer electrons from P700 to the iron-sulfur clusters was determined at low temperature by EPR spectroscopy. When the samples were frozen in darkness and illuminated at 15 K, the relative spin concentrations of reduced F₅₇₀ (g = 2.05, 1.94, 1.85) and F₇₃₀ (g = 2.07, 1.92, 1.88) were identical to those for PS I complexes isolated from the wild type (Fig. 6). The ratio of F₅₇₀ to F₇₃₀ reduced was also identical in the mutants and the wild type. Thus, the absence of phylloquinone in the A1 site does not effect low temperature electron transfer from A₀ to the terminal iron-sulfur clusters. When PS I complexes from the *menA* and *menB* mutants were subjected to photoaccumulation conditions by freezing the sample during illumination, F₅₇₀ and F₇₃₀ were completely reduced, as shown by the presence of an interaction spectrum (*g*-values of 2.05, 1.94, 1.92, and 1.88) with total spin concentrations similar to that of the wild type. It should be noted that the quantum yield cannot be determined in these studies because multiple turnovers of the PS I complex occur during continuous illumination. Nevertheless, the quantitative reduction of F₅₇₀ and F₇₃₀ does indicate that the entire population of mutant PS I complexes is competent in electron transport.

**Flavodoxin Reduction Rates in PS I Complexes**—Although...
The lower rates of whole-chain electron transfer in the menA and menB mutant cells can be explained by a lower PS I content per cell, there still remains the possibility that the efficiency electron transfer in individual PS I complexes is altered by the absence of phylloquinone. Steady-state rates of electron transfer were determined in PS I trimers by measuring the rate of flavodoxin reduction with cytochrome c₅₅ as electron donor as a function of light intensity (Fig. 7). The rates at saturating light intensity were determined by treating light as a substrate in a Michaelis-Menten kinetic analysis. The maximal rate of flavodoxin reduction that could be sustained was found to be 8460 μmol mg Chl⁻¹ h⁻¹ in the wild type PS I complexes, 7128 μmol mg Chl⁻¹ h⁻¹ in the PS I complexes of the menA mutant, and 6948 μmol mg Chl⁻¹ h⁻¹ in the PS I complexes of the menB mutant. Assuming 100 Chl per P700 in all PS I complexes, these maximal rates of electron transport correspond to 235 e⁻ PS I⁻¹ s⁻¹ in the wild type, 198 e⁻ PS I⁻¹ s⁻¹ in the menA mutant, and 193 e⁻ PS I⁻¹ s⁻¹ in the menB mutant. As shown in Fig. 7, the light saturation dependence of the electron transfer rates in the PS I complexes of the menA and menB mutants strains is similar to that for the wild type complexes, indicating that the relative quantum efficiencies of the PS I complexes are not affected by the mutations. These results show that despite the absence of phylloquinone in the A₁ site, electron transfer throughputs in PS I complexes isolated from the menA and menB mutants are 82–84% as efficient as in PS I complexes isolated from the wild type strain.

**DISCUSSION**

Although the phylloquinone biosynthetic pathway in cyanobacteria has not been previously described, the nucleotide sequence of the Synechocystis sp. PCC 6803 genome shows the existence of homologues of the menA, menB, menC, menD, menE, menF (entC), and menG genes, which code for enzymes involved in menaquinone biosynthesis in other bacteria. Because they encode enzymes that function near the end of the biosynthetic pathway, we focused exclusively on the menA and menB genes in this study. The menB gene of E. coli codes for 1,4-dihydroxy-2-naphthoic acid synthase, which catalyzes the formation of the two-ring system by converting o-succinylbenzoyl-coenzyme A to 1,4-dihydroxy-2-naphthoic acid. Menaquinone differs from phylloquinone by the presence of a partly unsaturated, C-40 isoprenyl tail rather than a mostly saturated, C-20 phylt side chain attached to the naphthoquinone nucleus. The menA gene of E. coli codes for 1,4-dihydroxy-2-naphthoate octaprenyl transferase, which catalyzes ligation of the C-40 isoprenyl chain to the C₅ position of the naphthoate moiety. The low degree of identity (17%) in the primary sequences of the MenA proteins of E. coli and Synechococcus sp. PCC 6803 is consistent with the difference in the substrate specificity of these enzymes.

Because of the structural similarity between menaquinones and phylloquinone, we worked from the premise that the menA and menB genes code for proteins that function in phylloquinone biosynthesis. To test this premise, we engineered mutants by targeted inactivation of the menA and menB genes in Synechocystis sp. PCC 6803. Southern blot hybridization and PCR analyses were used to confirm the absence of a complete menA or menB gene in the mutants. HPLC/MS and gas chromatography/MS showed that the membranes of the menA and menB mutant strains do not contain detectable levels of phylloquinone. Hence, one firm conclusion of this study is that the menA and menB homologues in the Synechocystis sp. PCC 6803 genome code for essential enzymes in the phylloquinone biosynthetic pathway. A corollary to this conclusion is that no other biosynthetic routes to phylloquinone exist beyond naphthoate synthase in Synechocystis sp. PCC 6803. The menA and menB mutants showed similar biochemical and physiological characteristics. Both lacked phylloquinone, and both contained plastoquinone-9 in their PS I complexes. These results further suggest that the phytol chain of phylloquinone is required for its stable assembly into PS I complexes in vivo. These observations confirm the participation of both the menA and menB gene products in the phylloquinone biosynthesis pathway in Synechocystis sp. PCC 6803.

To study their physiology and growth characteristics, as well as the role of phylloquinone in photosynthetic electron transfer, the menA and menB mutant strains were grown under a variety of conditions. Both mutant strains grew photautotrophically at low to moderate light intensities (20 and 40 μE m⁻² s⁻¹) but failed to grow either photautotrophically or photomixotrophically when the light intensity exceeded 100 μE m⁻² s⁻¹. Because photomixotrophic growth occurs in the mutants at high light intensities when atrazine is present, excess reductant produced by PS II is proposed to be the cause of the failure to grow at high light intensities. Atrazine binds competitively to the Q₅ site in PS II, blocks the high rate of damaging reductant and/or oxidant formation, and allows the cells to survive the toxic effect of light and to use glucose as the source of reduced carbon. We have found that although the amount of PS II is unchanged relative to the wild type, the amount of functional PS I per cell in the menA and menB mutants is approximately 50% lower than in the wild type. The observed phototoxicity may therefore be an indirect effect caused by an imbalance of the rates of electron transport between PS II and PS I. Indeed, we have recently obtained several second-site suppressor mutants that allow a phylloquinone-less mutant to grow photomixotrophically under high light intensity.² Most of these mutants have reduced PS II activity, thereby supporting our postulate that the PS II toxicity is responsible for the inability of the phylloquinone-less mutants to grow under high light intensity. The cause of reduction in the PS I level in the menA and menB mutant cells could be a decreased rate of assembly or an increased turnover rate of PS I complexes. However, an examination of the degradation rate of the PS I apoproteins in the mutant and wild type strains did not show significant differences (data not shown). We therefore suggest

² P. Chitnis and J. Golbeck, unpublished results.
that the absence of phylloquinone affects the rate of assembly of PS I complexes by influencing one or more steps involved in its biogenesis.

The ability of the menA and menB mutants to grow at low to moderate light intensities agrees with the finding that the absence of phylloquinone did not abolish room temperature photosynthetic electron transfer activity through PS I complexes isolated from these mutants. The mutant strains contained less PS I than the wild type on a per cell basis; additionally, the steady-state rates of electron transfer from cytochrome $c_6$ to flavodoxin in PS I complexes from the mutants were high but were 82–84% of the wild type rate. Therefore, the lower rate of whole-chain electron transfer in the mutant cells is a combination of both effects. In PS I complexes isolated from the menA and menB mutants, electron transfer from P700 to the terminal iron-sulfur clusters is quantitative at cryogenic temperatures. However, in PS I complexes in which phylloquinone has been partially extracted using solvents, the maximum amount of irreversible charge separation after a large number of flashes is independent of the number (0, 1, or 2) of phylloquinone molecules per PS I complex (28). Hence, single turnover optical studies of P700 turnover at room temperature will be necessary to confirm the slightly lower quantum efficiency of PS I electron transfer in the menA and menB mutants.

The inescapable conclusion from this study is that phylloquinone is not required for efficient electron transfer in PS I at either room or cryogenic temperatures. We considered two explanations for the high rates of PS I activity. One is that the mutant PS I complexes differ from the solvent-extracted PS I complexes in allowing room temperature as well as low temperature electron transfer in the absence of phylloquinone. This bypass may be direct or it may involve a redox-active amino acid in the transmembrane domain of the PsaA and PsaB polypeptides. The second possibility is that a foreign quinone has been recruited into the A1 site and that it may participate in electron transfer from A0 to Fx. This quinone, which may be identical to the plastoquinone-9 identified on the basis of the solvent extraction studies described here, may substitute for phylloquinone in the A1 site, thereby promoting electron transfer to Fx and $F_A/F_B$. Spectroscopic evidence supporting the presence of plastoquinone-9 in the A1 site and its participation in forward electron transfer is provided in the second paper of this series (33).
