

Measurement of the redox state of the plastoquinone pool in cyanobacteria

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Here, we developed a method for measuring the *in vivo* redox state of the plastoquinone (PQ) pool in the cyanobacteria *Synechocystis* sp. PCC 6803. Cells were illuminated on a glass fiber filter, PQ was extracted with ethyl acetate and determined with HPLC. Control samples with fully oxidized and reduced photoactive PQ pool were prepared by far-red and high light treatments, respectively, or by blocking the photosynthetic electron transfer chemically before or after PQ in moderate light. The photoactive pool comprised 50% of total PQ. We find that the PQ pool of cyanobacteria behaves under light treatments qualitatively similarly as in plant chloroplasts, is less reduced during growth under high than under ambient CO₂ and remains partly reduced in darkness.

Keywords: cyanobacteria; plastoquinone; *Synechocystis* sp. PCC6803

In cyanobacteria, the plastoquinone (PQ) pool functions in both the photosynthetic and respiratory electron transfer chains (see [1] for a review). In photosynthetic electron transfer, the first electron accepting PQ molecule Q_A of photosystem II (PSII) transfers electrons one by one to the second PQ called Q_B. In the Q_B pocket, oxidized PQ is reduced to plastoquinone (PQH₂) and then exchanged with an oxidized PQ from the PQ pool of the thylakoid membrane. PQH₂ is oxidized by the cytochrome *b₆/f* complex (Cyt *b₆/f*) that further transfers one of the electrons to PSI *via* plastocyanin or cytochrome *c₆* (Cyt *c₆*) while the second electron returns to PQ *via* the Q-cycle. Two protons are released to the thylakoid lumen per each electron transferred to PSI. In addition to its function in the linear photosynthetic electron transfer reactions, the PQ pool participates in cyclic electron transfer around PSI. The NAD(P)H dehydrogenase NDH-1 accepts electrons from ferredoxin and

delivers them to the PQ pool, pumping protons from the cytosol to the thylakoid lumen [2–4].

In respiratory electron transfer, succinate dehydrogenase acts as a major electron donor for the PQ pool [5], but NDH-1 and NDH-2 complexes may continue to reduce the PQ pool in the dark after redox equilibration of the succinate and PQ pool [6,7]. Respiratory oxidation of PQH₂ occurs *via* three types of terminal oxidases [8]. Cytochrome *bd*-quinol oxidase and an alternative respiratory terminal oxidase catalyze direct oxidation of PQH₂ whereas an *aa₃* type cytochrome *c* oxidase catalyzes oxidation of Cyt *c₆* [9–11].

In plants, the redox state of the PQ pool acts as a central source of signals that lead to both short and long-term acclimation to light quality and quantity [12]. The PQ pool is assumed to function in light signaling in cyanobacteria as well [13], although cyanobacterial state transitions appear not to depend on the redox state of the PQ pool [14]. The function

Abbreviations

Chl, chlorophyll; Cyt *b₆/f*, cytochrome *b₆/f* complex; Cyt *c₆*, cytochrome *c₆*; DBMIB, 2,5-dibromo-*t*-isopropyl-3-methyl-1,4-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PQ, plastoquinone; PQH₂, plastoquinone; PSI, photosystem I; PSII, photosystem II.

of the PQ pool in the middle of the two photosystems suggests that the PQ pool can be reduced by light favoring PSII over PSI and oxidized by light favoring PSI. Furthermore, bright light is expected to reduce the PQ pool, as PSII can feed electrons to the PQ pool more rapidly than Cyt b_6f and the Calvin-Benson cycle can remove electrons from the pool [15,16].

The determination of the *in vivo* redox state of the PQ pool has turned out to be difficult in cyanobacteria. Typically, indirect methods based on chlorophyll (Chl) *a* fluorescence have been applied [9], but the fluorescence methods actually measure the redox state of the primary quinone Q_A , not the state of the PQ pool. A quinone electrode has been used, but the results are considered to be only relative [17]. A HPLC method originally developed for plant leaves [18] was modified to measure the redox state of the PQ pool *in vivo* in cyanobacteria [19]. In the method, an extracted PQ sample containing PQ and PQH₂ is divided to two parts, one of which is reduced with sodium borohydride to obtain a fully reduced sample. In addition to the target samples, control samples from cyanobacteria containing a fully reduced or fully oxidized PQ pool are needed because only a fraction of PQ is found in the PQ pool. The redox state of the PQ pool is then obtained by comparing the ratio PQH₂ (no addition)/PQH₂ (after reduction with NaBH₄) in the target sample to the same ratio in the control samples.

The control samples can be obtained with chemical or light treatments that fully reduce or oxidize the PQ pool [13,18–22]. The herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) inhibits reduction of PQ by PSII and therefore the PQ pool can be assumed to be maximally oxidized in illuminated, DCMU-treated cyanobacteria. On the other hand, 2,5-dibromo-*t*-isopropyl-3-methyl-1,4-benzoquinone (DBMIB) inhibits the oxidation of PQH₂ by Cyt b_6f , which leads to maximum reduction of the PQ pool in illuminated, DBMIB-treated cyanobacteria. In the present study, the results obtained with the chemical method (DBMIB/DCMU) were compared with results obtained with the illumination method [high light/far red light (HL/FR)].

The results obtained by Schuurmans *et al.* [19] suggested that the redox state of the PQ pool of *Synechocystis* sp. PCC6803 remains nearly the same in darkness, under 625-nm orange light and in 625-nm light supplemented with 730-nm FR light. The results were surprising, as 625-nm light strongly favors PSII due to absorption by phycobilisomes [23]. Thus, the results of Schuurmans *et al.* [19] suggested that the redox state of the PQ pool of cyanobacteria is a target

of homeostatic regulation, instead of functioning as a source of a regulatory signal.

In the earlier method [19], a mixture (1 : 1, V : V) of methanol and petroleum ether was used for the extraction of PQ from cyanobacteria. We developed the extraction method further by layering the cyanobacteria to a filter and applying the light treatments to the layered cyanobacteria, and by using ethyl acetate for extraction. The results show that the use of filter-layered cells and extraction of PQ with ethyl acetate, continuing the light treatment throughout the extraction, provide a reliable method to measure *in vivo* redox state of the PQ pool. Furthermore, the behavior of the redox state of the PQ pool under different light treatments was found to be qualitatively similar in cyanobacteria as in plant chloroplasts.

Materials and methods

Thirty-millilitre liquid cultures of the glucose-tolerant strain of *Synechocystis* sp. PCC6803 were grown in 100-mL Erlenmeyer flasks under constant illumination, photosynthetic photon flux density (PPFD) 40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, at 32 °C. The BG-11 medium was supplemented with 20 mM Hepes-NaOH pH 7.5, and cells were either grown in ambient air (AIR) or in air enriched with 3% CO₂, as described earlier [24]. For experiments, the concentration of cells both in AIR and 3% CO₂ was 0.8–1.2 measured as OD at 730 nm. Chl *a* was measured spectrophotometrically from methanol extracts [25].

The redox state of the PQ pool was measured from cells directly under growth conditions or in the dark, after 2 h of dark treatment in the growth temperature and atmosphere. To oxidize the PQ pool, the cells were illuminated for 10 min in the presence of 20 μM DCMU or the cells were illuminated for 4 min at 710 nm at the photon flux density (PFD) of 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ defined with a Corion interference filter (10 nm half width at half maximum), as indicated. To reduce the PQ pool, the cells were illuminated in the presence of 50 μM DBMIB for 5 min or untreated cells were illuminated at the PPFD of 2000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for 30 s, as indicated.

HPLC analysis of PQ and PQH₂ was performed with LiChroCART (Sigma, St. Louis, MO, USA) C18 reversed-phase column (LiChrospher 100, 125 × 4 mm, 5 μm ; Sigma) using an Agilent (Santa Clara, CA, USA) 1100 series device with a diode array detector (G1315B) and fluorescence detector (G1321A), and using the mobile phase of methanol : hexane (34:2, v:v). The flow rate was 0.75 mL·min⁻¹, the temperature of the column was maintained at 25 °C, and the injection volume was 20 μL . PQ was detected with absorption at 255 nm using the diode array detector, and PQH₂ was detected with fluorescence detector (I_{ex} = 290 nm, I_{em} = 330 nm).

For the estimation of the *in vivo* redox state of the PQ pool, 3 mL of cells were layered on a 1.2 μ m glass microfiber filter by vacuum filtration, the light treatments were applied to the cyanobacteria layered on the filter, and then PQ was rapidly extracted from the filter by grinding in a mortar with 2 mL of ice-cold ethyl acetate for 10 s. The specific illumination was continued during the extraction. The extract was filtered through a 0.2 μ m polytetrafluoroethylene filter, dried with N_2 , the pellet was dissolved to 100 μ L of methanol and the sample was divided to two parts. PQ was reduced to PQH₂ in one part by adding 1 μ L of 250 mM NaBH₄ just prepared in methanol. The relative amount of PQH₂ in each sample was measured with HPLC using fluorescence intensity at 330 nm with excitation at 290 nm. The relative amount of PQ, obtained from absorbance at 255 nm, was used as a quality control to check the completeness of the reduction with NaBH₄. The percentage of photoactive PQH₂ in the PQ pool of a target sample was calculated from the equation

$$\text{Percentage} = 100 \times (F_{\text{TARGET}} - F_{\text{OX}}) / (F_{\text{RED}} - F_{\text{OX}}), \quad (1)$$

where F is the percentage of PQH₂ in a sample to the total amount of PQ in the sample (see Eqn 2). The subscripts TARGET, OX and RED refer to the target sample and the control samples with a fully oxidized and a fully reduced of photoactive PQ pool, respectively. F is calculated as the ratio between the HPLC peak area (A) of PQH₂ divided by the peak area of PQH₂ of the same sample after reduction with NaBH₄.

$$F = A_{\text{NO_ADDITION}} / A_{\text{NaBH}_4} \quad (2)$$

The peak area of PQH₂ after reduction with sodium borohydride (A_{NaBH_4}) represents the total amount of PQ in the sample. A small amount, representing < 5% of total PQ, always remains in the oxidized form after reduction by NaBH₄ (Fig. 1).

For estimation of the amount PQ in cyanobacteria, 10 mL of cells ($OD_{730} = 0.8\text{--}1.2$) was layered on a 1.2 μ m glass microfiber filter by vacuum filtration and then extracted by 5 mL of ethyl acetate by grinding in a mortar. The procedure was repeated three times to extract all PQ. The extract was filtered through a 0.2 μ m polytetrafluoroethylene filter, dried with N_2 , the pellet was dissolved to 200 μ L of methanol and the sample was divided to two parts. The amount of PQ was measured with both fluorescence at 330 nm with excitation at 290 nm and with absorbance at 255 nm. For measurement of PQ with fluorescence, 2 μ L of 250 mM NaBH₄ just prepared in methanol was added to one of part to totally reduce PQ to PQH₂. The amount of PQH₂ in this part is proportional to peak area of PQH₂ (with retention time about 8.2 min)

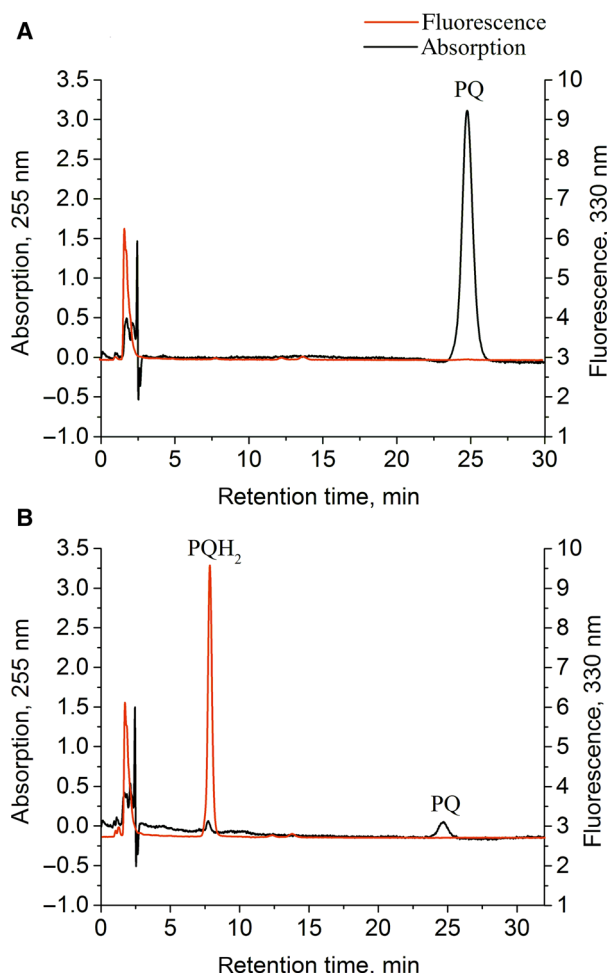


Fig. 1. Chromatograms of PQ (A) and PQH₂ (B) standards. The PQH₂ standard was obtained by reduction of the PQ-9 standard with 5 mM NaBH₄.

measured by fluorescence at 330 nm. For measurement of PQ with absorbance, 2 μ L of 100 mM KOH in water was added to another part; addition of KOH leads to rapid oxidation of PQH₂ [26]. The amount of PQ in this part is proportional to the peak area of PQ (with retention time about 29.3 min) measured by absorbance at 255 nm.

The HPLC peak areas were related to absolute amounts of PQH₂ and PQ by comparing to a PQ standard. To prepare the PQ standard, PQ-9 was isolated from pumpkin thylakoid membranes on a preparative scale using an alumina column-based method [27]. After extraction with an alumina column, PQ was additionally purified using an HPLC-based method [18] with some modifications. Briefly, the HPLC purification was performed with LiChroCART (Sigma) C₁₈ reversed-phase column (LiChrospher 100, 125 \times 4 mm, 5 μ m; Sigma) using an Agilent 1100 series device with absorption and fluorescence detectors (Agilent Technologies), and using an isocratic solvent system (methanol : hexane, 34 : 2 v : v). The flow rate was

0.75 mL·min⁻¹, the temperature of the column was maintained at 25 °C, and the injection volume was 40 µL. PQ-9 was detected with absorption at 255 nm. Pure PQ-9 was collected according to the retention times of the corresponding peaks on the chromatogram using Analyt-FC (Agilent Technologies) collector. Finally, the pure PQ-9 in methanol/hexane was dried under N₂ stream and dissolved with methanol. The concentration of pure PQ was measured using its molar absorption coefficient, $\gamma_{255} = 17\,940\text{ M}^{-1}\text{cm}^{-1}$ [28]. PQH₂-9 was prepared by reducing pure PQ-9 with NaBH₄. HPLC chromatograms of PQ and PQH₂ standards are shown in Fig. 1.

Results and Discussion

Typical chromatograms of extracts from cells of the cyanobacterium *Synechocystis* sp. PCC6803 are shown in Fig. 2. Quality checking with 255 nm absorption showed that ~95% of PQ was in the reduced form in samples reduced with NaBH₄ (Fig. 2B); the reason why a small amount remains as oxidized PQ is not known. The amount of PQ in *Synechocystis* cells was determined from the area of the 330 nm PQH₂ fluorescence peak after addition of NaBH₄ (Fig. 2D). The amount of PQ remaining after addition of NaBH₄, calculated from the remaining PQ absorbance peak (Fig. 2B), was included. The amount of PQ was also estimated from absorption at 255 nm by measuring the PQ peak area after addition of KOH. The HPLC peak areas were compared with a PQ standard. *Synechocystis* cells grown under AIR contained 26.45 ± 1.06 and those grown under 3% CO₂ contained 32.72 ± 0.31 PQ molecules per 1000 Chl *a* molecules, when the amount of PQ was measured with fluorescence after reduction of PQ with NaBH₄. The amount of PQ measured with absorption after oxidation induced by the addition of KOH was slightly higher (Table 1), possibly due to different sensitivity of the setup for fluorescence and absorption. Earlier results [19] suggest that the PQ/Chl ratio decreases with cell density of the culture from 67 to 19 per 1000 Chl *a* in *Synechocystis* sp. PCC6803. The present results are in the same range, but other growth conditions in addition to the cell density may affect the result. Interestingly, the Chl (*a* + *b*) to PQ ratio of plant chloroplasts is in the same range [18] despite of the big differences between the antennae of plants and cyanobacteria.

The measurement of the redox state of the PQ pool with HPLC requires reliable control samples in which the PQ pool is either fully reduced or fully oxidized. For this, *Synechocystis* sp. PCC6803 cells were illuminated in the presence of DCMU and in the presence

of DBMIB. The reduction level of total PQ was $24.8 \pm 1.6\%$ in DCMU-treated samples and $67.8 \pm 4.3\%$ in DBMIB-treated samples (Fig. 3), indicating that the PQ pool that can be reduced or oxidized with the combined treatment with chemicals and light, comprised 43.0% of total PQ, or 13.3 PQ molecules per 1000 Chls. This result is very similar as in plant leaves, where 30–40% of total PQ has earlier been found to belong to the photoactive pool [18,29].

To test an inhibitor-less method, HPLC measurements were also done with cyanobacteria treated with 710-nm FR light to oxidize the PQ pool and with high-intensity white light to reduce it. Similar results were obtained with this method ($30.5 \pm 4.1\%$ PQH₂ with FR light and $72.1 \pm 5.1\%$ with HL, Fig. 3), indicating that 41.6% of total PQ, or 12.9 PQ molecules per 1000 Chls, belong to the photoactive pool. This consensus of the two series of results is particularly important, as it shows that the modified HPLC method can be used to probe the redox state of the PQ pool under arbitrary illumination conditions. Furthermore, the results show that HL and far-red treatments can be used to poise the PQ pool of cyanobacteria to the reduced and oxidized conditions, respectively.

The physical identity of the non-photoactive PQ of cyanobacteria remains to be resolved. In plants, PQ is synthesized in the chloroplast [30,31] and non-photoactive PQ of plants is composed of PQ found in plastoglobuli and in the chloroplast envelope [18,32]. In cyanobacteria, alternative respiratory electron transfer functioning in the cytoplasmic membrane employs PQ-specific enzymes [33], strongly suggesting that cytoplasmic membrane contains non-photoactive PQ. Furthermore, electron micrographs showing the presence of lipid bodies resembling plastoglobuli [34], as well as protein homologs of plant plastoglobulins [35] in *Synechocystis* suggest that cyanobacteria might have plastoglobuli.

Cyanobacteria are often cultivated in an atmosphere containing a high percentage of CO₂. Growth of *Synechocystis* cells in high CO₂ increases the size of the phycobilisome antenna, and enhances the photosynthetic linear electron transfer reactions and the dark respiration [24]. Due to these physiological changes, the size of the photoactive PQ pool might be different in cells grown in high CO₂, compared to cells growth in AIR. We found that cells grown under 3% CO₂ contained 36.0 PQ molecules per 1000 Chls, and the photoactive pool comprised 47.9% of total PQ (Fig. 3) or 17.3 PQ/1000 Chl according to the DBMIB/DCMU method. The HL/FR method yielded a PQ pool size comprising 55.2% of total PQ (Fig. 3) or 19.9 PQ/1000 Chl. Thus, the ratio of the amount of photoactive

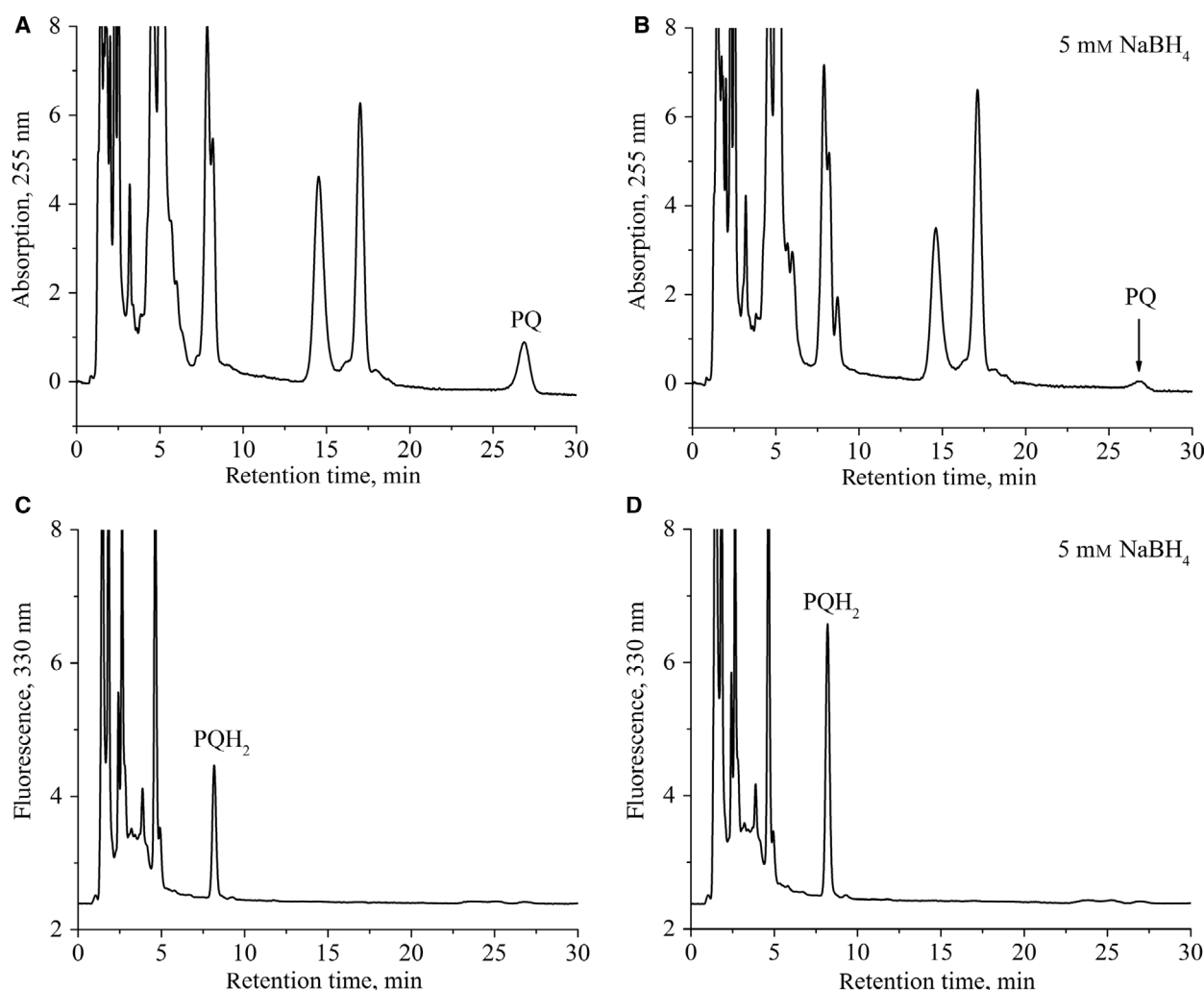


Fig. 2. Typical chromatograms. Absorbance at 255 nm, used for determination of the amount of PQ from the area of the peak at 26.9 min (A, B) and fluorescence at 330 nm, used for the determination of the amount of PQH₂ at 8.2 min (C, D). Chromatograms of the untreated samples (A, C) and in the presence of 5 mM NaBH₄ (B, D).

Table 1. Amount of PQ in *Synechocystis* sp. PCC6803 grown under AIR and under 3% CO₂, measured with HPLC. The amount of PQ was measured both after reduction by NaBH₄ with 330 nm fluorescence and after KOH-induced oxidation with 255 nm absorbance. The HPLC peak areas were related to absolute amounts by comparing with a PQ standard. The values represent the mean and SEM from four independent biological replicates.

Addition	PQ molecules per 1000 Chl <i>a</i> molecules	
	Air	3% CO ₂
5 mM NaBH ₄	26.45 ± 1.06	32.72 ± 0.31
2 mM KOH	29.32 ± 1.33	35.94 ± 0.8

PQ to the amount of Chl was 30–54% larger in cells grown in high CO₂ than in cells grown in air. This result may suggest an increase in the amount of PQ

per photosynthetic reaction centers but may also be explained by a lower PSI:PSII ratio at high CO₂.

The redox state of the PQ of the photoactive PQ pool in growth conditions and in the dark was calculated by comparing the ratio PQH₂/(PQH₂ + PQ) in the target samples to same ratio in control samples with fully reduced or oxidized PQ pool. At the studied moderate PPFD of 40 μmol·m⁻²·s⁻¹, a high concentration of CO₂ lowered the percentage of PQH₂ in the PQ pool from 45% in air to 14% in high CO₂ (Table 2; calculated using the control samples obtained with the DBMIB/DCMU treatments) or from 32% in air to 18% in high CO₂ (Table 2; calculated using the control samples obtained with the HL/FR light treatments). A small fraction of the PQ pool was found to remain reduced after two hours in the dark both under

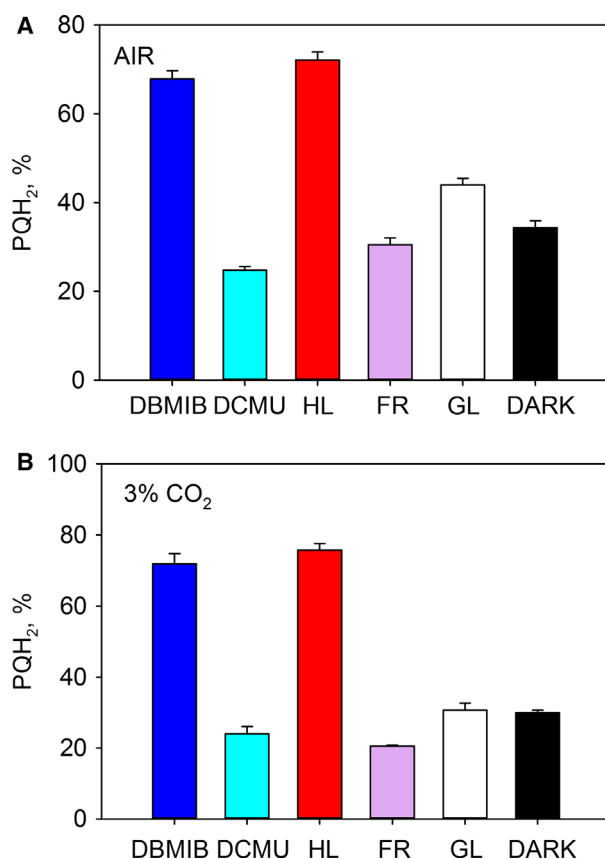


Fig. 3. The redox state of total PQ in cells of *Synechocystis* sp. PCC6803 under growth light (GL), darkness (DARK), during illumination with 710 nm FR light, during illumination in the presence of DCMU, during a HL pulse and during illumination in the presence of DBMIB. The cells were grown under AIR or under air supplemented with 3% CO₂, as indicated. Each bar represents an average of 3–11 independent biological replicates and the error bars show SEM.

air and under 3% CO₂ (Table 2). Interestingly, the PQ pool of *Synechocystis* appears to remain at a very similar reduction state in the dark as the PQ pool of plant chloroplasts, where approximately one fourth of PQ remains reduced in the dark [18,29]. A fraction of the PQ pool was found to remain reduced in the dark also in cyanobacteria [19].

Comparison of the control samples prepared with the HL pulse/FR treatment with those prepared with the DBMIB/DCMU method (Fig. 3) might suggest that DCMU treatment oxidizes a larger fraction of the PQ pool than 710-nm FR light and that a HL pulse reduces a larger fraction than DBMIB treatment. However, this comparison showed a statistically significant difference ($P = 0.011$) only for the comparison of DCMU and FR treatments of cyanobacteria grown under air, suggesting that the two methods for preparing the control samples are equivalent. Furthermore, the similarity of data obtained with the HL pulse/FR method and with the DBMIB/DCMU method data indicate that the side effects of DBMIB, including its ability to compete with 2,6-dichloro-*p*-benzoquinone for binding to the Q_B site of PSII [36], ability to slowly donate electrons to plastocyanin [37], or its long-term adverse effects [38] do not invalidate the efficiency of DBMIB in blocking oxidation of PQH₂ by cyt b₆f.

The cyanobacterial PQ pool was found to become oxidized under far-red light, in agreement with the expected behavior of the PQ pool in wavelengths favoring PSI. The result suggests that the redox state of the PQ pool responds to the balance of the electron transfer rates of the two photosystems in a fundamentally similar way in cyanobacteria as in plants. The result also suggests that the respiratory electron transfer mechanisms that both reduce and oxidize the PQ pool [5–11] do not rapidly respond to the HL and

Table 2. Percentage of PQH₂ in the PQ pool in *Synechocystis* sp. PCC6803 grown under AIR and under 3% CO₂, measured with HPLC. The control samples for fully reduced and fully oxidized PQ pool were obtained with the DBMIB/DCMU method or with the HL/FR light method, as indicated. The numbers in parentheses indicate the assumed full reduction and oxidation. The numbers represent an average \pm SEM estimate obtained by dividing the SEM of the experimental result by the fractional size of the PQ pool (0–1), as determined from the control samples. The number of independent biological replicates was 3–11.

Cells grown under:	Air		3% CO ₂	
	DBMIB	HL	DBMIB	HL
PQ pool reduced with:	DBMIB	HL	DBMIB	HL
PQ pool oxidized with:	DCMU	FR	DCMU	FR
PPFD 40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	44.6 \pm 3.5	32.4 \pm 3.6	13.9 \pm 4.1	18.2 \pm 3.5
Darkness	22.3 \pm 3.5	9.3 \pm 3.6	12.4 \pm 1.4	16.9 \pm 1.3
DBMIB, light	(100)	89.8 \pm 4.6	(100)	93.0 \pm 5.2
DCMU, light	(0)	–13.8 \pm 2.0	(0)	6.2 \pm 3.6
HL pulse	109.9 \pm 4.4	(100)	108.1 \pm 3.8	(100)
FR light	13.3 \pm 3.6	(0)	–7.1 \pm 0.5	(0)

far-red treatments used to induce reduction and oxidation of the PQ pool, respectively.

The oxidation of the PQ pool with far-red light contrasts with the conclusion of Schuurmans *et al.* [19] who suggested that the PQ pool is under metabolic regulation. One reason for the different results can be that we used far-red light to oxidize the PQ pool whereas a mixture of red and far-red was used by Schuurmans *et al.* [19]. However, it is possible that the methanol:petroleum ether extraction used by Schuurmans *et al.* [19] leads to oxidation of PQ during the extraction. The results of Kruk and Karpinski [18] demonstrate that extraction with ethyl acetate prevents both reduction of PQ by reductants present in plant cells and oxidation of PQH₂ during the analysis.

The method was also tested with another cyanobacterium, *Synechococcus* sp. PCC 7942. The cyanobacteria were grown in similar conditions as *Synechocystis*, under normal air. The DBMIB/DCMU method was used for the reduction and oxidation of the PQ pool. In *Synechococcus* sp. PCC 7942, only 23% of PQ was found to belong to the photoactive pool, and 60% of the PQ pool was reduced in the growth conditions. These data may suggest a permanent species-specific difference, as *Synechocystis* can grow both autotrophically and heterotrophically whereas *Synechococcus* sp. PCC 7942 is an obligate autotroph. Earlier analysis of Chl *a* fluorescence quenching in six cyanobacterial species suggested that the dark reduction state of the PQ pool varies between species [39]. However, it is also possible that the size and dark reduction state of the photoactive PQ pool depend on the metabolic state of the organism, and in *Synechococcus* sp. PCC 7942, similar growth conditions favored a smaller pool that is more reduced in the dark than in *Synechocystis*.

Conclusions

The method developed here for the measurement of the redox state of the PQ pool in cyanobacteria preserves the *in vivo* redox state of the PQ pool and allows the estimation of this important parameter in various conditions. The results show that a similar fraction of total PQ belongs to the photoactive pool in cyanobacteria as in plants and indicate that the PQ pool responds to light treatments in a fundamentally similar way in cyanobacteria as in plants.

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Author contributions

SK did the experiments with help from TTs, SK analyzed the data, ET and TTy designed the study, KT supervised TTs. TTy, ET and SK wrote the paper with contributions from all authors.

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