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Alternative photosynthetic electron flow to oxygen in marine *Synechococcus*

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Abstract

Cyanobacteria dominate the world's oceans where iron is often barely detectable. One manifestation of low iron adaptation in the oligotrophic marine environment is a decrease in levels of iron-rich photosynthetic components, including the reaction center of photosystem I and the cytochrome b_6f complex [R.F. Strzepek and P.J. Harrison, Photosynthetic architecture differs in coastal and oceanic diatoms, Nature 431 (2004) 689–692.]. These thylakoid membrane components have well characterised roles in linear and cyclic photosynthetic electron transport and their low abundance creates potential impediments to photosynthetic function. Here we show that the marine cyanobacterium *Synechococcus* WH8102 exhibits significant alternative electron flow to O_2, a potential adaptation to the low iron environment in oligotrophic oceans. This alternative electron flow appears to extract electrons from the intersystem electron transport chain, prior to photosystem I. Inhibitor studies demonstrate that a propyl gallate-sensitive oxidase mediates this flow of electrons to oxygen, which in turn alleviates excessive photosystem II excitation pressure that can often occur even at relatively low irradiance. These findings are also discussed in the context of satisfying the energetic requirements of the cell when photosystem I abundance is low.

Keywords: Cyanobacteria; Photosystem; Oxidase; Iron; Oxygen; Electron transport; Alternative electron transport; Synechococcus WH80102

1. Introduction

Synechococcus sp. dominate phytoplankton populations over much of the world's oceans and are important contributors to global primary productivity [2]. The availability of *Synechococcus* WH8102 in pure culture and the sequencing of its genome make this cyanobacterium an ideal model for integrating genomic, molecular and physiological information. Such studies are vital for addressing key issues in oceanic research [3]. We are particularly interested in physiological adaptations and acclimation responses of phytoplankton that enable them to survive in the oligotrophic oceans, where there is a scarcity of nutrients, particularly Fe (Fe^{2+}/Fe^{3+}).

Fe is an abundant component of the photosynthetic apparatus, and organisms that live in the nutrient-poor, open-ocean gyres must tailor cellular metabolism to this low Fe environment. Diatoms have adapted to low Fe levels by significantly lowering the cellular content of the Fe-rich photosynthetic electron transport components, which include cytochrome b_6f ($cytb_6f$) and photosystem I (PSI) [1]. Most cyanobacteria in terrestrial and freshwater environments maintain ratios of photosystem II (PSII):PSI that are below unity [4]. Having a low PSII:PSI ratio serves two fundamental functions for cyanobacteria. First, the major light-harvesting antenna, the phycobilisome (PBS), is

Abbreviations: $cytb_6f$, cytochrome b_6f; PSI, photosystem I; PSII, photosystem II; PBS, phycobilisome; PTOX, plastoquinol terminal oxidase; ETR, electron transport rate; PQ, plastoquinone/plastoquinol; Pgal, propyl gallate

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mostly associated with PSII, and light energy harvested by this complex drives photosynthetic electron flow from water to plastoquinone (PQ). Maintaining a low PSII:PSI ratio ensures that PSI turnover does not limit PSII electron flow, which is important for minimizing the potentially phototoxic effects that can result from excessive PSII excitation pressure [4]. Second, photosynthetic cyclic electron flow around PSI is critical for meeting the energetic and growth demands of cells by allowing for maintenance of an appropriate ATP:NADPH quotient in the light [5]. PSI is required for cyclic electron flow, which generates a trans-thylakoid H^+ gradient that is used for ATP synthesis. Therefore, low levels of PSI represent a significant obstacle to effective photosynthetic function.

Freshwater cyanobacteria respond to Fe depletion by lowering the relative abundance of PSI and forming an additional light harvesting antenna around the remaining PSI [6,7]. This light harvesting antenna, which is composed of the IsiA chlorophyll-binding protein, increases the absorption cross section and turnover of PSI, thereby alleviating potential problems caused by the decreased levels of PSI. Furthermore, moderate- and low-light adapted *Prochlorococcus* sp., cyanobacteria that dominate certain regions of the oligotrophic oceans, synthesize Pcb proteins in response to iron-deprivation which, like IsiA, serves as an antenna protein for PSI [8]. In contrast, based on full genome sequencing, the marine, open-ocean cyanobacterium *Synechococcus* WH8102 contains neither *isiA* nor *pcb* genes, making it likely that this organism has evolved other mechanisms for surviving the iron-poor oceanic environment, where PSI levels may be depleted.

One mechanism by which some marine cyanobacteria could avoid the potentially damaging consequences of having low PSI, in an environment in which excess excitation is common, would be to enlist pathways for electron transport that are independent of PSI. A number of alternative electron sinks operate upstream of PSI, some of which utilize O_2 as the terminal electron acceptor. These sinks include the plastoquinol terminal oxidase (PTOX) [9], cytochrome oxidase [10] and the alternative quinol oxidases [11].

Here we show that the model marine cyanobacterium, *Synechococcus* WH8102 compensates for low relative levels of PSI by enlisting O_2 as a major electron acceptor downstream of PSII, at the level of the intersystem electron transport chain. Based on inhibitor studies, the enzyme responsible for mediating this alternative electron flow is an oxidase with characteristics of PTOX.

2. Materials and methods

2.1. Growth conditions

Axenic cultures of *Synechococcus* sp. WH8102 were grown photoautotrophically on SN Medium [12] at 20 °C. Cultures were grown to mid-exponential growth phase and maintained under a diel light cycle described previously [13]. The peak light intensity for the simulated natural light cycle was 15 μmol quanta $m^{-2}s^{-1}$.

2.2. P700 assay

Total membranes were prepared according to England and Evans [14]. Chlorophyll *a* concentrations were estimated from the absorbance of methanol extracts at 665 nm [15]. P700 concentration was determined using a laboratory constructed split beam spectrophotometer as previously described [4]. Light-induced difference spectra of the reduced-minus-oxidized forms of the P700 reaction center of PSI were recorded in triplicate and the amplitude of the absorbance change at 700 nm following photooxidation of P700 was used to determine functional reaction centre concentration.

2.3. PE curves

Photosynthesis versus Irradiance (PE) relationships were determined using a modified ^{14}C-bicarbonate incorporation technique [16,17]. 24×2 ml subsamples were incubated for 1 h with ^{14}C-bicarbonate, at 24 °C, over a range of irradiances from 0–1000 μmol quanta $m^{-2}s^{-1}$. Incorporation was determined by measuring the radioactivity in the samples following their acidification with HCl to drive off all inorganic carbon that had not been fixed. Oxygen evolution was measured using a Clark-type electrode in the presence of 5 mM $NaHCO_3$ according to [18].

2.4. Fluorescence spectroscopy

Low temperature fluorescence emission spectra (77 K) were recorded with a single-beam fluorometer (Photon Technology International, New Brunswick, NJ). Samples were submerged in liquid nitrogen in state 1 following light adaptation. Excitation was provided at 435 nm (2.8 μmol quanta $m^{-2}s^{-1}$; bandwidth=5 nm) and fluorescence emission was measured at every 1 nm (bandwidth=1 nm) between 650 and 750 nm.

Pulse amplitude modulated fluorescence was recorded at the growth temperature of the culture using a water-PAM (Walz, Effeltrich, Germany). Samples were dark adapted in the sample chamber for a minimum of 10 min prior to all measurements. For anoxic conditions, cultures were vigorously bubbled with argon gas for 1 min in a laboratory-made, sealed cuvette. A further period of dark respiration was required for full anoxia to be reached. Propyl gallate dissolved in ethanol was added to a final concentration of 1 mM from a freshly prepared 100 mM stock solution.

The actual photochemical efficiency of PSII at any given actinic irradiance was calculated as Fm'-Fs/Fm' and represented as Y(II) according to the nomenclature of Walz.

2.5. Dual measurements of PSII and PSI photochemical efficiency

Simultaneous measurements of PSII and PSI photochemical efficiency were made with the Dual-PAM-100, P700 and chlorophyll fluorescence measuring system (Walz, Effeltrich, Germany). PSII photochemical efficiency was measured essentially as described above. PSI photochemical efficiency (YI) was determined by monitoring the oxidized form of PSI reaction center chlorophyll, P700, using the absorbance peak at 830 nm. Y(I) was calculated according to [19].

2.6. P700 oxidation reduction kinetics

P700 redox changes were measured with a LED-based spectrophotometer (JTS 10, Biologic, France) described elsewhere [20]. Continuous light was provided by a green LED array (emission peak 520 nm, 30 nm full width at half maximum) delivering up to 450 μmol quanta $m^{-2}s^{-1}$. Measuring flashes were provided by red LED (Luxeon, Lumileds, USA), filtered at 705 nm (10 nm full width at half maximum). The time resolution of the instrument was 10 μs.

3. Results

3.1. The marine cyanobacterium Synechococcus WH8102 has low levels of PSI

A decrease in the level of PSI as a potential adaptation to low Fe appears to occur for *Synechococcus* WH8102. As shown in Fig. 1A, a low temperature fluorescence emission spectrum,

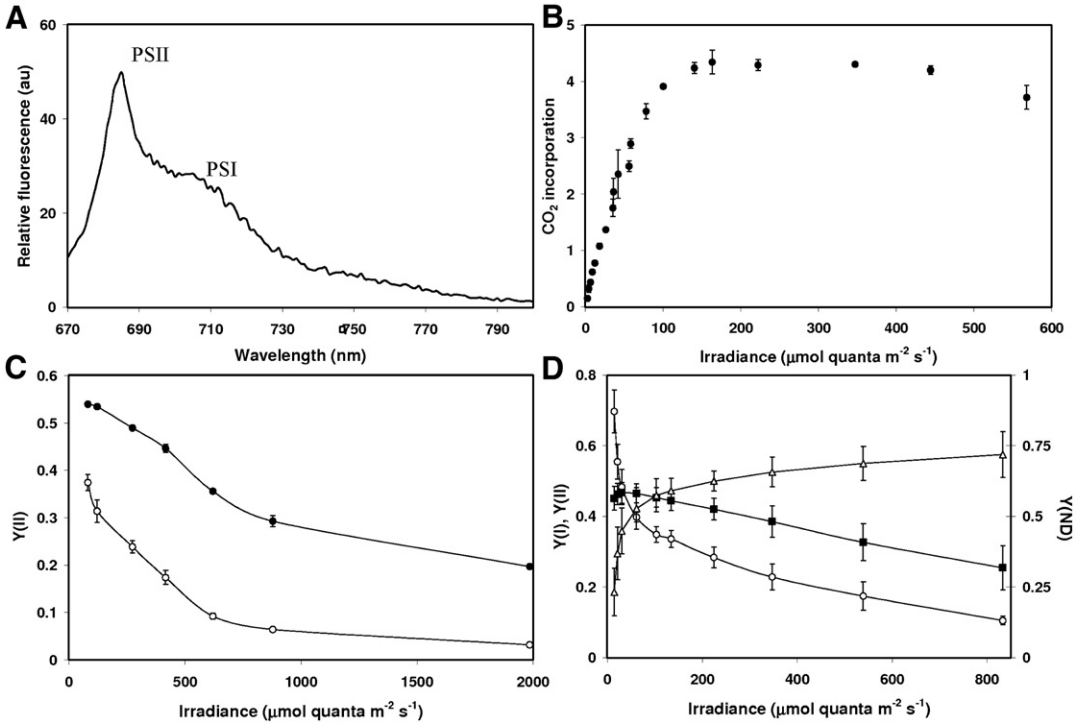

Fig. 1. (A) 77 K fluorescence emission spectra were recorded for light-adapted cells. The excitation wavelength was 435 nm and emission was scanned from 650 nm to 750 nm. PSII emission peak is at 685 nm while PSI emission forms a broad shoulder from 705 nm to 725 nm. (B) PE curves were generated to show the light dependence of $^{14}CO_2$ fixation expressed as incorporation of μgC per μg Chl per hour. A photosynthetron was used to generate a light gradient and the temperature was controlled at 24 °C. (C) PSII photochemical efficiency (Y(II)) was measured using pulse amplitude modulated (PAM) fluorometry and calculated as Fm'-Fs/Fm'. Closed circles are untreated cells, open circles are treated with 1 mM pgal. (D) Simultaneous measurements of PSII and PSI photochemical efficiency (Y(II) and Y(I) respectively) were performed using a dual PAM 100 (Walz, Effeltrich, Germany). Closed squares are Y(II), open circles are Y(I), open triangles are Y(ND), which represents donor side limitation of PSI. All measurements were performed in triplicate (±s.e.), with the exception of the PE curve (B), which was performed in duplicate (±s.d.).

with excitation at 435 nm, has unusually low emission associated with PSI relative to PSII. This may be an indication of low PSI content relative to PSII. However, it is possible for the long wavelength PSI fluorescence emitters to be absent, despite high levels of PSI [21]. In addition, the PSII in Fig. 1A only displays fluorescence at 685 nm and lacks the 695 nm fluorescence peak typically associated with PSII fluorescence at 77 K. Therefore we cannot rule out the possibility that the fluorescence peak at 685 nm contains a contribution from a source other than PSII. To gain a more direct measurement of PSI content, we used the light-induced difference change of the PSI reaction centre P700 (ΔA_{700}) in isolated thylakoids of *Synechococcus* WH8102. We determined a chlorophyll to P700 ratio of 319 for *Synechococcus* WH8102 (Table 1), compared to values for freshwater cyanobacteria of 139.86 (*Synechocystis* PCC6803) and 160.44 (*Anabaena* PCC7120), measured using the same spectroscopic technique. The chlorophyll to P700 values for the freshwater cyanobacteria are also consistent with previous measurements [4]. This result suggests that the relative abundance of PSI in *Synechococcus* WH8102 is unusually low, even though the medium that we used to culture the cyanobacterium had a relatively high Fe concentration.

3.2. Synechococcus WH8102 uses alternative electron sinks

To assess the potential for alternative electron transport upstream of PSI as a mechanism for coping with low PSI abundance, we employed a combination of measurements designed to assess PSII and PSI photochemical efficiency, along with the cell's capacity for CO_2 incorporation under different intensities of illumination.

Evidence for an alternative pathway for electron flow in *Synechococcus* WH8102 is observed when comparing light response curves for CO_2 fixation (Fig. 1B) with the photochemical efficiency of PSII (Y(II) (Fig. 1C). The PE curve presented in Fig. 1B represents linear electron transport from water to CO_2, while the light response curve for Y(II) that is shown in Fig. 1C represents electron flow from water to all available electron sinks. While CO_2 incorporation saturates at relatively low-light (~150 μmol quanta $m^{-2}s^{-1}$), Y(II) remains

Table 1
Chlorophyll to P700 ratios for marine and freshwater cyanobacteria

Strain	Chlorophyll/P700
Synechococcus WH8102	319
Synechocystis PCC 6803	139.86
Anabaena PCC 7120	160.44

P700 content was measured on isolated thylakoids using the light-induced, reduced-minus-oxidized difference spectrum at λ700 nm. Thylakoid membranes were partially solubilised in 0.01–0.02% SDS and measurements were recorded in the presence of 2 mM ascorbate and 100 μM methyl viologen. To ensure full reduction of P700 in the dark, samples are allowed to equilibrate in the reaction mixture for 1–2 min in the dark, followed by continuous saturating illumination to fully oxidize P700.

high even at the highest irradiance used (1985 μmol quanta $m^{-2}s^{-1}$), suggesting continuation of PSII electron transport at light levels that saturate CO_2 fixation. This discrepancy between CO_2 fixation and PSII photochemistry can best be explained by significant alternative sinks that accept electrons downstream of PSII (but prior to the consumption of electrons in CO_2 fixation).

As shown in Fig. 1C, the addition of 1 mM pgal led to large decreases in Y(II) at all irradiance levels, relative to untreated cells, suggesting a role for a quinol oxidase, possibly PTOX, in alternative electron flow. Inhibitors of other terminal oxidases, including KCN, azide (cytochrome oxidase) and salicylhydroxamic acid (alternative oxidase) had only small effects on Y (II) (data not shown), further implicating PTOX in alternative electron transport in *Synechococcus* WH8102.

To determine whether electrons originating from PSII photochemistry feed alternative electron sinks upstream of PSI, simultaneous measurements of PSII and PSI photochemistry were conducted. As shown in Fig. 1D, Y(I) decreases rapidly with increasing irradiance while, at the same time, Y(II) remains high, suggesting that PSII electron transport remains high as electron transport through PSI is decreasing. Decreases in PSI photochemical efficiency may result from either donor side limitation, with P700 remaining oxidized, or acceptor side limitation, when the acceptor side of PSI remains reduced. P700 measurements taken with the dual PAM can distinguish between acceptor side (Y(NA)) and donor side (Y(ND)) limitation of PSI. While acceptor side limitation remained low across the entire range of irradiance levels used (data not shown), PSI becomes significantly donor side limited (increasing YND)) at very low irradiance levels (Fig. 1D), despite Y(II) remaining high. These results suggest that PSI is deprived of electrons even though PSII is still performing photochemistry. Hence, electrons originating from PSII must be extracted from the intersystem electron transport chain (the electron transport carriers that separate PSI from PSII).

3.3. Alternative electron transport operates upstream of PSI

To confirm that the alternative electron transport pathway extracted electrons upstream of PSI, we monitored P700 redox kinetics following exposure to a brief period of actinic irradiance in the absence and presence of pgal. If a pgal-sensitive oxidase functioned to outcompete P700 for electrons originating from PSII, then P700 should become more reduced when the cells are exposed to actinic light in the presence of pgal (compared to in the absence of pgal). P700 kinetics were monitored spectroscopically using the 700 nm absorption band associated with P700 in its oxidized form. As shown in Fig. 2A, in the absence of pgal P700 becomes quickly oxidized immediately following the onset of low actinic irradiance (50 μmol quanta $m^{-2}s^{-1}$). Following a very slight re-reduction, P700 remains oxidized throughout the light treatment, becoming fully reduced immediately after the actinic light is turned off. In contrast, in the presence of pgal, P700 becomes quickly oxidized immediately following exposure to actinic light and then re-reduces over the course of the light treatment.

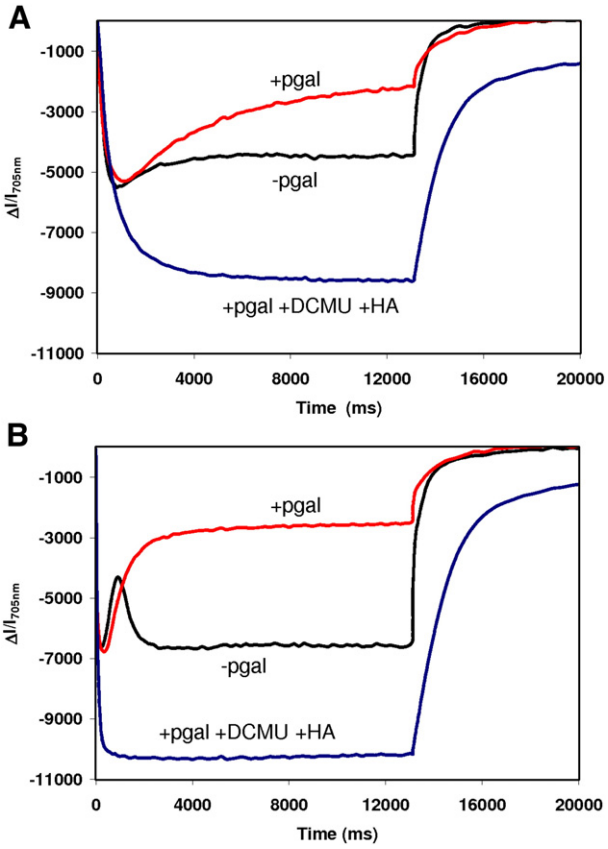

Fig. 2. P700 redox changes were measured during actinic light treatment. Continuous light was provided by a green LED array (emission peak 520 nm) at (A) 50 μmol quanta $m^{-2}s^{-1}$ and (B) 450 μmol quanta $m^{-2}s^{-1}$. In Figures. A and B black lines represent untreated samples, red lines represent samples treated with 1 mM pgal and blue lines represent samples treated with 1 mM pgal, 20 μM DCMU and 1 mM hydroxylamine.

The effect of pgal on the redox state of P700 during exposure to moderately high actinic light (450 μmol quanta $m^{-2}s^{-1}$), was even more dramatic (Fig. 2B). In the absence of pgal P700 oxidizes very quickly following the onset of actinic light. After 150–200 ms P700 starts to re-reduce. However, this re-reduction is reversed approximately 500 ms later and P700 is quickly re-oxidized. In contrast, in the presence of pgal during exposure of the cells to moderately high actinic light, P700 initially follows the same pattern of oxidation and re-reduction observed for the untreated sample. However, the re-oxidation observed for the untreated sample after 700–800 ms in the light is eliminated when pgal is present during the light exposure, and P700 continues to become reduced, reaching a steady state after a further 200 ms. These data have also been replicated using the 830 nm absorption band associated with oxidized P700 (data not shown). This ability to re-reduce P700 following pgal treatment clearly indicates that a pgal-sensitive electron valve upstream of PSI is able to compete with P700 for electrons.

To test whether PSII was the source of the electron flow for P700 reduction, in pgal treated cells, we used DCMU and hydroxylamine to inhibit PSII function, in addition to pgal. We then monitored P700 redox kinetics using the same actinic light

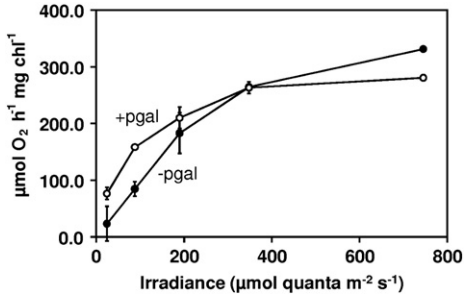

Fig. 3. Oxygen evolution was measured in the absence (closed circles) and presence of 1 mM pgal (open circles). Measurements were recorded in duplicate (±s.d.) at room temperature in the presence of 5 mM sodium bicarbonate.

treatments (Fig. 2A and B) as described above. In the absence of PSII electron flow (DCMU and hydroxylamine), P700 became oxidized following exposure to both actinic irradiance levels and remained oxidized throughout the light treatment. This result suggested that the electrons involved in the partial re-reduction of P700 were derived from PSII and that the pgal-sensitive pathway for electron flow competes with P700 for PSII-derived electrons. The result was identical whether DBMIB, which inhibits the reduction of the cytb$_6$f complex by PQ, was included in the reaction (data not shown). Furthermore, the finding that P700 becomes reduced during actinic light treatment in the presence of pgal alone suggested that the inhibitor had negligible effects on linear electron flow from water to P700.

If P700 is more readily reduced in the presence of pgal than in its absence, pgal should accelerate linear electron flow from water to the acceptor side of PSI. To test this we monitored the relationship between increasing actinic light treatment and oxygen evolution in the presence and absence of pgal. As shown in Fig. 3, at light-limiting irradiances the rate of oxygen evolution appeared to be significantly higher in the presence of pgal, compared to cells not exposed to the inhibitor. These results suggest that the pgal-dependent increase in P700 reduction results in increased linear electron transport. However, oxygen evolution rates saturated at similar levels in both untreated and treated samples, presumably because electron flow on the acceptor side of PSI ultimately became limiting.

3.4. Oxygen acts as a terminal electron acceptor

To determine whether O_2 accepts electrons from the photosynthetic electron transport chain of *Synechococcus* WH8102, O_2 was removed from cultures by purging them with argon gas and PAM fluorescence measurements were recorded following light induction. Since the culture was supplemented with sodium bicarbonate (see Materials and methods), an electron acceptor at the level of CO_2 was still available. Under oxic conditions, continuous actinic light resulted in a slow rise in fluorescence until a new higher steady state was established, as shown in Fig. 4. This light-dependent rise in fluorescence has been ascribed to a state 2 (dark) to state 1 (light) transition [22]. In cyanobacteria, state transitions involve the coupling of PBS antenna to PSII [23], and probably reflect the redistribution of PBS between PSII and PSI; in state 2 a mobile portion of the PBS antenna may be associated with PSI while in state 1 it is predominantly associated with PSII. However, a poorly defined chlorophyll component, often termed spillover, is also a constituent of the state transition [24]. State transitions are regulated by the redox state of the PQ pool [25]. In the dark, the PQ pool in most cyanobacteria is reduced via a thylakoid-associated respiratory electron transport pathway [22]. A reduced PQ pool favors state 2, in which the PBS is dissociated from PSII, and perhaps becomes associated with PSI, resulting in low PAM fluorescence levels. Upon illumination, the PQ pool becomes oxidized, a consequence of extraction of electrons from PQ by the operation of PSI and any other electron outlet. The accumulation of oxidized PQ stimulates a transition to state 1 in which the PBS becomes coupled to PSII. In state 1, the fluorescence yield is higher than in state 2, which explains the light-dependent rise in maximal fluorescence apparent in Fig. 4. Furthermore, as seen from the variable fluorescence during the saturating pulse at the end of the actinic light period, when the cells are in state 1, a large proportion of the PSII reaction centers are still opened, even though the light level is nearly 2000 µmol quanta $m^{-2}s^{-1}$.

In dramatic contrast, Fig. 5A shows that anoxia causes an extremely rapid, light-dependent decrease in fluorescence upon exposure of the cells to 1985 µmol quanta $m^{-2}s^{-1}$. This decline is similar in character to non-photochemical quenching (NPQ) that has been described for iron-starved freshwater cyanobacteria [26,27], although this decrease in fluorescence yield may also reflect an initial, rapid light-dependent shift toward state 2. This quenching was quickly reversed when anoxic cells were allowed to recover in the dark. In addition to the light-induced fluorescence quenching, the fluorescence yield (variable fluorescence) of cells in actinic light under anoxic conditions following a saturating light pulse was markedly decreased (Fig. 5A, second saturating pulse from the left and marked with

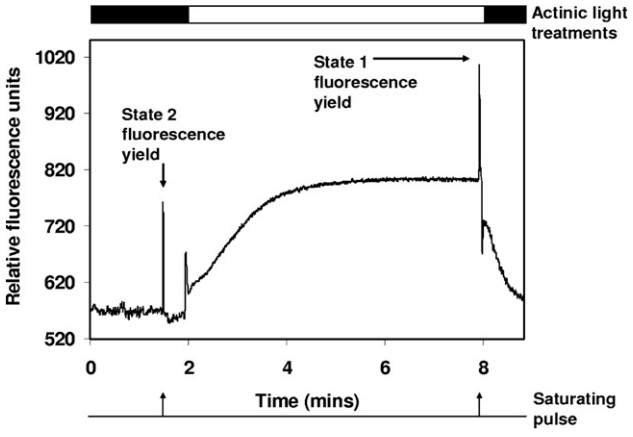

Fig. 4. Photosynthetic electron transport and state transitions were monitored using PAM fluorescence. The actinic light treatment was at 1985 µmol quanta $m^{-2}s^{-1}$. The fluorescence was monitored under ambient O_2 tension. Saturating pulses of light of 800 ms duration, marked with arrows prior to the imposition of actinic light (1.5 min) and just before terminating the actinic light (8 min), were at ~6,000 µmol quanta $m^{-2}s^{-1}$.

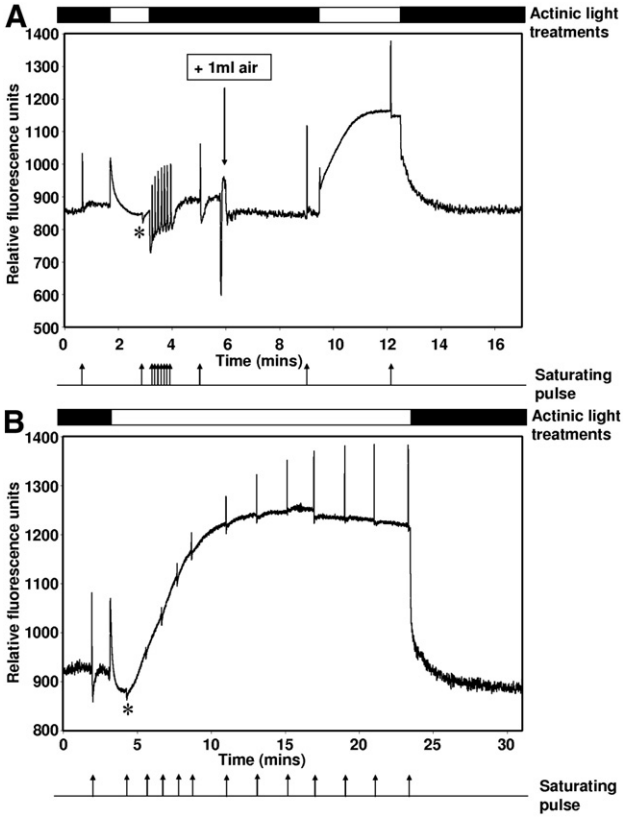

Fig. 5. Photosynthetic electron flow and state transitions were monitored using PAM fluorescence in the absence of O_2. In (A) and (B) the fluorescence was monitored under anoxic conditions after purging the cell suspensions with argon gas for 1 min, which was delivered via a needle through the rubber stopper of a home made cuvette. The rubber stopper was made air-tight with silica gel following removal of the needle. A further 5–10 min of dark respiration was required to achieve full anoxia, which was necessary to observe the characteristic light-dependent quenching of fluorescence. O_2 was re-introduced into the cell suspension through delivery of 1 ml of air (indicated at +1 ml air) using a needle and syringe (A). In (B), the cuvette remained sealed throughout the recording of the fluorescence trace. The asterisk in both (A) and (B) highlights the saturating light pulse during actinic irradiance, showing no variable fluorescence early after exposing the cells to light.

an asterisk), indicating the closure of PSII reaction centers. Like the relaxation of the fluorescence quenching, variable fluorescence also quickly recovered in the dark.

The introduction of O_2 to cells maintained under anoxic conditions in the dark quickly and fully reverses the fluorescence profile of *Synechococcus* WH8102 to that of pre-anoxic cells. Fig. 5A shows that briefly bubbling an anoxic culture with air (indicated as +1 ml air) restores variable fluorescence and the light-dependent increase in fluorescence; the profile is comparable to that shown in Fig. 4. Furthermore, if the cuvette is kept sealed under anoxic conditions, light treatment initially induces quenching, but after O_2 begins to accumulate in the cuvette as a consequence of PSII activity, presumably involving a relatively minor electron flow to CO_2, the PQ pool becomes oxidized through the activity of a terminal oxidase and this in turn could trigger a state transition and an associated rise in fluorescence, as observed in Fig. 5B. The escalating O_2 tension and the re-opening of PSII traps as a consequence of oxidase activity also allows for recovery of variable fluorescence in the light (observed between 10 and 25 min in actinic light). This recovery of variable fluorescence and the increase in steady state fluorescence are significantly inhibited by the presence of pgal (data not shown). Again, these data demonstrate that O_2 is a major terminal electron acceptor in marine cyanobacterial photosynthesis and that it controls the redox state of the PQ pool.

4. Discussion

The data presented here demonstrate that in open-ocean *Synechococcus*, O_2 can act as a major electron acceptor for photosynthetic electron transport prior to PSI. It appears that electrons are removed from the intersystem photosynthetic electron transport chain by an oxidase. We can find no evidence, using various inhibitors, for the involvement of cytochrome oxidase or the SHAM-sensitive alternative oxidase in this alternative electron flow. However, an oxidase sensitive to pgal, possibly PTOX, appeared to be highly active in extracting electrons from the intersystem electron transport system and combining them with H^+ and O_2 to generate water. While there is evidence for a minor role for PTOX in alternative photosynthetic electron flow in the higher plant species *Ranunculus glacialis* [9], the major role for PTOX is thought to be in carotenoid desaturation (reviewed in [28]). No previous biochemical data concerning PTOX activity in marine cyanobacteria has been presented. However there is strong evidence from genomic sequences for its existence in organisms that inhabit the oligotrophic oceans. Extensive sequence analysis of recombinant DNA libraries generated from cell samples collected from the Sargasso Sea revealed the presence of numerous potential PTOX genes [29,30]. In addition, genes encoding PTOX homologues were identified on the complete genomes of three marine cyanobacteria, including *Synechococcus* WH8102 [31].

In contrast to an apparent low PTOX activity in eukaryotic phototrophs, the data presented here strongly suggest that PTOX, or another pgal-sensitive oxidase, functions in marine cyanobacteria to maintain PSII in a highly oxidized state across a range of physiological irradiance levels. This oxidase activity would be critical when electron transport becomes limited by PSI activity and would maintain a highly oxidized pool of PSII when the cells experience high irradiance levels during the dramatic fluctuations in prevailing open-ocean light conditions.

Together, the data presented here suggest that high levels of electron flow to O_2 may reflect environmental conditions encountered by *Synechococcus* species in the iron-poor, oligotrophic oceans. These organisms are mostly confined to the turbulent upper layers of the water column in which very large changes in irradiance occur over relatively short (seconds) timescales due to intermittent cloud cover and the generation of caustics following the focusing of light by surface waves [32]. Utilizing oxidases such as PTOX as a highly active, integral component of electron transport has clear photoprotective advantages for organisms that must live in chronic, low Fe high light environments. In eukaryotic phototrophs, PTOX contains

two iron atoms per molecule [28]. Cytb$_6$f and PSI, on the other hand, contain 6 and 12 atoms of Fe per complex, respectively [1]. Hence, co-opting PTOX (and/or other oxidases) for alternative electron flow could dramatically decrease the cellular Fe requirement by allowing for a significant reduction in PSI levels and possibly cytb$_6$f levels, depending on the location of the oxidase in the electron transport chain, without augmenting photodamage that would result from increased PSII excitation pressure. Furthermore, in addition to protecting PSII by promoting linear electron flow, cytb$_6$f and PSI also increase the proton motive force by promoting the Q-cycle (via cytb$_6$f) and PSI electron cycling, ensuring the establishment of a large *trans*-thylakoid ΔpH and the generation of proper ATP/NADPH levels. Satisfying the requirements of the cell for ATP is vital for survival of oxygenic phototrophs [5].

These considerations raise the question of how the ATP:NADPH quotient is maintained in an organism with low relative amounts of PSI. The answer may also depend upon the role of PTOX (or another oxidase) in creating a water-to-water pseudocycle of electrons around PSII. While this cycle does not generate reductant, theoretical considerations suggest that it could conserve energy in the form of a substantial trans-thylakoid ΔpH. In eukaryotes, PTOX is associated with the stromal (cytoplasmic equivalent in bacteria) face of the thylakoid membrane [28]. The splitting of water by PSII releases H^+ into the thylakoid lumen while electrons extracted from water can be used by PTOX to regenerate water through the reduction of O_2 and the consumption of H^+ ($4H^+ + O_2 + 4e^-$ yield 2 H_2O) in the cytoplasm of the cell. Therefore the combination of PSII water splitting in the lumen and oxidase-mediated H^+-consuming activity in the cytoplasm could generate a large ΔpH, which may, at least in part, serve a similar role in proton motive force generation as the Q-cycle and PSI cyclic electron transport, and at the same time require a greatly reduced Fe budget.

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