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Investigating oxygen evolution in the absence of photosystem I in the cyanobacterium *Synechocystis* 6803

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Introduction

The photosynthetic Z-scheme describes the pathway of linear electron transport from H_2O to $NADP^+$ in higher plants, algae and cyanobacteria. The pathway requires the photosystem II (PSII) and photosystem I (PSI) reaction center complexes, the cytochrome bf complex, the peripheral protein ferredoxin-$NADP^+$-oxidoreductase, and the mobile electron carriers plastoquinone, plastocyanin (or cytochrome c_6), and ferredoxin. Although the Z-scheme serves as the dominant pathway for linear electron transport in the light reactions of photosynthesis, there have been numerous observations indicating alternative electron transport pathways in which PSII can support electron transport in the absence of PSI (*e.g.*, Smart et al., 1991; Arnon, 1995; Greenbaum *et al*., 1995; Bennoun, 1998; Cournac *et al*., 2000). Although some of the early observations depended on mutants that were not truly free of PSI (*e.g.*, Greenbaum *et al*., 1995), several true PSI deletion mutants have been shown to evolve oxygen (*e.g.*, Smart *et al.*, 1991). Here we report our progress investigating electron transport from H_2O to as yet unknown electron acceptors in cells of *Synechocystis* 6803 that completely lack the PSI reaction center.

Materials and methods

Photosystem I deletion mutants of *Synechocystis* 6803 created by deleting part of the *psaAB* operon were kind gifts from Drs. W. Vermaas and H. Pakrasi. WT cells were grown in BG11 medium in the presence of 5 mM glucose at 30 ^{0}C under 50 μE m^{-2} s^{-1} fluorescence light. The ΔPSI mutant strains were grown in the presence of 5 mM glucose at 30 ^{0}C under 1~3 μE m^{-2} s^{-1} fluorescence light. Rates of O_2 evolution and uptake were measured by a Clark-type electrode using whole cells at 30 ^{0}C (Mannan et al., 1991). The rate of O_2 evolution data given in Table 1 were corrected for respiration by assuming the rate of respiration was the same in the dark as in the light. The rate of dark respiration was less than 60% of the rate of light driven O_2 evolution, so cells exhibited net oxygen evolution in the light. Hydrogen evolution and uptake were measured using a Clark type electrode (Wang, 1980). Flash induced P700 redox changes were measured using a kinetic spectrophotometer (Mannan *et al*., 1996). The concentration of O_2 evolving PSII centers was determined by measuring O_2 evolution induced by single turnover flashes (Nedbal *et al*., 1991). Rates of CO_2 fixation were measured using ^{14}C-labeled bicarbonate as described by Eckardt *et al.* (1997).

Results

ΔPSI Mutants have no detectable P700 activity. The absence of PSI in the ΔPSI mutant strains was confirmed by 77 K fluorescence emission and western blot using antibodies raised against the PsaA and PsaB proteins (Shen *et al*., 1993; H. Pakrasi, personal communication 1999). To verify that there was no active PSI centers we measured P700 oxidation and re-reduction kinetics in thylakoid membranes prepared from ΔPSI mutant cells (data not shown). To determine the detection limit of our instrument, we added a small fraction of WT membranes to ΔPSI membranes. The data demonstrate that the concentration of active PSI in the ΔPSI mutant is less than 1% of the WT cells.

ΔPSI mutants exhibit a net O_2 evolution. The ΔPSI mutants sustain steady state rates of DCMU-sensitive, net O_2 evolution for more than an hour (Fig. 1) at a rate comparable to WT cells (Table 1). In the presence artificial PSII electron acceptors (FeCN & DCBQ) the WT and ΔPSI mutant cells exhibit the similar rates of O_2 evolution (data not shown).

Table 1. Comparison of O_2 evolution rates of *Synechocystis* 6803 WT and ΔPSI cells. O_2 evolution rates were measured in BG11 medium at 30 ^{0}C. WT and ΔPSI mutant were grown in the presence of 5 mM glucose.

Whole Cells of *Synechocystis* 6803	WT	ΔPSI
Chlorophyll : PSII ratio	490 ± 25	78 ± 4
O2 evolution rate (mmol O_2/mol chl s)	78 ± 10	130 ± 34
O_2 evolution rate (mol O_2/mol PSII.s)	38 ± 5	10 ± 3
Light intensity giving 50% maximal O_2 evolution rate w/o additions ($\mu E/m^2$ s)	150	100

Oxygen evolution in ΔPSI cells is insensitive to inhibition of the cytochrome bf complex by DBMIB. Oxygen evolution in WT cells is inhibited by DBMIB (I_{50} = 1 μM), which binds to the cytochrome bf complex. In contrast, O_2 evolution in the ΔPSI mutant is relatively insensitive (I_{50} = 10-20 μM). The inhibition observed at high concentrations of DBMIB is due to direct inhibition of PSII at the Q_B site. The relative insensitivity of the ΔPSI cells to DBMIB indicates that the cytochrome bf complex is not involved in electron transport from H_2O. This observation, together with chlorophyll fluorescence data showing that the plastoquinone pool is reduced in the light, indicates that an unidentified enzyme that oxidizes plastoquinol is involved in the O_2 evolution pathway in the ΔPSI cells.

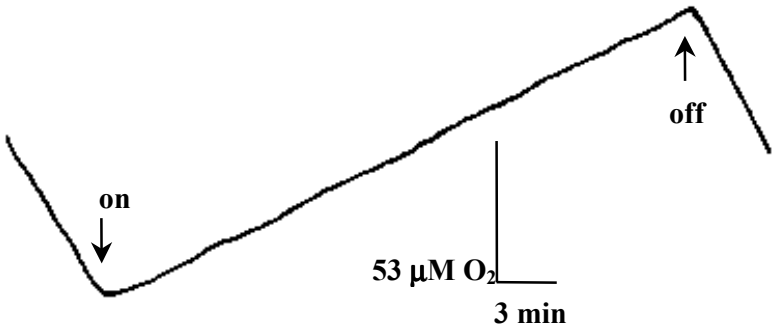


Fig. 1. O_2 evolution of *Synechocystis* 6803 cells lacking photosystem I (ΔPSI). O_2 evolution was measured in BG11 at 30 ^{0}C at a chlorophyll concentration of 1.3 μM.

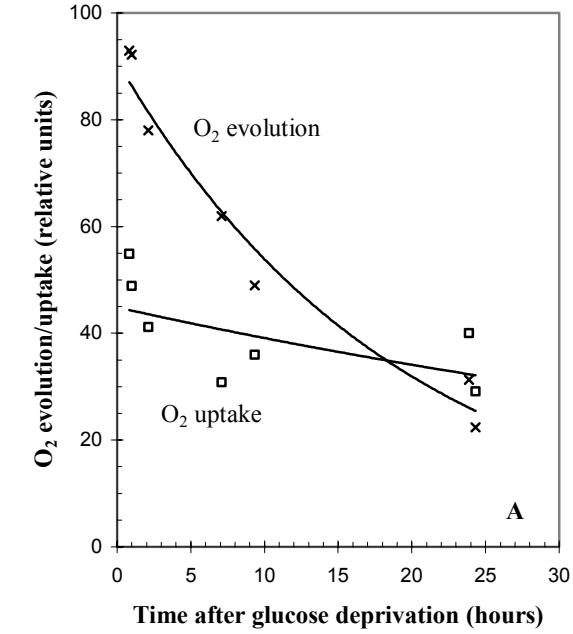
Oxygen evolution in ΔPSI cells is inhibited by KCN. Oxygen evolution in ΔPSI cells is highly sensitive to KCN (I_{50} ~ 5 μM), indicating that one of the enzymes in the electron transport pathway binds cyanide. Oxygen evolution in WT cells is relatively insensitive to KCN (I_{50} = 50 μM).

O_2 is not the terminal electron acceptor in the O_2 evolution pathway in ΔPSI cells. Although in thylakoid membranes O_2 molecules can be an efficient electron acceptor via the Mehler reaction or chlororespiration (Bennoun, 1998), neither of these reactions can support a net O_2 evolution as shown in Fig. 1.

Hydrogen ions are not the terminal electron acceptor in the O_2 evolution pathway in ΔPSI cells. Under some condition algae and cyanobacteria can produce H_2 gas by reducing protons. We tested for this possibility in the ΔPSI cells and found no light driven H_2 evolution (data not shown).

ΔPSI cells fix CO_2. Isotope studies using $NaH^{14}CO_3$ show that ΔPSI mutants fix CO_2 upon illumination in a DCMU-sensitive pathway (data not shown). However, simultaneous measurements of O_2 evolution and CO_2 fixation show that only 20% of the electrons removed from H_2O are used to reduce CO_2, leaving 80% of the electrons unaccounted for.

Glucose is required for O_2 in ΔPSI cells. The rate of O_2 evolution and respiration in ΔPSI cells decreases as the cells are deprived of glucose. Addition of glucose to the reaction medium restores O_2 evolution and respiration within two minutes (Fig. 2). This observation raises the possibility that intermediates of the Krebs cycle may served as electron acceptors in the ΔPSI O_2 evolving pathway. Interestingly, we cannot detect similar activity in WT cells. We are currently using chromatographic techniques to identify carbon compounds that may be serving as intermediate or terminal electron acceptors.



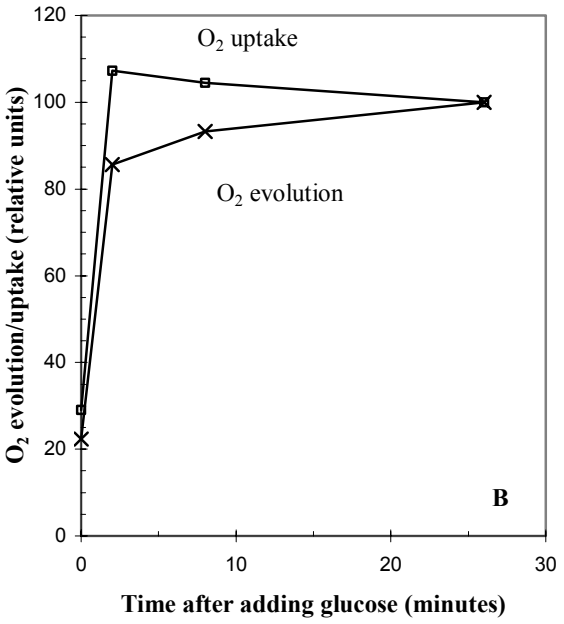


Fig. 2. (A) O_2 evolution and uptake rates of ΔPSI mutant cells resuspended in medium lacking glucose. **(B)** Recovery of O_2 evolution and uptake upon the addition of glucose to glucose-starved ΔPSI mutant cells.

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