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Analysis of PSI and PSII driven electron transport during steady-state photosynthesis in the cyanobacterium *Synechococcus* PCC7942

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Introduction

The inorganic carbon concentrating mechanism (CCM) of cyanobacteria is supported by the active, inward transport of CO_2 and HCO_3^-. Intracellular concentrations of Ci greater than 1000 times those in the external medium, resultant from the activity of the CCM, ensure that CO_2 fixation is saturated (Badger and Gallagher 1987). The energisation of CO_2 and HCO_3^- transport in cyanobacteria is thought to be dependent upon the flow of electrons from PSII to PSI. It was shown in *Synechocystis* PCC 6803 that NDH-1 complexes can participate in cyclic electron transport around PSI via the re-reduction of plastoquinone and that these complexes were involved in some way in CO_2 uptake (Mi *et al.* 1992, Ogawa 1991). This prompted some to speculate that CO_2 uptake was coupled to cyclic electron transport by a 'CA-like' conversion of CO_2 to HCO_3^- mediated by a specialised NDH-1 complex (Kaplan and Reinhold 1999). In the same organism NDH-1 complexes containing NDHD3 or NDHD4 subunits were shown to be necessary for CO_2 transport. A double mutant (NDHD3-/NDHD4-) which had no CO_2 uptake showed normal P700+ oxidation kinetics suggesting that cyclic electron transport might not participate directly in CO_2 uptake (Ohkawa *et al*. 2000). The present study was undertaken to provide insight into the nature of photosynthetic electron flux in the cyanobacterium, *Synechococcus* PCC 7942, under conditions where photosynthesis was driven primarily by CO_2 or HCO_3^- transport to delineate the contributions of linear and cyclic electron flows in the energisation of these Ci fluxes. A novel methodology for the combined measurement of the quantum yields of PSI and PSII and photosynthetic gas exchange is described.

Materials and Methods

Growth and Preparation of Cells

The freshwater, unicellular cyanobacterium *Synechococcus* PCC 7942 (R2) was grown continuously under photoautotrophic conditions at 70 µmol photons . m^{-2} . s^{-1} in liquid media as described elsewhere (Sültemeyer *et al*., 1998). The cultures were bubbled with air enriched with 2 % CO_2. Prior to experiments, the cultures were bubbled vigorously for 14 hours with air containing 20 ppm CO_2 to induce the CO_2 concentrating mechanism. Preparation of cells for experiments was as described elsewhere (Sültemeyer *et al*. 1998). The cells were resuspended in the assay medium (BG-11, pH 7.8) to a concentration of between 100-250 µg Chl a . mL^{-1} and stored in the dark on the bench for a period of no more than 2 hours.

Chlorophyll a Fluorescence and P700 Measurements

Changes in P700 absorbance were monitored using a modulated PAM flourometer equipped with an EDP700-DW emitter-detector unit (Walz, Effeltrich). Fluorescence was monitored simultaneously with a custom-built emitter-detector unit coupled to a PAM fluorometer. Assays (2 mL) of the quantum yields of photosystems I and II were made according to the protocol of Klughammer and Schreiber (1994). For light response experiments shown in Fig. 1, cells were supplemented with 2 mM $NaHCO_3$ prior to illumination with actinic light and actinic light intensity was varied with the use of neutral density filters. In some experiments the fluorescence and P700 measuring systems were used in conjunction with a membrane inlet mass spectrometer in order to acquire simultaneous measurements of photosynthetic gas exchange. This system was essentially as described above with some modification. In this system the 2.5 mL cell suspension was illuminated from the top and the custom-built emitter detector unit for fluorescence measurements was replaced with an ED-101 unit (Walz, Effeltrich).

Preparation of CO_2 Solutions

A solution of $^{13}CO_2$ was prepared by injecting 10 µL of 1 N HCl into a 10 mM solution of $NaH^{13}CO_3^-$ in a small glass vial such that virtually no headspace remained. This reduced the pH from 9 to 2.5. These solutions were tightly capped until use.

Quantum Yield Definitions

The quantum yield of photosystem II was defined according to Genty *et al.* (1989):

$$\phi PSII = (Fm' - Fs)/ Fm'$$

where Fm' is the maximum fluorescence yield obtained during a saturating flash and Fs is the steady-state fluorescence yield. The quantum yield of photosystem I was defined according to Klughammer and Schreiber (1994):

$$\phi PSI = (A_{max} - A_{ss})/ \Delta A_{\ 100\%\ red \rightarrow 100\%\ ox}$$

where A_{ss} and A_{max} represent the relative amounts of P700 absorbance before and during a saturating flash, respectively. The denominator represents the maximum P700 absorbance change from darkness induced with a combination of far-red light and saturating white light and is proportional to the total number of active PS1 centres (Klughammer and Schreiber, 1994).

Results

The rates of electron transport varied according to the conditions under which the measurements were made. In the presence of saturating Ci, whole chain electron transport (WCET) was maximised (Fig. 1, Panel A). Conversely, when the rate of photosynthesis was severely limited by the rate of HCO_3^- injection, the overall rate of WCET was likewise limited and the extent of cyclic electron transport was proportionately greater under these conditions (Fig. 1, Panels A and B). Under severe Ci limitation the $\phi PSI/\phi PSII$ ratio increased to 4 indicating a relatively higher electron flux around PSI. Thus, meaningful and reliable estimates of electron transport rates under different experimental conditions could be made with this system.

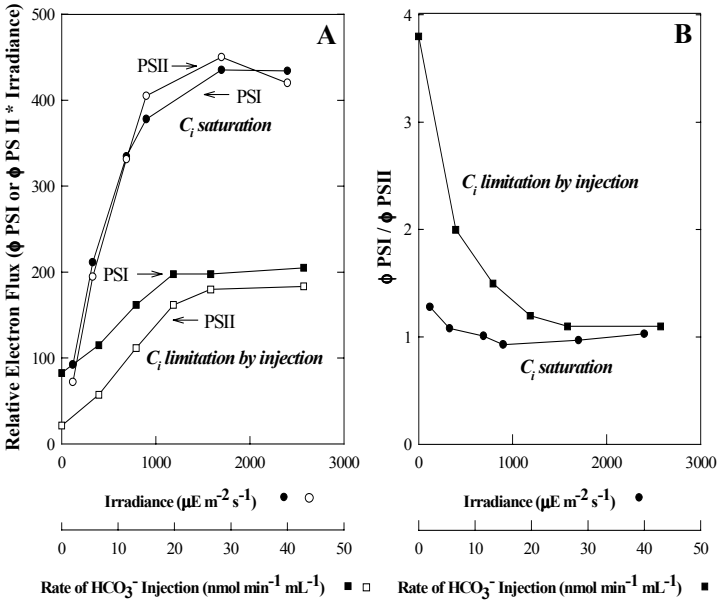

Figure 1. A, Response of relative electron flux through PSI and PSII to light and HCO_3^- injection in cells of *Synechococcus* PCC 7942 adapted to 20 ppm CO_2. Cells (15 μg^{-1} Chl · mL^{-1}) were supplemented with 2 mM HCO_3^- and the desired irradiance was obtained with the use of neutral density filters (circles). For HCO_3^- injection experiments, cells were allowed to reach the CO_2 compensation point at which time HCO_3^- was injected to the desired rate with an infusion pump (squares). B, ϕPS1/ϕPS2 ratios calculated from the data shown in Panel A.

The relationship between ϕPSI and ϕPSII can provide insight into the relative amounts of cyclic and linear electron transport proceeding when photosynthesis is changing. It is readily apparent that there was no greater engagement of PSI during CO_2-driven photosynthesis compared to the HCO_3^- treatment shown in Fig. 2. Under both conditions, the ratio ϕPSI/ϕPSII ranged from approximately 6-10 indicating that there was considerable cyclic electron flux around PSI under CO_2 limitation but no more so than when under HCO_3^- limitation.

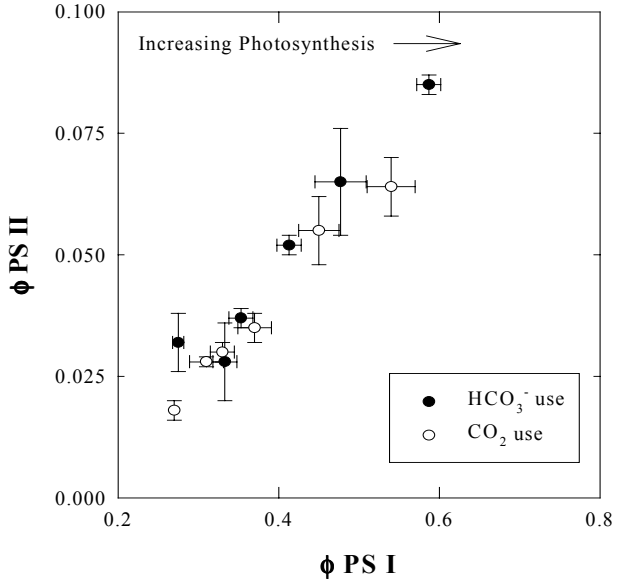

Figure 2. The relationship between ϕ PSI and ϕ PSII during CO_2 or HCO_3^- driven photosynthesis in 20 ppm CO_2 adapted cells of *Synechococcus* PCC 7942. Measurement of quantum yields were made according to the protocol described in Material sand Methods. CO_2 solutions were made by acidifying 10 mM solutions of $NaHCO_3$. Values are the means ± s.e. of 3 determinations.

Discussion

The system described above is useful for making measurements of steady-state photosynthetic electron flux in *Synechococcus*. This is demonstrated by Fig. 1 where reliable estimates of the rates of relative electron transport through photosystems I and II were made under conditions where the rate of photosynthesis was increased by increasing the irradiance (light-limited) or by increasing the rate of HCO_3^- injection (Ci-limited). Under conditions of light limitation, the V_{max} of linear electron transport was high and, under these conditions, electron flux through both photosystems should be closely matched. This was observed in *Synechococcus* PCC7942 and confirmed the reliability of the ϕPSI estimate. When the rate of electron transport was limited by keeping the rate of HCO_3^- injection low, cyclic electron transport was proportionately greater than under conditions of light limitation. It was desirable to apply the measurement system to *Synechococcus* PCC7942 cells in which both high affinity HCO_3^- and CO_2 transport systems had been induced to see if differences in the relative amounts of linear and cyclic electron transport could be seen. The response of ϕPSI and ϕPSII to Ci did not depend on the form in which it was presented to the cells. The relative amounts of linear and cyclic electron transport appear to be very similar when photosynthesis is driven by HCO_3^- or CO_2 in cells of *Synechococcus* PCC7942 with a high affinity for Ci. This suggests that CO_2 transport is energised in a similar manner to HCO_3^- transport. Recent models of CO_2 transport suggested that energisation occurred via cyclic electron transport around PSI catalysed by specialised NDH-1 complexes (Kaplan and Reinhold, 1999). If this mechanism for CO_2 transport were the sole one operating in cells with high affinity for Ci, then under conditions where photosynthesis is driven primarily by CO_2 transport there should be a much greater engagement of PSI, relative to PSII, than when photosynthesis is driven by HCO_3^-. This was clearly not the case. Since it is now clear that multiple systems for CO_2 and HCO_3^- transport exist in *Synechococcus* PCC7942*,* targeted mutagenesis of these systems and the subsequent analysis of the mutants should provide insight into which systems depend upon cyclic or linear electron transport for energisation.

References

Badger MR and Gallagher A (1987) *Aust. J. Plant Physiol*. **14,** 189-201.
Genty B, Briantais J-M and Baker NR (1989) *Biochim. Biophys. Acta* **990**, 87-92.
Kaplan A and Reinhold L (1999) *Annual Rev. Plant Physiol Plant Mol. Biology* **50**, 539-570.
Klughammer C and Schreiber U (1994) *Planta* **192**, 261-268.
Mi H, Endo T, Schreiber U, Ogawa T and Asada K (1992) *Plant Cell Physiol*. **33,** 1233-1237.
Ogawa T (1991) *Proc Natl Acad Sci U S A* **88,** 4275-4279.
Ohkawa H, Pakrasi H and Ogawa T (2000) *J. Biol. Chem*. **275**, 31630-31634
Price GD, Sultemeyer D, Klughammer B, Ludwig M and Badger MR (1998) *Can. J. Bot* **76**: 973-1002.
Sültemeyer D, Klughammer B, Badger MR and Price GD. (1998) *Plant Physiology* **116,** 183-192.