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# Investigating changes in the redox state of Photosystem I at low pH

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ABSTRACT

Changes in the redox state of Photosystem I (PSI) were studied in spinach leaf discs suspended in buffers of different pH (pH 7.5, 6.5, 5.5 and 4.5). By measuring absorbance changes at 820 nm, it was observed that under normal conditions, the electrons were supplied by Photosystem II (PSII) for the photo-oxidation of P700 while in the presence of DCMU when electrons coming from PSII are blocked, cyclic electron flow (CEF) around PSI was the major source for the absorbance changes observed at 820 nm. This was supported by complete inhibition in the reduction of both single turnover (ST) area and multiple turnover (MT) area, in the presence of DCMU, which is generally filled up by the electrons coming from PSII. In the absence of DCMU, the intersystem electron pool or plastoquinone (PQ) pool was increased at low pH which was probably due to enhanced cyclic electron flow around PSI. Our results also suggest that at low pH, in the absence of DCMU, the major contribution for faster dark re-reduction of $P700^+$ is attributed mainly by PSII and CEF PSI while in the presence of DCMU, the significant contribution is provided by CEF PSI and other stromal components.



## 1. Introduction

Oxygen-evolving photosynthesis operates with the involvement of two photosystems (PSI or P700 and PSII or P680). Light energy absorbed by antenna pigments is transferred to the photosystem reaction centres and is converted to assimilative power (ATP and NADPH) via a series of electron transporters [1], a major part of which is used for $CO_2$ assimilation. Photosynthetic electron transport is comprised of non-cyclic electron transport from $H_2O$ to $NADP^+$, cyclic electron transport from reduced ferredoxin (Fd) or NADPH recycling to plastoquinone (PQ) or the cytochrome *b6f* complex, and a number of $O_2$ consuming alternative pathways. The function of non-cyclic electron transport has been well studied, but the physiological function of PSI cyclic electron transport has not yet been clarified. Depending on the physiological state of the plant and the environmental conditions, alternative pathways of electron flow provide considerable regulatory flexibility in terms of NADPH/ATP ratios. Effective regulation is essential for survival under light-limited conditions as well as for protection against damage by excess radiation [2].

Excitation energy captured by Photosystem I (PSI) antenna pigment is efficiently transferred to the reaction centre (RC), where either charge separation takes place or the energy is dissipated in form of heat and fluorescence. After this charge separation, a positive charge resides on a special chlorophyll dimer called P700. In studies on the PSI trapping kinetics, $P700^+$ is usually chemically reduced to re-open the RCs. Photosystem I plays a major role in the light harvesting reaction of photosynthesis. The common view is that the P700 is the primary electron donor, after charge separation the released electron is transferred along the electron transport chain: $A_0$ (Chl a), $A_1$ (phylloquinone), and the $Fe_4S_4$ clusters $F_X$, $F_A$, and $F_B$, reviewed in Brettel [3]. Alternatively, it has been proposed that the accessory chlorophylls, located in the proximity of P700, are instead the primary electron donor, while P700 only gets oxidized in the secondary electron transfer step [4,5]. If PSI is in its natural environment, i.e., associated with the thylakoid membrane in cyanobacteria or chloroplasts, the electron from $F_B$ is donated to ferredoxin (or flavodoxin), while the hole on $P700^+$ is filled by an electron coming from plastocyanin [6].

In photosynthesis, light driven electron flow leads to translocation of protons into the thylakoid lumen by two means: first by splitting of water and second by transfer of protons across the thylakoid membrane leading to generation of proton motive force (pmf) and thus causing acidification of the lumen. It functions both to drive the synthesis of ATP as well as initiate processes that down-regulate photosynthesis.

Low pH has been shown to have a role in driving state transitions [7], inhibiting the donor and the acceptor side of PSII [8] and causing structural re-organization in the thylakoid membranes [9]. Jajoo et al. [10] suggested that low pH induces regulation of excitation energy between the two photosystems and this

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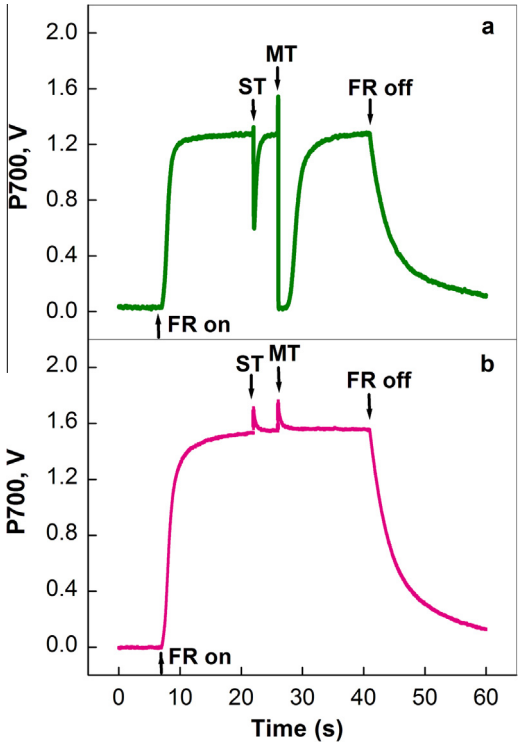


**Fig. 1.** A typical light induced P700 transients measured as $\Delta A_{820}$. After reaching a steady-state level of P700 oxidation ($P700^+$) by far-red (FR) illumination, single turnover (ST) and multiple turnover (MT) flash pulses of red saturating light were applied, (A) in the absence and (B) in the presence of DCMU. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

regulation of energy is induced by the protonation of PsbS protein leading to spillover of excitation energy towards PSI. Further, by using a proton blocker, e.g. DCCD, it was shown that low pH does regulate distribution of excitation energy between the two photosystems [11]. Based on the measurement of light response curves, enhancement in the rate of cyclic electron flow around PSI at low pH was suggested [12].

P700 can be readily oxidized upon illumination by far red light (FR) that is preferentially absorbed by PSI pigments. Its oxidized form displays a broad absorbance peak around 810–840 nm [13] and is relatively stable. It is oxidized upon illumination, with the electron being transferred to ferredoxin, eventually leading to NADP reduction and $CO_2$ fixation. It is re-reduced via the intersystem electron transport chain by PSII activity, with electrons originating from water splitting. P700 absorbance changes provide very similar information on PSI (state of acceptor and donor sides, fluorescence quantum yield etc.) as chlorophyll fluorescence provides on PSII.

This work demonstrates low pH induced redox changes in P700, in the absence and in the presence of DCMU. The various PSI kinetic studies like the photo-oxidation of PSI, ratios of the reduction areas of multiple turn (MT)/single turn (ST) ($MT_{area}/ST_{area}$) and the dark re-reduction kinetics ($t_{1/2}$) of P700 at low pH have been studied. The redox properties of P700 have not been investigated earlier under low pH conditions.

## 2. Material and methods

### 2.1. Plant material

Spinach (*Spinacea oleracea*) leaf discs.

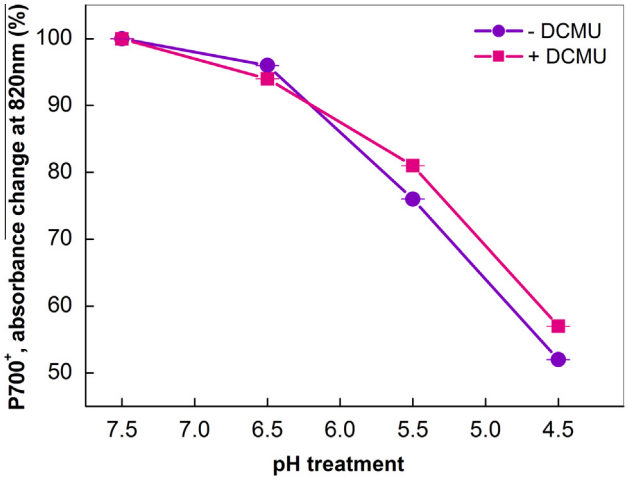


**Fig. 2.** Changes in the steady state level of P700 photo-oxidation in the absence and presence of DCMU, measured at different pH (pH 7.5, 6.5, 5.5 and 4.5) in spinach leaf disc. Each data represents the mean ± SD of three independent replicates.

### 2.2. pH treatment to spinach leaf disc

Fresh spinach leaves were brought from the market and cut into small discs which were transferred to the Petri plates containing 50 mM buffer solution of different pH. For pH 7.5 (HEPES-NaOH), for pH 6.5 and 5.5 (MES-NaOH) and for pH 4.5 (Sodium acetate–Acetic acid) buffer was used. Leaf discs were incubated at respective pH buffer in dark for 4 h.

### 2.3. DCMU treatment to spinach leaf disc

The leaf discs were immersed in DCMU solution (the DCMU concentration was 200 μM, and the solution contained 1% ethanol, which was used to dissolve the DCMU, as described in Toth et al. [14]). The duration of the treatment was ~4 h, and it was carried out in complete darkness. Following the treatment, the leaf discs were removed from DCMU solution (in darkness), wiped and left in air for 15 min before measurement.

### 2.4. P700 transient measurement

Far red (FR) light induced oxidation of P700 to $P700^+$ was determined in pH treated leaves at room temperature by Dual-PAM-100 (Heinz Walz Effeltrich, Germany). It is equipped with a dual wavelength (830 nm/875 nm) emitter detector ED-P700DW unit connected to a computer with control software. The redox state of P700 was evaluated as the absorbance change around 820 nm ($\Delta A_{830-875}$) due to the cation radical ($P700^+$). The transient reduction of $P700^+$ signal after application of single turn (ST) and multiple turn (MT) saturating flashes of red saturating light were used for estimation of the apparent intersystem electron ($e^-$) pool size [15,16]. MT saturating flashes (50 ms) and ST saturating flashes (50 μs) were applied. The complementary area between the oxidation curve of P700 after ST and MT excitation and the stationary level of $P700^+$ under FR illumination represent the ST and MT areas respectively. They were used for estimation of the functional pool size of intersystem electrons on a P700 reaction centre which was determined as: $e^-/P700 = MT_{area}/ST_{area}$ [17,18]. After switching off the far red light the re-reduction of P700 takes place. From the re-reduction curves of P700, decay kinetics ($t_{1/2}$) was calculated using origin 6.1.