BRIEF COMMUNICATION

Proton concentration in the thylakoid membranes can regulate energy distribution between the two photosystems

T. TONGRA, S. BHARTI, and A. JAJOO*

School of Life Science, Devi Ahilya University, Indore 452 017, M.P., India

Abstract

The aim of our study was to investigate the role of protons in regulating energy distribution between the two photosystems in the thylakoid membranes. Low pH-induced changes were monitored in the presence of a proton blocker, N,N'-dicyclohexylcarbodiimide (DCCD). When thylakoid membranes were suspended in a low-pH reaction mixture and incubated with DCCD, then a decrease in the fluorescence intensity of photosystem II (PSII) was observed, while no change in the intensity of photosystem I (PSI) fluorescence occurred according to the measured fluorescence emission spectra at 77 K. Since low pH induced distribution of energy from PSII to PSI was inhibited in the presence of DCCD, we concluded that pH/proton concentration of the thylakoid membranes plays an important role in regulating the distribution of the absorbed excitation energy between both photosystems.

Additional key words: light-harvesting complex; luminal pH; nonphotochemical quenching; oxygen consumption; oxygen evolution; spinach.

Photosynthetic reactions in plants and green algae fix CO2 by converting solar energy into electrochemical energy with the contribution of sequentially functioning PSII and PSI. In the nature, unexpected changes in light intensity could lead to overexcitation of the photosystems. Photosynthetic efficiency is one of the key factors which regulate plant growth and development. To balance the rate of photosynthesis under changing environmental conditions, several mechanisms have been evolved, such as protective, nonphotochemical quenching (NPQ), state transitions, and etc. Among these mechanisms, quenching of chlorophyll (Chl) fluorescence (qe), a feedback regulatory process of PSII dissipates excessive light energy safely as heat on luminal acidification of the thylakoid membranes (Tokutsu and Minagawa 2013).

In higher plants, induction of qE depends on activation of the xanthophyll cycle (Horton et al. 1996, Niyogi et al. 1997) as well as on the sensing of luminal acidification or proton concentration by PsbS, a protein homologous to light harvesting complex (LHC) proteins (Li et al. 2004). Electron microscopic studies have revealed that PsbS protonation regulates the macro-organisation of PSII complexes in the grana membranes of higher plant chloroplasts (Kereiche et al. 2010). A role of PsbS in regulating cyclic electron flow by controlling the tight coupling between cytochrome b/f and PSI was proposed (Roach and Krieger-Liszkay 2012). Protonation of PsbS drives concerted conformational changes in light harvesting complex of PSII (LHCCI) leading to qE (Kiss et al. 2008). Direct interactions of LHCs of PSII with PsbS monomers have been recently shown to regulate NPQ in Arabidopsis (Wilk et al. 2013). This sensing of pH by PsbS is a natural device to protect PSI from damage. Protonation of PsbS induces a change in the macro-organization of the thylakoid membranes (Horton et al. 2008, Kereiche et al. 2010) that results in the aggregation of LHCCI proteins and/or induces conformational change(s) in LHCCI, generating energy-quenching sites in the LHCCI aggregates (Johnson et al. 2011) and establishing a low-fluorescence state that is measurable as NPQ. Thus both PsbS protein and zeaxanthin are thought to have crucial roles in qE quenching in higher plants. It is also known that the LHC plays a vital role in the energy distribution between the two photosystems (Allen and Forsberg 2001, Cardol et al. 2003). LHC is not a static structure but it is able to interact with both the photosystems to maximize photosynthetic efficiency (Iwai et al. 2010).

Received 22 January 2014, accepted 4 April 2014.

*Corresponding author; phone: 91-731-2477166, fax: 91-731-4263453, e-mail: anjanajajoo@hotmail.com

Abbreviations: DCCD – N,N'-dicyclohexylcarbodiimide; NPQ – nonphotochemical quenching; qE – energy-dependent quenching.

Acknowledgement: Supports from University Grant Commission, India (No. F.5-26/2007(BSR) to T. Tongra and from Department of Science and Technology, India (INT/RFBR/P-173) to A. Jajoo are thankfully acknowledged.
It is well known that illumination of dark-adapted chloroplasts leads to the alkalization of stroma and acidification of thylakoid lumen and generates proton gradient and electrical potential difference across the thylakoid membrane (Kramer et al. 1999). Low pH in thylakoid lumen is a result of proton release from water molecules in the oxygen-evolving complex and proton influx from stroma which is mediated by plastoquinone. It is evident that the pH of the medium significantly affects the proton flux across the thylakoid membranes and also suggests that under physiological condition, the luminal pH in chloroplasts declines to around pH 5.5. At this pH, a fine tuning of the other electron transport components occurs to ensure proper functioning of PSII (Kramer et al. 1999, Waloszek and Wieckowski 2004).

Energy distribution between the two photosystems is regulated by a number of factors including light intensity (Wollman 2001, Tikkanen et al. 2012) and ionic environment of the thylakoid membrane (Jajoo et al. 1998, Singh-Rawal et al. 2010). To maintain maximal rates of photosynthesis at limiting light intensities, plants have evolved a mechanism that enables them to optimize the balance of incoming light energy between PSI and PSII (Bergantino et al. 2003, Tikkanen et al. 2012) by the mechanism called “state transitions” (Minagawa 2011).

In higher plants, effects of low pH on photosynthetic machinery are well documented. At low luminal pH, protonation of carboxyl groups of PSII Chl a/b antenna proteins induces conformational change in the PSII antenna which in turn generates energy-quenching centers (Horton et al. 1996). It was hypothesized that low luminal pH might have a role to drive state transitions (Singh-Rawal et al. 2010) and it induces structural reorganization in the thylakoid membranes (Jajoo et al. 2012, 2014). The PsbS monomer to dimer ratio in isolated thylakoid membranes varies with luminal pH, where at acidic pH, the monomeric and the active form for NPQ prevails (Bergantino et al. 2003).

DCCD has been shown to (1) bind to the lumen-exposed carboxyl groups of antenna proteins (Horton et al. 1996, Walters et al. 1996, Jahns and Heyde 1999) and (2) inhibit the pH-induced fluorescence quenching (Ruban et al. 1998, Liu et al. 2008). DCCD binds to the acidic sites close to the lumen surface (as found for CP26), and putative Chl-binding sites in the hydrophobic interior (as for CP29) (Ruban et al. 1998). At the site of water oxidation, DCCD stops the extrusion of protons into the lumen and at the site of plastoquinone reduction, it stops the uptake of protons from the medium (Jahns et al. 1988). DCCD also alters the pH dependence of violaxanthin de-epoxidase activity (Jahns and Heyde 1999). Thus it is widely used as a proton blocker.

Singh-Rawal et al. (2010) suggested that acidification of lumen leads to the protonation of specific glutamate residues that induces a conformational change in LHCCI resulting in the detachment of LHCCI from PSII and its migration and attachment towards PSI. In other words, all these observations suggest that protons in the lumen bind to certain residues (may be PsbS or LHCCI or some others) and thus control various elements of nonphotochemical quenching. In this study, we blocked the proton binding sites by treating the thylakoids with a proton blocker DCCD. In such case, free binding sites are not available for protonation of any of the residues and thus energy distribution caused by low pH-induced changes should not be observed. The work presented in this paper provides a new insight regarding the mode of action of proton concentration in thylakoid membranes.

Thylakoid membranes were prepared from fresh market spinach (Spinacia oleracea) in the following manner: The spinach leaves were washed with distilled water, dried, deveined, and then kept overnight in a refrigerator for dark incubation. Approximately 100 ml of isolation medium (0.33 M sucrose, 50 mM HEPES-NaOH, pH 7.5, 1 mM MgCl₂, and 10 mM NaCl) was used per 20 g of spinach leaves. Homogenate was then filtered through four layers of nylon gauze and four layers of cheese cloth. Filtrate was then centrifuged at 7,500 × g for 8 min at 4°C. The pellet was suspended in shock medium (50 mM HEPES-NaOH, pH 7.5, 1 mM MgCl₂, 10 mM NaCl) and kept in the dark for 3 min. The homogenate was again centrifuged at 7,500 × g for 8 min at 4°C. The pellet was resuspended in isolation medium (pH 7.5). The Chl concentration of fresh membranes was measured following Porra et al. (1989) and the suspension was kept in liquid nitrogen till further use.

Thylakoids were treated at different pH (7.5, 6.5, and 5.5) by suspending the membranes in isolation medium of different pH for 30 min in the dark. DCCD was added (from an 20 mM ethanolic stock solution to give a final concentration of 50 µM) in 2 ml of the reaction mixture of respective pH containing 20 µg Chl of thylakoid membranes. Incubation of thylakoids with DCCD was carried out at room temperature for 15–20 min in the dark. All measurements were performed five times and averaged spectra were presented.

Chl a fluorescence emission spectra at low temperature (77 K) were recorded in a spectrofluorimeter (FP-6300 model, Jasco, Japan) equipped with a liquid nitrogen dewar container with a continuous flow of nitrogen gas at room temperature from beneath to avoid formation of ice crystals from the water vapour. The reaction mixture and measurement conditions are described in Singh-Rawal et al. (2010).

PSII activity was measured in terms of [µmol(O₂ evolved) mg(Chl)⁻¹ h⁻¹]. The PSI activity was measured in terms of [µmol(O₂ consumed) mg(Chl)⁻¹ h⁻¹] by monitoring N, N, N, N-tetramethyl-p-phenylenediamine (TMPD) to methyl viologen (MV) reaction. Both measurements were carried out using a Clarke-type oxygen electrode (Hansatech, King Lynn, UK). Reaction mixture and measurement conditions are as described in Singh-Rawal et al. (2010).

The redox state of P700 was monitored by measuring the absorbance changes at 820 nm using PAM-100
Decrease in fluorescence of PSII with the high proton

state transition took place (Singh-Rawal et al. 2010). Low-temperature fluorescence emission spectra of the thylakoid membranes treated with different pH (7.5, 6.5, and 5.5) were measured in spinach thylakoid membranes treated with different pH (7.5, 6.5, and 5.5). As evident from Fig. 1C, lowering of the bulk pH of the thylakoid membranes resulted in a consistent decrease in the fluorescence emission at 730 nm.

An increase in the F730/F685 ratio suggested that PSI received more energy at the expense of PSII, or in other words, the rate of further excitation of PSI increased. However, in the presence of DCCD, no change in PSI fluorescence was observed in the fluorescence emission spectra (Fig. 1B). Decrease in PSI fluorescence might occur because of activation of energy-dependent fluorescence quenching (qE) which is a type of NPQ. However, in the presence of DCCD, no change in PSI fluorescence indicated that PSI had received extra energy. Probably DCCD treatment led to covalent modification in carboxyl groups of LHCIIs subunits which are involved in protonation. Further rearrangement of the molecule finally forms the stable dicyclohexyl-N-acyl urea (Azzi et al. 1984). Hence the ion-binding sites accommodating the structure have no bound H+ available for transport because of neutralization of the conserved carboxylic groups.

DCCD has been proposed to influence the ring structure in a way that mimics the arginine residue in the subunit (Pogoryelov et al. 2009, Mizutani et al. 2012).

Fig. 1C represents fluorescence excitation spectra (77 K) at 730 nm at each pH value, normalized at 710 nm. The results showed that the thylakoid membranes treated at pH 6.5 and 5.5 received more excitation energy from Chls absorbing at around 683–684 and 654–655 nm, indicating that the absorption cross-section of the PSI light-harvesting complex (LHCl) had increased because of the association of LHCII. Furthermore, no change in PSI fluorescence excitation spectra was observed with DCCD treatment (Fig. 1D).

To confirm the results obtained with 77 K fluorescence emission measurements, rates of electron transport through PSII and PSI were monitored at different pH (Table 1). It is clear that at low pH, rates of electron transfer through PSII decreased, while that of PSI increased. Increased rate of re-reduction of P700 also suggested that PSI is getting more electrons at low pH. However, in the presence of DCCD, pH-induced effects

concentration/acidification of thylakoid membranes suggests a low pH inducible mechanism in PSII that dissipates excess light energy, measured as the qE. Another probability is that binding of both protons and xanthophylls to specific sites in the PSII antenna causes a conformational change within LHClIs that switches PSII units into a quenched state, generating energy-quenching site(s) in the LHClII and establishing a low fluorescence state that is measurable as NPQ (Horton et al. 2005, Johnson et al. 2011, Tokutsu and Minagawa 2013). Low pH can also lead to accumulation of P680. The result is that whatever amount of energy is trapped by LHClIs it is not utilized properly in the photochemical reaction of PSII.

Fig. 1. Low-temperature (77 K) fluorescence emission spectra of the thylakoid membranes treated with different pH (7.5, 6.5, and 5.5) without addition of DCCD (A) and in the presence of DCCD (B). Low-temperature fluorescence excitation spectra of the thylakoid membranes treated with different pH (7.5, 6.5, and 5.5) without addition of DCCD (C) and in the presence of DCCD (D). The emission spectra have been normalized at 710 nm, while the excitation spectra have been normalized at 705 nm.
were observed almost to the same extent. Although it is known that DCCD inhibits PSII and Cyt b/f, rates of PSI were not found to be inhibited in the presence of DCCD. This is because of the use of artificial electron donors and acceptors which have their site of action beyond Cyt b/f.

Our finding suggests that low pH (higher proton concentration) in the thylakoid membranes controls energy distribution in favour of PSI. If the binding of protons is inhibited by the use of a proton binder such as DCCD, pH induced effects were not observed. However, whether the change in energy redistribution is a result of spillover or state transitions is yet to be explored. Further work on mutants which cannot perform state transitions (e.g., stn7) may resolve this issue.

### References


---

**Table 1. Change in the rates of electron transport of PSII and PSI under different pH conditions in the presence and absence of N,N’-dicyclohexylcarbodiimide (DCCD).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate of oxygen evolution [μmol(O2 evolved) mg(Chl)-1 h]-1</th>
<th>Rate of oxygen consumption [μmol(O2 consumed) mg(Chl)-1 h]-1</th>
<th>P700+ reduction kinetics [s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.5</td>
<td>257 ± 12</td>
<td>345 ± 15</td>
<td>16.2 ± 0.5</td>
</tr>
<tr>
<td>pH 7.5 + DCCD</td>
<td>249 ± 10</td>
<td>337 ± 15</td>
<td>15.7 ± 0.4</td>
</tr>
<tr>
<td>pH 5.5</td>
<td>188 ± 12</td>
<td>490 ± 14</td>
<td>11.7 ± 0.2</td>
</tr>
<tr>
<td>pH 5.5 + DCCD</td>
<td>176 ± 12</td>
<td>463 ± 15</td>
<td>12.2 ± 0.4</td>
</tr>
</tbody>
</table>

ERRATUM DOI: 10.1007/s11099-014-0054-5

Erratum to: Accumulation of photosynthetic pigments in Larix decidua Mill. and Picea abies (L.) Karst. cotyledons treated with 5-aminolevulinic acid under different irradiation

N. MAXIMOVÁ*+ and Ľ. SLOVÁKOVÁ**

Institute of High Mountain Biology, University of Žilina, 05956 Tatranská Javorina 7, Slovak Republic*
Department of Plant Physiology, Faculty of Natural Sciences, Comenius University, Mlynská dolina, 84215 Bratislava, Slovak Republic**

Erratum to: Photosynthetica
DOI: 10.1007/s11099-014-0019-8

The acknowledgements were completed in the corrected version:

Acknowledgements: This work was supported by Structural Funds of EU Project of the Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic, Bratislava, ITMS No. 26110230078 and the Slovak Research and Development Agency (APPV-20-020805). We thank Y. Fujita, J.-D. Rochaix, and B. Grimm for providing the primary antibodies used in this work. We thank MSc Robin Rigg for English correction.

The publisher and authors apologize for these errors and for inconveniences they may have caused.

The online version of the original article can be found under doi: 10.1007/s11099-014-0019-8

*Corresponding author; e-mail: maximova@uniza.sk