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Salt shock induces state II transition of the photosynthetic apparatus in dark-adapted *Dunaliella salina* cells

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Abstract

Previous evidence indicates that hypoosmotic shock decreases the photosynthetic rate and induces a state I transition of the photosynthetic apparatus in the dark-adapted halotolerant green alga, *Dunaliella salina*. The present study investigated the regulation of state transition by salt shock, another stressor *D. salina* commonly encounters. An upward-shift in the external NaCl concentration decreased the photosynthetic rate, the photosystem II (PSII) maximal fluorescence, and the respiratory rate of *D. salina*. An ATP synthase inhibitor, an uncoupler, or salt shock decreased intracellular ATP content and induced phosphorylation of the major light harvesting chlorophyll *a*/*b* proteins in dark-adapted *D. salina* cells. Furthermore, salt shock increased cyclic electron flow around photosystem I (PSI), as indicated by the enhancement in the post-illumination transient increase in chlorophyll fluorescence. These results suggest that salt shock induces state II transition in dark-adapted *D. salina* cells and that ATP content depression is likely involved in the regulation. Together, salt shock and hypoosmotic shock exert opposite effects on state transition and may decrease the photosynthetic rate via different mechanisms.
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Keywords: ATP content; LHCII phosphorylation; Salt shock; State transition; *Dunaliella salina*

1. Introduction

Photosynthesis in plants is mediated via photosystem I (PSI) and photosystem II (PSII), both of which use light energy to drive redox processes. When plants are exposed to transient short-term changes in light intensity and quality, the imbalance in excitation rates of PSII and PSI influence the redox state of intersystem electron carriers (e.g. the plastoquinone and the cytochrome b_6f complex) which then initiates a compensatory state transition mechanism (Allen, 1992; Haldrup et al., 2001; Allen and Forsberg, 2001; Aro and Ohad, 2003). The reduced plastoquinone pool induces state II transition by activating membrane-bound kinases that phosphorylate the major light harvesting chlorophyll *a*/*b* proteins (LHCII). The phosphorylated LHCII migrates from the PSII to PSI domains, where it preferentially provides PSI with light energy. State I transition occurs as a consequence of the oxidation of the plastoquinone pool and inactivation of LHCII kinase, resulting in LHCII dephosphorylation and its reassociation with PSII (Allen, 1992; Haldrup et al., 2001; Allen and Forsberg, 2001; Aro and Ohad, 2003). In this way, state transitions balance the energy distribution between PSI and PSII and, thus, optimize light utilization.

In dark-adapted *Chlamydomonas* cells, ATP depression can induce state II transition associated with LHCII phosphorylation via chlororespiration-related reduction of plastoquinone, whereas the restoration to state I requires net ATP synthesis (Bulté et al., 1990). Under state II conditions, phosphorylated LHCII is accompanied by migration of the cytochrome b_6f complex from the PSII to PSI domains, thus increasing cyclic electron flow and ATP synthesis upon re-illumination (Bulté et al., 1990; Vallon et al., 1991; Finazzi et al., 1999, 2002). Together, these previous reports indicate that state transition in *Chlamydomonas* responds to intracel-

Abbreviations: Chl, chlorophyll; DCCD, dicyclohexylcarbodiimide; LHCII, light harvesting chlorophyll *a*/*b* binding proteins; PSI, photosystem I; PSII, photosystem II

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lular ATP demands (Wollman, 2001; Rochaix, 2002; Zer and Ohad, 2003).

The general cell structure of halotolerant green alga *Dunaliella salina* resembles that of *Chlamydomonas*, but differs in that *D. salina* lacks a rigid cell wall (Ben-Amotz and Avron, 1973; Gilmour et al., 1985) and, most notably, adapts to salinity ranging from 50 mM to 5 M by regulating intracellular glycerol content (Ben-Amotz and Avron, 1973; Gilmour et al., 1985). Although Harrison and Allen (1993) have investigated how changes in light regime regulate the state transition in the halotolerant green alga, the effects of other environmental factors on state transition remain unknown. Recently, we reported that hypoosmotic shock induced a state I transition in dark-adapted *D. salina*. The present experiments investigated the effects of salt shock, another osmotic stress encountered by *D. salina*, on state transition by determining the changes in ATP content, LHCII phosphorylation level, and chlorophyll fluorescence.

2. Materials and methods

2.1. Plant materials and experimental treatments

D. salina cells were grown in an artificial hypersaline medium containing 1.5 M NaCl at 25 °C with continuous shaking (100 rpm) under 12 h light (fluorescent lamp, 70 μmol photons $m^{-2} s^{-1}$)/12 h dark conditions (Liu and Shen, 2004a). When the concentration of chlorophyll (Chl) was approximately 12 μg/ml, as determined by the method of Arnon (1949), *D. salina* cells were placed in the dark without shaking for approximately 3 h. Unless otherwise indicated, salt shock was induced by incubating dark-adapted cells (acclimated to 1.5 M NaCl) in artificial hypersaline media containing 2.5 M NaCl. Cells were incubated at 25 °C for 20 min without shaking. Treatments with dicyclohexylcarbodiimide (DCCD, 5 μM) or nigericin (5 μM) were performed by incubating dark-adapted cells in the presence of either reagent for 20 min without shaking.

2.2. Photosynthesis and respiration measurement

Oxygen evolution by photosynthesis and uptake by respiration were measured at 25 °C with a Clark-type oxygen electrode as described previously (Liu and Shen, 2004a). The reaction medium was the same as the medium used for resuspending *D. salina* cells. The samples used for the measurement of oxygen evolution and uptake contained 6 and 22 μg Chl/ml. A light of 1000 μmol photons $m^{-2} s^{-1}$ was used for O_2 evolution measurement.

2.3. ATP content measurement

Harvested *D. salina* cells were suspended in 50 mM Tricine–NaOH (pH 8.0) and then killed with boiling water to make ATP released. ATP content was measured with a luciferin–luciferase assay, as previously described (Liu and Shen, 2004a).

2.4. Chlorophyll fluorescence measurement

Chlorophyll fluorescence was measured with a pulse amplitude modulation (PAM) chlorophyll fluorimeter (Walz, Effeltrich, Germany) (Mi et al., 1997; Deng et al., 2003). The modulated measuring beam (1 μs duration, 1.6 kHz, peak emission at 650 nm) was set at 0.05 μmol photons $m^{-2} s^{-1}$. Actinic irradiation (20 s duration, 250 μmol photons $m^{-2} s^{-1}$) was provided by a halogen lamp. For measurement of maximal fluorescence (F_m), a saturating pulse (1000 μmol photons $m^{-2} s^{-1}$) was provided by a LED-array cone (high-power LED lamp, Walz).

2.5. Thylakoid membrane isolation and immunoblot analysis

Thylakoid membranes were isolated as previously described (Kim et al., 1993; Liu and Shen, 2004a). In brief, dark-adapted *D. salina* cells were collected and suspended in sonication buffer (100 mM Tris–HCl, pH 6.8, 5 mM $MgCl_2$, 0.2% polyvinyl pyrrolidone K30, 3 mM aminocaproic acid, 1 mM aminobenzamidine and 0.2 mM phenylmethanesulfonyl fluoride) and then disrupted by sonication for 90 s. Unbroken cells and other large fragments were removed by centrifugation at 3000 × *g* for 3 min at 4 °C. Samples were then centrifuged at 40000 × *g* for 20 min at 4 °C, and the pellets were solubilized in 0.5 M Tris–HCl (pH 6.8), 7% SDS, 20% glycerol and 2 M urea, and then incubated at 50 °C for 30 min. Unsolubilized materials were removed by centrifugation at 3000 × *g* for 5 min. Thylakoid membrane proteins (2.5 μg Chl per sample) were resolved by SDS–PAGE in a 15% resolving gel and a 5% stacking gel, both of which contained 4 M urea. SDS–PAGE-resolved thylakoid membrane proteins were transferred electrophoretically to a Hybond™ ECL™ nitrocellulose membrane (Amersham Pharmacia) with a semi-dry transfer cell (Amersham Pharmacia) for immunoblot analysis. Phosphorylated LHCII was recognized with rabbit polyclonal anti-phosphothreonine (P-Thr) (Zymed) (Rintamäki et al., 1997).

3. Results

We reported previously that hypoosmotic shock decreases photosynthesis of *D. salina* (Liu and Shen, 2004a). The present results indicate that salt shock (an increase in NaCl concentration from 1.5 to 2.5 M) for 20 min in darkness decreased the oxygen evolution by photosynthesis by approximately 19% when they were placed under light (Fig. 1A). Hypoosmotic shock was observed to exert stimulatory effect on respiration (Liu and Shen, 2004a). In contrast, salt shock decreased the respiratory rate by approximately 35% (Fig. 1B).

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